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### Determination of Indole-3-Acetic Acid (AUXIN) Levels in Plant Species Utilizing Phylogenetic Investigation

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**Abstract:** Plant hormones are chemical messengers that are made in one spot in the body and convey their message in an entirely unexpected place in the body. Auxin is a plant hormone that is responsible for many aspects of plant growth. Studies in experimental biology and biochemical and molecular biological findings relieve that the AMI1 gene family encoding indole-3-acetamide hydrolase is common in the plant kingdom. Phylogenetic analysis is used to analyze the auxin level in any plant provided any nucleotide sequence. An iterative method is used to analyze whether the AMI1 or any gene is present in the given sequences or not. Multiple sequence alignment algorithms are used for the comparison of the plant sequence. Gene sequences and nucleotide sequences which are used for the work is retrieved from NCBI or any Gene bank database. In order to construct the phylogenetic tree Phylogene.fr online tool is used for analyzing the protein sequences.

**Keyword:** Hormones; Auxin; Phylogenetic tree; AMI1 gene; IAA; FAST.

#### 1. INTRODUCTION

Plant hormones are created normally by plants and are vital for directing their own development. They act by controlling or changing plant development procedures, for example, arrangement of leaves and blooms, lengthening of stems, advancement and maturing of natural product. In cutting edge agribusiness, individuals have built up the advantages of broadening the utilization of plant hormones to control development of different plants. Whenever characteristic or engineered substances utilized as a part of this way, they are called Plant Growth Regulators. The use of plant development controllers in farming has begun in the 1930s in the USA. Ethylene, an actually happening substance, is one of the primary plant development controllers being found and utilized effectively to enhance blossom generation in pineapple. Its lethal impacts to people are low. Engineered substances that copy such actually happening plant hormones were additionally delivered; from that point forward the utilization of plant development controllers has been becoming fundamentally and turning into a noteworthy part in cutting edge horticulture. The five major classes are Auxin, Cytokinins, Ethylene, Abscisic Acid and Gibberellin.

In 1880, Charles Darwin suggested that some plant development reactions are directed by a matter which transmits its impact starting with one a player in the plant then onto the next (Darwin and Darwin, 1880). This substance used to be termed Auxin which is now recognized as Indole-3-acetic Acid (IAA) (Kögl and Kostermans, 1934; Went and Thimann, 1937). This phytohormone auxin is a key controller of numerous materials of plant development and growth including cell division and prolongation, separation, tropisms, apical predominance, senescence, abscission, and blossoming (Woodward and Bartel, 2005; Teale et. al., 2006). Albeit diverse plant species may have one of a kind techniques and alterations to advance their metabolic pathways, it would appear to be sensible that plants would share developmentally moderated center components for auxin biosynthesis since IAA is a major substance in the vegetation cycle. Two noteworthy pathways for IAA biosynthesis have been proposed in plants: the tryptophan (Trp)- independent and Trp-dependent pathways (Woodward and Bartel, 2005; Chandler, 2009; Normanly, 2010).

In Trp-independent IAA biosynthesis, a few pathways have been hypothesized (Woodward and Bartel, 2005; Pollmann et. al., 2006a; Chandler, 2009; Mano et. al., 2010; Normanly, 2010; Zhao, 2010):

- The indole-3-acetamide (IAM) pathway;
- The indole-3-pyruvic corrosive (IPA) pathway;
- The tryptamine (TAM) pathway; and
- The indole-3-acetaldoxime (IAOX) pathway.

The different studies demonstrate that the finest chance for the notable pathway of IAA biosynthesis is the IAM pathway or IPA pathway. Numerous analyses prescribe that the generation of IAM in plant cells is basic in IAA biosynthesis. IAM, as a metabolic middle of the road of Auxin biosynthesis is familiar in every plant species. In the IAM pathway, indole-3-acetamide hydrolase, encoded by the AMI1 gene, is generally dispersed in the plant kingdom. By breaking down the statement of the IAM biosynthesis genes, together with AMI1 gene, it is conceivable to at long last decide how, when and where Auxin is integrated in plants.

