STUDIES TO FIND *ETR1-1* GENE SEQUENCE SIMILARITIES IN FLOWER CROPS AND VARIABILITY TO POSTHARVEST ETHYLENE PRODUCTION IN ANTHURIUMS AND ORCHIDS

Thesis submitted in part fulfillment of the requirements for the degree of ***Master of Science (Horticulture)*** *to the Tamil Nadu agricultural university, Coimbatore-641 003*

By

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CERTIFICATE

This is to certify that the thesis entitled "**STUDIES TO FIND ETR1-1 GENE SEQUENCE SIMILARITIES IN FLOWER CROPS AND VARIABILITY TO POSTHARVEST ETHYLENE PRODUCTION IN ANTHURIUMS AND ORCHIDS**" submitted in part fulfillment of the requirements for the award of the degree of **Master of Science (Horticulture)** to the Tamil Nadu Agricultural University, Coimbatore is a record of **bonafide** research work carried out by **Mr.S.Arvind Kumar** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

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(S. ARVIND KUMAR)

ABSTRACT

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Floriculture is a billion dollar industry, and earns foreign exchange to the country. The use of refrigerated storage and reefers for transport involves exorbitant rates. The postharvest loss of flowers was established to be due to a plant hormone – Ethylene. A family of ethylene receptors was found in mutants of *Arabidopsis thaliana*. Mutants possessing this receptor were found to be ethylene insensitive. The gene responsible for this action was later found to be ETR1.

Since a skepticism existed whether Anthuriums could have a longer shelf life than Orchids, the presence of ETR1 in Anthuriums was predicted. Orchids were used for comparison in the study.

To realize the objectives of this study, experiments were conducted on postharvest ethylene evolution of *Anthurium andreanum* and *Dendrobium* and similarity search for ETR1 or ethylene antisense gene on flower crops were also carried out during the year 2001-2002 at Horticultural College and Research Institute, Coimbatore.

Among the sixteen Anthuriums, three genotypes viz., Bonfire orange, Merengue and Linda demol released no ethylene at third week. They did not release ethylene even when the sample size was increased to 10 flowers for three weeks. Dendrobiums evolved more postharvest ethylene than Anthuriums at third week. The highest postharvest ethylene was released by Spic white (4.139 ppm/100g) Dendrobium at third week. The critical stage for postharvest ethylene release was found to be around second week for Dendrobiums and around third week for Anthuriums.

Cluster analysis of the genotypes revealed that Dendrobiums produced high ethylene, Anthurium genotypes Tinora, Rosetta, Temptation and Honduras produced moderate ethylene and genotypes Bonfire orange, Merengue and Linda demol belonged to the low ethylene producing category.

The DNA sequence similarity search using BLAST for ETR1 gene (antisenescence gene or ethylene antisense gene) yielded 80 results with E-value less than 0.001. The BLAST search for ETR1 revealed 17 flower crop sequences with E-value ranging from 3e-66 to 4e-22 indicating a high similarity of flower sequences to ETR1.

Among the 17 flower crop sequences found in BLAST search, the lowest identity value of 79 per cent was found in *Petunia hybrida* ETR1-1 mRNA, while the highest identity value of 86 per cent was recorded in *Rosa hybrida* ETR2 gene. The BLAST search for ETR1 had less complexity regions or repeat sequences indicated by high K-value of 0.711. The ETR1 sequence search in BLAST resulted in a positive/positive strand values for all flower crops indicating a forward only search between query and database sequences.

The BEAUTY predicted amino acid sequences for ETR1 found in the database produced 50 significant alignments. The class of ETR1 protein determined by CATH was found to be Class 3 ($\alpha + \beta$), meaning that it contained mainly α – helices and β- strands. The Secondary structure of protein determined by PREDATOR

revealed that ETR1 was composed of 41.11 per cent α-helix, 18.56 per cent β- strand and the rest being random coils which was confirmed using RASTOP and SPDBV.

The phylogenetic tree produced by TREE TOP exhibited that ETR1 of *Pelargonium hortorum* was closely related to ETR1 of *Arabidopsis thaliana,* while ETR2 of *Rosa hybrida* was distantly related to the ETR1 sequence suggesting that it involved a greater mutation process during evolutionary development.

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INTRODUCTION

CHAPTER I

INTRODUCTION

All over the world, the floricultural sector is experiencing rapid changes. Due to globalization and its effect on income development in the different regions of the world, we see a growing per capita consumption in most countries. At the same time, competition is increasing worldwide. Besides the traditional centers of production (USA, Japan, Italy, The Netherlands, Columbia), new production centers are developing. In Latin America and Africa, production is increasing very quickly. In Asia, countries like India, China, Vietnam, etc., seem to be moving in the direction of more intensive horticulture. In the traditional centres, the total area under production will remain stable or increase slightly. Productivity will go up in these centres. Although it is to be expected that consumption will grow in the near future, competition on the world market will increase. Supply is growing quicker than demand. A fierce competition on certain markets will be the result. In this competitive trade, unless costs are cut down, there is no future scope for trade (Meyerowitz *et al* ., 2001).

The world trade in floriculture was 25 billion dollars in 1990 and it rose to US$ 35billion in 1995 and it is expected to reach $ 60 billion during 2002. Though the current floriculture trade is a meager 1 per cent of the world, the international floriculture industry is having a different perception about India that as a result of political changes in India, many entrepreneurs are changing to floricultural activities, which have become a high priority for the Indian government. Certain areas of India have great potential for floriculture because of sufficient winter and summer sunshine (APEDA, 2000).

This view about India with respect to international flower trade has lead to the starting of a number of floriculture industries. Though they are technically successful, in efficient production, they could not succeed commercially due to increased costs and thereby lack of competitiveness.

The cost increase is mainly attributed to postharvest losses and in preventing it the industry has to spend a lot on refrigerated storage and transport, as temperature is the major factor in inducing ethylene production which in turn causes senescence. A flower at 30°C will respire 45 times faster than one at 0°C and consequently will have a shorter life span. Flowers must be stored and handled at low temperatures, close to 0°C being the best. A "cold chain" from producer to retailer is essential, including cold storage at the airport.

Ethylene has been implicated in leaf abscission, senescence and fruit ripening besides in seed dormancy, seedling growth, and flower initiation. Ethylene is a plant hormone whose biosynthesis is induced by environmental stress such as oxygen deficiency, wounding, pathogen invasion and flooding. This aspect of ethylene plays a critical role in post harvest deterioration of flowers.

A number of approaches have been taken in an attempt to control ethylene biosynthesis to thereby control postharvest losses. Expression of an antisense RNA to ACC synthase inhibits fruit ripening in tomato plants. The use of an antisense TOM13 (ACC oxidase) gene in transgenic plants and altered fruit ripening and leaf senescence in tomatoes expressing an antisense ethylene-forming enzyme were reported in many studies. In a second approach, ethylene biosynthesis was reportedly modulated by expressing an ACC deaminase in plant tissue to lower the level of ACC available for conversion to ethylene (Meyerowitz *et al* ., 2001).

While a substantial amount of information has been gathered regarding the biosynthesis of ethylene, very little is known about how ethylene controls plant development. Although several reports indicate that a high affinity binding site for ethylene is present in plant tissues, such receptors have not been identified.

Studies in *Arabidopsis thaliana* have provided evidence that ethylene perception in plants is mediated by a family of receptors, including the ETR1 gene (Meyerowitz *et al* ., 2001), though ETR1 was the first ethylene receptor to be identified in plants (Bleecker *et al.* 1988). Further studies done for ethylene-insensitive seedlings and cloning by sequence similarity indicated that

reported. Many of these mutants also failed to respond to ethylene (Bleecker *et al.*, 1988).

Once the ETR nucleic acid is identified, it can be cloned and, if necessary, its constituent parts recombined to form the entire ETR nucleic acid. Once isolated from its natural source, e.g., contained within a plasmid or other vector or excised there from as a linear nucleic acid segment, the ETR nucleic acid can be further used as a probe to identify and isolate other ETR nucleic acids. It can also be used as a "precursor" nucleic acid to make modified ETR nucleic acids and proteins.

For example, the mutation responsible for the phenotype observed in the Never-ripe mutant is sufficient to confer a dominant ethylene insensitivity phenotype on the wild-type plant. The transformation of flower crops with this modified ETR nucleic acid may be expected to confer the dominant ethylene insensitivity phenotype on such transformed plant cells.

Alternatively, the precursor nucleic acid can be one wherein one or more of the nucleotides of a wild-type ETR nucleic acid have already been modified. Thus, for example, the Arabidopsis thaliana ETR nucleic acid can be modified at specific location to form a modified nucleic acid containing the substitution of that codon with a codon encoding an amino acid. This modified ETR nucleic acid may act as a precursor nucleic acid to introduce a second modification.

Deletions within the ETR nucleic acid can also be contemplated. For example, an ETR nucleic acid can be modified to delete that portion encoding the putative transmembrane or intracellular domains. The thus formed modified ETR nucleic acid when expressed within a plant cell may produce only an amino-terminal portion of the ETR protein, which is potentially capable of binding ethylene, either directly or indirectly, to modulate the effective level of ethylene in plant tissue.

In addition, the modified ETR nucleic acid can be identified and isolated from a mutant plant having a dominant or recessive phenotype characterized by an altered

response to ethylene. Such mutant plants can be spontaneously arising or can be induced by well known chemical or radiation mutagenesis techniques followed by the determination of the ethylene response in the progeny of such plants. Examples of such mutant plants which occur spontaneously include the Never ripe mutant of tomato and the ethylene insensitive mutant of carnation. Thus, modified ETR nucleic acids can be obtained by recombinant modification of wild-type ETR nucleic acids or by the identification and isolation of modified ETR alleles from mutant plant species.

Based upon the foregoing, it is clear that the genetic basis and molecular mechanism of ethylene interaction with plants has not been clearly delineated. Given the wide range of functions regulated by ethylene and the previous attempts to control ethylene function by regulating its synthesis, it would be desirable to have an alternate approach to modulate growth and development in various plant tissues such as fruits, vegetables and flowers by altering the interaction of ethylene with plant tissue.

Bioinformatics is the precursor of biotechnology today because a wealth of biotechnology data produced in earlier works is stored in huge computerized databases and this ocean of information can be mined for usable knowledge. Astounding advancements in computer technology facilitated by internet has created super computing power of unprecedented scale through collaborative computing of the internet. This has fueled the explosion of bioinformatics tools as well as business collaborations involving bioinformatics resulting in mutual benefit to the IT industry as well as researchers in biology. In this context, use of bioinformatics for mining the DNA sequencing data to find a match for the ETR1 or ethylene antisense gene in flower crops will enable the floriculture industry to spend less on postharvest measures by use of genetic tools to control postharvest losses; thereby the industry can become competitive in the international trade.

Accordingly, a study was formulated with the following objectives.

- To find out if the Anthuriums and Dendrobiums possess natural variability to postharvest ethylene release.

- To find out flower crop nucleic acid sequences comprising an ethylene response (ETR) nucleic acid with high similarity to ERT1 sequence of *Arabidopsis thaliana.*
- To find out the protein secondary structure of ETR1 to know about the amino acid composition of the sequence.
- To find out the phylogenetic relationship of genes of flower crops similar to ETR1 with ETR1
- To study the effectiveness of various tools of bioinformatics and find applications for ETR1 sequence.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Postharvest losses in horticultural crops are physiologically controlled by a single factor – Ethylene. Recent advances in biotechnology and the explosion of biological information through internet have lead the biological information revolution. As a result of this, a huge volume of nucleic acid and amino acid sequence information are catalogued in computer databases. ETR1 is one such sequence of *Arabidopsis* which controls ethylene production. The recent IT revolution has created a wealth of bioinformatics tools available in the public domain for free, so that one can find applications for these sequences.

Studying variability for this ethylene production and finding matching sites for ETR1 sequence in other crop species could find an application for this tool as well as the sequence database. The literature relevant to such aspects as postharvest ethylene production, variability for ethylene production, ETR1 gene sequence and application of bioinformatics tools and related software are reviewed here.

2.1. Role of ethylene in plants

Ethylene, a hydrocarbon i.e., C_2H_4 is a gas and is produced to some extent in every cell of higher plants. Though it is also produced in animals, it acts as a hormone only in plants. Neljubov, a Russian plant physiologist is credited with the discovery that ethylene is a biologically active gas. The role of ethylene and its physiological implications as suggested by different authors are explained below.

Abeles (1972) reported a list of ethylene regulated phenomena, viz., breaking of dormancy, regulation of swelling and elongation, hypertrophy, induction of adventitious roots, epinasty, hook closure, inhibition of leaf expansion, control of flower induction, exudation, ripening, senescence and abscission.

Bleecker and Kende (2000) revealed that ethylene appears to be involved in mediating the response to stress and also mediated defense response to some pathogens and to suppress them to others.

Bleecker *et al.* (1988) suggested that treatment of etiolated seedlings of *Arabidopsis thaliana* with ethylene evoked the 'triple response' symptoms. These include exaggerated apical hook, radial swelling of hypocotyls and inhibition of hypocotyls elongation (Sakai *et al.*, 1998; Chang and Shockey, 1999; Hirayama and Alonso, 2000; Johri and Mitra, 2001).

2.2. Ethylene biosynthesis

Abeles (1972) proposed the probability of many precursors of ethylene in higher plants. These included methionine, linoleic acid, β-alanine, propanol, ethanol, organic acids, acrylic acid, thiomalic acid, glycerol, sucrose, glucose and acetic acid.

It was reported that tissues fed with methionine produced more ethylene than control. Later it was established that methionine was the precursor for ethylene.

Taiz and Zeiger (1991) reported a salvage pathway in the ethylene cycle where the methylthio group was preserved through every revolution of the cycle at the cost of one ATP molecule. Thus, high rates of ethylene biosynthesis can be maintained even when the methionine pool was small.

Redundancy of genes encoding ACC synthase and ACC oxidase yielded relatively few insights into ethylene synthesis. Screening of mutants in *Arabidopsis* yielded plants that overproduced ethylene *i.e.*, eto1, eto2 and eto3. These mutants had elevated ACC synthase activities (Bleecker and Kende, 2000).

2.3. Postharvest ethylene

Based on ethylene production at the time of ripening, fresh produce are classified as climacteric and non-climacteric. Fruits that produce a burst of ethylene as they ripen are classified as climacteric and those that do not produce ethylene are termed non-climacteric. A similar phenomenon is noticed in flowers. Upon

pollination, there is a spurt in ethylene production that causes senescence and abscission of petals. Several authors, as mentioned below, have elucidated the role of post harvest ethylene.

Abeles (1972) propounded that translocation of ethylene from stem to the fruit was not necessary to cause ripening. The fruit made its own ethylene and the ability to respond was dependent on the changes in rate of production. The effect of ethylene was accumulative. Therefore, a continuous exposure to a low concentration of ethylene throughout marketing can cause significant harm (Wills *et al.*, 2000).

Bleecker and Kende (2000) revealed that ethylene induced senescence in flowers showed fading of flowers and abscission of leaves and petals.

Jenny Jobling (2000) reported that ethylene concentration between 0.017 and 0.06 ppm during marketing of fresh produce had the potential to cause a 10-30 per cent loss in shelf life of produce.

2.4. Variability to ethylene production

Plant senescence is a genetic programmed process. Ethylene production varies with the development of the plant. Ethylene production rate is high at the time of pollination. It is reduced subsequently and there is again a burst of ethylene production during senescence. Several authors have studied this variability in ethylene production during the senescence cascade. Their findings have been listed below.

Woltering (1990) suggested that ethylene production was the trigger of senescence. In *Cymbidium* orchid flowers, removal of pollinia or anther cap (emasculation) was known to advance the senescence process. During postharvest life, flowers may lose the anther cap due to handling. A photoacoustic detection system showed the existence of a small peak in ethylene production immediately after emasculation. This emasculation induced senescence was presumed to be due to disruption in cytokinin flow between anther cap and the column. 1-aminocyclopropane-1-carboxylic acid (ACC) was also involved.

Clark *et al.* (1997) highlighted the importance of self-pollination in inducing ethylene production. Geranium florets showed a dramatic rise in ethylene production, followed by abscission within four hours. Neither wounding of the stigma, pollination with tetraploid pollen nor heat-killed self-pollen could elicit as much ethylene production and petal abscission as self-pollination.

Langston and Jones (1999) studied the post pollination signaling and senescence in *Petunia*. Following pollination, a climacteric peak of ethylene production was detected in wild-type corollas at 36 hours after pollination, which initiated senescence of corolla.

Linstrom *et al.* (1999) insisted on the role of pistil in producing ethylene that ultimately travels to, or signals the petals to produce ethylene and senesce. Ethylene and ACC increased sequentially in the styles, ovaries and petals following pollination. The pollination signal in carnation reached the ovary by 12 hours and the petals by 14 to 16 hours after pollination. They also identified the synthesis of ACC in petunia pollen. An ACC synthase gene was expressed in pollen.

Production rate of ethylene declined with increased maturity stages. Small fruits of Tomato produced higher ethylene compared to medium or large sized fruits. Fruits stored with calyx produced less ethylene than those that were stored without calyxes (Abdul *et al.*, 2000).

Dervinis *et al.* (2000) conducted an experiment with six cultivars of Geranium. They were germinated and grown in the dark in the presence of ACC. Of the six cultivars tested, Ringo 2000 Salmon, Multibloom Lavender and Elite White were the least ethylene sensitive. Florets were self-pollinated to test for cultivar differences in ethylene synthesis. Ethylene production was promoted in self-pollinated florets compared to non-pollinated florets. The data also suggested that genetic variability exists among Geraniums for both ethylene sensitivity and biosynthesis.

Yamasaki *et al.* (2000) investigated the action mechanism of ethylene in the induction of femaleness of cucumber flowers. A greater accumulation of CS-ETR2 and CS-ERS mRNA in gynoecious cucumber plants was predicted to be due to the higher level of endogenous ethylene. Gynoecious cucumber plants produced more ethylene than monoecious ones.

Porat *et al.* (1996) reiterated that pollination induced senescence was well known in orchids. A study on ethylene sensitivity in *Phalaenopsis* orchid revealed that, at the day of opening the flowers were sensitive to ethylene and this sensitivity declined during the flowers life span and increased again at senescence.

Hwang *et al.* (2001) transformed *Petunia hybrida* plants with a BO ERS gene from *Brassica oleraceae* var. *botrytis*. Transgenic plants produced 3 times more ethylene indicating that flower longevity resulted from a reduced sensitivity to ethylene.

Jones (2002) reported the sensitivity to ethylene increase as carnation flowers mature. Six stages of flowers following ethylene treatment were investigated. Styles, ovaries and petals were investigated. Ovaries were the first floral organs that had increased ethylene production. Styles had highest ethylene production during stage VI, which corresponded to the stage at which the style was receptive to pollination. It was observed that there was a transcript abundance of DC ACS1, DC ACS2 genes in all floral organs as flowers matured.

2.5. Quantification of ethylene

Modern research on ethylene production by plant tissues and on the biological activity of ethylene began with the introduction of gas chromatography (Burg and Stolwijk, 1959). This new analytical technique permitted accurate and rapid determination of trace amounts of ethylene. How various scientists used this technique, has been explained below.

determination of trace amounts of ethylene. How various scientists used this technique, has been explained below.

Bleecker *et al.* (1988) studied measurement of ethylene biosynthesis in *Arabidopsis thaliana*. Excised leaves were treated with air or ethylene in flow-through chambers for 12 hours and placed thereafter in 10 ml glass culture tubes sealed with serum vial caps. After 30 minutes, 1 ml samples were taken from the head space of the sealed tubes and ethylene was quantified with gas chromatography, Ethylene production was expressed in nanomoles per hour per gram of fresh weight.

Ievins (1996) measured ethylene biosynthesis in Pine needles. 0.5 g of freshly detached needles was placed in 10 ml bottles with 1 ml of incubation media. Bottles were closed with stoppers and incubated in light for appropriate intervals of time. Gas samples were collected at 1 hour intervals with a gas tight hypodermic syringe. Ethylene was analysed with a Shimadzu GC-9, equipped with FID and an alumina column. Nitrogen was used as carrier gas. Ethylene peak was identified by retention time of standard gas samples.

Fjeld *et al.* (1995) investigated ethylene production from branchlets of English holly (*Ilex aquifolium*) treated with ethylene concentrations of 0 ppm, 0.01 ppm, 0.1 ppm and 1 ppm. Exposure period was from 12 to 144 hours. Ethylene production was examined after 24 and 96 hours by enclosing branchlets in gas tight glass containers for 12 hours, after which ethylene concentration was determined by gas chromatography. Ethylene concentrations as low as 0.01 to 0.1 ppm induced leaf abscission.

Yamasaki *et al.* (2000) examined the time course evolution of ethylene from two cucumber plants. Excised shoot apices were enclosed in an 18.2 ml vessel and sealed with a rubber stopper. After incubation at 25°C for 16 hours, 1 ml of head gas was withdrawn using a gas tight syringe and injected into gas chromatograph (Chromatopac C-R4A, Shimadzu) equipped with FID and an activated alumina column for the measurement of ethylene.

Tieman *et al.* (2000) transformed tomato plants with the LeETR4 gene. He then compared the ethylene production from transformed and non-transformed plants. Fruit ethylene production was determined with gas chromatography.

Shanmugasundaram (2001) quantified ethylene production in polyethylene packed Banana fruits treated with ethylene absorbents. Gas chromatograph (Varian CP-3800) with FID and Poropak-Q as column were used. 500 µL of gas sample from polyethylene bags was injected for analysis. Sampling technique of gas samples was standardized.

2.6. The ETR1 gene

Mutants that exhibited variation in the triple response were evaluated by Bleecker *et al.* (1988). The study predicted the probability of the existence of an ethylene receptor in *Arabidopsis*. Subsequent research proved that the N-terminal contained the ethylene binding domain. The signal transduction pathway was found to be similar to the two-component system established in prokaryotes. Now, it has been proved that the ETR1 gene is a part of a multigene family in *Arabidopsis*. Genes exhibiting sequence homology to ETR1 have been identified in Tomato, Petunia, Banana, Melons and Orchids. All information pertaining to this gene, as deduced by various authors has been elaborated below.

2.6.1. Evolutionary ancestry

Schaller and Bleecker (1995) identified a sequence with homology to the ethylene-binding domain of ETR1 in a gene from cyanobacterium *Synechocystis*. It was determined that this sequence codes for a functional ethylene binding protein. It was proved from a knock-out mutation in the *Synechocystis* gene that the protein likely functions as a copper sensor. They hypothesized that this copper sensor had the right chemistry to bind ethylene and was recruited in evolution to serve as the ethylene input domain in the plant ethylene receptor.

Chang and Shockey (1999) endorsed this view, as they found a sequence similar to ETR1 in the genome of the cyanobacterium, which binds ethylene, suggesting a bacterial origin for the ethylene receptors.

2.6.2. Role of mutation

Bleecker *et al.* (1988) isolated mutants with altered responses to ethylene in *Arabidopsis*. Inhibition of hypocotyl elongation in dark grown seedlings was taken as the cue. Seedlings that had grown more than 1 cm after 4 days were selected as potential ethylene insensitive mutants. The mutation was designated as etr to indicate that it was a mutant allele of the ETR gene.

The genetic basis of ethylene insensitivity was examined in the etr mutant. Segregation of ethylene insensitivity in the F2 progeny was also consistent with a 3:1 ratio. Hence, it was confirmed that ethylene insensitivity was due to a dominant mutation.

Plants homozygous for the etr mutation were similar in appearance to their wild-type, indicating that the mutation does not interfere with major developmental processes.

Schaller and Bleecker (1995) suggested the significance of etr1-1 mutation as it represented the mutation of a Cys residue in the second transmembrane domain to Tyr, which resulted in a plant insensitive to ethylene. When Cys was converted to Tyr or Ser, the mutant proteins showed no ethylene binding in yeast.

Mutant alleles of ETR1 resulted from point mutations in the ethylene binding domain. The dominant insensitivity observed in *Arabidopsis* plants was due to disruption in ethylene binding, thus locking the protein in a form unable to sense ethylene.

Sakai *et al.* (1998) isolated etr2 (ethylene response 2) mutant from an EMS mutagenized population. Similar to the etr1 mutants, the etr2-1 allele was genetically dominant over wild-type.

Hall *et al.* (1999) studied the difference between four mutant alleles in *Arabidopsis*. The etr1-1 and etr1-4 mutations completely eliminated ethylene binding. etr1-3 mutation severely reduced ethylene binding. etr1-2 mutation did not disrupt ethylene binding in yeast. They further explored the relationship between ethylene binding and dominant insensitivity. They tested whether novel mutations in ETR1 that abolished ethylene binding in yeast could confer ethylene insensitivity to plants transformed with these mutant genes.

In *Arabidopsis*, mutants can be classified into three groups. Ethylene insensitive mutants – etr1, etr2, ein3, ein4, ein5, ein6. Constitutive ethylene response mutants – eto1, 2, 3 and ctr2. Tissue specific ethylene response mutants – hls1 and eir1 (Hirayama and Alonso, 2000).

Hall *et al.* (2000) observed that single loss- of- function mutants in four of the five ETR1 family members showed normal sensitivity to ethylene, in *Arabidopsis*.

Tieman *et al.* (2000) reported that a semidominant mutation in the NR gene of tomato resulted in the phenotype of the Tomato Never ripe (Nr) mutant.

Johri and Mitra (2001) identified two classes of mutants in *Arabidopsis*. Those which were insensitive to ethylene (ein or etr1) and those which showed the triple response constitutively, even in the absence of ethylene (eto1 and ctr1).

2.6.3. The ethylene receptor family of *Arabidopsis*

Bleecker *et al.* (1988) reported that a single receptor for ethylene may be present in all tissues of *Arabidopsis* and it is possible that mutation directly affects this receptor.

Schaller and Bleecker (1995) suggested that the discovery of ETR1 protein expressed in yeast was capable of directly binding ethylene, indicating that ETR1 protein was the bonafide receptor for ethylene.

Theologis (1995) elucidated that when ETR1 was first described, it seemed to be the only receptor. But then a second ethylene sensor, ERS was isolated from *Arabidopsis*.

It remains to be explained, why several ethylene receptors are required. It could be possible that ETR1, ETR2 and ERS contributed differently to ethylene signal transduction. It was thought that each gene may have specific activities in certain tissues, and individual genes had separate functions. Thus, ETR2 was suggested to have a tissue-specific function (Sakai *et al.*, 1998).

Chang and Shockey (1999) reported that the predicted proteins of tomato *i.e.*, LeETR1, LeETR2 and LeETR3 belonged to the ETR1 like subfamily. LeETR4 and LeETR5 belonged to the ETR2 like subfamily.

Bleecker and Kende (2000) explained the existence of an ethylene receptor family. This family was divided into two subfamilies based on structural similarities. The ETR1 like subfamily consisted of ETR1 and ERS1. The ETR2 like subfamily included ETR2, EIN4 and ERS2 (Johri and Mitra, 2001 and Hwang *et al*, 2002).

Hall *et al.* (2000) expressed ambiguity as to how each ethylene receptor isoform contributed to ethylene perception and signaling. Though genetic evidence indicated that the proteins were functionally redundant, it was suggested that the five isoforms might not possess entirely equivalent activities.

Tieman *et al.* (2000) provided the explanation by studying transgenic lines in Tomato. Lines with reduced NR mRNA levels exhibited normal ethylene sensitivity but elevated levels of LeETR4 mRNA. Over expression of NR in lines with lowered LeETR4 gene expression eliminated the ethylene sensitive phenotype. They confirmed that a functional compensation mechanism existed between members of the ethylene receptor family.

2.6.4. Structure of ETR1

Schaller and Bleecker (1995) cloned the ETR1 gene, which was found to encode a polypeptide with a hydrophobic NH2 – terminus responsible for membrane localization and a COOH – terminal region with homology to the histidine kinases and response regulators of bacteria.

Sakai *et al.* (1998) suggested the hybrid structure, specific to ETR1. ETR1 had both the putative histidine kinase domain and the receiver domain, in the same protein. This structure also existed in some bacterial sensors.

They also showed that ETR2 (isoform of ETR1) had a high sequence similarity to ETR1 (71 per cent similarity). A weaker sequence similarity in the putative histidine kinase domains was observed between ETR2 and ETR1 (58 per cent similarity). The receiver domain of ETR2 had 66 per cent similarity to that of ETR1.

Chang and Shockey (1999) reported that the ethylene receptor family was characterized by an amino-terminal ethylene binding domain, followed by a GAF-related domain and a histidine kinase domain. Some members of the family also had a carboxy-terminal receiver domain. The function of the GAF-related domain in the ethylene receptors was unknown.

Dieckmann *et al.* (1999) reported the intricacies of the structure of signal receiver domain of ETR1. The ethylene binding site was demonstrated to reside in the N-terminal 165 residues. The cytoplasmic, C-terminal had 400 residues of ETR1. They also endorsed that ETR1 was a hybrid two-component system.

The monomeric form of ETR resembled the known structure of bacterial receiver domain. ETR formed a homodimer in solution and in the crystal. Dimerization was mediated by the C-terminus, which formed an extended β sheet. The loop immediately following the active site adopted an exceptional conformation.

Hall *et al.* (2000) reported that the subfamily II genes contained a fourth hydrophobic segment at their N termini and it was unclear if this hydrophobic stretch

of amino acids served as a signal sequence or a fourth transmembrane domain, which could have implications for altering the ethylene binding site.

They also studied the structure of ERS1, an isoform of ETR1. ERS1 encoded a protein with 67 per cent identity to ETR1. ERS1 lacked the response regulator domain found in ETR1, ETR2 and EIN4. Another difference between ERS1 and ETR1 was that mRNA expression of ERS1 was ethylene inducible, whereas ETR1 mRNA expression was not.

Tieman *et al.* (2000) divided the ETR1 protein into three domains. The amino-terminal sensor domain contained three putative transmembrane segments and was shown to bind ethylene when expressed in yeast. The second domain exhibited homology to histidine kinases in bacteria. The third domain, the response regulator, received phosphate from the histidine kinase domain at aspartate residue. A region between the membrane spanning and histidine kinase domain also exhibited homology to GAF domain. (Hall *et al*, 2000 and Johri and Mitra, 2001)

They also predicted that the LeETR5 gene in Tomato could have a fourth N-terminal hydrophobic region and also lacked the histidine kinase domain that was shown to be autophosphorylated.

Dervinis *et al.* (2000) isolated and characterized ethylene receptor cDNAs from six Petunia cultivars. One was demonstrated to be a novel type, which lacked histidine kinase domain, and therefore it was suggested that this domain might not be essential for ethylene signal transduction.

A detailed display of every domain of the ETR1 protein with a self-explanatory diagram was established using the Pfam software. (URL: http://www.sanger.ac.uk/Software/Pfam.)

2.6.5. Location of ETR1 gene

Bleecker *et al.* (1988) crossed a plant homozygous for etr onto a line carrying ten phenotypic markers, to obtain a chromosomal location for the etr mutation.

Linkage analysis of 176 F2 plants indicated that the etr mutation was located in chromosome 1 of *Arabidopsis*.

Schaller and Bleecker (1995) reported that analysis of the protein from *Arabidopsis* and expressed as a recombinant protein in yeast indicated that the protein was associated with membranes as a covalently-linked homodimer in the cell.

Gorsel and Jovin (1998) used Fluorescence Resonance Energy Transfer (FRET) to visualize the localization of the ETR1 protein. FRET based microscopy methods combined with new probes were used for visualization of ETR1 protein. It was proved that the receptor was located in the plasma membrane of the cell.

Chang and Shockey (1999) deduced that the N-terminal of ETR1 was found in the plasma membrane, while the C-terminal of the protein was found in the cytoplasm.

Hirayama and Alonso (2000) ratified this view and reported that ETR1 and its related proteins were localized in the plasma membrane.

Hwang *et al.* (2002) gave an elaborate explanation on the location of the ethylene receptor family on the *Arabidopsis* chromosomes. ETR1 and ERS2 were located on chromosome 1, ERS1 on chromosome 2, EIN4 and ETR2 on chromosome 3.

The location of ETR1 on chromosome 1 of *Arabidopsis* was confirmed using the bioinformatics tool – Map viewer (URL: www.ncbi.nlm.nih.gov/mapviewer/).

2.6.6. Mode of action

Bleecker *et al.* (1988) predicted that ethylene binding in plants involved the interaction with a transition metal complex.

Schaller and Bleecker (1995) suggested that the ETR1 protein was normally active in the absence of ethylene and it negatively regulated the response pathway.

Binding of ethylene inactivated the receptor, resulting in expression of the response pathways.

Chang and Shockey (1999) identified a negative regulator of ethylene responses, CTR1, that acted downstream of the ethylene receptors. They elucidated the signal transduction pathway formulated on the basis of cloned *Arabidopsis* genes (Hwang *et al.*, 2002).

The membrane localized ethylene binding sites require a copper factor and the delivery of copper required a copper transporter RAN1. In the absence of ethylene, the receptors repressed responses possibly through the direct activation of the downstream negative regulator, CTR1.Binding of ethylene, inhibited receptor action of CTR1, followed by phosphotransfer to an attached receiver. In the absence of activated CTR1, the EIN2 integral membrane domain activated the carboxy terminal domain of EIN2. The C-terminal domain of EIN2 activated EIN3 (a member of the EIN3 transcription factors). EIN3 a positive regulator induced expression of an EREBP transcription factor called ERF1. ERF1 – a positive regulator caused ethylene – response gene induction.

From the above loss-of-function mutations, it was deduced that the dominant receptor mutations, which conferred ethylene insensitivity were gain-of-function alleles.

However, some ambiguous factors still remained unanswered, in the signal transduction pathway. In the second step of the two component mechanism, transfer of the phosphate to a receiver domain, was yet to be demonstrated.

The role of EIN2 in the pathway was elaborated. Signal propagation from CTR1 to the nucleus required EIN2. EIN2 was membrane associated, but lacked detectable metal transport activity. It was predicted that the amino-terminal domain served as a sensor. Overexpression of EIN2's hydrophilic C-terminal domain conferred a number of constitutive ethylene responses and it appeared that ethylene

regulation required EIN2 N-terminal domain. (Bleecker and Kende, 2000; Hirayama and Alonso, 2000 and Johri and Mitra, 2001)

Bleecker and Kende (2000) suggested that the utility of ethylene as a signal molecule depended on the ability of cells to monitor the changing concentrations of ethylene and transduction of this information into physiological responses, appropriate to the cell type.

Hirayama and Alonso (2000) highlighted the role played by copper in ethylene perception and transduction in *Arabidopsis*. They reported that metal-deficient ethylene receptors were non-functional, resulting in a constitutively activated signaling pathway. It was also predicted that the plant cells controlled ethylene receptor activity by modulating copper supply. Upon onset of senescence, the copper level dropped in *Arabidopsis* leaves. It was hypothesized that senescing leaves were more sensitive to ethylene, due to depletion of copper supply.

The role of EIN2 in the pathway was also studied. EIN2 function was required for the transduction of ethylene signal from CTR1 to the transcription factor EIN3. A loss-of-function mutation in EIN2 completely blocked ethylene response. They also endorsed the view that both N-terminal and C-terminal played a coherent role in signal transduction.

Hwang *et al.* (2002) reported that signaling was initiated when the His protein kinase, modulated by the environmental stimulus, autophosphorylated its conserved His residue. The phosphoryl group was transferred to a conserved Asp residue on the response regulator.

2.6.7. ETR1 – Shortcomings

Bleecker *et al.* (1988) reported that seeds carrying the etr mutation showed very low germination, compared to wild-type seeds. The germination of dormant seeds of wild-type but not of mutant plants could be induced by ethylene treatment.

Langston and Jones (1999) studied the process of senescence in wild-type and Etr1-1 Petunia flowers. Senescence of wild-type flowers started with wilting at petal margins followed by complete wilting of the corolla. Etr1-1 flower senescence was characterized by drying of petal margins and subsequent drying of entire corolla without wilting.

Clark *et al.* (1999) observed delayed floral senescence at varying degrees in transgenic Petunias that varied with production environment. Flowers grown in cooler greenhouse environments showed greater delay in pollination induced and natural flower senescence than those grown in warmer environments. Fruit ripening was delayed and adventitious root formation was significantly reduced in transgenic etr1 plants compared to wild-type. They suggested the requirement of tissue-specific ethylene insensitivity to extend flower life.

Hall *et al.* (1999) hypothesized that increased dosage of wild-type alleles in triploid lines of *Arabidopsis*, led to the partial recovery of ethylene sensitivity, which indicated that dominant ethylene insensitivity involved either interactions between wild-type and mutant receptors or competition between mutant and wild-type receptors for downstream effectors.

Gubrium *et al.* (2000) discovered the obvious benefits and problems that evolved by making a plant completely insensitive to ethylene. Hence, there was a need to engineer ethylene insensitivity in Petunia, only in specific parts of the plant i.e., ethylene insensitivity in the flower only. Using a promoter from the APETALA1 gene from *Arabidopsis*, they tied to confer ethylene insensitivity only at flowering and only in floral tissues. The results are under scrutiny.

Claire Granger (2001) examined two transgenic ethylene insensitive varieties of Petunia. It was found that, although both lines possessed the desirable early flowering feature, one line required reduced culture temperature to exhibit the phenotype. Both lines exhibited a delay in flower-senescence when compared to wild-type, but the extent of the delay was genotype-specific. More worrisome was the

finding that both lines exhibited a marked delay in fruit ripening and a significant reduction in the rooting of stem cuttings.

2.7. Horticultural crops and ETR1

Wilkinson *et al.* (1995) first reported a ripening-impaired tomato mutant Never-ripe (Nr). This gene encoded a protein with homology to ETR1 of *Arabidopsis* but lacked the response regulator domain. A single amino-acid change in the sensor domain conferred ethylene insensitivity when expressed in transgenic tomato plants.

Yamasaki *et al.* (2000) isolated three ethylene receptor related genes, CS-ETR1, CS-ETR2 and CS-ERS from Cucumber (*Cucumis sativus*) plants. CS-ETR1, CS-ETR2 and CS-ERS exhibited 90, 71, 79 per cent amino acid sequence similarities to *Arabidopsis* ETR1, ETR2 and ERS1 respectively.

Tieman *et al.* (2000) reported that Tomato contained a family of ethylene receptors, designated LeETR1, LeETR2, NR, LeETR4 and LeETR5 with homology to the *Arabidopsis* ETR1 ethylene receptor. LeETR4 was a negative regulator of the ethylene signal transduction pathway. NR lacked the response regulator domain found in the other LeETRs. LeETR4 also contained a 24 – aa amino-terminal extension that was not present in NR.

Dervinis *et al.* (2000) isolated two cDNAs from Geranium, Ph ETR1 and Ph ETR2. These genes shared 78 and 79 per cent identity with ETR1 from *Arabidopsis* respectively. These genes were expressed in Geranium florets long before they were receptive to pollination. Results indicated that the amount of Ph ETR1 and Ph ETR2 mRNA was not indicative of the level of sensitivity of Geranium florets to ethylene.

Wu *et al.* (2001) isolated a Banana ethylene receptor cDNA from Banana cDNA library. The cDNA clone encoded a 535 residue polypeptide named Mh-ERS1. Comparison between Mh-ERS1 and ETR1 from *Arabidopsis* showed more than 70 per cent similarity, but no responsive domain existed in the C-terminus of Mh-ERS1.

Wu *et al.* (2001) highlighted the role of EIN3 as a transcription factor in the ethylene signal transduction pathway. An orchid cDNA encoding an EIN3 homolog designated as PEIN3 was obtained from *Phalaenopsis* flower buds and subjected to sequence analysis. The deduced amino acid sequences shared 55 to 60 per cent identity to that of *Arabidopsis* EIN3.

All genes that show sequence homology to ETR1 were also analyzed and exhibited using Basic Local Alignment Search Tool (BLAST). (URL: www.ncbi.nlm.nih.gov/BLAST/).

2.8. Bioinformatics tools

Bioinformatics derives knowledge from computer analysis of biological data. These can consist of the information stored in the genetic code, but also experimental results from various sources, patient statistics and scientific literature. Research in bioinformatics includes method development for storage, retrieval, and analysis of the data. Bioinformatics is a rapidly developing branch of biology and is highly interdisciplinary, using techniques and concepts from informatics, statistics, mathematics, chemistry, biochemistry, physics and linguistics. It has many practical applications in different areas of biology and medicine. Various authors have developed softwares for analysis of biological data. Their findings have been reported below.

A basic introduction to bioinformatics was provided. Information regarding databases, evolutionary biology, protein modeling, genome mapping and applications of bioinformatics was elucidated (URL: www.ncbi.nlm.nih.gov/).

Luscombe *et al.* (2001) reported that the recent flood of data from genome sequences and functional genomics gave rise to a new field, bioinformatics, which combined elements of biology and computer science. A definition for this new field was proposed and research pursued in this field was explained.

Zakerin and Sharan (2001) gave a comprehensive explanation of all tools and databases extensively used in bioinformatics. Information regarding DNA databases, Protein databases, Text based search, Statistical estimators and multiple sequence alignments have been elaborated.

Gwynne and Heebner (2002) stressed on the intensifying influence of information technology in life sciences. They highlighted two important roles played by information technology. First, the combination of data collection and archiving. Second, simulations of biological processes, which range from modeling of chemical processes and of proteins all the way up to predictive modeling of cellular pathways and the effects of drugs on tissues.

2.8.1. Software for analysis

Altschul *et al.* (1990) described a sequence alignment heuristic method – Basic Local Alignment Search Tool (BLAST) that was developed for protein alignments in comparison to FASTA, which was developed for DNA sequences.

