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Transcriptomic response of plants to external biotic and abiotic factors:

- Defense against pathogens in *Olea europaea* (L.)
- Functional adaptation along environmental clines in *Posidonia oceanica* (L) Delile

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CHAPTER 1

GENERAL INTRODUCTION AND SCOPE OF THESIS

1-1 ADAPTATION AT ENVIRONMENT

Phenotypic plasticity occurs when variability in an environmental stimulus leads individuals of the same genotype to develop into alternative phenotypes (Stearns, 1989).

Because phenotypic plasticity can be an adaptation to variable environments, it is becoming increasingly recognized that understanding its regulation and evolution may offer general insights into the genetic and developmental bases of morphological evolution (Pigliucci, 1996)

The interaction between genotype and phenotype has often been conceptualized by the following relationship: *genotype + environment → phenotype*, a slightly more nuanced version of the relationships is: *genotype + environment + random-variation → phenotype.*

Genotypes often have much flexibility in the modification and expression of phenotypes. In many organisms phenotypes can be very different under varying environmental conditions. Phenotypic variation among individuals can range from being largely of genetic origin, to being largely caused by environmental variation; extreme conditions can influence both the expression of phenotypic variability and the extent to which it is genetically determined (Jablonka and Lamb 1995; Parsons, 1996).

Environmental changes can directly affect the genetic information carried by organisms and the expression of variation in characters. Environmental effects have been detected by looking at the way different conditions influence mutation rates, recombination rates, the stability of an organism's development, and the way genes interact with the environment to produce phenotypes.

Rapid changes under extremes can alter traits other than those under selection; these results from the phenomenon of "pleiotropy" where the same gene influences more than one trait: e.g. gene controlling levels of a hormone are expected to have pleitropic effects because hormones tend to influence multiple physiological processes (Hoffmann and Parsons 1997).

Tolerance range is also correlated with phenotypic plasticity, defined as the extent to which the phenotype associated with a particular genotype can be altered by the environment. A high level of plasticity means that environmental factors have a strong influence on the particular phenotype that develops. If there is little plasticity, the phenotype of an organism can be reliably predicted from knowledge of the genotype, regardless of environmental peculiarities during life-cycle.

Genes controlling the expression of novel characters can be selected once the environment is stressful even if effects of these genes are not evident under optimal environmental conditions. Continued selection of the novel characters could favor genes that lead to the expression of novelties even in the absence of a stress; in these conditions the natural selection acts on phenotypes to favor the emerging of the genotypes better adapted to particular range of ecological conditions.

Natural populations show a range of variation in phenotypic traits among individuals. Traits that improve competitive ability are thereby selected, and these increase in frequency in a population. The population evolves as traits improving competitive ability are favored, and evolutionary change is largely driven by competitive interactions between individuals (Hofmann and Parsons 1997).

Presence of variations among individuals suggests that populations have the potential to evolve and adapt when they encounter unfavourable conditions, but during periods of severe stressful populations can reduce in size and age structure. In extreme cases, stresses may result in the extinction of populations and reduction in the area occupied by a species, and this is truer in populations that live at the boarders of species distributions where conditions can be often marginal to survival (Hofmann and Parsons 1997). These changes can have evolutionary consequences by influencing the ability of populations to adapt to the stressful conditions and to other environmental changes.

Environmental changes can cause the physical fragmentation of species habitat, and when populations remain isolated, population size is reduced. Geographical isolation has a strong influence on the gene flow among populations. The reduction of genetic exchange caused the increasing of inbreeding in a population, small populations are also more prone to loose genetic variation by the process of “genetic drift”. Small, inbreed and less genetically diverse populations are more fragile to external impacts and less capable to adapt to new conditions. From another point of view, genetic isolation among populations can favor the emerging of “ecotypes” or groups of individuals better adapted at the new ecological range.

Understanding why an organism occupies a particular geographic and environmental range could allow predictions of the response to environmental changes and ecological studies of populations

living at geographic and environmental extremes can allow the identification of relevant factors constraining species distribution

About the term "Stress"

Biologists used the word "stress" in different ways and different contexts with reference to several forces; generically they refer to stress as a disturbance of the normal steady state (homeostasis) of a biological system.

For Hofmann and Parson (1991), two components are involved in dealing with stress, "the external and internal forces that are applied to organisms and change in biological systems that occur as a consequence of these forces" (Hofmann and Parson, 1991). The degree of stress caused by an environment can only be evaluated in relation to the organism or population experiencing this environment. The term "stress" is moreover used to indicate either the environmental or the biological component. From an evolutionary perspective the environmental force and the biological response should be viewed as integrative.

Stress effects can be analyzed at different biological levels, namely molecular, physiological, and both in single individuals and in populations. Sensitivity at one level does not necessary have to become manifest at another. Phenotypic plasticity, for example, may prevent biochemical changes from being revealed at the individual level, but this process itself may be costly and may be revealed as a change in fitness. Finally, the use of the term "stress" is often associated with the intensity of the stress. The environment is considered to be stressful only if the response it caused exceeds an arbitrary threshold, or when more than a certain fraction of the population is affected.

The interest to study environmental stress has been stimulated by the development in molecular genetics techniques. These have revealed that most organisms evolved sophisticated mechanisms to cope with different environmental stresses such as heat shock proteins to counteract thermal and other stresses, mixed function of enzymes like superoxide dismutase, catalase, glutathione-S-transferase, which contributes somehow to stress tolerance, and peculiar enzymatic mechanisms to fight biotic attacks. A genomic approach to stress-studies allows both fine-scale investigation of response at specific stressor and enlightening of the molecular networks that are involved in the homeostasis' restoring

In the last century global warming and anthropogenic pressure, with consequences such as climatic shifts, chemical pollution and habitat destruction, represent increasing threats for the ecosystems on this planet as well as for the survival of the human community.
Therefore understanding the mechanisms underlying responses of organisms at stressor is of paramount importance to evaluate rate of species plasticity.

Acclimation and response to stress

The perception of stressful conditions will determine its transduction and subsequent responses, including various changes in the activity of single enzymes or changes in several metabolic pathways
Extreme of abiotic factors can cause death when the physiological tolerance limits of organisms are exceeded. These limits can be very different for different species and for different developmental stage of the same organisms. Tolerance range is also correlated with phenotype plasticity, defined as the extent to which the phenotype associated with a particular genotype can be altered by the environment. A high level of plasticity means that environmental factors have a strong influence on the particular phenotype that develops. If there is little plasticity, the phenotype of an organism can be reliably predicted from knowledge of the genotype, regardless of environmental peculiarities during life-cycle.

Plants are particularly exposed to their physical environments, as they cannot move away from disadvantageous surroundings and have to cope with the stress on site, then plants have develop fine-scale mechanisms to protect itself from biotic and abiotic stressors.
Among the most important factors which can affect plant development and survival, we can include light, temperature, chemical pollutants such as heavy metals, and interaction with pathogens and herbivores.

Light

Light affects all aspects during development and growth of plant. Plant responses to light occur in the context of multiple developmental processes, including seed germination, seedling photomorphogenesis, phototropism, gravitropism, chloroplast movement, shade avoidance, circadian rhythms and flower induction (Jiao, 2007). The major threat concerning light limitation is shading as it limits significantly the quantity of light perceived by the plant, in particular red and blue wavelengths photosyntheticaly active radiations.

Plants have adopted the ability to sense multiple parameters of ambient light signals, including light quantity (fluency), quality (wavelength), direction and duration. Light signals are perceived through at least four distinct families' of photoreceptors, which include phytochromes, cryptochromes, phototropins and unidentified ultraviolet B (UVB) photoreceptors (Jiao, et al. 2007 review)

Temperature

The effect of temperature varies extensively, from freezing to hot and cold and freezing conditions are two relevant factors limiting the distribution of plant species. Heat shock proteins were first identified as a set of proteins that exhibit strong induction when cells experience a rapid rise in temperature of 10°C or more and subsequently were found in plants. Since their discovery, the fundamental function of most of the major heat shock proteins has been determined, and the majority has been recognized as being molecular chaperones (Miernyk, 1997).

Moreover an important part of the thermal adjustment is vernalization or photoperiodic response, which might reflect a complex genetic network response.

Heavy-metal tolerance

There are many areas of the world were anthropogenic activities have increased to a greater or lesser extent by heavy metals. Heavy metals, such as cadmium, selenium, mercury, arsenic etc, are micronutrients essential for plant growth, which can become toxic when present in excess in soil (Clements 2006).

Plants respond to heavy metal toxicity in a variety of different way: responses include immobilization, exclusion, chelation and compartmentalization of the metal ions, and the expression of more general stress response mechanisms such as ethylene and stress proteins which are implicated in metal tolerance in plants (Cobbett et al. 2000).

A common mechanism for heavy metal detoxification in plant is the chelation of the metal by a ligand with metal-binding proteins as Metallothioneins (MTs). Metallothioneins are low-molecular-weight, cysteine-rich metal-binding proteins found in a wide variety of organisms including bacteria, fungi and all eukaryotic plant and animal species. MTs bind essential and non-essential heavy metals, and plants appear to contain many different form of these proteins which can to perform distinct roles in the metabolism of different metal ions metal, likely participate in the uptake, transport, and regulation of metal in biological systems also they are implicated in the

detoxification of metal excesses. The effect of metals on the expression of Metallothionein varies with the plant species in certain such as rice (Hsieh et al,1995) was enhanced by certain metals; on the contrary in other species (Okomura et al, 1991) Metallothionein gene expression decrease by copper treatment.

Plant resistant to herbivores and pathogens

Plants' defense include morphological structures such as thorns, and chemical compounds able to struggle herbivores and pathogens. Through the course of evolution, plants have become nature's organic chemists par excellence, and collectively synthesize a plethora of secondary metabolites to defend themselves against herbivores and microorganisms and adapt to different types of abiotic environmental stresses. Traditionally, plant defense compounds are grouped into preformed defense compounds (phytoanticipins) forming the first chemical barrier to herbivore and pathogen attack and defense compounds synthesized in response to herbivore or pathogen attack (phytoalexins) (Morant et al, 2008).

In addition to their direct effects on pests and pathogens some secondary metabolites may also be important in defence-related signal transduction (Bouarab *et al*., 2002). The ability of plants to carry out *in vivo* combinatorial chemistry by mixing, matching and evolving the gene products required for secondary metabolite biosynthetic pathways is likely to have been key for their survival.

1.2- STUDY ON ADAPTATION: MOLECULAR APPROACHES

Biological questions concerning how individuals, populations and ecosystems respond to environmental changes or biotic stress can be seen in different way.

Investigations on biochemical, physiological, morphological and evolutionary mechanisms that underlie stress tolerance need very different approaches (fig1.2.1).

Molecular genetics approach chooses a key gene activated in response to specific factors to decipher the function and the mechanism underlying activation/silencing of these genes.

However, abiotic stress tolerance is a complex trait and, although large numbers of genes have been identified to be involved in the abiotic stress response by genetic approaches, there are large gaps in our understanding of the phenotypic traits involved in the responses.

The recent availability of the genome sequences of certain non-model plant species has enabled the use of strategies, such as genome-wide expression profiling, to identify the genes associated with the stress response. Gene transcription profiling, in particular, is one important step toward identifying those genes and metabolic pathways that underlie ecologically important traits, such as stress tolerance.

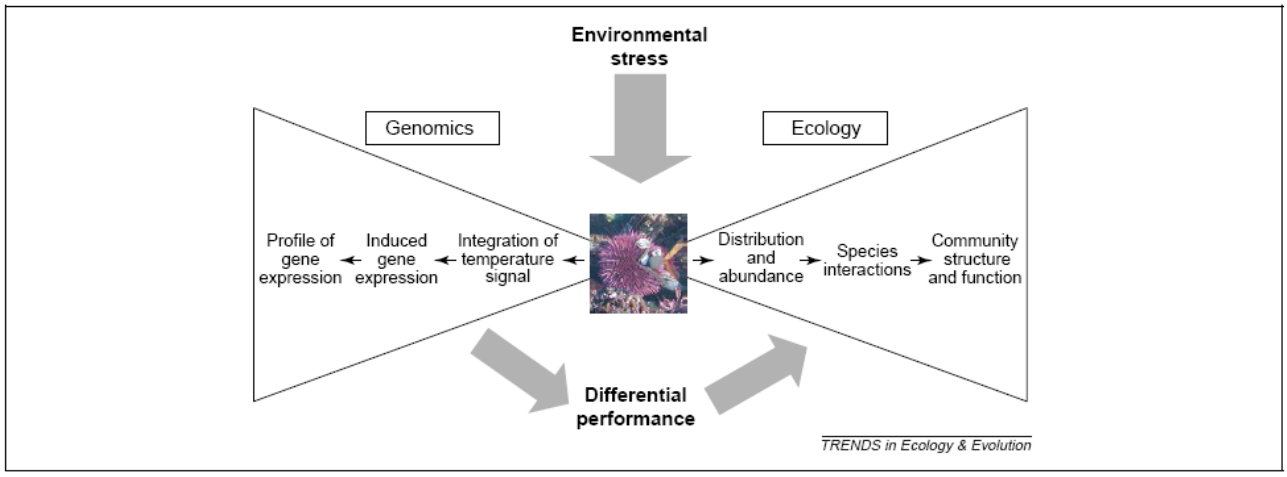


Fig 1.2.1 Conceptual diagram for integration of genomics, physiology and ecology in the study of environmental stress. *Modified by Hoffman et al, 2005*

1.3-SCOPE OF THESIS

In this thesis I studied two different plant species, the oleaceae *Olea europaea* L. and the Mediterranean seagrass *Posidonia oceanica* L. (*Delile*), to explore the components and hierarchical structure of the functional adaptation processes that are activated during the plant response to biotic stressors (in *O. europaea*) and to different environmental conditions (in *P. oceanica*). I used a "bottom-up" approach to study the plant defence against pathogens in *O. europaea*; and a "top-down" approach to identify the genetic basis for functional adaptation in *P. oceanica.*

CHAPTER 2

TARGET SPECIES

In the following chapter we selected some information on the biology of *Olea europaea L*. and *Posidonia (L.) Delile*. We mainly focused on those topics that may hopefully enhance the comprehension of experiments, questions and results dealt with this thesis.

2.1 *OLEA EUROPAEA* L.

Biological aspects

Olea europaea is member of the family Oleaceae, order of Lamiales, which includes about 10 families for a total of about 11,000 species.

The Olive trees is a plant with over-century longevity, which has persistent leaves and, on the wild status, it starts to bear fruits after a long juvenile period preserving high rate of fruit production for many years.

Leaves are narrowly elliptic to oblong or “laceolate”, 1-8 cm long and 0.3- 2 cm wide, with glabrous upper surface and lower surface moderately to densely grayish-green. Flowers are hermaphroditic and reunited in a small cluster, commonly called "mignola”. Nevertheless a good part of the Italian varieties is auto-sterile; therefore the fertilization is primarily heterogamous.

Drupes are green when immature, becoming black or brownish at maturity, sub-globose to narrowly ellipsoid, 6-19 mm long (Baldini 1993).

Fig, flower, tree, fruits Carolea. Source for tree e fruits pictures www.oleadb.it

Genetic resource

Hundreds of varieties (cultivar) of olives currently exist, characterized by different ratios between core and pulp and therefore from a middle content of oil varying from 18 to 27%.

The high number of existing cultivars is due to modifications of the genotype, become of mutations fixed for by vegetative or for intersection spontaneous and following dissemination, or for fluctuation of varietal characters following environmental conditions. *Olea europaea* is a diploid species (2n = 2x = 46), predominantly allogamous, with a genome size of about 1,800 Mb, although several studies report cases of polyploidy among variety that may have played a major role in the diversification of the olive complex (Besnard, 2008) .

In spite of the economical importance and metabolic peculiarities of this species, very few data are available on genome composition.

2 .2 *POSIDONIA OCEANICA* L. *(DELILE)*

Seagrasses are aquatic angiosperms, belonging to the Monocotyledons, subclass Alismatidae, which are confined to the marine environment and are adapted to develop the whole lifecycle completely submerged. The seagrasses form a distinct ecological groups but not a unique taxonomic group: this implies that the various seagrass families do not necessarily have to be closely related (den Hartog, e Kuo 2006). The evolutionary convergence of morphological and physiological characters among species is driven by the pressure of marine environmental constrains and increases in some cases the difficulties for the precise systematic distinction between species. The almost 60 presently recognized seagrass species are polyphyletic and belong to four different families, Potomagetonaceae, Zosteraceae, Hydrocharitaceae and Cymodoceaceae. Molecular phylogenetic data, i.e. from the plastid gene encoding for Ru-BisCO large subunit (Les et al, 1997) (fig 2..2.1), suggest that the return into the sea occurred at least three times independently, within the families of Zosteraceae, Posidoniaceae/Cymodoceaceae, and Hydrocharitaceae.

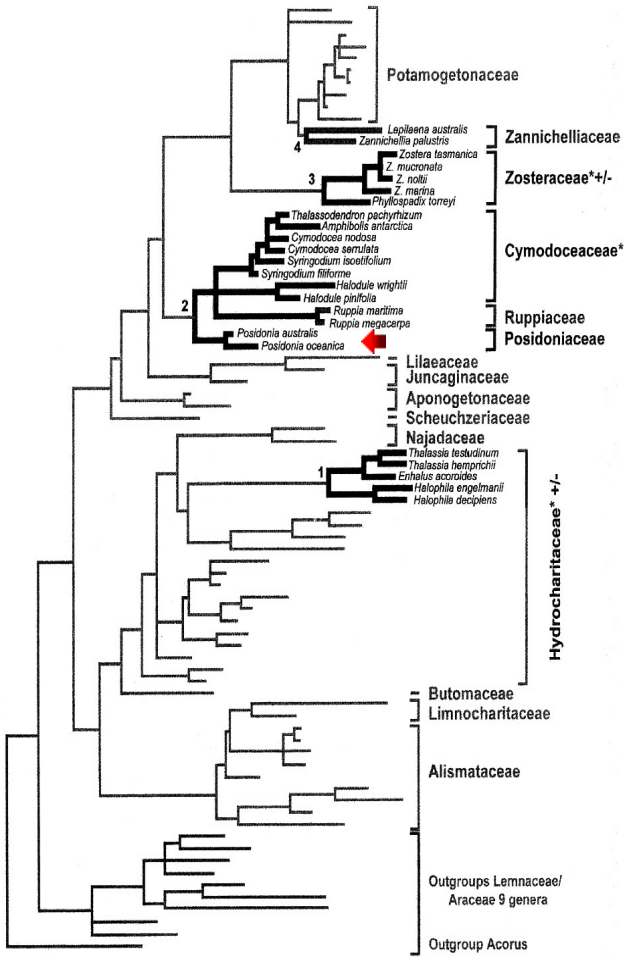


Fig2.2.1 Maximum parsimony *rbc*L cladogram of species from the 15 families typically recognized in the monocotyledon subclass Alismatidae with representatives from two outgroup families. The degree of internal support is indicated by bootstrap values. Values for nodes with <50% bootstrap support are excluded. (Les, Cleland e Waycott, 1997)

Seagrasses posse's similar organs and tissues as the other flowering plants, but in contrast with terrestrial monocotyledons, seagrasses have to live in marine or highly saline environments and this has profoundly influenced their morphology and anatomy (fig2.2.2). Theirs unique life-style has brought to the development of unique characteristics. In particular, the most aknowledged are the following:

- Life in estuarine or marine environment, and nowhere else.
- Underwater pollination with specialized pollen.
- Production of seeds underwater and seed dispersal by both biotic and abiotic agents.
- Presence of specialized leaves with a reduced cuticle and epidermis which lacks stomata and which represent the main photosynthetic tissue.
- Presence of roots capable of growing in anoxic environment and important in the nutrient transfer processes.
- Presence of a rhizome or underground stem with an important anchoring role.

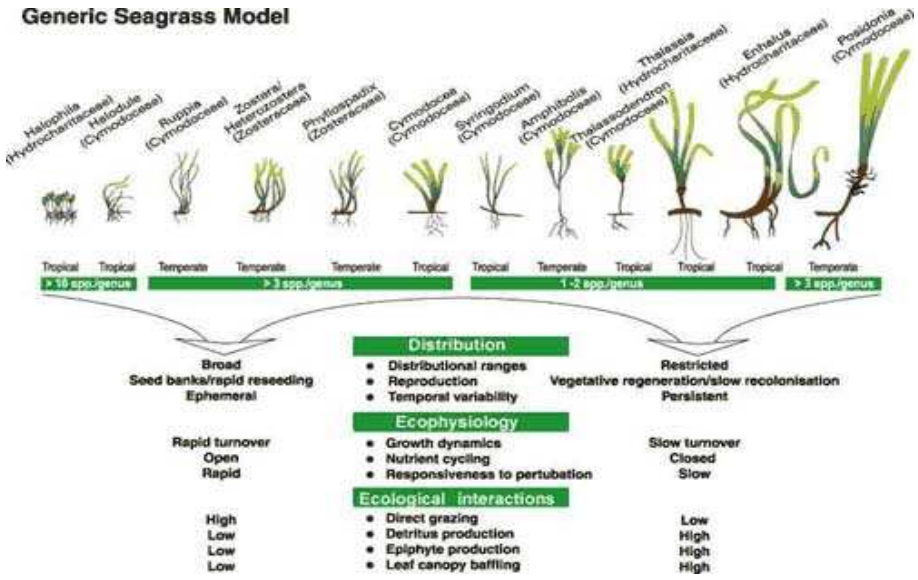


fig 2.2.2 Generic Seagrass model which categories seagrasses on the basis of growth forms.

Seagrasses exert a strong influence on the coastal environment and provide spatial structures and resources for rich associated communities. Given their multi-functional role and because of their widespread decline, seagrasses have been described in the UNCED Action Paper as an ecosystem that must be given priority for protection

The global species diversity of seagrasses is low, approximately 60 species, but species can have ranges that extend for thousands of kilometers of coastline. The importance of seagrasses as a key species in shallow coastal waters all over the world including polar areas (Green and Short, 2003) emerged over the last quarter of the 20th Century. Temperate seagrasses play a major role in providing habitat for commercially, recreationally and ecologically important fish and shellfish as well as food for many water fowl species (Green and Short, 2003). Seagrass species in temperate areas are also important in reducing wave and current energy and trapping and binding of fine grained sediments. The close habitat linkage between seagrasses and bivalve species is evident in many temperate parts of the world. Tropical seagrasses are important in their interactions with mangroves and coral reefs (Green and Short, 2003).

Seagrass meadows have been considered as high-value natural ecosystems compared with terrestrial habitats (Costanza et al.,1997). A measure of Productivity Value of seagrass ecosystems has been reported for the global area of 200 x106 ha as 3.801x109 \$ yr^{-1} (total value), the value per hectare being 19.004 \$ yr^{-1} (tropical forest 2.007 \$ ha^{-1} yr^{-1}).

The importance of seagrass systems can be summarized in a set of axioms, which have become familiar to planners and conservationists, now often referred to as “ecosystem services” (Costanza et al. Nature 1997). The parameters utilized to calculate the reported values refer to the monetary resopurces which would be necessary to replace the seagrass meadows “ecosystem services” for one year. Ecosystem services of seagrass meadows are the following:

- Seagrasses are important primary producers, converting sunlight and carbon dioxide efficiently into organ form;
- Seagrasses supply organic food to a variety of dependent food webs;
- Seagrasses stabilized the seabed in which they grow;
- Seagrasses structure the seabed on which they grow into a complex environment which provides places form many organisms to exist;
- Seagrasses act as the nursery ground for many commercially-caught species.

Most studies show that seagrass meadows are being lost rapidly in developed and developing parts of the world (Short et al. 2006b), with only occasional efforts at mitigation and restoration. Seagrass losses have been attributed to a broad spectrum of anthropogenic and natural causes: the effects of global climate change on seagrasses are difficult to document, but whether they manifest as sea level change, heat stress, radiation exposure, or increased storm activity, all largely diminish seagrass habitat, distribution and diversity (Short et al, 1999).

Moreover in many parts of the world, recent changes in species distributions have occurred as the result of human alteration of the physical environment or transport of species from their endemic locations (Short et al 2007). The global decrease of seagrass ecosystem has been quantified in a recent research (Waycott et al 2009): this analysis demonstrates that, over the entire period analyzed, 1879-2006, there was a mean decline in seagrass area of 1.5% yr^{-1}. (Waycott et al 2009). The same research reveals that the rate of decline has accelerated over the past 8 decades, and the largest losses occurred after 1980 (Waycott et al 2009).

Posidonia oceanica

The genus *Posidonia* comprises nine species: *P. oceanica* is endemic of Mediterranean Sea and its distribution is restricted to this basin; the others eighth *Posidonia* species occur along the Australian coasts. Mediterranean and Australian species of *Posidonia* are not genetically close. The divergence in their DNA sequences (Waycott et al., 2000) indicates that the separation of the two groups took place at a relatively early time in the history the seagrasses, probably during the Late Eocene.

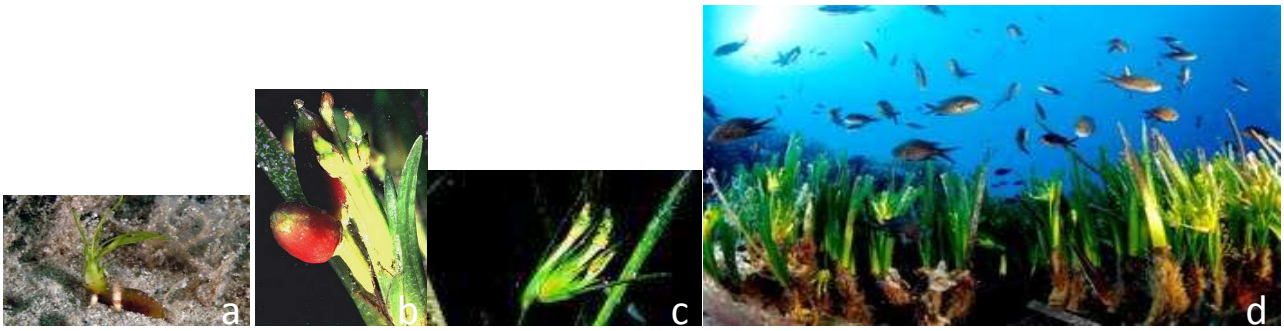


fig2.2.3 Posidonia oceanica: *a)* plantula *b)*fruits *c)* flower and *d)* meadow

Posidonia oceanica is a clonal plant, monoecius, hermaphroditic with irregular flowering and fruiting (fig 2.3a;b;c) characterized by slow rhizome elongation rates, a high dispersal potential of reproductive structures and drifting vegetative fragments and long persistence of genotypes (Procaccini et al. 2003). *Posidonia oceanica* forms extensive monospecific meadows that can persist *in situ* for millennia, building an organogenic structure (i.e. matte) (fig 2.3d) that rises for meters above the sediment level (Mateo et al. 1997; Procaccini et al. 2003). *Posidonia oceanica* has been present in the Mediterranean Sea since the Miocene (Pérès 1985) and survived during the last glaciation in refugia from which it re-colonized the basin (Pérès 1984). It has been hypothesized that the plant had its climatic optimum around 4000 to 8000 yr ago, not being well adapted to the present conditions of the basin (Pérès 1984). *Posidonia* meadows are extremely sensitive to medium-high levels of disturbance. *P. oceanica* is in regression in particular along the Western Mediterranean coasts, due to external impacts mainly acting on thermal regime and light availably, to the presence of pollutants and competition with introduced seaweed species.

Besides general trends of increased coastal erosion and decreased water clarity, possibly related to global climatic changes, the high impact of human communities along the coast can also account for the recorded meadow regression (Short & Wyllie-Echeverria 1996).