Many experiments by biologists have proved that the presence of AMI1 gene says that the plants have some level of Auxin present in them. The aim of this research is to build up an approach to decide/discover the nearness of AMI1 gene in any given DNA sequence of any plant species. The level of Auxin is determined by finding the nearness of AMI1 or PLT1 gene sequence in any nucleotide sequences of plants using phylogenetic analysis. Everyday state of affairs in research is that a biologist having a particular sequence of curiosity in hand they need to find the similar sequences and analyze it with different sequences for this they usually use Phylogenetic tree. The Basic Local Alignment Search Tool (BLAST) is the most widely used set of programs for this purpose. A phylogenetic tree is constructed using Neighbor–Joining (NJ) method. Phylogene.fr tool is used to construct the tree.

Yoshihiro Mano at .el. study relieves the importance of the plants hormone Auxin i.e. IAA. According to the study the indole-3-acetamide (IAM) and the indole-3- pyruvic acid (IPA) pathways are best pathway. In the IAM pathway, AMI1 gene which was identified in *Arabidopsis thaliana*, this gene has some level of auxin present in it and it is widely distributed in plant cell. In the TAM pathway the function of YUCs is unknown. By using analyzing the expression of the IAM biosynthesis genes, along with AMI1 gene it is feasible to find the how, when and where auxin is synthesized in plants.

Yoshihiro Mano at .el. study relieves that NtAMI1 gene in *Nicotiana tabacum* will encode the indole-3-acetamide hydrolase, which indicates the Auxin presence in plant kingdom. This work also shows the different AMI1 genes and TOC64 genes with their similarity using Phylogenetic tree.

Mary K. Kuhner et al. compared the five methods of phylogenetic tree estimation: parsimony, compatibility, maximum likelihood, Fitch-Margoliash, and neighbor joining. The study revealed that all the approaches analyzed take part in quite just right and can also be usual to get better or nearly proper tree from a competently tremendous data set.

Phylogene.fr is a free website that is used by many analysts to analyze the biological sequences using phylogenetic analysis. The site is user friendly and also has many options which help in reconstruction and analysis of phylogenetic trees. The site provides three modes – one click, advanced and A la Carte.

BLAST-Explorer is a system that uses the Basic Local Alignment Search Tool algorithm which is used to compare the biological sequences. It helps to remove the gaps, calculate E - value and substitution matrix. BLAST can be utilized as a part of the development of arrangement data sets for the further reconstruction phylogenetic trees.

## 2. MATERIALS AND METHODS

The proposed work deals with inputting different sequences retrieve from DDBJ/GenBank/EMBL and the gene sequences formulated as given in the table below:

**Table 1**  
**Plant AMI1 proteins (Mano, Y et. al., 2012)**

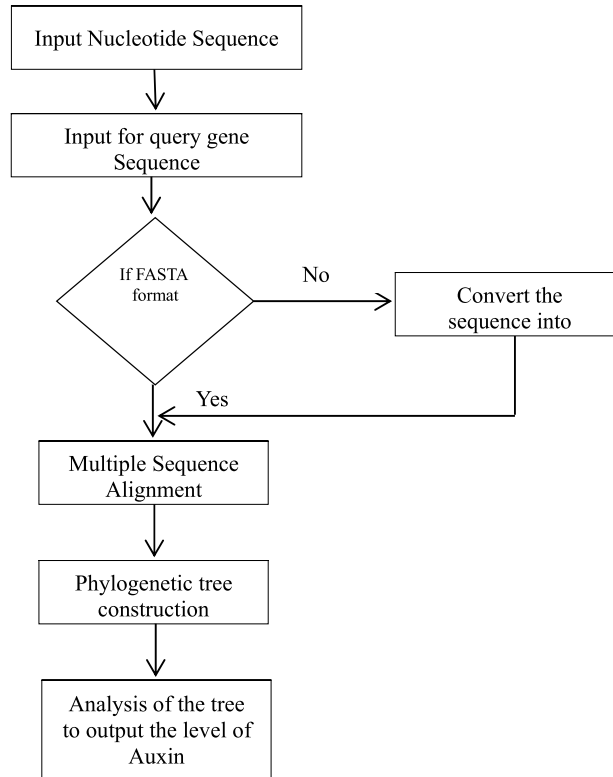
<i>Accession Number</i>	<i>Number of deduced amino acids</i>	<i>Similarity</i>	<i>E-Value</i>
B6ZLB8 (NtAMI1)	425	100	2.3e-174
B9RF45	500	94.8	3.0e-119
A7PSC3	433	94.6	3.6e-124
B9I7X1	427	94.1	1.6e-117
Q9FR37 (AtAMI1)	425	91.9	3.3e-110
Q7XTK3	435	89.6	9.6e-99
C4IZS6	444	89.2	3.5e-100
Q01ML1	434	88.7	7.0e-93