Altschul *et al.* (1997) developed an improved version of BLAST called Gapped BLAST and PSI-BLAST. Gapped BLAST triggered the extension of word hits combined with a new heuristic that generated gapped alignments. PSI-BLAST was a method that automatically combined statistically significant alignments produced by BLAST into a position-specific score matrix and searching the database using this matrix.

Thompson *et al.* (1997) developed software for multiple sequence alignments and for construction dendrograms. This was an improvement over the CLUSTAL W package. While CLUSTAL W was command-based software, CLUSTAL X was GUI based software.

Hillis (1997) gave an explanation on phylogenetic analysis and its importance. An important tool throughout biology for comparing information about genes, individuals, populations and species. Phylogenetic analysis was used to estimate the

historical relationships among the genes or species and to depict these relationships in the form of a branching diagram, known as a phylogenetic tree.

Sonnhammer *et al.* (1998) reported that Pfam contained multiple alignments and hidden Markov model based profiles of complete protein domains. The definition of domain boundaries, family members and alignment was done semi-automatically based on expert knowledge, sequence similarity, other protein family databases and the ability of HMM-profiles to correctly identify and align the members.

Laskowski (2001) elucidated the features of the software PDBsum. A web-based database that provided pictorial summary of the key information on each macromolecular structure deposited in the PDB. Images of the structure, annotated plots of each protein chain's secondary structure, detailed structure analyses generated by the PROMOTIF programme, summary PROCHECK results and schematic diagrams of protein-ligand and protein-DNA interactions. Interactive viewing in 3D was also possible. PDBsum is updated whenever new sequences are entered into the Protein Data Bank (PDB).

Ron Shamir (2001) explained all the sequence alignment softwares and the steps involved in operating them. These included, FASTA, BLAST, Substitution matrices, Improved BLAST searches etc.

Pethururaj (2002) highlighted the advance options and additional features of BLAST Enhanced Alignment Utility (BEAUTY) and Power BLAST. Power BLAST had options for masking repetitive elements and low complexity subsequences. It was also capable of processing sequences of any length.

Bateman *et al.* (2002) explained Pfam – software that had a collection of protein families available via the web and in flat file form. The multiple sequence alignments around which Pfam families were built are important tools for understanding protein structure and function and this formed the basis for techniques such as secondary structure prediction, fold recognition and phylogenetic analysis.

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

Experiments were conducted to study the variability among *Anthurium andreanum* and *Dendrobium* genotypes for postharvest ethylene productivity. As ethylene production was controlled genetically by ETR1 gene (antisenescence gene) and as the nucleotide sequence information for this gene and its mutant ETR1-1 is available in the public domain, various tools of bioinformatics were applied to this sequence. These studies were carried out at the Department of Floriculture and Landscaping, Horticultural College and Research Institute, TNAU, Coimbatore – 641003 during 2000-2002.

The methodology consisted of two parts. The first part was the study of variability for postharvest ethylene production among Anthurium and Dendrobium genotypes. The second part consisted of application of bioinformatics tools on ETR1 gene (antisenescence gene) sequence. The procedures of the experiments are listed below.

3.1. Variability to Ethylene Production

The experiment was performed in Completely Randomized Design (CRD) with sixteen *Anthurium andreanum* and three *Dendrobiums* (for comparison) with three replications in each variety. Preliminary estimation trials were conducted, to determine if the varieties exhibited variability in ethylene production, to perfect the sampling technique.

Ethylene production was studied over a period of time, to determine variability after the flower was excised from the mother plant. Hence, ethylene production was estimated at one-week intervals for three consecutive weeks. This coincided with the period when the flowers were fresh upto the stage at which flowers showed wilting symptoms.

Sixteen Anthurium and three Dendrobium varieties were collected at the Botanical Garden, TNAU, Coimbatore for the study, as mentioned in the list below.

Table.1. List of varieties and treatment details

Treatment	Variety	Treatment	Variety	Treatment	Variety
T1	Sikkim Red	T8	Red Dragon	T15	Tinora
T2	Sunshine Orange	T9	Glamor	T16	B 13
T3	Temptation	T10	Linda Demol	T17 (Orchid)	Thai Hybrid
T4	Bonfire Orange	T11	Leema White	T18 (Orchid)	Sonia 17
T5	Merengue	T12	Sunset Orange	T19 (Orchid)	Spic White
T6	Honduras	T13	Gloria		
T7	Rosetta	T14	Linda		

3.1.1. Sampling Technique

The polyethylene cover selected for covering the flowers had a length of 53cm, breadth of 36cm, with a thickness of 400 gauge.

Two flowers in each of the genotype of Anthurium and one spike in each of the variety of Orchid flowers were packed in these covers, as soon as they were excised from the mother plant. Anthurium flowers were packed along with their pedicels. Orchid flowers were packed at the rate of one spike per cover.

The covers were sealed using an electric bag sealer. Assurance was made that the covers were free of leakages. The initial weight of the flowers was recorded. The packed flowers were maintained at room temperature.

500µL of gas sample was drawn from the polybag using 1000µL gas tight GC micro-syringe, at weekly intervals for the estimation of ethylene in the polybag.

3.1.2. Quantification of Ethylene

Varian Analytical Instruments, GC model CP-3800, gas chromatograph was used for estimation of ethylene from sample. Nitrogen was used as carrier gas, hydrogen as fuel and zero air as oxidant was supplied. A constant gas flow of 30ml/min. was maintained by the EFC of the instrument. FID detector was used with Poropak-Q as column. The FID temperature was set at 220°C and column oven temperature maintained at 60°C for 5 minutes. The injector temperature was maintained at 200°C. Pure ethylene gas standard from standard cylinder was used for standardisation. 500µL of gas sample was withdrawn from the package using a gas-tight glass syringe and was injected for analysis. Ethylene concentration was calculated from the area count from the standard graph and expressed as ppm. This procedure was repeated at weekly intervals for three weeks and the ethylene content was estimated. The ethylene data recorded was analyzed in CRD. Further, a clustering analysis using JOIN and K-MEANS was done to group the genotypes according to the postharvest ethylene evolution data.

Based on the cluster analysis performed on the ethylene data of the above experiment, five Anthuriums evolving less or no ethylene were screened again with two Dendrobiums for confirmation. They are listed below.

Table.2. List of Anthurium varieties selected for bunch treatment

Anthurium
B 13
Bonfire Orange
Sunset Orange
Linda Demol
Merengue

To determine if these varieties showed ethylene production when packed in large numbers, a bunch of flowers comprising 10 flowers of Anthurium/cover were packed. These flowers were maintained at room temperature for three weeks with three replicates each, and ethylene production was estimated by injecting 500μL of gas into the gas chromatograph. The ethylene data collected was analyzed in CRD.

3.2. Bioinformatics Experiment

A bioinformatics study was undertaken to investigate the properties of the ETR1 gene, its structure, mode of action and crops that showed sequence similarity, as ETR1 was found to be involved in controlling ethylene production. Since all the software are available in the public domain through internet, the purpose and procedures to access these software are illustrated below. The bioinformatics tools are grouped as those specific for location, nucleotide sequence, amino acid sequence, structure prediction and macromolecular movements.

3.2.1. Location Specific Search

3.2.1.1. Map Viewer

This tool utilizes a chromosome-specific search on the organisms that have been sequenced so far. This includes humans, mouse, fruit fly and mouse-ear cress. Since, there were predictions that etr1 gene was located on chromosome1 of *Arabidopsis*, this tool was used for its conformation.

Method

The chromosome view (called Map View) shows one or more detailed maps for a single chromosome. It displays a list of the elements on the master map, and allows the maps to be viewed in progressively greater levels of detail.

Since, etr1 gene was predicted to be present in chromosome1 of *Arabidopsis*, this chromosome was selected for the study. The gene name was typed in the search slot to determine the exact position of this gene on chromosome1 of Arabidopsis.

The URL to access this tool is : http:// www.ncbi.nlm.nih.gov/cgi-bin/Entrez/map_search

3.2.2. NUCLEOTIDE SEARCH

3.2.2.1. Basic Local Alignment Search Tool (BLAST)

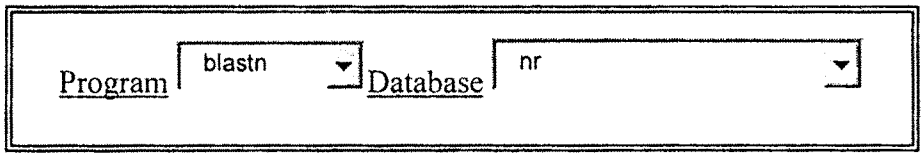
This tool was developed by Altschul *et al* (1990). This had an advantage over the tool FASTA in that, BLAST was faster and used local alignment search in contrast to FASTA that used global alignment. This tool exhibited the HSPs (High Scoring Pair) that had a score above the threshold. Since, the results were more exact, BLAST was the widely used search tool.

BLAST was used in this study to find nucleotide sequences in the database that showed homology to the ETR1 sequence. The following steps were followed and the necessary parameters were maintained.

Step 1. Choose the program to use and the database to search.

Programs specific for nucleotide search, amino acid search, reading frame search are available in the program menu. Since, a nucleotide search was employed in the experiment, the **blastn** program was selected.

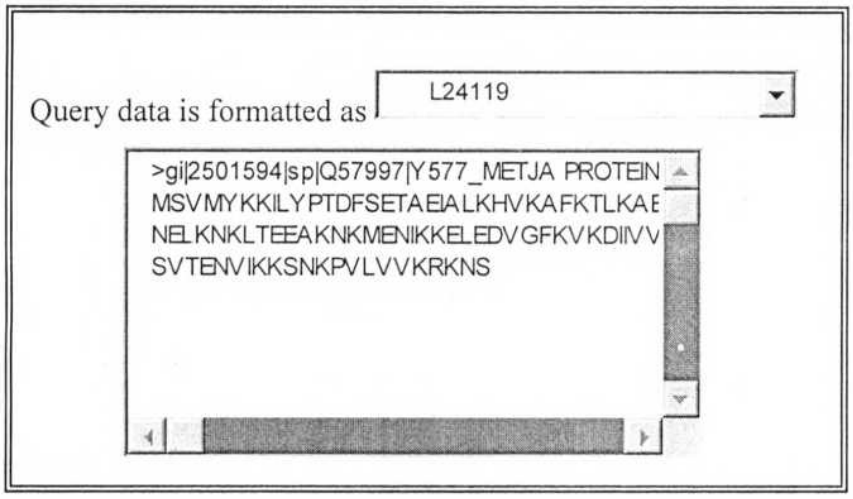
Organism specific databases and Expressed Sequence Tag (EST) databases are available in Genbank. The **non-redundant** database represents all the sequences irrespective of the organism (whole databank search). Hence, this database was selected.



Step 2. Input the data.

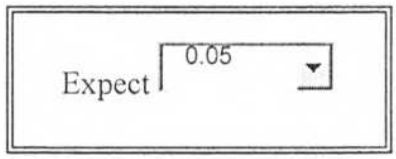
The sequence can be loaded in FASTA format or by typing the accession number in the space provided. FASTA format denotes the algorithm developed by Lipman and Pearson (1988). This format is represented by a '>' sign, followed by the name of the gene and the name of the organism/plant.

The accession number of the ETR1 sequence was obtained from the genbank database using the Entrez search tool. In the experiment, the accession number **L24119** was typed in the space as shown below.



Step 3. Set the program options or choose defaults.

The program options were modified based on the requirements of the experiment. The E value denotes the probability that a hit occurs by chance. To obtain hits with high scores, the E value should be maintained below 1. For this study, an E value of 0.05 was used.



Step 4. Selection of Substitution Matrix.

A matrix is used in this tool to convert the biological data into scores. This score determines the HSPs that are displayed in the output. Percent Accepted Mutation (PAM)

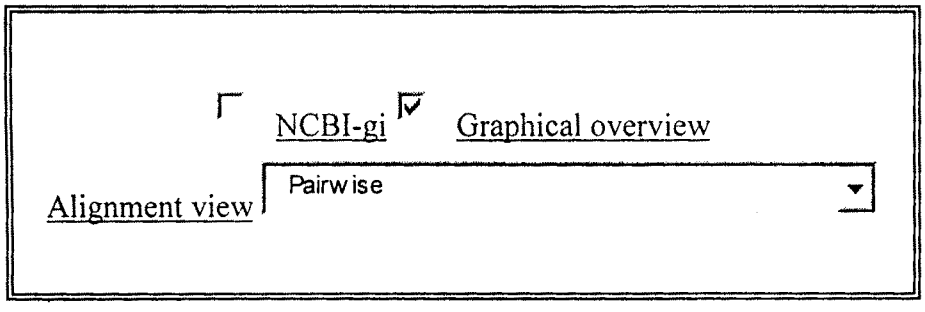
matrix and Blocks Substitution Matrix (BLOSUM) are the two types of matrices that are widely used. The default BLOSUM 62 matrix was used in this study.

* Matrix	Gap existence cost	Per residue gap cost	Lambda ratio
PAM30	9	1	0.87
PAM70	10	1	0.87
BLOSUM80	10	1	0.87
BLOSUM62	11	1	0.85

Step 5. Set the output formatting options

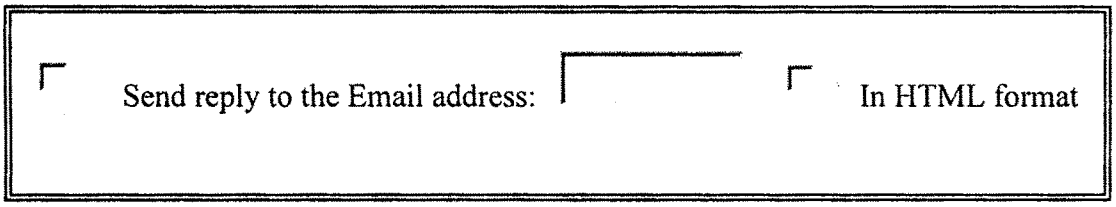
All sequences that showed homology above the threshold can be displayed in three formats. The graphical representation had a color key that denoted sequences with varying similarity to the query sequence. The tabular representation displayed the genbank identifier number, the name of the organism/plant, the score obtained by a particular alignment and the E value of that alignment. The pairwise alignment view represented the number of gaps and gap length between the query sequence and the sequence from the database.

In the experiment, all three output formats were selected for display and the results were analyzed.



Step 6. Perform the search

The search was performed and results were obtained in HTML format.

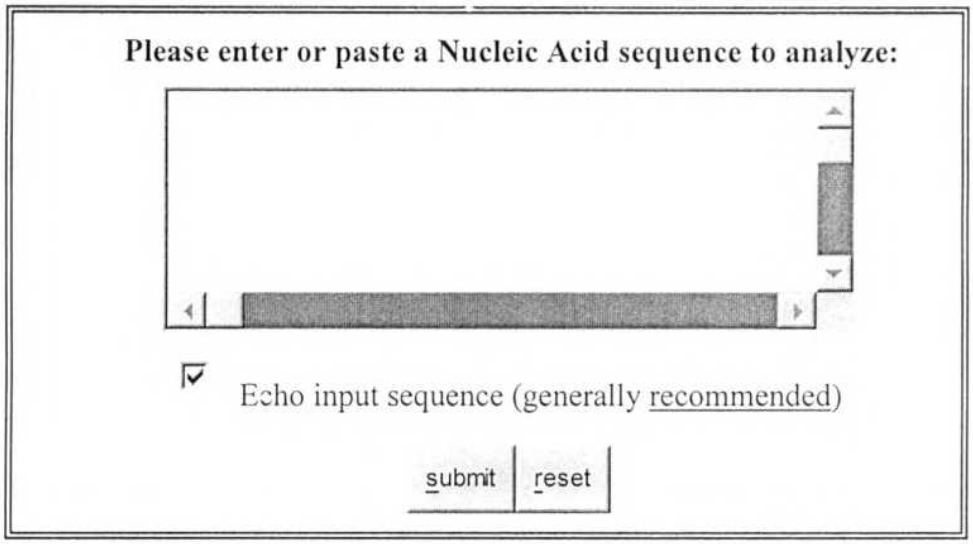


The URL to access this tool is : http:// www.ncbi.nlm.nih.gov/BLAST/

3.2.2.2. PROMOTER SCAN

Promoter Scan was designed to find putative eukaryotic Pol II promoter sequences in primary sequence data. Promoter Scan is used to find regions in primary DNA sequence that might be good candidate regions to further test for promoter functionality. The program recognizes approximately 70% of primate promoter sequences, with a false positive rate of about one in every 14,000 bases.

This tool was employed in the experiment to hypothetically determine the putative promoter regions of the ETR1 nucleotide sequence, which would facilitate the nucleotide sequencing of this gene in Anthurium. The nucleotide sequence of ETR1 was pasted in the space as seen below. This is a simple tool and does not involve parameter settings. The 'submit' button was clicked to view the results.

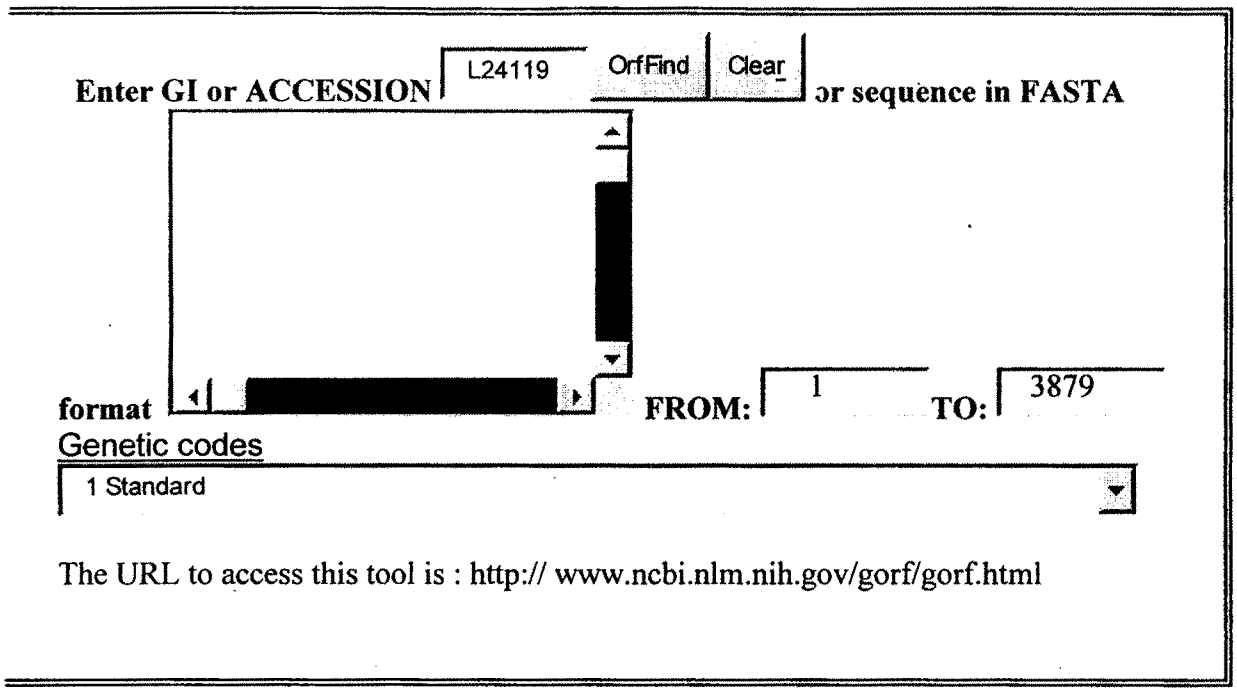


The URL to access this tool is : http:// www.molbiol.ox.ac.uk/promoterscan.htm

3.2.2.3. ORF FINDER

The Open Reading Frame (ORF) denotes the region between the start codon and the terminating codon that encodes a potential polypeptide. While, identifying this region could take months in the laboratory, the ORF finder predicts this region in a sequence in a few seconds. The ORF Finder is a graphical analysis tool that finds all open reading frames of a selectable minimum size in a user's sequence or in a sequence already in the database. This tool identifies all open reading frames using the standard or alternative genetic codes. The ORFs between a selected number of nucleotide sequences can be determined by typing the region required in the 'From' and 'To' slots. The deduced amino acid sequence can be saved in various formats and searched against the sequence database using the WWW BLAST server.

This tool was included in the study to determine the prospective reading frames present in the ETR1 sequence. The output can be obtained either by loading the nucleotide sequence in FASTA format or by typing the accession number in the slot as shown below. The standard code was selected in the search. The accession number i.e., L24119 was typed and results obtained.



3.2.3. Amino Acid Search

3.2.3.1. BLAST Enhanced Alignment Utility (BEAUTY)

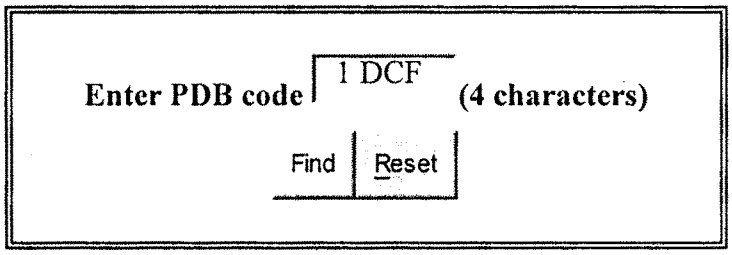
This is an improvement over the existing BLAST tool. In contrast to BLAST which only displays the sequence alignment between the query and the database sequence, this tool displays the function of the sequence in the database. This tool also filters the low complexity regions thus making this tool a more effective one.

To have an enhanced BLAST search this tool was included in the study. The amino acid sequence was pasted in the space provided. The E value was maintained at 0.05. Graphical, table and pairwise alignments were displayed.

3.2.3.2. PDB sum

A software developed by Laskowski (2001), this tool provides a holistic pictorial representation of the desired protein, present in the Protein Data Bank (PDB). All information pertaining to a protein viz., structure, literature, CATH code, 3D view and links to other software are available. This tool was used in the study to unearth the secondary structure and to avail the links present. The receiver domain of the ETR1

secondary structure and to avail the links present. The receiver domain of the ETR1 protein was stored in PDB. The PDB ID code for this was **1 DCF.** This PDB code was entered into the input space, displayed below.

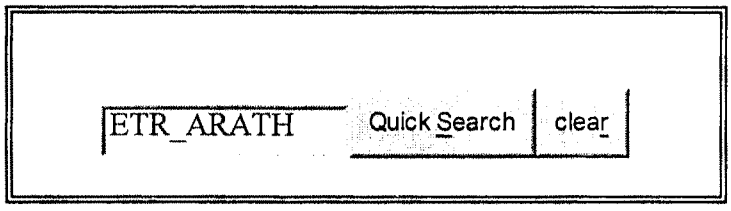


The URL to access this tool is : http:// www.biochem.ucl.ac.uk/bsm/pdbsum/

3.2.3.3. SWISS-PROT

SWISS-PROT is a protein knowledgebase established in 1986. The SWISS-PROT protein knowledgebase consists of sequence entries. Two classes of data i.e., core data and annotation are available for each sequence entry. This comprises function of the protein, post-translational modifications, domains and sites, secondary structure, quartenary structure and similarity to other proteins.

To determine the core data and annotation of ETR1 the information in this databank was used. The SWISS-PROT code for ETR1 was **ETR_ARATH.** This code was typed in the space provided and results were obtained.



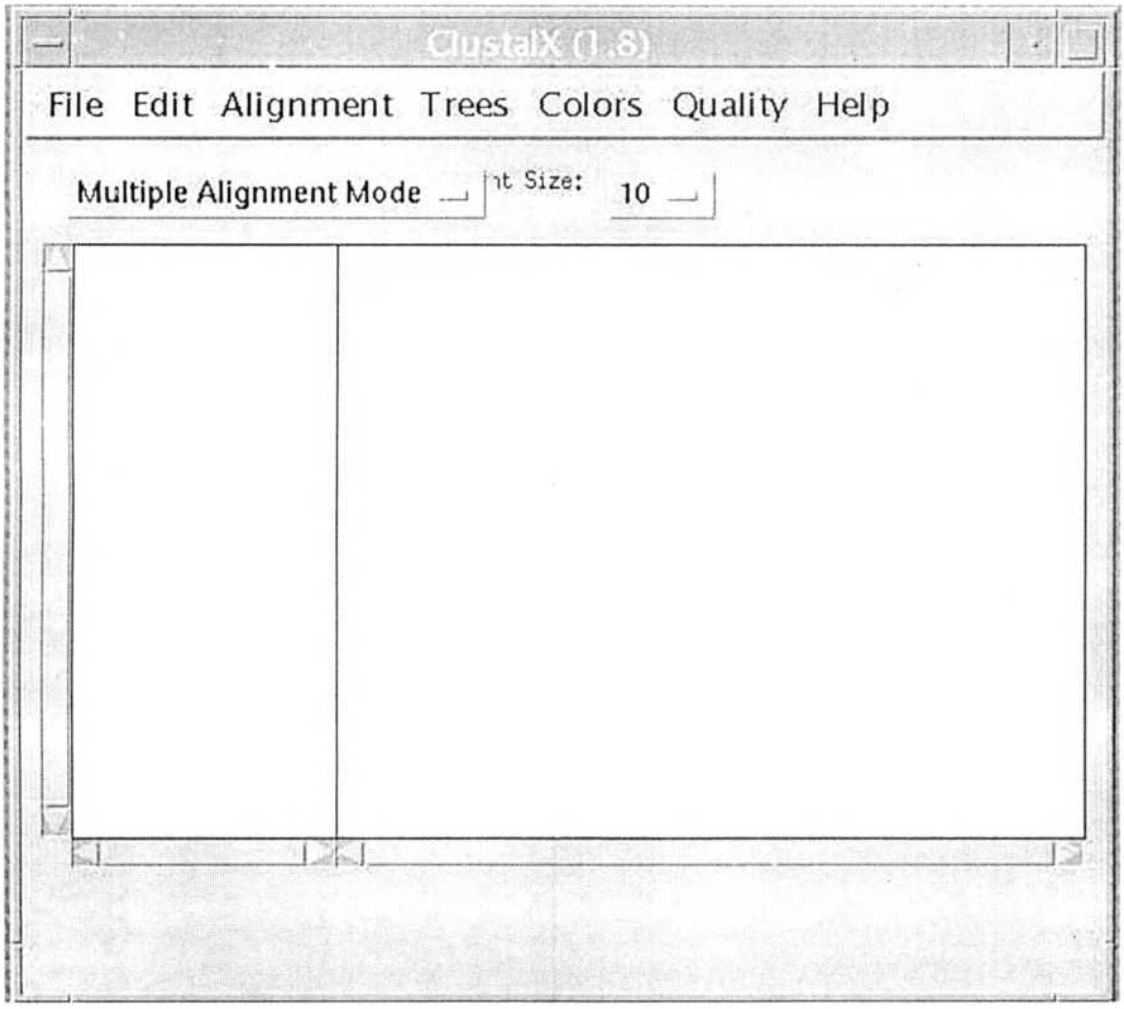
The URL to access this tool is : http:// www.expasy.ch/sprot/

3.2.3.4. CLUSTAL X

CLUSTAL X, a multiple alignment search tool was developed by Thompson *et al* (1997) as an upgradation over the already existing CLUSTAL W software which was command based. This tool consists of two modes i.e., the multiple alignment mode and

the profile alignment mode. In the experiment, this tool was used to reveal the per cent similarity between sequences from *Arabidopsis* and other horticultural crops. Amino acid sequences from cucumber, rose, banana, geranium and tomato were loaded in FASTA format.

The opening window of this program is displayed below.



Sequence Input

Seven formats are automatically recognised: NBRF/PIR, EMBL/SWISSPROT, Fasta, Clustal (*.aln), GCG/MSF (Pileup), GCG9 RSF and GDE flat file. In the study, the sequences were loaded in FASTA format.

Sequence / Profile Alignments

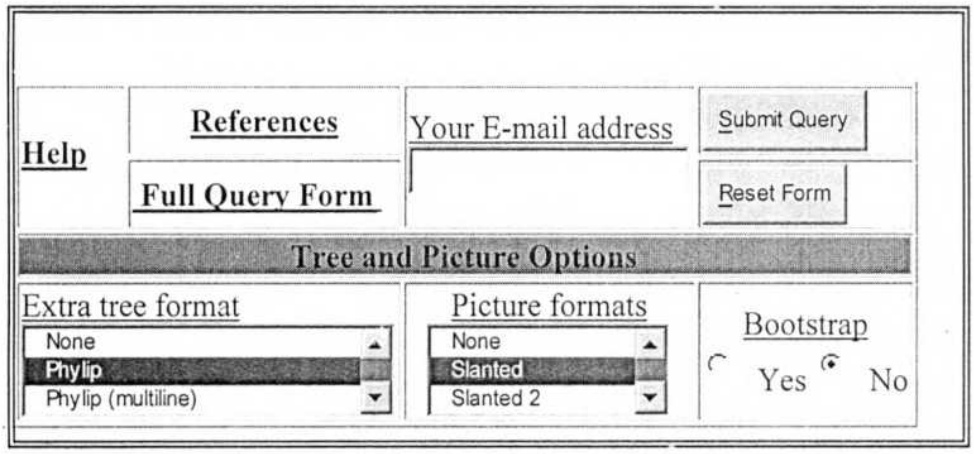
The multiple alignment mode and profile alignment mode were used in the study. The former was used to align multiple sequences and the latter was used to compare two sequences.

The URL to access this tool is : http:// www-igbmc.u-rasbg.fr/BioInfo/ClustalX/Top.html

3.2.3.5. TREE TOP

Similar to CLUSTAL X, this tool displays the multiple sequence alignments and the phylogenetic tree. A phylogenetic tree elucidates the distance by which two sequences are related. This tree is established with the use of a distance matrix.

This tool was included in the study to deduce a phylogenetic relationship between the sequences obtained from horticultural crops. The amino acid sequences of horticultural crops that showed homology to ETR1, were selected and loaded in FASTA format. Phylip tree output format was selected.



The URL to access this tool is : http://www.genebee.msu.su/services/phtree_reduced.html

3.2.3.6. BIOEDIT

A versatile bioinformatics tool, Bioedit has many applications. It is used to determine multiple sequence alignments, nucleic acid/amino acid composition, molecular weight of protein and display of phylogenetic tree.

This tool was included in this study, to find the above mentioned properties of the ETR1 sequence and its allied sequences from other horticultural crops. The respective sequences were loaded in FASTA format by using the 'Open' option in the 'FILE' menu. Subsequent analysis of each of the proteins was carried out by working with various menu options available on the main page.

The URL to access this tool is : http:// www.mbio.ncsu.edu/BioEdit/bioedit.html

3.2.4. Structure Prediction Tools

3.2.4.1. CATH – Protein Structure Classification

The CATH database is a hierarchical domain classification of protein structures in the Brookhaven protein databank. All non-protein, model, and "C-alpha only" structures are not classified in CATH. Only crystal structures solved to resolution better than 3.0 angstroms are considered, together with NMR structures. There are four major levels in this hierarchy; Class, Architecture, Topology (fold family) and Homologous superfamily.

Class, derived from secondary structure content, is assigned for more than 90% of protein structures automatically. Architecture, which describes the gross orientation of secondary structures, independent of connectivities, is currently assigned manually. The topology level clusters structures according to their toplogical connections and numbers of secondary structures. The homologous superfamilies cluster proteins with highly similar structures and functions. The assignments of structures to toplogy families and homologous superfamilies are made by sequence and structure comparisons.

This tool was used to determine the structure classification of ETR1 protein. The CATH code for ETR1 was found to be **3.40.50.3000** and it was typed in the search slot. The class, architecture, topology and homologous superfamilies were illustrated.

The URL to access this tool is : http:// www.biochem.ucl.ac.uk/bsm/cath/

3.2.4.2. Pfam - Protein Families Database

Pfam was developed by Sonnhammer *et al* (1998) to provide accurate descriptions of protein domains. The structure of an established SWISS-PROT sequence or the structure of a new sequence can be determined with this tool.

This tool was employed in the study to reveal the domain structures of ETR1 protein and to confirm these results with the hypothesized structure. Since, this structure was stored in the database, the SWISS-PROT code was typed in the search slot.

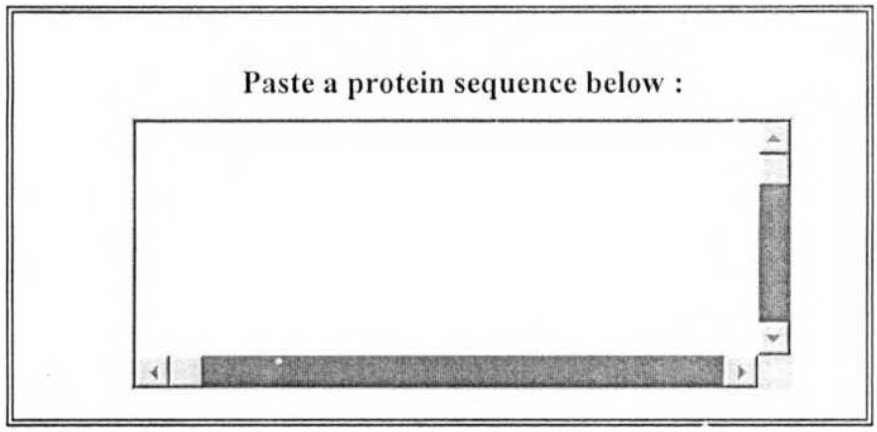
The URL to access this tool is : http://www.sanger.ac.uk/Software/Pfam

3.2.4.3. CDART -Conserved Domain Architecture Retrieval Tool

The conserved domains in a protein sequence play a significant role in determining its structure and hence its function. These domains are also not easily affected by mutation. To determine the conserved domains in the ETR1 protein sequence and to determine the sequences that showed homology to the conserved domains of ETR1 from various organisms, this tool was used in the experiment.

The amino acid sequence of ETR1 protein in FASTA format was pasted in the space as shown below.

To find a protein sequence's domain architecture, enter its accession or the sequence in FASTA format:



The URL to access this tool is : http:// www.ncbi.nlm.nih.gov/Structure/lexington/ lexington.cgi?cmd=rps

3.2.4.4. Color Protein Sequence

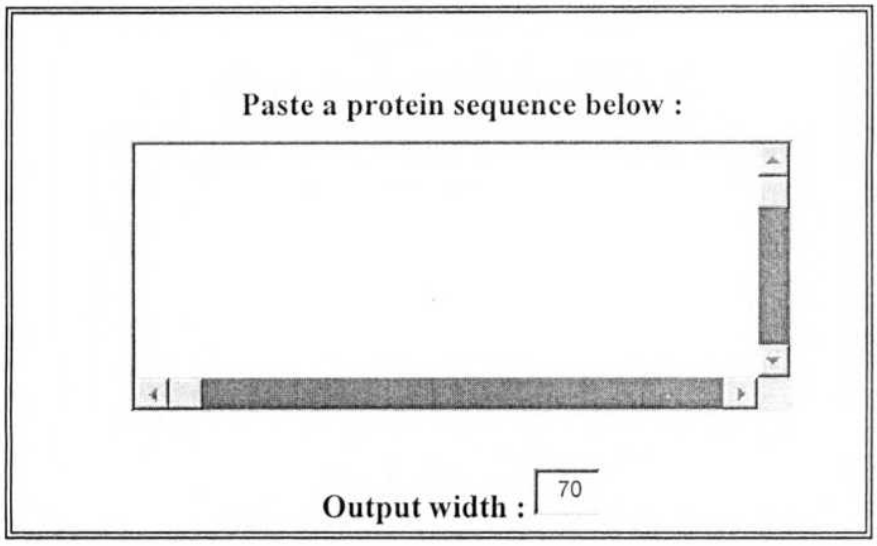
It is a simple tool that permits the user to color the residues of a sequence. The user can select a predefined residue set for coloring. This algorithm uses a threshold value for residue number to color the region of interest.

In the study, this tool was included to isolate the hydrophobic and hydrophilic regions of the ETR1 protein sequence by coloring. The following parameters were maintained.

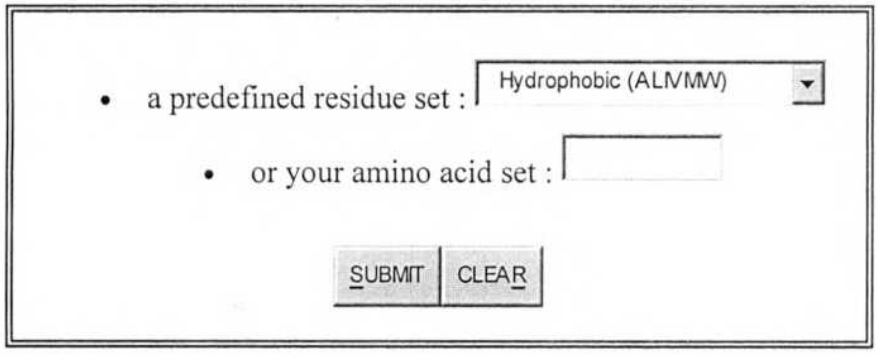
Amino acid sequence pasted in the space provided.

Output width maintained at default value of 70.

The predefined residue set option was clicked and 'hydrophobic' and 'hydrophilic' were selected separately.



Color :



The URL to access this tool is : http:// pbil.ibcp.fr/cgi-bin/ npsa_automat.pl?page=npsa_color.html

3.2.4.5. PREDATOR

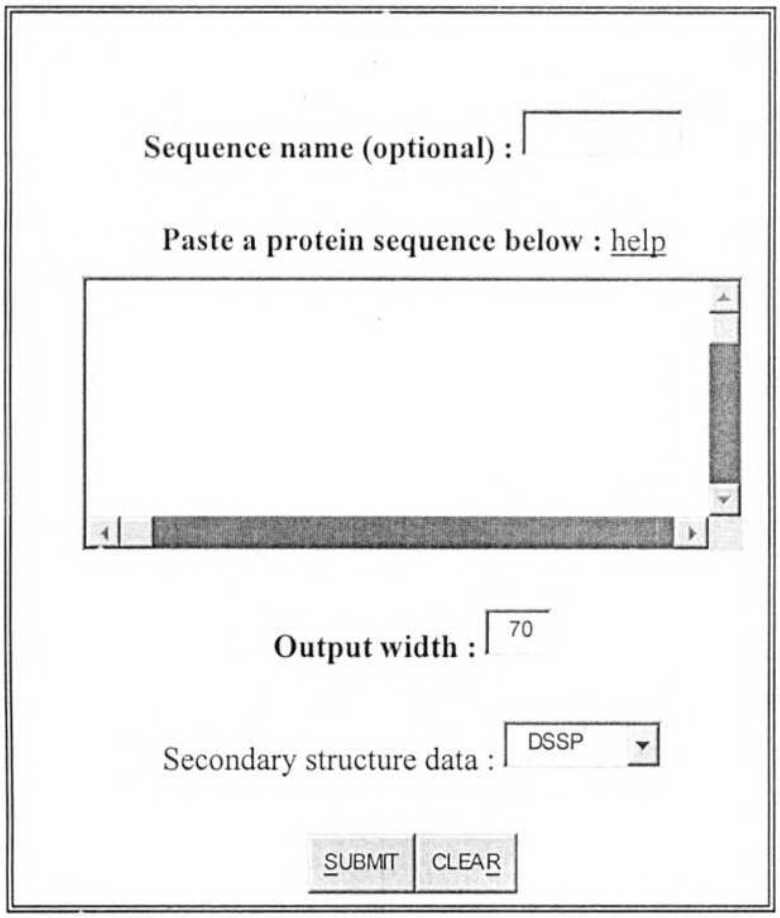
Predator is a secondary structure prediction method based on recognition of potentially hydrogen-bonded residues in a single amino acid sequence. This tool provides a 2D display of the secondary structure of the desired protein.

Parameters

The user can choose between two database files **stride.dat** and **dssp.dat**. These files contain propensity tables, secondary structural assignments and thresholds for two secondary structure assignment methods from tertiary structure : STRIDE and DSSP.

Predator method

The amino acid sequence was pasted in the space as seen below. The output width was maintained at the default value of 70. DSSP database was selected for analysis.



The URL to access this tool is : http:// pbil.ibcp.fr/cgi-bin/ npsa_automat.pl?page=npsa_predator.html

3.2.4.6. SOPMA - Self-Optimized Prediction Method With Alignment

Similar to PREDATOR, this tool also predicts the 2D structure of the desired protein. An advantage in this tool is that, the percentage of helices, sheets, coils and loops is also illustrated.

This tool was included in the study to determine the secondary structure of ETR1 protein and to determine the percent of helices and sheets. This tool was used as a basis for the 3D structure prediction of the ETR1 protein.

Parameters

The similarity threshold parameter is the threshold below which a subject peptide is rejected when it's compared with a query peptide of the sequence. The window size parameter sets the length of the peptides to use. The number of conformational states to be predicted can be selected: 3 or 4.

The amino acid sequence of ETR1 was pasted in the space provided and the prediction threshold was maintained at 3.

The URL to access this tool is : http:// pbil.ibcp.fr/cgi-bin/ npsa_automat.pl?page=npsa_sopma.html

3.2.4.7. SWISS – PDB Viewer

The structure of a protein is imperative in determining its function. A small change in the protein conformation can cause mutations and diseases. Hence, the secondary structure of proteins plays a pivotal role in protein function. The unraveling of secondary structure, was performed using NMR spectroscopy and X-ray diffraction methods. This could take months or years to predict the structure. Now, many secondary

structure prediction tools are available. These tools are based on the principle that, if a sequence has homology to a sequence in a database, then the new protein performs the same function as the one in the database.

To reveal the 3D structure and other characteristics i.e., electrostatic potential, side chains, amino acid arrangement on the protein, water molecules etc. of ETR1 protein this tool was used.