Genetic structure of Posidonia oceanica in Mediterranean basin

Existing population genetic data on *Posidonia oceanica* have been obtained using neutral microsatellite markers (Procaccini and Waycott 1998, Alberto et al , 2003), which allowed a good understanding of the meadows genetic structure and population connectivity (Arnoud-Haond et al, 2005) at the basin scale. These studies showed that within the Mediterranean basin three main population groups were present. The first one including the North-Tyrrhenian populations, north Sardinia, Corsica and Médès Island; the second one with the South- Tyrrhenian populations, South Spain, Pantelleria, Malta and Tunis; the third one with the Adriatic and Aegean populations. Recent analysis shows the existence of a further quarter group including populations of the Sicily Channel (Arnoud-Haond et al 2007; Serra et al, 2009 in press). Serra et al, (2009 *in press*) also show that the distinction existing between *Posidonia oceanica* populations growing in the Western and in Eastern Mediterranean basin results from past vicariance events and is maintained at present by low levels of gene flow. Theoretical seed dispersal follows a West-East direction but most of them do not reach suitable substrate for settling along the coast because are transported in open water areas. Nevertheless, the two population groups do not seem to be reproductively isolated, but are maintained in contact by the transition zone represented by meadows at the longitude of the South-Eastern tip of Sicily and of the Malta Island in the Sicily Strait. Meadows in the transition zone present the higher genetic diversity observed and signature of recent admixture from different gene pools. *Posidonia oceanica* meadows in Sicily do generally show high genetic polymorphism, as also suggested by the frequent record of flowers and beached fruits along the coasts of the Island. Nonetheless very low genotypic richness has been found in meadows either isolated from the main patters of local gene flow (Serra et al., 2009 in press). Low genetic variability has also been found at the geographic boundaries of the species distribution, in isolated North Adriatic populations, where distinct meadows seem to be represented by single clones (Ruggiero et al, 2002), and in the Marmara Sea, where meadows, established since the Middle Holocene, seem to be isolated from the Mediterranean since the catastrophic intrusion of brackish water into the Marmara Sea and the strong and persistent flow coming from the Black Sea, occurred between 10,000 and 5,300 yrs BP

(Meinesz et al, 2009).The enhanced meadow isolation, together with the existing low genetic polymorphism and small population size found in *P. oceanica* (Procaccini et al. 2001) increase risk of survival of this species in the Mediterranean under environmental challenges (Arnaund-Haond et al 2007, Procaccini 2007). Populations living near or at the edges of their distributional ranges will be the first to suffer the environmental changes, and in the absence of local adaptation may be disappear; however populations at edge may have evolved ecophysiological adaptations, (e.g. *P.oceanica* at the costal lagoon Stagnone di Marsala, Sicily lives at extreme salinity values, Tommasello et al, 2009), and the development of selective markers will enable to identified genes underlying adaptation which can represent the baiss for the development of a new set of genetic/genomic tools for proactive monitoring, restoration and conservation of seagrasses ecosystems.

3.1 PLANT DEFENSE IN *OLEA EUROPAEA*

Introduction

Many plant defense compounds are stored in a non-active glucosylated form to chemically stabilize and increase the solubility of the defense compound, to render it suitable for storage in the vacuole, and to protect the plant from the toxic effects of its own defense system (Jones and Vogt, 2001).

It has been established that the β-glucosidase and its glycoside substrate system have a role in the response against herbivores and pathogen attacks, and their different localization seems to be physiologically important, because some products of hydrolysis are also toxic to host cells. Upon cell disruption, caused for example by a chewing insect, the defense compounds are bioactivated via hydrolysis of the glucosidic linkage catalyzed by b glucosidases. In intact plant tissue, the β-glucosidases are stored separately from the substrates. This two-component system, of which each of the individual components is chemically inert, provides plants with an immediate chemical defense against attacking herbivores and pathogens (Morant et al, 2008**) (**fig 3.1.1)

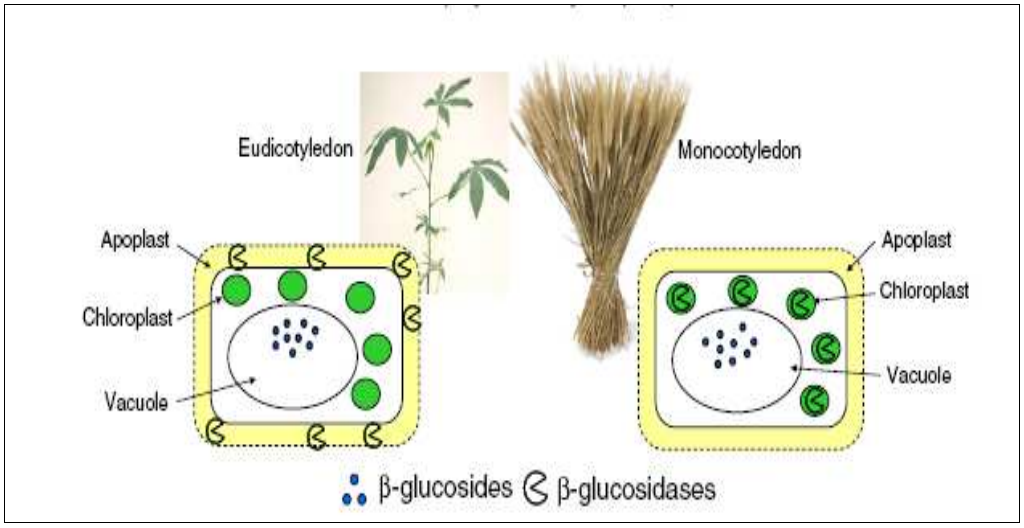


Fig3.1 Model for subcellular compartmentalization of β-glucosidase and glycoside substrate system in plants. *Modified from Morant et al, 2008*

The biological activity of the defense compounds is attributed to their hydrolysis products, and therefore the separation of glucosides and β-glucosidases into different (sub) cellular compartments in intact tissue is a critical feature of the two component defense systems. As illustrated in Fig. 3.1, the glucosides are stored in the vacuole (among the others: Oba et al., 1981; Gruhnert et al., 1994). Subcellular localization of expressed β-glucosidases differ among monocotyledenous and eudicotyledenous plants: monocotyledenous β-glucosidases contain an N-terminal transit peptide resulting in localization to plastids (Gusmayer et al., 1994); in eudicotyledons, the β-glucosidases contain an N-terminal signal peptide that results in direction through the secretory pathway via the endoplasmatic reticulum and in co-translational glycosylation (Cairns et al., 2000;). Their final localization might be either apoplastic or intracellular in protein bodies (Swain et al., 1992; Poulton et al, 1994).

In Oleaceae the β-glucosidases is involved in a constitutive defence system against herbivores and insect attacks by realizing the bioactive molecules produced by oleuropein hydrolysis (Mazzuca t al, 2006). In *Ligustrum*, a genus of the Oleaceae family, the oleuropein-degradative-β-glucosidase activity has been detected in purified chloroplast fractions of leaf extracts (Mazzuca et al.,2002). Chloroplasts of *Olea europaea* leaf and fruit parenchyma cells also contain large amounts of an oleuropein-degradative-β-glucosidase which specifically hydrolyses the bitter phenol oleuropein
(Konno et al. 1999)
A rising of oleuropein-degradative-β-glucosidase activity was detected in the olive fruit pulp following a biomimed insect attack as the early response of tissue damages (Mazzuca et al., 2008). All these findings suggested that chloroplast -β-glucosidase is related to leaf and fruit defence mechanism by storing and releasing the toxic chemical; the damage of cells and tissues by pests bring in contact -β-glucosidase and oleuropein with the consequent release of toxic aglycones which either deterred herbivores or inhibited the entry, growth and spread of phytophathogens (Konno et al. 1999). The oleuropein degradative-β-glucosidase is expressed in different isoforms, able to segregate in two different cell compartments. They are also differentially expressed in the cells of the outer and inner mesocarp tissues. Since the GH 1 enzyme family is encoded by nuclear genes (Esen 1993) , the chloroplast isoform must moves from the cytoplasm, where it is synthesized, into the chloroplast organelle by the cleavage of the transit peptide sequences located in the N-terminus of the bglucosidase protein precursor (Mieszczak et al., 1992)

By means of in situ enzyme assay, performed in olive fruit tissues, It was found enzymatic hydrolysis in the chloroplasts of the outer mesocarp cells (Fig. a1) and in the small cytoplasm oil droplets of the inner mesocarp cells (Fig. b2). By means of proteomic approach three enzyme isoforms were detected in leaf tissue whose the expression seems to be related to the photosynthetic competence, since they are not expressed in young immature leaf parenchyma, but strongly expressed in mature and aged leaves. Even in fruits the three enzyme isoforms were differentially expressed at different stage of ripening. These results suggest that at least three different β-glucosidases, are highly specific to oleuropein. The efficiency of this system could be responsible for the different susceptibility of olive grove to infestation (Mazzuca et al, 2008 *in press*)

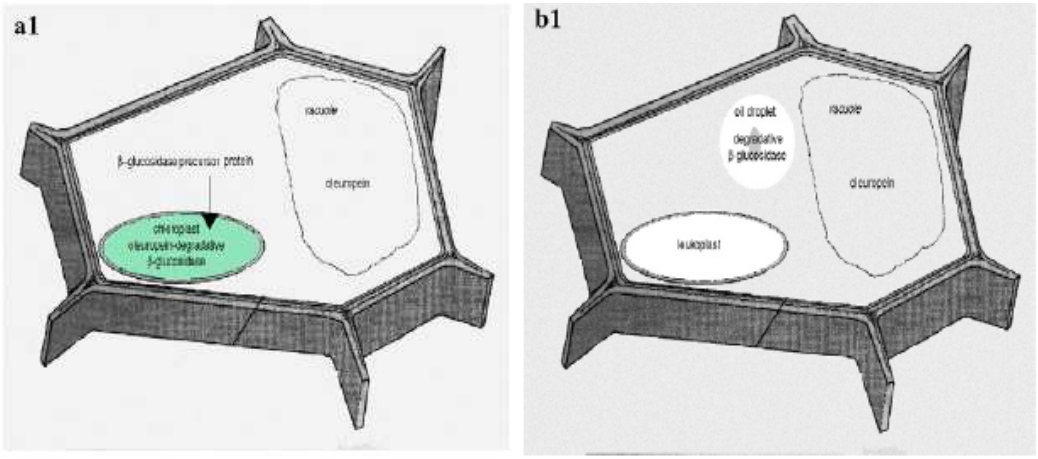


Fig3.2 Models of cell compartmentalization of oleuropein-degradative β-glucosidase and oleuropein s in outer mesocarp cells (a1) and in the inner mesocarp cells (b1) In the outer mesocarp cell the oleuropein is stabilised in the vacuole of cells, the enzyme is located inside the chloroplasts. A β-glucosidase precursor protein is also in the cytoplasm, before going in the chloroplast organelle: alternatively, in the cell of the inner the oleuropein degradative β-glucosidase is localized inside the core of oil droplets and the oleuropein is stay in the vacuole compartment.*Modified from Mazzuca et al, 2006*

Glycoside hydrolases (EC 3.2.1.-) are a widespread group of enzymes which hydrolyse the glycosidic bonds between two or more carbohydrates or between a carbohydrate and a non-carbohydrate moieties. The completion of genome sequences of model plants *Arabidopsis thaliana*, which has 48 predicted β-glucosidases, and rice, which has about 50 predicted ß-glucosidases,

indicates that in plants these enzymes are encoding by multigene family The high ß-glucosidase multiplicity in plants is not surprising because plants produce hundreds of different ß-linked glucoconjugates (i.e. substrates), and they need a corresponding enzyme repertoire to hydrolyze these glucoconjugates specifically. The IUB-MB Enzyme nomenclature of glycoside hydrolases (GHs) is based on their substrate specificity and occasionally on their molecular mechanism; such a classification does not reflect the structural features of these enzymes. At least 100 families are currently available on CAZYweb server (http://www.cazy.org /CAZY/index.html). On the basis of their three-dimensional structure, GHs can be grouped into clans of related structure (Henrissat et al.,1997). Because there is a direct relationship between sequence and folding similarities, such a classification reflects the structural features of these enzymes better than their sole substrate specificity, helps to reveal the evolutionary relationships between these enzymes, and provides a convenient tool to derive mechanistic information

β-glucosidases belonging to the family 1 glycoside hydrolases, catalyze the hydrolysis of the glucosidic bond between the anomeric carbon (C1 of the glucose) and the glucosidic oxygen by a mechanism in which the anomeric configuration of the glucose is retained (Davies and Henrissat, 1995). The catalytic mechanism is illustrated in fig. 3.2. Two conserved glutamic acid residues serve as a catalytic nucleophile and a general acid/base catalyst, respectively. In retaining b-glucosidases, the catalytic glutamic acid residues are situated on opposite sides of the b-glucosidic bond of the docked substrate at a distance of _5.5 Å (Davies and Henrissat, 1995). As the initial step in catalysis, the nucleophile performs a nucleophilic attack at the anomeric carbon, which results in formation of a glucose–enzyme intermediate. In this process, aglicone departure is facilitated by protonation of the glucosidic oxygen by the acid catalyst. During the second catalytic step (deglucosylation), a water molecule is activated by the catalytic base to serve as a nucleophile for hydrolysis of the glucosidic bond and release of the glucose (Davies and Henrissat, 1995). Under suitable conditions, β-glucosidases can perform a transglucosylation in which the covalently bound glucose in the enzyme– glucose intermediate is transferred to an alcohol or a second sugar group. Even though this reaction has not been observed to occur in vivo, b-glucosidases such as cassava (*Manihot esculenta*) linamarase (b-glucosidase specific for the cyanogenic glucosides linamarin and lotaustralin) are used for industrial production of alkyl b-glucosides and show great industrial application potentials (Svasti et al., 2003; Hommalai et al., 2005).

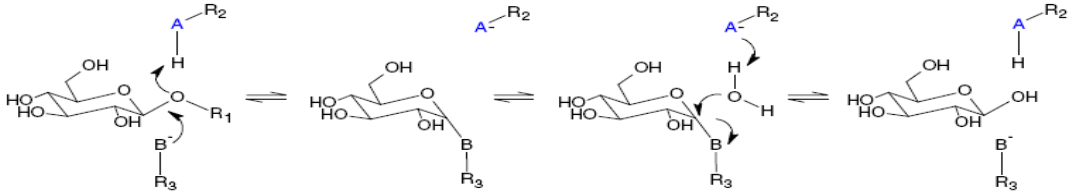


Fig. 3.3. Reaction mechanism of retaining b-glucosidases. A glutamic acid residue in the conserved TFNEP motif (Davies and Henrissat, 1995) serves as a general acid/basecatalyst (AH) while a glutamic acid residue in the I/VTENG motif (Davies and Henrissat, 1995) serves as a nucleophile (B). The reaction cycle is specified in the text. Modified from Morant et al 2008.

Phylogenetic relationship and structural similarity between β-glucosidases involved in plant defense

The β-glucosidases involved in hydrolysis of defense compounds upon tissue disruption form three distinct clusters in the phylogenetic tree shown in fig. 3.4: eudicotyledenous O-β-glucosidases, dicotyledenous S-β-glucosidases (myrosinases) and monocotyledenous O-β-glucosidases. Although separated into three different clusters, these b-glucosidases show a high degree of amino acid sequence identities and very similar overall tertiary structures. (Morant et al., 2008).

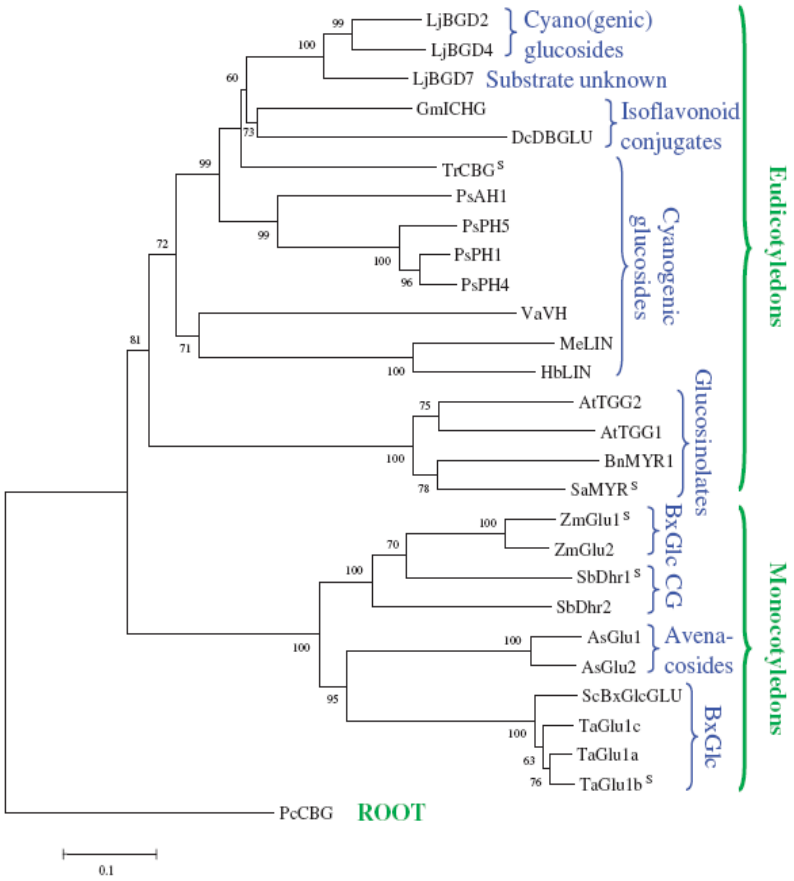


Fig. 3.4 eudicotyledenous O-β-glucosidases, dicotyledenous S-β-glucosidases (myrosinases) and monocotyledenous O-β-glucosidases. Although separated into three different clusters, these b-glucosidases show a high degree of amino acid sequence identities and very similar overall tertiary structures (Morant et al 2008).

Genomic features

At the moment, for *Olea europaea* only one nucleotide sequence encoding for putative GH1 (ACCESSION N°AAL93619.1) have been obtained from mRNA extracted from leaves (*cultivar* "Koroneiki". To date, no studies have specifically focused on the identification of individual functions of the ß-glucosidase isozymes in *Olea europaea*. An EST database which brings about 22,904 gene clusters becomes recently available (Alagna et al 2009).

The specific aim of this section of my work was to obtain preliminary data on DNA and cDNA sequence and organization of the putative members of GH1-family from *Olea europaea* cv "Carolea", a grove very susceptible to olive fly infestation, using a PCR approach in order to provide a basis for studying their functioning and expression.

3.1 MATERIALS AND METHODS

Genomic DNA and total RNA was extracted using a C-TAB method (Chang et al.1993 *mod*.) from fresh leaves and fruits. In order to remove contaminant DNA, RNA samples were treated with DNase I. Almost 3 μg of total RNA were used as a template to synthesize first-strand cDNA with Protoscript First Strand cDNA synthesis Kit (BioLabs), according to the manufacture's protocol. Genomic DNA and cDNA were amplified with a set of primers (*Obglc-F*; *Obglc-R ;tab1*) designed on conserved regions of β-glucosidase sequences of eudicotyledons, in particular based on the sequence of Olea bglc-mRNA cv Koroneiki GenBank Accession No. AY083162, in order to obtain fragments overlapping all bglc CDS region and the flanking regions (Tab 1). PCR products, selected according to the hypothetical molecular size assessed on Agarose gel against a molecular size standard, were cut from the gel and cleaned with QIAquick Gel Extraction Kit (QUIAGEN). Eluted fragments were sequenced with Beckman Coulter CEQ2000 .and electropherograms were analyzed with SequenceScanner v1.0 and manually cleaned for reading errors. Cleaned sequences were aligned using the ClustalW algoritm (Thompson et al., 1994), run in the BioEdit 5.0.9 software package (Hall,1999) .

Tab3.1 Primers design primers sequences (*Obglc-F*; *Obglc-R*). Primers Obglc- 1-6 were designed based on nucleotidic sequence of *bglc*-mRNA cv Koroneiki GenBank Accession No. AY083162 Primers Obglc- E-N were designed based on respectively: T(L/F)NEP and V(Y/I)TENG conserved strength among eucotyledons β-glucosidases.

PRIMER CODE	SEQUENCE 5'→3'	Tm	REGION	CONSERVED MOTIF
Obglc- 1F	TTA CAC TAC TTC AAA GAG CAC C	62	5' UTR	
Obglc- 1R	TCC TTT ATT GGA ACA TAA CGA G	60	3' UTR	
Obglc- 5F	TTG GAT TAC CTT GAA TGA GCC	60	CDS	
Obglc- 5R	GTG CCG GGA TTT CCA TAC T	58	CDS	
Obglc- 6F	ATA TGT TCAA GGA CGA TGT TGT C	64	CDS	
Obglc- EF	TGG ATT ACC TTG AAT GAG CCA TG	66	CDS	TLNEP
Obglc- ER	CAT GGC TCA TTC AAG GTA ATC CA	66	CDS	TLNEP
Obglc- Nf	ATA TAC ATC ACC GAG AAC GGA	60	CDS	TENG
Obglc- Nr	TCC GTT CTC GGT GAT GTA TAT	60	CDS	TENG

Proteomic and bioinformatic tools allowed us to identify the enzyme isoforms and their timing of expression in developing organs. To reach these goals we compared the biomolecular data with the immunogold analyses in leaf cells at different stages of leaf development and during fruit ripening, by identification of sequence region encoding for amino acid strength recognized by the antibody against the β-glucosidase from *Polygonum tinctorium*

Database searching for to identified other putative member of β-glucosidases family in Olea, genomic sequence of Obglc Carolea was used as query against Olea EST database (www.oleadb.it) to search homology sequence

3.2 RESULT

Polymorphism analysis of cDNA sequences Carolea and Koroneiki

After the PCR amplification of cDNA Carolea using specific primers an amplicones of 1795 bp was obtained. The sequence obtained from cDNA Carolea were alignment with *bglc* AY083162 obtained from cultivar Koroneiki. Along the alignment were scored sixteen polymorphic positions (Tab 3.2.1). There are nine 9 putative SNP (alignment position 43, 90, 507, 1020, 1511, 1624, 1723, 1798, 2194) between cDNA Carolea and AY083162.1. Among these, six are in the CDS (90, 507, 1020, 1511, 1624, 1723) one in the 5'UTR (43) two in the 3'UTR (1798, 2194) . In in the 3'UTR region also there are two deletions. After the conceptual translation of nucleotide sequence bglc Carolea using BESTORF Softberry (http://linux1.softberry.com/), the deduced amino acid sequences were aligns with AY083162.1. Five amino acid substitutions were identified between Carolea and Koroneiki protein sequence (Tab 3.2.2).

Tab 3.2.1 Polymorphic position of bglc sequence among mRNA sequence Carolea and were scored 9 putative SNP and 2 Deletions (see text).

	SNP P 43	*SNP* P 90	*SNP* P 507	*SNP* P 1020	*SNP* P 1511	*SNP* P 1624	*SNP* P 1723	*SNP* P 1798	*SNP* P 2194	*DEL* P 2236	*DEL* P 2244
AY083162.1	C	A	A	A	A	C	C	T	C	T	A
cDNA Carolea	T	T	T	T	C	T	A	C	T	-	-

Tab 3.2.2 List of amino acid substitutions between BGCL protein sequences deduced from cDNA Carolea and Koroneiki

	P15	P91	P 177	P231	P421
AY083162.1 Koroneiki	S	F	Q	K	R
BESTORF_ cDNA Carolea	T	Y	K	Q	K

Identification of region sequence encoding for BGLC extracted from olea tissue .Amino acidic strength recognized by the antibody against the β-glucosidase from *Polygonum tinctorium*

Tab 3.2.3 Amino acidic sequence of SPOT1 and SPOT2 recognized by the antibody against the β-glucosidase from *Polygonum tinctorium*

	AMINO ACIDIC SEQUENCE SPOT	
EPITOPO 1	VVVMKKLGLKAYRFSLSWPRIG	SPOT 1
EPITOPO 2	HYHQEHLY	SPOT 2

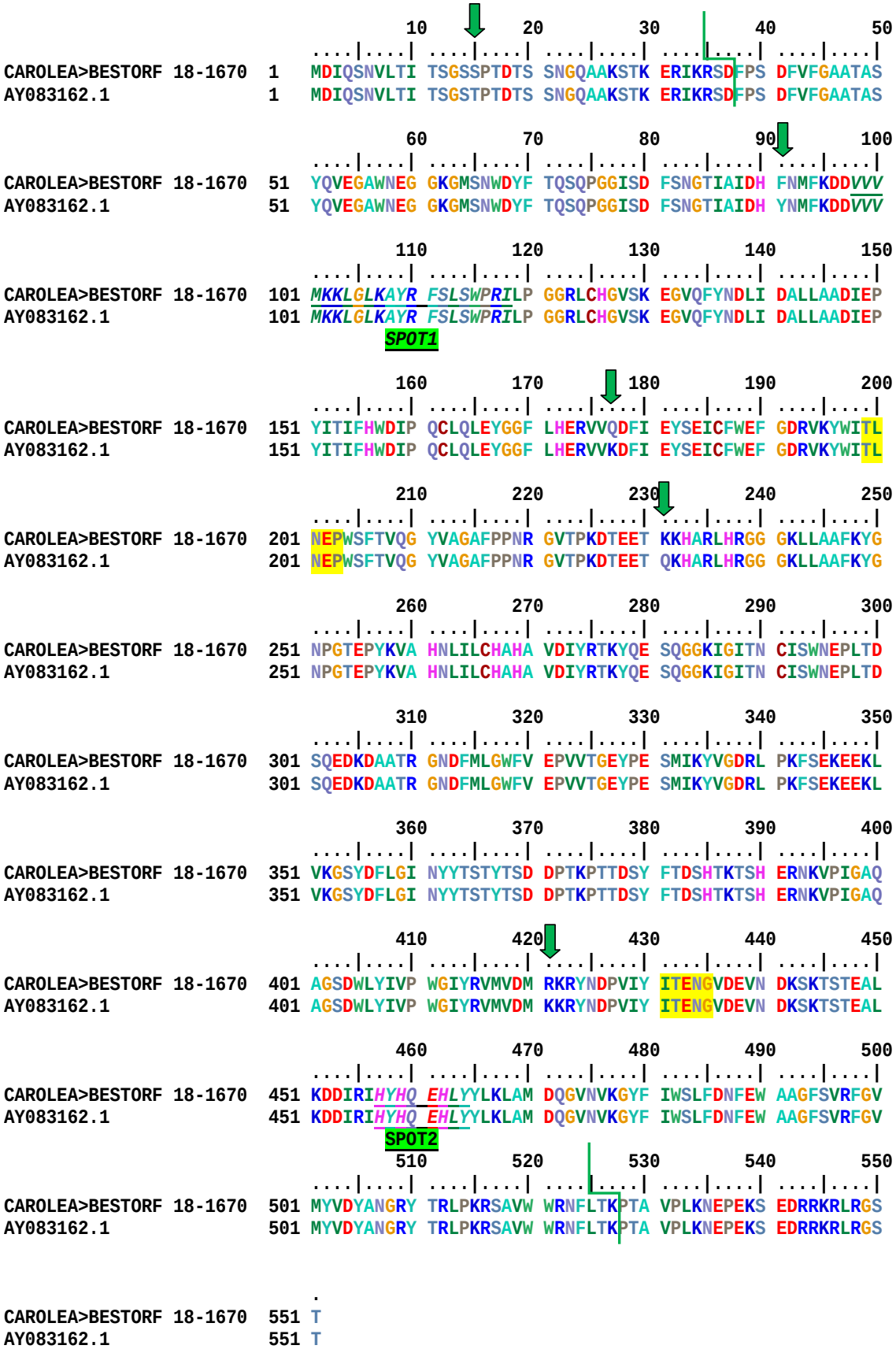


Fig alignment bglc cDNA Carolea and AY083162.1. allows shows polymorphic position, green line region Glyco_hydro_1 (start position aa 38 end position 527), yellow catalytic site

Partial genetic structure of β-glucosidase in Olea europaea

After the PCR reaction using O*bglc*- 1F Obglc- 5R specific primers, a fragment of approximately 1638 bp was obtained. The putative genomic partial sequence of O*bglc* Carolea was aligns with sequences of cDNA obtained from Carolea

Alignment of genomic sequences and cDNA coverage a region comprise between 23-1667 nt of cDNA sequence.Five polymorphic positions between cDNA and gDNA were scored: three insertions in P 798, 828, 829; two inversions Purine/Pyrimidine in P 819 and Pyrimidine/Purine in P 812 (Tab 3.2.3)

Tab. 3.2.3 List of polymorphic position scored among genomic and mRNA sequence

	INS P306	*INV* P320	*INV* P762	*INS* P336	*INS* P337
GENOMIC Carolea	-	C	A	-	-
mRNA Carolea	G	G	T	A	A

On the basis of comparison between bglc genomic sequence with the cDNA sequence, the partial genomic structure was established (fig 3.2.1).