These genes mainly used for the query were NtAMI1 gene found in *Nicotiana tabacum* and AtAMI1 gene found in *Arabidopsis thaliana*. The proposed work for analyzing the gene level is as follows:

- Get the accession number from NCBI and copy the nucleotide sequence in FASTA format to Phylogene.fr.
- If the sequence is not in FASTA reformat it
- Comparison of the sequences using pairwise alignment algorithm.
- Construction of the phylogenetic tree for the aligned sequences.
- Finally analyzed report is displayed to the user.

Figure 1 gives the complete steps of the proposed methodology.

The phylogene.fr has 3 modes: one click, advanced and A la Carte mode. Advanced mode is used in this work so that we can change the setting of the program and also work on the nonaligned sequences. The Sequences were aligned with MUSCLE configured for highest accuracy. The aligned sequences are used for the phylogenetic tree construction. Since dicots plants have more growth problems regarding cell elongation and apical meristem. For this work we have used Tomato, Beans, Pumpkin, Coriander, Spinach and Cabbage since it is most commonly grown dicot plants by framers who have less land for growing.



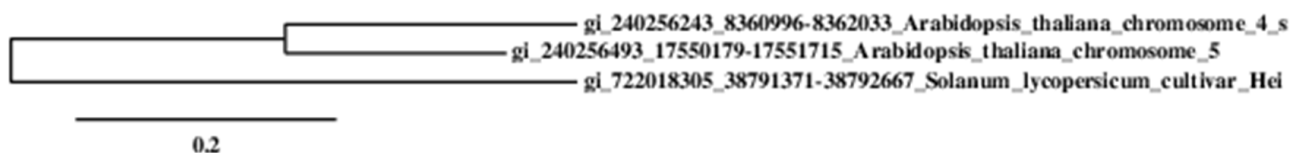
**Figure 1: The process of progressive steps**

The dataset gathered essentially are from NCBI and UniProtKB where distinctive biologists contributed the sequences.

### 3. DISCUSSION AND RESULTS

#### Solanum Lycopersicum

In tomato fruits the factors which affect the growth is majorly the environmental factor i.e., temperature and light. Since tomato fruit is very sensitive to environmental conditions. There are many studies which reveal that there are different processes to control the growth during fruit ageing. It is well established that in better vegetation, such as tomato uses both Trp-independent and Trp-dependent pathways to synthesize IAA. The level of auxin is observed high, the embryo supplies the auxin necessary for continued fruit growth. The auxin responsive protein IAA4 AtAux2-11 gene of Arabidopsis Thaliana was responsive for the cell elongation in Tomato fruit set (Saglam et. al., 2000). In cloning of new members of the tomato AUX/IAA family by PCR amplification was based on some conserved protein domains. The IAA1 auxin responsive protein of Arabidopsis Thaliana which is used in the expression indicates a potential involvement of IAA1 in vascular development (Mito, N., & Bennett, A. B. (1995)). The phylogenetic tree shown in Figure 2 with scale 0.2 indicates there is an auxin high level present in tomatoes.



**Figure 2: Phylogenetic analysis of the IAA genes of Arabidopsis Thaliana and Solanum Lycopersicum Genome**

### Brassica oleracea var. capitata

The soil and fertilizer are important growth factor for cultivating cabbage. The climatic factors such as light, temperature, rainfall etc influences the growth and development of cabbage plant. The vegetative growth of cabbage goes into 4 stages from leaf generation till folding of leaves. At this stage development BcpLH gene was expressed preferentially in folding leaves. The major influential growth factor of cabbage plant is climate; hence the plant undergoes a lot of stress including drought and high salinity. To enhance these stress tolerance Aux genes is used. The experiments show that BcpLH gene was increased when cabbage plants were sprayed with IAA (Ku et. al., 2009). The Xyloglucan endo-transglycosylases (XETs) encoded by xyloglucan endo-transglycosylases/hydrolase (XTH) genes are responsible for cell modification. Xyloglucan endotransglycosylaseprecursor (XET16A) mRNA, mRNA sequence of Brassica oleracea is necessary for cell elongation in cabbage plants. The phylogenetic tree shown in Figure 3 with scale 0.2 indicates there is an auxin high level present in cabbages.