SPDBV Method

The 'window' menu on the main page was selected.

ETR1 amino acid sequence was loaded in fasta format.

The 3D structure of ETR1 protein was displayed.

Manipulation of the protein structure could be performed by using the menu options available on the main page.

The URL to access this tool is : http:// www.expasy.ch/spdbv/

3.2.4.8. RASTOP

This was an updated version of the 3D structure prediction tool RASMOL. Released in Oct. 2002 this tool has additional options to manipulate the protein structure.

This software accepts sequences only in the pdb/rasmol/rastop formats. The pdb format of the receiver domain of ETR1 was obtained from the PDB (www.pdblite.org). This sequence was loaded in the software and the 3D structure of the receiver domain was displayed. Deleting/adding residues to the structure, thus changing the conformation was performed using this tool. Structure details like bond angles, number of residues in a selected region of the protein, backbone structure etc. were determined with this software.

The URL to access this tool is : http:// www.geneinfinity.org/rastop/

The URL to access this tool is : http:// www.geneinfinity.org/rastop/

3.2.4.9. VAST - Vector Alignment Search Tool

Analogous to the BLAST search for nucleotide sequences, there was a need to develop software to determine sequences that had structural similarity to a query sequence. VAST search is a service that allows searching for structural neighbors starting with a set of 3D-coordinates specified by the user. This service is meant to be used with newly determined protein structures that are not yet part of MMDB. Structure neighbors for proteins already in MMDB have been pre-computed and can simply be looked up from MMDB's structure summary pages.Protein structure neighbors in Entrez are determined by direct comparison of 3-dimensional protein structures with the VAST algorithm. Each of the more than 18,000 domains in MMDB is compared to every other one. From the MMDB structure summary pages, retrieved via Entrez, structure neighbors are available for protein chains and individual structural domains.

This tool was employed in the study to determine sequences from other organisms that showed structural similarity to the ETR1 sequence. The link to this site was achieved through the PDBsum site. By clicking on the 'VAST' icon on the PDBsum main page, the link was established and results obtained.

The URL to access this tool is : http:// www.ncbi.nlm.nih.gov/Structure/VAST/vast.shtml

3.2.5. Macromolecular Movements Search

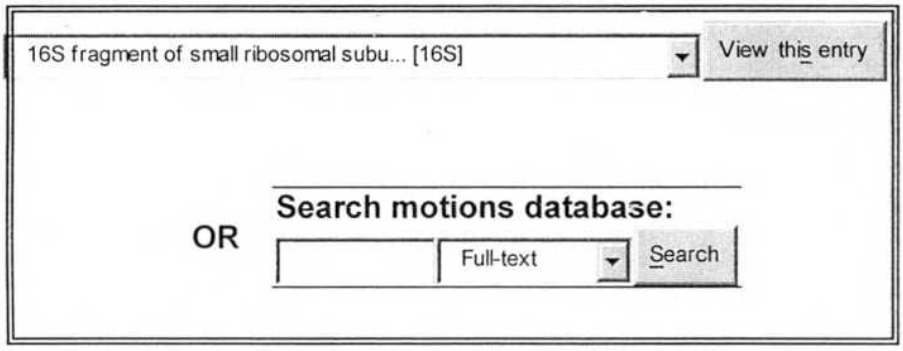
3.2.5.1. Database of Macromolecular Movements

The movements exhibited by proteins play a pivotal role in exhibiting the function of the protein. Also, these movements are important in protein-ligand interaction. Hence, a database specific for macromolecular movements was established by Gerstein at the

university of Yale. Using the options available, the movement of an unknown sequence can also be determined.

This tool was used to test if the ETR1 sequence was similar to the CheY sequence of bacteria. The protein motion of CheY protein was determined from this database. This was achieved by selecting the options available on the main page.

View entry:



The URL to access this tool is : http:// molmovdb.mbb.yale.edu/MolMovDB/

3.3. Statistical Analysis

Statistical procedures as prescribed by Panse and Sukhatme (1978) was followed wherever applicable using AgRes software, available at HC & RI, TNAU, Coimbatore. Further, a cluster analysis was performed using JOIN as well as K-MEANS algorithms for the ethylene production data at weekly intervals.

Clustering was used as a data reduction technique to group the genotypes of Anthurium and Dendrobium for variability to ethylene production. The least ethylene production group from the study was identified and the genotypes in this cluster was used for further study with large quantity of flowers to study their ethylene evolution properties and to confirm the results of the earlier experiment with larger number of genotypes. The clustering procedure was run as per the algorithm suggested by Sneath and Sokal (1973). The software used was SysStat.

COMPUTING INFRASTRUCTURE

The computing facility available at Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore-3 was used for the study. The facility consisted of Dual P III Windows server with nine nodes running windows 2000/98 operating system. The internet connection was facilitated by a fast 128 kbps ISDN connection distributed through dedicated router.

The website used for the application of various bioinformatics tools are listed in the Table.3.

Table.3. Bioinformatics tools used in the study

Bioinformatics tools	URL Address
MAP VIEWER	www.ncbi.nlm.nih.gov/cgi-bin/Entrez/map_search
BLAST	www.ncbi.nlm.nih.gov/BLAST/
PROMOTER SCAN	www.molbiol.ox.ac.uk/promoterscan.htm
ORF FINDER	www.ncbi.nlm.nih.gov/gorf/gorf.html
BEAUTY	www.ncbi.nlm.nih.gov/BEAUTY/
PDB SUM	www.biochem.ucl.ac.uk/bsm/pdbsum/
SWISS-PROT	www.expasy.ch/sprot/
CLUSTAL X	www-igbmc.u-strasbg.fr/BioInfo/ClustalX/Top.html
TREE TOP	www.genebee.msu.su/services/phtree_reduced.html
BIOEDIT	www.mbio.ncsu.edu/BioEdit/bioedit.html
CATH	www.biochem.ucl.ac.uk/bsm/cath/
Pfam	www.sanger.ac.uk/Softwares/Pfam
CDART	www.ncbi.nlm.nih.gov/Structure/lexington/lexington.cgi?cmd=rps
COLOR PROTEIN	pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_color.html
PREDATOR	pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_predator.html
SOPMA	pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html
SPDBV	www.expasy.ch/spdbv/
RASTOP	www.geneinfinity.org/rastop/
VAST	www.ncbi.nlm.nih.gov/Structure/VAST/vast.shtml
MACROMOLECULAR MOVEMENTS	molmovdb.mbb.yale.edu/MolMovDB/

Plate.1. Gas Chromatography Laboratory

EXPERIMENTAL RESULTS

CHAPTER IV

EXPERIMENTAL RESULTS

Experiments were conducted to study the variability among *Anthurium andreanum* and *Dendrobium* genotypes for postharvest ethylene evolution and application of various bioinformatics tools on the ETR1 gene (antisenescence gene) to find applications for the gene in controlling postharvest losses in flower crops. The results of the experiments are presented below.

4.1. VARIABILITY TO ETHYLENE PRODUCTION

Postharvest ethylene production was recorded in 16 *Anthurium andreanum* and three *Dendrobium* varieties at weekly intervals for three weeks and the results are presented in the Table 4 . There was no ethylene detected during the first week. Only four Anthuriums and all the three Dendrobiums recorded ethylene evolution at second week. All, except three Anthuriums viz., Bonfire orange, Merengue and Linda demol recorded ethylene evolution during the third week. Ethylene evolution could not be recorded beyond third week due to pathogenic deterioration.

In general, Dendrobiums produced more ethylene than Anthuriums. In the second week, the highest ethylene was recorded in Thai hybrid (1.926 ppm/100g) followed by Spic white (0.822ppm/100g) and Sikkim red (0.420ppm/100g). In the third week, it was found that Spic white recorded the highest ethylene of 4.139ppm/100g, an increase of approximately five times, while it was only a slight increase to 2.269ppm/100g at third week in the case of Thai hybrid. In general, all the Dendrobiums recorded the highest ethylene in third week. Among the Anthuriums, Temptation recorded the highest ethylene (0.602ppm/100g) followed by Leema white (0.584ppm/100g) and Sikkim red (0.556ppm/100g)

The lowest ethylene in the third week was recorded in B 13 (0.031ppm/100g). There was no ethylene evolution detected in three Anthuriums viz., Bonfire orange, Linda demol and Merengue at third week

Compared to other varieties, ethylene production was found to be higher at second week in Thai Hybrid (1.926 ppm), followed by Spic White (0.822 ppm) and Sikkim Red (0.420 ppm), whereas at third week the ethylene production was higher in Spic White (4.139 ppm), followed by Thai Hybrid (2.269 ppm) and Sonia 17 (1.911 ppm).

Table 4. Post harvest ethylene production in *Anthurium andreanum* and *Dendrobium* at weekly intervals

Treatment	Variety	Ethylene evolution (ppm/100 g)		
		I week	II week	III week
T1	Sikkim red	0	0.420	0.556
T2	Sunshine	0	0.010	0.093
T3	Tempt	0	0.290	0.602
T4	Bonfire	0	0.000	0.000
T5	Merengue	0	0.000	0.000
T6	Honduras	0	0.126	0.464
T7	Rosetta	0	0.000	0.095
T8	Reddragon	0	0.000	0.067
T9	Glamor	0	0.000	0.089
T10	Linda demol	0	0.000	0.000
T11	Leema	0	0.000	0.584
T12	Sunset	0	0.000	0.106
T13	Gloria	0	0.000	0.428
T14	Linda	0	0.000	0.258
T15	Tinora	0	0.000	0.319
T16	B 13	0	0.000	0.031
T17	Thai hybrid	0	1.926	2.269
T18	Sonia 17	0	0.309	1.911
T19	Spic white	0	0.822	4.139
SEd			0.3081	0.0246
CD(p=0.05)			0.0414	0.0499

Five anthuriums were selected for low postharvest ethylene evolution based on the cluster analysis and ethylene evolution for the five varieties was recorded at third week, by increasing the sample size to ten flowers. The results are presented in the Table 5.

Table 5. Post harvest ethylene evolved at 3rd week

Treatment	Variety	ppm
T_1	Bonfire orange	0.000
T_2	Merengue	0.000
T_3	Linda demol	0.000
T_4	Sunset orange	0.294
T_5	B 13	4.107
SEd		0.0653
CD(p=0.05)		0.4393

It was found that Bonfire orange, Merengue and Linda demol did not record any ethylene while enormous increase in ethylene was found in B 13 (4.107ppm/100g).

4.2. CLUSTER ANALYSIS

A cluster analysis was performed for the ethylene production data with K-MEANS algorithm. The distance matrix was Euclidean distance and single linkage (nearest neighbor) method was used. The first run was with number of clusters = 5. The first cluster had nine genotypes. The ethylene levels in this cluster ranged from 0.00 to 0.11 with a mean of 0.11ppm/100g. Cluster two had one genotype which had highest ethylene of 4.13 ppm/100g. Cluster three had two genotypes with a cluster mean of 2.08 ppm/100g. The other genotypes were distributed in clusters four and five.

K-MEANS algorithm was run again with number = 7 setting to see if the nine low ethylene producing genotypes got separated. The results of this analysis presented in Table 6 showed that the same nine genotypes were in cluster one of this analysis. Only members of the clusters no.3 and 4 got separated as clusters 3, 4, 6 and 7.

Table 6. Cluster analysis for postharvest ethylene production data of *Anthurium andreanum* and *Dendrobium* – five clusters

Cluster 1						
Members		**Statistics**				
Variety	**Distance**	**Variable**	**Minimum**	**Mean**	**Maximum**	**Std. Devn.**
Sunshine Orange	0.04	ETH	0.00	0.05	0.11	0.04
Bonfire Orange	0.05					
Merengue	0.05					
Rosetta	0.04					
Red Dragon	0.01					
Glamor	0.04					
Linda Demol	0.05					
Sunset Orange	0.05					
B 13	0.02					
Cluster 2						
Spic white (Orchid)	0.00	ETH	4.13	4.13	4.13	0.00
Cluster 3						
Thai hybrid	0.18	ETH	1.90	2.08	2.26	0.18
Sonia 17	0.18					
Cluster 4						
Sikkim Red	0.03	ETH	0.43	0.53	0.60	0.07
Temptation	0.07					
Honduras	0.06					
Leema White	0.06					
Gloria	0.10					
Cluster 5						
Linda	0.03	ETH	0.26	0.29	0.32	0.03
Tinora	0.03					

Table 7. Cluster analysis for ethylene production data – seven clusters.

Cluster 1						
Members		Statistics				
Variety	**Distance**	**Variable**	**Minimum**	**Mean**	**Maximum**	**Std. Devn.**
Sunshine Orange	0.04	ETH	0.00	0.05	0.11	0.04
Bonfire Orange	0.05					
Merengue	0.05					
Rosetta	0.04					
Red Dragon	0.01					
Glamor	0.04					
Linda Demol	0.05					
Sunset Orange	0.05					
B 13	0.02					
Cluster 2						
Spic White	0.00	ETH	4.13	4.13	4.13	0.00
Cluster 3						
Thai hybrid	0.00	ETH	2.26	2.26	2.26	0.00
Cluster 4						
Sikkim Red	0.02	ETH	0.55	0.58	0.60	0.02
Temptation	0.02					
Leema White	0.00					
Cluster 5						
Linda	0.03	ETH	0.26	0.29	0.32	0.03
Tinora	0.03					
Cluster 6						
Sonia 17	0.00	ETH	1.90	1.90	1.90	0.00
Cluster 7						
Honduras	0.02	ETH	0.43	0.44	0.46	0.02
Gloria	0.02					

As cluster 1 had low ethylene evolving genotypes in both the runs, JOIN algorithm of cluster analysis was run with nearest neighbor (single linkage) for Euclidean distance. The dendrogram produced is presented in Fig.34. The dendrogram showed the 9 low ethylene evolving genotypes in three groups. Bonfire orange, Merengue and Linda Demol in one group with the lowest distance of 0.000. B

13 alone was present as next group with a distance of 0.031. Sunset orange, Glamor, Rosetta, Sunshine orange and Red dragon were present in the third group.

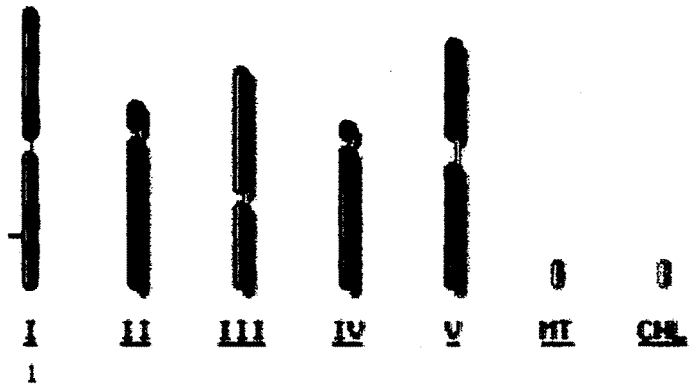
The dendrogram was used to identify five low postharvest ethylene producing genotypes for testing ethylene production using a bunch of flowers – an increased sample size. For this purpose, the five lowest ethylene producing types were identified from the dendrogram viz., Bonfire orange, Merengue, Linda demol, Sunset orange and B 13.

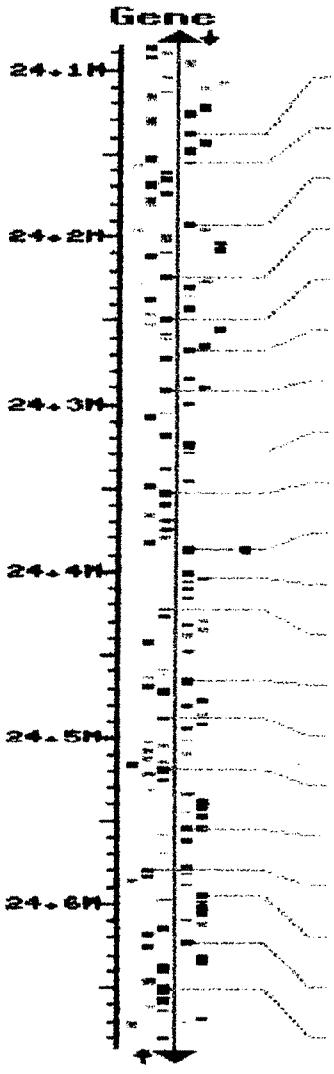
4.3. BIOINFORMATICS EXPERIMENT

4.3.1. LOCATION SPECIFIC SEARCH

4.3.1.1. MAP VIEWER

The map view shows one or more detailed maps for a specific chromosome. In the present study, to identify the exact location of etr1 gene on chromosome1 of Arabidopsis, the mapviewer tool was used. The results are depicted for both, whole chromosome and a deeper view of the chromosome. The red dot on the chromosome indicates the location of etr1 gene.





4.3.2. NULEOTIDE SEARCH

4.3.2.1. BLAST - Basic Local Alignment Search Tool

BLAST was used to determine sequences that showed similarity to ETR1 sequence. The query length of ETR1 was 3879 bp. This sequence was searched against the *nr* database which comprised 1,377,978 sequences. The summary of the search results are presented in the Table 8. The E-value denotes the probability by which a hit occurs by chance. Lower E-value settings will produce highly significant hits. Hence, the E-value for this search for ETR1 was maintained at 0.05. The search summary showed a computed K-value of 0.711 and lambda ratio of 1.37. The search produced 80 sequences with E-value less than 0.05. The search with the above parameter settings produced results which are listed in Table 9.

Table 8. Summary of BLAST search result for ETR1

Feature	Value
Expect value	0.05
Lambda ratio	1.37
K value	0.711
Gap penalty existence	5.00
Gap penalty extension	2.00
Number of successful extensions	125846
Number of sequences better than .5.0e-02	80
Length of the query	3879
Length of database	6,515,211,940
Effective HSP length	22
Effective length of query	3857
Effective length of database	6,484,896,424
Effective search space	25012245507368
Effective search space used	25012245507368
Expect value	0.05
Lambda ratio	1.37
K value	0.711
Gap penalty existence	5.00
Gap penalty extension	2.00

The score values of sequences of all crops are listed in Table 9. The results showed that the search yielded 80 sequences in various crops with significant matching (E-value less than 0.05) alignments. The highest score value (7162) was for *Arabidopsis thaliana* 1BAC ETR1 sequence and it had the lowest E-value of 0. In all, four Arabidopsis sequences were listed and all of these had an E-value of 0. The score value for *Arabidopsis* sequences ranged from 7162 to 1243.

After *Arabidopsis*, the score value dropped sharply to 476 in *Brassica oleraceae* ETR1 sequence which had an E-value of e-130. The lowest score among the 80 sequences was foumd in *Prunus mume* mRNA ethylene receptor with an E-value of 0.001. *Mangifera indica* ethylene receptor had a moderate score of 289 with an E-value of 1e-74. *Musa acuminate* ethylene receptor had a low score of 72 with an E-value of 6e-09.

Table 9. Score values of crops producing significant alignments

Identifier number	*Gene*	*Score*	*E-value*
gi|12324388|gb|AC020665.6|AC020665	Arabidopsis thaliana chr...	7162	0.0
gi|409704|gb|L24119.1|ATHETR1A	Arabidopsis thaliana ETR1 ge...	7162	0.0
gi|22330452|ref|NM_105305.2|	Arabidopsis thaliana chromosom...	1889	0.0
gi|20271013|gb|AF494374.1|	Arabidopsis lyrata subsp. lyrata...	1243	0.0
gi|2896028|gb|AF047476.1|AF047476	Brassica oleracea ethylen...	476	e-130
gi|2655060|gb|AF022727.1|AF022727	Nicotiana tabacum ethylen...	387	e-104
gi|4164158|dbj|AB015496.1|	Passiflora edulis mRNA for ethyl...	321	4e-84
gi|18496058|emb|AJ420193.1|FSY420193	Fagus sylvatica partia...	315	2e-82
gi|18252318|gb|AF386509.1|AF386509	Pyrus communis putative ...	311	4e-81
gi|21666554|gb|AF396830.1|	Prunus persica ethylene receptor...	303	9e-79
gi|18252350|gb|AF386525.1|AF386525	Pyrus communis putative ...	295	2e-76
gi|7407122|gb|AF227742.1|AF227742	Mangifera indica ethylene...	289	1e-74
gi|6841074|gb|AF124527.1|AF124527	Prunus persica ethylene r...	285	2e-73
gi|2738022|gb|U87238.1|BOU87238	Brassica oleracea putative ...	278	5e-71
gi|17646112|gb|AF145972.1|AF145972	Petunia x hybrida ethyle...	262	3e-66
gi|3411050|gb|AF032448.1|AF032448	Malus domestica ethylene ...	252	3e-63
gi|1163080|gb|U41103.1|SLU41103	Lycopersicon esculentum ETR...	248	5e-62
gi|2852990|gb|AF043084.1|AF043084	Lycopersicon esculentum e...	248	5e-62
gi|18252340|gb|AF386520.1|AF386520	Pyrus communis putative ...	236	2e-58
gi|15131528|emb|AJ297511.1|FRX297511	Fragaria x ananassa mR...	230	1e-56
gi|17646116|gb|AF145974.1|AF145974	Petunia x hybrida ethyle...	226	2e-55
gi|6136813|dbj|AB026498.1|	Cucumis sativus mRNA for ethylen...	222	3e-54
gi|7547006|gb|AF243474.1|AF243474	Vitis vinifera putative e...	212	2e-51
gi|5006605|gb|AF141929.1|AF141929	Pelargonium x hortorum et...	210	1e-50
gi|3641253|gb|AF054806.1|AF054806	Cucumis melo var. reticul...	206	2e-49
gi|11611625|dbj|AB052228.1|	Cucumis melo var. reticulatus C...	206	2e-49
gi|5006603|gb|AF141928.1|AF141928	Pelargonium x hortorum et...	204	6e-49
gi|17646118|gb|AF145975.1|AF145975	Petunia x hybrida ethyle...	186	1e-43
gi|17646114|gb|AF145973.1|AF145973	Petunia x hybrida ethyle...	186	1e-43
gi|2852992|gb|AF043085.1|AF043085	Lycopersicon esculentum e...	182	2e-42
gi|1272245|gb|U47279.1|SLU47279	Lycopersicon esculentum eth...	182	2e-42
gi|11935115|gb|AF311942.1|AF311942	Carica papaya ethylene r...	180	9e-42

gi|21310082|gb|AF380127.1|	Rosa hybrid cultivar ethylene re...	178	3e-41
gi|20378350|gb|AF441283.1|	Rosa hybrid cultivar ethylene-re...	170	8e-39
gi|7208794|emb|AJ276294.1|CSI276294	Citrus sinensis partial...	163	2e-36
gi|6906699|dbj|AB031028.1|	Prunus mume PM-ER1 mRNA for ethy...	163	2e-36
gi|2745901|gb|AF039746.1|AF039746	Pisum sativum ERS-like et...	155	5e-34
gi|8570033|dbj|AB035806.1|	Dianthus caryophyllus DC-ETR1 mR...	151	8e-33
gi|3123665|emb|AJ005829.1|PSA5829	Pisum sativum mRNA for et...	147	1e-31
gi|18539221|dbj|AB055429.1|	Delphinium 'MagicFountains dark...	137	1e-28
gi|18539223|dbj|AB055430.1|	Delphinium 'MagicFountains dark...	129	3e-26
gi|18496060|emb|AJ420194.1|FSY420194	Fagus sylvatica mRNA f...	121	7e-24
gi|4138852|gb|AF098272.1|AF098272	Vigna radiata ethylene re...	121	7e-24
gi|14572557|gb|AY040228.1|	Malus x domestica ethylene recep...	119	3e-23
gi|4416485|gb|AF127220.1|AF127220	Rosa hybrida ethylene rec...	115	4e-22
gi|22036193|gb|AF316534.1|	Prunus persica ethylene-responsi...	111	7e-21
gi|21632786|gb|AY061640.1|	Prunus persica putative ethylene...	111	7e-21
gi|15054445|gb|AY043031.1|	Oryza sativa subsp. indica putat...	111	7e-21
gi|2281704|gb|AF013979.1|AF013979	Oryza sativa ethylene res...	111	7e-21
gi|18405553|ref|NM_129658.1|	Arabidopsis thaliana chromosom...	98	1e-16
gi|20196931|gb|AC002409.3|	Arabidopsis thaliana chromosome ...	98	1e-16
gi|15450903|gb|AY054532.1|	Arabidopsis thaliana ethylene re...	98	1e-16
gi|15131530|emb|AJ297512.1|FRX297512	Fragaria x ananassa mR...	98	1e-16
gi|1046224|gb|U21952.1|ATU21952	Arabidopsis thaliana ethyle...	98	1e-16
gi|21206702|gb|AY103624.1|	Zea mays PCO067639 mRNA sequence	96	4e-16
gi|2707333|gb|AF037368.1|AF037368	Cucumis melo putative eth...	94	2e-15
gi|10280983|dbj|AB049128.1|	Cucumis melo var. reticulatus C...	94	2e-15
gi|20386498|gb|AF500121.1|	Persea americana ethylene respon...	92	6e-15
gi|18252334|gb|AF386517.1|AF386517	Pyrus communis putative ...	90	2e-14
gi|20135555|gb|AY083169.1|	Malus x domestica ethylene recep...	88	1e-13
gi|18252330|gb|AF386515.1|AF386515	Pyrus communis putative ...	88	1e-13
gi|18252344|gb|AF386522.1|AF386522	Pyrus communis putative ...	84	2e-12
gi|4092525|gb|AF092088.1|AF092088	Citrus sinensis putative ...	84	2e-12
gi|4164160|dbj|AB015497.1|	Passiflora edulis mRNA for ethyl...	84	2e-12
gi|6136815|dbj|AB026499.1|	Cucumis sativus mRNA for ethylen...	84	2e-12
gi|4650820|gb|AF055894.1|AF055894	Phalaenopsis sp. 'True La...	76	4e-10
gi|4154358|gb|AF113541.1|AF113541	Phalaenopsis sp. 'KCbutte...	76	4e-10
gi|5566245|gb|AF159172.1|AF159172	Rosa hybrida ethylene rec...	74	1e-09
gi|19032290|dbj|AB070652.1|	Passiflora edulis PeERS2 mRNA f...	74	1e-09
gi|6425100|gb|AF113748.1|MBER2	Musa acuminata ethylene rece...	72	6e-09

gi|7652765|gb|AF039921.2|AF039921	Nicotiana tabacum ethylen...	70	2e-08
gi|1122444|gb|U38666.1|SLU38666	Solanum lycopersicum putati...	70	2e-08
gi|984156|emb|Z54099.1|LEETRMRNA	L.esculentum mRNA for ethy...	70	2e-08
gi|2353689|gb|U63291.1|RPU63291	Rumex palustris ethylene re...	60	2e-05
gi|2243157|emb|Y08359.1|RPERS1	R.palustris mRNA for ethylen...	60	2e-05
gi|2896030|gb|AF047477.1|AF047477	Brassica oleracea ethylen...	58	9e-05
gi|2662474|gb|AF034770.1|AF034770	Dianthus caryophyllus put...	58	9e-05
gi|15029365|gb|AF394914.1|AF394914	Rosa hybrid cultivar eth...	54	0.001
gi|4210923|gb|AF051938.1|AF051938	Solanum tuberosum ethylen...	54	0.001
gi|6906701|dbj|AB031029.1|	Prunus mume PM-ER2 mRNA for ethy...	54	0.001

The summaries of sequence similarity with alignments are presented in Table 10. In all, 49 significant alignments were found. The score value ranged from 7162 in *Arabidopsis thaliana* ETR1 to 111 in *Oryza sativa* ethylene responsive factor mRNA. The identities was highest (100%) in *Arabidopsis thaliana* chromosome 1 CHR1v07142002 followed by *Arabidopsis thaliana* chromosome 1 BAC T27F4.

Table 10. Summary of identities and sequence alignments with ETR1

Identifier number	Gene	Length	Score	E-value	Identities	Strand
gi|12324388|gb|AC020665.6|AC020665	Arabidopsis thaliana chromosome 1 BAC T27F4	85702	7162 bits (3613)	0.0	3641/3655 (99%)	Plus / Plus
gi|409704|gb|L24119.1|ATHETR1A	Arabidopsis thaliana ETR1 gene	3879	7162 bits (3613)	0.0	3641/3655 (99%)	Plus / Plus
gi|22330452|ref|NM_105305.2|	Arabidopsis thaliana chromosome 1 CHR1v07142002	4137	1889 bits (953)	0.0	953/953 (100%)	Plus / Plus
gi|20271013|gb|AF494374.1|	Arabidopsis lyrata subsp. lyrata ethylene receptor 1 (ETR1) gene	2019	1243 bits (627)	0.0	754/795 (94%)	Plus / Plus
gi|2896028|gb|AF047476.1|AF047476	Brassica oleracea ethylene receptor (ETR1) mRNA	2208	476 bits (240)	e-130	741/908 (81%)	Plus / Plus
gi|2655060|gb|AF022727.1|AF022727	Nicotiana tabacum ethylene receptor ETR1 homolog mRNA	2574	387 bits (195)	e-104	710/881 (80%)	Plus / Plus

gi|4164158|dbj|AB015496.1|	Passiflora edulis mRNA for ethylene receptor	2715	321 bits (162)	4e-84	477/582 (81%)	Plus / Plus
gi|18496058|emb|AJ420193.1|FSY420193	Fagus sylvatica partial mRNA for ethylene receptor 1 (ers1 gene)	1207	315 bits (159),	2e-82	435/527 (82%)	Plus / Plus
gi|18252318|gb|AF386509.1|AF386509	Pyrus communis putative ethylene receptor (ETR1a) mRNA	2720	311 bits (157)	4e-81	475/581 (81%)	Plus / Plus
gi|21666554|gb|AF396830.1|	Prunus persica ethylene receptor (ETR) gene	6726	303 bits (153)	9e-79	485/596 (81%)	Plus / Plus
gi|18252350|gb|AF386525.1|AF386525	Pyrus communis putative ethylene receptor (DETR1a) gene	4239	295 bits (149)	2e-76	473/581 (81%)	Plus / Plus
gi|7407122|gb|AF227742.1|AF227742	Mangifera indica ethylene receptor mRNA	2610	289 bits (146)	1e-74	407/494 (82%)	Plus / Plus
gi|6841074|gb|AF124527.1|AF124527	Prunus persica ethylene receptor (ETR1)	2540	285 bits (144)	2e-73	483/596 (81%)	Plus / Plus
gi|2738022|gb|U87238.1|BOU87238	Brassica oleracea putative ethylene receptor (BOETR1) gene	621	278 bits (140)	5e-71	293/344 (85%)	Plus / Plus
gi|17646112|gb|AF145972.1|AF145972	Petunia x hybrida ethylene receptor (ETR1-1) mRNA	2564	262 bits (132)	3e-66	617/778 (79%)	Plus / Plus
gi|3411050|gb|AF032448.1|AF032448	Malus domestica ethylene receptor (ETR1)	2817	252 bits (127)	3e-63	472/587 (80%)	Plus / Plus
gi|1163080|gb|U41103.1|SLU41103	Lycopersicon esculentum ETR1 homolog	2681	248 bits (125)	5e-62	311/373 (83%)	Plus / Plus
gi|2852990|gb|AF043084.1|AF043084	Lycopersicon esculentum ethylene receptor homolog (ETR1) mRNA	2659	248 bits (125)	5e-62	311/373 (83%)	Plus / Plus
gi|18252340|gb|AF386520.1|AF386520	Pyrus communis putative ethylene receptor (DETR1b) gene	4089	236 bits (119)	2e-58	470/587 (80%)	Plus / Plus
gi|15131528|emb|AJ297511.1|FRX297511	Fragaria x ananassa mRNA for ethylene receptor (etr1 gene)	2773	230 bits (116	1e-56	410/508 (80%)	Plus / Plus
gi|17646116|gb|AF145974.1|AF145974	Petunia x hybrida ethylene receptor (ETR1-3)	2903	226 bits (114)	2e-55	399/494 (80%)	Plus / Plus

gi|6136813|dbj|AB026498.1|	Cucumis sativus mRNA for ethylene receptor CS ETR1	2924	222 bits (112)	3e-54	445/556 (80%)	Plus / Plus
gi|7547006|gb|AF243474.1|AF243474	Vitis vinifera putative ethylene receptor (ETR1)	2654	212 bits (107)	2e-51	278/335 (82%)	Plus / Plus
gi|5006605|gb|AF141929.1|AF141929	Pelargonium x hortorum ethylene receptor homolog (PhETR2)	2656	210 bits (106)	1e-50	277/334 (82%)	Plus / Plus
gi|3641253|gb|AF054806.1|AF054806	Cucumis melo var. reticulatus ethylene receptor (ETR1)	2696	206 bits (104)	2e-49	443/556 (79%)	Plus / Plus
gi|11611625|dbj|AB052228.1|	Cucumis melo var. reticulatus Cm-ETR1 mRNA for ethylene receptor	2340	206 bits (104)	2e-49	443/556 (79%)	Plus / Plus
gi|5006603|gb|AF141928.1|AF141928	Pelargonium x hortorum ethylene receptor homolog (PhETR1)	2646	204 bits (103)	6e-49	268/323 (82%)	Plus / Plus
gi|2852992|gb|AF043085.1|AF043085	Lycopersicon esculentum ethylene receptor homolog (ETR2) mRNA	2688	182 bits (92)	2e-42	290/356 (81%)	Plus / Plus
gi|1272245|gb|U47279.1|SLU47279	Lycopersicon esculentum ethylene receptor (ETR1)	2390	182 bits (92)	2e-42	290/356 (81%)	Plus / Plus
gi|11935115|gb|AF311942.1|AF311942	Carica papaya ethylene receptor mRNA	2187	180 bits (91)	9e-42	211/251 (84%)	Plus / Plus
gi|21310082|gb|AF380127.1|	Rosa hybrid cultivar ethylene receptor mRNA	797	178 bits (90)	3e-41	330/410 (80%)	Plus / Plus
gi|20378350|gb|AF441283.1|	Rosa hybrid cultivar ethylene-response ETR1	795	170 bits (86)	8e-39	329/410 (80%)	Plus / Plus
gi|7208794|emb|AJ276294.1|CSI276294	Citrus sinensis partial mRNA for ethylene receptor (ETR-1 gene)	1240	163 bits (82)	2e-36	121/134 (90%)	Plus / Plus
gi|6906699|dbj|AB031028.1|	Prunus mume PM-ER1 mRNA for ethylene receptor	608	163 bits (82)	2e-36	157/182 (86%)	Plus / Plus
gi|2745901|gb|AF039746.1|AF039746	Pisum sativum ERS-like ethylene receptor (ERS1)	2092	155 bits (78)	5e-34	221/266 (83%)	Plus / Plus

gi|8570033|dbj|AB035806.1|	Dianthus caryophyllus DC- ETR1	897	151 bits (76)	8e-33	265/328 (80%)	Plus / Plus
gi|3123665|emb|AJ005829.1|PSA5829	Pisum sativum mRNA for ethylene receptor	2131	147 bits (74)	1e-31	220/266 (82%)	Plus / Plus
gi|18539221|dbj|AB055429.1|	Delphinium 'MagicFountains dark blue' ERS mRNA for ethylene receptor	2224	137 bits (69)	1e-28	219/269 (81%)	Plus / Plus
gi|18539223|dbj|AB055430.1|	Delphinium 'MagicFountains dark blue' ERS mRNA for ethylene receptor	2231	129 bits (65)	3e-26	218/269 (81%)	Plus / Plus
gi|18496060|emb|AJ420194.1|FSY420194	Fagus sylvatica mRNA for ethylene receptor 1 (ers1 gene)	2553	121 bits (61)	7e-24	214/265 (80%)	Plus / Plus
gi|4138852|gb|AF098272.1|AF098272	Vigna radiata ethylene response sensor (ERS1) mRNA	2477	121 bits (61)	7e-24	199/245 (81%)	Plus / Plus
gi|14572557|gb|AY040228.1|	Malus x domestica ethylene receptor (etr) mRNA	1999	119 bits (60)	3e-23	258/324 (79%)	Plus / Plus
gi|4416485|gb|AF127220.1|AF127220	Rosa hybrida ethylene receptor (ETR2) gene	1299	115 bits (58)	4e-22	106/122 (86%)	Plus / Plus
gi|22036193|gb|AF316534.1|	Prunus persica ethylene-responsive sensor (ERS) gene,	4633	111 bits (56)	7e-21	161/196 (82%)	Plus / Plus
gi|21632786|gb|AY061540.1|	Prunus persica putative ethylene receptor (ERS1) mRNA	1616	111 bits (56)	7e-21	161/196 (82%)	Plus / Plus
gi|15054445|gb|AY043031.1|	Oryza sativa subsp. indica putative ethylene receptor gene	7330	111 bits (56)	7e-21	131/156 (83%)	Plus / Plus
gi|2281704|gb|AF013979.1|AF013979	Oryza sativa ethylene responsive factor (OSERS) mRNA	2234	111 bits (56)	7e-21	131/156 (83%)	Plus / Plus

The sequence alignment of *Arabidopsis thaliana* chromosome 1 BAC T27F4 gene showing the alignment and gaps are represented by 'n' as shown in Fig.1. There

was almost no gap, as the identity score was 99 per cent. The query (ETR1) sequence is matched against the subject sequence in the database. The numbers at the beginning and end positions in each alignment is shown in Fig.1.