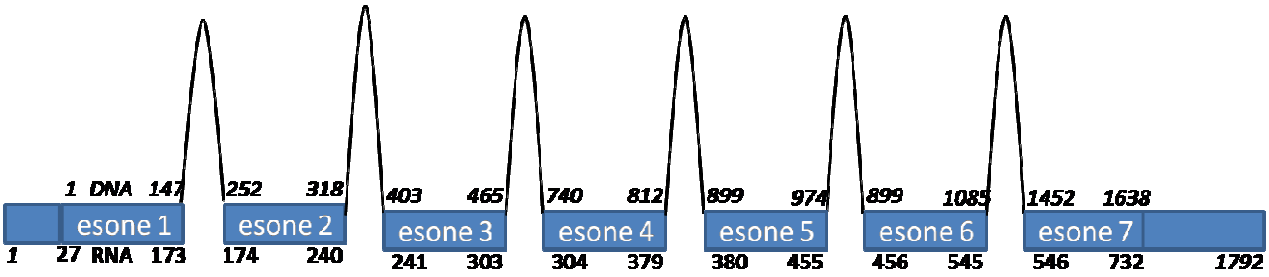
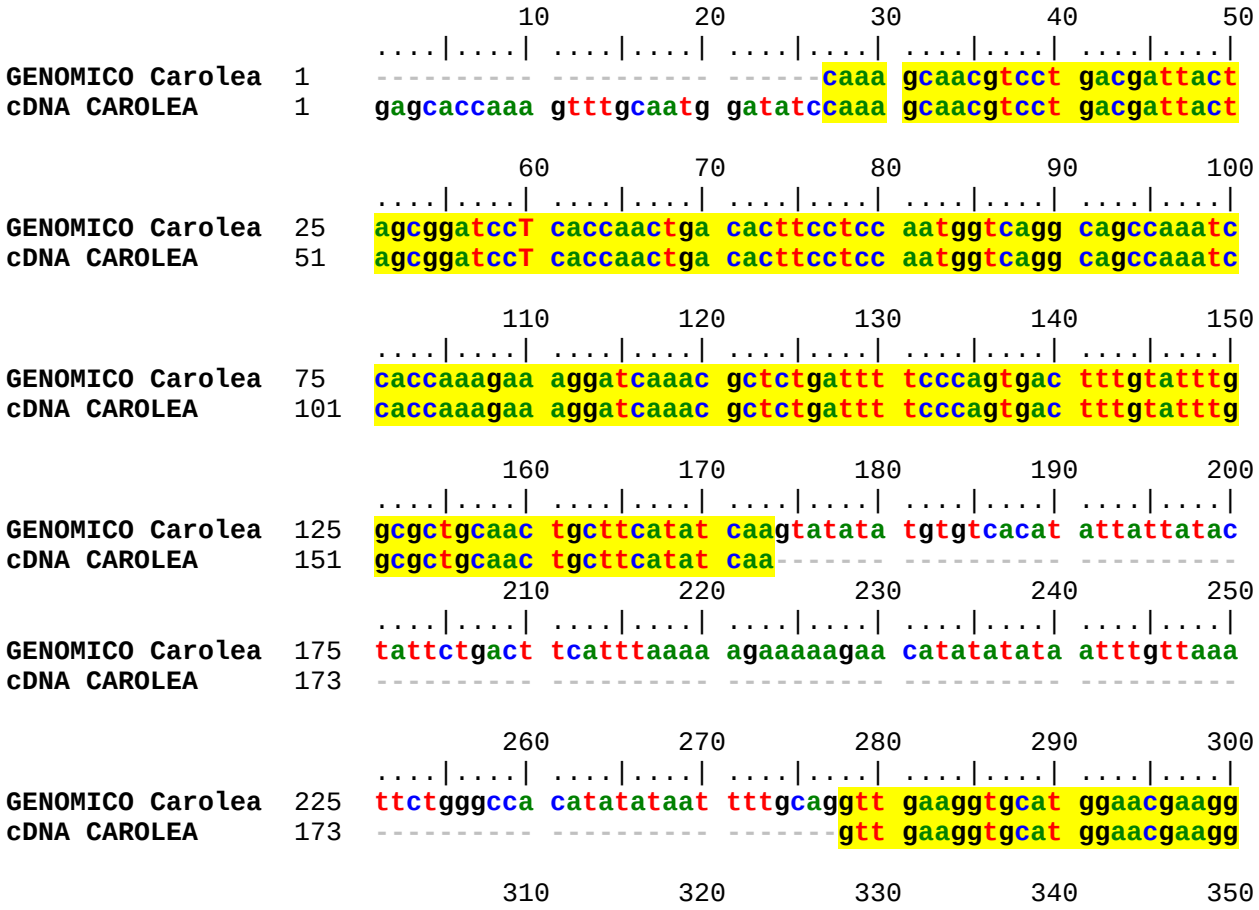


Fig 3.2.1 Partial genomic structure of β glucosidases. The gene consist at least of seven exons and six introns

Alignment bglc genomic sequence and cDNA carolea



```
                    ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO Carolea  275 aggtaaaggc atgagtaatt gggattactt tacacagagt caaccaggtc
cDNA CAROLEA      197 aggtaaaggc atgagtaatt gggattactt tacacagagt caac------

                            360        370        380        390        400
                    ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO Carolea  325 tcctcttttt ccaatctttt catgaatata ttatatagtg ttccatgtct
cDNA CAROLEA      240 ---------- ---------- ---------- ---------- ----------


                            410        420        430        440        450
                    ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO Carolea  375 attgctgaga atatgtttat tggactggca ggtggtattt ccgactttag
cDNA CAROLEA      240 ---------- ---------- --------ca ggtggtattt ccgactttag

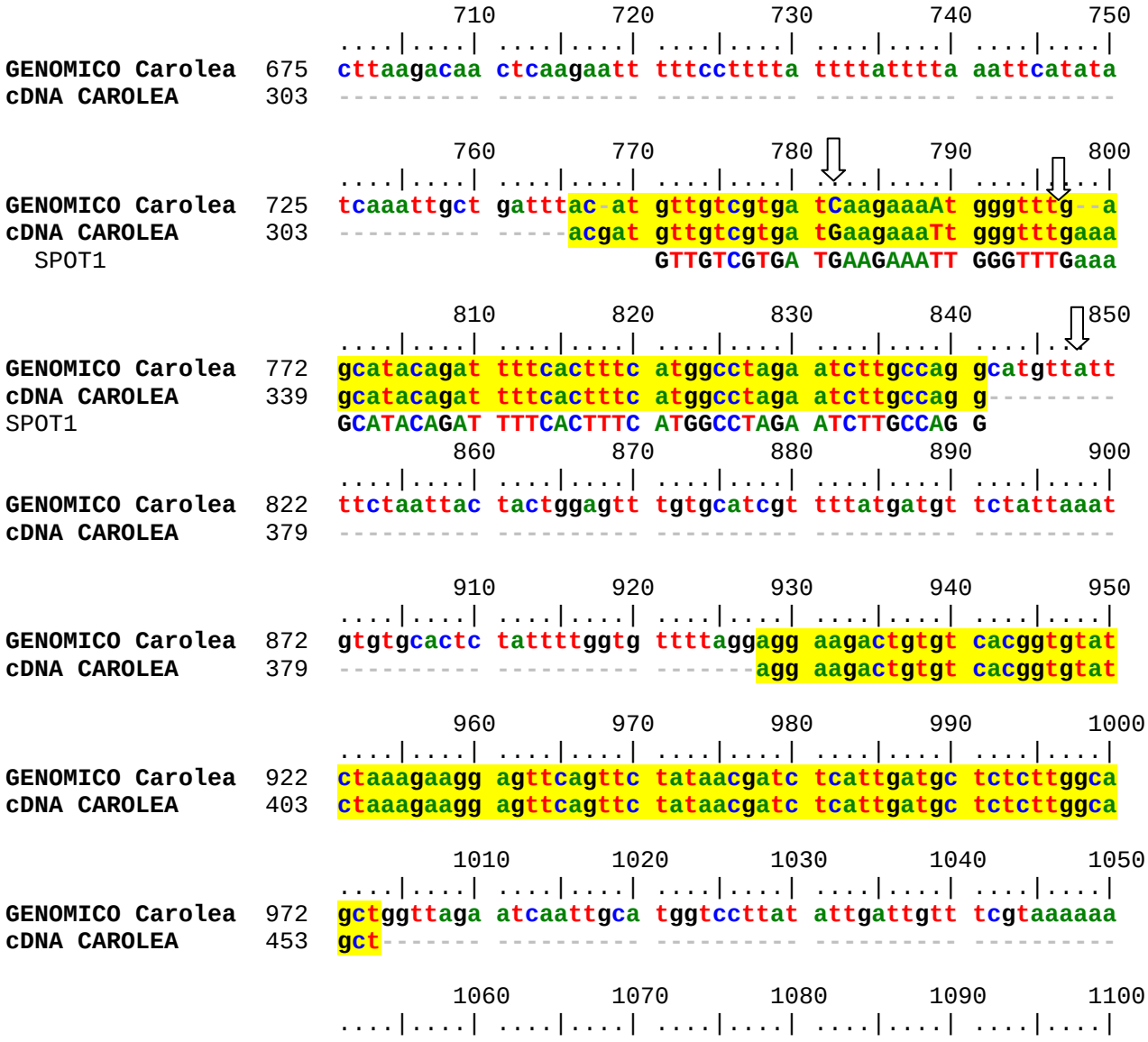
                            460        470        480        490        500
                    ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO Carolea  425 caatggcact attgcaattg atcacttTaa tatgttcaag gtatactttc
cDNA CAROLEA      263 caatggcact attgcaattg atcacttTaa tatgttcaag g---------

                            510        520        530        540        550
                    ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO Carolea  475 agtaatattt actatcncat gctctcgagc gagttagata aattcaactt
cDNA CAROLEA      303 ---------- ---------- ---------- ---------- ----------

                            560        570        580        590        600
                    ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO Carolea  525 ttttaactg gcttgtacgc taatatctat gttacnttcg tattaaacat
cDNA CAROLEA      303 ---------- ---------- ---------- ---------- ----------

                            610        620        630        640        650
                    ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO Carolea  575 ggaatttaga tgaattaaaa aagaaactta aaataaaaat tccgaatttn
cDNA CAROLEA      303 ---------- ---------- ---------- ---------- ----------

                            660        670        680        690        700
                    ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO Carolea  625 nncttcattt tgctttactc atgaaaaagt tttccatnat tccttttact
cDNA CAROLEA      303 ---------- ---------- ---------- ---------- ----------
```


710 720 730 740 750
GENOMICO Carolea 675 cttaagacaa ctcaagaatt tttccttta ttttatttta aattcatata
cDNA CAROLEA 303
760 770 780 790 800
GENOMICO Carolea 725 tcaaattgct gatttac-at gttgtcgtga tCaagaaaAt gggttt-g--a
cDNA CAROLEA 303 acgat gttgtcgtga tGaagaaaTt gggtttgaaa
SPOT1 GTTGTCGTGA TGAAGAAATT GGGTTTGaaa
810 820 830 840 850
GENOMICO Carolea 772 gcatacagat tttcactttc atggcctaga atcttgccag gcatgttatt
cDNA CAROLEA 339 gcatacagat tttcactttc atggcctaga atcttgccag g
SPOT1 GCATACAGAT TTTCACTTTC ATGGCCTAGA ATCTTGCCAG G
860 870 880 890 900
GENOMICO Carolea 822 ttctaattac tactggagtt tgtgcatcgt tttatgatgt tctattaaat
cDNA CAROLEA 379
910 920 930 940 950
GENOMICO Carolea 872 gtgtgcactc tattttggtg ttttaggagg aagactgtgt cacggtgtat
cDNA CAROLEA 379 agg aagactgtgt cacggtgtat
960 970 980 990 1000
GENOMICO Carolea 922 ctaaagaagg agttcagttc tataacgatc tcattgatgc tctcttggca
cDNA CAROLEA 403 ctaaagaagg agttcagttc tataacgatc tcattgatgc tctcttggca
1010 1020 1030 1040 1050
GENOMICO Carolea 972 gctggttaga atcaattgca tggtccttat attgattgtt tcgtaaaaaa
cDNA CAROLEA 453 gct
1060 1070 1080 1090 1100

```
GENOMICO Carolea  1022 actattattg cataaatcat atttcatatt atcttctcac tttctgtcga
cDNA CAROLEA      455  ---------- ---------- ---------- ---------- ----------

                          1110       1120       1130       1140       1150
                     ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO Carolea  1072 tccctttgt gcagacatag agccatatat aactatcttt cactgggata
cDNA CAROLEA      455  ---------- ---gacatag agccatatat aactatcttt cactgggata

                          1160       1170       1180       1190       1200
                     ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO Carolea  1122 ttccccaatg tttgcaacta gagtatggtg gcttcctaca tgaaagagtt
cDNA CAROLEA      493  ttccccaatg tttgcaacta gagtatggtg gcttcctaca tgaaagagtt

                          1210       1220       1230       1240       1250
                     ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO Carolea  1172 gtgtaagtaa ctcaatcttt catcctatgg cacattacta agatatttat
cDNA CAROLEA      543  gtg------- ---------- ---------- ---------- ----------

                          1260       1270       1280       1290       1300
                     ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO Carolea  1222 tgaacctgaa aagtgtatcc agattttaaa aaaataaaaa ttaaataata
cDNA CAROLEA      545  ---------- ---------- ---------- ---------- ----------

                          1310       1320       1330       1340       1350
                     ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO Carolea  1272 ataatcattt gctccaacat tataaaagag aaaatacaaa atttttattt
cDNA CAROLEA      545  ---------- ---------- ---------- ---------- ----------

                          1360       1370       1380       1390       1400
                     ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO Carolea  1322 aaaaataaaa taaaattgat tacaatttta gtaaaatggt ggacaatctt
cDNA CAROLEA      545  ---------- ---------- ---------- ---------- ----------

                          1410       1420       1430       1440       1450
                     ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO Carolea  1372 ctacaaagat taaagttcca gcttaacaag ttctaccata ttttgatcta
cDNA CAROLEA      545  ---------- ---------- ---------- ---------- ----------

                          1460       1470       1480       1490       1500
```

```
                    ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO Carolea    1422 ttgagccatt ttttgtgtct ataatacagg Caggatttta ttgagtattc
cDNA CAROLEA        545  ---------- ---------- ---------- Caggatttta ttgagtattc

                             1510       1520       1530       1540       1550
                    ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO Carolea    1472 tgagatttgc ttctgggaat ttggtgatcg ggtgaaatat tggattacct
cDNA CAROLEA        566  tgagatttgc ttctgggaat ttggtgatcg ggtgaaatat tggattacct

                             1560       1570       1580       1590       1600
                    ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO Carolea    1522 tgaatgagcc atggtccttt actgttcaag gatatgtggc tggTgctttt
cDNA CAROLEA        616  tgaatgagcc atggtccttt actgttcaag gatatgtggc tggTgctttt

                             1610       1620       1630       1640       1650
                    ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO Carolea    1572 ccgcccaatc gtggtgtaac tccgaaagat actgaagaaa ctAaaaagca
cDNA CAROLEA        666  ccgcccaatc gtggtgtaac tccgaaagat actgaagaaa ctAaaaagca

                             1660       1670       1680       1690       1700
                    ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO Carolea    1622 tgcccgtctt catagag
cDNA CAROLEA        716  tgcccgtctt catagaggtg gaggaaaact tctagctgct ttcaagtatg
```

As has been observed in fig (3.2.1) the gene bglc comprise at least seven exons, which were interrupted by six introns (Tab3.2.4): first intron long 103bp star with a short repetitive sequence (GTATATATGTG), G+C content = 23.08% A+T content = 76.92%, second intron is long
84 bp star , A+T content = 66.67%,

Tab3.2.4 principal featujre of intron sequence of bglc

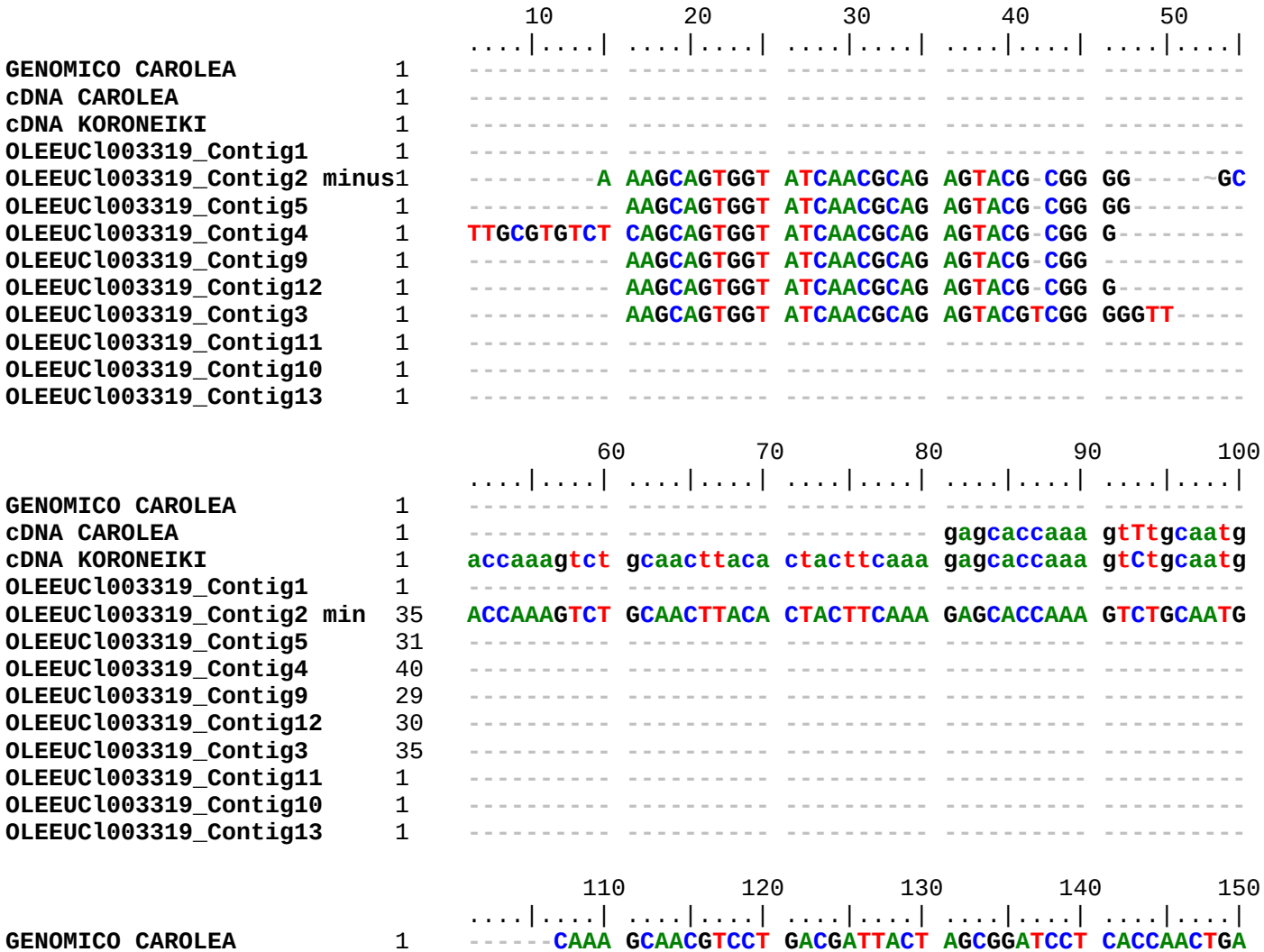
INTRON	JUNCTION	LENGTH	% A+T content	note
INTRON 1	A/G	103	76.92%	GTATATATGTG
INTRON 2	C/C	84	66.67%	CAGGT *flanking region*
INTRON 3	G/A	274	73.36%	
INTRON 4	G/A	86	68.60%	
INTRON 5	T/G	110	70.00%	
INTRON 6	G/C	277	76.53%	

Using blastn analysis was founded positive match for 10 EST clusters (tab 3,2.5). Clusters which have homology with Obglc Carolea were aligned to detect putative mRNA variants (see in appendix complete alignment).

Tab3.2.5: List of EST clusters producing significant alignments with O*bglc* Carolea

EST cluster producing significant alignments:	Score (bits)	EValue
>OLEEUCl003319|Contig10	954	0.0
>OLEEUCl003319|Contig11	391	e^{-111}
>OLEEUCl003319|Contig2	353	e^{-100}
>OLEEUCl003319|Contig12	305	$1e^{-085}$
>OLEEUCl003319|Contig3	268	$3e^{-074}$
>OLEEUCl003319|Contig13	168	$2e^{-044}$
>OLEEUCl003319|Contig9	125	$2e^{-031}$
>OLEEUCl003319|Contig6	26	0.16
>OLEEUCl003319|Contig5	26	0.16
>OLEEUCl003319|Contig7	24	0.64

Alignment Between bglc Genomic Carolea, mRNA Carolea mRNA Koroneiki, EST sequence which have positive homology against Genomic Carolea



```
cDNA CAROLEA                 21   gatatccaaa gcaacgtcct gacgattact agcggatccT caccaactga
cDNA KORONEIKI               51   gatatccaaa gcaacgtcct gacgattact agcggatccA caccaactga
OLEEUCl003319_Contig1        1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min    85   GATATCCAAA GCAACGTCCT GACGATTACT AGCGGATCCT CACCAACTGA
OLEEUCl003319_Contig5        31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4        40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9        29   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12       30   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3        35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11       1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10       1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig13       1    ---------- ---------- ---------- ---------- ----------

                                        160        170        180        190        200
                                   ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA             45   CACTTCCTCC AATGGTCAGG CAGCCAAATC CACCAAAGAA AGGATCAAAC
cDNA CAROLEA                 71   cacttcctcc aatggtcagg cagccaaatc caccaaagaa aggatcaaac
cDNA KORONEIKI               101  cacttcctcc aatggtcagg cagccaaatc caccaaagaa aggatcaaac
OLEEUCl003319_Contig1        1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min    135  CACTTCCTCC AATGGTCAGG CAGCCAAATC CACCAAAGAA AGGATCAAAC
OLEEUCl003319_Contig5        31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4        40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9        29   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12       30   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3        35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11       1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10       1    ---------- ---------- ---CCAAATC CACCAAAGAA AGGATCAAAC
OLEEUCl003319_Contig13       1    ---------- ---------- ---------- ---------- ----------

                                        210        220        230        240        250
                                   ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA             95   GCTCTGATTT TCCCAGTGAC TTTGTATTTG GCGCTGCAAC TGCTTCATAT
cDNA CAROLEA                 121  gctctgattt tcccagtgac tttgtatttg gcgctgcaac tgcttcatat
cDNA KORONEIKI               151  gctctgattt tcccagtgac tttgtatttg gcgctgcaac tgcttcatat
OLEEUCl003319_Contig1        1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min    185  GCTCTGATTT TCCCAGTGAC TTTGTATTTG GCGCTGCAAC TGCTTCATAT
OLEEUCl003319_Contig5        31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4        40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9        29   ---------- ---------- ---------- ---------- ----------
```

```
OLEEUCl003319_Contig12     30   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3      35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11     1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10     28   GCTCTGATTT TCCCAGTGAC TTTGTATTTG GCGCTGCAAC TGCTTCATAT
OLEEUCl003319_Contig13     1    ---------- ---------- ---------- ---------- ----------

                                       260        270        280        290        300
                                .....|.....| .....|.....| .....|.....| .....|.....| .....|.....|
GENOMICO CAROLEA           145  CAAGTATATA TGTGTCACAT ATTATTATAC TATTCTGACT TCATTTAAAA
cDNA CAROLEA               171  caa------- ---------- ---------- ---------- ----------
cDNA KORONEIKI             201  caa------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig1      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  235  CAA------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig5      31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      29   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12     30   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3      35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11     1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10     78   CAAGTATATA TGTGTCACAT ATTATTATAC TATTCTGACT TCATTTAAAA
OLEEUCl003319_Contig13     1    ---------- ---------- ---------- ---------- ----------

                                       310        320        330        340        350
                                .....|.....| .....|.....| .....|.....| .....|.....| .....|.....|
GENOMICO CAROLEA           195  AGAAAAAGAA CATATATATA ATTTGTTAAA TTCTGGGCCA CATATATAAT
cDNA CAROLEA               173  ---------- ---------- ---------- ---------- ----------
cDNA KORONEIKI             203  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig1      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  237  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig5      31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      29   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12     30   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3      35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11     1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10     128  AGAAAAAGAA CATATATATA ATTTGTTAAA TTCTGGGCCA CATATATAAT
OLEEUCl003319_Contig13     1    ---------- ---------- ---------- ---------- ----------

                                       360        370        380        390        400
```

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                                  ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA                  245  TTTGCAGGTT GAAGGTGCAT GGAACGAAGG AGGTAAAGGC ATGAGTAATT
cDNA CAROLEA                      173  -------gtt gaaggtgcat ggaacgaagg aggtaaaggc atgagtaatt
cDNA KORONEIKI                    203  -------gtt gaaggtgcat ggaacgaagg aggtaaaggc atgagtaatt
OLEEUCl003319_Contig1             1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min         237  -------GTT GAAGGTGCAT GGAACGAAGG AGGTAAAGGC ATGAGTAATT
OLEEUCl003319_Contig5             31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4             40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9             29   ---------- ---------- -----GAAGG AGGTAAAGGC ATGAGTAATT
OLEEUCl003319_Contig12            30   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3             35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11            1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10            178  TTTGCAGGTT GAAGGTGCAT GGAACGAAGG AGGTAAAGGC ATGAGTAATT
OLEEUCl003319_Contig13            1    ---------- ---------- ---------- ---------- ----------

                                           410        420        430        440        450
                                  ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA                  295  GGGATTACTT TACACAGAGT CAACCAGGTC TCCTCTTTTT CCAATCTTTT
cDNA CAROLEA                      217  gggattactt tacacagagt caac------ ---------- ----------
cDNA KORONEIKI                    247  gggattactt tacacagagt caac------ ---------- ----------
OLEEUCl003319_Contig1             1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min         281  GGGATTACTT TACACAGAGT CAACCAGGTG GTATTTCCGA CTTTAGCAAT
OLEEUCl003319_Contig5             31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4             40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9             55   GGGATTACTT TACACAGAGT CAAC------ ---------- ----------
OLEEUCl003319_Contig12            30   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3             35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11            1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10            228  GGGATTACTT TACACAGAGT CAACCAGGTC TCCTCTTTTT CCAATCTTCT
OLEEUCl003319_Contig13            1    ---------- --CACAGAGT CAAC------ ---------- ----------

                                           460        470        480        490        500
                                  ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA                  345  CATGAATATA TTATATAGTG TTCCATGTCT ATTGCTGAGA ATATGTTTAT
cDNA CAROLEA                      240  ---------- ---------- ---------- ---------- ----------
cDNA KORONEIKI                    270  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig1             1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min         331  GGCACTATTG CAATTGATCA CTTTAATATG TTCAAGGACG ATGTTGTCGT
OLEEUCl003319_Contig5             31   ---------- ---------- ---------- ---------- ----------
```

```
OLEEUCl003319_Contig4       40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9       78   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12      30   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3       35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10      278  CATGAATATA TTATATAGTG TTCCATGTCT ATTGCTGAGA ATATGTTTAT
OLEEUCl003319_Contig13      12   ---------- ---------- ---------- ---------- ----------

                                        510        520        530        540        550
                                   ....|....| ....|....| ....|....| ....|....| ....|....|

