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Mini Review

Development and Evaluation of Bioinformatics Methods a Successful Way to Design Plants for Genetic Modification

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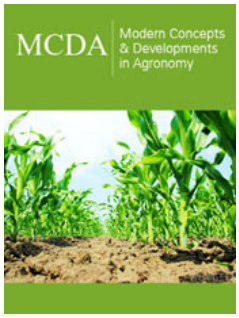

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Abstract

Bioinformatics is an interdisciplinary field that combines the biology sciences, computer science and mathematics, which has gained a unique place in the life sciences. Bioinformatics has provided researchers with a very new and reliable solution for the forthcoming research in the fields of gene sequencing, genome studies and peripheral analysis, gene expression, and protomix. Another common application of bioinformatics is the identification of mononucleotide polymorphisms (SNPs) and candidate genes. Such identifications are often made with the aim of better understanding the genetic basis of diseases, matching and establishing desirable properties (especially in agricultural species), or recognizing differences between populations. Bioinformatics also seeks to better understand the structural principles of nucleic acids and protein sequences in protomix science. Gene expression studies are one of the most important characteristics that can be considered for bioinformatics, which has seen rapid growth in genetic engineering and biotechnology. For example, gene transfer with the aim of increasing expression in order to manipulate a specific biochemical pathway for the accumulation of a particular compound or metabolite, this approach can be well evaluated with the help of bioinformatics in silico environment. In this mini review, an attempt has been made to study some bioinformatics experiments, such as the phylogenetic tree and the anatomical and developmental expression of the *GSA* gene. This study can provide a good perspective for future laboratory studies for the researcher.

Keywords: Protomix; Bioinformatics; Environment; Phylogenetic tree

Introduction

The results of the phylogenetic tree help to identify the all-relatives gene. Actually, genetic evolution is a scientific process that allows history to be determined the evolution of groups or sequences through the trees of genetic evolution. In fact, by examining the phylogenetic tree, it is possible to discover gene function, tracing gene origin and identifying relatives one organism is provided. Phylogenetic study using molecular data such as DNA sequence for genes and amino acid sequence for proteins is very common not only in the field of evolutionary biology but also in the wide fields of molecular biology. The methods for phylogenetic study are improving along with the evolution of computer knowledge. Thus, there are many methods to infer phylogenetic tree, and many programs for each method are presented [1]. The results of the phylogenetic tree indicate less genetic distance between the members of a family.

Identifying the evolutionary relationships of a gene and recognizing the orthologous of that gene, examining the level of gene expression in different developmental and anatomical stages of the plant, determining the expression relationships between genes in addition to

creating a comprehensive and clear picture of how the genome works, is a guaranty procedure for plant genetic engineering improves the desired traits. It is clear that an important and initial step in creating genetic change is study of bioinformatics and the function of the gene in differentiation and anatomical stages and how the expression of this gene is related to other genes. To do this, the first step is to use relatively new tools with much higher efficiency in comprehensive collection and analysis. There are many tools for analyzing data in public databases, one of which is Genevestigator, introduced in 2004. Genevestigator is a database and web browser with data mining capabilities for microarray data. Equipped with express analysis tools. Online analysis tools allow a wide range of information about gene expression during developmental or anatomical stages to explore patterns of gene expression. According to the website Genevestigator, the rank of a reference among all gene expression tools in the field of plant biology in all scientific journals were more than 2000 cases (related to this site).