Table 11. Summary of sequencc alignment of *A. thaliana* 1BAC T27F4 with ETR1

Identifier number	Gene	Length	Score	E-value	Identities	Strand
gi|12324388|gb|AC020665.6|AC020665	Arabidopsis thaliana chromosome 1 BAC T27F4	85702	7162 bits (3613)	0.0	3641/3655 (99%)	Plus / Plus

Fig 1. Sequence alignment of *A. thaliana* 1 BAC T27F4 with ETR1

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Query: 225   gtaagaacgaagaagaagtgttaaacccaaccaattttgacttgnnnnnnngcttcaacg 284
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 35790 gtaagaacgaagaagaagtgttaaacccaaccaattttgacttgaaaaaaagcttcaacg 35849

Query: 285   ctcccctttttctccttctccgtcgctctccgccgcgtcccaaatccccaattcctcctct 344
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 35850 ctcccctttttctccttctccgtcgctctccgccgcgtcccaaatccccaattcctcctct 35909

Query: 345   tctccgatcaattcttcccaagtaagcttcttcttcctcgattctctcctcagattgttt 404
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 35910 tctccgatcaattcttcccaagtaagcttcttcttcctcgattctctcctcagattgttt 35969

Query: 405   cgtgacttctttatatatattcttcacttccacagttttcttctgttgttgtcgtcgatc 464
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 35970 cgtgacttctttatatatattcttcacttccacagttttcttctgttgttgtcgtcgatc 36029

Query: 465   tcaaatcatagagattgattaacctaattggtctttatctagtgtaatgcatcgttatta 524
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 36030 tcaaatcatagagattgattaacctaattggtctttatctagtgtaatgcatcgttatta 36089

Query: 525   ggaactttaaattaagatttaatcgttaatttcatgattcggattcgaattttactgttc 584
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 36090 ggaactttaaattaagatttaatcgttaatttcatgattcggattcgaattttactgttc 36149

Query: 585   tcgagactgaaatatgcaacctatttttttcgtaatcgttgtgatcgaattcgattcttca 644
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 36150 tcgagactgaaatatgcaacctatttttttcgtaatcgttgtgatcgaattcgattcttca 36209

Query: 645   gaatttatagcaattttgatgctcatgatctgtctacgctacgttctcgtcgtaaatcga 704
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 36210 gaatttatagcaattttgatgctcatgatctgtctacgctacgttctcgtcgtaaatcga 36269

Query: 705   agttgataatgctatgtgtttgttacacaggtgtgtgtatgtgtgagagaggaactatag 764
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 36270 agttgataatgctatgtgtttgttacacaggtgtgtgtatgtgtgagagaggaactatag 36329

Query: 765   tgtaaaaaattcataatggaagtctgcaattgtattgaaccgcaatggccagcggatgaa 824
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 36330 tgtaaaaaattcataatggaagtctgcaattgtattgaaccgcaatggccagcggatgaa 36389

Query: 825   ttgttaatgaaataccaatacatctccgatttcttcattgcgattgcgtatttttcgatt 884
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 36390 ttgttaatgaaataccaatacatctccgatttcttcattgcgattgcgtatttttcgatt 36449

Query: 885   cctcttgagttgatttactttgtgaagaaatcagccgtgtttccgtatagatgggtactt 944
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 36450 cctcttgagttgatttactttgtgaagaaatcagccgtgtttccgtatagatgggtactt 36509
```

```
Query: 945   gttcagtttggtgcttttatcgttctttgtggagcaactcatcttattaacttatggact 1004
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 36510 gttcagtttggtgcttttatcgttctttgtggagcaactcatcttattaacttatggact 36569

Query: 1005  ttcactacgcattcgagaaccgtggcgcttgtgatgactaccgcgaaggtgttaaccgct 1064
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 36570 ttcactacgcattcgagaaccgtggcgcttgtgatgactaccgcgaaggtgttaaccgct 36629

Query: 1065  gttgtctcgtgtgctactgcgttgatgcttgttcatattattcctgatcttttgagtgtt 1124
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 36630 gttgtctcgtgtgctactgcgttgatgcttgttcatattattcctgatcttttgagtgtt 36689

Query: 1125  aagactcgggagcttttcttgaaaaataaagctgctgagctcgatagagaaatgggattg 1184
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 36690 aagactcgggagcttttcttgaaaaataaagctgctgagctcgatagagaaatgggattg 36749

Query: 1185  attcgaactcaggaagaaaccggaaggcatgtgagaatgttgactcatgagattagaagc 1244
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 36750 attcgaactcaggaagaaaccggaaggcatgtgagaatgttgactcatgagattagaagc 36809

Query: 1245  actttagatagacatactattttaaagactacacttgttgagcttggtaggacattagct 1304
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 36810 actttagatagacatactattttaaagactacacttgttgagcttggtaggacattagct 36869

Query: 1305  ttggaggagtgtgcattgtggatgcctactagaactgggttagagctacagctttcttat 1364
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 36870 ttggaggagtgtgcattgtggatgcctactagaactgggttagagctacagctttcttat 36929

Query: 1365  acacttcgtcatcaacatcccgtggagtatacggttcctattcaattaccggtgattaac 1424
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 36930 acacttcgtcatcaacatcccgtggagtatacggttcctattcaattaccggtgattaac 36989

Query: 1425  caagtgtttggtactagtagggctgtaaaaatatctcctaattctcctgtggctaggttg 1484
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 36990 caagtgtttggtactagtagggctgtaaaaatatctcctaattctcctgtggctaggttg 37049

Query: 1485  agacctgtttctgggaaatatatgctaggggaggtggtcgctgtgagggttccgcttctc 1544
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 37050 agacctgtttctgggaaatatatgctaggggaggtggtcgctgtgagggttccgcttctc 37109

Query: 1545  caccttttctaattttcagattaatgactggcctgagctttcaacaaagagatatgctttg 1604
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 37110 caccttttctaattttcagattaatgactggcctgagctttcaacaaagagatatgctttg 37169

Query: 1605  atggttttgatgcttccttcagatagtgcaaggcaatggcatgtccatgagttggaactc 1664
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 37170 atggttttgatgcttccttcagatagtgcaaggcaatggcatgtccatgagttggaactc 37229

Query: 1665  gttgaagtcgtcgctgatcaggttttacattgctgagaatttctcttctttgctatgttc 1724
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 37230 gttgaagtcgtcgctgatcaggttttacattgctgagaatttctcttctttgctatgttc 37289

Query: 1725  atgatcttgtctataacttttcttctcttattataggtggctgtagctctctcacatgct 1784
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 37290 atgatcttgtctataacttttcttctcttattataggtggctgtagctctctcacatgct 37349

Query: 1785  gcgatcctagaagagtcgatgcgagctagggaccttctcatggagcagaatgttgctctt 1844
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 37350 gcgatcctagaagagtcgatgcgagctagggaccttctcatggagcagaatgttgctctt 37409

Query: 1845  gatctagctagacgagaagcagaaacagcaatccgtgcccgcaatgatttcctagcggtt 1904
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 37410 gatctagctagacgagaagcagaaacagcaatccgtgcccgcaatgatttcctagcggtt 37469

Query: 1905  atgaaccatgaaatgcgaacaccgatgcatgcgattattgcactctcttccttactccaa 1964
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Sbjct: 37470 atgaaccatgaaatgcgaacaccgatgcatgcgattattgcactctcttccttactccaa 37529

Query: 1965  gaaacggaactaaccccctgaacaaagactgatggtggaaacaatacttaaaagtagtaac 2024
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 37530 gaaacggaactaaccccctgaacaaagactgatggtggaaacaatacttaaaagtagtaac 37589

Query: 2025  cttttggcaactttgatgaatgatgtcttagatctttcaaggttagaagatggaagtctt 2084
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Query: 2085  caacttgaacttgggacattcaatcttcatacattatttagagaggtaacttttgaacag 2144
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Sbjct: 37650 caacttgaacttgggacattcaatcttcatacattatttagagaggtaacttttgaacag 37709

Query: 2145  ctctatgtttcataagtttatactatttgtgtacttgattgtcatattgaatcttgttgc 2204
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Sbjct: 37710 ctctatgtttcataagtttatactatttgtgtacttgattgtcatattgaatcttgttgc 37769

Query: 2205  aggtcctcaatctgataaagcctatagcggttgttaagaaattacccatcacactaaatc 2264
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 37770 aggtcctcaatctgataaagcctatagcggttgttaagaaattacccatcacactaaatc 37829

Query: 2265  ttgcaccagatttgccagaatttgttgttggggatgagaaacggctaatgcagataatat 2324
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 37830 ttgcaccagatttgccagaatttgttgttggggatgagaaacggctaatgcagataatat 37889

Query: 2325  taaatatagttggtaatgctgtgaaattctccaaacaaggtagtatctccgtaaccgctc 2384
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Sbjct: 37890 taaatatagttggtaatgctgtgaaattctccaaacaaggtagtatctccgtaaccgctc 37949

Query: 2385  ttgtcaccaagtcagacacacgagctgctgacttttttgtcgtgccaactgggagtcatt 2444
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Sbjct: 37950 ttgtcaccaagtcagacacacgagctgctgacttttttgtcgtgccaactgggagtcatt 38009

Query: 2445  tctacttgagagtgaaggttattatcttgtatcttgggatcttataccatagctgaaagt 2504
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Query: 2505  atttcttaggtcttaattttgatgattattcaaatataggtaaaagactctggagcagga 2564
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Query: 2565  ataaatcctcaagacattccaaagattttcactaaatttgctcaaacacaatctttagcg 2624
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Query: 2625  acgagaagctcgggtggtagtgggcttggcctcgccatctccaagaggtttgagccttat 2684
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Query: 2685  taaaagacgnnnnnnnccaacttttttcttgtcttctgtgttgttaaaagtttactcataa 2744
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Query: 2745  gcgtttaatatgacaaggtttgtgaatctgatggagggtaacatttggattgagagcgat 2804
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Query: 2805  ggtcttggaaaaggatgcacggctatctttgatgttaaacttgggatctcagaacgttca 2864
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Sbjct: 38370 ggtcttggaaaaggatgcacggctatctttgatgttaaacttgggatctcagaacgttca 38429

Query: 2865  aacgaatctaaacagtcgggcataccgaaagttccagccattccccgacattcaaatttc 2924
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Query: 2985  ctttgcaaaatctctcgccttacttcttgcaaatgcagatattggcgtttagaaaaaacg 3044
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Sbjct: 38550 ctttgcaaaatctctcgccttacttcttgcaaatgcagatattggcgtttagaaaaaacg 38609

Query: 3045  caaatttaatcttatgagaaaccgatgattattttggttgcagggtaagtagaatggtga 3104
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Sbjct: 38610 caaatttaatcttatgagaaaccgatgattattttggttgcagggtaagtagaatggtga 38669

Query: 3105  cgaagggacttcttgtacaccttgggtgcgaagtgaccacggtgagttcaaacgaggagt 3164
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Sbjct: 38670 cgaagggacttcttgtacaccttgggtgcgaagtgaccacggtgagttcaaacgaggagt 38729

Query: 3165  gtctccgagttgtgtcccatgagcacaaagtggtcttcatggacgtgtgcatgcccgggg 3224
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 38730 gtctccgagttgtgtcccatgagcacaaagtggtcttcatggacgtgtgcatgcccgggg 38789
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```
Query: 3225  tcgaaaactaccaaatcgctctccgtattcacgagaaattcacaaaacaacgcccccaac 3284
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Query: 3285  ggccactacttgtggcactcagtggtaacactgacaaatccacaaaagagaaatgcatga 3344
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Query: 3345  gctttggtctagacggtgtgttgctcaaacccgtatcactagacaacataagagatgttc 3404
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Query: 3405  tgtctgatcttctcgagccccgggtactgtacgagggcatgtaaaggcgatggatgcccc 3464
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Query: 3465  atgccccagaggagtaattccgctcccgccttcttctcccgtaaaacatcggaagctgat 3524
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 39030 atgccccagaggagtaattccgctcccgccttcttctcccgtaaaacatcggaagctgat 39089

Query: 3525  gttctctggtttaattgtgtacatatcagagattgtcggagcgttttggatgatatctta 3584
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Sbjct: 39090 gttctctggtttaattgtgtacatatcagagattgtcggagcgttttggatgatatctta 39149

Query: 3585  aaacagaaagggaataacaaaatagaaactctaaaccggtatgtgtccgtggcgatttcg 3644
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Sbjct: 39150 aaacagaaagggaataacaaaatagaaactctaaaccggtatgtgtccgtggcgatttcg 39209

Query: 3645  gttatagaggaacaagatggtggtggtataatcataccatttcagattacatgtttgact 3704
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Sbjct: 39210 gttatagaggaacaagatggtggtggtataatcataccatttcagattacatgtttgact 39269

Query: 3705  aatgttgtatccttatatatgtagttacattcttataagaatttggatcgagttatggat 3764
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 39270 aatgttgtatccttatatatgtagttacattcttataagaatttggatcgagttatggat 39329

Query: 3765  gcttgttgcgtgcatgtatgacattgatgcagtattatggcgtcagctttgcgccgctta 3824
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 39330 gcttgttgcgtgcatgtatgacattgatgcagtattatggcgtcagctttgcgccgctta 39389

Query: 3825  gtagaacaacaacaatggcgttacttagtttctcaatcaacccgatctccaaaac 3879
              |||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 39390 gtagaacaacaacaatggcgttacttagtttctcaatcaacccgatctccaaaac 39444
```

Among the 80 crop sequences showing close matches to ETR1, there were 17 sequences of flower crops. The score values of these 17 flower crops are listed in Table 12. The highest score value among the 17 flower crops was found in *Petunia hybrida* (ETR1-1) mRNAwhich had a score value of 262 with an E-value of 3e-66. The lowest score value was found in *Rosa hybrida* ethylene receptor which had a score of 54 with the highest E-value of 0.001.

Among the 49, there were 12 flower crop sequences, the summary of which is presented in Table 13. Among the flower crops, the score value ranged from 262 (bit-132) in *Petunia hybrida* ethylene receptor (ETR1-1) to 115(bit-580 in *Rosa hybrida* ethylene receptor ETR2 gene. The identities in flower crops ranged from 86% in *Rosa hybrida* ETR2 to 79% in *Petunia hybrida* ETR1-1 mRNA.

Among the 17 flower crop sequences with significant alignment, there were four of *Petunia hybrida*, five of *Rosa hybrida* and two in each of *Dianthus caryophyllus* and *Phalaenopsis* orchid.

Table 12. Sequences of flower crops producing significant alignments

Identifier Number	Gene	Score bits	E-value
gi|17646112|gb|AF145972.1|AF145972	Petunia x hybrida ethyle...	262	3e-66
gi|17646116|gb|AF145974.1|AF145974	Petunia x hybrida ethyle...	226	2e-55
gi|5006605|gb|AF141929.1|AF141929	Pelargonium x hortorum et...	210	1e-50
gi|5006603|gb|AF141928.1|AF141928	Pelargonium x hortorum et...	204	6e-49
gi|17646118|gb|AF145975.1|AF145975	Petunia x hybrida ethyle...	186	1e-43
gi|17646114|gb|AF145973.1|AF145973	Petunia x hybrida ethyle...	186	1e-43
gi|21310082|gb|AF380127.1|	Rosa hybrid cultivar ethylene re...	178	3e-41
gi|20378350|gb|AF441283.1|	Rosa hybrid cultivar ethylene-re...	170	8e-39
gi|8570033|dbj|AB035806.1|	Dianthus caryophyllus DC-ETR1 mR...	151	8e-33
gi|18539221|dbj|AB055429.1|	Delphinium 'MagicFountains dark...	137	1e-28
gi|18539223|dbj|AB055430.1|	Delphinium 'MagicFountains dark...	129	3e-26
gi|4416485|gb|AF127220.1|AF127220	Rosa hybrida ethylene rec...	115	4e-22
gi|4650820|gb|AF055894.1|AF055894	Phalaenopsis sp. 'True La...	76	4e-10
gi|4154358|gb|AF113541.1|AF113541	Phalaenopsis sp. 'KCbutte...	76	4e-10
gi|5566245|gb|AF159172.1|AF159172	Rosa hybrida ethylene rec...	74	1e-09
gi|2662474|gb|AF034770.1|AF034770	Dianthus caryophyllus put...	58	9e-05
gi|15029365|gb|AF394914.1|AF394914	Rosa hybrid cultivar eth...	54	0.001

Table 13. Summary of identities and sequence alignments of flower crops with ETR1

Identifier number	Gene	Length	Score	E-value	Identities	Strand
gi|17646112|gb|AF145972.1|AF145972	Petunia x hybrida ethylene receptor (ETR1-1) mRNA	2564	262 bits (132)	3e-66	617/778 (79%)	Plus / Plus
gi|17646116|gb|AF145974.1|AF145974	Petunia x hybrida ethylene receptor (ETR1-3) mRNA	2903	226 bits (114)	2e-55	399/494 (80%)	Plus / Plus
gi|5006605|gb|AF141929.1|AF141929	Pelargonium x hortorum ethylene receptor homolog (PhETR2)	2656	210 bits (106)	1e-50	277/334 (82%)	Plus / Plus
gi|5006603|gb|AF141928.1|AF141928	Pelargonium x hortorum ethylene receptor homolog (PhETR1)	2646	204 bits (103)	6e-49	268/323 (82%)	Plus / Plus
gi|17646118|gb|AF145975.1|AF145975	Petunia x hybrida ethylene receptor (ETR3) mRNA	1415	186 bits (94)	1e-43	394/494 (79%)	Plus / Plus

gi|17646114|gb|AF145973.1|AF145973	Petunia x hybrida ethylene receptor (ETR1-2) mRNA	2567	186 bits (94)	1e-43	457/578 (79%)	Plus / Plus
gi|21310082|gb|AF380127.1|	Rosa hybrid cultivar ethylene receptor mRNA	797	178 bits (90)	3e-41	330/410 (80%)	Plus / Plus
gi|20378350|gb|AF441283.1|	Rosa hybrid cultivar ethylene-response ETR1 mRNA	795	170 bits (86)	8e-39	329/410 (80%)	Plus / Plus
gi|8570033|dbj|AB035806.1|	Dianthus caryophyllus DC-ETR1 mRNA	897	151 bits (76)	8e-33	265/328 (80%)	Plus / Plus
gi|18539221|dbj|AB055429.1|	Delphinium 'MagicFountains dark blue' ERS mRNA for ethylene receptor	2224	137 bits (69)	1e-28	219/269 (81%)	Plus / Plus
gi|18539223|dbj|AB055430.1|	Delphinium 'MagicFountains dark blue' ERS mRNA for ethylene receptor	2231	129 bits (65)	3e-26	218/269 (81%)	Plus / Plus
gi|4416485|gb|AF127220.1|AF127220	Rosa hybrida ethylene receptor (ETR2) gene	1299	115 bits (58)	4e-22	106/122 (86%)	Plus / Plus

The alignment sequences of 12 flower crops are presented in Figures 2 to 13 illustrating the position of the gaps in the alignment indicated by 'n'. The query (ETR1) sequence is matched against the subject (sequence in database). The numbers at the beginning and end positions in the respective sequences.

Table 14. Summary of sequence alignment of *Petunia hybrida* (ETR1-1) mRNA with ETR1

Identifier number	*Gene*	*Length*	*Score*	*E-value*	*Identities*	*Strand*
gi|17646112|gb|AF145972.1|AF145972	Petunia x hybrida ethylene receptor (ETR1-1) mRNA	2564	262 bits (132)	3e-66	617/778 (79%)	Plus / Plus

Fig.2. Sequence alignment of Petunia x hybrida ethylene receptor (ETR1-1) mRNA with ETR1

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Query: 910  agaaatcagccgtgtttccgtatagatgggtacttgttcagtttggtgcttttatcgttc 969
            ||||||| || || ||||||||||||||||| || || |||||||||||| ||||| ||||
Sbjct: 216  agaaatcggcagtttttccgtatagatgggtgctcgtgcagtttggtgcgtttatagttc 275

Query: 970  tttgtggagcaactcatcttattaacttatggactttcactacgcattcgagaaccgtgg 1029
            |||||||||| || |||||||||| |||||||| |  ||| ||||| | || ||  |||
Sbjct: 276  tttgtggagcgacacatcttattaatttatggacatcgactgcgcatacaaggactctgg 335

Query: 1030 cgcttgtgatgactaccgcgaaggtgttaaccgctgttgtctcgtgtgctactgcgttga 1089
            |  | ||||||||||| || |||||  | || ||||| || || ||||| || ||  | |
Sbjct: 336  caatagtgatgactactgctaaggttctcactgctgtggtatcatgtgcaacggctctca 395

Query: 1090 tgcttgttcatattattcctgatcttttgagtgttaagactcgggagcttttcttgaaaa 1149
            ||||||| || || ||||| ||| | || ||||| || ||| |||||||| ||||||||||
Sbjct: 396  tgcttgtgcacatcattccggatttattaagtgtcaaaactagggagctattcttgaaaa 455

Query: 1150 ataaagctgctgagctcgatagagaaatgggattgattcgaactcaggaagaaaccggaa 1209
            | ||||| || ||||| ||| | |||||||||| | ||||| || || || || || || | |
Sbjct: 456  acaaagcggcagagcttgatcgtgaaatgggacttattcggacacaagaggagacgggta 515

Query: 1210 ggcatgtgagaatgttgactcatgagattagaagcactttagatagacatactattttaa 1269
            |  |||| || |||||||| |||||||| || || ||  | |||||||||||| ||||| |
Sbjct: 516  gatatgttaggatgttgacgcatgagatcaggagtaccctcgatagacataccattttga 575
```

```
Query: 1270 agactacacttgttgagcttggtaggacattagctttggaggagtgtgcattgtggatgc 1329
Sbjct: 576  agactacacttgttgagctaggaagagcattgggattagaaggtgtgcattgtggatgc 635

Query: 1330 ctactagaactgggttagagctacagctttcttatacacttcgtcatcaacatcccgtgg 1389
Sbjct: 636  caactcgtactggactggagcttcaactttcgtacactctgcgtcatcaaaatccagttg 695

Query: 1390 agtatacggttcctattcaattaccggtgattaaccaagtgtttggtactagtagggctg 1449
Sbjct: 696  gatttacagtacctatacagcttcctgtaattaatcaagttttcagtacaaatcggctg 755

Query: 1450 taaaaatatctcctaattctcctgtggctaggttgagacctgtttctgggaaatatatgc 1509
Sbjct: 756  taaaaatatcaccaaattctcctgttgcaaggcttcgacctg---ctggcaagtacatgc 812

Query: 1510 taggggaggtggtcgctgtgagggttccgcttctccacctttctaattttcagattaatg 1569
Sbjct: 813  ctggagaggtggttgctgtcagggtccctcttctgcatctctcaaattttcagataaatg 872

Query: 1570 actggcctgagctttcaacaaagagatatgctttgatggttttgatgcttccttcagata 1629
Sbjct: 873  attggcctgaactttctactaagcgctacgcattgatggttttgatgcttccttccgata 932

Query: 1630 gtgcaaggcaatggcatgtccatgagttggaactcgttgaagtcgtcgctgatcaggt 1687
Sbjct: 933  gtgcaagacaatggcatgcccacgtgttggagcttgttgaagtggtagctgatcaggt 990
```

Table 15. Summary of sequence alignment of *Petunia hybrida*(ETR1-3)mrna with ETR1

Identifier number	*Gene*	*Length*	*Score*	*E-value*	*Identities*	*Strand*
gi|17646116|gb|AF145974.1|AF145974	Petunia x hybrida ethylene receptor (ETR1-3) mRNA	2903	226 bits (114)	2e-55	399/494 (80%)	Plus / Plus

Fig.3. Sequence alignment of Petunia x hybrida ethylene receptor (ETR1-3) mRNA with ETR1

```
Query: 891  gagttgatttactttgtgaagaaatcagccgtgtttccgtatagatgggtacttgttcag 950
Sbjct: 193  gagttgatatactttgttaagaagtcggctgtttttccatatagatgggttcttgtgcag 252

Query: 951  tttggtgcttttatcgttctttgtggagcaactcatcttattaacttatggactttcact 1010
Sbjct: 253  tttggtgctttcatagttctttgtggagcaacacatcttattaacttatggacatttagt 312

Query: 1011 acgcattcgagaaccgtggcgcttgtgatgactaccgcgaaggtgttaaccgctgttgtc 1070
Sbjct: 313  atgcatacaaggactgtggccgtagtgatgactactgcaaaggtattgaccgcgctggtg 372

Query: 1071 tcgtgtgctactgcgttgatgcttgttcatattattcctgatcttttgagtgttaagact 1130
Sbjct: 373  tcatgtataactgctctcatgcttgtccacatcattcctgatttattaagtgtcaaaact 432

Query: 1131 cgggagcttttcttgaaaaataaagctgctgagctcgatagagaaatgggattgattcga 1190
Sbjct: 433  agggaactattcttgaagaaaaaagctgcacagcttgatcgtgaaatgggtattattcgg 492

Query: 1191 actcaggaagaaaccggaaggcatgtgagaatgttgactcatgagattagaagcacttta 1250
Sbjct: 493  actcaagaggagacaggtagacatgttagaatgctaactcatgaaatccgaagcactctt 552

Query: 1251 gatagacatactatttaaagactacacttgttgagcttggtaggacattagctttggag 1310
Sbjct: 553  gatagacatactatttaaagactacacttgttgagctaggaagaacattggccctggaa 612

Query: 1311 gagtgtgcattgtggatgcctactagaactgggttagagctacagctttcttatacactt 1370
Sbjct: 613  gagtgtgcattatggatgccaacacgtactggactagagcttcaactttcatacactctt 672
```

```
Query: 1371 cgtcatcaacatcc 1384
            ||||| ||| ||||
Sbjct: 673  cgtcaccaaaatcc 686
```

Table 16. Summary of sequence alignment of *Pelargonium hortorum* (ETR2) with ETR1

Identifier number	*Gene*	*Length*	*Score*	*E-value*	*Identities*	*Strand*
gi|5006605|gb|AF141929.1|AF141929	Pelargonium x hortorum ethylene receptor homolog (PhETR2) mRNA	2656	210 bits (106)	1e-50	277/334 (82%)	Plus / Plus

Fig.4. Sequence alignment of *Pelargonium hortorum* (ETR2) with ETR1

```
Query: 1759 aggtggctgtagctctctcacatgctgcgatcctagaagagtcgatgcgagctagggacc 1818
            ||||||| || ||||| |||||||||||| || |||||||||||||| | || |||||||
Sbjct: 1082 aggtggcagttgctctttcacatgctgctattttagaagagtcgatgagggcaagggacc 1141

Query: 1819 ttctcatggagcagaatgttgctcttgatctagctagacgagaagcagaaacagcaatcc 1878
            |||||||||||||| |||||||| |||||  | || ||  ||||||||||||||||||| |
Sbjct: 1142 ttctcatggagcaaaatgttgcacttgacatggccaggagagaagcagaaacagcaattc 1201

Query: 1879 gtgcccgcaatgatttcctagcggttatgaaccatgaaatgcgaacaccgatgcatgcga 1938
            |||| |||||||||||| | || || |||||||||| ||| |||| || ||||||||||
Sbjct: 1202 gtgctcgcaatgatttcttggctgtcatgaaccatgagatgagaactcccatgcatgcga 1261

Query: 1939 ttattgcactctcttccttactccaagaaacggaactaacccctgaacaaagactgatgg 1998
            |||||||||| ||||| ||||| || ||||| || || || || || || | |||||||
Sbjct: 1262 ttattgcactttcttcattactacaggaaactgagctgacacccgagcagcgcctgatgg 1321

Query: 1999 tggaaacaatacttaaaagtagtaacctttggcaactttgatgaatgatgtcttagatc 2058
            ||||||| || | |||||||| |||||||||| ||| | || ||||| || || ||||
Sbjct: 1322 tggaaactgtattgaaaagtagcaacctttggctactcttataaatgacgtgttggatc 1381

Query: 2059 tttcaaggttagaagatggaagtcttcaacttga 2092
            | || ||| |||||||||| || ||||||||||
Sbjct: 1382 tctccaggctagaagatgggagccttcaacttga 1415
```

Table 17. Summary of sequence alignment of *Pelargonium hortorum* (ETR1) with ETR1

Identifier number	*Gene*	*Length*	*Score*	*E-value*	*Identities*	*Strand*
gi|5006603|gb|AF141928.1|AF141928	Pelargonium x hortorum ethylene receptor homolog (PhETR1) mRNA	2646	204 bits (103)	6e-49	268/323 (82%)	Plus / Plus

Fig.5. Sequence alignment of *Pelargonium hortorum* (ETR1) with ETR1

```
Query: 1767 gtagctctctcacatgctgcgatcctagaagagtcgatgcgagctagggaccttctcatg 1826
            ||||| || |||||||||||| ||  |||||||||||||| | || ||||| ||||| |||
Sbjct: 1106 gtagcactttcacatgctgctatattagaagagtcgatgagggcaagggatcttcttatg 1165

Query: 1827 gagcagaatgttgctcttgatctagctagacgagaagcagaaacagcaatccgtgcccgc 1886
            |||||||||||| || |||||  | || ||  |||||||||||||||||||| ||||| |||
Sbjct: 1166 gagcagaatgtggcacttgacatggccaggagagaagcagaaacagcaattcgtgctcgc 1225

Query: 1887 aatgatttcctagcggttatgaaccatgaaatgcgaacaccgatgcatgcgattattgca 1946
            ||||| ||| | || |||||||||||||| ||| |||| || |||||||| || ||||||
Sbjct: 1226 aatgacttcttggctgttatgaaccatgagatgagaactcctatgcatgcaataattgca 1285

Query: 1947 ctctcttccttactccaagaaacggaactaacccctgaacaaagactgatggtggaaaca 2006
            || |||||||||||| || ||||| || || ||  |||| ||  | ||||||||||||||
Sbjct: 1286 ctttcttccttactacaggaaactgatctgacatctgagcagcgcctgatggtggaaact 1345
```

```
Query: 2007 atacttaaaagtagtaacctttggcaactttgatgaatgatgtcttagatctttcaagg 2066
           ||| | |||||| ||||||||||||||| ||| | || |||||||| |||||||| ||||||
Sbjct: 1346 atattaaaaagcagtaacctttggctactcttataaatgatgtgttagatctgtcaagg 1405

Query: 2067 ttagaagatggaagtcttcaact 2089
           | |||||||| ||||||||||
Sbjct: 1406 cttgaagatgggagtcttcaact 1428
```

Table 18. Summary of sequence alignment of *Petunia hybrida*(ETR3)mrna with ETR1

Identifier number	*Gene*	*Length*	*Score*	*E-value*	*Identities*	*Strand*
gi|17646118|gb|AF145975.1|AF145975	Petunia x hybrida ethylene receptor (ETR3) mRNA	1415	186 bits (94)	1e-43	394/494 (79%)	Plus / Plus

Fig.6. Sequence alignment of *Petunia hybrida* (ETR3) mRNA with ETR1

```
Query: 891  gagttgatttactttgtgaagaaatcagccgtgtttccgtatagatgggtacttgttcag 950
           |||||| | |||||||| |||||||||| || ||||||||||||||||| || || |||
Sbjct: 197  gagttggtatactttgtccagaaatcagcagtttttccgtatagatgggtgctcgtgcag 256

Query: 951  tttggtgctttttatcgttctttgtggagcaactcatcttattaacttatggactttcact 1010
           |||||||| ||||| |||||||||||||| || |||||||||| |||||||| |  |||
Sbjct: 257  tttggtgcgtttatagttctttgtggagcgacacatcttattaatttatggacatcgact 316

Query: 1011 acgcattcgagaaccgtggcgcttgtgatgactaccgcgaaggtgttaaccgctgttgtc 1070
           |||||  | || ||  ||||  | |||||||||| || |||||  | || ||||| ||
Sbjct: 317  gcgcatacaaggactctggcaatagtgatgactactgctaaggttctcactgctgtggta 376

Query: 1071 tcgtgtgctactgcgttgatgcttgttcatattattcctgatcttttgagtgttaagact 1130
           || ||||| || ||  | |||||||| || || ||||| ||| | |||||||| || |||
Sbjct: 377  tcatgtgcaacggctcttatgcttgtgcacatcattccggatttattgagtgtcaaaact 436

Query: 1131 cgggagcttttcttgaaaaataaagctgctgagctcgatagagaaatgggattgattcga 1190
           ||||| | |||||||||||| ||||| || ||||| ||| ||||||||||| | |||||
Sbjct: 437  agggagttattcttgaaaaacaaagcggcagagcttgatcgagaaatgggacttattcgg 496

Query: 1191 actcaggaagaaaccggaaggcatgtgagaatgttgactcatgagattagaagcacttta 1250
           || || || || || || ||  |||| || |||||||| |||||||| || || ||  |
Sbjct: 497  acacaagaggagacgggtagatatgttaggatgttgacgcatgagatcaggagtaccctc 556

Query: 1251 gatagacatactattttaaagactacacttgttgagcttggtaggacattagctttggag 1310
           |||||||||||| ||||| ||||||||||||||||||||| || ||  |||| |  || ||
Sbjct: 557  gatagacataccattttgaagactacacttgttgagctaggaagagcattgggattagaa 616

Query: 1311 gagtgtgcattgtggatgcctactagaactgggttagagctacagctttcttatacactt 1370
           |||||||||||||||||||| ||| | |||||  | ||||| || ||||| || || ||
Sbjct: 617  gagtgtgcattgtggatgccaactcgtactggactggagcttcaactttcgtacactcta 676

Query: 1371 cgtcatcaacatcc 1384
           ||||||||| ||||
Sbjct: 677  cgtcatcaaaatcc 690
```

Table 19. Summary of sequence alignment of *Petunia hybrida* (ETR1-2) with ETR1

Identifier number	*Gene*	*Length*	*Score*	*E-value*	*Identities*	*Strand*
gi|17646114|gb|AF145973.1|AF145973	Petunia x hybrida ethylene receptor (ETR1-2) mRNA	2567	186 bits (94)	1e-43	457/578 (79%)	Plus / Plus

Fig.7. Sequence alignment of *Petunia hybrida* (ETR1-2) with ETR1

```
Query: 807  caatggccagcggatgaattgttaatgaaataccaatacatctccgatttcttcattgcg 866
           |||||||||||| ||||| |||||||||||| || || ||  | || ||||| ||||| ||
Sbjct: 113  caatggccagctgatgagttgttaatgaagtatcagtatctatctgatttttcatcgca 172
```

```
Query: 867  attgcgtatttttcgattcctcttgagttgatttactttgtgaagaaatcagccgtgttt 926
Sbjct: 173  cttgcttatttctctatccccgtcgagttggtatactttgtccagaaatcagcagttttt 232

Query: 927  ccgtatagatgggtacttgttcagtttggtgcttttatcgttctttgtggagcaactcat 986
Sbjct: 233  ccgtatagatgggtgctcgtgcagtttggtgcgtttatagttctttgtggagcgacacat 292

Query: 987  cttattaacttatggactttcactacgcattcgagaaccgtggcgcttgtgatgactacc 1046
Sbjct: 293  cttattaatttatggacatcgactgcgcatacaaggactctggcaatagtgatgactact 352

Query: 1047 gcgaaggtgttaaccgctgttgtctcgtgtgctactgcgttgatgcttgttcatattatt 1106
Sbjct: 353  gctaaggttctcactgctgtggtatcatgtgcaacggctcttatgcttgtgcacatcatt 412

Query: 1107 cctgatcttttgagtgttaagactcgggagcttttcttgaaaaataaagctgctgagctc 1166
Sbjct: 413  ccggatttattaagtgtcaaaactagggagttattcttgaaaaacaaagcggcagagctt 472

Query: 1167 gatagagaaatgggattgattcgaactcaggaagaaaccggaaggcatgtgagaatgttg 1226
Sbjct: 473  gatcgagaaatgggacttattcggacacaagaggagacgggtagatatgttaggatgttg 532

Query: 1227 actcatgagattagaagcactttagatagacatactattttaaagactacacttgttgag 1286
Sbjct: 533  acgcatgagatcaggagtaccctcgatagacataccattttgaagactacacttgttgag 592

Query: 1287 cttggtaggacattagctttggaggagtgtgcattgtggatgcctactagaactgggtta 1346
Sbjct: 593  ctaggaagagcattgggattagaagagtgtgcattgtggatgccaactcgtactggactg 652

Query: 1347 gagctacagctttcttatacacttcgtcatcaacatcc 1384
Sbjct: 653  gagcttcaactttcgtacactctacgtcatcaaaatcc 690
```

Table 20. Summary of sequence alignment of Rosa hybrid ethylene receptor with ETR1

Identifier number	*Gene*	*Length*	*Score*	*E-value*	*Identities*	*Strand*
gi|21310082|gb|AF380127.1|	Rosa hybrid cultivar ethylene receptor mRNA	797	178 bits (90)	3e-41	330/410 (80%)	Plus / Plus

Fig.8. Sequence alignment of Rosa hybrid ethylene receptor with ETR1

```
Query: 942  cttgttcagtttggtgcttttatcgttctttgtggagcaactcatcttattaacttatgg 1001
Sbjct: 1    cttgtgcagtttggtgcttttattgtattgtgtggagcgacacatcttattaacttatgg 60

Query: 1002 actttcactacgcattcgagaaccgtggcgcttgtgatgactaccgcgaaggtgttaacc 1061
Sbjct: 61   acttttagcgtgcattcaagaactgtggccatagtaatggctactgccaaggttttcact 120

Query: 1062 gctgttgtctcgtgtgctactgcgttgatgcttgttcatattattcctgatcttttgagt 1121
Sbjct: 121  gctgcggtgtcatgtgctactgccctcatgcttgttcatattattcctgatttactgagt 180

Query: 1122 gttaagactcgggagcttttcttgaaaaataaagctgctgagctcgatagagaaatggga 1181
Sbjct: 181  gttaaaactagagagttattcctgaagaacaaggctgcagagctcgacagagaaatgggt 240

Query: 1182 ttgattcgaactcaggaagaaaccggaaggcatgtgagaatgttgactcatgagattaga 1241
Sbjct: 241  ttgattcgtacacaggaagaaacaggtagacatgtcaggatgctgacgcatgagatcaga 300

Query: 1242 agcactttagatagacatactattttaaagactacacttgttgagcttggtaggacatta 1301
Sbjct: 301  agcactcttgatcgacatactatactgaaaaccacacttgttgaattaggtagaattttg 360

Query: 1302 gctttggaggagtgtgcattgtggatgcctactagaactgggttagagct 1351
Sbjct: 361  acattggaagagtgtgccttgtggatgccaacacgcactgggttggagct 410
```

Table 21. Summary of sequence alignment of Rosa hybrid (ETR1) with ETR1

Identifier number	*Gene*	*Length*	*Score*	*E-value*	*Identities*	*Strand*
gi|20378350|gb|AF441283.1|	Rosa hybrid cultivar ethylene-response ETR1 mRNA	795	170 bits (86)	8e-39	329/410 (80%)	Plus / Plus

Fig.9. Sequence alignment of Rosa hybrid (ETR1) with ETR1

```
Query: 942  cttgttcagtttggtgcttttatcgttctttgtggagcaactcatcttattaacttatgg 1001
            ||||||||||||||||||||||||  ||  | ||||||||  || ||||||||||||||||||
Sbjct: 1    cttgttcagtttggtgcttttattgtattgtgtggagcgacacatcttattaacttatgg 60

Query: 1002 actttcactacgcattcgagaaccgtggcgcttgtgatgactaccgcgaaggtgttaacc 1061
          |||||  |    | |||| ||||| |||||  | || ||| |||| || ||||| || ||
Sbjct: 61   acttttagcgtgtattcaagaactgtggccatagtaatggctactgccaaggttttcact 120

Query: 1062 gctgttgtctcgtgtgctactgcgttgatgcttgttcatattattcctgatcttttgagt 1121
          ||||  || || ||||||||||  | ||||||||||||||||||||||| |  |||||
Sbjct: 121  gctgcggtgtcatgtgctactgccctcatgcttgttcatattattcctgatttactgagt 180

Query: 1122 gttaagactcgggagcttttcttgaaaaataaagctgctgagctcgatagagaaatggga 1181
          ||||| ||| | ||| | ||| |||| || || ||||| ||||||||  ||||||||||
Sbjct: 181  gttaaaactagagagttattcctgaagaacaaggctgccgagctcgacagagaaatgggt 240

Query: 1182 ttgattcgaactcaggaagaaaccggaaggcatgtgagaatgttgactcatgagattaga 1241
          ||||||||  || ||||||||||| || || ||||| || ||| |||| |||||||| |||
Sbjct: 241  ttgattcgtacacaggaagaaacaggtagacatgtcaggatgctgacgcatgagatcaga 300

Query: 1242 agcactttagatagacatactattttaaagactacacttgttgagcttggtaggacatta 1301
          ||||||| | ||| |||||| |||  | || || ||||||||||  | |||||  |  ||
Sbjct: 301  agcactcttgatcgacatattatactgaaaaccacacttgttgaattaggtagaattttg 360

Query: 1302 gctttggaggagtgtgcattgtggatgcctactagaactgggttagagct 1351
           | ||||| |||||||| |||||||||| ||  | ||||||||  |||||
Sbjct: 361  acattggaagagtgtgccttgtggatgccaacacgcactgggttggagct 410
```

Table 22. Summary of sequence alignment of *Dianthus caryophyllus* (ETR 1) with ETR1

Identifier number	*Gene*	*Length*	*Score*	*E-value*	*Identities*	*Strand*
gi|8570033|dbj|AB035806.1|	Dianthus caryophyllus DC-ETR1 mRNA	897	151 bits (76)	8e-33	265/328 (80%)	Plus / Plus

Fig.10. Sequence alignment of *Dianthus caryophyllus* (ETR 1) with ETR1

```
Query: 1057 taaccgctgttgtctcgtgtgctactgcgttgatgcttgttcatattattcctgatcttt 1116
          |||| ||||||||||||||||  |||||  | ||||| || |||||||||| ||||| |
Sbjct: 98   taactgctgttgtctcgtgtgtgactgctcttatgctcgtacatattattcccgatctat 157

Query: 1117 tgagtgttaagactcgggagcttttcttgaaaaataaagctgctgagctcgatagagaaa 1176
          | |||||||| ||| ||||  | |||||||| || || || ||  |||| ||||||||||
Sbjct: 158  taagtgttaaaactagggaattgttcttgaagaacaaggccgcacagctggatagagaaa 217

Query: 1177 tgggattgattcgaactcaggaagaaaccggaaggcatgtgagaatgttgactcatgaga 1236
          ||||  | || || || || |||||||| ||| | |||||||||||| |||| |||||||
Sbjct: 218  tgggtctcatccgcacacaagaagaaactggacgacatgtgagaatgctgacacatgaga 277

Query: 1237 ttagaagcactttagatagacatactattttaaagactacacttgttgagcttggtagga 1296
          | ||||| ||| | || ||||| || ||| | ||||| || |||||||||||  |||||||
Sbjct: 278  tcagaagtactcttgacagacacacgattcttaagacaactcttgttgagctgggtagga 337

Query: 1297 cattagctttggaggagtgtgcattgtggatgcctactagaactgggttagagctacagc 1356
          |  |||||||||| |||||||| |||||||||||||  | || ||||| || || ||||
Sbjct: 338  ctctagctttggaagagtgtgcgttgtggatgcctacacgtacagggttggaacttcagc 397
```

```
Query: 1357 tttcttatacacttcgtcatcaacatcc 1384
            || |||| ||||| ||||| ||| ||||
Sbjct: 398  ttgcttacacactccgtcagcaatatcc 425
```

Table 23. Summary of sequence alignment of Delphinium 'Magic fountains dark blue'(ERS) with ETR1

Identifier number	*Gene*	*Length*	*Score*	*E-value*	*Identities*	*Strand*
gi|18539221|dbj|AB055429.1|	Delphinium 'MagicFountains dark blue' ERS mRNA for ethylene receptor	2224	137 bits (69)	1e-28	219/269 (81%)	Plus / Plus

Fig.11. sequence alignment of Delphinium 'Magic fountains dark blue'(ERS) with ETR1

```
Query: 1062 gctgttgtctcgtgtgctactgcgttgatgcttgttcatattattcctgatcttttgagt 1121
            |||||||| || |||||||| ||| ||||||||||||| |||||||||||||  |||||
Sbjct: 331  gctgttgtatcttgtgctacagcgctgatgcttgttcacattattcctgatctactgagt 390

Query: 1122 gttaagactcgggagcttttcttgaaaaataaagctgctgagctcgatagagaaatggga 1181
            ||||| ||  | ||||| || ||||| ||||| ||||  || || ||||| || |||||
Sbjct: 391  gttaaaacaagagagctatttttgaagaataaggctgaagaacttgatagggagatgggt 450

Query: 1182 ttgattcgaactcaggaagaaaccggaaggcatgtgagaatgttgactcatgagattaga 1241
             | |||||||| || |||||||| |||||||||| |||||| | || ||||| ||| |
Sbjct: 451  cttattcgaacacaagaagaaactggaaggcatgtaagaatgctaacgcatgaaattcgt 510

Query: 1242 agcactttagatagacatactatttttaaagactacacttgttgagcttggtaggacatta 1301
            || ||  | || || ||||| || || || |||||||||||||||||| ||  |||| |||
Sbjct: 511  agtacacttgacaggcataccatattgaaaactacacttgttgagctgggccggacttta 570

Query: 1302 gctttggaggagtgtgcattgtggatgcc 1330
            | ||||||||| ||||||||||||||||
Sbjct: 571  ggtttggaggaatgtgcattgtggatgcc 599
```

Table 24. Summary of sequence alignment of Delphinium 'Magic fountains dark blue'(ERS) with ETR1

Identifier number	*Gene*	*Length*	*Score*	*E-value*	*Identities*	*Strand*
gi|18539223|dbj|AB055430.1|	Delphinium 'MagicFountains dark blue' ERS mRNA for ethylene receptor	2231	129 bits (65)	3e-26	218/269 (81%)	Plus / Plus