GENOMICO CAROLEA            395  TGGACTGG-- ---------- ---------- ---------- ----------
cDNA CAROLEA                240  ---------- ---------- ---------- ---------- ----------
cDNA KORONEIKI              270  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig1       1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min   381  GATGAAGAAA TTGGGTTTGA AAGCATACAG ATTTTCACTT TCATGGCCTA
OLEEUCl003319_Contig5       31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4       40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9       78   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12      30   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3       35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10      328  TGGACTGG-- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig13      12   ---------- ---------- ---------- ---------- ----------
```

3.3-DISCUSSIONS

In our experiments for gene search, we have found one mRNA sequence and one gene sequence encoding for member of BGLC family in Olea genome.

Alignment of genomic sequences and cDNA coverage a region comprise between 23-1667 nt of cDNA sequence. Analysis of polymorphism revealed that among mRNA sequence and genomic sequence there are polymorphic sites and these suggest the existence of mechanisms of post – transcriptional modifications on bglc mRNA sequence that can

BGLC gene was organized in at least of seven exons and six introns.

In order to identify on nucleotide sequences conserved amino acidic region, we have identified nucleotide strength encoding for epitope recognized by anti-β-glucosidase antibody. Interesting, sequence of epitope 1 is encoding only by exon four the region encoding for amino acid strength recognized by the spot two is in a region do not coverage by genomic sequence. Blast searching against protein database using the sequence of exon four as a query shows positive match for most β-glucosidase of plants. This data suggest that exon four encode for a conserved region in β-glucosidase of plants. Instead the same blast searching for the region recognized by the second spot do not found positive match, and then the amino acid strength recognized by the epytope is specific of *Olea europaea.*

By blastn analysis of genomic sequence against olea EST database for homology sequence were scored ten different EST cluster which have positive match with genome sequence.

Alignment comparison between genomic sequence and cDNA Carolea and ESTs sequence in database suggest that there are at least two different genes encoding for β-glucosidase family.

CHAPTER 4

FUNCTIONAL ADAPTATION IN *POSIDONIA OCEANICA*

Introduction

Seagrass loss has been attributed to a suite of environmental and anthropogenic factors, but still little is known about the mechanism and the causes of this global decline. The Mediterranean coastline is undergoing fast environmental changes, which are imposing a strong selective pressure to the long living *P. oceanica* meadows, particularly the ones at the extremes of the environmental and geographic limits of the species. The broad focus of this study is the identification of the extent and of the genetic basis of *P .oceanica* ecological and physiological plasticity, in order to forecast molecular genetics mechanisms that may confer plasticity and tolerance at new environmental scenarios.

Fine- scale local adaptation

Local adaptation in response to fine-scale spatial heterogeneity is well documented in terrestrial ecosystems where there is a large and growing body of empirical evidence for local adaptation to fine-scale environmental heterogeneity in plants (reviewed in Linhart and Grant 1996). In contrast, in marine environments local adaptation of plants and other clonal marine organisms has rarely been documented or rigorously explored (e.g. Ayre 1995, Hämmerli 2002, Sherman et al., 2008). Despite the apparent uniformity of the marine environment, due to its continued structure, it can change largely at local scales. Populations that live in different areas can develop specific traits to respond to local conditions of light and nutrients availability, hydrodynamics, temperature, and salinity.

A clear genetic structure has been found at a Mediterranean scale in *P. oceanica* (see Chapter 2). Genetic clusters do not seem to represent distinct taxonomic groups and no clear data exist on the relation between different morphologies, especially as regards as leaf size, and genetic and functional differences.

At the single meadow scale, several studies carried out at *Lacco Ameno* (Ischia, Gulf of Naples) showed that *Posidonia* growing along the bathymetric gradient presents differences in several morphological and physiological traits such as chlorophyll content (Pirc, 1986), photosynthetic rate (Lorenti et al 1995) and life cycle shift (Buia e Mazzella 1991). Moreover, a genetic analysis based

on neutral microsatellite loci (Migliaccio et al, 2005) carried out at the same meadows confirmed that clones growing at different depths are genetically distinct. The genetic distinction, probably related to the above mentioned life cycle shift, with delayed ripening of reproductive structures in the deep stands, could have led to divergent adaptation to the different environmental conditions experienced by the two stands in particular during summer months, when the termocline is stable. Another case of possible local adaptation in *Posidonia* is represented by *P. oceanica* patches growing within the coastal lagoon named Stagnone di Marsala (Sicily): genetic analysis demonstrated the existence of a genetic distinction between plants growing outside and inside the lagoon where plants suffer from the extreme salinity and temperature, especially in summer (Tommasello et al, 2009).
These data open the field to examine if plants experiencing strong differences in environmental conditions, do really develop inheritable functional adaptations to their current conditions.

Main Environmental parameters

Light availability

Seagrass are sensitive to changes in light availability across a range of spatial scales, including individual leaf responses, shoot-scale responses and alteration of the meadow structure (Olesen et al 2002). Seagrass photosynthesis, and thereby, their growth, survival and depth distribution are directly linked to underwater irradiance (Dennison et al 1993).

Underwater light intensity is attenuated exponentially with water depth according to the Lambert–Beer equation ($I_z = I0 \times e^{-} Kd \times Z$), wherein light attenuation with increasing water depth is associated with absorption and scattering processes. Moreover, reduced underwater irradiance, may be caused by epiphytic and planktonic algal accumulations from excess anthropogenic nutrients, as well as by regional weather patterns (e.g. extreme storms and altered rainfall patterns). These processes are graphically summarized in Fig. 4.1 (Ralph et al 2007).

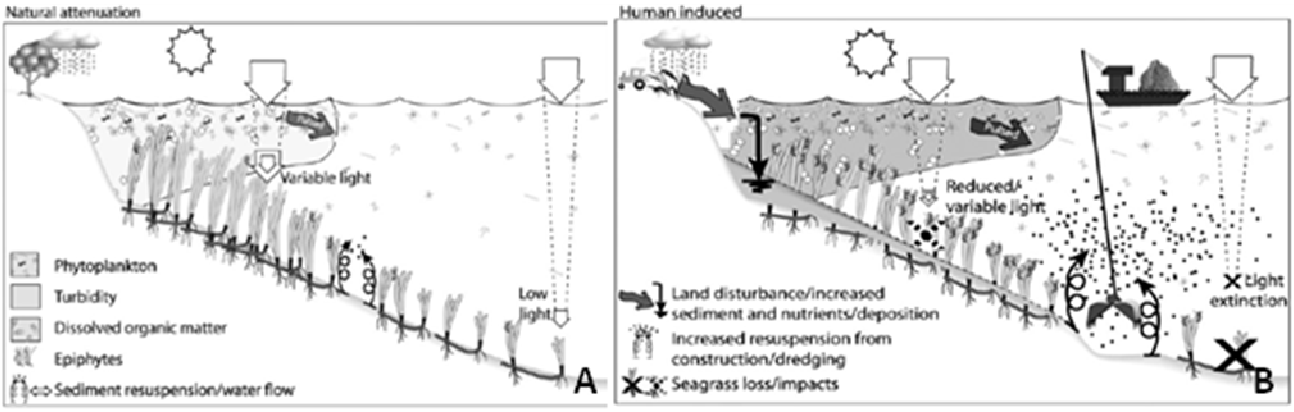


Fig 4.1 Conceptual model of light reduction and impact on seagrass with the following as the legend. *Panel A* Growth of seagrasses is strongly influenced by the optical quality of the water, including suspended sediments, dissolved organic matter and phytoplankton, which vary strongly according to run-off, as well as growth of microalgae on the leaves. *Panel B* Human activities, both one the land and in-water have led to changes in water quality that are reducing the light available to seagrasses. *Modified by Ralf et al. 2007*

The sensibility of seagrasses to reduction in light availability is attributed to their minimum light requirements (Dennison et al,1993), traditionally estimated as percent light at maximal depth limit using Kd, the light attenuation coefficient (Duarte, 1991; Dennison et al.,1993).

Minimum light requirements for seagrasses (2–37% Lee et al, 2007) are much higher than those of macroalgae and phytoplankton (about 1–3% SI; Duarte, 1991; Dennison et al., 1993).

Each seagrass species has unique physiological and morphological adaptations to light availability, and then the minimal light requirements should vary among different species (Dennison et al., 1993); for *P. oceanica* minimum light requirement is estimated in 9.2- 16 % (Dennison et al, 1993; Ruiz et al, 2001; Olesen,et al 2002).

Minimum light requirements may also be determined by establishing the relationship between underwater irradiance and photosynthetic production using photosynthesis-irradiance (*P-I*) curves which may provide valuable information for light requirements necessary to sustain optimal growth (Dunton et al, 1994). The application of P-I models to assess seagrass light requirements is complicated by variability.

Seasonal variance is found for P-I parameters, which are influenced by temperature, inter- and intra-specific differences (Lee et al, 2007). A single species growing under different environmental conditions has also been shown to possess different light requirements (Lee et al, 2007). Plants collected near their maximum depth limits have higher photosynthetic efficiencies (α) and lower light requirements for saturated photosynthesis than plants growing in shallower waters (Masini and Manning, 1997). Seagrasses can enhance light harvesting efficiencies through photo-acclimation

during low light conditions (West, 1990; Olesen and Sand-Jensen, 1993; Olesen et al., 2002), thus plants growing near their depth limit may have higher photosynthetic efficiencies. Lower I_c and I_{sat} and greater photosynthetic efficiencies have also been observed in seagrasses inhabiting inner harbors, where light availability is reduced through high turbidities, in comparison to less turbid outer harbor stations (Ruiz and Romero, 2003). The plants in the inner harbor were likely subjected to light-limitation, and changes in photosynthetic parameters possibly represent photo-acclimatization responses, allowing seagrasses to maximize carbon fixation under light limited conditions (Alcoverro et al., 1998; Ruiz and Romero, 2003).

Consistent with the P–I relationship, a seagrass growth response to light is described by a hyperbolic function with saturation kinetics (Peralta et al., 2002). Studies on *Zostera marina* (Short et al., 1995) show a linear growth response to light availability. This linear response, which is in contrast to hyperbolic functions with light saturation levels, suggests that plants in that study used all available light (up to full sunlight) to increase productivity (Short et al., 1995).

Seagrasses may exhibit several physiological and morphological responses to light reduction. The magnitude and time required to initiate a response may depend on species, light intensity and duration, and interactions between environmental conditions (e.g. temperature and nutrient availabilities; Longstaff and Dennison, 1999). In *Halophila pinifolia*, physiological responses to light reductions include changes in amino acid content, chlorophyll (chl) levels, and $\delta^{13}C$ occurred well before morphological changes are observed (e.g. biomass, shoot density, and canopy height; Longstaff and Dennison, 1999).

Photoadaptive responses in seagrasses to reductions in irradiance can result in the decrease of several plant traits, including chlorophyll a: b ratios and chlorophyll content (Pirc, 1986 ; Peralta et al., 2002). At reduced light conditions, seagrass performance can be enhanced through a number of photo-acclimatation responses that maximize the efficiency of light harvesting (Olesen et al., 2002) The acclimatation responses may be achieved through increased pigment content and greater leaf area per unit leaf biomass (increasing light absorption efficiencies; Lee and Dunton, 1997; Olesen et al., 2002) or in reduction of shoot-density during low light conditions and are possibly an effective acclimation response to reduce self-shading within the canopy (Olesen et al., 2002).

Temperature

Growth rates in seagrasses exhibit clear seasonal trends, with increasing growth during spring and summer, and decreasing growth during fall and winter (Lee and Dunton, 1996). It has been

suggested that these seasonal growth patterns are regulated by insulation and temperature or via an interaction of both. Because irradiance and temperature are related, it is difficult to separate these effects. Therefore, based on studies involving water temperature with seasonal growth patterns, numerous researchers consider temperature a primary factor controlling seasonal growth. Optimal growth temperatures for temperate species range from 11.5 °C to 26 °C, whereas the temperatures for tropical/subtropical species range from 23 °C to 32 °C. For *P. oceanica* authors report 32°C as optimal temperature for photosynthesis (Drew et al, 1978). Photosynthesis (examined using P–I curves P_{max}, I_k, I_c, and respiration rates) usually increases with increasing water temperature. The optimum temperature for photosynthesis can vary with underwater irradiance (Bulthuis, 1987). This implies that seagrasses growing in low light conditions have lower optimum temperatures for photosynthesis than plants in high light conditions. Plants at higher temperatures likely need more light to maintain positive carbon balances than those at lower temperatures. Thus, photosynthetic production in seagrasses is more susceptible to high water temperatures at reduced light conditions. Consequently, reductions in underwater light may be more harmful to seagrasses during summer rather than in winter (review Lee et al, 2007).

The role of temperature on seagrass growth is considerably more complicated than its effect on photosynthesis. The importance of temperature on nutrient availability and uptake, leaf senescence, nutrient partitioning within the plants, and respiration will contribute to the optimal temperature for seagrasses. Both respiration and photosynthesis increase with increasing water temperatures, but respiration usually increases more than photosynthesis at progressively higher temperatures, thus leading to a reduction in net photosynthesis (Pérez and Romero, 1992; Masini and Manning, 1997). From the available data, the optimum temperatures for both growth and photosynthesis were variable with seagrass species, but the optimum temperatures for growth were usually lower than those for photosynthesis, and for *P. oceanica* optimum growth is reported at 15.5±2.5°C (Lee, et al 2007).

Anthropogenic impact

Human impacts have a significant effect on the decline of seagrass ecosystems most notably manifesting in the near absence of seagrasses in industrialized ports and other areas of intense human coastal development (Short and Wyllie-Echeverria, 1996; Orth et al., 2006), with direct and indirect effects. Direct impacts include dredging, filling, land reclamation, dock and jetty construction, and some fisheries and aquaculture practices. Indirect impacts include nutrient and

sediment loading from the watershed, removal of coastal vegetation and hardening of the shoreline, and pollution.

Posidonia oceanica, as all sessile organisms, cannot easily move if environmental conditions change and become close to the tolerance limit of the species. Moving for seagrasses involve seed dispersal and settling with re-establishment of a new meadow in a more suitable area, a stochastic process which requires long time particularly for a slow growing species such as *P. oceanica*. It becomes than important to possess an adequate genetic background to adapt to environmental changes, in particular in the scenario of global climatic changes such as what we are facing today. The broad focus of this section of my thesis is to contribute in building the body of genome resources necessary to progress in the application of ecogenomic approaches in *Posidonia oceanica*.

The following approaches have been carried out:

.

- The genome size has been estimated using a flow cytometric approach. Flow cytometry is widely applied in the determination of nuclear DNA content and ploidy level in many organisms. An accurate determination of genome size provides basic information for breeders and molecular genetics (Leen et al, 2005). Results will be interpreted in relation to results obtained from EST and SSH libraries.

- The first seagrass specific EST-Database (DrZompo: http://drzompo.uni.muester.de/; see genomic resource) has been built in collaboration with research groups at Munster University and at the IFM-Geomar in Kiel (Germany).

- A SSH cDNA library has been built up between two different depths (-5 and -25 m) in *Lacco Ameno* meadow (Ischia, Gulf of Naples), from plants growing above and below the summer thermocline located about -15m depth, in order to select specific stress-responsive genes and genes associated with different light and temperature regimes.

4.1-MATERIAL AND METHODS

4.1.1 Estimation of the Genome Size

In collaboration with Dott.ssa R. Casotti (Functional and Evolutionary Ecology Lab, SZN) we have estimated the Genome size of *P. oceanica* via flow cytometry.

Sample preparation

Preparation of suspensions of intact nuclei for estimation of absolute DNA amounts has been almost universally performed following the method of Galbraith et al. (1983).
Given the different chemical composition and diversity of plant tissues, there is no general isolation buffer that works with all plants, although several protocols have been described for isolation of nuclei (Galbraith et al., 1983; Doležel et al., 1989; Louriero et al, 2007). We tested two nuclear isolation buffers developed by Louriero (Louriero et al 2007), the general purpose buffer (GPB) and the woody plant buffer (WPB) (See appendix for buffers composition)

Approximately 50–80 mg of non-meristematic non-pigmented basal part of leaf tissue was used for sample preparation. 1 mL of buffer solution was added to a Petri dish containing the plant tissue, which was chopped using a sharp razor blade for approx. 60 s. The resulting homogenate was filtered through a 80-mm nylon filter to remove large debris. Nuclei were stained with propidium iodide PI (50 ug/ml final concentration), and RNase (10 mg/l final concentration) was added to nuclear suspension to prevent staining of double-stranded RNA. For the standard CTN (calf thymocyte nuclei) was added (final concentration of 1 ul/ml). Nuclear suspension was incubated on ice and analyzed after 10 min, 20 min, 45min.

Flow cytometric analysis

DNA content of nuclei was measured by relative fluorescence with a FACScalibur flow cytometer (Becton, Dickinson, USA) .Instrument was set as following: E00 260-597-460 x 1 lin (LOG 312) 400 Threshold FL2 62 Low FR.

The following parameters were evaluated in each sample: relative fluorescence intensity of PI-stained nuclei (FL) and half peak coefficient of variation (CV) of the G0/G1 peak (to estimate nuclei integrity and variation in DNA staining).

Measurements of relative fluorescence intensity of stained nuclei were performed on a linear scale and typically, 5000–20 000 nuclei were analyzed for each sample (Galbraith et al., 1998). The absolute DNA amount of a sample is calculated based on the values of the G1 peak means.

The nuclear DNA content of each species was calculated according to the formula (Galbraith et al., 1998):

$$\text{Samples DNA content (pg)} = \frac{\text{sample G1 peak mean}}{\text{Standard G1 peak mean}} \times \text{standard DNA content (pg)}$$

Conversion of mass values into numbers of base pairs was done according to the factor 1 pg ¼ 978 Mbp (Doležel al., 2003)

4.1.2 Building of Suppression Subtractive Hybridization (SSH) Library

Samples collection

Shoots were collected by SCUBA diving in July, after the stabilization of the summer thermocline, from a meadow located in Lacco Ameno, Ischia (Gulf of Naples). Sampling was performed at two different depths, -5m (shallow station) and -25m (deep station; fig 4.2). Leaf tissue from about 40 shoots for each station, was cleaned from epiphytes and shock frozen in liquid nitrogen on the boat soon after collection. The main environmental parameters obtained during sampling are summarized in table 4.1. Values have been obtained using a Seabird Seacat Probe, operated from the boat and connected to a wired computer onboard.

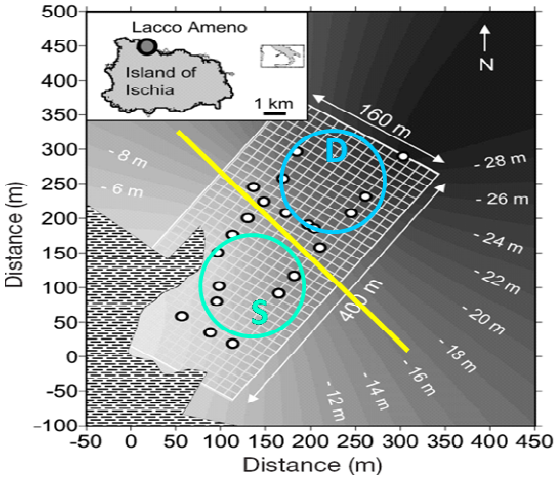


Fig4.1.1 Sampling area *yellow line* summer Thermocline circle green Shallow station, blue circle Deep station

Tab 4. 1 Values of temperature, salinity and PAR (Photosynthetically active radiation) collected during shoots sampling. Depths are indicated (*green star* Shallow station, *blue star* Deep station). Seabird Seacat Probe, Lacco Ameno Ischia, July 2007, h ~14:00.

Prof(m)	Temp (°C)	Salin (PSU)	PAR (μM/m^2/sec)
0-	27.55	37.79	960
5	26.84	37.78	703
10	24.03	37.75	491
15	22.24	37.74	355
20	20.02	37.78	230
25	18.99	37.78	100
30	18.12	37.79	50

Since our expectation were that the main differences between shallow and deep plants would be present mainly in the photosynthetic performances, the principal physiological parameters of photosynthetic efficiency have been measured during sampling (thanks to M. Lorenti and M.C. Buia, SZN), by means of a submerged Pulse Amplitude Modulated fluorometery (PAM), that offers a rapid *in situ* way to develop sub lethal physiological indicators of stress, detecting a response to light reduction within days.
These will allow to compare genetic data on photosynthetic performances obtained by the SSH library with physiological parameters of plants collected at the same time.

Chlorophyll fluorescence (fluorescence yield) can be measured and used to calculate the proportion of photons absorbed by the photosynthetic PSII reaction centers (i.e. the quantum yield), since fluorescence is inversely correlated to photosynthetic efficiency. In light adapted plants, these photons are used for photosynthetic electron transport, described as the effective quantum yield (DF/Fm¢).

RNA isolation

Total RNA was isolated from leaf tissue and meristematic portions of shoots using a modified hexadecyltrimertihyl bromide (CTAB) method (Chang at al, 1993 *mod*). Lithium was used for precipitation of total RNA (see appendix). The total RNA supplied was analyzed for its integrity by

agarose gel electrophoresis. PolyA$^+$ mRNA was isolated from the total RNA using Dynabeads mRNA Purification kit (DYNAL BIOTECH) following the manufactures' instructions.

Library Construction

To identify genes involved in the adaptation at light and temperature regimes, two reciprocal SSH libraries were constructed to identify genes preferentially expressed in both shallow and deep conditions.

The SSH experiment was carried out for FORWARD and REVERSE subtractions.

Shallow library (FORWARD subtraction, S) was carried out with shallow mRNA as a tester pool and deep mRNA as a driver pool. At the same time, in the Deep library (REVERSE subtraction, D) deep mRNA was used as a tester pool and shallow mRNA as a driver pool.

The construction of the forward and reverse SSH libraries was performed using the PCR-select cDNA subtraction kit (Clontech, Palo Alto, CA) and following the procedure suggested by the manufacturer (fig4.2).

Equal amounts (2 µg) of polyA$^+$ mRNA from each of the tester and driver populations was converted to double-stranded cDNA by reverse transcription followed by digestion with *Rsa*I to produce shorter blunt ended fragments. The digested tester cDNA was subdivided into two populations, each of which was ligated with a different adaptor provided in the cDNA subtraction kit (Clontech).

Ligation efficiency was evaluated by PCR. Following ligation, a series of two hybridization steps was performed.