GSA is a gene involved in ALA biosynthesis pathway. ALA is a precursor for all tetrapyrrole as example chlorophyll, heme, siroheme, Vitamin B_{12}, phytochromobilin. These components have critical functions in living cell, as pigments (chlorophyll, phycobiliproteins), light receptor (Phytochrome), prosthetic group of many different proteins (like cytochromes, hemoglobin, myoglobin, and leghemoglobin) and enzymes (Like Catalase, Ascorbate peroxidase (or APX), Peroxidase (POD) and so on). Nowadays, ALA has received wide attention for its widespread use in agriculture, forestry and medicine. ALA at low concentrations (30-100 mg l^{-1}) increases photosynthesis, growth, development, yield and productivity, also promoted fruit color appearance [2-4] and quality and taste of products in treated plants [4] under both normal and stressful conditions. ALA also improves nutrient uptake [5,6], antioxidant characteristics [7] and osmotic equilibrium and water use efficiency in plants. Numerous studies have shown that ALA has increased the resistance of different plants to a wide range of biotic [8] and abiotic stresses such as herbicides [9], shade [10], heat [11], cold [12], chilling [13], drought [14], salt [15], and heavy metals [16], waterlog [17], nutrient deficiency [5] and UV-B [18]. ALA also accelerates seed germination [19], and as a new plant growth regulator (PGR) and growth-promoter [20], also a potential role in tetrapyrrole-mediated retrograde signaling and exploited the direct impact of ALA biosynthesis on nuclear gene expression (NGE) [21]. ALA at high concentrations is known as an environmentally friendly and biodegradable herbicide or insecticide [22,23]. Furthermore, ALA has wild application in medical, nutrition and cosmetics [24,25], for example in cancer Photodynamic Diagnosis (PDD), Photodynamic Therapy (PDT) [26], antimicrobial drug [27] and improvement sleep [28], oral verrucous hyperplasia [29], and other clinical uses. Vitamin B_{12} is one of ALA byproducts. It uses as an anti-pernicious anemia factor, growth factor. ALA has sleep improvement without negative side effects like dry mouth, dizziness, continued drowsiness lasting into the next day and reminiscence problems [28]. ALA is a potential treatment for negative emotional barriers like stress and mood which various components of it are hopefulness, loneliness, and motivation [30,31]. ALA was prevention of renal injury, such as ischemia-reperfusion injury, by antioxidant effect [32]. ALA prevent chemotherapy nephrotoxicity for cisplatin without compromising the anticancer efficacy of it [33]. According to the literature data in the effect of ALA compound and its potential role in plants, which is the result of the activity of the *GSA* gene, it was decided to select this gene as a model for bioinformatics studies.

Database search identified one gene encoding *GSA* in tobacco. The microarray data available in the database (https://genevestigator.com/gv/plant.jsp) revealed anatomical and developmental highly expression patterns for GSA gene.

Bioinformatic analysis

By blasting the *GSA* gene sequences at nucleotide BLAST search programs available at the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi), other *GSA* homologous sequences were found. The cladogram that shows the phylogenetic relationship between the GSA nucleotide sequences was created by using the Neighbor-Joining option present on MAGA7 software (Molecular Evolutionary Genetics Analysis) [34], Bootstrap values from 1000 were used for the creation of the cladogram. Also, determining evolutionary relationships and calculating the distance matrix by MAGA7 software.

Microarray data available through Genevestigator (https://www.genevestigator) for *GSA* gene were analyzed. Genevestigator software was used for compere expression of *GSA* gene in two similar species of *M. sativa* and *M. truncatula* in different developmental stages and anatomical parts also survey orthologs of the *GSA* gene in *M. truncatula* and others bioinformatic analysis.

Result

Phylogenetic investigation of *GSA* gene

GSA gene have been found in photosynthetic organisms and in many eubacteria as no photosynthetic organisms. *GSA* gene and are highly conserved across all groups including higher plants and in bryophytes, cyanobacteria and many eubacteria. The phylogenetic tree was constructed based on cDNA and RNA sequences of 73 different *GSA* genes. As shown in Figure 1, there was indicated ten different groups of *GSA* gene in this phylogenetic tree which *GSA* gene of *Medicago* belongs to the Fabaceae Family. This clearly shows that Fabaceae species have more similar *GSA* gene than other species therefore evolutionarily are more similar to other plant groups. Also, the *GSA* gene of tobacco is more closely related to the *GSA* gene of the Solanaceae Family. Phylogenic tree was designed by MEGA7 software (Figure 1).