Fig.12. sequence alignment of Delphinium 'Magic fountains dark blue'(ERS) with ETR1

```
Query: 1062 gctgttgtctcgtgtgctactgcgttgatgcttgttcatattattcctgatcttttgagt 1121
            |||||||| || ||||| || ||| |||||||||||||| ||||||||||||||  |||||
Sbjct: 360  gctgttgtatcttgtgcgacagcgctgatgcttgttcacattattcctgatctactgagt 419
Query: 1122 gttaagactcgggagcttttcttgaaaaataaagctgctgagctcgatagagaaatggga 1181
            ||||| ||  | ||||| || ||||| ||||| ||||  || || ||||| || |||||
Sbjct: 420  gttaaaacaagagagctatttttgaagaataaggctgaagaacttgatagggagatgggt 479
Query: 1182 ttgattcgaactcaggaagaaaccggaaggcatgtgagaatgttgactcatgagattaga 1241
             | |||||||| || |||||||| |||||||||| |||||| | || ||||| ||| |
Sbjct: 480  cttattcgaacccaagaagaaactggaaggcatgtaagaatgctaacgcatgaaattcgt 539
Query: 1242 agcactttagatagacatactatttttaaagactacacttgttgagcttggtaggacatta 1301
            || ||  | || || ||||| || || || |||||||||||||||||| ||  |||| |||
Sbjct: 540  agtacacttgacaggcataccatattgaaaactacacttgttgagctgggccggacttta 599

Query: 1302 gctttggaggagtgtgcattgtggatgcc 1330
            | ||||||||| ||||||||||||||||
Sbjct: 600  ggtttggaggaatgtgcattgtggatgcc 628
```

Table 25. Summary of sequence alignment of *Rosa hybrida* (ETR2) with ETR1

Identifier number	*Gene*	*Length*	*Score*	*E-value*	*Identities*	*Strand*
gi|4416485|gb|AF127220.1|AF127220	Rosa hybrida ethylene receptor (ETR2) gene	1299	115 bits (58)	4e-22	106/122 (86%)	Plus / Plus

Fig.13. Sequence alignment of *Rosa hybrida* (ETR2) with ETR1

```
Query: 1566 aatgactggcctgagctttcaacaaagagatatgctttgatggttttgatgcttccttca 1625
            ||||||||||||||||| |||||||| | ||||||||||||| |||||||| |||||
Sbjct: 256  aatgactggcctgagctctcaacaaaacgctatgctttgatggtgttgatgctcccttcc 315

Query: 1626 gatagtgcaaggcaatggcatgtccatgagttggaactcgttgaagtcgtcgctgatcag 1685
            |||||||||||||||||||| | |||||||| || || ||||| || || ||||| |||
Sbjct: 316  gatagtgcaaggcaatggcatatgcatgagttagagctggttgaggtagttgctgaccag 375

Query: 1686 gt 1687
            ||
Sbjct: 376  gt 377
```

4.3.2.2. PROMOTER SCAN

This tool was used to find the putative promoter regions in the ETR1 sequence which would facilitate the gene sequencing process. The results are as shown in Fig.14

Fig.14. Processed sequence of ETR1 as displayed by Proscan.

```
1       AAAGATAGTATTTGTTGATAAATATGGGGATATTTATCCTATATTATCTG
51      TATTTTTCTTACCATTTTTACTCTATTCCTTTATCTACATTACGTCATTA
101     CACTATCATAAGATATTTGAATGAACAAATTCATGCACCCACCAGCTATA
151     TTACCCTTTTTTATTAAAAAAAAACATCTGATAATAATAACAAAAAAATT
201     AGAGAAATGACGTCGAAAAAAAAAGTAAGAACGAAGAAGAAGTGTTAAAC
251     CCAACCAATTTTGACTTGAAAAAAAGCTTCAACGCTCCCCTTTTCTCCTT
301     CTCCGTCGCTCTCCGCCGCGTCCCAAATCCCCAATTCCTCCTCTTCTCCG
351     ATCAATTCTTCCCAAGTAAGCTTCTTCTTCCTCGATTCTCTCCTCAGATT
401     GTTTCGTGACTTCTTTATATATATTCTTCACTTCCACAGTTTTCTTCTGT
451     TGTTGTCGTCGATCTCAAATCATAGAGATTGATTAACCTAATTGGTCTTT
501     ATCTAGTGTAATGCATCGTTATTAGGAACTTTAAATTAAGATTTAATCGT
551     TAATTTCATGATTCGGATTCGAATTTTACTGTTCTCGAGACTGAAATATG
601     CAACCTATTTTTTCGTAATCGTTGTGATCGAATTCGATTCTTCAGAATTT
651     ATAGCAATTTTGATGCTCATGATCTGTCTACGCTACGTTCTCGTCGTAAA
701     TCGAAGTTGATAATGCTATGTGTTTGTTACACAGGTGTGTGTATGTGTGA
751     GAGAGGAACTATAGTGTAAAAAATTCATAATGGAAGTCTGCAATTGTATT
801     GAACCGCAATGGCCAGCGGATGAATTGTTAATGAAATACCAATACATCTC
851     CGATTTCTTCATTGCGATTGCGTATTTTTCGATTCCTCTTGAGTTGATTT
901     ACTTTGTGAAGAAATCAGCCGTGTTTCCGTATAGATGGGTACTTGTTCAG
951     TTTGGTGCTTTTATCGTTCTTTGTGGAGCAACTCATCTTATTAACTTATG
1001    GACTTTCACTACGCATTCGAGAACCGTGGCGCTTGTGATGACTACCGCGA
1051    AGGTGTTAACCGCTGTTGTCTCGTGTGCTACTGCGTTGATGCTTGTTCAT
1101    ATTATTCCTGATCTTTTGAGTGTTAAGACTCGGGAGCTTTTCTTGAAAAA
1151    TAAAGCTGCTGAGCTCGATAGAGAAATGGGATTGATTCGAACTCAGGAAG
1201    AAACCGGAAGGCATGTGAGAATGTTGACTCATGAGATTAGAAGCACTTTA
1251    GATAGACATACTATTTTAAAGACTACACTTGTTGAGCTTGGTAGGACATT
1301    AGCTTTGGAGGAGTGTGCATTGTGGATGCCTACTAGAACTGGGTTAGAGC
1351    TACAGCTTTCTTATACACTTCGTCATCAACATCCCGTGGAGTATACGGTT
```

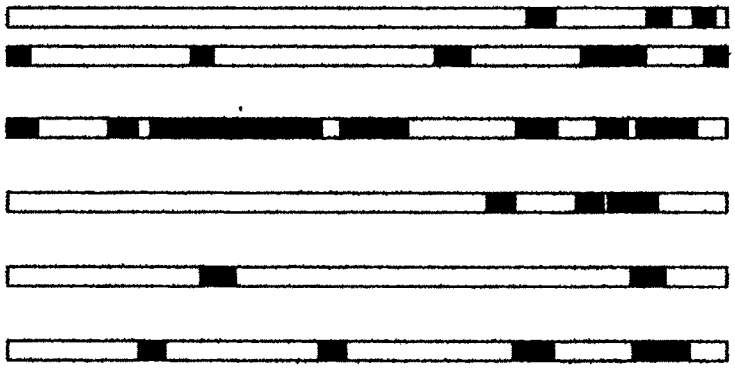
```
1401   CCTATTCAATTACCGGTGATTAACCAAGTGTTTGGTACTAGTAGGGCTGT
1451   AAAAATATCTCCTAATTCTCCTGTGGCTAGGTTGAGACCTGTTTCTGGGA
1501   AATATATGCTAGGGGAGGTGGTCGCTGTGAGGGTTCCGCTTCTCCACCTT
1551   TCTAATTTTCAGATTAATGACTGGCCTGAGCTTTCAACAAAGAGATATGC
1601   TTTGATGGTTTTGATGCTTCCTTCAGATAGTGCAAGGCAATGGCATGTCC
1651   ATGAGTTGGAACTCGTTGAAGTCGTCGCTGATCAGGTTTTACATTGCTGA
1701   GAATTTCTCTTCTTTGCTATGTTCATGATCTTGTCTATAACTTTTCTTCT
1751   CTTATTATAGGTGGCTGTAGCTCTCTCACATGCTGCGATCCTAGAAGAGT
1801   CGATGCGAGCTAGGGACCTTCTCATGGAGCAGAATGTTGCTCTTGATCTA
1851   GCTAGACGAGAAGCAGAAACAGCAATCCGTGCCCGCAATGATTTCCTAGC
1901   GGTTATGAACCATGAAATGCGAACACCGATGCATGCGATTATTGCACTCT
1951   CTTCCTTACTCCAAGAAACGGAACTAACCCCTGAACAAAGACTGATGGTG
2001   GAAACAATACTTAAAAGTAGTAACCTTTTGGCAACTTTGATGAATGATGT
2051   CTTAGATCTTTCAAGGTTAGAAGATGGAAGTCTTCAACTTGAACTTGGGA
2101   CATTCAATCTTCATACATTATTTAGAGAGGTAACTTTTGAACAGCTCTAT
2151   GTTTCATAAGTTTATACTATTTGTGTACTTGATTGTCATATTGAATCTTG
2201   TTGCAGGTCCTCAATCTGATAAAGCCTATAGCGGTTGTTAAGAAATTACC
2251   CATCACACTAAATCTTGCACCAGATTTGCCAGAATTTGTTGTTGGGGATG
2301   AGAAACGGCTAATGCAGATAATATTAAATATAGTTGGTAATGCTGTGAAA
2351   TTCTCCAAACAAGGTAGTATCTCCGTAACCGCTCTTGTCACCAAGTCAGA
2401   CACACGAGCTGCTGACTTTTTTGTCGTGCCAACTGGGAGTCATTTCTACT
2451   TGAGAGTGAAGGTTATTATCTTGTATCTTGGGATCTTATACCATAGCTGA
2501   AAGTATTTCTTAGGTCTTAATTTTGATGATTATTCAAATATAGGTAAAAG
2551   ACTCTGGAGCAGGAATAAATCCTCAAGACATTCCAAAGATTTTCACTAAA
2601   TTTGCTCAAACACAATCTTTAGCGACGAGAAGCTCGGGTGGTAGTGGGCT
2651   TGGCCTCGCCATCTCCAAGAGGTTTGAGCCTTATTAAAAGACGTTTTTTT
2701   CCAACTTTTTCTTGTCTTCTGTGTTGTTAAAAGTTTACTCATAAGCGTTT
2751   AATATGACAAGGTTTGTGAATCTGATGGAGGGTAACATTTGGATTGAGAG
2801   CGATGGTCTTGGAAAAGGATGCACGGCTATCTTTGATGTTAAACTTGGGA
2851   TCTCAGAACGTTCAAACGAATCTAAACAGTCGGGCATACCGAAAGTTCCA
2901   GCCATTCCCCGACATTCAAATTTCACTGGACTTAAGGTTCTTGTCATGGA
2951   TGAGAACGGGTTAGTATAAGCTTCTCACCTTTCTCTTTGCAAAATCTCTC
3001   GCCTTACTTCTTGCAAATGCAGATATTGGCGTTTAGAAAAAACGCAAATT
3051   TAATCTTATGAGAAACCGATGATTATTTTGGTTGCAGGGTAAGTAGAATG
3101   GTGACGAAGGGACTTCTTGTACACCTTGGGTGCGAAGTGACCACGGTGAG
3151   TTCAAACGAGGAGTGTCTCCGAGTTGTGTCCCATGAGCACAAAGTGGTCT
3201   TCATGGACGTGTGCATGCCCGGGGTCGAAAACTACCAAATCGCTCTCCGT
3251   ATTCACGAGAAATTCACAAAACAACGCCACCAACGGCCACTACTTGTGGC
3301   ACTCAGTGGTAACACTGACAAATCCACAAAAGAGAAATGCATGAGCTTTG
3351   GTCTAGACGGTGTGTTGCTCAAACCCGTATCACTAGACAACATAAGAGAT
3401   GTTCTGTCTGATCTTCTCGAGCCCCGGGTACTGTACGAGGGCATGTAAAG
3451   GCGATGGATGCCCCATGCCCCAGAGGAGTAATTCCGCTCCCGCCTTCTTC
3501   TCCCGTAAAACATCGGAAGCTGATGTTCTCTGGTTTAATTGTGTACATAT
3551   CAGAGATTGTCGGAGCGTTTTGGATGATATCTTAAAACAGAAAGGGAATA
3601   ACAAAATAGAAACTCTAAACCGGTATGTGTCCGTGGCGATTTCGGTTATA
3651   GAGGAACAAGATGGTGGTGGTATAATCATACCATTTCAGATTACATGTTT
3701   GACTAATGTTGTATCCTTATATATGTAGTTACATTCTTATAAGAATTTGG
3751   ATCGAGTTATGGATGCTTGTTGCGTGCATGTATGACATTGATGCAGTATT
3801   ATGGCGTCAGCTTTGCGCCGCTTAGTAGAACAACAACAATGGCGTTACTT
3851   AGTTTCTCAATCAACCCGATCTCCAAAAC
```

From the 3879 base pairs processed, this tool revealed that no promoter regions existed in the ETR1 sequence.

4.3.2.3. ORF FINDER

ORF finder was used to determine potential ORF regions in the ETR1 sequence. The result is depicted below in six frames. The Fig.15 indicates the the exact region of ORFs with their respective residue lengths.

Fig.15. ORF in six frames for ETR1



Frame	from	to	Length
+3	780	1700	921
+3	1803	2159	357
+2	3098	3448	351
+3	3399	3728	330
-3	3383	3679	297
-1	3253	3513	261
+3	2754	2969	216
-3	2735	2947	213
+2	2312	2500	189
-2	3369	3548	180
-2	1056	1232	177
+3	3183	3344	162

Frame	from	to	Length
+1	2803	2964	162
+3	558	710	153
-1	2596	2742	147
-3	716	862	147
-1	3073	3216	144
-3	1682	1825	144
+3	24	167	144
+1	3454	3585	132
+2	2	124	123
+1	3706	3825	120
+2	998	1111	114
+2	3767	3878	113

The green regions marked on the frames denotes the ORF regions in the ETR1 sequence.

4.3.3. Amino Acid search

4.3.3.1 BEAUTY – BLAST Enhanced Alignment Utility

To determine the function of the sequence identified in the database, this tool was used. A total of 738 amino acids were searched against the *nr* database of 173,394 sequences. The summary results of the search are listed in Table 26.

The K-value was found to be 0.047 and the lambda ratio was 0.270. The number of HSPs greater than 10 without gaps was 63 and the effective HSP length was 56.

Table 26. The search summary produced in BEAUTY search for ETR1.

Parameter	Value
E value	10
Lambda ratio	0.322
K value	0.136
Matrix	Blosum 62
Gap penalty existence	11
Gap penalty extension	1
Number of hits to database	63069271
Number of sequences	173394
Number of extensions	2393976
Number of successful extensions	5612
Number of sequences better than 10	203
Number of HSPs better than 10.0 without gapping	63
Number of HSPs successfully gapped in prelim test	140
Number of HSPs that attempted gapping in prelim test	5299
Number of HSPs gapped (non-prelim)	236
Length of query	738
Length of database	50782800
Effective HSP length	56
Effective length of query	682
Effective length of database	41072736
Effective search space	28011605952

The results of the analysis are presented in the Table 27. The analysis produced 50 significant alignments. Among these ETR1 protein recorded the highest score of 1388 with an E-value of 0.0 while sensor –like histidine kinase had the lowest score of 69. ETR1 protein homolog eTAE1 of Tomato had a high score value of 1122 with an E-value of 0.0, which was second only to ETR1 protein.

Table 27. Sequences producing significant alignments with ETR1

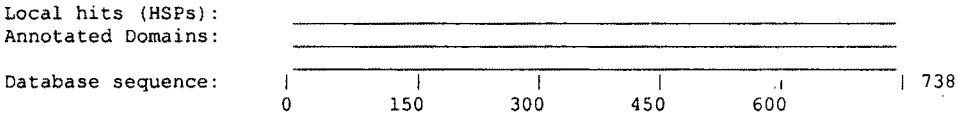
Identifier Number	Protein	Score (bits)	E-value
gi|1352397|sp|P49333|ETR1_ARATH	ETR1 PROTEIN >gi|625975|pir||A48...	1388	0.0
gi|7488993|pir||S71783	ETR1 protein homolog cTAE1 - tomato >gi|1...	1122	0.0
gi|7489052|pir||T07847	probable ethylene-response protein ETR1 -...	1012	0.0
gi|7488992|pir||T07794	ethylene receptor - tomato (strain UC82-B...	769	0.0
gi|7484950|pir||T00758	ethylene response sensor T20B5.14 - Arabi...	744	0.0
gi|7488991|pir||T07026	ethylene receptor - tomato (strain Ailsa ...	576	e-163
gi|281611|pir||B41863	two-component regulatory protein lemA - Ps...	169	2e-41
gi|1346440|sp|P48027|GACS_PSESY	SENSOR PROTEIN GACS >gi|151329|g...	169	2e-41
gi|939724|gb|AAA87840.1|	(U30858) putative sensor kinase; regula...	166	2e-40
gi|463195|gb|AAA20829.1|	(L30101) pectate lyase [Pseudomonas vir...	166	2e-40
gi|808104|gb|AAA82924.1|	(U25692) regulator of multiple function...	163	2e-39
gi|114830|sp|P26607|BARA_ECOLI	SENSOR PROTEIN BARA >gi|78846|pir...	155	5e-37
gi|40951|emb|CAA37397.1|	(X53315) arcB [Escherichia coli]	147	9e-35
gi|1168485|sp|P22763|ARCB_ECOLI	AEROBIC RESPIRATION CONTROL SENS	145	4e-34
gi|728989|sp|P40330|BVGS_BORPA	VIRULENCE SENSOR PROTEIN BVGS PRE	145	5e-34
gi|728990|sp|P16575|BVGS_BORPE	VIRULENCE SENSOR PROTEIN BVGS PRE	144	1e-33
gi|115160|sp|P26762|BVGS_BORBR	VIRULENCE SENSOR PROTEIN BVGS PRE nik-	143	1e-33
gi|7493968|pir||T18359	1 protein - Neurospora crassa >gi|126...	142	4e-33
gi|11359571|pir||T47214	nik-1 protein [imported] - Neurospora cr...	142	4e-33
gi|421019|pir||JU0221	EvgS protein - Escherichia coli >gi|216554...	138	8e-32
gi|1708886|sp|P54302|LUXQ_VIBHA	SENSOR PROTEIN LUXQ >gi|1363400|...	137	1e-31
gi|1237202|emb|CAA65612.1|	(X96869) histidine kinase [Dictyostel...	134	1e-30
gi|1136289|gb|AAC47300.1|	(U42597) histidine kinase A [Dictyoste...	126	3e-28
gi|461403|gb|AAA98756.1|	(L29642) sensor kinase [Pseudomonas flu...	122	4e-27
gi|147525|gb|AAA24503.1|	(M28242) capsule synthesis regulator co...	116	3e-25
gi|147528|gb|AAA24505.1|	L11272) putative [Escherichia coli]	116	3e-25
gi|1122856|emb|CAA63920.1|	(X94231) TorS [Escherichia coli]	115	5e-25
gi|7470834|pir||S76650	sensory transduction histidine kinase sll...	114	1e-24
gi|130130|sp|P23545|PHOR_BACSU	ALKALINE PHOSPHATASE SYNTHESIS SE	109	2e-23
gi|285403|pir||A41860	tetracycline resistance element regulator...	106	2e-22
gi|226292|prf||1505375A	vir gene [Bordetella pertussis	104	7e-22
gi|1168486|sp|P44578|ARCB_HAEIN	AEROBIC RESPIRATION CONTROL SENS	102	3e-21
gi|7470847|pir||S74399	sensory transduction histidine kinase slr...	100	2e-20
gi|625679|pir||A36929	virulence regulatory protein VsrB - Pseudo...	100	2e-20
gi|585701|sp|P37894|PLEC_CAUCR	NON-MOTILE AND PHAGE-RESISTANCE P...	100	2e-20
gi|7469649|pir||S76588	hypothetical protein - Synechocystis sp. ...	98	1e-19
gi|2507379|sp|P20169|DSPA_SYNY3	DRUG SENSORY PROTEIN A >gi|32202...	94	2e-18
gi|466195|sp|P35164|RESE_BACSU	SENSOR PROTEIN RESE >gi|629125|pi...	94	2e-18
gi|420780|pir||A46414	histidine protein kinase divJ - Caulobacte...	90	2e-17
gi|585055|sp|Q03228|DIVJ_CAUCR	HISTIDINE PROTEIN KINASE DIVJ	90	2e-17
gi|7470845|pir||S76370	sensory transduction histidine kinase slr...	87	2e-16
gi|730806|sp|P39664|SPHS_SYNP7	SENSOR PROTEIN SPHS >gi|421361|pi...	82	6e-15
gi|7469378|pir||S75974	hypothetical protein – Synechoc`ystis sp. ...	80	2e-14
gi|1145263|gb|AAA84961.1|	(U37008) SocD [Myxococcus xanthus	76	3e-13
gi|7475968|pir||F70089	two-component sensor histidine kinase hom...	75	8e-13

gi|1055348|gb|AAB38750.1|	(U38917) sensor kinase PhoR [Synechoco...	75	1e-12
gi|11465858	hypothetical chloroplast ORF 26. [Porphyra purpurea]...	73	4e-12
gi|6322044	histidine kinase osmosensor that regulates an osmosen...	72	5e-12
gi|7469878|pir||S76113	hypothetical protein sll0337 - Synechocys...	70	3e-11
gi|1731204|sp|P54883|SEX3_MYCLE	SENSOR-LIKE HISTIDINE KINASE SEN...	69	6e-11

The sequence alignment of six significant proteins with ETR1 are shown in Figures 16 to 21

Fig.16. Sequence alignment of ETR1 protein with ETR1

Identifier number	*Gene*	*Score*	*E-value*	*Identities*	*Positives*
gi|1352397|sp|P49333|ETR1_ARATH	ETR1-Arabidopsis thaliana	1388 bits (3553)	0.0	706/738 (95%)	706/738 (95%)



Annotated Domains	
DM00074: TRANSMITTERDOMAIN	347..594
DM00037: RESPONSEREGULATOR	596..729
Domain: TRANSMITTER DOMAIN (POTENTIAL).	332..572
Domain: RECEIVER DOMAIN (POTENTIAL).	607..728
Transmembrane region: POTENTIAL.	26..43
Transmembrane region: POTENTIAL.	53..76
Transmembrane region: POTENTIAL.	83..106
phosphorylation site: (AUTO-) (BY SIMILA	353
phosphorylation site: (BY SIMILARITY).	659
mutagenized site: A->V: IN ETR1-3; ETHYL	31
mutagenized site: I->F: IN ETR1-4; ETHYL	62
mutagenized site: C->Y: IN ETR1-1; ETHYL	65
mutagenized site: A->T: IN ETR1-2; ETHYL	102
GAF: GAF domain	158..312
signal: Histidine kinase	353..582
response_reg: Response regulator receive	610..725
PD003916:	11..176
PD186128:	179..280
PD004120: P73184(2) Q55434(2) Q55445(2)	282..340
PD000142: NTRB(9) PHYA(7) PHYB(5)	353..433
PD000064: NTRB(9) SP22(7) PHOR(4)	441..580
PD088652: ETR1_ARATH	582..608
PD000039: CHEY(8) NTRC(8) PHOB(6)	610..728

```
Query: 1   MEVCNCIEPQWPADELLMKYQYISDFFIAIAYFSIPLELIYFVKKSAVFPYRWVLVQFGA 60
           MEVCNCIEPQWPADELLMKYQYISDFFIAIAYFSIPLELIYFVKKSAVFPYRWVLVQFGA
Sbjct: 1   MEVCNCIEPQWPADELLMKYQYISDFFIAIAYFSIPLELIYFVKKSAVFPYRWVLVQFGA 60

Query: 61  FIVLCGATHLINLWTFTTHSRTVALVMTTAKVLTAVVSCATALMLVHIIPDLLSVKTREL 120
           FIVLCGATHLINLWTFTTHSRTVALVMTTAKVLTAVVSCATALMLVHIIPDLLSVKTREL
Sbjct: 61  FIVLCGATHLINLWTFTTHSRTVALVMTTAKVLTAVVSCATALMLVHIIPDLLSVKTREL 120

Query: 121 FLKNKAAELDREMGLIRTQEETGRHVRMLTHEIRSTLDRHTILKTTLVELGRTLALEECA 180
           FLKNKAAELDREMGLIRTQEETGRHVRMLTHEIRSTLDRHTILKTTLVELGRTLALEECA
Sbjct: 121 FLKNKAAELDREMGLIRTQEETGRHVRMLTHEIRSTLDRHTILKTTLVELGRTLALEECA 180

Query: 181 LWMPTRTGLELQLSYTLRHQHPVEYTVPIQLPVINQVFGTSRAVKISPNSPVARLRPVSG 240
           LWMPTRTGLELQLSYTLRHQHPVEYTVPIQLPVINQVFGTSRAVKISPNSPVARLRPVSG
Sbjct: 181 LWMPTRTGLELQLSYTLRHQHPVEYTVPIQLPVINQVFGTSRAVKISPNSPVARLRPVSG 240

Query: 241 KYMLGEVVAVRVPLLHLSNFQINDWPELSTKRYALMVLMLPSDSARQWXXXXXXXXXXA 300
           KYMLGEVVAVRVPLLHLSNFQINDWPELSTKRYALMVLMLPSDSARQW          A
Sbjct: 241 KYMLGEVVAVRVPLLHLSNFQINDWPELSTKRYALMVLMLPSDSARQWHVHELELVEVVA 300
```

```
Query: 301 DQVAVALSHAAILEESMRARDLLMEQNVALDLXXXXXXXXXXXXXNDFLAVMNHEMRTPMH 360
           DQVAVALSHAAILEESMRARDLLMEQNVALDL             NDFLAVMNHEMRTPMH
Sbjct: 301 DQVAVALSHAAILEESMRARDLLMEQNVALDLARREAETAIRARNDFLAVMNHEMRTPMH 360

Query: 361 AIIALSSLLQETELTPEQRLMVETILKSSNLLATLMNDVLDLSRLEDGSLQLELGTFNLH 420
           AIIALSSLLQETELTPEQRLMVETILKSSNLLATLMNDVLDLSRLEDGSLQLELGTFNLH
Sbjct: 361 AIIALSSLLQETELTPEQRLMVETILKSSNLLATLMNDVLDLSRLEDGSLQLELGTFNLH 420

Query: 421 TLFREVLNLIKPIAVVKKLPITLNLAPDLPEFVVGDEKRLMQIILNIVGNAVKFSKQGSI 480
           TLFREVLNLIKPIAVVKKLPITLNLAPDLPEFVVGDEKRLMQIILNIVGNAVKFSKQGSI
Sbjct: 421 TLFREVLNLIKPIAVVKKLPITLNLAPDLPEFVVGDEKRLMQIILNIVGNAVKFSKQGSI 480

Query: 481 SVTALVTKSDTRAADFFVVPTGSHFYLRVKVKDSGAGINPQDIPKIFTKFAQTQSLATRX 540
           SVTALVTKSDTRAADFFVVPTGSHFYLRVKVKDSGAGINPQDIPKIFTKFAQTQSLATR
Sbjct: 481 SVTALVTKSDTRAADFFVVPTGSHFYLRVKVKDSGAGINPQDIPKIFTKFAQTQSLATRS 540

Query: 541 XXXXXXXXAISKRFVNLMEGNIWIESDGLGKGCTAIFDVKLGISERSNESKQSGIPKVPA 600
                   AISKRFVNLMEGNIWIESDGLGKGCTAIFDVKLGISERSNESKQSGIPKVPA
Sbjct: 541 SGGSGLGLAISKRFVNLMEGNIWIESDGLGKGCTAIFDVKLGISERSNESKQSGIPKVPA 600

Query: 601 IPRHSNFTGLKVLVMDENGVSRMVTKGLLVHLGCEVTTVSSNEECLRVVSHEHKVVFMDV 660
           IPRHSNFTGLKVLVMDENGVSRMVTKGLLVHLGCEVTTVSSNEECLRVVSHEHKVVFMDV
Sbjct: 601 IPRHSNFTGLKVLVMDENGVSRMVTKGLLVHLGCEVTTVSSNEECLRVVSHEHKVVFMDV 660

Query: 661 CMPGVENYQIALRIHEKFTKQRHQRPLLVALSGNTDKSTKEKCMSFGLDGVLLKPVSLDN 720
           CMPGVENYQIALRIHEKFTKQRHQRPLLVALSGNTDKSTKEKCMSFGLDGVLLKPVSLDN
Sbjct: 661 CMPGVENYQIALRIHEKFTKQRHQRPLLVALSGNTDKSTKEKCMSFGLDGVLLKPVSLDN 720

Query: 721 IRDVLSDLLEPRVLYEGM 738
           IRDVLSDLLEPRVLYEGM
Sbjct: 721 IRDVLSDLLEPRVLYEGM 738
```

Fig.17. Sequence alignment of *L. esculentum*(ETR1) protein with ETR1

Identifier number	*Gene*	*Score*	*E-value*	*Identities*	*Positives*
>gi|7488993|pir||S71783	ETR1 homolog [Lycopersicon esculentum]	1122 bits (2870)	0.0	572/741 (77%)	638/741 (85%)

```
Query: 1   MEVCNCI-EPQWPADELLMKYQYISDFFIAIAYFSIPLELIYFVKKSAVFPYRWVLVQFG 59
           +E CNCI +PQ PAD+LLMKYQYISDFFIA+AYFSIP+ELIYFVKKSAVFPYRWVLVQFG
Sbjct: 15  VESCNCIIDPQLPADDLLMKYQYISDFFIALAYFSIPVELIYFVKKSAVFPYRWVLVQFG 74

Query: 60  AFIVLCGATHLINLWTFTTHSRTVALVMTTAKVLTAVVSCATALMLVHIIPDLLSVKTRE 119
           AFIVLCGATHLINLWTF  H+R VA+VMTT K LTA+VSC TALMLVHIIPDLLSVKTRE
Sbjct: 75  AFIVLCGATHLINLWTFNMHTRNVAIVMTTPKALTALVSCITALMLVHIIPDLLSVKTRE 134

Query: 120 LFLKNKAAELDREMGLIRTQEETGRHVRMLTHEIRSTLDRHTILKTTLVELGRTLALEEC 179
           LFLK KAA+LDREMG+IRTQEETGRHVRMLTHEIRSTLDRHTILKTTLVELGRTLALEEC
Sbjct: 135 LFLKKKAAQLDREMGIIRTQEETGRHVRMLTHEIRSTLDRHTILKTTLVELGRTLALEEC 194

Query: 180 ALWMPTRTGLELQLSYTLRHQHPVEYTVPIQLPVINQVFGTSRAVKISPNSPVARLRPVS 239
           ALWMPTRTGLELQLSYTLRHQ+PV  TVPIQLPVINQVFGT+  VKISPNSPVARLRP +
Sbjct: 195 ALWMPTRTGLELQLSYTLRHQNPVGLTVPIQLPVINQVFGTNHVVKISPNSPVARLRP-A 253

Query: 240 GKYMLGEVVAVRVPLLHLSNFQINDWPELSTKRYALMVLMLPSDSARQWXXXXXXXXXXX 299
           GKYM GEVVAVRVPLLHLSNFQINDWPELSTKRYALMVLMLPSDSARQW
Sbjct: 254 GKYMPGEVVAVRVPLLHLSNFQINDWPELSTKRYALMVLMLPSDSARQWHVHELELVEVV 313

Query: 300 ADQVAVALSHAAILEESMRARDLLMEQNVALDLXXXXXXXXXXXXXNDFLAVMNHEMRTPM 359
           ADQVAVALSHAAILEESMRARDLLMEQNVALDL             NDFLAVMNHEMRTPM
Sbjct: 314 ADQVAVALSHAAILEESMRARDLLMEQNVALDLARREAEMAVRARNDFLAVMNHEMRTPM 373

Query: 360 HAIIALSSLLQETELTPEQRLMVETILKSSNLLATLMNDVLDLSRLEDGSLQLELGTFNL 419
           HAIIALSSLLQET+LTPEQRLMVETILKSSNLLATL+NDVLDLSRLEDGSLQL++GTFNL
Sbjct: 374 HAIIALSSLLQETDLTPEQRLMVETILKSSNLLATLINDVLDLSRLEDGSLQLDIGTFNL 433

Query: 420 HTLFREVLNLIKPIAVVKKLPITLNLAPDLPEFVVGDEKRLMQIILNIVGNAVKFSKQGS 479
           H LFREV +LIKPIA VKKL +TL+L+ DLPE+V+GDEKRLMQI+LN+VGNAVKFSK+G+
Sbjct: 434 HALFREVHSLIKPIASVKKLFVTLSLSSDLPEYVIGDEKRLMQILLNVVGNAVKFSKEGN 493

Query: 480 ISVTALVTKSDT----RAADFFVVPTGSHFYLRVKVKDSGAGINPQDIPKIFTKFAQTQS 535
           +S++A V KSD+     RA +FF VP+ +HFYLRV++KD+G GI PQDIP +F+KF Q+Q+
Sbjct: 494 VSISAFVAKSDSLRDPRAPEFFAVPSENHFYLRVQIKDTGIGITPQDIPNLFSKFTQSQA 553
```

```
Query: 536 LATRXXXXXXXXXAISKRFVNLMEGNIWIESDGLGKGCTAIFDVKLGISERSNESKQSGI 595
          LAT          AI KRFVNLMEG+IWIES+GLGKG TAIF +KLGI  R+NESK   +
Sbjct: 554 LATTNSGGTGLGLAICKRFVNLMEGHIWIESEGLGKGSTAIFIIKLGIPGRANESKLPFV 613

Query: 596 PKVPAIPRHSNFTGLKVLVMDENGVSRMVTKGLLVHLGCEVTTVSSNEECLRVVSHEHKV 655
           K+PA     +F GLKVLVMDENGVSRMVTKGLL HLGC+VTTV S +ECLRVV+HEHKV
Sbjct: 614 TKLPANHTQMSFQGLKVLVMDENGVSRMVTKGLLTHLGCDVTTVGSRDECLRVVTHEHKV 673

Query: 656 VFMDVCMPGVENYQIALRIHEKFTKQRHQRPLLVALSGNTDKSTKEKCMSFGLDGVLLKP 715
           V MDV M G++ Y++A+ IHE+F K RH RPL+VAL+GNTD+ TKE CM  G+DGV+LKP
Sbjct: 674 VIMDVSMQGIDCYEVAVVIHERFGK-RHGRPLIVALTGNTDRVTKENCMRVGMDGVILKP 732

Query: 716 VSLDNIRDVLSDLLEPRVLYE 736
           VS+  +R VLS+LLE  V+ E
Sbjct: 733 VSVYKMRSVLSELLEHGVVLE 753
```

Fig.18. Sequence alignment of *L. esculentum* ethylene receptor with ETR1

Identifier number	*Gene*	*Score*	*E-value*	*Identities*	*Positives*
>gi|7489052|pir||T07847	ethylene receptor [Lycopersicon esculentum]	1012 bits (2589)	0.0	518/703 (73%)	584/703 (82%)

```
Query: 32  YFSIPLELIYFVKKSAVFPYRWVLVQFGAFIVLCGATHLINLWTFTTHSRTVALVMTTAK 91
           YFSIP+EL+YFV+KSAVFPYRWVLVQFGAFIVLCGATHLINLWT T H+RTVA+VMTTAK
Sbjct: 1   YFSIPIELVYFVQKSAVFPYRWVLVQFGAFIVLCGATHLINLWTSTPHTRTVAMVMTTAK 60

Query: 92  VLTAVVSCATALMLVHIIPDLLSVKTRELFLKNKAAELDREMGLIRTQEETGRHVRMLTH 151
             TA VSCATA+MLVHIIPDLLSVKTRELFLKNKAAELDREMGLIRTQEETGR+VRMLTH
Sbjct: 61  FSTAAVSCATAVMLVHIIPDLLSVKTRELFLKNKAAELDREMGLIRTQEETGRYVRMLTH 120

Query: 152 EIRSTLDRHTILKTTLVELGRTLALEECALWMPTRTGLELQLSYTLRHQHPVEYTVPIQL 211
           EIRSTLDRHTILKTTLVELGR L LEECALWMPTRTG+ELQLSYTL HQ+PV +TVPIQL
Sbjct: 121 EIRSTLDRHTILKTTLVELGRALQLEECALWMPTRTGVELQLSYTLHHQNPVGFTVPIQL 180

Query: 212 PVINQVFGTSRAVKISPNSPVARLRPVSGKYMLGEVVAVRVPLLHLSNFQINDWPELSTK 271
           PVINQVF  + AVKISPNS VARLRP   KY+ GEVVAVRVPLLHLSNFQ NDWPELS K
Sbjct: 181 PVINQVFSANCAVKISPNSAVARLRPTR-KYIPGEVVAVRVPLLHLSNFQTNDWPELSPK 239

Query: 272 RYALMVLMLPSDSARQWXXXXXXXXXXXADQVAVALSHAAILEESMRARDLLMEQNVALD 331
            YALMVLMLPS+SARQW           ADQVAVALSHAAILEESMRARDLL+EQNVALD
Sbjct: 240 SYALMVLMLPSNSARQWHVHELELVDVVADQVAVALSHAAILEESMRARDLLIEQNVALD 299

Query: 332 LXXXXXXXXXXXXNDFLAVMNHEMRTPMHAIIALSSLLQETELTPEQRLMVETILKSSNL 391
           L             NDFL VMNHEMRTPMHA++ALSSLLQE+EL PEQRLMVETILKSSNL
Sbjct: 300 LARREAETAVRARNDFLGVMNHEMRTPMHAVVALSSLLQESELIPEQRLMVETILKSSNL 359

Query: 392 LATLMNDVLDLSRLEDGSLQLELGTFNLHTLFREVLNLIKPIAVVKKLPITLNLAPDLPE 451
           LATL+NDVLDLSRLEDGSLQL++GTFNLH LFREVLNLIKP+A VKKL +TL+L+ D PE
Sbjct: 360 LATLINDVLDLSRLEDGSLQLDVGTFNLHALFREVLNLIKPVAAVKKLFVTLSLSSDFPE 419

Query: 452 FVVGDEKRLMQIILNIVGNAVKFSKQGSISVTALVTKS----DTRAADFFVVPTGSHFYL 507
             +GDEKRLMQI+LN+VGNAVKFS++GS+SV+A+  KS    D RA +FF V + +HFYL
Sbjct: 420 VAIGDEKRLMQILLNVVGNAVKFSEEGSVSVSAVNAKSESLIDPRAPEFFPVQSENHFYL 479

Query: 508 RVKVKDSGAGINPQDIPKIFTKFAQTQSLATRXXXXXXXXXAISKRFVNLMEGNIWIESD 567
           RV+VKD+G+GINPQD PK+F KFAQ Q  AT+          AI KRFVNLMEG+IWIES+
Sbjct: 480 RVQVKDTGSGINPQDFPKLFCKFAQNQEPATKNSAGTGLGLAICKRFVNLMEGHIWIESE 539

Query: 568 GLGKGCTAIFDVKLGISERSNESKQSGIPKVPAIPRHSNFTGLKVLVMDENGVSRMVTKG 627
           G+GKG TAIF VKLGI  R NESK       +PA       F GLKVLVMD+NG SRMVTK
Sbjct: 540 GVGKGSTAIFIVKLGIPGRLNESKLPFTAGLPANHMQMTFQGLKVLVMDDNGFSRMVTKS 599

Query: 628 LLVHLGCEVTTVSSNEECLRVVSHEHKVVFMDVCMPGVENYQIALRIHEKFTKQRHQRPL 687
           LLVHLGC+VTT+ S +ECLR+++ EHKV+ MD  + G+  Y +A+ +HEKF K R +RPL
Sbjct: 600 LLVHLGCDVTTIGSGDECLRILTREHKVLIMDASITGMNCYDVAVSVHEKFGK-RLERPL 658

Query: 688 LVALSGNTDKSTKEKCMSFGLDGVLLKPVSLDNIRDVLSDLLE 730
           +VAL+GNTD+ TKE C+  G+DGV+LKPVS+D +R VLS LLE
Sbjct: 659 IVALTGNTDQVTKENCLRVGMDGVILKPVSIDKMRSVLSGLLE 701
```