For the first hybridization, an excess of driver was added to each tester, denatured, and allowed to anneal. The concentrations of high- and low-abundance target single-stranded molecules in the tester were normalized due to the second-order kinetics of hybridization and the suppression PCR effect genes. In the second hybridization step, the two reaction products from the first hybridization were mixed with each other and with fresh denatured driver cDNA. The populations of normalized and subtracted single-stranded target cDNAs anneal with each other, forming double-stranded hybrids with different adaptor sequences at their 5' ends. The adaptor ends were then filled with DNA polymerase and the subtracted molecules were specifically amplified by nested PCR using adaptor-specific primer pairs.The target sequences in the tester also become significantly enriched for differentially expressed

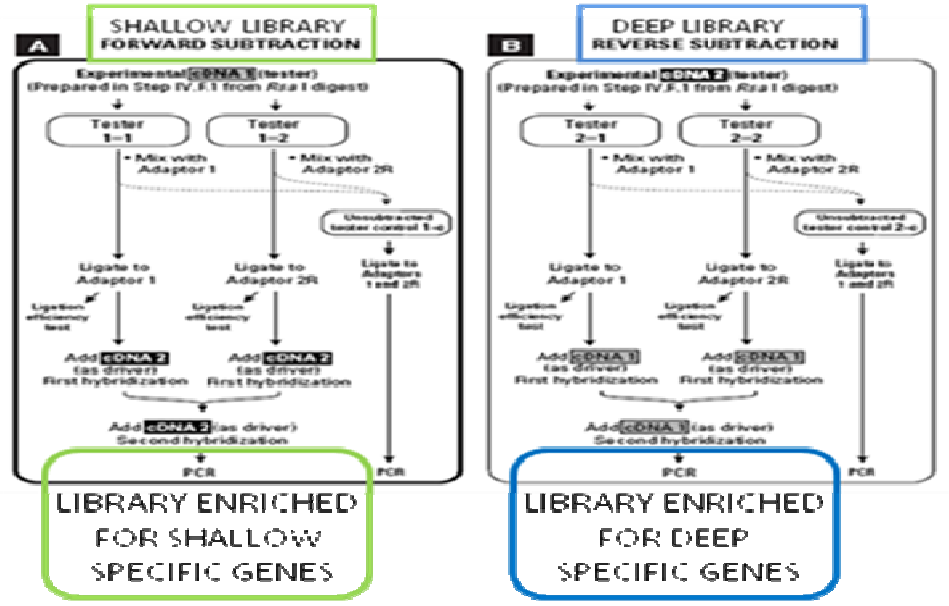


fig 4.1.2 PCR-selected cDNA Subtraction Protocol: tester cDNA each must be ligated to the appropriate adaptors. *Panel A*: the forward subtraction. *Panel B*: see text for explanation *Modified from Clontech PCR select cDNA subtraction kit user*

Library Cloning and EST Sequencing

The two resulting subtractive libraries have been cloned separately in pCR2.1 TOPO (Invitrogen) prior to transferring into TOP F' cells (Invitrogen).

Clones were plated on LB media supplemented with ampicilina (50 µg/ml; LBamp), every single colonies were picked into 96-well plates containing LBamp, and grown overnight.

About twenty 96-well plates for each library were screened in PCR to identified positive recombinant colony. Every single colony has been amplified using specific primers of the TOPO vectors: T7 forward and M13 reverse. PCR products has been analyzed on 1.5% agarose/EtBr gel in 1X TAE buffer and the colonies that produced amplicones longer than 500bp have been selected for the sequencing. Insert length of in the selected clones ranged between 500 and 1000 bp. Replicates of selected colonies were stored in LBamp 15% glicerolo at -80°C.

In order to generate ESTs from the constructed libraries, 1920 random clones, 960 from shallow and 960 from the deep library, were selected randomly and sequenced in double-pass, at MPI Molecular Genetics in Berlin (Germany).

The bioinformatics' analysis of EST data sequences was performed thanks to a collaboration with Erich Bornberg-Bauer and Jenny Gof the Westfälische Wilhelms University Institute for Evolution and Biodiversity Evolutionary Bioinformatics Group, University of Müester (Germany). The total of 3840 sequences, were trimmed removing the low quality regions, the vector, the adapter, and the poly-A/T fragments. Only the EST raw sequences longer than 100 nucleotides entered the assembly step and were classified into the Tentative Unigenes (TUG).

Successfully trimmed EST reads were then assembled into tentative gene clusters using CAP3 (Huang and Madan 1999). Two parameters were specified: there was no reverse orientation of sequence reads, and one read of good quality is sufficient to build a consensus at a given position.

TUGs assembly includes both batches of *Posidonia* sequences arisen from deep and shallow libraries.

4.1.2.3 Dr. Zompo a EST Seagrasses Database

Insert PDF Paper

Wissler, L., et al., *Dr. Zompo: an online data repository for Zostera marina and Posidonia oceanica ESTs.* Database, 2009.

4.2-RESULTS

4.2.1 Estimation of the Genome Size

Testing the two buffers GPB and WPB revealed that easily scorable and reliable peaks were obtained only using the WPB while the other buffer did not allow to preserve intact nuclei. All the experiments were than performed using the WPB buffer.
Fluoresce values obtained for two replicate mix including P. *oceanica* and CTN standard are shown in table 4.2.

Values are stable along the three incubation times (Tab 4.2.1). Conversion of recorded values gave a DNA content of 3.7 pg/nucleus for CTN while for Posidonia the DNA content has been estimated to be of 3.7*2.2 = **8.14** pg/cell.

Number of bases has been calculate according to the conversion value of 1pg= 978Mb. The resulting size in bp for the *P. oceanica* genome was 7.96 x 109 bp.

Tab 4.2.1 Ratios of Peaks: Flow cytometry measures after three incubation times (Posidonia higher than CTN)

Time of staining	*Mix 1*	*Mix 2*	*Average*
10 min	2.112	2.236	2.174
20 min		2.176	
45 min	2.167		2.171
			2.172

4.2.2 Subtractive Hybridization (SSH) Library

Pulse Amplitude Modulated fluorometery (PAM)

Photosynthetic efficiency parameters collected during sampling are shown in table xxx. Different lines refer to replicates during sampling. A clear difference exists between shallow and deep shoots in most of the parameters collected, although the only significant value at 0.01 is E_k, the saturating irradiances, which is higher at the shallow station. The quasi dark and the ETR_{max} (maximum electron transport rates) estimates are only close to be significant at 0.05, sand higher in the deep station, while the alpha (photosynthetic efficiencies) value is not significant.

quasi dark[1]		α[2]		ETRmax[3]		Ek[4]	
adimensional		µmol electrons m^{-2} sec^{-1}/µmol photons m^{-2} sec^{-1}		µmol electrons m^{-2} sec^{-1}		µmol photons m^{-2} sec^{-1}	
25m	5m	25m	5m	25m	5m	25m	5m
0.677	0.684	0.323	0.333	3.3	9	10	27
0.728	0.711	0.328	0.365	5.9	6.1	17	17
0.73	0.73	0.355	0.381	6.4	8	18	21
0.725	0.655	0.354	0.306	5.6	6.7	16	22
0.721	0.704	0.357	0.318	8.7	7	22	22
0.726	0.739	0.395	0.376	6.5	7.9	18	21
0.736	0.738	0.362	0.382	4.5	8.4	12	22
0.743	0.617	0.374	0.344	7.8	11	17	32
0.769	0.677	0.385	0.377	9.9	11.7	20	31

[1] p= 0.052; [2]p= 0.659; [3]p= 0.055; [4]p= 0.003

Data Analyses and Bioinformatics

The total number of good quality sequences after trimming and deletion of short sequences was 2643. The final assembly of shallow and deep ESTs includes 486 Tentative Unigene with a successful mapping of the 2279 ESTs. 364 ESTs could not be assigned to the TUGs library because they did not fulfill the criteria specified above (fig 4.2.2.1).

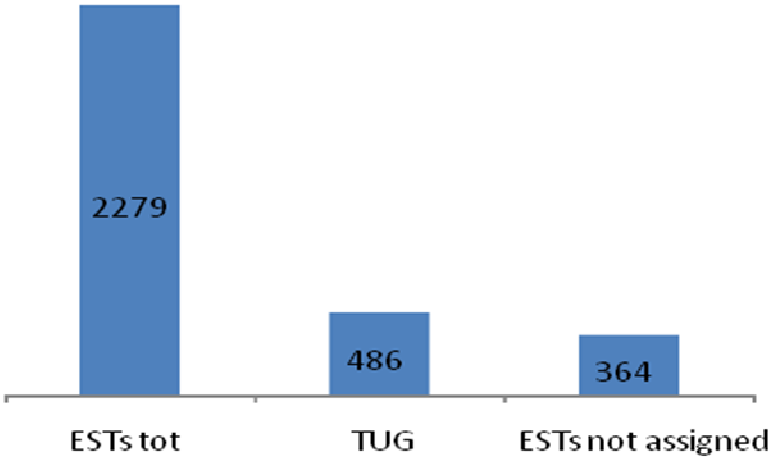


Fig. 4.2.2.1 - Histogram of the distribution of EST sequences in TUG. After the final assembly 2279 ESTs are included in 486 Tentative Unigene while 364 ESTs could not be assigned to any TUG.

Tentative Unigene Annotation

To infer putative functions of the identified tentative unigenes, a search was made against the non-redundant (nr) databases of NCBI Gene Ontology (GO), SWISSPROT and NR -NCBI using BLASTX algorithm with an Expect-value threshold of 0.001, and against Dr. Zompo database using BLASTN algorithm (fig . 4.2.2.2).

Protein annotation against the first three public databases mentioned above gave in total 290 clones (60%) classified into putative known functions and unclassified proteins, and 197 clones (40%) without homologies with known transcripts.

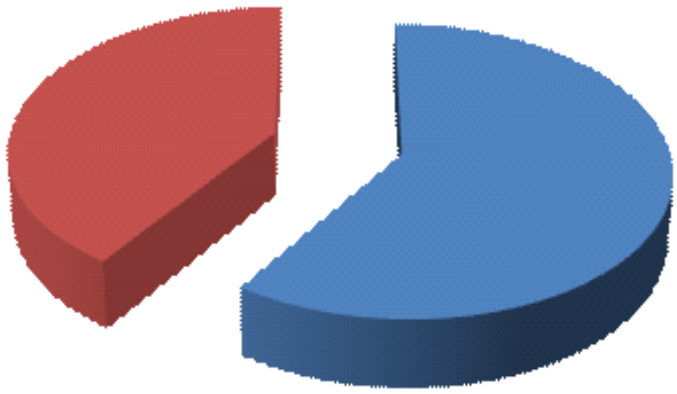


Fig. . 4.2.2.2- Pie-chart on the categories of the 486 TUGs with or without positive blast against known genes (Expect-value threshold of 0.001). *Red slice* TUG which have positive match. *Blue slice* TUG which do no have positive match witch any known Gene.

Annotating the 486 TUGs identified against Dr. Zompo database, only for 185 (38%) an homology was found (see App). The remaining 301 TUGs did not match with any EST present in Dr. Zompo (fig . 4.2.2.3).

Fig. 4.2.2.3- Pie chart on the categories of the 486 TUGs with or without positive blast against ESTs in the Dr. Zompo database. *Red slice* TUG which have positive match in Dr. Zompo. *Yellow slice* TUG which did not match with sequences in Dr. Zompo

Among the 486 TUGs identified, 202 TUGs have been found in the Shallow library and 312 TUGs have been found in the Deep library. Only 28 TUGs (5.86% of the total) were present in both libraries, while 177 TUGs were present only in the S-library and 282 TUGs were present only in the D-library (fig 4.2.2.4)

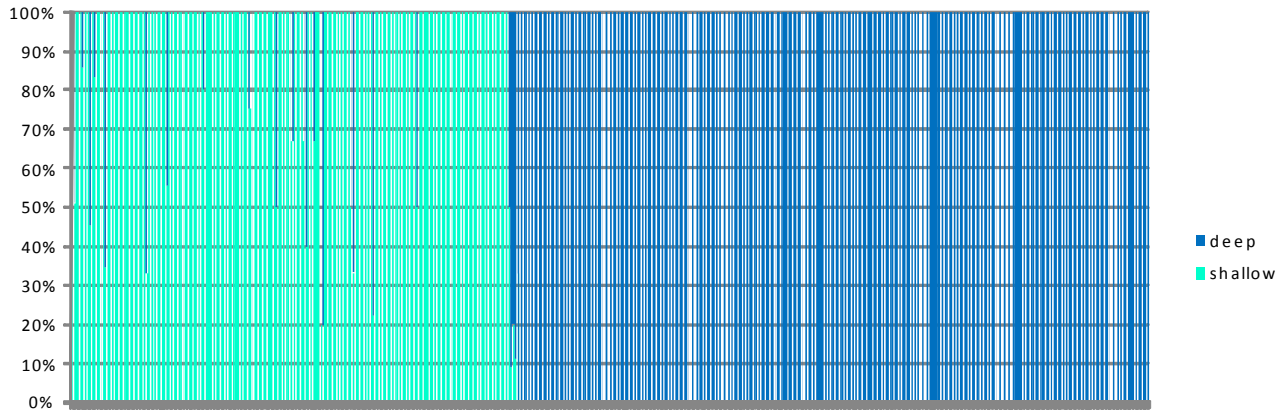


Fig. 4.2.2.4- Schematic illustration of the TUG partition between Shallow and Deep libraries. *Light-blu bars*:TUGs which have clones only in Shallow library; *Blu bars*: TUGs which have clones only in the D-library; *Double-color bars*: TUGs which have clones in both libraries.

TUGs which have homology for known proteins were grouped into thirty-three functional categories according to their best annotation (fig. 4.2.2.5). Functional categories were arbitrarily identified. The most represented functional category was the proteasome (dark green in fig. 4.2.2.5), followed by the proteins of primary metabolism (dark red in fig. 4.2.2.5) and by the chlorophyll a, b proteins (orange in fig. 4.2.2.5). Proteins for molecular functions and cell components were also present in high percent. Proteins from the other categories were roughly similar in abundance.

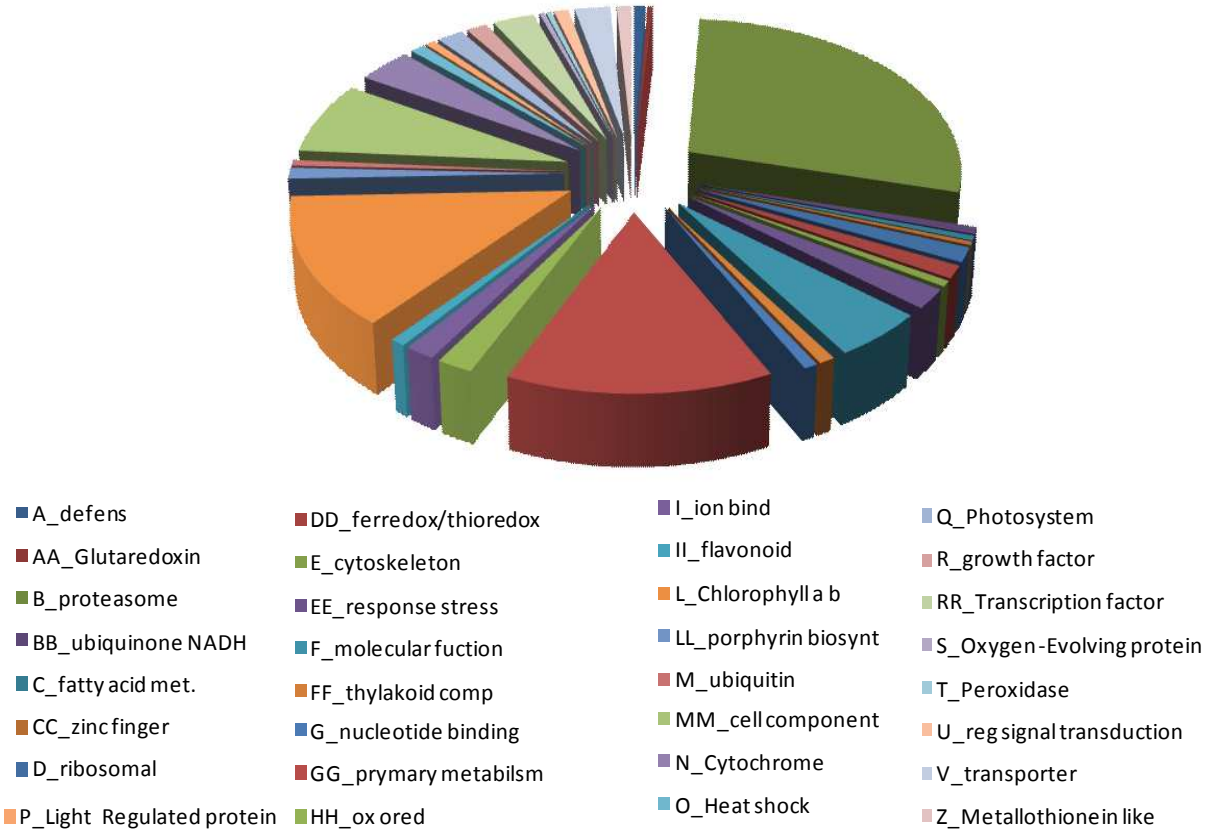


Fig 4.2.2.5- Pie chart with all the TUGs which showed homologies with known proteins. TUGs were grouped into thirty-three functional categories according to their best annotations.

In order to assess the existence of differences in the relative abundance of functional categories between the S- and D-libraries, TUGs were also grouped separately using the same thirty-three functional categories defined in the legend of Fig. 4.2.2.5. The existence of differences between the two libraries is already evident at a first look (fig. 4.2.2.6). There is a striking difference in the abundance of the proteins of the proteosome (the annotation as *putative* Proteasome subunit alpha is only based on homologies with *Posidonia* EST sequence using blastN against Dr. Zompo), which

represent about the 40% of the whole the S-library, while the more abundant categories in the D-library
include primary metabolism and chlorophyll a, b proteins.

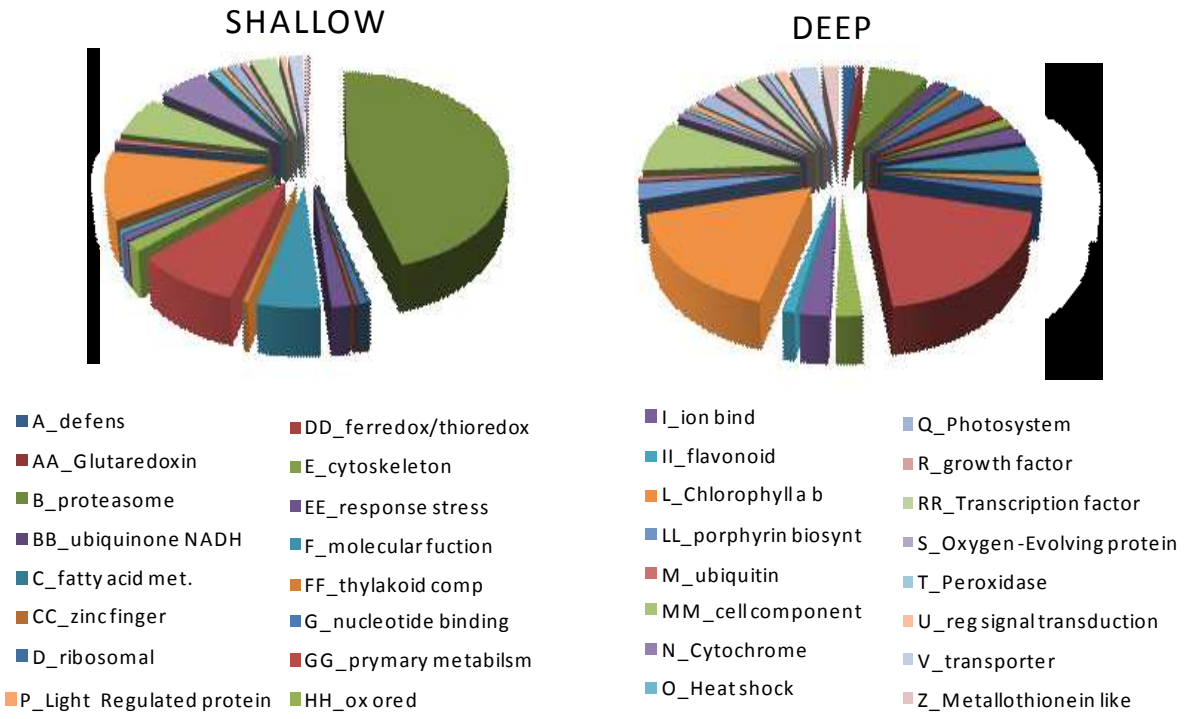


Fig. 4.2.2.6- Pie chart with all the TUGs which showed homologies with known proteins. TUGs were grouped into thirty-three functional categories according to their best annotations. Shallow library: left, Deep library: right.

Grouping clones in even more general functional categories it is possible to better appreciate the high abundance, in shallow conditions, of clones associated to protein turnover (Fig4.2.2.7a,b), which represent the 47% of the clones annotated in the S-library (Fig. 4.9a). Stress-defense and photosynthesis related proteins are instead more relatively abundant in the D-library (15% and 24%, respectively; Fig. 4.2.2.7b). The lower percent of the other functional categories in the S-library could be biased by the high number of clones annotated as Proteasome subunit alpha. To test that, we have excluded the 'Protein turnover' category, and re-calculated the percent presence of the remaining categories. Stress-defense and photosynthesis categories remain more represented in the D-library (data not shown).

Looking more in detail at single clones belonging to functional categories, clones more abundant in the S-library in comparison to the D-library include proteins involved in protein turnover as:

Proteasome subunit alpha, E3 ubiquitin (F-box protein), ATP-dependent Clp protease proteolytic subunit; protein involved in stress defense as Heat shock cognate 70 kDa protein and also proteins involved in hormone signal transduction as ETO1-like protein, Ethylene-responsive transcription factor, Ketol-acid reductoisomerase, and Acyl-CoA-binding protein (Table 4.2.2.1).

Clones that were more abundant in the D-library, instead, include proteins involved in photosynthetic pathway as Cholorohyll a-b- binding proteins, Cytochromes c/b, Photosystem I/II,;Oxygen-evolving enhancer protein; protein involved in stress defense as Universal stress protein, Zinc-finger –protein, Metallothionein-like protein and protein such as A-, Caffeoyl-CoA O-methyltransferase, Aquaporin PIP2 and S-norcoclaurine synthase involved in basal metabolism but also in stress response.

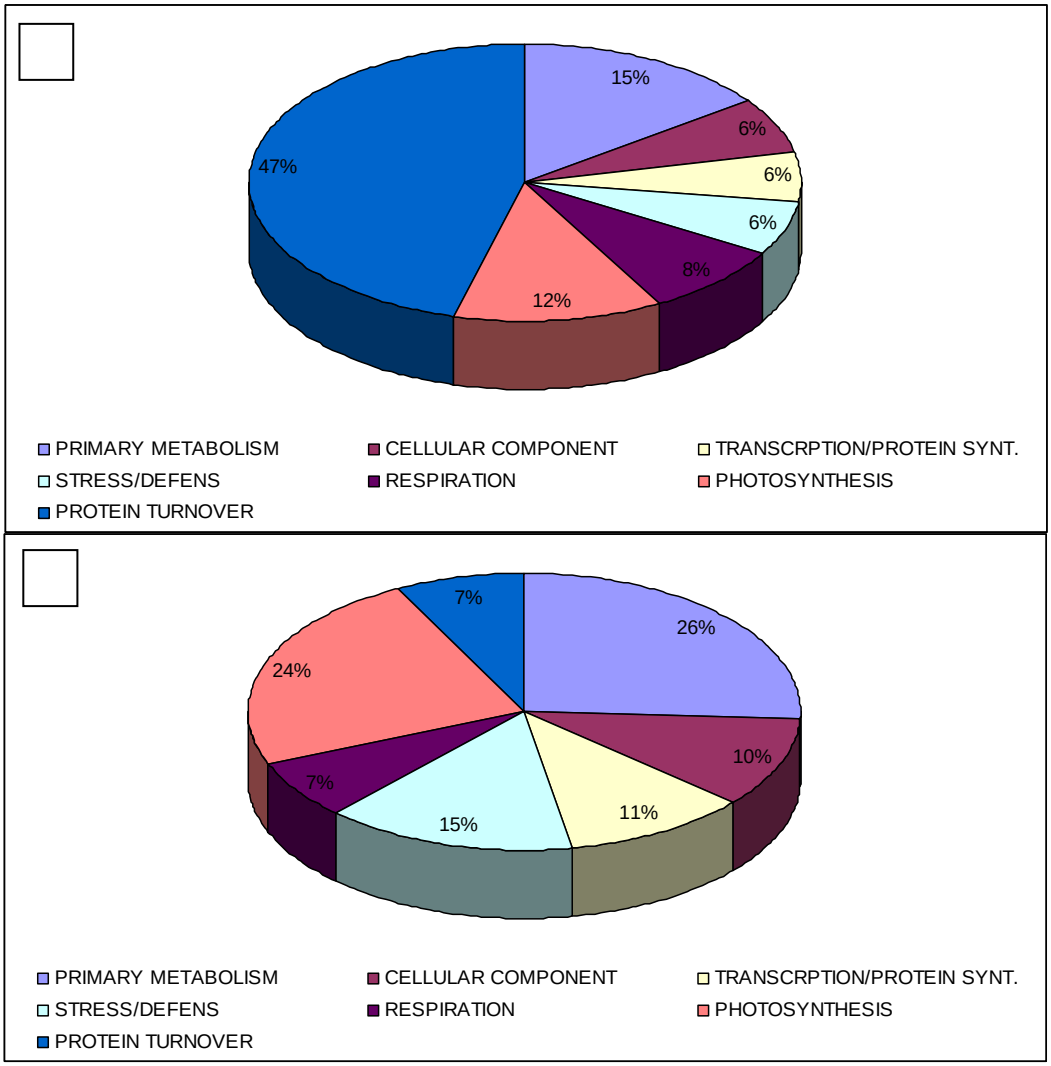


Fig. 4.2.2.7– Representation of the percent presence of all the TUGs annotated in the shallow (a) and in the deep (b) libraries, grouped in wide functional categories.

Among all the 202 TUGs identified in the Shallow library, 105 TUGs are presented with only 1 clone, 46 TUGs with 2 clones, 20 TUGs with 3 clones, 12 TUGs with 4 clones, 7 TUGs with 5 clones, and 11 TUGs with more than 6 clones. Among the latter group, five of them (Contig 205, 18, 209, 341 and 349) did not match with any known protein (Table 4.2.2.1).

Among the 312 TUGs identified in Deep library: 157 TUGs are presented with only 1 clone, 98 TUGs with 2 clones, 29 TUGs with 3 clones, 11 TUGs with 4 clones, 5 TUGs with 5 clones, and 11 TUGs with more than 6 clones. Among the latter group, four of them (Contig 217 , 142 , 246 and 259) did not match with any known protein (Table 4.2.2.2).

Tab 4.2.2.1- List of Shallow TUGs. For each TUG the number of clone founded in library, the best annotation in Swissprot and the presence/absence of hits in Dr. Zompo are indicated. Only TUGs represented by more than 2 clones are listed here, complete list of TUGs is given in the Appendix.

TUG	# clones	SWISS-PROT	hit	eValue	EST Dr. Zompo Hit
Contig205	190	Proteasome subunit alpha type-2 putative			Yes
Contig132	43	Chlorophyll a-b binding protein 16, chloroplastic (Nicotiana tabacum)	P27492	8.61E-87	Yes
Contig18	24	Proteasome subunit alpha type-2 putative			Yes
Contig444	10	F-box protein At5g67140 (Arabidopsis thaliana)	Q9FH99		No
Contig209	8	No Swissprot-Hit			No
Contig95	7	Heat shock cognate 70 kDa protein 1 (Arabidopsis thaliana)	P22953	2.84E-10	Yes
Contig248	6	Cytochrome c oxidase polypeptide VIb (Schizosaccharomyces pombe)	O94581	2.52E-07	Yes
Contig284	6	Photosystem II 10 kDa polypeptide, chloroplastic (Nicotiana tabacum)	Q40519	1.67E-35	Yes
Contig341	6	No Swissprot-Hit			Yes
Contig250	6	Ketol-acid reductoisomerase, chloroplastic (Arabidopsis thaliana)	Q05758	1.36E-60	No
Contig449	6	No Swissprot-Hit			No
Contig386	5	Chlorophyll a-b binding protein 37, chloroplastic (Petunia sp.)	P12062	1.38E-77	Yes
Contig142	5	No Swissprot-Hit			Yes
Contig365	5	Cytochrome b6-f complex iron-sulfur subunit, chloroplastic (Fritillaria agrestis)	O49078	1.43E-37	Yes
Contig130	5	No Swissprot-Hit			No
Contig198	5	No Swissprot-Hit			No
Contig36	5	ATP-dependent Clp protease proteolytic subunit (Azoarcus sp.)	Q5P161	1.80E-22	No
Contig434	5	ETO1-like protein 1 (Arabidopsis thaliana)	Q9ZQX6	2.43E-88	No
Contig162	4	Regulator of ribonuclease activity A (Ralstonia eutropha)	Q0K9J4	1.80E-42	Yes
Contig287	4	Protein TIFY 3B putative			Yes
Contig382	4	No Swissprot-Hit			Yes

Contig391	4	Probable ATP synthase 24 kDa subunit, mitochondrial (Arabidopsis thaliana)	Q9SJ12	3.71E-27	Yes
Contig7	4	Acyl-CoA-binding protein (Brassica napus)	Q39315	1.25E-14	Yes
Contig116	4	No Swissprot-Hit			No
Contig168	4	No Swissprot-Hit			No
Contig208	4	No Swissprot-Hit			No
Contig362	4	No Swissprot-Hit			No
Contig415	4	No Swissprot-Hit			No
Contig422	4	No Swissprot-Hit			No
Contig44	4	Putative U-box domain-containing protein 53 (A.thaliana)	Q9LU47	1.27E-33	No
Contig97	3	Aquaporin PIP2-5 (Zea mays)	Q9XF58	2.56E-52	Yes
Contig229	3	Light-regulated protein putative			Yes
Contig149	3	Proteasome subunit alpha type-2 putative			Yes
Contig327	3	Ammonium transporter 1 member 1 (A. thaliana)	P54144	3.16E-16	Yes
Contig337	3	Ethylene-responsive transcription factor 6 (A. thaliana)	Q8VZ91	3.45E-17	Yes
Contig384	3	Phytosulfokines putative			Yes
Contig61	3	Acyl-CoA-binding protein (Fritillaria agrestis)	O22643	2.16E-06	Yes
Contig110	3	No Swissprot-Hit			No
Contig118	3	Translocase of chloroplast 159, chloroplastic (A.thaliana)	O81283	4.76E-27	No
Contig146	3	No Swissprot-Hit			No
Contig163	3	No Swissprot-Hit			No
Contig215	3	No Swissprot-Hit			No
Contig230	3	No Swissprot-Hit			No
Contig298	3	Chalcone synthase (Arabis alpina)	Q9SEP4	1.37E-33	No
Contig314	3	No Swissprot-Hit			No
Contig315	3	No Swissprot-Hit			No
Contig331	3	No Swissprot-Hit			No
Contig370	3	Cytochrome P450 76A1 (Fragment) (Solanum melongena)	P37121	1.16E-35	No

Contig417	3	No Swissprot-Hit			No
Contig428	3	Cysteine-rich receptor-like protein kinase 2 (A. thaliana)	Q9CAL3	2.19E-07	No
Contig486	3	Cytochrome b (Coracias caudata)	Q9ZZD4	1.51E-27	No

Tab 4.2.2.2- List of Deep TUGs. For each TUG the number of clone founded in library, the best annotation in Swissprot and the presence/absence of hits in Dr. Zompo are indicated. Only TUGs represented by more than 2 clones are listed here, complete list of TUGs is given in the Appendix.

TUG	# clones	SWISS-PROT	hit	eValue	EST Dr. Zompo Hit
Contig132	44	Chlorophyll a-b binding protein 16, chloroplastic (Nicotiana tabacum)	P27492	8.61E-87	Yes
Contig205	10	Proteasome subunit alpha type-2 putative			Yes
Contig386	10	Chlorophyll a-b binding protein 37, chloroplastic (Petunia sp.)	P12062	1.38E-77	Yes
Contig217	10	No Swissprot-Hit			No
Contig142	9	No Swissprot-Hit			Yes
Contig42	8	Cytochrome c oxidase subunit 1 (Petromyzon marinus)	Q35536	1.61E-11	No
Contig421	7	S-norcoclaurine synthase (Thalictrum flavum subsp. glaucum)			Yes
Contig97	6	Aquaporin PIP2-5 (Zea mays)	Q9XF58	2.56E-52	Yes
Contig182	6	Protochlorophyllide reductase, chloroplastic (Cucumis sativus)	Q41249	1.16E-20	Yes
Contig376	6	Universal stress protein A-like protein (Arabidopsis thaliana)	Q8LGG8	2.98E-07	Yes
Contig246	6	No Swissprot-Hit			No
Contig259	6	No Swissprot-Hit			No
Contig262	5	Caffeoyl-CoA O-methyltransferase (Populus tremuloides)	Q43095	1.11E-10	Yes
Contig447	5	Metallothionein-like protein type 3 (Malus domestica)	O24059	1.31E-12	Yes
Contig231	5	Centrin-2 putative			Yes
Contig304	5	No Swissprot-Hit			Yes
Contig275	5	No Swissprot-Hit			No
Contig271	4	Photosystem I reaction center subunit III, chloroplastic (Arabidopsis thaliana)	Q9SHE8	3.55E-06	Yes
Contig274	4	Protein sym1 (Aspergillus fumigatus)			Yes
Contig286	4	No Swissprot-Hit			Yes
Contig31	4	Ferredoxin (Sambucus nigra)	P00226	1.13E-14	Yes
Contig81	4	Oxygen-evolving enhancer protein 3, chloroplastic (Oryza sativa subsp. indica)	P83646	3.91E-36	Yes

Contig438	4	29 kDa ribonucleoprotein A, chloroplastic (Nicotiana sylvestris)	Q08935	1.51E-34	No
Contig442	4	Soluble hydrogenase 42 kDa subunit (Anabaena cylindrica)	P16421	2.77E-11	No
Contig469	4	Signal peptide peptidase-like 2B (Mus musculus)	Q3TD49	2.62E-13	No
Contig68	4	No Swissprot-Hit			No
Contig70	4	No Swissprot-Hit			No
Contig84	4	1-deoxy-D-xylulose-5-phosphate synthase (Rhizobium meliloti)	Q92RJ1	6.09E-46	No
Contig310	3	Chlorophyll a-b binding protein of LHCII type III, chloroplastic (Hordeum vulgare)	P27523	1.00E-32	Yes
Contig144	3	No Swissprot-Hit			Yes
Contig169	3	BEL1-like homeodomain protein 6 putative			Yes
Contig17	3	Ferredoxin-thioredoxin reductase catalytic chain putative			Yes
Contig194	3	BI1-like protein (Arabidopsis thaliana)	Q94A20	9.53E-12	Yes
Contig219	3	Magnesium-protoporphyrin IX monomethyl ester [oxidative] cyclase 2, chloroplastic (Chlamydomonas reinhardtii)	Q9AR22	1.78E-21	Yes
Contig226	3	NADH-ubiquinone oxidoreductase subunit 8 (Reclinomonas americana)	O21233	1.81E-24	Yes
Contig279	3	Putative syntaxin-24 (Arabidopsis thaliana)	Q9C615	1.25E-06	Yes
Contig312	3	Ferredoxin-thioredoxin reductase, catalytic chain (Porphyra yezoensis)	Q1XDA1	2.94E-38	Yes
Contig313	3	40S ribosomal protein S15 (Picea mariana)	O65059	1.04E-60	Yes
Contig332	3	No Swissprot-Hit			Yes
Contig349	3	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 7 putative			Yes
Contig363	3	Putative mitochondrial import receptor subunit TOM40 homolog 2 (Arabidopsis thaliana)	Q9SX55	2.17E-56	Yes
Contig385	3	60S acidic ribosomal protein P2 putative			Yes
Contig441	3	S-norcoclaurine synthase putative			Yes
Contig452	3	Glyceraldehyde-3-phosphate dehydrogenase A, putative			Yes
Contig108	3	No Swissprot-Hit			No
Contig190	3	No Swissprot-Hit			No

Contig216	3	Desiccation protectant protein Lea14 homolog (Glycine max)	P46519	2.53E-30	No
Contig239	3	No Swissprot-Hit			No
Contig282	3	No Swissprot-Hit			No
Contig290	3	No Swissprot-Hit			No
Contig33	3	Putative metal tolerance protein C3 putative			No
Contig356	3	No Swissprot-Hit			No
Contig373	3	No Swissprot-Hit			No
Contig395	3	No Swissprot-Hit			No
Contig409	3	Probable 6-phosphogluconolactonase 2 (Oryza sativa subsp. japonica)	Q6Z4H0	8.79E-14	No
Contig423	3	No Swissprot-Hit			No
Contig472	3	Probable serine/threonine-protein kinase WNK4 (A.thaliana)	Q9LVL5	4.13E-09	No

It should be noticed that the difference between the two libraries is both in the number of clones belonging to a particular TUG, and also in the proteins composition of the same functional categories or metabolic pathway.

Looking for example at the photosynthesis-related proteins, it is striking that in the D-library there are 19 different TUGs encoding for Chlorophyll a-b binding proteins, whereas only 7 different TUGs were present in the Shallow one (Fig. 4.2.2.8).

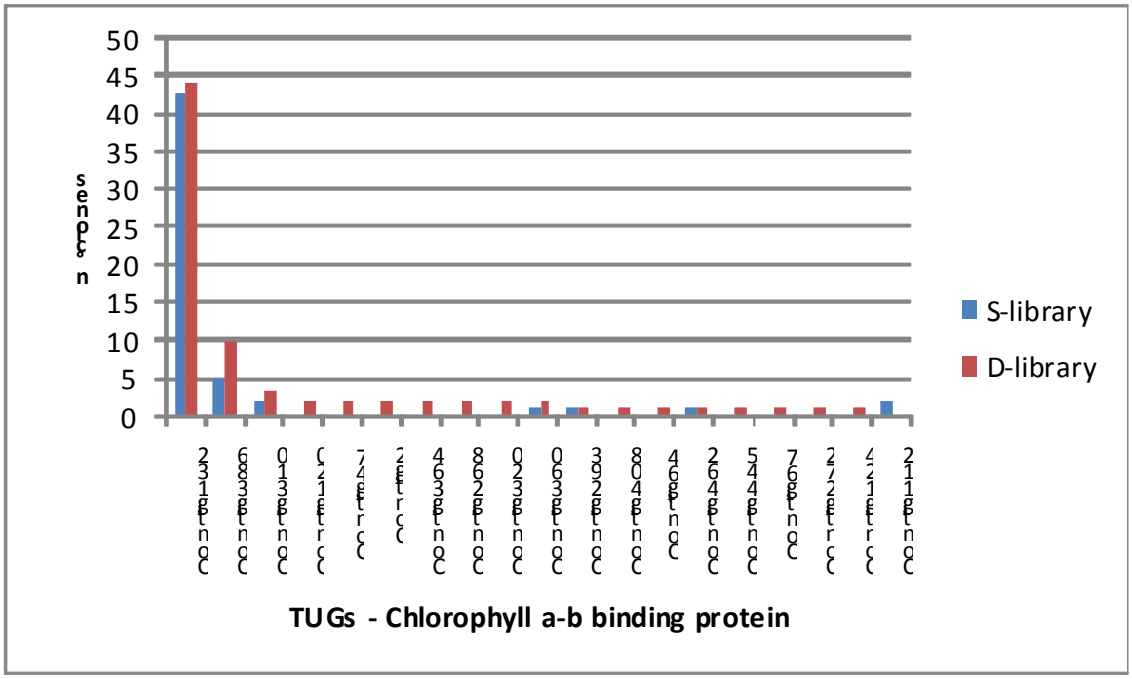
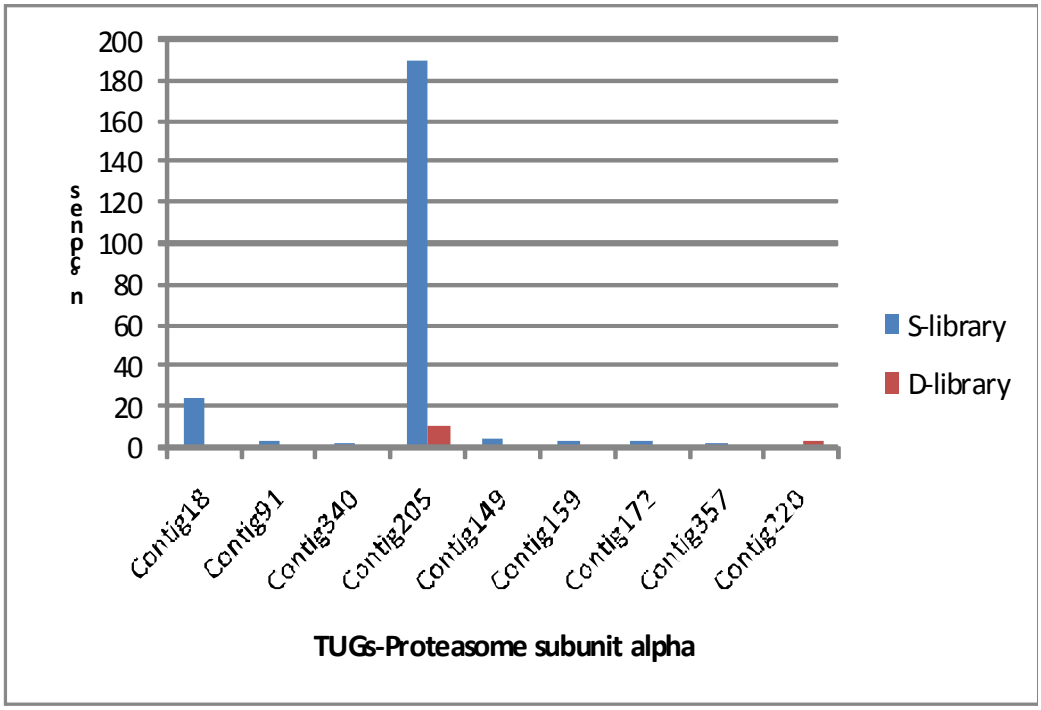


Fig. 4.2.2.8

This is also true for Proteasoma-related proteins, where in the S-library there are 8 different TUGs encoding for Proteasome sub-unit α, while only 2 different TUGs were present in the Deep one (fig4.2.2.9)



4.2.2.9

Other proteins with the exception of Proteasome sub-unit α (see above), related to protein turnover in particular with the Ubiquitin /26S proteasome complex are more abundant in the S-library where there are 7 different TUGs, than in the D-library where are 3 TUG. Interesting proteins are represented in one library and are absent in the other.(fig4.2.2.10), such as TUG 44, 389, 444, 126, 105, 199 encoding for proteins with regulatory functions.

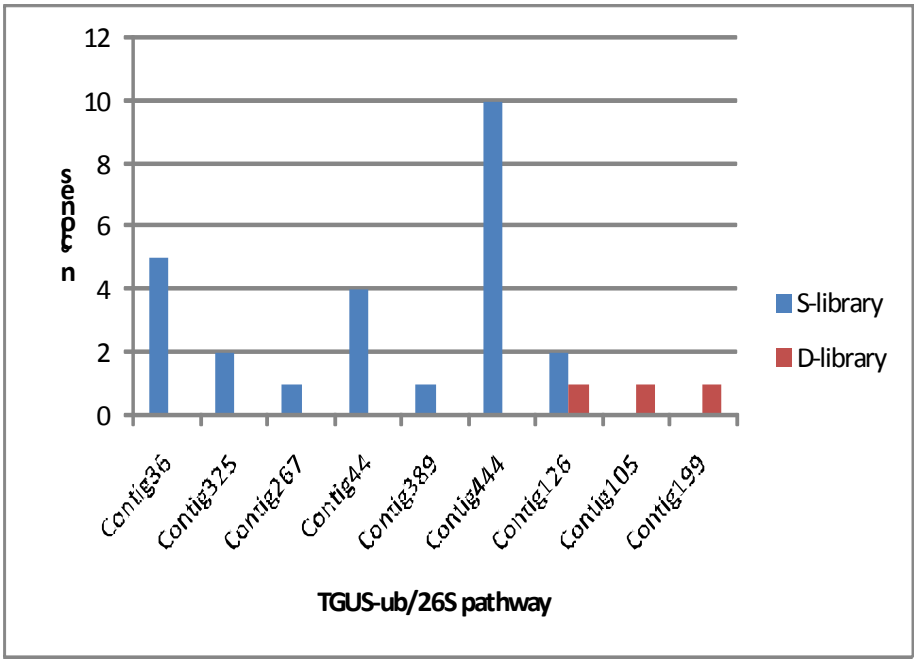


fig4.2.2.10

Other proteins related to light harvesting complex, follow the same trend of Chlorophyll a-b binding proteins. There are 6 different TUGs in the D library, and only 2 in the S-library (fig4.2.2.11).

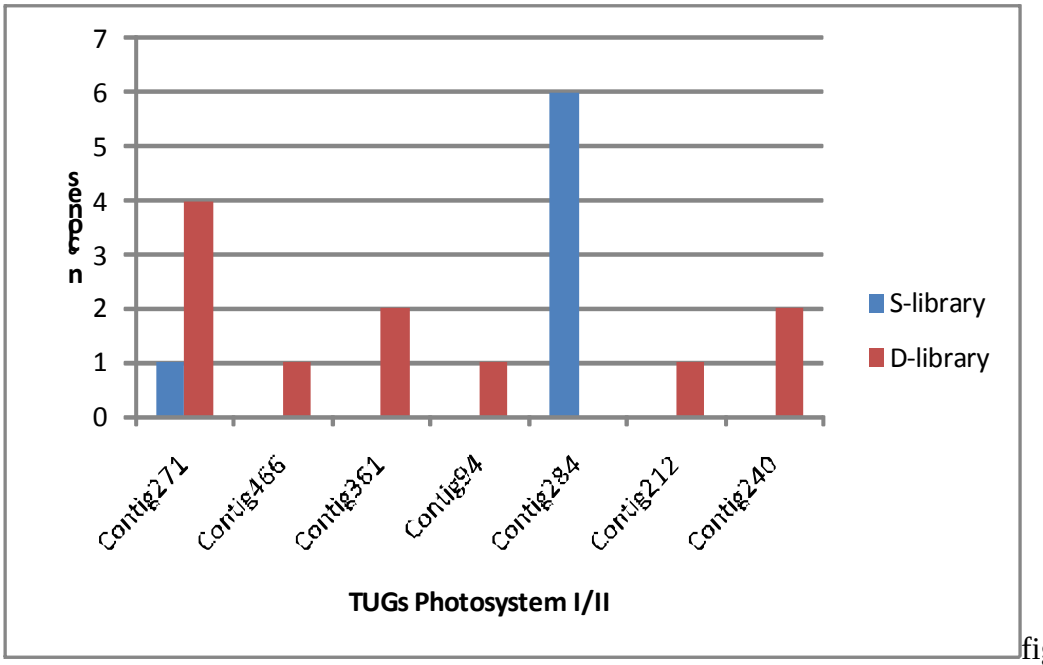


fig4.2.2.11

Proteins related to electrons carrier transport have the opposite trend. There are more different TUGs in Shallow then in Deep, with 5 TUGs in the S-library and 3 in the D-library (fig4. 2.2.12)

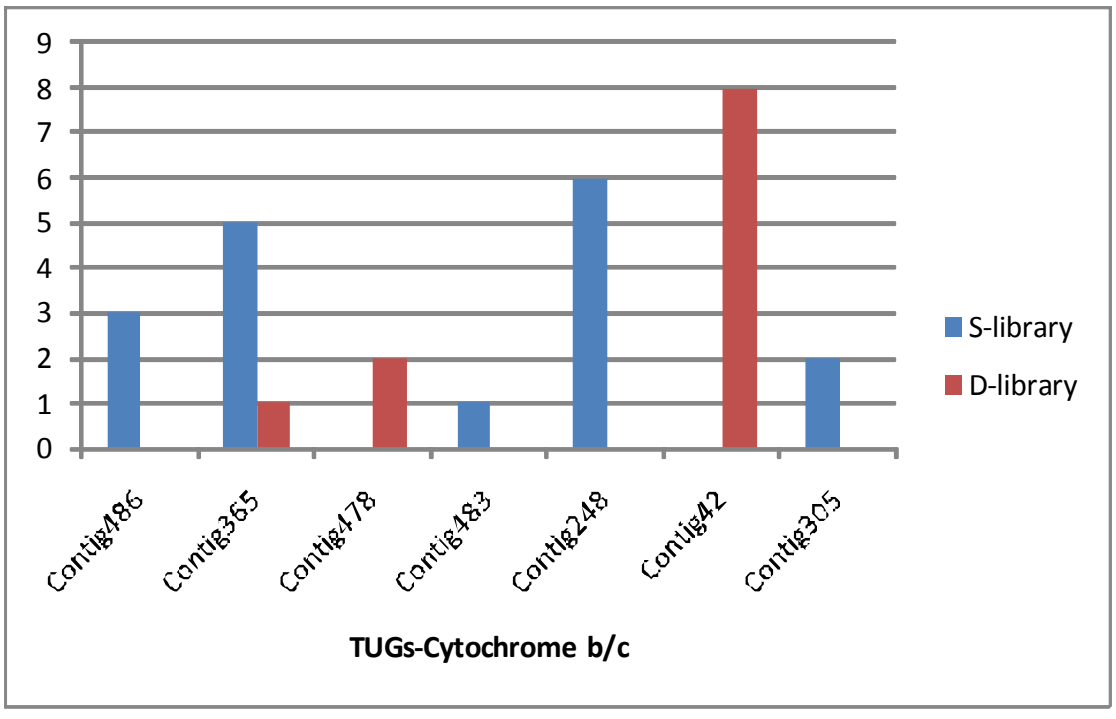


fig4. 2.2.12

Among proteins involved in metal binding- proteins, it is striking that in the D-library there are 6 different TUGs encoding for metal ion-binding proteins, whereas only 2 different TUGs were

present in the Shallow one. TUGs present in the S-library encode only for Metallothionein-like proteins (TUG 447, 319), whereas in D-library besides Metallothionein-like protein (TUG 447, 319,88) there are also Selenium binding protein (TUG 161), Blue copper protein (TUG 388) and putative metal tolerance proteins (TUG 330 , 33). (fig4. 2.2.13).

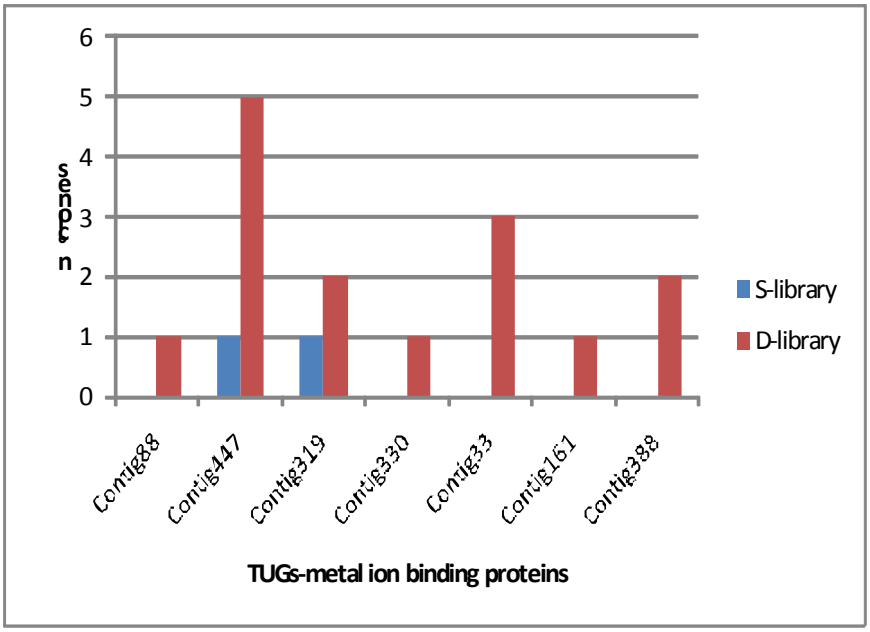


fig4. 2.2.13

Looking at proteins implicated in detoxification processes, in D-library these are represented by 37 TUGs while only 8 TUG are present in the S-library (Fig 4. 2.2.14). Different types of proteins are associated to this category, such as Calmodulin (TUG 473, 474), Cytochrome P450 (TUG 370, 77,30,45), Ferredoxin/thioredoxin (TUG 31, 147, 17, 312, 372, 241, 334, 107), metal binding proteins (TUG 447, 319, 88, 330, 33, 161, 456,349), Oxygen-evolving enhancer protein (TUG 296, 83, 81), Peroxidase (139, 152) and Peroxisomal protein (TUG 291, 457) Zinc finger protein (TUG 82, 49, 261, 468,) and NADH-ubiquinone (TUG 226, 349).

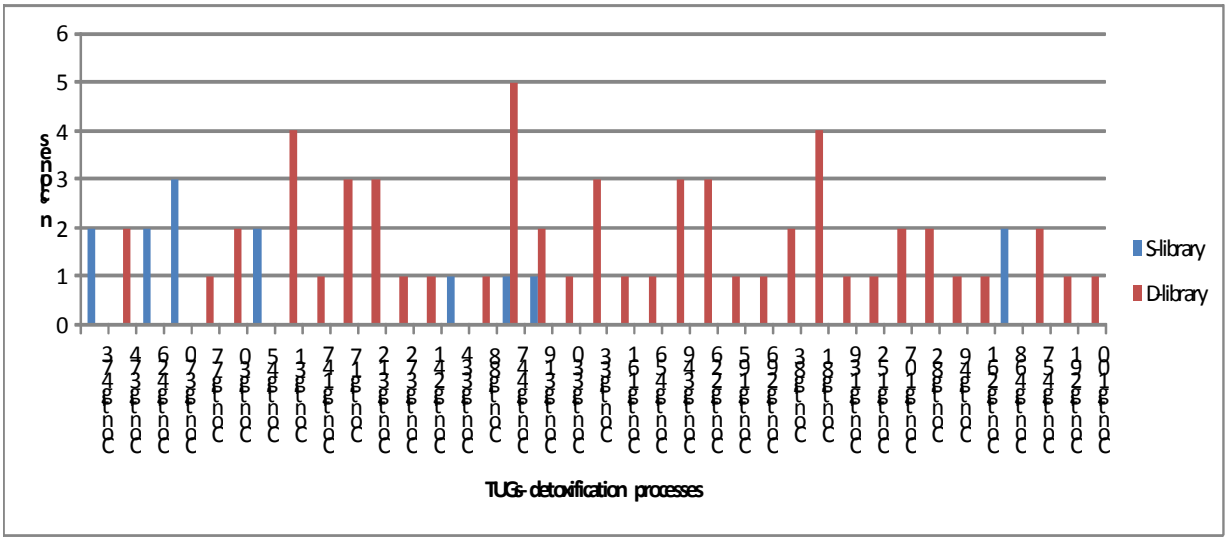


Fig 4. 2.2.14

Other TUGs for protein involved in generic stress and defense responses are more abundant in D-library, where 7 TUGs are present (Defensin Tk-AMP - TUG348, Desiccation protectant and salt tolerance proteins - TUG 216, 448, universal stress protein A-like - TUG 376, 55, and Heat shock cognate 70 kDa protein - TUG 344, 448) then in S-library, where there is only one TUG (95) encoding for Heat shock cognate 70 kDa protein. (Fig4. 2.2.15)

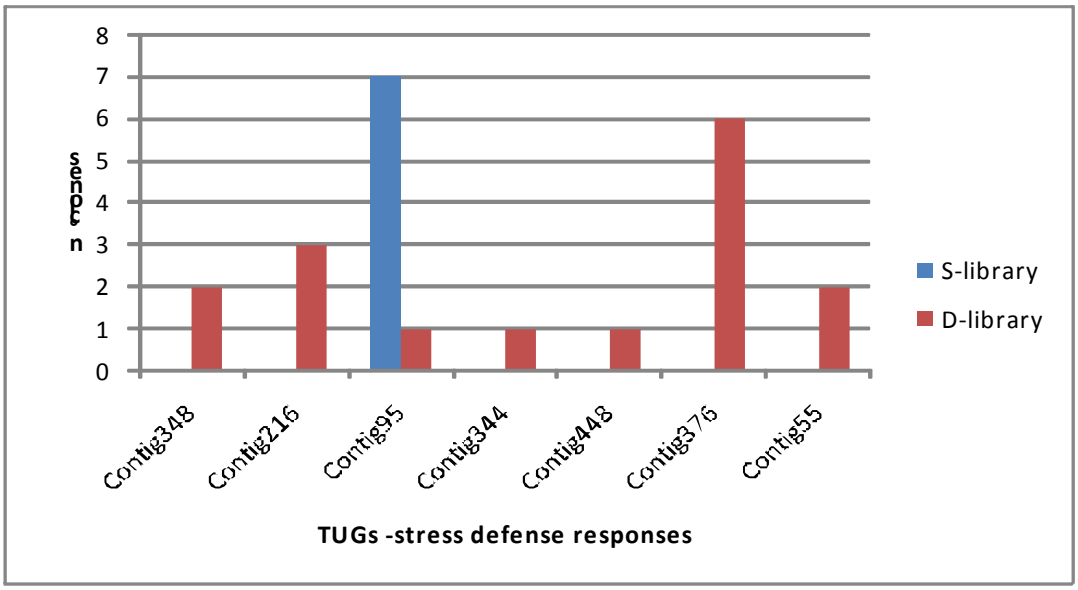


Fig4. 2.2.15

4.3-DISCUSSIONS