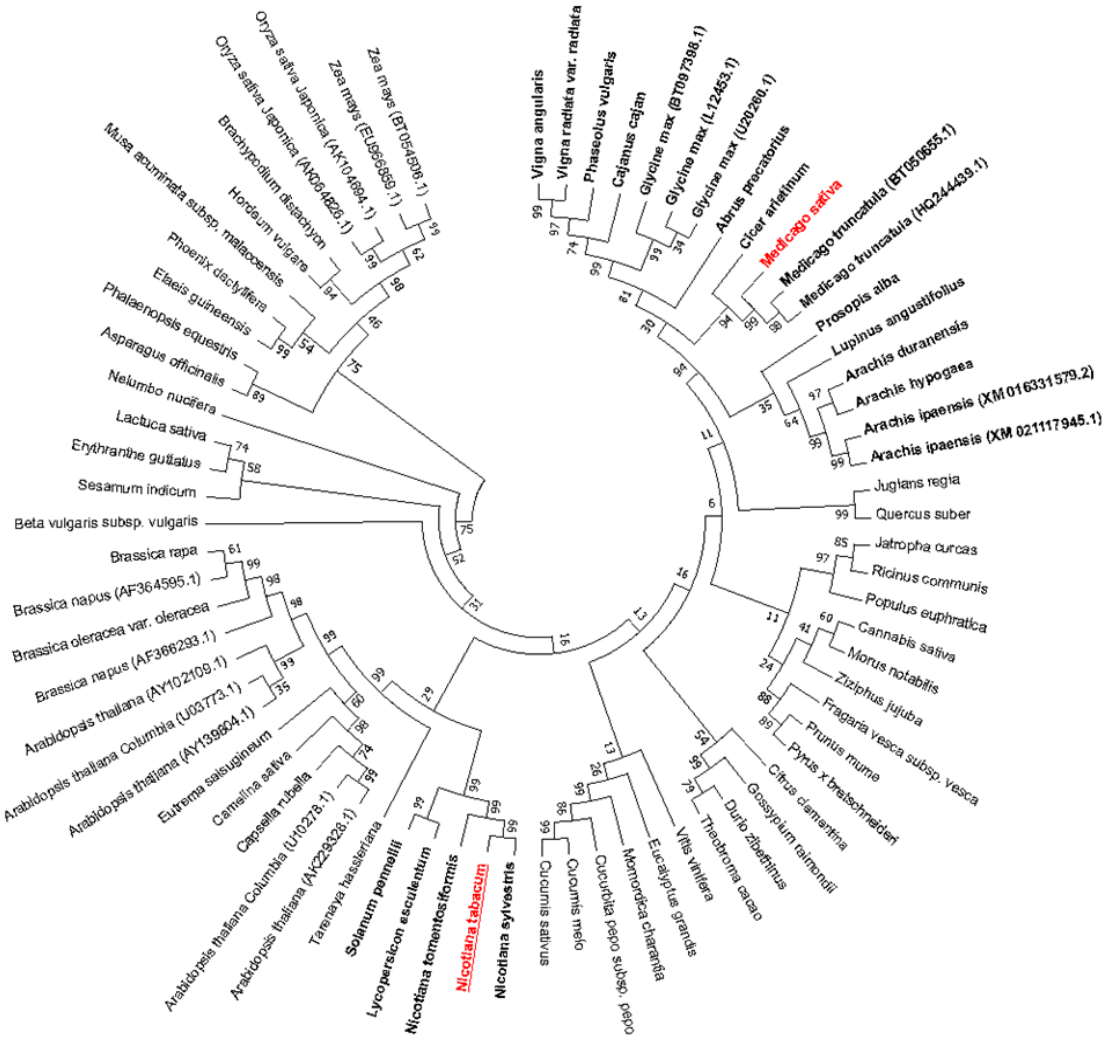

Figure 1: Unrooted phylogenetic tree of the *GSA* genes. Cladogram showing the relationship of the *GSA* genes in 73 terrestrial plant species. The alignments of sequences were performed using ClustalW method and grouping them using Neighbor-Joining method. Bootstrap values from 1000 pseudo-replicates were used for the creation of the cladogram and to determine the accuracy of the phylogenetic tree. phylogenic tree was designed by MEGA7. Ten different clades are illustrious among this species. Clads whose receiver and donor genes are similar indicated bolded. *M. sativa* as gene donor is indicated in red and Nicotiana tobacco as gene receiver is indicated in red underline.