Fig.19. Sequence alignment of *L. esculentum* ethylene receptor with ETR1

Identifier number	*Gene*	*Score*	*E-value*	*Identities*	*Positives*
>gi|7488992|pir||T07794	ethylene receptor [Lycopersicon esculentum]	769 bits (1964)	0.0	396/607 (65%)	465/607 (76%)

```
Query: 1   MEVCNCIEPQWPADELLMKYQYISDFFIAIAYFSIPLELIYFVKKSAVFPYRWVLVQFGA 60
           ME C+CIE   P  +LL+KYQY+SDFFIA+AYFSIPLELIYFV KSA FPYRWVL+QFGA
Sbjct: 1   MESCDCIEALLPTGDLLVKYQYLSDFFIAVAYFSIPLELIYFVHKSACFPYRWVLMQFGA 60

Query: 61  FIVLCGATHLINLWTFTTHSRTVALVMTTAKVLTAVVSCATALMLVHIIPDLLSVKTREL 120
           FIVLCGATH I+LWTF  HS+TVA+VMT +K+LTA VSC TALMLVHIIPDLLSVKTREL
Sbjct: 61  FIVLCGATHFISLWTFFMHSKTVAVVMTISKMLTAAVSCITALMLVHIIPDLLSVKTREL 120

Query: 121 FLKNKAAELDREMGLIRTQEETGRHVRMLTHEIRSTLDRHTILKTTLVELGRTLALEECA 180
           FLK +A ELD+EMGLI  QEETGRHVRMLTHEIRSTLDRHTILKTTLVELGRTL L ECA
Sbjct: 121 FLKTRAEELDKEMGLIIRQEETGRHVRMLTHEIRSTLDRHTILKTTLVELGRTLDLAECA 180

Query: 181 LWMPTRTGLELQLSYTLRHQHPVEYTVPIQLPVINQVFGTSRAVKISPNSPVARLRPVSG 240
           LWMP + GL LQLS+ L +  P+  TVPI LP+IN++F +  A++J   +P+AR+R   G
Sbjct: 181 LWMPCQGGLTLQLSHNLNNLIPLGSTVPINLPIINEIFSSPEAIQIPHTNPLARMRNTVG 240

Query: 241 KYMLGEVVAVRVPLLHLSNFQINDWPELSTKRYALMVLMLPSDSARQWXXXXXXXXXXA 300
           +Y+  EVVAVRVPLLHLSNF  NDW ELST+ YA+MVL+LP +  R+W           A
Sbjct: 241 RYIPPEVVAVRVPLLHLSNF-TNDWAELSTRSYAVMVLVLPMNGLRKWREHELELVQVVA 299

Query: 301 DQVAVALSHAAILEESMRARDLLMEQNVALDLXXXXXXXXXXXXNDFLAVMNHEMRTPMH 360
           DQVAVALSHAAILE+SMRA D LMEQN+ALD+             NDFLAVMNHEMRTPMH
Sbjct: 300 DQVAVALSHAAILEDSMRAHDQLMEQNIALDVARQEAEMAIRARNDFLAVMNHEMRTPMH 359

Query: 361 AIIALSSLLQETELTPEQRLMVETILKSSNLLATLMNDVLDLSRLEDGSLQLELGTFNLH 420
           A+IAL SLL ET+LTPEQR+M+ETILKSSNLLATL+NDVLDLSRLEDG L+LE GTFNLH
Sbjct: 360 AVIALCSLLLETDLTPEQRVMIETILKSSNLLATLINDVLDLSRLEDGILELENGTFNLH 419

Query: 421 TLFREVLNLIKPIAVVKKLPITLNLAPDLPLFVVGDEKRLMQIILNIVGNAVKFSKQGSI 480
            + RE +NLIKPIA +KKL ITL LA DLP   VGD KRL+Q +LN+ GNAVKF+K+G I
Sbjct: 420 GILREAVNLIKPIASLKKLSITLALALDLPILAVGDAKRLIQTLLNVAGNAVKFTKEGHI 479

Query: 481 SVTALVTK----SDTRAADFFVVPTGSHFYLRVKVKDSGAGINPQDIPKIFTKFAQTQSL 536
           S+ A V K     D    + F +P+   FYLRV+V+D+G GI+PQDIP +FTKFA+++
Sbjct: 480 SIEASVAKPEYARDCHPPEMFPMPSDGQFYLRVQVRDTGCGISPQDIPLVFTKFAESRPT 539

Query: 537 ATRXXXXXXXXXAISKRFVNLMEGNIWIESDGLGKGCTAIFDVKLGISERSNESKQSGIP 596
           + R          AI +RF+ LM+GNIWIES+G GKG T  F VKLGI    N      +P
Sbjct: 540 SNRSTGGEGLGLAICRRFIQLMKGNIWIESEGPGKGTTVTFVVKLGICHHPN-----ALP 594

Query: 597 KVPAIPR 603
            +P  PR
Sbjct: 595 LLPMPPR 601
```

Fig.20. Sequence alignment of A. thaliana(ERS) protein with ETR1

Identifier number	*Gene*	*Score*	*E-value*	*Identities*	*Positives*
>gi|7484950|pir||T00758	ethylene response sensor [Arabidopsis thaliana]	744 bits (1899)	0.0	386/597 (64%)	459/597 (76%)

```
Query: 1   MEVCNCIEPQWPADELLMKYQYISDFFIAIAYFSIPLELIYFVKKSAVFPYRWVLVQFGA 60
           ME C+C E     D+LL+KYQYISD  IA+AYFSIPLELIYFV+KSA FPY+WVL+QFGA
Sbjct: 1   MESCDCFETHVNQDDLLVKYQYISDALIALAYFSIPLELIYFVQKSAFFPYKWVLMQFGA 60

Query: 61  FIVLCGATHLINLWTFTTHSRTVALVMTTAKVLTAVVSCATALMLVHIIPDLLSVKTREL 120
           FI+LCGATH INLW F  HS+ VA+VMT AKV  AVVSCATALMLVHIIPDLLSVK REL
Sbjct: 61  FIILCGATHFINLWMFFMHSKAVAIVMTIAKVSCAVVSCATALMLVHIIPDLLSVKNREL 120

Query: 121 FLKNKAAELDREMGLIRTQEETGRHVRMLTHEIRSTLDRHTILKTTLVELGRTLALEECA 180
           FLK KA ELDREMGLI TQEETGRHVRMLTH IR TLDRHTIL+TTLVELG+TL LEECA
Sbjct: 121 FLKKKADELDREMGLILTQEETGRHVRMLTHGIRRTLDRHTILRTTLVELGKTLCLEECA 180
```

```
Query: 181 LWMPTRTGLELQLSYTLRHQHPVEYTVPIQLPVINQVFGTSRAVKISPNSPVARLRPVSG 240
           LWMP+++GL LQLS+TL H+  V  +VPI LP+IN++F +++A+ I  + P+A++ P  G
Sbjct: 181 LWMPSQSGLYLQLSHTLSHKIQVGSSVPINLPIINELFNSAQAMHIPHSCPLAKIGPPVG 240

Query: 241 KYMLGEVVAVRVPLLHLSNFQINDWPELSTKRYALMVLMLPSDSARQWXXXXXXXXXXA 300
           +Y   EVV+VRVPLLHLSNFQ +DW +LS K YA+MVL+LP+D AR+W           A
Sbjct: 241 RYSPPEVVSVRVPLLHLSNFQGSDWSDLSGKGYAIMVLILPTDGARKWRDHELELVENVA 300

Query: 301 DQVAVALSHAAILEESMRARDLLMEQNVALDLXXXXXXXXXXXXNDFLAVMNHEMRTPMH 360
           DQVAVALSHAAILEESM ARD LMEQN ALD              NDFLAVMNHEMRTPMH
Sbjct: 301 DQVAVALSHAAILEESMHARDQLMEQNFALDKARQEAEMAVHARNDFLAVMNHEMRTPMH 360

Query: 361 AIIALSSLLQETELTPEQRLMVETILKSSNLLATLMNDVLDLSRLEDGSLQLELGTFNLH 420
           AII+LSSLL ETEL+PEQR+M+ETILKSSNL+ATL++DVLDLSRLEDGSL LE   F+L
Sbjct: 361 AIISLSSLLLETELSPEQRVMIETILKSSNLVATLISDVLDLSRLEDGSLLLENEPFSLQ 420

Query: 421 TLFREVLNLIKPIAVVKKLPITLNLAPDLPEFVVGDEKRLMQIILNIVGNAVKFSKQGSI 480
            +F EV++LIKPIA VKKL   L L+ DLP + +GDEKRLMQ ILNI+GNAVKF+K+G I
Sbjct: 421 AIFEEVISLIKPIASVKKLSTNLILSADLPTYAIGDEKRLMQTILNIMGNAVKFTKEGYI 480

Query: 481 SVTALVTKSDT----RAADFFVVPTGSHFYLRVKVKDSGAGINPQDIPKIFTKFAQTQSL 536
           S+ A + K ++     + +FF V + SHFYL V+VKD+G GI+ QDIP +FTKF Q ++
Sbjct: 481 SIIASIMKPESLQELPSPEFFPVLSDSHFYLCVQVKDTGCGIHTQDIPLLFTKFVQPRTG 540

Query: 537 ATRXXXXXXXXXAISKRFVNLMEGNIWIESDGLGKGCTAIFDVKLGISERSNESKQS 593
             R          A+ KRFV LM G +WIES+GL KGCTA F ++LGI    + S  S
Sbjct: 541 TQRNHSGGGLGLALCKRFVGLMGGYMWIESEGLEKGCTASFIIRLGICNGPSSSSGS 597
```

Fig.21. Sequence alignment of *L. esculentum* ethylene receptor with ETR1

Identifier number	*Gene*	*Score*	*E-value*	*Identities*	*Positives*
>gi|7488991|pir||T07026	ethylene receptor [Lycopersicon esculentum]	576 bits (1468)	e-163	302/489 (61%)	360/489 (72%)

```
Query: 119 ELFLKNKAAELDREMGLIRTQEETGRHVRMLTHEIRSTLDRHTILKTTLVELGRTLALEE 178
           ELFLK +A ELD+EMGLI  QEETGRHVRMLTHEIRSTLDRHT LKTTLVELGRTL L E
Sbjct: 3   ELFLKTRAEELDKEMGLIIRQEETGRHVRMLTHEIRSTLDRHTTLKTTLVELGRTLDLAE 62

Query: 179 CALWMPTRTGLELQLSYTLRHQHPVEYTVPIQLPVINQVFGTSRAVKISPNSPVARLRPV 238
           CALWMP + GL LQLS+ L +  P+  TVPI LP+IN++F +  A++I   +P+AR+R
Sbjct: 63  CALWMPCQGGLTLQLSHNLNNLIPLGSTVPINLPIINEIFSSPEAIQIPHTNPLARMRNT 122

Query: 239 SGKYMLGEVVAVRVPLLHLSNFQINDWPELSTKRYALMVLMLPSDSARQWXXXXXXXXXX 298
            G+Y+  EVVAVRVPLLHLSNF  NDW ELST+ YA+MVL+LP +  R+W
Sbjct: 123 VGRYIPPEVVAVRVPLLHLSNFT-NDWAELSTRSYAVMVLVLPMNGLRKWREHELELVQV 181

Query: 299 XADQVAVALSHAAILEESMRARDLLMEQNVALDLXXXXXXXXXXXXNDFLAVMNHEMRTP 358
            ADQVAVALSHAAILE+SMRA D LMEQN+ALD+              NDFLAVMNHEMRTP
Sbjct: 182 VADQVAVALSHAAILEDSMRAHDQLMEQNIALDVARQEAEMAIRARNDFLAVMNHEMRTP 241

Query: 359 MHAIIALSSLLQETELTPEQRLMVETILKSSNLLATLMNDVLDLSRLEDGSLQLELGTFN 418
           MHA+IAL SLL ET+LTPEQR+M+ETILKSSNLLATL+NDVLDLSRLEDG L+LE GTFN
Sbjct: 242 MHAVIALCSLLLETDLTPEQRVMIETILKSSNLLATLINDVLDLSRLEDGILELENGTFN 301

Query: 419 LHTLFREVLNLIKPIAVVKKLPITLNLAPDLPEFVVGDEKRLMQIILNIVGNAVKFSKQG 478
           LH + RE +NLIKPIA +KKL ITL LA DLP   VGD KRL+Q +LN+ GNAVKF+K+G
Sbjct: 302 LHGILREAVNLIKPIASLKKLSITLALALDLPILAVGDAKRLIQTLLNVAGNAVKFTKEG 361

Query: 479 SISVTALVTK----SDTRAADFFVVPTGSHFYLRVKVKDSGAGINPQDIPKIFTKFAQTQ 534
            IS+ A V K     D    + F +P+   FYLRV+V+D+G GI+PQDIP +FTKFA+++
Sbjct: 362 HISIEASVAKPEYARDCHPPEMFPMPSDGQFYLRVQVRDTGCGISPQDIPLVFTKFAESR 421

Query: 535 SLATRXXXXXXXXAISKRFVNLMEGNIWIESDGLGKGCTAIFDVKLGISERSNESKQSG 594
             + R          AI +RF+ LM+GNIWIES+G GKG T  F VKLGI    N
Sbjct: 422 PTSNRSTGGEGLGLAICRRFIQLMKGNIWIESEGPGKGTTVTFVVKLGICHHPN-----A 476

Query: 595 IPKVPAIPR 603
           +P +P  PR
Sbjct: 477 LPLLPMPPR 485
```

4.3.3.2. PDB sum

This tool was used to determine all information pertaining to the ETR1 protein. The PDB code 1DCF was used in the search. The rasmol 3D structure is shown in Fig.22.

PDB id: 1dcf

Transferase

Title: *Crystal structure of the receiver domain of the ethylene receptor of arabidopsis thaliana*

Structure: *Etr1 protein. Chain: a. Fragment: receiver domain. Engineered: yes*
Source: *Arabidopsis thaliana. Thale cress. Expressed in: escherichia coli.*

Resolution: 2.50Å. **R-factor:** 0.222. **R-free:** 0.271.

Authors: H.J.Muller-Dieckmann, A.Grantz, S.H.Kim

Date: 04-Nov-99

PDB header records

Enzyme class from PDB file: **E.C.2.7.3.-** E.C.->PDB

SWISS-PROT code: **ETR1_ARATH** Swiss-prot Pfam

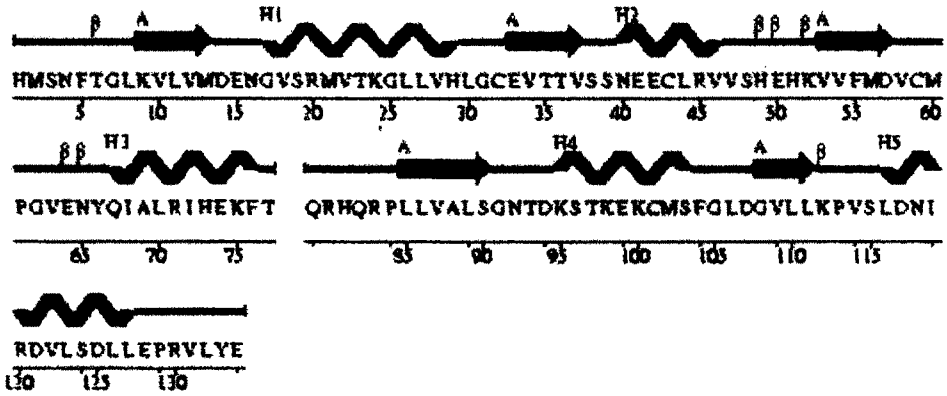
Fig.22. 3D structure of ETR1 protein as predicted by Rasmol

Molecule(s) in PDB file 1dcf:

Chain A (133 residues)

CATH structural classification (1 domain)

Links	CATH no.	Class	Architecture
CATH DBS	**3.40.50.3000** -> *Alpha Beta 3-Layer(aba) Sandwich*		



4.3.3.3. SWISS – PROT

This is a protein database and was used in the study to determine the function of the individual domains, features, sequence details, cross-references are as shown in Tables 28 to 32.

The database illustrated many features of ETR1 protein.

Function : May act early in the ethylene signal transduction pathway, possibly as an ethylene receptor, or as a regulator of the pathway.

Cofactor : Binds one copper ion per dimmer.

Subunit : Homodimer; disulfide-linked

Subcellular location : Endoplasmic reticulum

Tissue specificity : Leaves, roots, stems, flowers and seedlings.

Similarity : Belongs to the ethylene receptor family.

NiceProt View of SWISS-PROT: P49333

Table 28. General information about ETR1

Entry name	**ETR1_ARATH**
Primary accession number	**P49333**
Secondary accession numbers	None
Entered in SWISS-PROT in	Release 33, February 1996
Sequence was last modified in	Release 33, February 1996
Annotations were last modified in	Release 41, June 2002

Table 29. Taxonomy of the protein

Protein name	**Ethylene receptor**
Synonym	**EC 2.7.3.-**
Gene name	**ETR1** or **AT1G66340** or **T27F4.9**
From	Arabidopsis thaliana (Mouse-ear cress) [TaxID: 3702]
Taxonomy	Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; eudicotyledons; core eudicots; Rosidae; eurosids II; Brassicales; Brassicaceae; Arabidopsis.

Table 30. Cross-references for ETR1

EMBL	L24119; AAA70047.1; -. AC020665; AAG52169.1; -.
InterPro	IPR003594; ATPbind_ATPase. IPR003018; GAF. IPR004359; HIS_KIN_sig. IPR003661; His_kinA. IPR001789; Response_reg.
Pfam	PF00072; response_reg; 1. PF00512; signal; 1. PF01590; GAF; 1. PF02518; HATPase_c; 1.
ProDom	PD000039; Response_reg; 1. [Domain structure / List of seq. sharing at least 1 domain].
SMART	SM00065; GAF; 1. SM00387; HATPase_c; 1. SM00388; HisKA; 1. SM00448; REC; 1.
PROSITE	PS50109; HIS_KIN; 1. PS50110; RESPONSE_REGULATORY; 1.

Implicit links to	BLOCKS; ProtoNet; ProtoMap; PRESAGE; DIP; ModBase; SWISS-2DPAGE.

Table 31. Features of ETR1 protein

Key	From	To	Length	Description
DOMAIN	350	585	236	HISTIDINE KINASE.
DOMAIN	611	729	119	RESPONSE REGULATORY.
TRANSMEM	23	43	21	*POTENTIAL.*
TRANSMEM	53	73	21	*POTENTIAL.*
TRANSMEM	92	112	21	*POTENTIAL.*
METAL	65	65		COPPER.
METAL	69	69		COPPER.
DISULFID	4	4		INTERCHAIN.
DISULFID	6	6		INTERCHAIN.
MOD_RES	353	353		PHOSPHORYLATION (AUTO-) (*BY SIMILARITY*).
MOD_RES	659	659		PHOSPHORYLATION (*BY SIMILARITY*).
MUTAGEN	4	4		C->S: PREVENTS DIMERIZATION BUT NOT ETHLENE BINDING.
MUTAGEN	6	6		C->S: PREVENTS DIMERIZATION BUT NOT ETHLENE BINDING.
MUTAGEN	31	31		A->V: IN ETR1-3; ETHYLENE INSENSITIVITY.
MUTAGEN	38	38		E->A: NO EFFECT ON ETHYLENE BINDING.
MUTAGEN	62	62		I->F: IN ETR1-4; ETHYLENE INSENSITIVITY.
MUTAGEN	65	65		C->Y,S: IN ETR1-1; NO COPPER BINDING AND ETHYLENE INSENSITIVITY.
MUTAGEN	69	69		H->A: NO COPPER BINDING AND ETHYLENE INSENSITIVITY.
MUTAGEN	79	79		H->A: NO EFFECT.
MUTAGEN	99	99		C->S: NO EFFECT ON DIMERIZATION OR ETHYLENE BINDING.
MUTAGEN	102	102		A->T: IN ETR1-2; ETHYLENE INSENSITIVITY.
MUTAGEN	104	104		M->A: NO EFFECT ON ETHYLENE BINDING.
MUTAGEN	107	107		H->A: NO EFFECT ON ETHYLENE BINDING.

Table 32. ETR1 Sequence information

Length: **738 AA**
Molecular weight: **82565 Da**
CRC64: **D6ED3C4BBA87E96E** [This is a checksum on the sequence]

```
10          20          30          40          50          60
|           |           |           |           |           |
MEVCNCIEPQ WPADELLMKY QYISDFFIAI AYFSIPLELI YFVKKSAVFP YRWVLVQFGA

70          80          90         100         110         120
|           |           |           |           |           |
FIVLCGATHL INLWTFTTHS RTVALVMTTA KVLTAVVSCA TALMLVHIIP DLLSVKTREL

130         140         150         160         170         180
|           |           |           |           |           |
FLKNKAAELD REMGLIRTQE ETGRHVRMLT HEIRSTLDRH TILKTTLVEL GRTLALEECA

190         200         210         220         230         240
|           |           |           |           |           |
LWMPTRTGLE LQLSYTLRHQ HPVEYTVPIQ LPVINQVFGT SRAVKISPNS PVARLRPVSG

250         260         270         280         290         300
|           |           |           |           |           |
KYMLGEVVAV RVPLLHLSNF QINDWPELST KRYALMVLML PSDSARQWHV HELELVEVVA

310         320         330         340         350         360
|           |           |           |           |           |
DQVAVALSHA AILEESMRAR DLLMEQNVAL DLARREAETA IRARNDFLAV MNHEMRTPMH

370         380         390         400         410         420
|           |           |           |           |           |
AIIALSSLLQ ETELTPEQRL MVETILKSSN LLATLMNDVL DLSRLEDGSL QLELGTFNLH

430         440         450         460         470         480
|           |           |           |           |           |
TLFREVLNLI KPIAVVKKLP ITLNLAPDLP EFVVGDEKRL MQIILNIVGN AVKFSKQGSI

490         500         510         520         530         540
|           |           |           |           |           |
SVTALVTKSD TRAADFFVVP TGSHFYLRVK VKDSGAGINP QDIPKIFTKF AQTQSLATRS

550         560         570         580         590         600
|           |           |           |           |           |
SGGSGLGLAI SKRFVNLMEG NIWIESDGLG KGCTAIFDVK LGISERSNES KQSGIPKVPA

610         620         630         640         650         660
|           |           |           |           |           |
IPRHSNFTGL KVLVMDENGV SRMVTKGLLV HLGCEVTTVS SNEECLRVVS HEHKVVFMDV

670         680         690         700         710         720
|           |           |           |           |           |
CMPGVENYQI ALRIHEKFTK QRHQRPLLVA LSGNTDKSTK EKCMSFGLDG VLLKPVSLDN

730
|
IRDVLSDLLE PRVLYEGM
```

4.3.3.4. PHYLOGENETIC TREE

A phylogenetic tree was established using the Tree Top software. Amino acid sequences of ETR1, ERS, ETR2, EIN4, ERS2 of *Arabidopsis*; ETR2 of *Brassica oleraceae*; ERS of *Citrus sinensis*; ERS2 of *Dianthus caryophyllus*; ERS, ETR1 and ETR2 of *Cucumis sativus*; ETR of *Cucumis melo*; ETR1 and ETR2 of *Lycopersicon esculentum*; ETR1 and ETR2 of *Pelargonium hortorum*; ERS of *Prunus persica*; ETR2 of *Rosa hybrida* were loaded in fasta format. The parameters maintained are presented in Table 33.

Table 33. Parameters maintained forTree Top search.

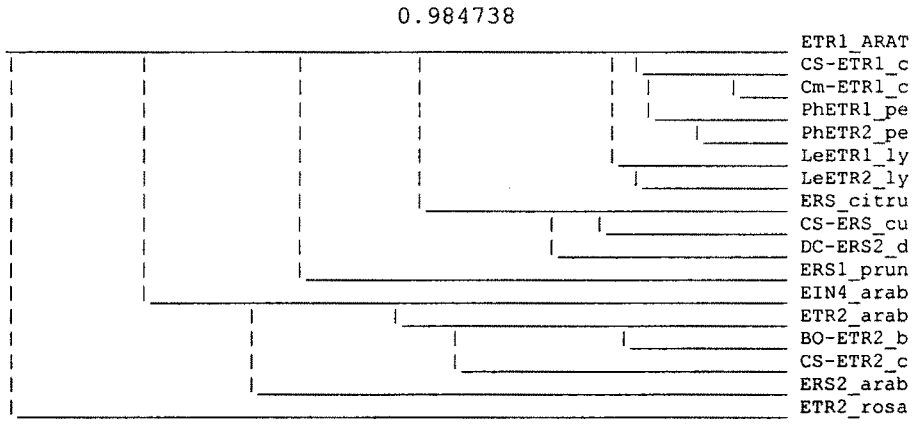
User e-mail address	arvikum@yahoo.com
Use homological fragments only	Yes
Scale	Random
Algorithm	Both Cluster and Topological
Start position	1
End position	10000
Gap penalty (in SD units)	1
Throw off columns with N	
Output Tree Type	PHYLIP
Picture width	640
Picture height	480
Max/min factor (cluster)	8
Max/min factor (topological)	8
Bootstrap Enable	No

The cluster diagram produced is shown in Fig.23. The tree diagram showed the link of all ETR1 sequences in *Arabidopsis*. *Pelargonium hortorum* was closely linked to A. thaliana ETR1. ETR2 of *Rosa hybrida* was farthest from ETR1 of *A. thaliana* (distance value of 0.984738)

Fig.23. Phylogenetic tree obtained with cluster algorithm

Phylogenetic Tree

Cluster Algorithm



Distance values of sequences similar to ETR1 of *A. thaliana*

```
(((((ETR1_ARAT:0.172371,((CS-ETR1_c:0.051424,Cm-
ETR1_c:0.051424):0.116380,(PhETR1_pe:0.103605,PhETR2_pe:0.103605):0.0
64198):0.004567):0.042771,(LeETR1_ly:0.175752,LeETR2_ly:0.175752):0.0
39389):0.244948,((ERS_citru:0.229834,CS-ERS_cu:0.229834):0.048238,DC-
ERS2_d:0.278072):0.182017):0.150304,ERS1_prun:0.610393):0.195289,((EI
N4_arab:0.479275,((ETR2_arab:0.193964,BO-
ETR2_b:0.193964):0.207177,CS-
ETR2_c:0.401141):0.078134):0.195009,ERS2_arab:0.674284):0.131398):0.1
79056,ETR2_rosa:0.984738);
```

4.3.3.5. BIOEDIT

BIOEDIT RESULTS

This tool was used to determine nucleotide compositions of ETR1 are displayed in Table 34 and Fig.24. The molecular weight of the single strand of ETR1 protein which had a length of 738 base pairs was found to be 224708 dalton while that of the double strand was 448684 dalton. The percent contribution of Guanine and Cytosine was 6.23, while Adenine and Thymine contributed 13.14% to the protein.

Table 34. Nucleotide composition of etr1 gene.

Nucleotide	Number	Molecular weight (%)
A	49	6.64
C	10	1.36
G	36	4.88
T	48	6.50
R	42	5.69
Y	12	1.63
W	7	0.95
S	50	5.78
K	37	5.01
M	25	3.39
D	29	3.93
H	22	2.98
V	71	9.62
N	300	40.65

The amino acid composition of ETR1 is presented in Table 35 and Fig.25. The molecular weight of the protein was found to be 82561.13 dalton. The amino acid Leucine contributed 13.55% of the molecular weight and was present in 100 numbers as evident in the Fig.25.

Table 35. Amino acid composition of ETR1 protein.

Amino Acid	Number	Molecular weight(%)
Ala A	49	6.64
Cys C	10	1.36
Asp D	29	3.93
Glu E	49	6.64
Phe F	27	3.66
Gly G	36	4.88
His H	22	2.98
Ile I	42	5.69
Lys K	37	5.01
Leu L	100	13.55
Met M	25	3.39
Asn N	27	3.66
Pro P	31	4.20
Gln Q	24	3.25
Arg R	42	5.69
Ser S	50	6.78
Thr T	48	6.50
Val V	71	9.62
Trp W	7	0.95
Tyr Y	12	1.63

Fig.24. Nucleotide Composition of ETR1 Protein

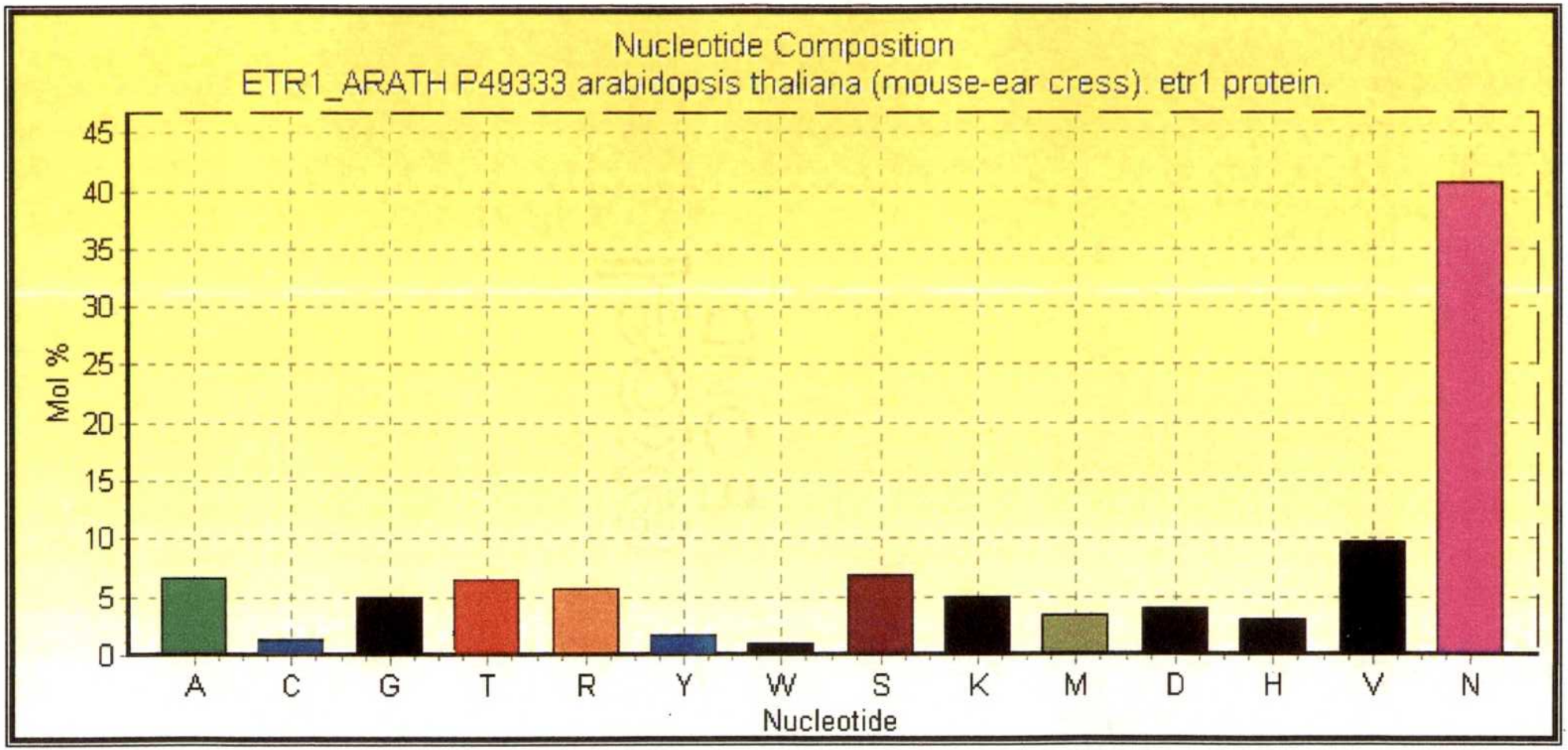
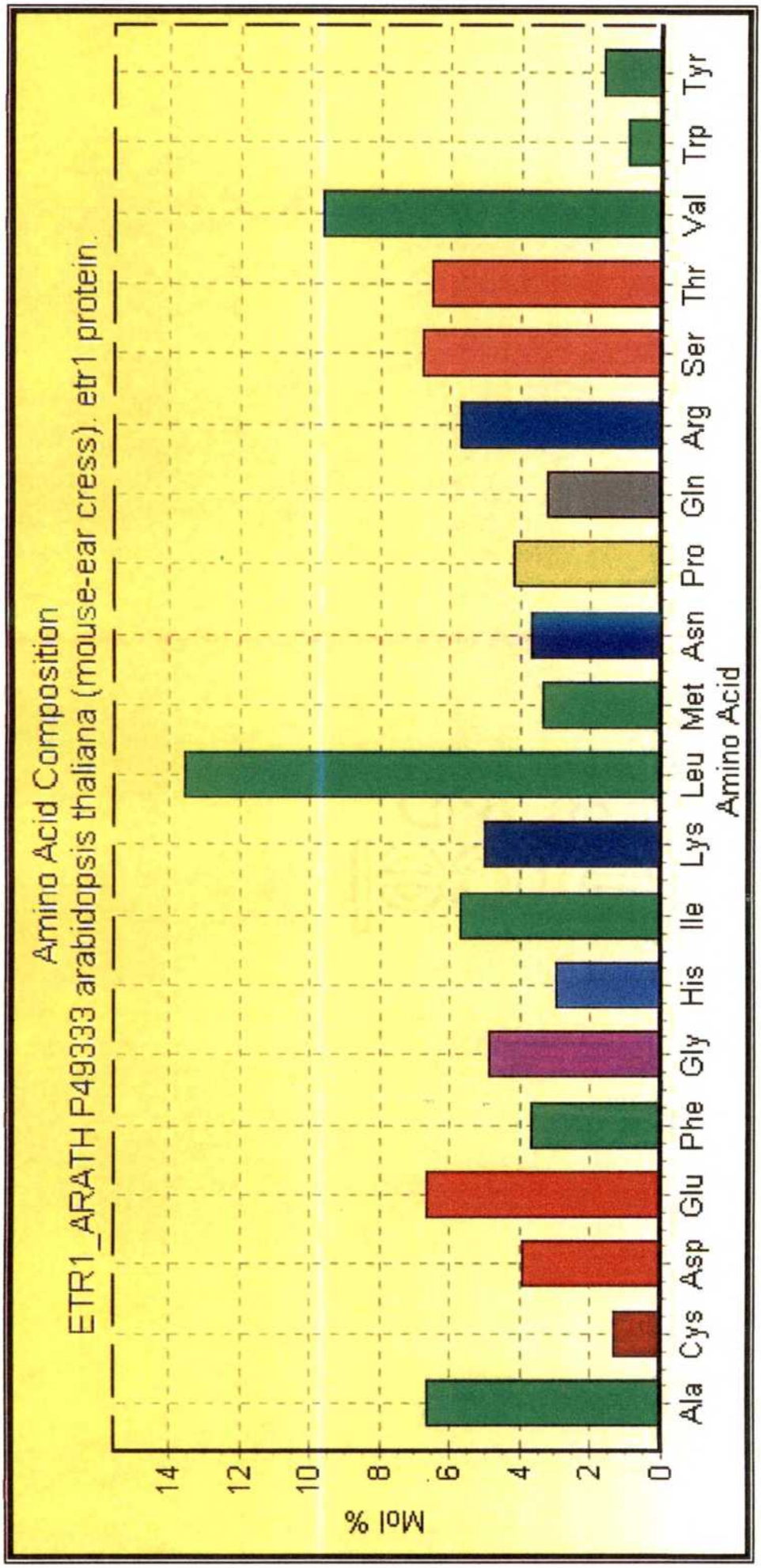


Fig.25. Amino acid Composition of ETR1 Protein



4.3.3.6. CATH – PROTEIN CLASSIFICATION DATABASE

This tool was used to determine the ETR1 protein classification pertaining to class, architecture, topology and superfamilies. The other members belonging to this family in each category were displayed in Tables 36 to 39.

The class representative was found to be 1rthA1 and the results of Class 3 are presented in Table 36.

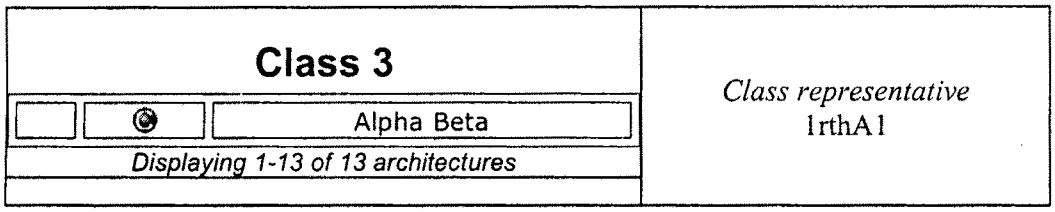


Table 36. Representatives of ETR1 Class 3 family

Code	Representative	Architecture description
3.10	1rthA1	*Roll*
3.15	1bp101	*Super Roll*
3.20	3daaA2	*Barrel*
3.30	1aa8A2	*2-Layer Sandwich*
3.40	1div01	*3-Layer(aba) Sandwich*
3.50	2hgf00	*3-Layer(bba) Sandwich*
3.60	1aorA1	*4-Layer Sandwich*
3.65	1ejdA1	*Alpha-beta prism*
3.66	1ospO3	*COMPLEX (IMMUNOGLOBULIN/LIPOPROTEIN)*
3.70	1plq00	*Box*
3.75	4jdwA0	*5-stranded Propeller*
3.80	2bnh00	*Horseshoe*
3.90	1tsg00	*Complex*

The architecture representative was found to be 1div01 and the results of the architecture family are presented in Table 37.

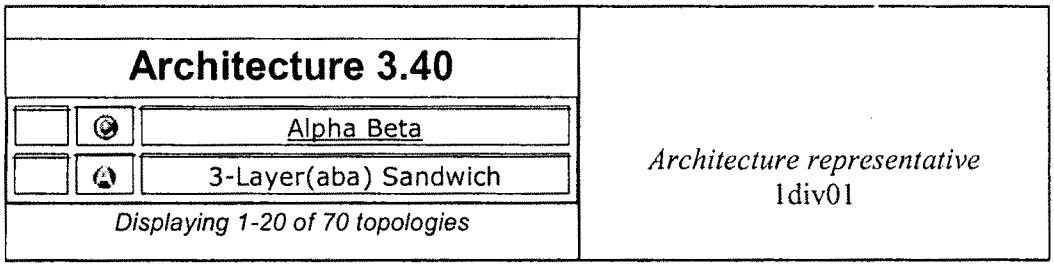


Table 37. Representatives of the ETR1 Architecture family

Code	Representative	Topology description
3.40.5	1div01	*Ribosomal Protein L9, domain 1*
3.40.10	1adn00	*DNA Methylphosphotriester Repair Domain*
3.40.20	1svq00	*Severin*
3.40.30	1aba00	*Glutaredoxin*
3.40.33	1cfe00	*Pathogenesis-related Protein P14a;*
3.40.35	1ble00	*Fructose Permease;*
3.40.47	1pxtA1	*Peroxisomal Thiolase, subunit A, domain 1*
3.40.50	2minB2	*Rossmann fold*
3.40.80	1lba00	*Lysozyme*
3.40.91	1cfr00	*RESTRICTION ENDONUCLEASE*
3.40.109	1nox00	*NADH Oxidase*
3.40.120	3pmgA3	*Alpha-D-Glucose-1,6-Bisphosphate, subunit A, domain 3*
3.40.140	1ctt02	*Cytidine Deaminase, domain 2*
3.40.190	1anf02	*D-Maltodextrin-Binding Protein, domain 2*
3.40.191	1inp03	*Inositol Polyphosphate Phosphatase, domain 3*
3.40.192	1lehA1	*Leucine Dehydrogenase, chain A, domain 1*
3.40.198	1cby00	*Delta-endotoxin Cytb*
3.40.210	1pvuA0	*PVUII Endonuclease, subunit A*
3.40.220	1lam01	*Leucine Aminopeptidase, subunit E, domain 1*
3.40.225	1fua00	*L-fuculose-1-phosphate Aldolase*

The topology representative of the family was found to be 2 minB2 and the results of the toplogy family of ETR1 are presented in Table 38 .

Topology 3.40.50 Alpha Beta 3-Layer(aba) Sandwich Rossmann fold	*Topology representative* 2minB2

Table 38. Representatives of ETR1 topology family

Code	Representative	Homologous Superfamily description
3.40.50.10	2minB2	*NITROGEN FIXATION*
3.40.50.20	1iow01	*LIGASE*
3.40.50.40	3ecaA2	*HYDROLASE*
3.40.50.50	1pkyA1	*PHOSPHOTRANSFERASE*
3.40.50.70	1poxA2	*TPP-binding domain*
3.40.50.80	2pia02	*Nucleotide-binding domain of ferredoxin-NADP reductase (FNR) module*
3.40.50.90	1opr00	*TRANSFERASE*
3.40.50.110	1c3jA2	*TRANSFERASE*
3.40.50.140	1ecl01	*TOPOISOMERASE*
3.40.50.150	1admA1	*TRANSFERASE (METHYLTRANSFERASE)*
3.40.50.170	1garA0	*TRANSFERASE (FORMYL)*
3.40.50.180	1chd00	*CARBOXYL METHYLESTERASE*
3.40.50.200	1thm00	*HYDROLASE(SERINE PROTEASE)*
3.40.50.220	1ordA1	*CARBOXY-LYASE*
3.40.50.261	1scuA2	*LIGASE (ATP-BINDING)*
3.40.50.270	1phr00	*PHOSPHOTYROSINE PROTEIN PHOSPHATASE*
3.40.50.280	1bmtA2	*METHYLTRANSFERASE*
3.40.50.300	1efuA1	*P-loop containing nucleotide triphosphate hydrolases*
3.40.50.360	5nul00	*ELECTRON TRANSPORT*
3.40.50.410	1lfaA0	*CELL ADHESION*

The homologous superfamily representative was found to be 3chy00 and the results of the homologous superfamily .3000 of ETR1 are presented in Table 39 .

Homologous Superfamily 3.40.50.3000	
Alpha Beta	*Homologous superfamily representative* 3chy00
3-Layer(aba) Sandwich	
Rossmann fold	
SIGNAL TRANSDUCTION PROTEIN	

Table 39. Representatives of the ETR1 homologous superfamily

Code	Representative	Sequence family description
3.40.50.3000.1	3chy00	*SIGNAL TRANSDUCTION PROTEIN*
3.40.50.3000.2	1dc7A0	*SIGNALING PROTEIN*
3.40.50.3000.3	1a04A1	*SIGNAL TRANSDUCTION PROTEIN*
3.40.50.3000.4	1tmy00	*CHEMOTAXIS*
3.40.50.3000.5	1b00A0	*GENE REGULATION*
3.40.50.3000.6	1qmpA0	*RESPONSE REGULATOR*
3.40.50.3000.7	1dcfA0	*TRANSFERASE*
3.40.50.3000.8	1a2oA1	*BACTERIAL CHEMOTAXIS*

4.3.3.7. Pfam – Protein Families Database

This tool was used to determine the structure of ETR1 protein. A picture illustrating the domains of the protein was obtained and presented in Fig. 26 and Table 40.