Flow cytometry (FCM) is widely applied in the determination of nuclear DNA content and ploidy level in many organisms. Plants produces many different cytosolic compounds that are released during nuclei isolation. These compounds interact with nuclear DNA and/or the fluorochrome, affecting sample quality and causing stoichiometric errors in DNA staining (Louriero et al, 2006). Given the different chemical composition and diversity of plant tissues a difficulty with flow cytometry is to find lysis buffers able to ensure the stability of nuclei throughout the experiment, protect DNA from degradation and facilitate stoichiometric. *Posidonia oceanica* produces several secondary metabolites such as tannins, which can interfere with staining and then with flow cytometer analysis. Hence, we were not surprised that among the two buffer used in our analysis, only WPB which contained PVP was able to preserve *Posidonia* nuclei by degradation.

The previously published 2C-values for *P. oceanica* were 5.6 pg DNA (Cavallini et al., 1995) and 7.27 pg DNA (Koce et al., 2003). Our measure confirms that *Posidonia* genome is very large in comparison with the size reported in literature for the other seagrass, such as *Z. noltii* 0.94 pg , *C. nodosa* 0.64 pg (Koce et al., 2003) measured with flow cytometry and Z. *marina* 1.22 pg, measured with Image cytometry (Koce et al., 2003). In comparison with the other monocot plants, *Posidonia* has a genome of medium size (Koce et al., 2003). As reported by Knight et al. (2005), most plants species have genome size under 5.0pg. This suggests an evolutionary advantage for small genomes and the finding that small genome size is linked to success in plants (Vinogradov, 2003; Knight et al., 2005) also seems to hold true for green algae. In plants large genome sizes have been linked to a higher chance of extinction (Vinogradov 2003), less species richness (Vinogradov 2003; Knight et al. 2005) and they are underrepresented in extreme environments (Knight & Ackerly 2002). Larger genomes are associated with longer cell and life cycles (Bennett 1972), so species with very small genome sizes could have the advantage of rapid growth, which would be a real advantage in colonizing new areas. Association of genome size with life style seems to apply well inthe seagrasses, since species as *Zostera* and *Cymodocea* have a small genome and adopt a “guerilla” growing strategy, whereas *Posidonia* has a large genome and is a plant with slow growing and long persistence (Ruggiero et al, 2005; Migliaccio et al, 2005)

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Analysis of EST data

In our EST collection, we identified several clones (>200) having homology to components of (Ub)Ubiquitin-26S proteasome pathway. In shallow library, in particular, we found: putative proteasome α sub unit, E3 ubiquitin-protein ligase, Chaperone protein, U-box and RING-box protein and a SCF E3 protein (fig3.3.1 and fig 3.3.2) that appear differentially expressed in comparison with deep library.

This degradation pathway involved in the removal of abnormal polypeptides throughout normal protein turnover, but also by targeting of appropriately normal proteins, this pathway provide at the degradation of enzymes and key regulatory factors, which are regulators of signal transduction. Thus,Ubiquitin-26S proteasome pathway making it one of the most elaborate regulatory mechanisms in plants allowing cells to respond rapidly to signal molecules and changes in environmental conditions (Hershko and Ciechanover, 1998; Gagne et al., 2004, Moon, 2004).

The 26S Proteasome is localized in the cytoplasm and in the nucleus of plant cells, and consists of a multisubunit complex composed by a catalytic core particle (20S) flanked by two regulatory particles (fig3.3.1). Each regulatory particle can be subdivided into a 'base' and a 'lid'. The 'lid' is responsible for the recognition of ubiquitin-tagged substrates. The base contains several subunits that work to unfold the substrate. As a whole, the regulatory particle serves as the gate into the interior of the core protease. The core protease consists of a stack of proteolytic α and β subunits surrounding a narrow chamber. It is here that the substrates are finally degraded into short peptides, after which the constituent amino acids can be recycled (Yang et al., 2004).

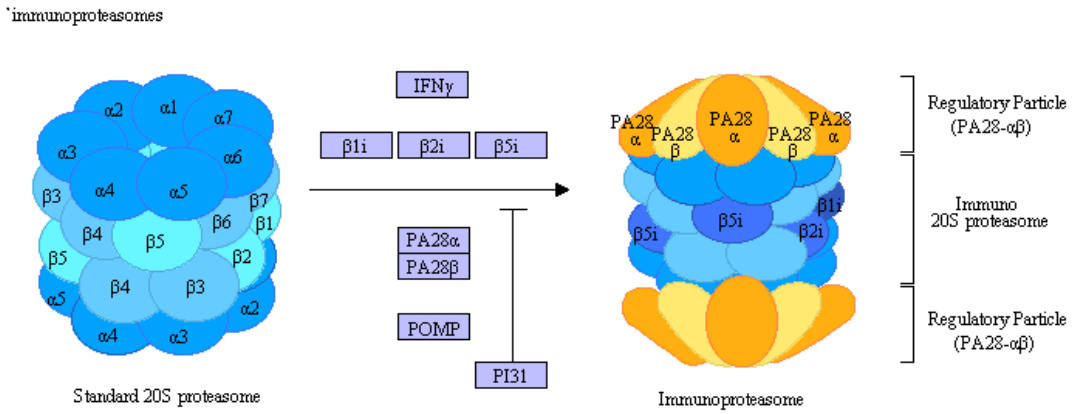


fig3.3.1 Proteasome complex subunits founded in SSH library are indicated

The general function of the ubiquitination pathway is to conjugate ubiquitin to Lys residues within substrate proteins, thus targeting them for degradation by the proteasome (Smalle and Vierstra, 2004). Three enzyme are involved in the "protein-targeting": an ubiquitin-activating enzyme (E1), an ubiquitin conjugating enzyme (E2) and an ubiquitin ligase (E3). E3 is a large gene family generally considered to be most important in specifying the substrate (Plants UBQ database http://plantsubq.genomics.purdue.edu/) Five E3 types have been described based on subnit composition: HECT, SCF, RING/U-box , CUL3-BTB and APC complexes (fig3.3.2),E3s can be further divided into single subunit RING/U-box E3s, and multisubunit RING E3s, which include the SCF, CUL3-BTB, and APC (fig3.3.2).

The SCF class of E3 ligases is the most thoroughly studied in plants. It is formed by four subunits: SKP1 (ASK in plants, SKP1 for *Arabidopsis*), CDC53 (or Cullin), F-box protein and RING-box. In this complex, the ASK and F-box are responsible of target recognitions (Smalle and Vierstra, 2004). In the *Arabidopsis* genome there are 21 ASKs (Takahashi et al., 2004), and more then 700 F-box proteins (Risseeuw et al., 2003).

The E3 ubiquitin ligases comprise a large and diverse family of proteins or protein complexes,

The combinatorial possibilities of ASKs and F-box proteins can result in remarkable variability and the potential to target a very large number of substrates. Known targets of the SCF include transcription factors, cell cycle regulators, and factors involved in development and signal transduction (Wang, 2003).

F-box proteins, named for the conserved 60–amino acid motif responsible for binding to ASK/SKP, represent the largest superfamily in *Arabidopsis*, comprising 2.7% of the *Arabidopsis* genome (Gagne et al., 2002). In almost all F-box proteins, the N terminus of the protein contains the F-box motif, and the remainder of the protein contains the protein–protein interaction domains required for substrate binding (Moon et al, 2004).There is evidence from yeast (Kominami et al.,1998), animals (Suzuki et al., 2000), and plants (Risseeuw et al., 2003) that some F-box proteins form heterodimers with other F-box proteins, which would lead to an even higher level of complexity.

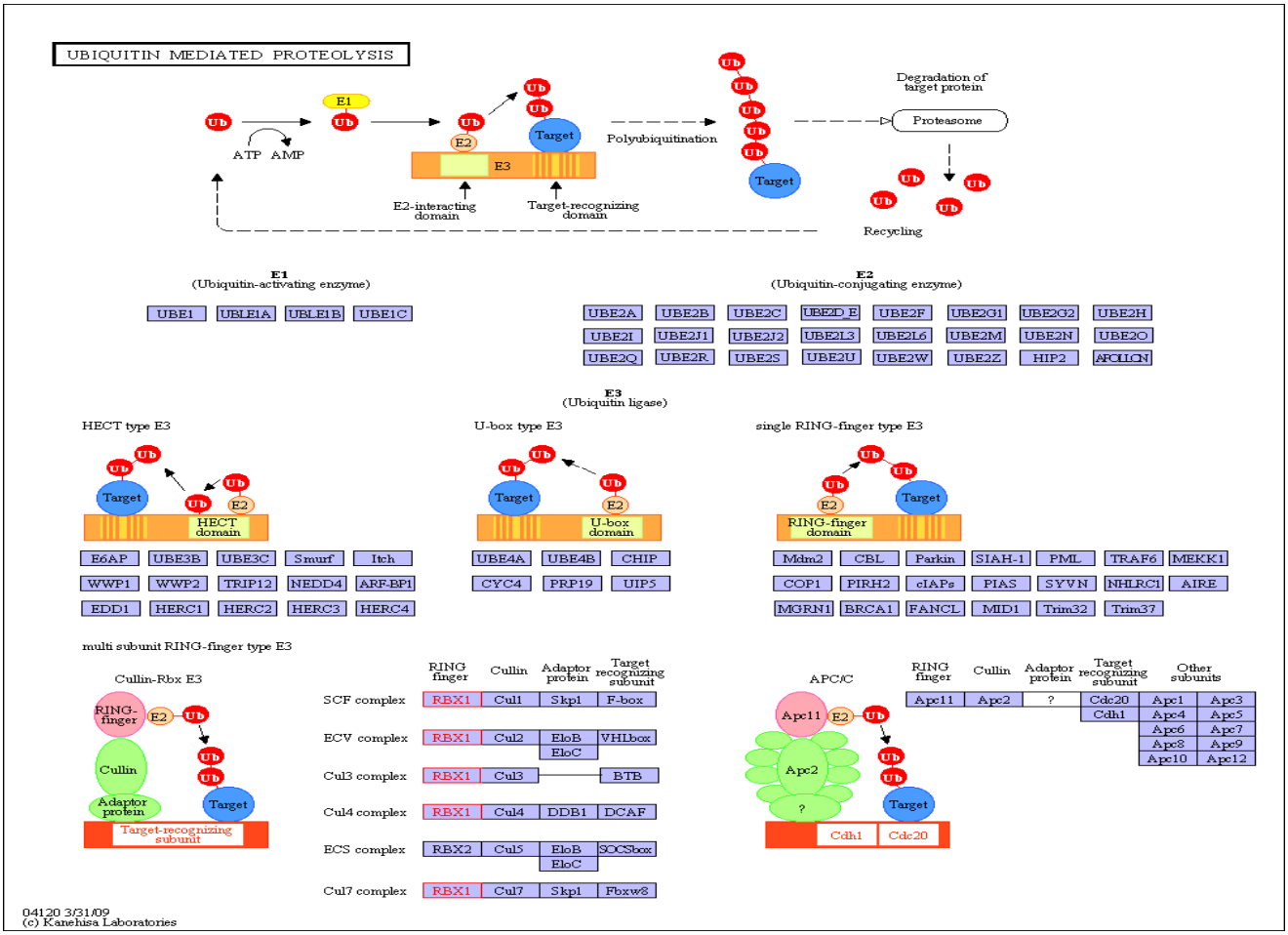


FIG 3.3.2 Rapresentation Pathway of Ubiquitin mediated proteolysis and E3 ubiquitin ligase types.Proteins founded in SSH library are indicated

The participation of SCFs in plant development is extensive, affecting processes such as hormone response, photomorphogenesis, circadian rhythms, floral development, and senescence (Plants UBQ database http://plantsubq.genomics.purdue.edu/).

At present, information exists on the functions of a relatively small number of F-box proteins. Most of these are involved in regulation of hormone signaling pathways. For some responses, the role of the SCF is to degrade repressors of hormone response (auxin, GA, and JA), whereas in response to ethylene, the SCF degrades positive regulators in the absence of the hormone.

Plants utilize hormones to control growth and development and to respond rapidly at ever changing environmental conditions. Without precise controls over both the production of and response to these hormones, plants would not be able to survive.

Ehylene is an important regulator of several plants processes; it play a role both on the regulation of endogenus rhytms such as seed germination, plant growth, leaf expansion, root hair formation, fruit ripening, timing of vegetative senescence both in the transduction of environmental signaling such as responses to abiotic stresses and pathogen attack (Wang et al., 2003; Potuschak et al., 2003).

The ethylene signaling cascades is mediated by Ethylene binding with ethylene receptors and then terminate in the activation of transcription factors that responded to ethylene (ERF) (Moon et al., 2004).

In shallow library we have identified as differential expressed two proteins implicated in the Ehylene signal trasduction: Ehylene-overproduction protein1, which Ethylene-responsive transcription factor 5and also we have found one clone for ethylene-receptor.

In the absence of ethylene, the receptors are in an active state and constitutively activate by protein kinase (CTR1). Therefore, binding of ethylene to the receptor inactivates protein kinase and then promote ethylene responses via activating the downstream EIN3/EILs transcription factors (Moon et al., 2004), which are vital transcription factors for mediating ethylene-regulated gene expression and associated morphological responses (Guo and Ecker, 2003).

Ethylene stabilizes its and that in the absence of ethylene; the protein is degraded by the proteasome; the ubiquitin E3tresponsible of degradation is a SCF type.

Moreover, the data strongly suggest that protein degradation via the ubiquitin/ 26S proteasome pathway is a control point of Ethylene signaling regulation.

Several studies support that F-box proteins such as SCF E3, are involved in phyA-mediated light signaling and in the regulation of circadian clock, so it is possible that SCF proteins degrades a repressor of light response in preparation for light signals at dawn (Harmon and Kay, 2003).

Ethylene plays a key role in timing regulation of many processes, including seed germination, leaf senescence, fruit ripening, and it is a positive regulator of senescence (Wang, 2003; Raab et al, 2009). Then the cross-regulation between ethylene biosynthesis and proteasome activity is a potential target pathway to allow *P.oceanica* response to light-signal at short time such as circadian clock and at long-time related to regulation of annual seasonality.

Clones abundant in Deep library include metabolic genes involved in glycolysis, oxidative phosphorylation, lignin biosynthesis, flavanoid biosynthesis, fatty acid regulation, carbon fixation and hormonal synthesis and protein associated with cell division and growth (see tab result). In particular, Caffeoyl CoA 3-O-methyltransferase, indicated as up-regulated during the secondary seasonal growing (Matsumoto, 2006), is differentially expressed in Deep library.

The strong differences in transcriptome composition between the two libraries in combination with (putative) differential expression of specific genes (as proteasoma in shallow and Caffeoyl-CO-A in deep) suggest that plants are in two different life cycle phases. This hypothesis is supported by previous studies (Buia e Mazzella, 1991) carried out in the same meadows (Lacco Ameno) where our samples have been collected, that illustrate a clear shift in life cycle for plant growing in shallow and deep station: in early summer shallow plants (5m) end their annual cycle and turn into the senescence, at the same time when plants of deep stands (25 m) are fully growing.

In plants light and temperature signaling drive timing of Seasonal and Circadian rhythm by a fine scale modulation of activation and repression of many transcription factors (review Jiao et al 2005)

Light-responsive transcription factors have been identified through screens for light-responsive *cis*-element (LRE)-binding proteins and through genetic analyses of mutants that are deficient in their response to specific types of light. Some of these transcription factors are regulated by just one type of light, whereas many more respond to a wide spectrum of light. Transcriptional regulation, post-translational modification and degradation of these transcription factors are all important in the light-regulated control of development. Recent studies (Moon et al., 2004 for a review) demonstrated the importance of ubiquitin-mediated proteolysis in light signaling and in particular the role of specific RING- ubiquitin E3.

Regulation in the expression level of some ubiquitin/26S proteins has also been found in *P. oceanica* during photo-acclimatation at low-light conditions (Mazzuca et al, 2009), this data also suggest that ubiquitin- mediated proteolysis in *P.oceanica* is a ordinary mechanism of gene regulation and may be react at different light impulse.

Then, genes drawn in cross-regulation between ethylene signaling, ubiquitin/26S proteolysis and light-responsive transcription factors are potential targets to investigate P.oceanica reaction to light-signal at short time, as circadian clock or under day-light varying, and in long-time ruling such as timing of annual seasonality.

Clones associated to the photosynthesis Pathway

In our EST collection we identified several components of the photosynthetic machinery (see tab) as elements of Photosystem I/II, several different Chlorophyll a-b binding factors and several Cytochrome c/b subunits (fig)

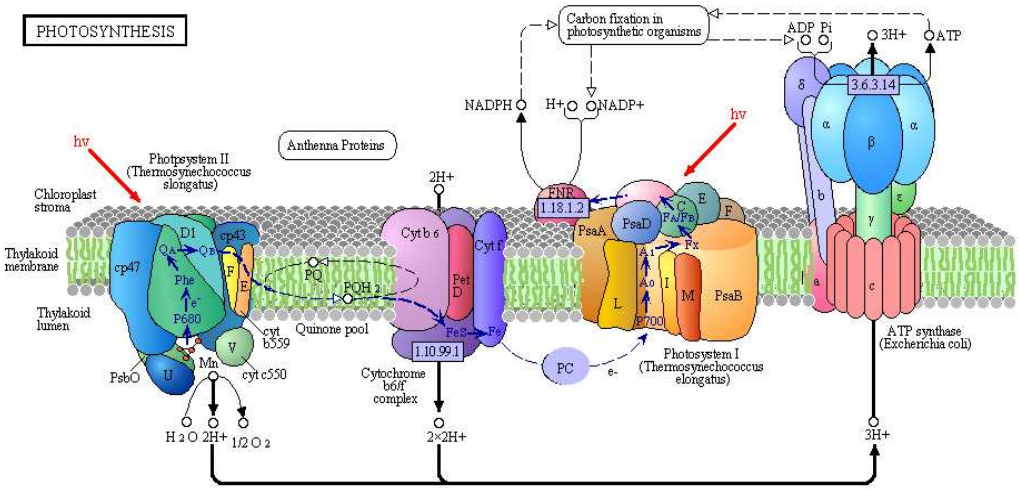


Fig Photosynthetic pathway

The amount of clones encoding for putative photosynthetic proteins is higher in Deep then in Shallow and this difference is mainly due to the presence in Deep library of many different TUG encoding for Chlorophyll-a-b binding proteins in comparison to Shallow TUG. Among these, only one protein (CHL 16) is expressed at same level in both conditions, the others CHLs being apparently specific for each of the two conditions.

Increase of Chlorophyll rate is a typical photo-adaptative response to low light conditions in seagrasses (Dennison et al 1986) where the ability to enhance light harvesting efficiencies through photo-acclimation is also well documented: experiments carried out with single species growing under different environmental conditions have revealed the capacity of plants to vary their light requirement (Ruiz, Romero, 2003). Our molecular data fit very well with the expectations: in low - light condition, as in Deep station, we have identified more clones for the photosynthetic machinery than in high-light conditions, and in addition, our data indicate that protein composition of light harvesting complex may change under different light conditions and that the photo-acclimatation process may occur through the utilization of different Chlorophyll a-b binding proteins able to capture light at different wavelength, allowing to maximize light-harvesting and consequently, carbon fixation.

Also our estimates of *Posidonia* photosynthetic response obtained during sampling are in accordance with expectations, showing that plants growing in the deep station near their maximum depth limits have higher saturation threshold (E_k) then plants growing in shallower waters.

In Shallow library a TUG encoding for an element of Photosystem II (10 kDa polypeptide) appears to be differentially expressed.

PSII complex contains the Reaction Center of the photosystem machinery. The pigments and proteins of the Reaction Center can be photochemically damaged by formation of the triplet state of $P680^+$during the normal photosynthetic activity. Damage to the Reaction Center of PSII occurs under all conditions, because PSII cannot accumulate $P680^+$ after 10^{-6}-10^{-7} turnovers can be inactivated. In order to repair polypeptides of the reaction center disassembly and re-synthesizing of all components is necessary. In this picture the high level of elements of PSII founded in Shallow in combination with the presence of high number of clones encoding for electrons carriers and Heat-shock proteins, can be related with the major rate of photodamage suffered by shallow plants in comparison with deep plants in particular during the summer time.

Clones associated at stress conditions

In our EST collection we been identified several proteins associated with stress (see tab appendix SSH); clones similar to proteins involved in plant defense or stress responses are also differentially expressed in Deep conditions, including stress regulated proteins, proteins associated to generic stress, universal stress proteins, A-like protein, protein; Metallothionein-like protein which are implicated in metal tolerance in plants (Cobbett et al. 2002); Catalase and Oxygen-evolving enhancer proteins that responds to reactive oxygen species (ROS) stress and are responsible for the breakdown of hydrogen peroxide to oxygen and water (Blokhina et al, 2003) and Cytochromes P450 family which are implicated in detoxification. Moreover in Deep are several TUGs encoding for Zinc finger domain stress-associated protein. All these elements suggest that deep plants are more sensitive at oxidative stress than plants growing in shallow station.

CHAPTER 5

GENERAL CONCLUSION

Molecular ecology also aims to elucidate the genetic basis of ecologically important phenotypic variation, and to understand the distribution of phenotypic and genotypic variation in natural populations in terms of fundamental evolutionary forces such as drift, selection, mutation, and migration

Developments from the fields of genomics and bioinformatics have great potential to help address numerous topics in molecular ecology, not only by providing sequence information and comparative data useful in designing markers, but also by opening up entirely new methods to study the genetic basis of adaptation. In particular the availability of expressed sequence tag (ESTs) for non-model species can help to resolve many technical problems related to extensive data collection.

ESTs can serve as a source of molecular markers, and can also provide an entrée into gene and genome-level questions, even for studies of non-model organisms that lack other sequence resources and have no history of functional genetics.EST collections can serve as a bridge between the genomic resources of model organisms and diverse species of interest to ecologists and evolutionary biologists and. Even if ESTs are not available from the organism under study, EST data from related organisms can be used in a variety of ways to study the ecology and evolution of diverse wild species.

EST collections sample the gene space of an organism by providing a snapshot of the transcribed mRNA population within a given set of tissues, developmental stages, environmental conditions and genotypes. During our work we have used EST data to obtain information about alternatively spliced forms of the same gene transcript (β-glucosidase) both about expression of set of thousand genes together (library).

EST have same limitation, in particular, transcripts that are in low abundance in the particular tissues sampled may not be sequenced at all. To resolve this we have used a Subtracted library that is able to enhance representatively of gene “rare” by suppression of common sequences.

Another that we have found is the contamination of EST by introns sequences that can sometimes Inferred by comparison to genomic sequences from related mRNA sequences’. Then it is not obvious distinguish alleles and alternative splice forms from paralogues using EST as a bases.

APPENDIX

PROTOCOLS

TOTAL – RNA PREPARATION FROM *OLEA EUROPAEA*/*POSIDONIA OCEANICA* LEAVES

Modified on the basis of TOTAL – RNA PREPARATION FROM POSIDONIA OCEANICA LEAVES from *Populus* or *Arabidopsis* leaves

Materials

- CTAB buffer
- CLOROPHORMIO : ALCOL ISOAMILICO (24:1) (CLO/ISO)
- B-MERCAPTOETANOLO
- 10 M LiCl
- 100% EtOH
- 75% EtOH
- H_20 RNase free (DEPC Treated)

Method

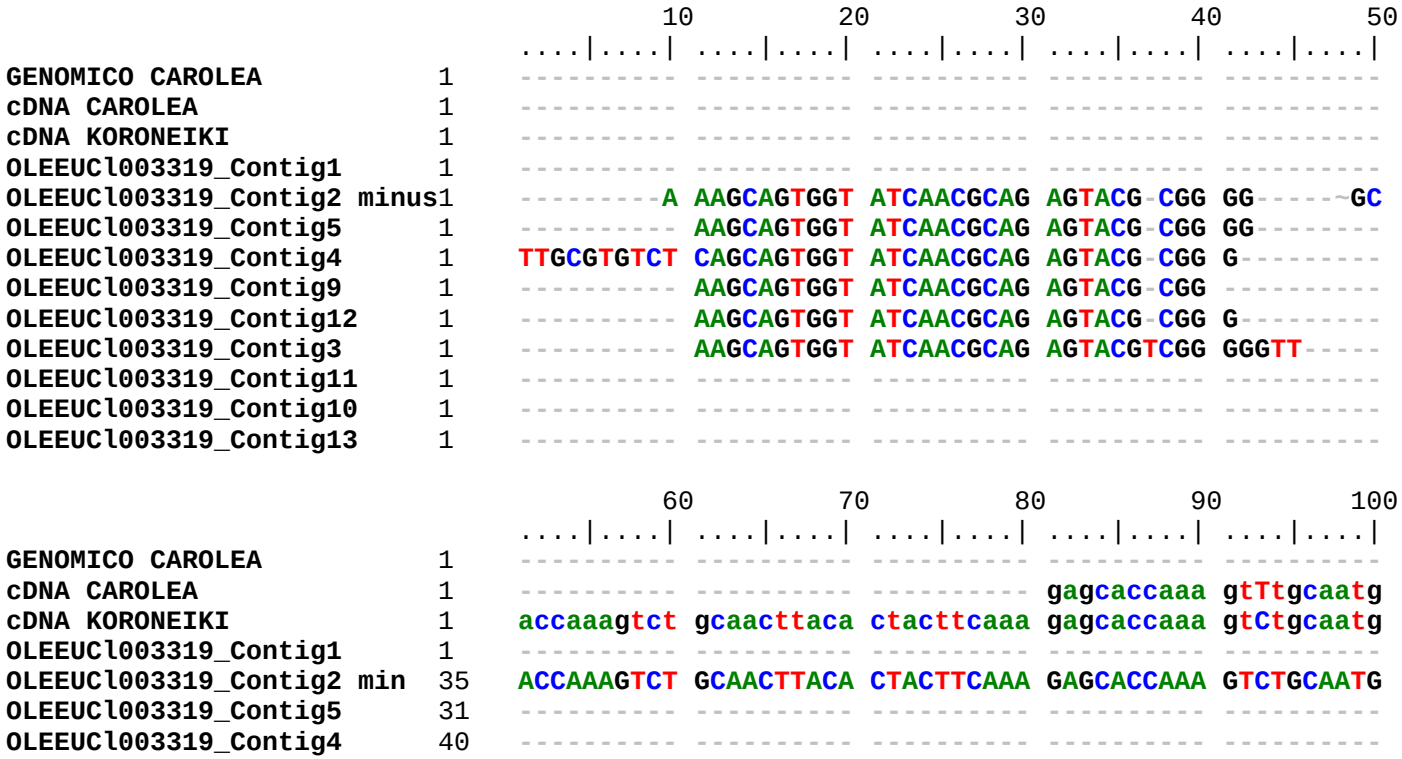
- Pre-warm 24 ml CTAB extraction buffer in falcon tubes at 65°C
- Weight 2-4g frozen leaves tissue and grind it in liquid nitrogen in a pre-cooled mortal to affine powder
- Divided the powdered plant material in four parts and transfer it into the falcon tubes, add at each tubes 8 ml CTAB + 40µl β-mercapto and mix all together thoroughly
- Incubate the suspension at 65°C for 10 min to lysate the cells completely
- Add 8ml CLO/ISO and centrifuge at 4000rpm at room T for 20 min (in swing-out rotor)
- Transfer the upper phase to a new tube and add 8ml CLO/ISO, repeat centrifuge
- Transfer the upper phase to a JA-20 tube add 1/4volume 10 M LiCl
- Precipitate 2-4h at -20°C (**not over-night**)
- Centrifuge the RNA at 11,000 rpm and 4° C for 30 min (Beckman JA-20 rotor)
- Remove the supernatant
- Wash pellet with 1ml 100% EtOH by centrifuging at 10,000 rpm for 5min
- Remove the supernatant and wash the pellet twice with 1ml 75% EtOH by centrifuging at 10,000 rpm for 5min
- Dry the pellet at 37°C or by vacuum for few minutes and re-suspend the RNA in 50 µl H_2O RNase free

BUFFER COMPOSITION FOR FLOW CYTOMETRY ANALYSIS

WPB Lysis Buffer (Louriero and Dolžel 2007)

0.2 TRIS –HCl (ph 7.5)
4mM $MgCl_2.6\ H_20$
2mM NaCl
10mM Sodio Metabisulfito
1% PVP-10 (*mod* 0,25% PVP-40)
1% (V/V) TRITON X- 100 PH 7.5

ALLIGNMENT SEQUENCES *bglc* Genomic Carolea , mRNA from Carolea leaves , mRNA AAL93619.1 , EST sequences oleaESTdatabase with positive macth



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OLEEUCl003319_Contig9      29  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12     30  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3      35  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11     1   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10     1   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig13     1   ---------- ---------- ---------- ---------- ----------

                                      110        120        130        140        150
                               ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1       ------CAAA GCAACGTCCT GACGATTACT AGCGGATCCT CACCAACTGA
cDNA CAROLEA               21  gatatccaaa gcaacgtcct gacgattact agcggatccT caccaactga
cDNA KORONEIKI             51  gatatccaaa gcaacgtcct gacgattact agcggatccA caccaactga
OLEEUCl003319_Contig1      1   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  85  GATATCCAAA GCAACGTCCT GACGATTACT AGCGGATCCT CACCAACTGA
OLEEUCl003319_Contig5      31  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      29  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12     30  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3      35  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11     1   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10     1   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig13     1   ---------- ---------- ---------- ---------- ----------

                                      160        170        180        190        200
                               ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           45  CACTTCCTCC AATGGTCAGG CAGCCAAATC CACCAAAGAA AGGATCAAAC
cDNA CAROLEA               71  cacttcctcc aatggtcagg cagccaaatc caccaaagaa aggatcaaac
cDNA KORONEIKI             101 cacttcctcc aatggtcagg cagccaaatc caccaaagaa aggatcaaac
OLEEUCl003319_Contig1      1   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  135 CACTTCCTCC AATGGTCAGG CAGCCAAATC CACCAAAGAA AGGATCAAAC
OLEEUCl003319_Contig5      31  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      29  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12     30  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3      35  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11     1   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10     1   ---------- ---------- ---CCAAATC CACCAAAGAA AGGATCAAAC
OLEEUCl003319_Contig13     1   ---------- ---------- ---------- ---------- ----------
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                                  210        220        230        240        250
                           ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           95   GCTCTGATTT TCCCAGTGAC TTTGTATTTG GCGCTGCAAC TGCTTCATAT
cDNA CAROLEA               121  gctctgattt tcccagtgac tttgtatttg gcgctgcaac tgcttcatat
cDNA KORONEIKI             151  gctctgattt tcccagtgac tttgtatttg gcgctgcaac tgcttcatat
OLEEUCl003319_Contig1      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  185  GCTCTGATTT TCCCAGTGAC TTTGTATTTG GCGCTGCAAC TGCTTCATAT