Comparison of expression of GSA gene in different developmental stage of *M. truncatula*

At different developmental stage in living organism each gene has different expression, and this cause differentiation [35]. Eight developmental stages of expression of *GSA M. truncatula* (MTR-3g118070) were studied (Figure 2). In germination stage, plant has lowest expression level among other developmental stages, about 15.4, then developed flower has a little more *GSA* expression. Seedling, main shoot (primary axis) growth, axillary shoot formation has medium level expression of *GSA*, almost 16, and highest level of expression related to flower emergence stage of plant that is about 17 then gene expression of flower and pods stage and mature pods stage are less than flower emergence stage respectively. Level of *GSA* gene expression in all stages of life is high, compared to expression of other genes in *M. truncatula*, because expression further 12 is considered high expression (Figure 2A). Percent of *GSA* expression potential in eight developmental stage of *M. truncatula* are investigated, according to Figure 2B, flower emergence, mature pods, flower and pods are exhibited 50-70 percent of gene expression potential whereas seedling, germination, main shoot growth (primary axis), axillary shoot formation, developed flower are exhibited 30-50 percent of *GSA* gene expression potential.

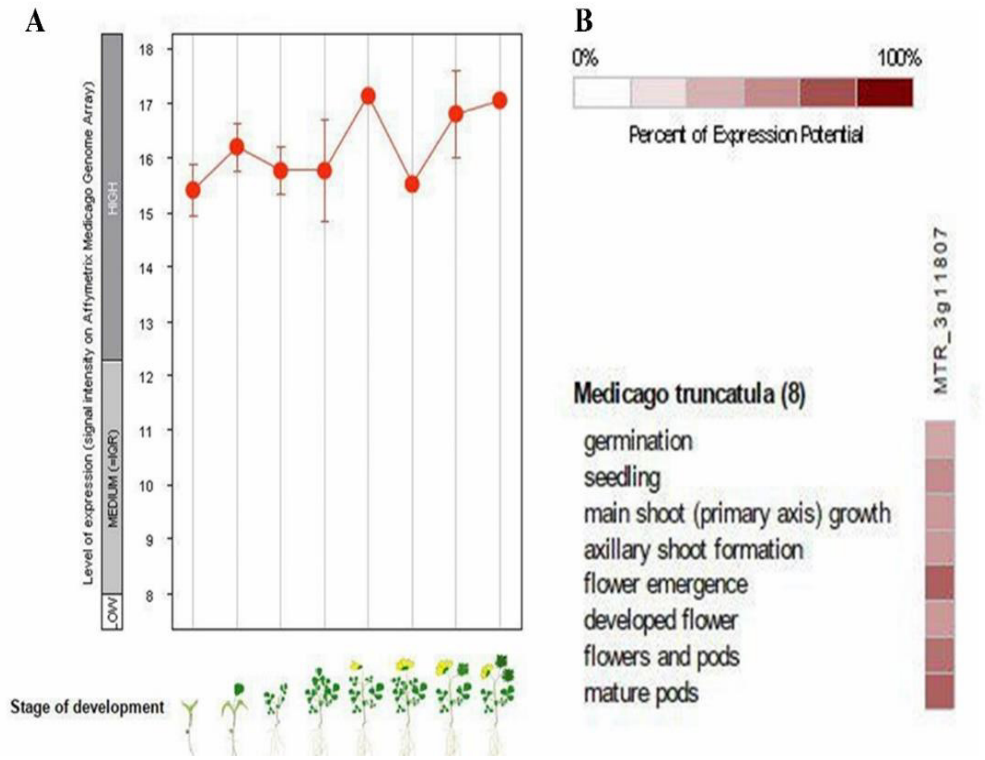

Figure 2: A. Level of *GSA* expression in eight developmental stages of *M. truncatula*. Germination, seedling, main shoot (primary axis) growth, axillary shoot formation, flower emergence, developed flower, flower and pods, mature pods respectively.
B. The percentage of GSA gene expression in the previous 8 steps.