Fig.26. Structure of ETR1 with domains as predicted by Pfam

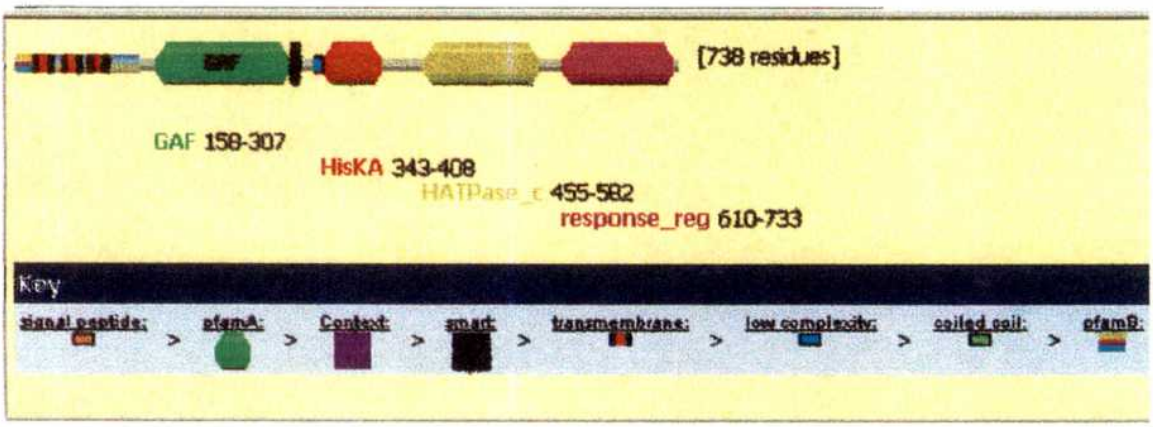


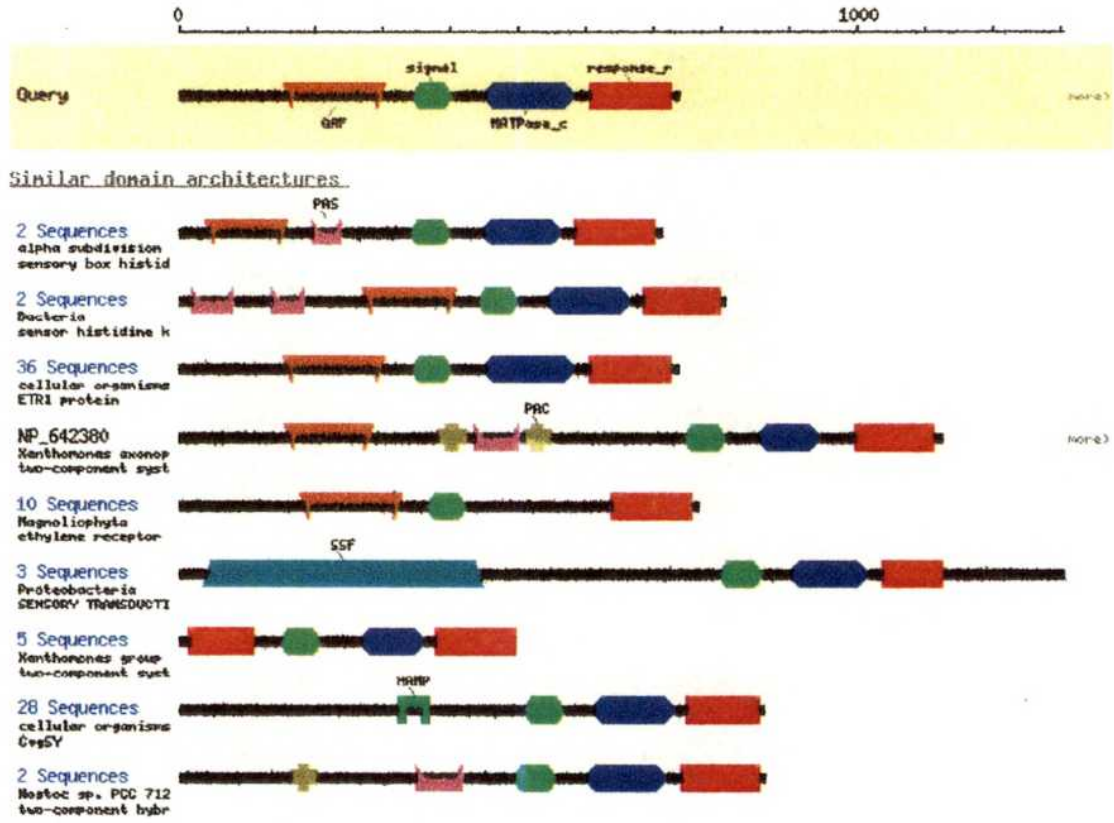
Table 40. Details of the ETR1 protein domains

Pfam Domains			Other Regions				
Domain	Start	End	Type	Source	Start	End	Score
Pfam-B_6677	4	23	transmembrane	tmhmm	21	43	
Pfam-B_472	24	140	transmembrane	tmhmm	53	75	
GAF	158	307	transmembrane	tmhmm	82	104	
HisKA	343	408	low complexity	seg	289	299	1.9400
HATPase_c	455	582	low complexity	seg	333	344	2.0800
response_reg	610	733	low complexity	seg	540	548	1.5300
Smart Domains							
Domain	Start	End					
GAF	158	317					
HisKA	343	408					
HATPase_c	455	583					
REC	610	725					

4.3.3.8. CDART – Conserved Domain Architecture Retreival Tool

The conserved domains of the ETR1 protein sequence and other sequences that showed homology to the ETR1 sequence are depicted in Fig.27.

Fig.27. Sequences showing conserved domain similarity to that of ETR1



	pfam00474	Sodium:solute symporter family.
	pfam01590	GAF domain. Domain present in phytochromes and cG...
	includes:	smart00065
	smart00304	HAMP (Histidine kinases, Adenylyl cyclases, Methy...
	includes:	pfam00672
	smart00387	Histidine kinase-like ATPases; Histidine kinase-,...
	includes:	pfam02518
	pfam00072	Response regulator receiver domain. This domain r...
	includes:	smart00448
	pfam00512	His Kinase A (phosphoacceptor) domain. Dimerisati...
	includes:	smart00388
	pfam00785	PAC motif. PAC motif occurs C-terminal to a subse...
	includes:	smart00086
	smart00091	PAS domain; PAS motifs appear in archaea, eubacte...
	includes:	pfam00989

4.3.3.9. COLOR PROTEIN SEQUENCE

This tool was used to differentiate the hydrophobic and hydrophilic regions of the ETR1 protein. The results are presented in Fig.28

Fig. 28. Hydrophobic and Hydrophilic regions of ETR1 protein.

Hydrophobic region

Color coding result : UNK_182030

```
        10        20        30        40        50        60        70
        |         |         |         |         |         |         |
MEVCNCIEPQWPADELLMKYQYISDFFIAIAYFSIPLELIYFVKKSAVFPYRWVLVQFGAFIVLCGATHL
INLWTFTTHSRTVALVMTTAKVLTAVVSCATALMLVHIIPDLLSVKTRELFLKNKAAELDREMGLIRTQE
ETGRHVRMLTHEIRSTLDRHTILKTTLVELGRTLALEECALWMPTRTGLELQLSYTLRHQHPVEYTVPIQ
LPVINQVFGTSRAVKISPNSPVARLRPVSGKYMLGEVVAVRVPLLHLSNFQINDWPELSTKRYALMVLML
PSDSARQWHVHELELVEVVADQVAVALSHAAILEESMRARDLLMEQNVALDLARREAETAIRARNDFLAV
MNHEMRTPMHAIIALSSLLQETELTPEQRLMVETILKSSNLLATLMNDVLDLSRLEDGSLQLELGTFNLH
TLFREVLNLIKPIAVVKKLPITLNLAPDLPEFVVGDEKRLMQIILNIVGNAVKFSKQGSISVTALVTKSD
TRAADFFVVPTGSHFYLRVKVKDSGAGINPQDIPKIFTKFAQTQSLATRSSGGSGLGLAISKRFVNLMEG
NIWIESDGLGKGCTAIFDVKLGISERSNESKQSGIPKVPAIPRHSNFTGLKVLVMDENGVSRMVTKGLLV
HLGCEVTTVSSNEECLRVVSHEHKVVFMDVCMPGVENYQIALRIHEKFTKQRHQRPLLVALSGNTDKSTK
EKCMSFGLDGVLLKPVSLDNIRDVLSDLLEPRVLYEGM
```

Total number of **ALIVMW** in sequence: 294

Hydrophilic region

Color coding result : UNK_183490

```
        10        20        30        40        50        60        70
        |         |         |         |         |         |         |
MEVCNCIEPQWPADELLMKYQYISDFFIAIAYFSIPLELIYFVKKSAVFPYRWVLVQFGAFIVLCGATHL
INLWTFTTHSRTVALVMTTAKVLTAVVSCATALMLVHIIPDLLSVKTRELFLKNKAAELDREMGLIRTQE
ETGRHVRMLTHEIRSTLDRHTILKTTLVELGRTLALEECALWMPTRTGLELQLSYTLRHQHPVEYTVPIQ
LPVINQVFGTSRAVKISPNSPVARLRPVSGKYMLGEVVAVRVPLLHLSNFQINDWPELSTKRYALMVLML
PSDSARQWHVHELELVEVVADQVAVALSHAAILEESMRARDLLMEQNVALDLARREAETAIRARNDFLAV
MNHEMRTPMHAIIALSSLLQETELTPEQRLMVETILKSSNLLATLMNDVLDLSRLEDGSLQLELGTFNLH
TLFREVLNLIKPIAVVKKLPITLNLAPDLPEFVVGDEKRLMQIILNIVGNAVKFSKQGSISVTALVTKSD
TRAADFFVVPTGSHFYLRVKVKDSGAGINPQDIPKIFTKFAQTQSLATRSSGGSGLGLAISKRFVNLMEG
NIWIESDGLGKGCTAIFDVKLGISERSNESKQSGIPKVPAIPRHSNFTGLKVLVMDENGVSRMVTKGLLV
HLGCEVTTVSSNEECLRVVSHEHKVVFMDVCMPGVENYQIALRIHEKFTKQRHQRPLLVALSGNTDKSTK
EKCMSFGLDGVLLKPVSLDNIRDVLSDLLEPRVLYEGM
```

Total number of **DEKNQRST** in sequence: 306

4.3.3.10. PREDATOR

This tool was used to predict the secondary structure of ETR1 sequence. The summary of structure components of ETR1 is presented in Table 41. The sequence

length of ETR1 was 738 amino acids and this search was posted against the DSSP database.

The secondary structure of ETR1 is shown below.

```
10        20        30        40        50        60        70
          |         |         |         |         |         |         |
MEVCNCIEPQWPADELLMKYQYISDFFIAIAYFSIPLELIYFVKKSAVFPYRWVLVQFGAFIVLCGATHL
ccccccccccccchhhhhhhhhhhhhhhheeecccchhhhhhhhhcccccceeeeeeeccceeeeecccee
INLWTFTTHSRTVALVMTTAKVLTAVVSCATALMLVHIIPDLLSVKTRELFLKNKAAELDREMGLIRTQE
eeeeeccccchhhhhhhhhhhhhhhchhhhhhhhhhhcccceeehhhhhhhhhhhhhhhhhhhhhchhhh
ETGRHVRMLTHEIRSTLDRHTILKTTLVELGRTLALEECALWMPTRTGLELQLSYTLRHQHPVEYTVPIQ
hhcccchhhhhhhhhhhhhhhhheeeeecccccchhhhhhhhhcccccccchhhhhhhhhhcccceeeeecccc
LPVINQVFGTSRAVKISPNSPVARLRPVSGKYMLGEVVAVRVPLLHLSNFQINDWPELSTKRYALMVLML
cccceeeecccceeeecccccceeccccccceeeeeeeeeeecccccccccccccccchhhhhhhhccc
PSDSARQWHVHELELVEVVADQVAVALSHAAILEESMRARDLLMEQNVALDLARREAETAIRARNDFLAV
ccchhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhccchhhh
MNHEMRTPMHAIIALSSLLQETELTPEQRLMVETILKSSNLLATLMNDVLDLSRLEDGSLQLELGTFNLH
hhhccccchhhhhhhhhhhhhhhchhhhhhhhhhhhhhccchhhhhhhhhhhheeecccccccccccccchh
TLFREVLNLIKPIAVVKKLPITLNLAPDLPEFVVGDEKRLMQIILNIVGNAVKFSKQGSISVTALVTKSD
hhhhhhhhhcccceeeeeeccccccccccccccchhhhhhhhhhhhhhccceeecccccceeeeeeecccc
TRAADFFVVPTGSHFYLRVKVKDSGAGINPQDIPKIFTKFAQTQSLATRSSGGSGLGLAISKRFVNLMEG
ccccceeecccceeeeeeeccccccccccchhhhhhcccccccccccccccchhhhhhhhhcccc
NIWIESDGLGKGCTAIFDVKLGISERSNESKQSGIPKVPAIPRHSNFTGLKVLVMDENGVSRMVTKGLLV
ceeeeccccccceeeeeccccccccccccccccccccccccccccccceeeeeeecccccccccccee
HLGCEVTTVSSNEECLRVVSHEHKVVFMDVCMPGVENYQIALRIHEKFTKQRHQRPLLVALSGNTDKSTK
eeceeeeecccceeeeeecceeeeeecccccchhhhhhhhhhhhhhhhhcceeeeeecccccchhh
EKCMSFGLDGVLLKPVSLDNIRDVLSDLLEPRVLYEGM
Hhhhccccccccccccccchhhhhhhhhccceeeeccc
```

The secondary structure showed the highest percent of 41.33 for random coil followed by Alpha helix with a percent contribution of 40.11. The rest (18.56%) is contributed by extended strand.

Table 41. Summary of structure composition of ETR1

Parameter	Percent contribution
Alpha helix (Hh)	296 is 40.11%
3_{10} helix (Gg)	0 is 0.00%
Pi helix (Ii)	0 is 0.00%
Beta bridge (Bb)	0 is 0.00%
Extended strand(Ee)	137 is 18.56%
Beta turn (Tt)	0 is 0.00%
Bend region (Ss)	0 is 0.00%
Random coil (Cc)	305 is 41.33%
Ambigous states (?)	0 is 0.00%
Other states	0 is 0.00%

4.3.3.11. SOPMA – Self-Optimized Prediction Method with Alignment

This tool was also used to predict the secondary structure of ETR1 protein. The following parameters were maintained during the search.

Table 42. Parameters maintained during SOPMA search of ETR1

Parameter	*Value*
Window width	17
Similarity threshold	8
Number of states	3

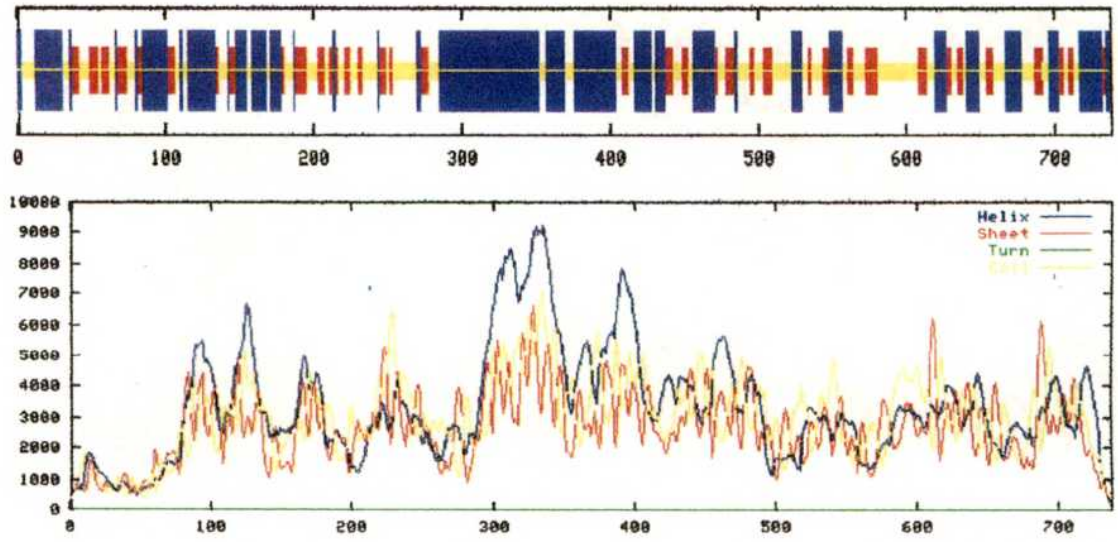
The secondary structure of ETR1 as predicted by SOPMA is shown below.

```
10         20         30         40         50         60         70
|          |          |          |          |          |          |
MEVCNCIEPQWPADELLMKYQYISDFFIAIAYFSIPLELIYFVKKSAVFPYRWVLVQFGAFIVLCGATHL
hhhh      hhhhhhhhhhhhhhhhhh    hheeeee     eeeeee  eeeee    he
INLWTFTTHSRTVALVMTTAKVLTAVVSCATALMLVHIIPDLLSVKTRELFLKNKAAELDREMGLIRTQE
eeeeee    hheeehhhhhhhhhhhhhhhhheeeee   hh   hhhhhhhhhhhhhhhhhhhee
ETGRHVRMLTHEIRSTLDRHTILKTTLVELGRTLALEECALWMPTRTGLELQLSYTLRHQHPVEYTVPIQ
    heeeehhhhhhhh   hhhhhhhhhh  hhhhhhhhee    heeeeeeee      eeeee
LPVINQVFGTSRAVKISPNSPVARLRPVSGKYMLGEVVAVRVPLLHLSNFQINDWPELSTKRYALMVLML
  eehhee   eeee   eeee      heeeee  ee          hhheeeee
PSDSARQWHVHELELVEVVADQVAVALSHAAILEESMRARDLLMEQNVALDLARREAETAIRARNDFLAV
    hhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhh
MNHEMRTPMHAIIALSSLLQETELTPEQRLMVETILKSSNLLATLMNDVLDLSRLEDGSLQLELGTFNLH
hhhh    hhhhhhhhhhhhh    hhhhhhhhhhhhhhhhhhhhhhhhhhhh    eeee    hh
TLFREVLNLIKPIAVVKKLPITLNLAPDLPEFVVGDEKRLMQIILNIVGNAVKFSKQGSISVTALVTKSD
hhhhhhhhhh  hhhhhhheeeee    eeee  hhhhhhhhhhhhhhhhee    eeeeeehh
TRAADFFVVPTGSHFYLRVKVKDSGAGINPQDIPKIFTKFAQTQSLATRSSGGSGLGLAISKRFVNLMEG
    eee    eeeeeee      hhhhhhh  ee        eeeehhhhhhhhh
NIWIESDGLGKGCTAIFDVKLGISERSNESKQSGIPKVPAIPRHSNFTGLKVLVMDENGVSRMVTKGLLV
 eeee    eeeeeeee                    eeeeee    hhhhhhhhee
HLGCEVTTVSSNEECLRVVSHEHKVVFMDVCMPGVENYQIALRIHEKFTKQRHQRPLLVALSGNTDKSTK
e   eeee  hhhhhhhhh    eeeee    hhhhhhhhhhh    eeeeee    hhh
EKCMSFGLDGVLLKPVSLDNIRDVLSDLLEPRVLYEGM
Hhhheee   eee   hhhhhhhhhhhhhhhheehhh
```

Table 43. Summary of structure composition of ETR1

Parameter	Percent contribution
Alpha helix (Hh)	315 is 42.68%
3_{10} helix (Gg)	0 is 0.00%
Pi helix (Ii)	0 is 0.00%
Beta bridge (Bb)	0 is 0.00%
Extended strand (Ee)	157 is 21.27%
Beta turn (Tt)	0 is 0.00%
Bend region (Ss)	0 is 0.00%
Random coil (Cc)	266 is 36.04%
Ambigous states (?)	0 is 0.00%
Other states	0 is 0.00%

Fig.29. Score curves for each predicted state of ETR1

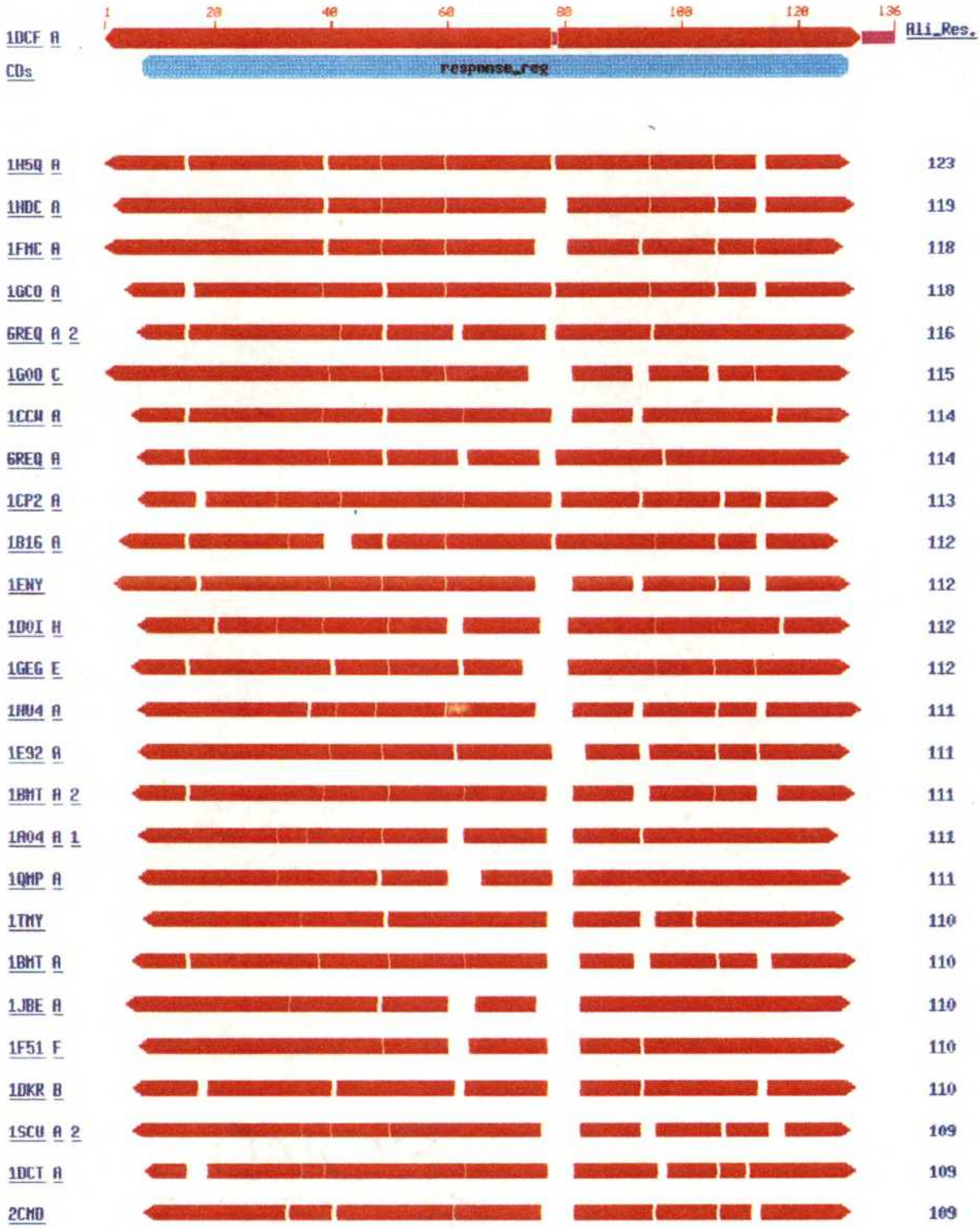


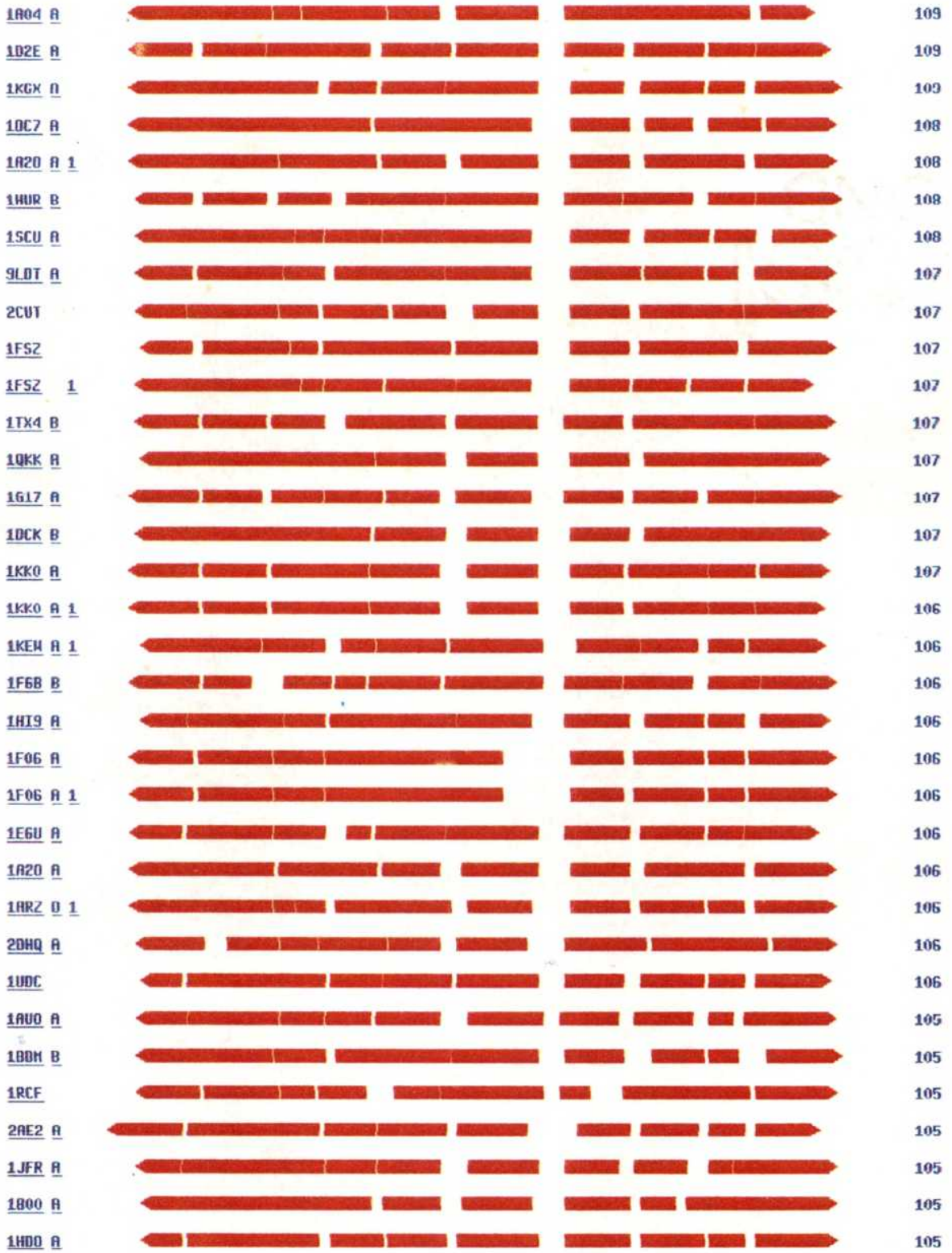
4.3.3.12. VAST – VECTOR ALIGNMENT SEARCH TOOL.

This tool was used to determine the sequences that showed structure similarity to the ETR1 structure. The results are illustrated in Fig.30.

The first part denotes the ETR1 sequence and subsequent red part denotes the structures similar to ETR1.

Fig.30. VAST search result for ETR1




1A04 A 109
1D2E A 109
1KGX A 109
1DC7 A 108
1A2O A 1 108
1HUR B 108
1SCU A 108
9LDT A 107
2CUT 107
1FSZ 107
1FSZ 1 107
1TX4 B 107
1QKK A 107
1G17 A 107
1DCK B 107
1KKO A 107
1KKO A 1 106
1KEW A 1 106
1F6B B 106
1HI9 A 106
1F06 A 106
1F06 A 1 106
1E6U A 106
1A2O A 106
1ARZ A 1 106
2DHQ A 106
1UDC 106
1AUO A 105
1BDM B 105
1RCF 105
2AE2 A 105
1JFR A 105
1B0O A 105
1HDO A 105

4.3.3.13. CLUSTAL X

This tool was used to determine the multiple sequence alignment of amino acid sequences of ETR1, ERS, ETR2, EIN4, ERS2 of *Arabidopsis*; ETR2 of *Brassica oleraceae*; ERS of *Citrus sinensis*; ERS2 of *Dianthus caryophyllus*; ERS, ETR1 and ETR2 of *Cucumis sativus*; ETR of *Cucumis melo*; ETR1 and ETR2 of *Lycopersicon esculentum*; ETR1 and ETR2 of *Pelargonium hortorum*; ERS of *Prunus persica*; ETR2 of *Rosa hybrida*. The results are as shown in Fig.31

The graph at the bottom of the page indicates the extent of conserved domains in the respective sequences. From Fig.31 it can be concluded that the extent of conserved regions in the sequence alignment is minimal.

4.3.4. MACROMOLECULAR MOVEMENTS DATABASE

The secondary structure of CheY protein was determined from this database. Since, ETR1 belonged to the homologous superfamily of CheY, it could be assumed that ETR1 also possesses a similar structure and motion. The secondary structure of CheY is depicted in Fig.32

Fig.31. Multiple sequence alignment as displayed in multiple alignment mode of CLUSTALX

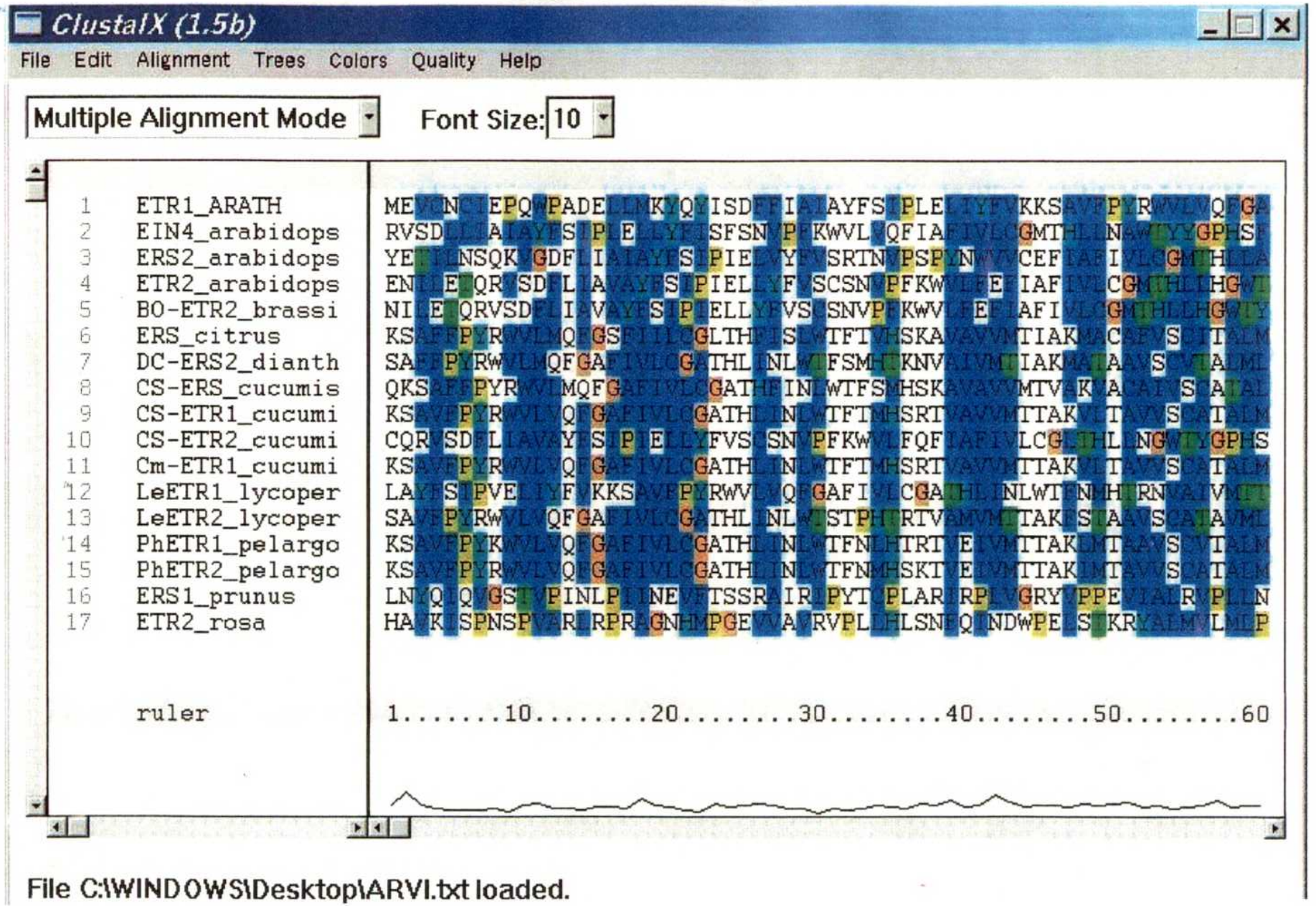
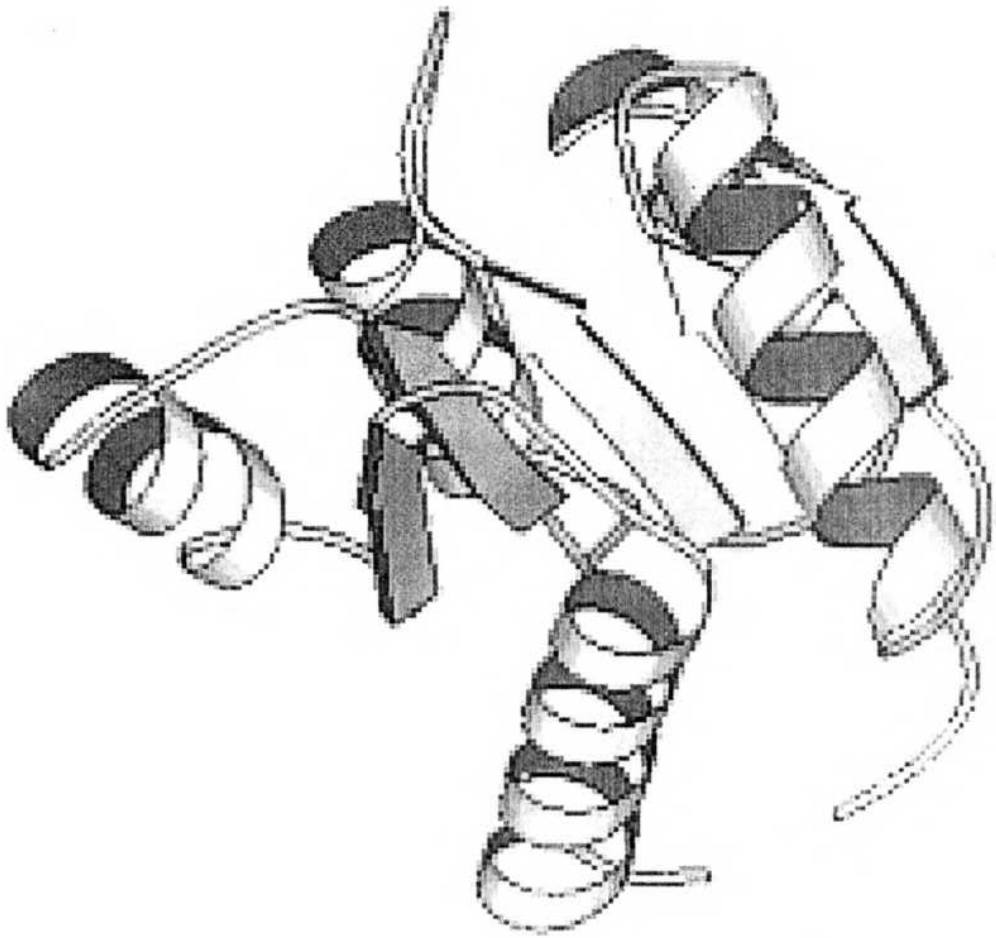


Fig.32. Structure of CheY protein as illustrated in the macromolecular movements database



DISCUSSION

CHAPTER V
DISCUSSION

In the recent decade, in India, there has been a tremendous euphoria about floriculture industry and people have been talking about export or foreign investment in this industry. But, now only a handful of such industries are successful. Majority of such industries faced not the production problem, but mainly postharvest problems. The harvested stems have to be airlifted and reach the destination within 48 hours. This warrants enormous inputs in the form of refrigeration outfits end to end including storage at airport. Though, our airports claim to have world class facilities for floriculture industry, the reality, however, is pathetic.

Though, a number of problems have been cited for these postharvest losses, the main culprit is ethylene. Its major response in plants is senescence of plant parts whereby flowers and leaves wither and fall. Besides, the major postharvest losses in other horticultural crops, especially fruits and vegetables, are mainly due to fruit ripening. And, again, this response is controlled by ethylene (Bleecker *et al.*, 1988). Thus, it is obvious that if we could control ethylene by some means, majority of postharvest losses could be prevented.

Traditionally, the floriculture industry used ethylene control agents, predominantly chemicals like $KMnO_4$. Later, many patented products were developed, like the use of TiO_2 coatings in containers with provisions for UV exposure to control ethylene. All these cost money and it is not economic for the floriculture industry.

In this paradox, a solution could be found in the form of application of biotechnology tools to control ethylene production in flower crops which in turn controls flower senescence. To aid such feasibility, Bleecker and Kende (2000)

reported a dominant mutant gene in wild plants of *Arabidopsis thaliana* which was later named as ETR1 or antisenescence gene for its ability to control ethylene synthesis in plants. Besides, a number of databases are available in the form of DNA and protein sequences of many horticultural crops. To mine this heap of sequence data and to find out if there is anything useful, we used tools. And the recent hype throughout the scientific and business world is about "Bioinformatics" which can provide such tools freely due to the astounding advancements in computing technology and the information revolution spearheaded by the internet.

Presently, a lot of sequence information is available about a lot of horticultural crops, in general, and a few flower crops. Though the biotechnology tools are available and a few applications – like "never – ripe" tomato were found, they could not become a commercial success. The "never – ripe" tomato was recalled from market since 1999. This was not due to the failure of technology, but it was due to social problems – like objections from environmentalists.

The technology may be successful in flower crops because people are used to dry and artificial flowers, and hence one cannot expect any opposition for the use of GM flowers. Further, a number of flower crops have been reported to possess varied responses to ethylene control (Meyerowitz *et al* ., 2001). Hence, there could be a possibility to genetically transformed flower crops for ethylene control.

In this context, an experiment was conducted to study the extent of variability to postharvest ethylene production in *Anthurium andreanum* and *Dendrobiums* to identify the low ethylene evolving genotypes. This could pave the way for identifying the presence of DNA sequences similar to ETR1 or antisenescence gene. Besides, bioinformatics exploration was also done to find out the applications for ETR1 gene

which could lead to low cost way of controlling postharvest losses in flower crops. The results of the study are discussed below.

5.1. Variability to Postharvest Ethylene Production

Ethylene has been recognized as a plant hormone since the turn of the century when its effect on pea seedling development was first described by Neljubov (1901). Since then, numerous reports have appeared which demonstrate that ethylene is an endogenous regulator of growth and development in higher plants. For example, ethylene has been implicated in seed dormancy, seedling growth, flower initiation, leaf abscission, senescence and fruit ripening. Ethylene is a plant hormone whose biosynthesis is induced by environmental stress such as oxygen deficiency, wounding, pathogen invasion and flooding.

Our particular interest in flower crops is that it induces senescence and abscission there by the flowers and leaves wither and fall.

In the present study, postharvest ethylene production was recorded in 16 *Anthuriums* and 3 *Dendrobiums* at weekly intervals. It was found that no evolution could be recorded in all the 19 genotypes during first week. However, all the three *Dendrobiums* recorded high ethylene levels, the highest (1.926 ppm 100 g^{-1}) being in Thai Hybrid. Among the *Anthuriums*, only 4 recorded ethylene, the highest being in Sikkim red (0.420 ppm 100 g^{-1}) which was higher than that of Sonia 17 (*Dendrobium*). During the third week also all the three *Dendrobiums* had the highest ethylene levels. However, in Spic white there was an enormous increase, almost five fold, in ethylene for second (0.822 ppm 100 g^{-1}) to third week (4.139 ppm 100 g^{-1}), while in Thai Hybrid it was only a slight increase in third week (2.269 ppm 100 g^{-1}) from second week (1.926 ppm 100 g^{-1}). This indicated that second week was the critical period for Thai Hybrid while it was the third week Spic white.