OLEEUCl003319_Contig5      31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      29   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12     30   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3      35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11     1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10     28   GCTCTGATTT TCCCAGTGAC TTTGTATTTG GCGCTGCAAC TGCTTCATAT
OLEEUCl003319_Contig13     1    ---------- ---------- ---------- ---------- ----------

                                  260        270        280        290        300
                           ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           145  CAAGTATATA TGTGTCACAT ATTATTATAC TATTCTGACT TCATTTAAAA
cDNA CAROLEA               171  caa------- ---------- ---------- ---------- ----------
cDNA KORONEIKI             201  caa------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig1      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  235  CAA------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig5      31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      29   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12     30   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3      35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11     1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10     78   CAAGTATATA TGTGTCACAT ATTATTATAC TATTCTGACT TCATTTAAAA
OLEEUCl003319_Contig13     1    ---------- ---------- ---------- ---------- ----------

                                  310        320        330        340        350
                           ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           195  AGAAAAAGAA CATATATATA ATTTGTTAAA TTCTGGGCCA CATATATAAT
cDNA CAROLEA               173  ---------- ---------- ---------- ---------- ----------
cDNA KORONEIKI             203  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig1      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  237  ---------- ---------- ---------- ---------- ----------
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OLEEUCl003319_Contig5       31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4       40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9       29   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12      30   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3       35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10      128  AGAAAAAGAA CATATATATA ATTTGTTAAA TTCTGGGCCA CATATATAAT
OLEEUCl003319_Contig13      1    ---------- ---------- ---------- ---------- ----------

                                        360        370        380        390        400
                                   ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA            245  TTTGCAGGTT GAAGGTGCAT GGAACGAAGG AGGTAAAGGC ATGAGTAATT
cDNA CAROLEA                173  -------gtt gaaggtgcat ggaacgaagg aggtaaaggc atgagtaatt
cDNA KORONEIKI              203  -------gtt gaaggtgcat ggaacgaagg aggtaaaggc atgagtaatt
OLEEUCl003319_Contig1       1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min   237  -------GTT GAAGGTGCAT GGAACGAAGG AGGTAAAGGC ATGAGTAATT
OLEEUCl003319_Contig5       31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4       40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9       29   ---------- ---------- -----GAAGG AGGTAAAGGC ATGAGTAATT
OLEEUCl003319_Contig12      30   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3       35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10      178  TTTGCAGGTT GAAGGTGCAT GGAACGAAGG AGGTAAAGGC ATGAGTAATT
OLEEUCl003319_Contig13      1    ---------- ---------- ---------- ---------- ----------

                                        410        420        430        440        450
                                   ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA            295  GGGATTACTT TACACAGAGT CAACCAGGTC TCCTCTTTTT CCAATCTTTT
cDNA CAROLEA                217  gggattactt tacacagagt caac------ ---------- ----------
cDNA KORONEIKI              247  gggattactt tacacagagt caac------ ---------- ----------
OLEEUCl003319_Contig1       1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min   281  GGGATTACTT TACACAGAGT CAACCAGGTG GTATTTCCGA CTTTAGCAAT
OLEEUCl003319_Contig5       31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4       40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9       55   GGGATTACTT TACACAGAGT CAAC------ ---------- ----------
OLEEUCl003319_Contig12      30   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3       35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10      228  GGGATTACTT TACACAGAGT CAACCAGGTC TCCTCTTTTT CCAATCTTCT
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OLEEUCl003319_Contig13     1    ---------- --CACAGAGT CAAC------ ---------- ----------

                                        460        470        480        490        500
                                ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           345  CATGAATATA TTATATAGTG TTCCATGTCT ATTGCTGAGA ATATGTTTAT
cDNA CAROLEA               240  ---------- ---------- ---------- ---------- ----------
cDNA KORONEIKI             270  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig1      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  331  GGCACTATTG CAATTGATCA CTTTAATATG TTCAAGGACG ATGTTGTCGT
OLEEUCl003319_Contig5      31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      78   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12     30   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3      35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11     1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10     278  CATGAATATA TTATATAGTG TTCCATGTCT ATTGCTGAGA ATATGTTTAT
OLEEUCl003319_Contig13     12   ---------- ---------- ---------- ---------- ----------

                                        510        520        530        540        550
                                ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           395  TGGACTGG-- ---------- ---------- ---------- ----------
cDNA CAROLEA               240  ---------- ---------- ---------- ---------- ----------
cDNA KORONEIKI             270  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig1      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  381  GATGAAGAAA TTGGGTTTGA AAGCATACAG ATTTTCACTT TCATGGCCTA
OLEEUCl003319_Contig5      31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      78   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12     30   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3      35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11     1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10     328  TGGACTGG-- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig13     12   ---------- ---------- ---------- ---------- ----------

                                        560        570        580        590        600
                                ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           402  ---------- ---------- ---------- ---------- ----------
cDNA CAROLEA               240  ---------- ---------- ---------- ---------- ----------
cDNA KORONEIKI             270  ---------- ---------- ---------- ---------- ----------
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OLEEUCl003319_Contig1      1   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  431 GAATCTTGCC AGGAGGAAGA CTGTGTCACG GTGTATCTAA AGAAGGAGTT
OLEEUCl003319_Contig5      31  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      78  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12     30  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3      35  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11     1   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10     335 ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig13     12  ---------- ---------- ---------- ---------- ----------

                                    610        620        630        640        650
                           ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           402 ---------- ---------- ---------- ---------- ----------
cDNA CAROLEA               240 ---------- ---------- ---------- ---------- ----------
cDNA KORONEIKI             270 ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig1      1   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  481 CAGTTCTATA ACGATCTCAT GGAACGAAGG AGGTAAAGGC ATGAGTAATT
OLEEUCl003319_Contig5      31  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      78  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12     30  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3      35  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11     1   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10     335 ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig13     12  ---------- ---------- ---------- ---------- ----------

                                    660        670        680        690        700
                           ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           402 ---------- ---------- ----CAGGTG GTATTTCCGA CTTTAGCAAT
cDNA CAROLEA               240 ---------- ---------- ----caggtg gtatttccga ctttagcaat
cDNA KORONEIKI             270 ---------- ---------- ----caggtg gtatttccga ctttagcaat
OLEEUCl003319_Contig1      1   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  531 GGGATTACTT TACACAGAGT CAACCAGGTG GTATTTCCGA CTTTAGCAAT
OLEEUCl003319_Contig5      31  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      78  ---------- ---------- ----CAGGTG GTATTTCCGA CTTTAGCAAT
OLEEUCl003319_Contig12     30  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3      35  ---------- ---------- ---------- ---------- ----------
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OLEEUCl003319_Contig11     1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10     335  ---------- ---------- ----TAGGTG GTATTTCCGA CTTTAGCAAT
OLEEUCl003319_Contig13     12   ---------- ---------- ----CAGGTG GTATTTCCGA CTTTAGCAAT

                                       710        720        730        740        750
                                .....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           429  GGCACTATTG CAATTGATCA CTTTAATATG TTCAAGGTAT ACTTTCAGTA
cDNA CAROLEA               267  ggcactattg caattgatca ctTtaatatg ttcaagg--- ----------
cDNA KORONEIKI             297  ggcactattg caattgatca ctAtaatatg ttcaagg--- ----------
OLEEUCl003319_Contig1      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  581  GGCACTATTG CAATTGATCA CTATAATATG TTCAAGGA-- ----------
OLEEUCl003319_Contig5      31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      105  GGCACTATTG CAATTGATCA CTTTAATATG TTCAAGG--- ----------
OLEEUCl003319_Contig12     30   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3      35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11     1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10     362  GGCACTATTG CAATTGATCA CTTTAATATG TTCAAGGTAT ACTTTCAGTA
OLEEUCl003319_Contig13     39   GGCACTATTG CAATTGATCA CTATAATATG TTCAAGGTAT ACTTTCAGTA

                                       760        770        780        790        800
                                .....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           479  ATATTTACTA TCNCATGCTC TCGAGCGAGT TAGATAAATT CAACTTTTTT
cDNA CAROLEA               303  ---------- ---------- ---------- ---------- ----------
cDNA KORONEIKI             333  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig1      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  618  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig5      31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      141  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12     30   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3      35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11     1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10     412  ATATTTACTA TGGCATGCTC TCGACGAGTT AGATAAATTC TAAACTTTTT
OLEEUCl003319_Contig13     89   ATATTTACTT ATGGCGATGC TCTCGACGGG TTTGATAAAT TCAACTTTTT

                                       810        820        830        840        850
                                .....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           529  TAACTGGCTT GTACGCTAAT ATCTATGTTA CNTTCGTATT AAACATGGAA
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cDNA CAROLEA                303  ---------- ---------- ---------- ---------- ----------
cDNA KORONEIKI              333  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig1       1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min   618  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig5       31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4       40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9       141  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12      30   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3       35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10      462  TTTAACTGGC TTGTGCGCTA ATATCTATGT TACTTCGTAT TAAACATGGA
OLEEUCl003319_Contig13      139  T--AACTT-- ---------- ---------- ---------- ----------

                                        860        870        880        890        900
                                ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA            579  TTTAGATGAA TTAAAAAAGA AACTTAAAAT AAAAATTCCG AATTTNNNCT
cDNA CAROLEA                303  ---------- ---------- ---------- ---------- ----------
cDNA KORONEIKI              333  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig1       1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min   618  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig5       31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4       40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9       141  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12      30   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3       35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10      512  ATTAGATGAA ATTAAAAAAG AAACTTAAAT AAAATTCCGA ATTTCCCTTC
OLEEUCl003319_Contig13      144  ---------- ---------- ---------- ---------- ----------

                                        910        920        930        940        950
                                ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA            629  TCATTTTGCT TTACTCATGA AAAAGTTTTC CATNATTCCT TTTACTCTTA
cDNA CAROLEA                303  ---------- ---------- ---------- ---------- ----------
cDNA KORONEIKI              333  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig1       1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min   618  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig5       31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4       40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9       141  ---------- ---------- ---------- ---------- ----------
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OLEEUCl003319_Contig12     30   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3      35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11     1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10     562  ATTTTGCTTA CTCATGAAAA GTT------- ---------- ----------
OLEEUCl003319_Contig13     144  ---------- ---------- ---------- ---------- ----------

                                       960        970        980        990        1000
                                  ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           679  AGACAACTCA AGAATTTTTC CTTTTATTTT ATTTTAAATT CATATATCAA
cDNA CAROLEA               303  ---------- ---------- ---------- ---------- ----------
cDNA KORONEIKI             333  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig1      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  618  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig5      31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      141  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12     30   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3      35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11     1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10     584  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig13     144  ---------- ---------- ---------- ---------- ----------

                                       1010       1020       1030       1040       1050
                                  ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           729  ATTGCTGATT T-ACA-TGTT GTCGTGATCA AGAAAATGGG TTTG--AGCA
cDNA CAROLEA               303  ---------- --acgatgtt gtcgtgatGa agaaaTtggg tttgaaagca
cDNA KORONEIKI             333  ---------- --acgatgtt gtcgtgatGa agaaaTtggg tttgaaagca
OLEEUCl003319_Contig1      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  618  ---------- ---CGATGTT GTCGTGATGA AGAAATTGGG TTTGAAAGCA
OLEEUCl003319_Contig5      31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      141  ---------- --ACGATGTT GTCGTGATGA AGAAATTGGG TTTGAAAGCA
OLEEUCl003319_Contig12     30   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3      35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11     1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10     584  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig13     144  ---------- ---------- ---------- ---------- ----------

                                       1060       1070       1080       1090       1100
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                                  ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA                  775  TACAGATTTT CACTTTCATG GCCTAGAATC TTGCCAGGCA TGTTATTTTC
cDNA CAROLEA                      342  tacagatttt cactttcatg gcctagaatc ttgccagg-- ----------
cDNA KORONEIKI                    372  tacagatttt cactttcatg gcctagaatc ttgccagg-- ----------
OLEEUCl003319_Contig1             1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min         656  TACAGATTTT CACTTTCATG GCCTAGAATC TTGCCAGG-- ----------
OLEEUCl003319_Contig5             31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4             40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9             180  TACAGATTTT CACTTTCATG GCCTAGAATC TTGGCCAG-- ----------
OLEEUCl003319_Contig12            30   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3             35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11            1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10            584  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig13            144  ---------- ---------- ---------- ---------- ----------

                                           1110       1120       1130       1140       1150
                                  ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA                  825  TAATTACTAC TGGAGTTTGT GCATCGTTTT ATGATGTTCT ATTAAATGTG
cDNA CAROLEA                      379  ---------- ---------- ---------- ---------- ----------
cDNA KORONEIKI                    409  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig1             1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min         693  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig5             31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4             40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9             217  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12            30   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3             35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11            1    ----------GGAGTTTGT GCATCGTTTT ATGATGTTCT ATTAAATGTG
OLEEUCl003319_Contig10            584  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig13            144  ---------- ---------- ---------- ---------- ----------

                                           1160       1170       1180       1190       1200
                                  ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA                  875  TGCACTCTAT TTTGGTGTTT TAGGAGGAAG ACTGTGTCAC GGTGTATCTA
cDNA CAROLEA                      379  ---------- ---------- ----aggaag actgtgtcac ggtgtatcta
cDNA KORONEIKI                    409  ---------- ---------- ----aggaag actgtgtcac ggtgtatcta
OLEEUCl003319_Contig1             1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min         693  ---------- ---------- ----AGGAAG ACTGTGTCAC GGTGTATCTA
OLEEUCl003319_Contig5             31   ---------- ---------- ---------- ---------- ----------
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OLEEUCl003319_Contig4      40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      217  ---------- ---------- ---GAGGAAG ACTGTGTCAC GGTGTATCTA
OLEEUCl003319_Contig12     30   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3      35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11     40   TGCACTCTAT TTTGGTGTTT TAGGAGGAAG ACTGTGTCAC GGTGTATCTA
OLEEUCl003319_Contig10     584  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig13     144  ---------- ---------- ---------- ---------- ----------

                                      1210       1220       1230       1240       1250
                                 ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           925   AAGAAGGAGT TCAGTTCTAT AACGATCTCA TTGATGCTCT CTTGGCAGCT
cDNA CAROLEA               406   aagaaggagt tcagttctat aacgatctca ttgatgcTct cttggcagct
cDNA KORONEIKI             436   aagaaggagt tcagttctat aacgatctca ttgatgcAct cttggcagct
OLEEUCl003319_Contig1      1     ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  720   AAGAAGGAGT TCAGTTCTAT AACGATCTCA TTGATGCACT CTTGGCAGCT
OLEEUCl003319_Contig5      31    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      245   AAGAAGGAGT TC-------- ---------- ---------- ----------
OLEEUCl003319_Contig12     30    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3      35    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11     90    AAGAAGGAGT TCAGTTCTAT AACGATCTCA TTGATGCACT CTTGGCAGCT
OLEEUCl003319_Contig10     584   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig13     144   ---------- ---------- ---------- ---------- ----------

                                      1260       1270       1280       1290       1300
                                 ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           975   GGTTAGAATC AATTGCATGG TCCTTATATT GATTGTTTCG TAAAAAAACT
cDNA CAROLEA               455   ---------- ---------- ---------- ---------- ----------
cDNA KORONEIKI             485   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig1      1     ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  769   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig5      31    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      256   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12     30    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3      35    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11     140   GGTTAGAATC AATTGCATGG TTCTTATATT GATTGTTTCG TAAAAAAACT
OLEEUCl003319_Contig10     584   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig13     144   ---------- ---------- ---------- ---------- ----------
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                                1310       1320       1330       1340       1350
                           ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1025 ATTATTGCAT AAATCATATT TCATATTATC TTCTCACTTT CTGTCGATCC
cDNA CAROLEA               455  ---------- ---------- ---------- ---------- ----------
cDNA KORONEIKI             485  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig1      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  769  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig5      31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      256  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12     30   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3      35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11     190  ATTATTGCAT AAATCA---- ---------- ----CAATTG GAATTAAGTA