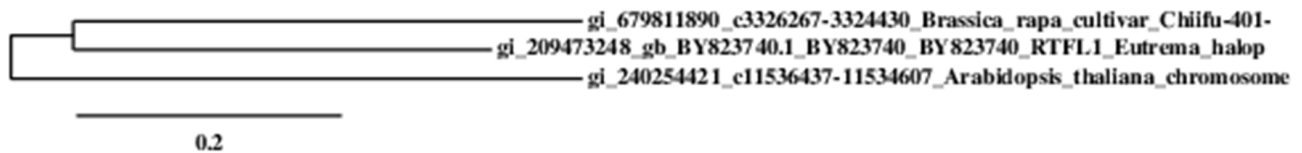


Figure 3: Phylogenetic analysis of the IAA genes of Arabidopsis Thaliana and Brassica oleracea Genome.

### Cucurbita pepo

With the environmental factors like temperature and light, soil and fertilizer are the major influential growth factors which will affect the growth of pumpkin plant. Cucurbitgene is a genomic tool used to squash breeding, transcriptome and genetic map. Cucurbita pepo unigene was identified and saved in a separate file ‘cucurbita unigenes’. Auxin is essential for increasing anchor root formation where it inturn increases the nutrition level and water uptake with the aid of expand in the well-being. On this work the unigene numbered CUTC019992 and CUTC009234 is used to analyze the auxin degree in pumpkin plant. The Phylogenetic analysis indicates that there’s an auxin level in pumpkins with a scale of 0.2 is shown in Figure 4.

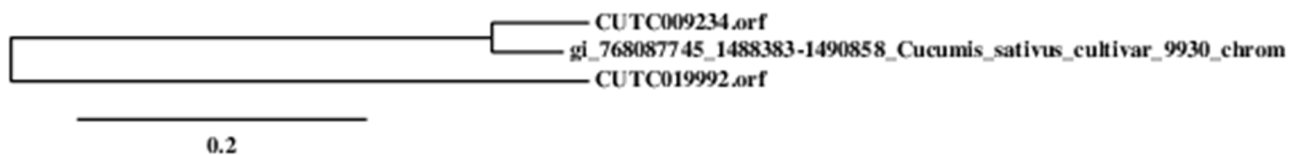


Figure 4: Phylogenetic analysis for Cucurbita pepo chromosome sequence and unigene

### Coriandrum Sativum (Coriander)

Coriandrum Sativum belongs to the family Apiaceae which is generally used as an ingredient in lots of dishes worldwide. Coriander is a photophilic plant, which needs 1000 hours of light for growing from sowing to harvesting. Using the complete genome of Coriandrum sativum chloroplast and comparing it with auxin inducible gene extracted from Arabidopsis thaliana and Arabidopsis lyrata. The Figure 5 shows the phylogenetic analysis with 0.2 scale will indicate that the auxin phyto hormone is present in coriander which is very essential for its growth.

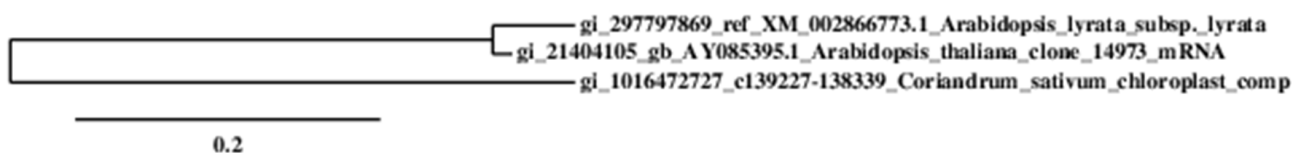


Figure 5: Phylogenetic analysis for Coriandrum Sativum chromosome sequence and Arabidopsis genes