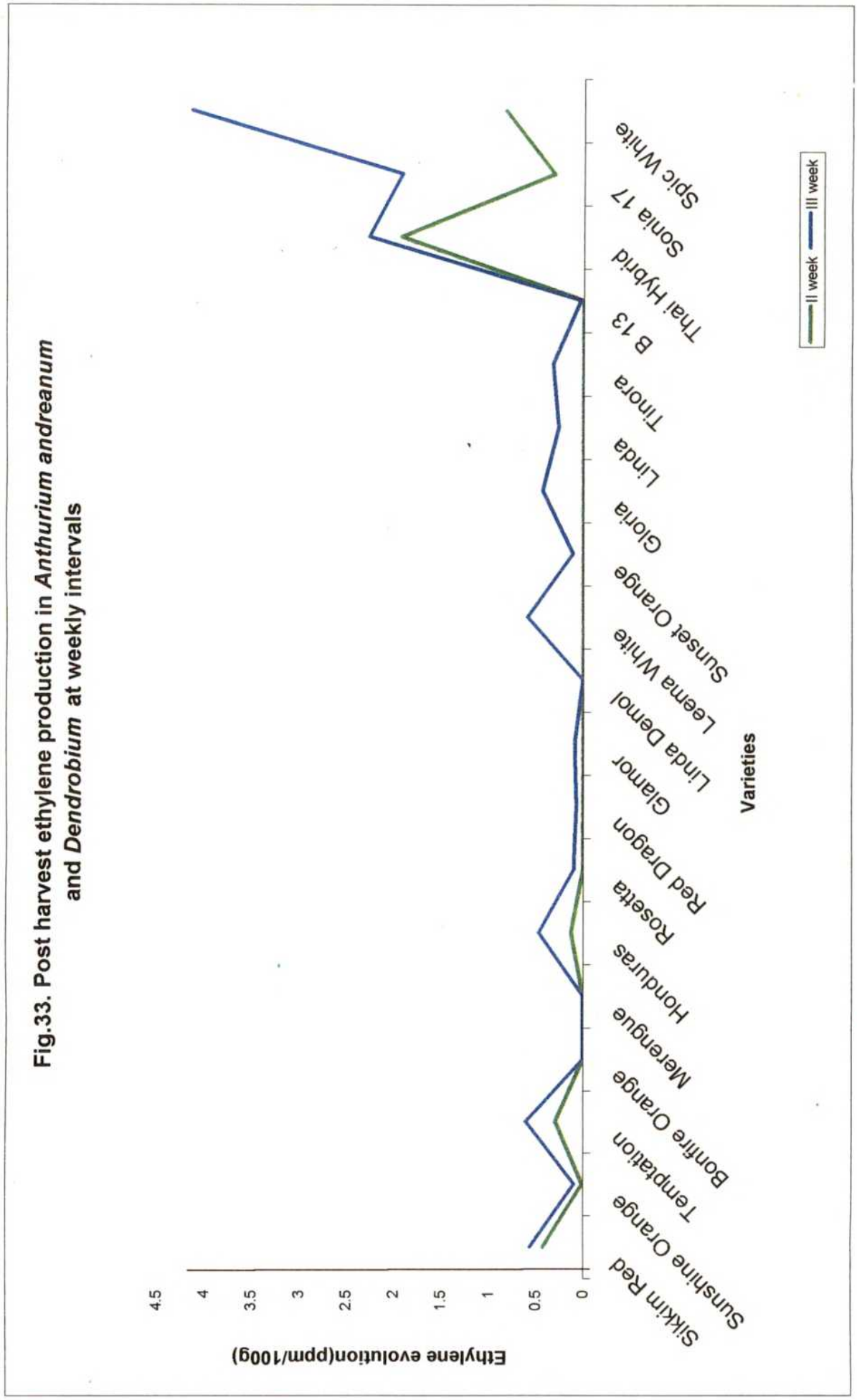


Fig.33. Post harvest ethylene production in *Anthurium andreanum* and *Dendrobium* at weekly intervals

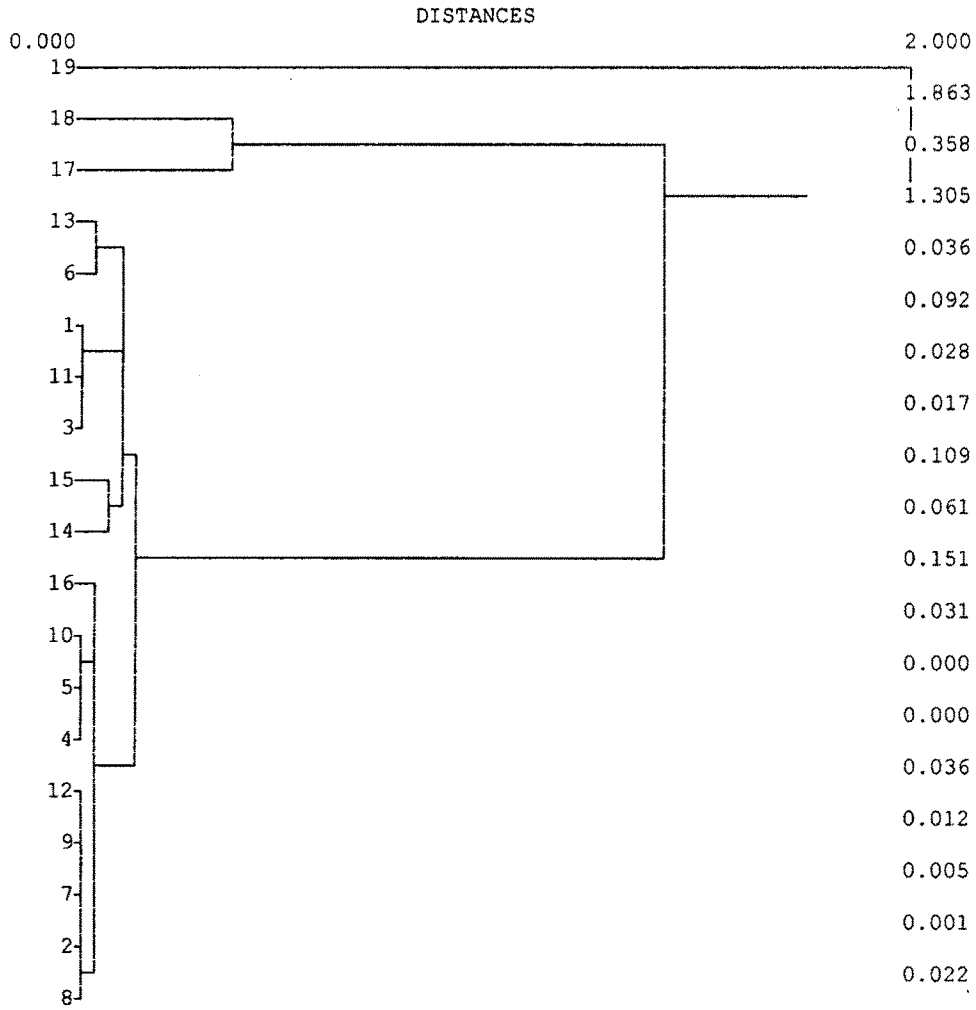
Among the Anthuriums, all except Bornfire orange, Merengue, and Linda Demol recorded ethylene at third week. Among these, Temptation recorded the highest ethylene followed by Leema white and the lowest in B 13 (0.031ppm 100 g^{-1}).

Plant senescence is the genetic programmed process. Ethylene production varied with the development of the plant. Hence, the level of precursor for ethylene production varied with the stage of plant development, which could influence the postharvest production of ethylene. In Cymbidium flowers, removal of anther cap (emasculation) was known to advance senescence (Woltering, 1990). During postharvest life flowers might loose anther cap due to handling. The detection of ethylene showed that production of ethylene shot up soon after emasculation. Huang *et al.* (2001) suggested that flower longevity resulted from a reduced sensitivity to ethylene.

In the present study, a cluster analysis was performed to find out the diversity for postharvest ethylene production. In the K- MEANS algorithm of cluster analysis, five clusters were formed first. Cluster 1 had 9 *Anthuriums* which recorded low or no ethylene at third week. The K – MEANS was run again for seven clusters to find out if the nine low ethylene evolving *Anthuriums* got separated. Surprisingly, all the 9 were found in cluster 1, here also.

To find out whether the low ethylene evolving *Anthuriums* were distinct from no ethylene evolving (at 3rd week) *Anthuriums*, the JOIN algorithm of the cluster analysis was run. The dendrogram produced is shown in Fig.34.

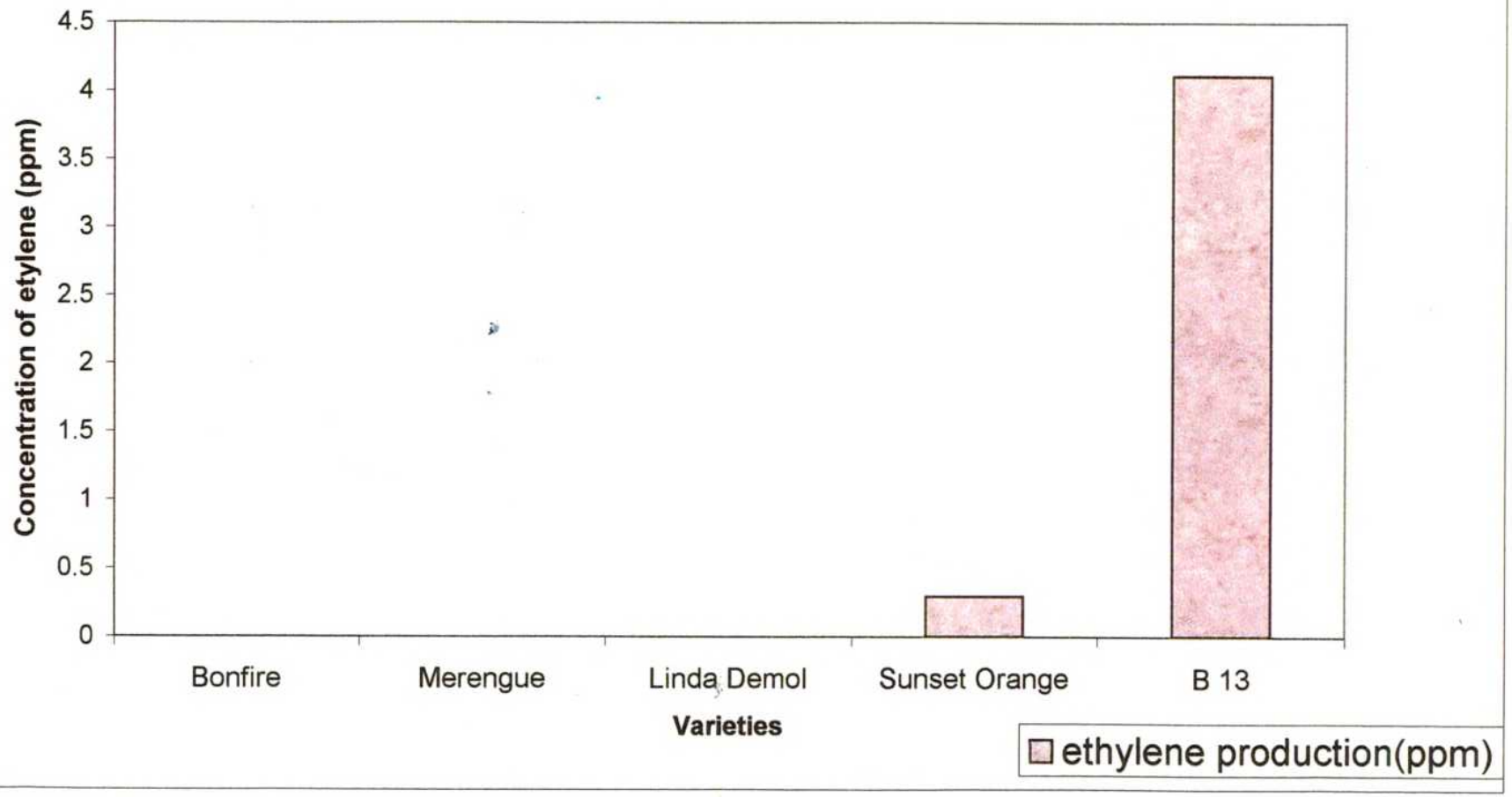
Fig. 34 . Dendrogram for Ethylene Production data



The dendrogram showed that the 'no ethylene' *Anthuriums* viz., Bornfire orange, Merengue and Linda Demol were in a group and got separated from other low ethylene evolving genotypes. The low ethylene B 13 was found separately attached to no – ethylene group, which again was attached to a group with the other 5 low ethylene yielding genotypes. Why B13 was found alone? To find an answer for this, another experiment was conducted with a bunch of 10 flowers in the polyethylene cover, with the 3 'no ethylene' types and B13 and Sunset orange (low ethylene). The 'no ethylene' types viz., Bonfire orange, Merengue and Linda Demol, as expected, produced no ethylene even when 5 times more flowers were used. However, it was a different story for B13 which produced enormous amounts of ethylene (4.107 ppm 100 g^{-1}) at third week, which was almost equivalent to that of Spic white (4.139 ppm 100 g^{-1}). And that could be the reason, why B13 stood alone in the dendrogram.

During these ethylene evolution experiments, an interesting phenomena was noted that the deterioration of *Anthuriums* was more due to pathogenic decay while it was more due to withering and senescence of flowers in the case of *Dendrobiums*. This is exhibited in Plates 2 to 4. In plate 3, 'no- ethylene' Bonfire orange, Merengue and Linda Demol are displayed. It could be seen from the photos that the deterioration was only decay and no wilting. In plate 2, high ethylene evolving *Dendrobiums* viz., Thai Hybrid and Sonia 17 are displayed, which shows wilting rather than decay. In plate 4, six moderate ethylene evolving *Anthuriums* are shown, depicting the pathogenic decay rather that senescence and showed less wilting. In general, it could be construed that *Anthuriums,* evolve less ethylene than *Dendrobiums* and that when there was low or no ethylene, the deterioration of flowers was more due to pathogenic decay rather than senescence. A similar view was suggested by Neljubov (1901) that wounding or pathogen invasion could enhance ethylene production.

Fig.35. Post harvest ethylene evolved in *Anthurium andreanum* at 3rd week



Once the answer was found to the question- if *Anthuriums* have variability for low postharvest ethylene evolution, and the answer as elucidated above was found to be 'yes', the next question is – what to do with this result?

Here comes the possibility of exploring the presence of genes responsible for ethylene control. In this context, antisenescence gene, a dominant mutant designated etr1 was reported in *Arabidopsis thaliana* (Bleecker *et al.*, 1988) which lacked a number of responses to ethylene that are present in wild-type plant, the major response being leaf, flower, fruit stalk senescence and fruit ripening. In the presence of etr1 gene, these responses of ethylene are controlled and hence etr1 or ethylene receptor gene is called 'antisenescence gene'. The DNA sequence of this gene is available in public domain. Besides, a lot of tools to manipulate these sequence data bases are also available in the public domain for free access through internet. Thus, there is a possibility to explore application for this antisenescence gene for utilization in controlling the postharvest ethylene production in horticultural crops, especially flower crops.

5.2. ETR 1

Genetic studies in *Arabidopsis thaliana* have provided evidence that ethylene perception in plants is mediated by a family of receptors, including the ETR1 gene. The ETR1 gene encodes a protein with homology to the two-component His Kinase regulators that control a variety of signaling cascades in prokaryotic systems and some eukaryotic systems (Chang *et al.*, 1993). While ETR1 was the first ethylene receptor to be identified in plants (Bleecker *et al.* 1988), additional screen for ethylene-insensitive seedlings and cloning by sequence similarity indicated that additional genes mediated ethylene sensitivity in various plants and, perhaps, they could be termed as mutants of ETR1 or ETR1-1 (Hall *et al.*, 1999). Hence, the

present bioinformatics study was formulated to find out the possibility of existence of ETR1 mutants or homologues in flower crops and other horticultural crops for comparison.

5.3. BLAST – Basic Local Alignment Search Tool

Variations within a family of related nucleic acid or protein sequences provide an invaluable source of information for evolutionary biology. As DNA sequencing became a common laboratory activity, genes with an important biological function could be sequenced with the hope of learning something about the biochemical nature of the gene product. An example was the *retrovirus – encoded r- sis* and *v – src* oncogenes, genes that cause cancer in animals. By comparing the predicated sequences of the viral products with all of the known protein sequences at the time, Doolittle *et al.* (1983) made the startling discovery that these genes appeared to be derived form cellular genes. Thus arose the field of sequence searching where the biotechnologists discovered that many organisms share similar genes that could be identified by their sequence similarity.

Finding a gene in a new organism (e.g. a flower crop) with a sequence similar to a model organism gene (*Arabidopsis thaliana*) provides a prediction that the new gene has the same function as in the model organism. Such searches are greatly facilitated by the programme BLAST (Altschul *et al.*, 1990). In the present study, such an exploration was deviced to search for the existence of high similarity homologues of ETR1 gene (antisenescence gene).

Searching a sequence database that is similar to a query sequence is the most common type of database similarity search. The search provides a list of database sequences with which the query sequence can be aligned. Once the list is available, additional searches may be performed using one of the initially found sequences as a

query sequence. In this manner, the search may be extended to find more distant relatives of the initial query sequence. BLAST automatically performs these functions very quickly when compared to other programmes like FASTA. Hence, BLAST was chosen as the software for similarity searching of ETR1 sequences in flowers, in the present study.

In the present study, ETR1 (length 3879 bp) was used as the query sequence. This sequence was searched in the *nr* database which comprised 1, 377, 978 sequences. The BLAST programme was configured to list the similarity matches with an E – value (expect value) less than 0.05.

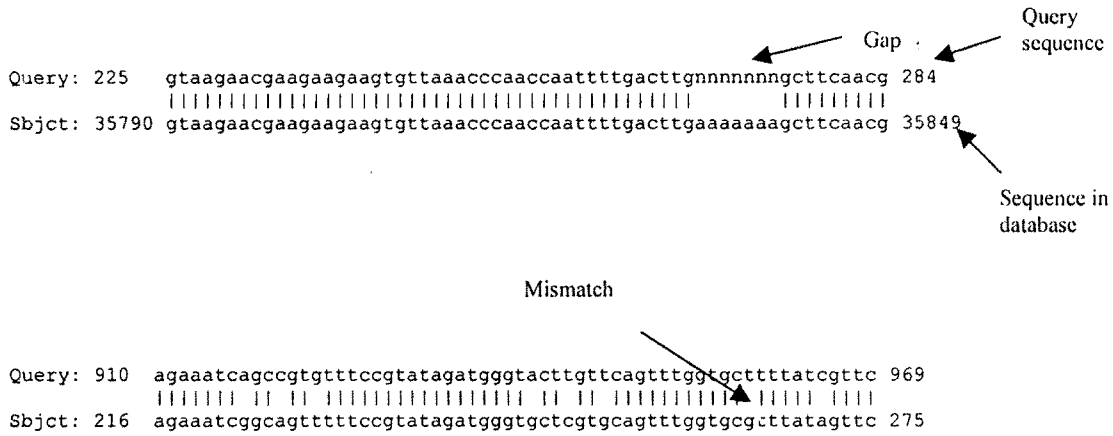
E-value of an alignment score is the probability that an alignment score as good as the one found would be observed between two random or unrelated sequences in a search of a database. The lower the E-value, the more significant the alignment between a pair of matching sequences. A cut-off value in the range of 0.01 – 0.05 is normally used.

In the present study, the search with the E-value setting of 0.05 resulted the eighty sequences with high similarity. Among these eighty, there were 17 flower crop sequences. The results showed that the highest score value of 7162 was recorded for the ETR1, because it was a key search. *Brassica oleraceae* ETR1 – mRNA produced the highest score value of 476 (bits – 240) with and E-value of e-130, which is highly significant. This indicated that this sequence could be a homologue or identical to ETR1 gene as E-values less than 1e-100 could be classified thus.

Locally optimal sequence alignments are called HSPs or High Scoring Segment Pairs (Altschul *et al.*, 1997). This is a larger stretch of a sequence, which has a larger score than the original. The score in the BLAST is calculated as follows.

The method starts by making a list of matching words. The matching between the query sequence and one in database sequence is illustrated in Fig.36.

Fig. 36 Gaps and mismatches depicted in the sequence alignment



As shown in Fig.36, there are types of aligned pairs of characters in each column of an alignment, indicated by vertical line as identical matches and mismatches without a vertical line and a gap indicated by 'n'. Using as an example of 1 for each type of match, the similarity score adds up all of the matches in the aligned sequences, and divides by the sum of the number of matches and number of mismatches (gaps are ignored). This is the most popular method of scoring similarities (Karlin and Altschul 1993).

The score or S-value is computed by adding up all the similarities. Approximately a value of +1 is used for matches, -3 for mismatches, -5 for gaps. (A gap is the maximal contiguous run of spaces in a single sequence within a given alignment. A gap penalty function is a function that measures the cost of a gap as a non-linear function of its length)

In the present study among the 17 flower crop sequences, the score value ranged from 262 to 254 and the E-value ranged from 3e-66 to 0.001 which was highly

significant. This indicated that majority if the sequences were related closely to ETR1 as they possessed moderately to high E-values, where the moderate critical E-value was 1e-40. In general, there was a wide distribution of scores and E-values indicating a large family of genes.

The BLAST search revealed that the final matched sequences, numbering 80, had less repeats of sequences as indicated by the high K-value of 0.771. Normally, the K-value ranges from 0-1 and the low K-value represented more repeated sequences found, while a high K-value indicated a lower number of repeated sequences (Rubin *et al.*, 2000).

The similarity of such results varied, as indicated by identities, from 100 percent in *Arabidopsis thaliana*, because it was a key search, to 79 percent in various crops. Getting results of more than 79% similarity is considered to be very high and hence there existed a possibility of using these found sequences in further studies.

In the present study, among the 17 flower crop sequences found, the BLAST listed only 12 based on the cut-off E-value of e-20 as suggested by Rubin *et al.* (2000).

In an all- against- all comparison in BLAST, significantly matched pairs of sequences may be paralogs that originated from a gene duplication event in this genome or the genome of an ancestor organism. Hence a conservative cut-off E-value of e-20 was suggested (Rubin *et al.*, 2000). The strand value was found to be plus/plus in all cases without exception indicating that all the searches were only in forward direction which in turn suggested high similarity.

Thus, the BLAST search revealed a very high possibility of using the ETR1 homologues found in various flowers such as *Petunia hybrida*, *Rosa hybrida*, *Dianthus caryophyllus* for further application in ethylene control.

5.4. Amino acid search – BEAUTY

Amino acid residues equivalent to those specifically identified in an ETR protein which can be modified to alter the ethylene response can also be readily identified in ETR proteins from other plant species. For example, equivalent amino acid residues to those identified in the ETR protein from *Arabidopsis thaliana* can be readily identified in other ETR proteins. An amino acid residue in a precursor ETR protein is equivalent to a particular residue in the ETR protein of *Arabidopsis thaliana* if it is homologous in position in either primary or tertiary structure to the specified residue of the *Arabidopsis* ETR protein.

In order to establish homology by way of primary structure, the primary amino acid sequence of a precursor ETR protein is directly compared by alignment with the primary sequence of the ETR protein from *Arabidopsis thaliana*. Such alignment is preferably of the amino-terminal domain and will take into account the potential insertion or deletion of one or more amino acid residues as between the two sequences so as to maximize the amino acid sequence homology. A comparison of a multiplicity of ETR protein sequences with that of *Arabidopsis thaliana* provides for the identification of conserved residues among such sequences which conservation is preferably maintained for further comparison of primary amino acid sequence. Based on the alignment of such sequences, the skilled artisan can readily identify amino acid residues in other ETR proteins which are equivalent to Ala – 102 and other residues in *Arabidopsis thaliana* ETR protein. Such equivalent residues are selected for modifications analogous to those of other modified ETR proteins which confer the desired ethylene responsive phenotype. Such modified ETR proteins are preferably made by modifying a precursor ETR nucleic acid to encode the corresponding substitution, insertion and/or deletion at the equivalent amino acid residue.

In the present study, BLAST Enhance Alignment Utility (Beauty) was used to search for the matching amino acid sequences. A total of 738 amino acids were searched. The relative locations of each HSP within query sequence with the sequence accession number are shown in Fig.37 .

Fig. 37 Locally-aligned regions (HSPs) with respect to query sequence

The BEAUTY search, in the present study produced 50 significant alignments with the E-value less than 6e-11.

5.5. Protein Classification

Proteins have been classified into families on the basis of sequence similarity. If the analysis reveals that the new protein is a member of a family that is predicted to have a structural fold, multiple sequence alignments of these proteins can be used for structural modeling.

In the present study, CATH was used to determine the ETR1 protein classification. The class of the protein was found to be 3, meaning $\alpha+ \beta$, i.e. it is mainly composed of α – helix and β strands. If two proteins share significant sequence similarity, they should also have similar three-dimensional structures. The similarity may be present throughout the sequence lengths or in one or more localized regions having relatively short patterns that may or may not be interrupted with gaps.

5.6. Prediction of Protein Secondary Structure

Accurate prediction as to whether α – helices, β strands and other secondary structure will form along the amino acid chain of proteins is one of the greatest challenges in sequence analysis. The COLOUR PROTEIN SEQUENCE programme was used to differentiate the protein into hydrophobic and hydrophilic regions. The most hydrophobic / hydrophilic regions as shown in Fig.28 are represented in red colour. In the present study, the total number of hydrophobic regions of ALIVMW amino acid was found to be 294, while that of DEKNQRST hydrophilic amino acids was found to be 306. The location of hydrophobic amino acids with in a predicted secondary structure can also be used to predict the location of the structure.

The ability to predict secondary structure also depends on identifying types of secondary structural elements in known structures and determining the location of

these elements. The main types of secondary structures that are examined are α – helices and β strands. The programme PREDATOR was used for this purpose. In the present study it was found that the ETR1 protein consisted of 41.11% α – helix and 18.56% β strands and the rest (41.33%) was random coil and there were 0% ambiguous states. The exact location of the α – helices and the β-strands in the receiver domain of the protein was determined with RASTOP and is illustrated in Plate 6. The side chains and α-carbons in the receiver domain of ETR1 protein was determined with SPDBV and illustrated in Plate 5.

5.7. Phylogenetic prediction

A phylogenetic analysis of a family of related nucleic acid or protein sequences is a determination of how the family might have been derived during evolution. The evolutionary relationships among the sequences are depicted by placing the sequences as outer branches on a tree.

In the present study, the programme TREE TOP was used for establishing a phylogenetic tree. The tree diagram produced by TREE TOP is shown in Fig.23. The tree diagram showed the close link of all ETR1 sequences of *Arabidopsis thaliana*. *Pelargonium hortorum* was linked closely to *Arabidopsis thaliana* ETR1. ETR2 of *Rosa hybrida* was far away from ETR1 of *Arabidopsis thaliana*. This indicated that ETR1 sequence of *Arabidopsis thaliana* and *Pelargonium hortorum* sequences are very closely related with high similarity of sequences. When a gene family is found in an organism or group of organisms, phylogenetic relationships among the genes can help to predict which ones might have an equivalent function. These functional predictions can then be tested by genetic experiments.

In the present study, in conclusion, it was found that ETR nucleic acids could be derived from any of the higher plants which are responsive to ethylene. ETR

nucleic acids con be isolated from flower crops like *petunia,* Roes, *Dianthus, Anthurium, Delphiniums, Dendrobium, Phalaenopsis* and other orchids. Particularly preferred plants may include lily, carnation, chrysanthemum, petunia, rose, geranium, gladioli and poinsettia. Besides, particularly suitable plants may include tomato, banana, kiwi fruit, avocado, melon, mango, papaya, apple, peach and other climacteric fruit plants. Non-climacteric species from which ETR nucleic acids can be isolated may include strawberry, raspberry, blackberry, blueberry, lettuce, cabbage, cauliflower, onion and grapes.

The study has applications in a wide variety of plants to obtain useful phenotypes. For example, it can be used to delay or prevent floral senescence and abscission during growth or during transport or storage as occurs in flower beds and in ornamental flowers (e.g. carnations, roses) that are either cut or not cut and ornamental (e.g. holly wreaths) plants. Other uses include the reduction or prevention of bitter – tasting phenolic compounds (iso coumarins) which are induced by ethylene for example in sweet potatoes, carrots, parsnip and brassica. The loss of flavor, firmness and/or texture as occurs in stored produce such as apples and watermelons, russet spotting (a postharvest disorder) which is ethylene induced in crisphead lettuce to promote male flower production and to increase plant size, e.g. by delaying the formation of flowers in ornamental bromeliads. Furthermore, a decrease in ethylene response can be used to delay disease developments such as the preventing of lesions and senescence in cucumbers infected with *Colletotrichum lagenasrim* and to reduce diseases in plants in which ethylene causes and increase in disease development, e.g., in grape fruit, plum, rose, carnation, strawberry, tomato, melon and ornamental plants. In addition, it can be used to reduce the effect of various environmental stresses which result in the biosynthesis of ethylene in plant tissue. For example, ethylene exists at

biologically detrimental levels in localized atmospheres due to fires, automobile exhaust and industry. In addition, it can be used to minimize the effect of ethylene synthesized in response to environmental stresses such as flooding, drought, oxygen deficiency, wounding (including pressure and bruising), chilling, pathogen invasion (by viruses, bacteria, fungi, insects, nematodes and the like), chemical exposure (e.g., ozone salt and heavy metal ions) and radiation (Meyerowitz *et al.* 1996).

Finally, the present study revealed that the *Anthuriums* possessed inherent variability for not producing post-harvest ethylene, which could be exploited for genetic manipulation. The bioinformatics study revealed that there were as many as 17 flower crop sequences that produced the ETR1 sequence units with high similarity indicating the possibility of genetic transformation in these crops by using these sequences in other related crops to control post-harvest ethylene evolution and for preventing the post harvest losses of flower crops.

SUMMARY

CHAPTER VI
SUMMARY

The findings of the experiments conducted on postharvest ethylene evolution of *Anthurium andreanum* and *Dendrobium* and similarity search for ETR1 or ethylene antisense gene on flower crops are summarized below.

1. Dendrobiums evolved more postharvest ethylene than Anthuriums at third week.
2. The highest postharvest ethylene was released by Spic white (4.139 ppm/100g) Dendrobium at third week.
3. Among the Anthuriums, three genotypes viz., Bonfire orange, Merengue and Linda demol released no ethylene at third week. They did not release ethylene even when the sample size was increased to 10 flowers for three weeks.
4. Among the rest of Anthuriums at third week, Temptation recorded the highest ethylene (0.602 ppm/100g) while the lowest was recorded in B 13.
5. Critical stage for postharvest ethylene release was found to be around second week for Dendrobiums and around third week for Anthuriums.
6. Postharvest deterioration beyond second week, in Dendrobiums, was found to be predominantly due to higher levels of postharvest ethylene release.
7. In Anthurium, postharvest deterioration beyond third week was found to be mainly due to pathogenic decay rather than senescence and withering, as they produced low or no postharvest ethylene.
8. In the cluster analysis for postharvest ethylene release data, all the 'low ethylene' and 'no ethylene' releasing Anthuriums were grouped together in K-means algorithm, while these got separated into three groups in JOIN algorithm where all 'no ethylene' types were clubbed together.

9. The DNA sequence similarity search using BLAST for ETR1 gene (antisenescence gene or ethylene antisense gene) yielded 80 results with E-value less than 0.001 indicating a very high similarity as the critical E-value was kept at 0.05.

10. The BLAST search for ETR1 yielded 17 flower crop sequences with E-value ranging from 3e-66 to 4e-22 indicating a high similarity of flower sequences to ETR1.

11. Among the 17 flower crop BLAST results, 8 had moderate E-values in the region of less than 1e-40 (though high similarity scores) indicating that these sequences were closely related to ETR1.

12. Among the 17 flower crop sequences found in BLAST search, the lowest identity value of 79 per cent was found in *Petunia hybrida* ETR1-1 mRNA, while the highest identity value of 86 per cent was recorded in *Rosa hybrida* ETR2 gene. Higher identity value means less mismatches or gaps, which is a real indication of similarity.

13. The BLAST search for ETR1 had less complexity regions or repeat sequences indicated by high K-value of 0.711.

14. The BLAST results for the ETR1 had the computed gap penalty of -5 and gap extension of -2. This high negative value indicated very high similarity of resulting sequences even under severe penalties.

15. The ETR1 sequence search in BLAST resulted in a positive/positive strand values for all flower crops indicating a forward only search between query and database sequences.

16. The BEAUTY predicted amino acid sequences for ETR1 found in the database produced 50 significant alignments. The K-value in this case was very low

(0.047) indicating high complexity regions or higher number of repeating sequences.

17. The class of ETR1 protein determined by CATH was found to be Class 3 (α + β), meaning that it contained mainly α – helices and β- strands.

18. The Secondary structure of protein determined by PREDATOR revealed that ETR1 was composed of 41.11 per cent α-helix, 18.56 per cent β- strand and the rest being random coils.

19. The phylogenetic tree produced by TREE TOP revealed that ETR1 of *Pelargonium hortorum* was closely related to ETR1 of *Arabidopsis thaliana,* while ETR2 of *Rosa hybrida* was found to be the farthest.

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WEBSITES

A science primer on bioinformatics. **(URL: www.ncbi.nlm.nih.gov/)**

BIOEDIT **www.mbio.ncsu.edu/BioEdit/bioedit.html**

BLAST **www.ncbi.nlm.nih.gov/BLAST/**

CATH **www.biochem.ucl.ac.uk/bsm/cath/**

CDART **www.ncbi.nlm.nih.gov/Structure/lexington/ lexington.cgi?cmd=rps**

CDD **www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml**

CLUSTALX **www-igbmc.u-strasbg.fr/BioInfo/ClustalX/Top.html**

Cn3D **www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.shtml**

COLOR PROTEIN **www.pbil.ibcp.fr/cgi-bin/ npsa_automat.pl?page =npsa_ color.html**

Identification of specified gene on the chromosome of sequenced genomes. **(URL: www.ncbi.nlm.nih.gov/mapviewer/)**.

MACROMOLECULAR MOVEMENTS **molmovdb. mbb. yale.edu/ Mol MovDB/**

MAP VIEWER **www.ncbi.nlm.nih.gov/cgi-bin/Entrez/map_search**

ORF FINDER **www.ncbi.nlm.nih.gov/gorf/gorf.html**

PDB SUM **www.biochem.ucl.ac.uk/bsm/pdbsum/**

PFAM **www.sanger.ac.uk/Softwares/Pfam/**

PREDATOR **www.pbil.ibcp.fr/cgi-bin/ npsa_automat. pl?page=npsa_ predator.html**

PROMOTER SCAN **www.molbiol.ox.ac.uk/promoterscan.htm**

RASTOP **www.geneinfinity.org/rastop/**

SOPMA **www.pbil.ibcp.fr/cgi-bin/ npsa_automat.pl?page=npsa_sopma.html**

SPDBV **www.expasy.ch/spdbv/**

SWISS-PROT **ww.expasy.ch/sprot/**

TREE TOP **www.genebee.msu.su/services/phtree_reduced.html**

VAST **www.ncbi.nlm.nih.gov/Structure/VAST/vast.shtml**

APEDA **www.apeda.com**

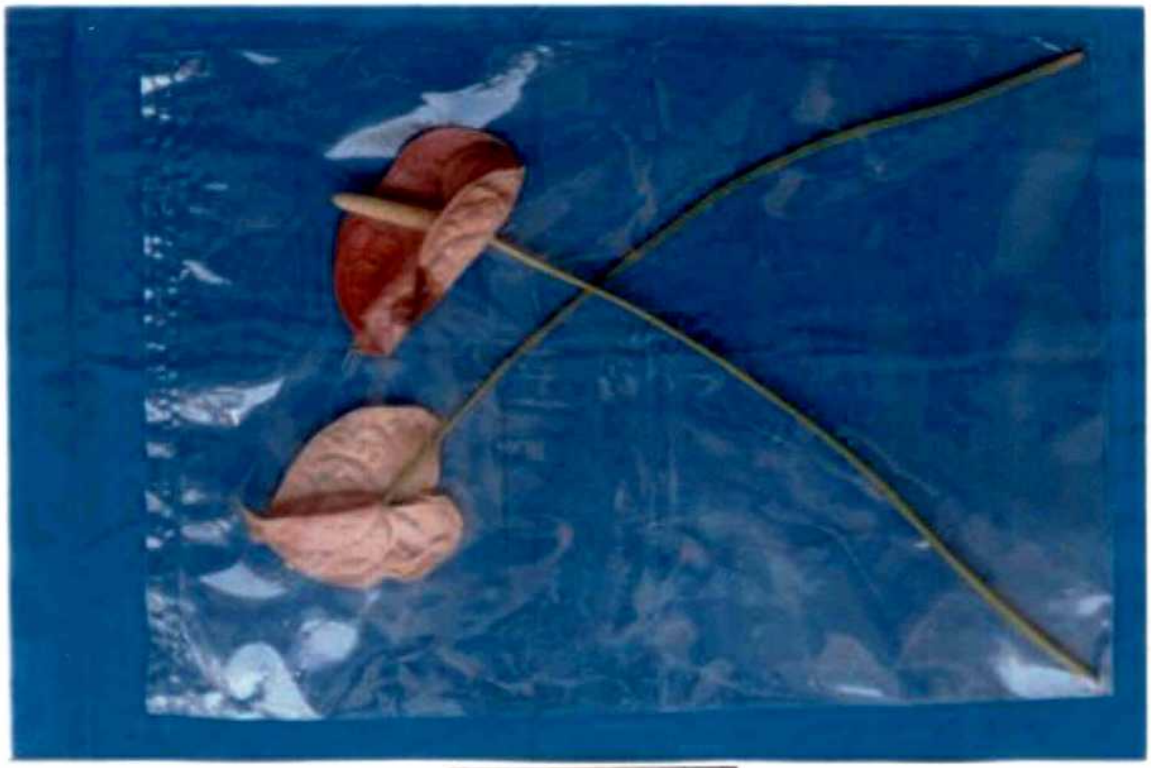
PLATES

Plate.2. High Ethylene evolving Dendrobiums

Thai Hybrid

Sonia 17

Plate.3. No – Ethylene evolving Anthuriums



Bonfire Orange

Linda

Merengue

Plate.4. Moderate Ethylene evolving Anthuriums

Sikkim Red

Temptation

Rosetta

Honduras

Tinora

Gloria

predicted by SPDBV

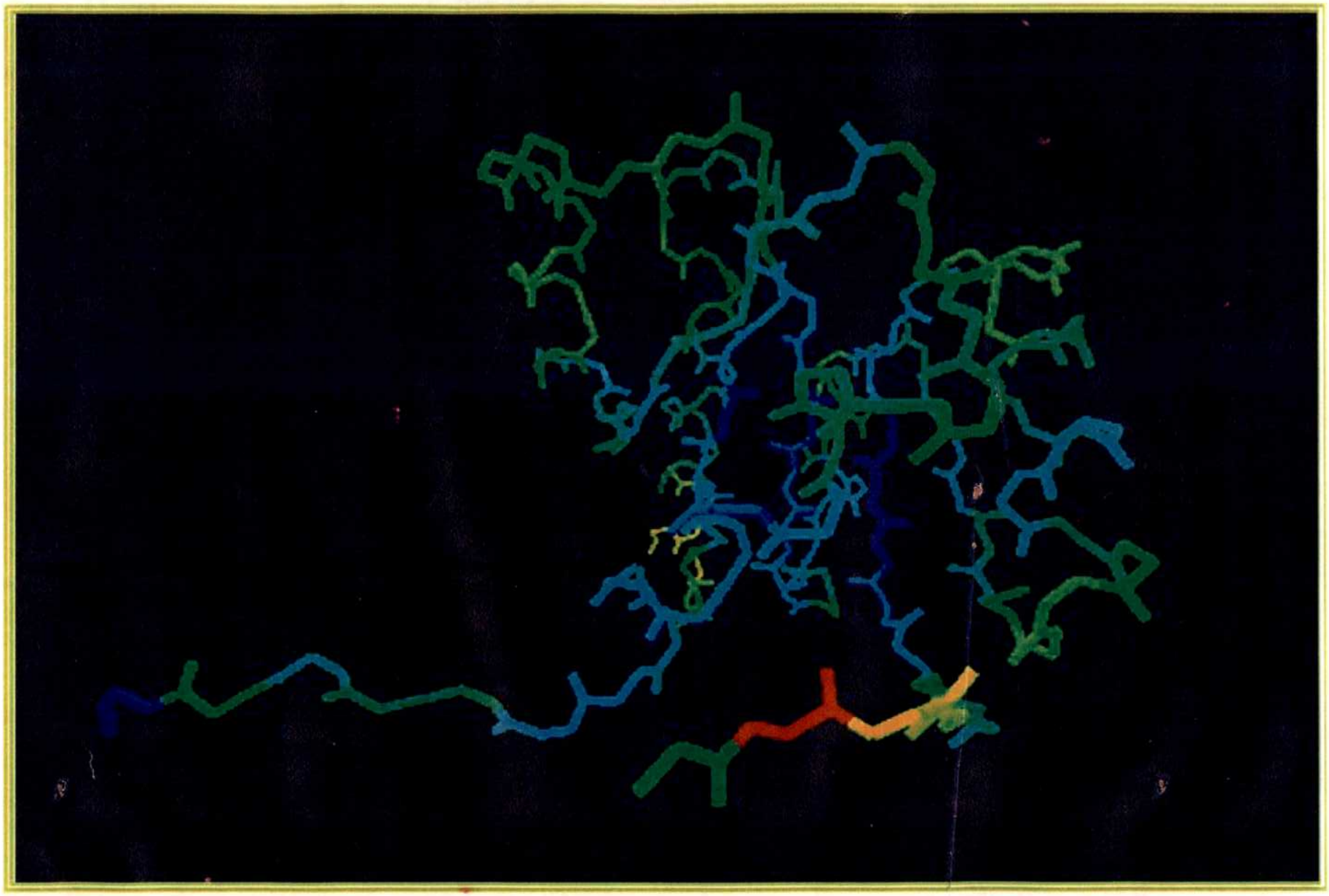


Plate.6. 3-D view of receiver domain of ETR1 protein as predicted by RASTOP