Comparison of expression of GSA gene in different anatomical parts of *M. truncatula*

In living organisms each gene has different expression at different anatomical parts [35]. Difference in gene expression at each group of cells cause formation of different type of cells that each one has the same genome, and this is the basis of differentiation. Thirty anatomical part of *M. truncatula* were investigated, results showed *GSA* gene expression potential in inflorescence, shoot apex, leaf and leaf culture, pod, seed, macro-sclereid layer, shoot, trifolate leaf, petiole and primary root, was about 70% to 85%, which among them inflorescence, shoot apex, leaf and leaf culture had the most *GSA* gene expression potential. In hairy root, nodule apical meristem cell, nodule distal infection zone cell, nodule proximal infection zone cell, uninfected cell from nodule fixation zone, root border cell, cell suspension culture, hypocotyl, radicle, radicle tip, flower, stem, internode, axillary bud expression is about 50% to 70%. In callus, infected cell from nodule fixation zone, testa (seed coat), axillary bod, roots and nodule *GSA* gene expression potential is about 35% to 50% which nodule, root, testa and infection cell from nodule fixation zone had lowest gene expression among them (Figure 3).

In this study, *Medicago sativa* was used as *GSA* gene donor because this species among C3 plants is very active photosynthetically) Gifford, [36] also as a main legume forage in the world due to its high nutritional value, high yield and wild adaptation [37]. So, *GSA* gene of Medicago sativa was candidate a good choice for study the physiological effects of increasing endogenous ALA in tobacco plant. To investigate this, first bioinformatics studies were performed, and the phylogenetic tree of this gene was studied in 73 species. This study showed the existence of 10 groups in this collection. *GSA* is ubiquitous in plants, existence of this gene in different families with the same function, shows that this gene plays key role in the plant, and this has led to its conservation in different species during evolution [35].

By examining the *GSA* gene among all plant species studied, Arabidopsis is most similar to *M. truncatula* in terms of sequence-based score and expression-based score. As a result, a suitable alternative for gene transfer for the overexpression of the *GSA* Arabidopsis gene is a more appropriate option than other species. Expressive studies of this gene at different differentiation and anatomical stages revealed that this gene is a high-expression gene in Medicago (Figure 1-3).