### Phaseolus vulgaris(Common Bean)

Phaseolus vulgaris common bean is generally cultivated worldwide. It is rich in protein and consumed in large. Experiments from unique biologists indicate naturally that bean seedlings synthesize IAA de novo in the first days of germination (Wang et. al.2012). Beans (Phaseolus vulgaris) use the sun's energy to make food and auxin is used to control how sunlight affects them. Auxin promotes growth of the bean plant from root to the tip of the plant. In this work the soya bean Glycine\_max\_v2.0, whole genome shotgun sequence is analyzed with two bean genotypes Phaseolus vulgaris cultivar G19833 chromosome 2, whole genome shotgun sequence with NCBI accession number NC\_016090.2 and Phaseolus vulgaris cultivar G19833 chromosome 7, whole genome shotgun sequence. The result was a phylogeny tree with a scale of 0.2 which indicates that Bean contains auxin naturally is shown in Figure 6.

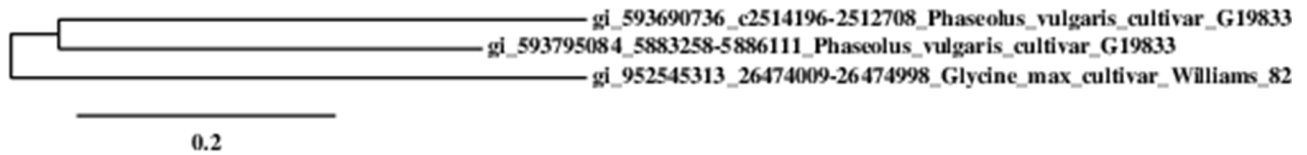


Figure 6: Phylogenetic analysis for Phaseolus vulgaris chromosome 2 sequence and genotypes

### Spinacia oleracea

Spinach is a perfect food loaded with tons of nutrients in a low calorie package. The growth issue of spinach relies on the best way we grow the plant and environmental conditions. In hot climate, seeds are sluggish to germinate and may not germinate in any respect. Heat also motivates the vegetation to bolt (go to seed) quickly, ruining the flavor of the crop. The endogenous auxin-like components have been analyzed within the shoot extracts of younger spinach seedlings, uncovered to photoperiodic induction. The plastid chromosome of spinach (Spinacia oleracea): complete nucleotide sequence was used for the analysis. *Vibrio tubiashii* is a larval shellfish pathogen which has related spinach gene hence *Vibrio tubiashii* with reference number NZ\_CP009355.1 and NCIMB 41619 is also used in the analysis. The Beta vulgaris subsp. vulgaris (sub-species: vulgaris) LOC104894514 is a gene of Vulgaris which is an auxin related gene. Using these genes with the complete nucleotide of spinach was used for the phylogenetic analysis Figure 7 which showed the scale of 0.2 which showed that there is an auxin level in the spinach.

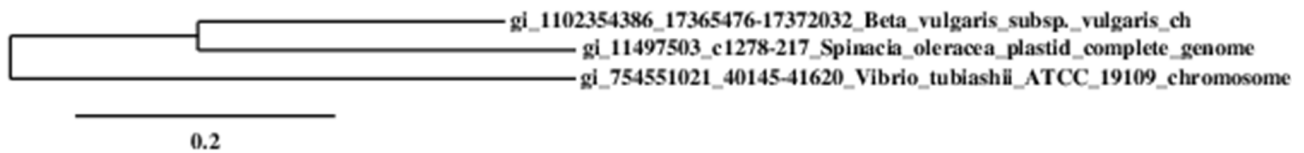


Figure 7: Phylogenetic analysis for Spinacia oleracea sequence and related genes

## 4. CONCLUSION

Phylogenetic is a study for finding the evolutionary relations between the species. The distance scale in all analysis shows 0.2 with which we can say the all the plants Tomato, Beans, Pumpkin, Coriander, Spinach and Cabbage have an auxin level in them. The AMI1 or TOC 64 gene of Arabidopsis thaliana is used for the analysis and we can conclude that the auxin is present in any nucleotide sequence of plants. Future genes recognized can be utilized to distinguish the level of the Plant hormones present in the plants.

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