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Phylogenetic tree some plants of family Zingiberaceae using VEGA ZZ program

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ABSTRACT

The phylogenetic tree analysis of the characteristics of organism based on gene sequences. Vega ZZ program is one of program which usefull for comparative analysis on the genetic characteristic of some plant of the Zingiberaceae family especially the closest genetic relationship with *Curcuma longa* and *Curcuma xanthorrhiza*, which is contain curcumin. This aim of research to determine genetic relationship on some plants from Zingiberaceae family particularly the closest genetic relationship with *Curcuma longa* and *Curcuma xanthorrhiza* so that the data obtained by other plants that can be a source of curcumin compound. The research was done by thirty DNA sequences downloaded from GenBank, further DNA sequences were compared to find family links using multiple alignment program with Clustal X. Then the reconstruction of the phylogenetic tree of genetic relationship which can be obtained by using NJ Plot visualization. The results obtained from this study are genetically thirty plants derived from Zingiberaceae family is divided into two groups indicated by the two branches to the phylogenetic tree. The first group consisted of *Alpinia shimadae* and *Alpinia formosana*. The second group is divided into two subgroups, namely first subgroup consisting of 20 plants and the second subgroup consists of 8 plants. In the second group in the first subgroup obtained *Curcuma sichuanensis* have a very close genetic relationship with *Curcuma longa* and *Curcuma xanthorrhiza*. The closeness of genetic relationship is supported by bootstrap values were quite high at 767.

Keywords : Zingiberaceae, In silico, Phylogenetic tree, Genetic comparisons, Vega ZZ programs

INTRODUCTION

Zingiberaceae family is the most widely grown crop in the tropics, especially Southeast Asia. This plant is important for natural resources for humans as a source of food, spices, dyes, perfumes, food preservatives, food coloring, and a source of herbal medicine¹. Some herbs are derived from Zingiberaceae family has been known to have medicinal properties, for example *Curcuma longa* and *Curcuma xanthorrhiza* [2].

Curcumin (diferuloylmethane) is a polyphenol derived from the *Curcuma longa* plant. Curcumin has been used extensively in Ayurvedic medicine, as it is nontoxic and exhibits a variety of therapeutic properties, including antioxidant, analgesic, anti-inflammatory and antiseptic activities³. Turmeric constituents include the three curcuminoids: curcumin (diferuloylmethane; the primary constituent and the one responsible for its vibrant yellow color), demethoxycurcumin, and bisdemethoxycurcumin, as well as volatile oils (tumerone, atlantone, and zingiberone), sugars, proteins, and resins. While numerous pharmacological activities, including antioxidant and antimicrobial properties, have been attributed to curcumin, this article focuses on curcumin's anti-inflammatory properties and its use for inflammatory conditions [4].

In the Origin of Species, Charles Darwin wrote (1859): "Maybe all the organic creature which have ever lived on this earth descended from some primordial form". Thus, if all living organisms descended from a common ancestor, in this theory it should be possible to determine the relationship of living beings (taxonomy) based on a number of their same properties. Phylogenetic relationships can be based on anatomical characteristics, proteins, DNA, or other molecules⁵. After Darwin, classifications were most often assumed without proof to be evolutionary: phenetics was taken to be identical to phylogenetics. As De Querioz noted, "...the relationships expressed in existing taxonomies were merely reinterpreted as the result of evolution, and evolutionary concepts were developed to justify existing methods" [6].

Studies about the differences, both at the level of gene alleles or kingdom, has demonstrated the characteristics and critical functions of the most common biological structure [7]. Evolutionary genomic studies take advantage of experiments that natural selection has been performing over millennia. In particular, inter-species comparative genomic analyses can highlight the genetic determinants of infection susceptibility or severity [8].

MATERIALS AND METHODS

2.1 Tools and Materials

The tools that we used on this research are, free software Vega ZZ, web-browser Google Chrome, GenBank (NCBI), Toshiba Satellite C640 Notebook with CPU specification Intel Pentium B940 processor @ 2.00 GHz 2.0 GB RAM, operation system is Windows 7 Ultimate. The DNA sequences that we used in this study is obtained from obtained from GenBank.

2.2 Working procedures

2.2.1 Clustal X

Open the program VEGA ZZ then selected *Bioinformatics* at the main menu. Selected Clustal X and loaded sequences files that have been previously downloaded. After that do alignment and selected *Tree* on the menu Clustal X. Selected *Bootstrap Draw-NJ Tree* and saved files (file extension: phb) [9].

2.2.2 NJPlot

Selected *Bioinformatics* at the main menu. After that selected NJPlot and open the file that we saved earlier. Observed phylogenetic tree [10].

RESULTS AND DISCUSSION

In this study, we compared thirty strands of DNA that are downloaded from GenBank [11]. DNA strand that is downloaded is derived from the family Zingiberaceae [12]. Strand DNA is *matK* DNA downloaded. The reason is *matK* DNA or *matK* phylogenetic gene has a high marks compared with other types of genes that used in this field [13].