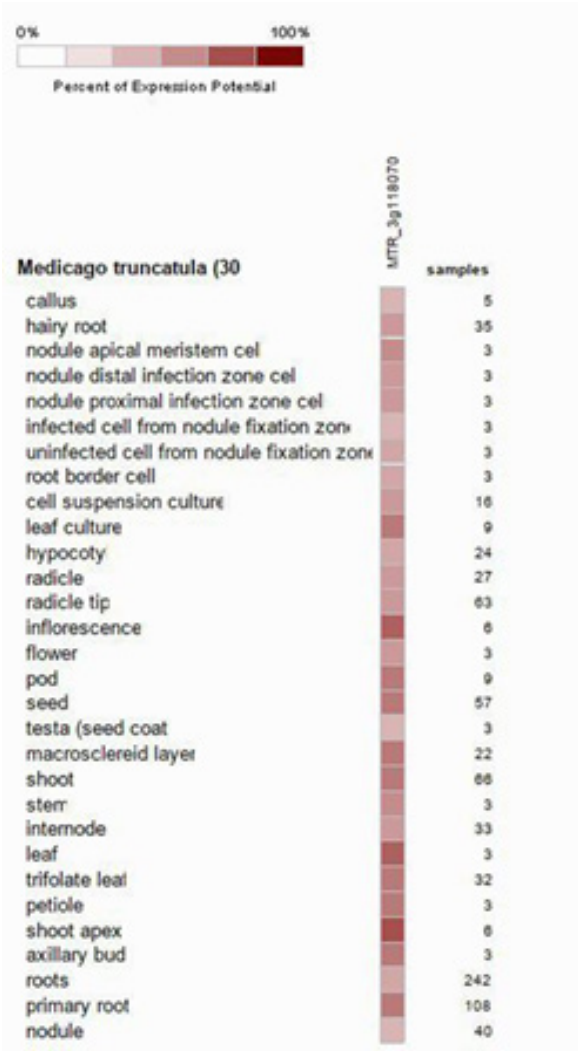

Figure 3: Percent of *GSA* expression potential in thirty anatomical stage of *M. truncatula.*

The most expression of *GSA* gene is seen in the stages of flower formation, flower pods and pod maturity because the plant is active in these stages and performs a lot of photosynthesis and therefore need to produce chlorophyll to expand photosynthetic tissues and compensate for the loss of chlorophylls that become out of the cycle. Also, the activation of mechanisms inactivation of species of ROS oxygen, for this activity of enzymes such as APX and POD and catalase is necessary, which is also present in the structure of these enzymes as a cofactor. In addition, the proteins and enzymes required by photosystem I, II pathway also have cytochromes in their structure, such as photosystem I complex and cytochrome $Cytb_6f$, which are also essential in their function. Only in the seed germination stage, less expression of *GSA* gene was seen than other stages, which indicates less need for chlorophyll production and other compounds requiring heme in this stage than other developmental stages of this plant (Figures 2A&2B).

Also, in different anatomical parts, the highest relative expression of *GSA* gene was seen in clusters, leaves, stem tips and leaf cultures, which indicates the high requirement of these cells to synthesize compounds, proteins and enzymes that in the structure of them tetrapyrroles are necessary. while the lowest relative expression of the *GSA* gene is in calluses, roots, nodes, and internodes, indicating less biosynthesis of compounds, proteins, and enzymes in which tetrapyrroles are used like chlorophyll, cytochrome, catalase and APX and POD in each of these anatomical parts.

Discussion

To over expression of a gene, effort should be on the best option among other organisms. For this reason, bioinformatics data is used to help choose the best option. In this way, the expression level of the relevant gene in the source organism should be high, so that by transferring it to the destination organism, the expression level of the relevant gene is even higher than before [38,39]. To do this, one strategy is to select a gene from an organism that the sequence of the interested gene is not exactly the same as the gene of the

target plant, and thus it is possible to identify and differentiate it in the plant of origin from the original gene, but at the same time this discrepancy should not be to the extent of gene expression cause conflict in the destination plant [40,41]. The second strategy, in the use of bioinformatics information is to study gene expression in computational space. In fact, the use of database information labels researcher to make the right choice in molecular programs in the laboratory by default about the desired gene or genes [42,43]. Studies of gene expression in different tissues as well as at different stages of development can reinforce the view that genetic manipulation can attain the expected achievement for the researcher. Therefore, studies showed that *GSA* gene of *M. sativa* is very high expression, therefore high expression of this gene cause increases chlorophyll concept and therefore the growth rate and biomass of *M. sativa* is higher than other plants [38,39]. So, in this study GSA gene of *M. sativa* selected as the best candidate for the source gene to transfer. As seen in the studies clearly, expression level of *GSA* gene in *M. truncatula* is high and it was included in the genes with high expression. Therefore, we used alfalfa gene to be transferred to tobacco, which has a high expression potential.

As perceived in the studies obviously, *GSA* gene expression is highly correlated with genes that are highly expressed during activation and development. By investigative the overexpression tobacco plant in *GSA* gene, higher expression of *GSA* was associated with higher levels of ALA content in these plants. The Molecular laboratories works also showed that tobacco *GSA* over expression plant had higher photosynthesis, growth rate and chlorophyll as well as elevated ALA content (Ghasemzadeh et al., not published yet).

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