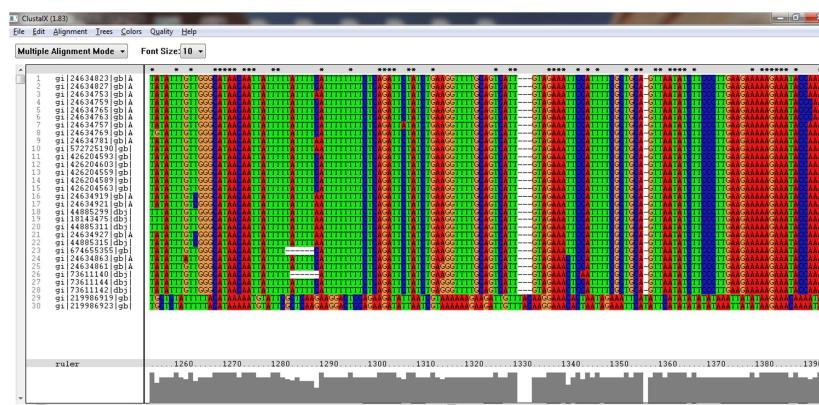


Figure 1. Alignment using ClustalX (gi24634823gbA = *Nicolaia elatior*, gi24634827gbA = *Etlingera yunnanensis*, gi24634753gbA = *Alpinia elegans*, gi24634759gbA = *Alpinia intermedia*, gi24634765gbA = *Alpinia pumila*, gi24634763gbA = *Alpinia officinarum*, gi24634757gbA = *Alpinia galanga*, gi24634769gbA = *Amomum glabrum*, gi24634781gbA = *Boesenbergia rotunda*, gi572725190gb = *Caulokaempferia alba*, gi426204593gb = *Boesenbergia longiflora*, gi426204603gb = *Boesenbergia maxwellii*, gi426204559gb = *Boesenbergia collinsii*, gi426204589gb = *Boesenbergia kingii*, gi426204563gb = *Boesenbergia hamiltonii*, gi24634919gbA = *Smithatris supraneanae*, gi24634921gbA = *Stahlianthus involucrat*, gi44885299dbjA = *Curcuma sichuanensis*, gi18143475dbjA = *Curcuma longa*, gi44885311dbjA = *Curcuma xanthorrhiza*, gi24634927gbA = *Zingiber corallinum*, gi44885315dbjA = *Zingiber officinale*, gi674655355gb = *Haniffia santubongensis*, gi24634863gbA = *Kaempferia rotunda*, gi24634861gbA = *Kaempferia pulchra*, gi73611140dbjA = *Kaempferia parviflora*, gi73611144dbjA = *Kaempferia marginata*, gi73611142dbjA = *Kaempferia galanga*, gi219986919gb = *Alpinia formosana*, gi219986923gb = *Alpinia shimadae*)

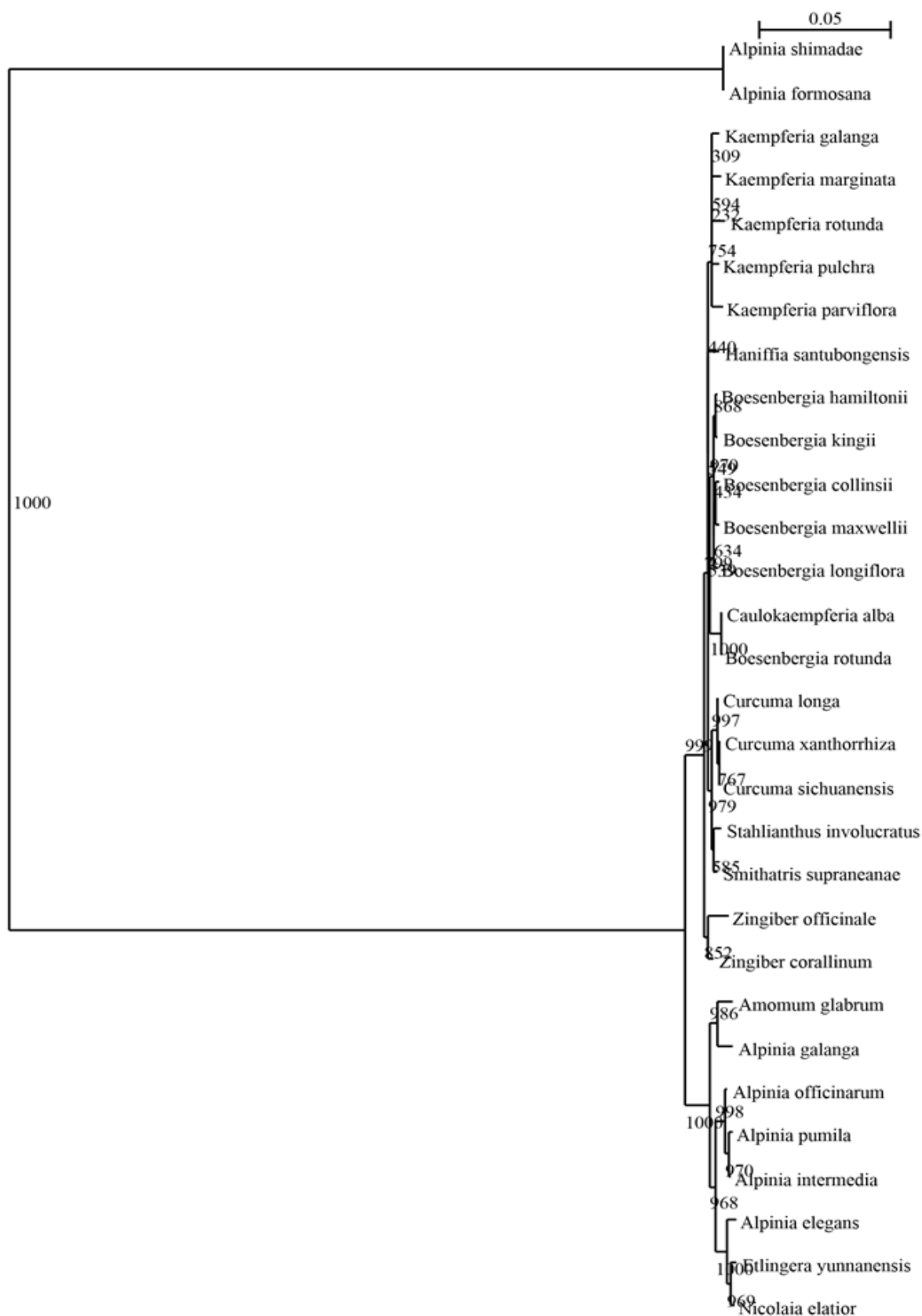


Figure 2. The phylogenetic tree resulting from *NJPlot*. The score on the branch shown the bootstrap value

To compare the strands of DNA, used Vega ZZ program with plug-in ClustalX and NJPlot [14]. ClustalX used for alignment of DNA strands while NJPlot used to create the phylogenetic tree of the alignment results. Furthermore this family tree that will be interpreted [15].

On the results of alignment, there are some gaps that is marked with dashed lines. The gaps mean that the alignment process is completely done. The gaps is also needed to make the identical or similar strand of DNA can perfectly on a line or branch [16].

The result of phylogenetic tree, there are several branches that determine the genetic relation between some plants. Genetic distances between some plants of the family Zingiberaceae can be expected with long horizontal branches of the phylogenetic tree, in accordance with the scale shown [17]. Bootstrap values on the stalk branch in the phylogenetic tree shows the accuracy of the outgroup [18]. The result, thirty plants derived from Zingiberaceae family are genetically divided into two groups shown by the two branches to the phylogenetic tree above that the first group consisting of *Alpinia shimadae* and *Alpinia formosana* and supported by a very high bootstrap value at 1000. Same as in the second group that also has a very high bootstrap value equal to the first group. The second group was divided into two subgroups, the first subgroup is represented by 20 plants, that is; *Boesenbergia rotunda*, *Caulokaempferia alba*, *Boesenbergia longiflora*, *Boesenbergia maxwellii*, *Boesenbergia collinsii*, *Boesenbergia kingii*, *Boesenbergia hamiltonii*, *Smithatris supraneanae*, *Stahlianthus involucratus*, *sichuanensis Curcuma*, *Curcuma longa*, *Curcuma xanthorrhiza*, *Zingiber corallinum*, *Zingiber officinale*, *Haniffia santubongensis*, *Kaempferia rotunda*, *Kaempferia pulchra*, *Kaempferia parviflora*, *Kaempferia marginata*, and *Kaempferia galanga*. While the second subgroup is represented by the remaining eight plants that is; *Nicolaia elatior*, *Etlingera yunnanensis*, *Alpinia elegans*, *Alpinia intermedia*, *Alpinia pumila*, *Alpinia officinarum*, *Alpinia galanga*, and *Amomum glabrum*.

The results of the phylogenetic tree above shows that there are two species of the genus *Alpinia* residing in large groups, that is; *Alpinia shimadae* and *Alpinia formosana* while 5 other species of the genus *Alpinia* is residing in a subgroup that is; *Alpinia elegans*, *Alpinia intermedia*, *Alpinia pumila*, *Alpinia officinarum*, and *Alpinia galanga*. Which showed that although in the same genus but not necessarily have close genetic relationship.

Plants are known to have a secondary metabolite of curcumin based on the literature is *Curcuma xanthorrhiza* and *Curcuma longa*. Both plants are residing in one subgroup that showed genetic relationship is close and supported by a quite high bootstrap values at 997. *Curcuma xanthorrhiza* very close to *Curcuma sichuanensis* supported by a quite high bootstrap values at 767. These results indicate that there is one plant closely related to the curcumin producing plants (*Curcuma* and turmeric) that is *Curcuma sichuanensis* that near *Curcuma xanthorrhiza* (*Curcuma*).

CONCLUSION

Based on the results of research conducted, it can be concluded that the plant in the family Zingiberaceae even within the same genus but are genetically not necessarily have a close genetic relation. And there is one plant that close to to the *Curcuma xanthorrhiza*, that is *Curcuma sichuanensis*.

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