OLEEUCl003319_Contig10     584  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig13     144  ---------- ---------- ---------- ---------- ----------

                                1360       1370       1380       1390       1400
                           ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1075 CTTTTGTGCA GACATAGAGC CATATATAAC TATCTTTCAC TGGGATATTC
cDNA CAROLEA               455  ---------- gacatagagc catatataac tatctttcac tgggatattc
cDNA KORONEIKI             485  ---------- gacatagagc catatataac tatctttcac tgggatattc
OLEEUCl003319_Contig1      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  769  ---------- GACATAGAGC CATATATAAC TATCTTTCAC TGGGATATTC
OLEEUCl003319_Contig5      31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      256  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12     30   ---------- ---------- ---------- ---------- --------TC
OLEEUCl003319_Contig3      35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11     222  CAAAATTTGG CTTGTGTGCT TCTCGGAATC ATATTTCATA TTATCA----
OLEEUCl003319_Contig10     584  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig13     144  ---------- ---------- ---------- ---------- ----------

                                1410       1420       1430       1440       1450
                           ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1125 CCCAATGTTT GCAACTAGAG TATGGTGGCT TCCTACATGA AAGAGTTGTG
cDNA CAROLEA               496  cccaatgttt gcaactagag tatggtggct tcctacatga aagagttgtg
cDNA KORONEIKI             526  cccaatgttt gcaactagag tatggtggct tcctacatga aagagttgtg
OLEEUCl003319_Contig1      1    ---------- ---------- ---------- ---------- ----------
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OLEEUCl003319_Contig2 min  810  CCCAATGTTT GCAACTAGAG TATGGTGGCT TCCTACATGA AAGAGTTGTG
OLEEUCl003319_Contig5      31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      256  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12     33   CCCAATGTTT GCAACTAGAG TATGGTGGCT TCCTACATGA AAGAGTTGTG
OLEEUCl003319_Contig3      35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11     267  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10     584  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig13     144  ---------- ---------- ---------- ---------- ----------

                                       1460       1470       1480       1490       1500
                                  ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1175 TAAGTAACTC AATCTTTCAT CCTATGGCAC ATTACTAAGA TATTTATTGA
cDNA CAROLEA               545  ---------- ---------- ---------- ---------- ----------
cDNA KORONEIKI             575  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig1      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  860  A--------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig5      31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      256  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12     83   A--------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3      35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11     267  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10     584  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig13     144  ---------- ---------- ---------- ---------- ----------

                                       1510       1520       1530       1540       1550
                                  ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1225 ACCTGAAAAG TGTATCCAGA TTTTAAAAAA ATAAAAATTA AATAATAATA
cDNA CAROLEA               545  ---------- ---------- ---------- ---------- ----------
cDNA KORONEIKI             575  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig1      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  860  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig5      31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      256  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12     83   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3      35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11     267  ---------- ---------- ---------- ---------- ----------
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OLEEUCl003319_Contig10     584  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig13     144  ---------- ---------- ---------- ---------- ----------

                                       1560       1570       1580       1590       1600
                                  ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1275 ATCATTTGCT CCAACATTAT AAAAGAGAAA ATACAAAATT TTTATTTAAA
cDNA CAROLEA               545  ---------- ---------- ---------- ---------- ----------
cDNA KORONEIKI             575  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig1      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  860  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig5      31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      256  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12     83   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3      35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11     267  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10     584  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig13     144  ---------- ---------- ---------- ---------- ----------

                                       1610       1620       1630       1640       1650
                                  ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1325 AATAAAATAA AATTGATTAC AATTTTAGTA AAATGGTGGA CAATCTTCTA
cDNA CAROLEA               545  ---------- ---------- ---------- ---------- ----------
cDNA KORONEIKI             575  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig1      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  860  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig5      31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      256  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12     83   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3      35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11     267  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10     584  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig13     144  ---------- ---------- ---------- ---------- ----------

                                       1660       1670       1680       1690       1700
                                  ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1375 CAAAGATTAA AGTTCCAGCT TAACAAGTTC TACCATATTT TGATCTATTG
cDNA CAROLEA               545  ---------- ---------- ---------- ---------- ----------
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cDNA KORONEIKI              575  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig1       1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min   860  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig5       31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4       40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9       256  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12      83   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig3       35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11      267  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10      584  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig13      144  ---------- ---------- ---------- ---------- ----------

                                      1710       1720       1730       1740       1750
                                .....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA            1425 AGCCATTTTT TGTGTCTATA ATACAGGCAG GATTTTATTG AGTATTCTGA
cDNA CAROLEA                545  ---------- ---------- -------Cag gattttattg agtattctga
cDNA KORONEIKI              575  ---------- ---------- -------Aag gattttattg agtattctga
OLEEUCl003319_Contig1       1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min   860  ---------- ---------- --------AG GATTTTATTG AGTATTCTGA
OLEEUCl003319_Contig5       31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4       40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9       256  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12      83   ---------- ---------- --------AG GATTTTATTG AGTATTCTGA
OLEEUCl003319_Contig3       35   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig11      267  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10      584  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig13      144  ---------- ---------- ---------- ---------- ----------

                                      1760       1770       1780       1790       1800
                                .....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA            1475 GATTTGCTTC TGGGAATTT- GG-TGATCGG GTGAAATATT GGATTACCTT
cDNA CAROLEA                569  gatttgcttc tgggaattt- gg-tgatcgg gtgaaatatt ggattacctt
cDNA KORONEIKI              599  gatttgcttc tgggaattt- gg-tgatcgg gtgaaatatt ggattacctt
OLEEUCl003319_Contig1       1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min   883  GATTTGCTTC TGGGAATTT- GG-TGATCGG GTGAAATATT GGATTACCTT
OLEEUCl003319_Contig5       31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4       40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9       256  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12      106  GATTTGCTTC TGGGAATTT- GG-TGATCGG GTGAAATATT GGATTACCTT
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OLEEUCl003319_Contig3      35   ---------- ---AATTTTG GTTGATCGGG TGAAATATTG GATTACCTTG
OLEEUCl003319_Contig11     267  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10     584  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig13     144  ---------- ---------- ---------- ---------- ----------

                                       1810       1820       1830       1840       1850
                                  ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1523 GAATGAGCCA TGGTCCTTTA CTGTTCAAGG ATATGTGGCT GGTGCTTTTC
cDNA CAROLEA               617  gaatgagcca tggtccttta ctgttcaagg atatgtggct ggTgctttтc
cDNA KORONEIKI             647  gaatgagcca tggtccttta ctgttcaagg atatgtggct ggCgctttтc
OLEEUCl003319_Contig1      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  931  GAATGAGCCA TGGTCCTTTA CTGTTCAAGG ATATGTGGCT GGCGCTTTTC
OLEEUCl003319_Contig5      31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      256  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12     154  GAATGAGCCA TGGTCCTTTA CTGTTCAAGG ATATGTGGCT GGCGCTTTTC
OLEEUCl003319_Contig3      73   AATGAGCCAT GGTCCTTTAC TGTTCAAGGA TATGTGGCTG GCGCTTTTCC
OLEEUCl003319_Contig11     267  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10     584  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig13     144  ---------- ---------- ---------- ---------- -------

                                       1860       1870       1880       1890       1900
                                  ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1573 CG-CCCAA-T CGTGGTGTAA CTCCGAAAGA TACTGAAGAA ACTAAAAAGC
cDNA CAROLEA               667  cg-cccaa-t cgtggtgtaa ctccgaaaga tactgaagaa actAaaaagc
cDNA KORONEIKI             697  cg-cccaa-t cgtggtgtaa ctccgaaaga tactgaagaa actCaaaagc
OLEEUCl003319_Contig1      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  981  CG-CCCAA-T CGTGGTGTAA CTCCGAAAGA TACTGAAGAA ACTCAAAAGC
OLEEUCl003319_Contig5      31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      256  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig12     204  CGTCCCAACT CGTGGTGTAA CTCCGAAAGA TACTGAAGAA ACTCAAAAGC
OLEEUCl003319_Contig3      123  G-CCCAA-TC GTGGTGTAAC TCCGAAAGAT ACTGAAGAAA CTCAAAAGCA
OLEEUCl003319_Contig11     267  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig10     584  ---------- ---------- ---------- ---------- --
OLEEUCl003319_Contig13     144

                                       1910       1920       1930       1940       1950
                                  ....|....| ....|....| ....|....| ....|....| ....|....|
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GENOMICO CAROLEA           1621 ATGCCCGTCT TCATAGAG
cDNA CAROLEA               715  atgcccgtct tcatagaggt ggaggaaaac ttctagctgc tttcaagtat
cDNA KORONEIKI             745  atgcccgtct tcatagaggt ggaggaaaac ttctagctgc tttcaagtat
OLEEUCl003319_Contig1      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  1029 ATGG------ ---------- ---------- ---------- -
OLEEUCl003319_Contig5      31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      256  ----
OLEEUCl003319_Contig12     254  ATGCCCGT-- ---------- -
OLEEUCl003319_Contig3      171  TGCCCGTCTT CATAGAGGTG GAGGAAAACT TCTAGCTGCT TTCAAGTATG
OLEEUCl003319_Contig11     267  ---------- ---------- --
OLEEUCl003319_Contig10     584
OLEEUCl003319_Contig13     144

                                      1960       1970       1980       1990       2000
                                 ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1638
cDNA CAROLEA               765  ggaaatcccg gcacggaacc atataaagtg gcacacaatt taatcctttg
cDNA KORONEIKI             795  ggaaatcccg gcacggaacc atataaagtg gcacacaatt taatcctttg
OLEEUCl003319_Contig1      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  1032
OLEEUCl003319_Contig5      31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      256
OLEEUCl003319_Contig12     261
OLEEUCl003319_Contig3      221  GAAATCCCGG CACGGAACCA TATAAAGTGG CACACAATTT AATCCTTTGT
OLEEUCl003319_Contig11     267
OLEEUCl003319_Contig10     584
OLEEUCl003319_Contig13     144

                                      2010       2020       2030       2040       2050
                                 ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1638
cDNA CAROLEA               815  tcatgcacat gctgtggaCa tatacagaac gaaatatcag gaaagtcagg
cDNA KORONEIKI             845  tcatgcacat gctgtggaTa tatacagaac gaaatatcag gaaagtcagg
OLEEUCl003319_Contig1      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  1032
OLEEUCl003319_Contig5      31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40   ---------- ---------- ---------- ---------- ----------
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OLEEUCl003319_Contig9      256
OLEEUCl003319_Contig12     261
OLEEUCl003319_Contig3      271  CATGCACATG CTGTGGACAT ATACAGAACG AAATAT----
OLEEUCl003319_Contig11     267
OLEEUCl003319_Contig10     584
OLEEUCl003319_Contig13     144

                                       2060       2070       2080       2090       2100
                                  ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1638
cDNA CAROLEA               865  gaggtaagat agggattaca aattgcatta gttggaatga gcctcttact
cDNA KORONEIKI             895  gaggtaagat agggattaca aattgcatta gttggaatga gcctcttact
OLEEUCl003319_Contig1      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  1032
OLEEUCl003319_Contig5      31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      256
OLEEUCl003319_Contig12     261
OLEEUCl003319_Contig3      306
OLEEUCl003319_Contig11     267
OLEEUCl003319_Contig10     584
OLEEUCl003319_Contig13     144

                                       2110       2120       2130       2140       2150
                                  ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1638
cDNA CAROLEA               915  gactctcaag aagataaaga tgctgctact agaggaaatg attttatgct
cDNA KORONEIKI             945  gactctcaag aagataaaga tgctgctact agaggaaatg attttatgct
OLEEUCl003319_Contig1      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  1032
OLEEUCl003319_Contig5      31   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      256
OLEEUCl003319_Contig12     261
OLEEUCl003319_Contig3      306
OLEEUCl003319_Contig11     267
OLEEUCl003319_Contig10     584
OLEEUCl003319_Contig13     144
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                                2160       2170       2180       2190       2200
                           ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1638
cDNA CAROLEA               965  tggatggttt gtggaaccag tggtaactgg agagtaccca gaaagtatga
cDNA KORONEIKI             995  tggatggttt gtggaaccag tggtaactgg agagtaccca gaaagtatga
OLEEUCl003319_Contig1      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  1032
OLEEUCl003319_Contig5      31   ---------- ---------- ---------- ----TACCCA GAAAGTATGA
OLEEUCl003319_Contig4      40   ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      256
OLEEUCl003319_Contig12     261
OLEEUCl003319_Contig3      306
OLEEUCl003319_Contig11     267
OLEEUCl003319_Contig10     584
OLEEUCl003319_Contig13     144

                                2210       2220       2230       2240       2250
                           ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1638
cDNA CAROLEA               1015 ttaaatatgt tggcgatcgc cttcctaaat tttctgaaaa agaggagaag
cDNA KORONEIKI             1045 ttaaatatgt tggcgatcgc cttcctaaat tttctgaaaa agaggagaag
OLEEUCl003319_Contig1      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  1032
OLEEUCl003319_Contig5      48   TTAAAAATGT TGGTGATCGC CTTCCTAAAT TTTCTGAAAA AGAGGAGAAG
OLEEUCl003319_Contig4      40   ---------- ---------- ---------- -GAGGAGAAG CTAGTGAAAG
OLEEUCl003319_Contig9      256
OLEEUCl003319_Contig12     261
OLEEUCl003319_Contig3      306
OLEEUCl003319_Contig11     267
OLEEUCl003319_Contig10     584
OLEEUCl003319_Contig13     144

                                2260       2270       2280       2290       2300
                           ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1638
cDNA CAROLEA               1065 ctagtgaaag gatcctacga ctttctaggc ataaactact acacgtctac
cDNA KORONEIKI             1095 ctagtgaaag gatcctacga ctttctaggc ataaactact acacgtctac
OLEEUCl003319_Contig1      1    ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  1032
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OLEEUCl003319_Contig5      98   CTAGTGAAAG GATCCTATGA CTTTCTAGGC ATAAACTACT ACACGTCTAC
OLEEUCl003319_Contig4      60   GATCCTACGA CTTTCTAGGC ATAAACTAAC TACACGTCTA CTTATACCAG
OLEEUCl003319_Contig9      256
OLEEUCl003319_Contig12     261
OLEEUCl003319_Contig3      306
OLEEUCl003319_Contig11     267
OLEEUCl003319_Contig10     584
OLEEUCl003319_Contig13     144

                                    2310       2320       2330       2340       2350
                           ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1638
cDNA CAROLEA               1115 ttataccagc gatgatccaa caaagccgac aactgatagt tactttact-
cDNA KORONEIKI             1145 ttataccagc gatgatccaa caaagccgac aactgatagt tactttact-
OLEEUCl003319_Contig1      1    ---------- ---------C AAAG-CCGAC AACTGATAGT TACTTGTAC-
OLEEUCl003319_Contig2 min  1032
OLEEUCl003319_Contig5      148  TTATACCAGC GATGATCCGA CAAAGCCGAC AACTGATAGT TACTTGACT-
OLEEUCl003319_Contig4      110  CGATGATCCA ACAAAGACCG ACAACTGATA GTTACTTTAC TTGATTCGCA
OLEEUCl003319_Contig9      256
OLEEUCl003319_Contig12     261
OLEEUCl003319_Contig3      306
OLEEUCl003319_Contig11     267
OLEEUCl003319_Contig10     584
OLEEUCl003319_Contig13     144

                                    2360       2370       2380       2390       2400
                           ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1638
cDNA CAROLEA               1164 gattcgcaca ctaagacttc acatgaacgc aataaggtgc ctattggtgc
cDNA KORONEIKI             1194 gattcgcaca ctaagacttc acatgaacgc aataaggtgc ctattggtgc
OLEEUCl003319_Contig1      30   TTTTGTTTAT TTCGCGCACT AAGACTTCAC ATGAACGCAA TAAGGTGCCT
OLEEUCl003319_Contig2 min  1032
OLEEUCl003319_Contig5      196  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      160  CACTAAGACT TC-------- ---------- ---------- ----------
OLEEUCl003319_Contig9      256
OLEEUCl003319_Contig12     261
OLEEUCl003319_Contig3      306
OLEEUCl003319_Contig11     267
OLEEUCl003319_Contig10     584
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OLEEUCl003319_Contig13     144

                                    2410       2420       2430       2440       2450
                                ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1638
cDNA CAROLEA               1214 acaggctggt tcggaTtggt tatacatagt tccatgggga atctacagag
cDNA KORONEIKI             1244 acaggctggt tcggaCtggt tatacatagt tccatgggga atctacagag
OLEEUCl003319_Contig1      80   ATTGGTGCAC AGGCTGGTTC GGATTGGTTA TACATAGTTC CATGGGGAAT
OLEEUCl003319_Contig2 min  1032
OLEEUCl003319_Contig5      196  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      171  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      256
OLEEUCl003319_Contig12     261
OLEEUCl003319_Contig3      306
OLEEUCl003319_Contig11     267
OLEEUCl003319_Contig10     584
OLEEUCl003319_Contig13     144

                                    2460       2470       2480       2490       2500
                                ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1638
cDNA CAROLEA               1264 ttatggt-ga tatga-gaaa aagatataat gatccggtta tatacatcac
cDNA KORONEIKI             1294 ttatggttga tatgaagaaa aagatataat gatccggtta tatacatcac
OLEEUCl003319_Contig1      130  TTACAGAGTT ATGGTTGATA TGAAGAAAAG ATATAATGAT CCAGTTATTT
OLEEUCl003319_Contig2 min  1032
OLEEUCl003319_Contig5      196  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      171  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      256
OLEEUCl003319_Contig12     261
OLEEUCl003319_Contig3      306
OLEEUCl003319_Contig11     267
OLEEUCl003319_Contig10     584
OLEEUCl003319_Contig13     144

                                    2510       2520       2530       2540       2550
                                ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1638
cDNA CAROLEA               1312 cgagaacgga gtggatgaag taaacgataa gtccaagact agtaccgaag
cDNA KORONEIKI             1344 cgagaacgga gtggatgaag taaacgataa gtccaagact agtaccgaag
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OLEEUCl003319_Contig1      180  ACATTACAGA GAATGGAGTG GATGAAGTAA ACGATAAGTC CAAGACTAGT
OLEEUCl003319_Contig2 min  1032
OLEEUCl003319_Contig5      196  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      171  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      256
OLEEUCl003319_Contig12     261
OLEEUCl003319_Contig3      306
OLEEUCl003319_Contig11     267
OLEEUCl003319_Contig10     584
OLEEUCl003319_Contig13     144

                                    2560       2570       2580       2590       2600
                                ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1638
cDNA CAROLEA               1362 ctctcaagga cgatataagg attcactatc accaagaaca tctctactac
cDNA KORONEIKI             1394 ctctcaagga cgatataagg attcactatc accaagaaca tctctactac
OLEEUCl003319_Contig1      230  ACCGAAGCTC TCAAGGATGA TATAAGGATT CACTATCACC AAGAACATCT
OLEEUCl003319_Contig2 min  1032
OLEEUCl003319_Contig5      196  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      171  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      256
OLEEUCl003319_Contig12     261
OLEEUCl003319_Contig3      306
OLEEUCl003319_Contig11     267
OLEEUCl003319_Contig10     584
OLEEUCl003319_Contig13     144

                                    2610       2620       2630       2640       2650
                                ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1638
cDNA CAROLEA               1412 cttaagctag cgatggatca aggtgtaaat gtgaagggtt acttcatatg
cDNA KORONEIKI             1444 cttaagctag cgatggatca aggtgtaaat gtgaagggtt acttcatatg
OLEEUCl003319_Contig1      280  CTACTACCTT AAGCTAGCGA TGGAGTAA-- ---------- -TTAATTCCT
OLEEUCl003319_Contig2 min  1032
OLEEUCl003319_Contig5      196  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      171  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      256
OLEEUCl003319_Contig12     261
OLEEUCl003319_Contig3      306
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OLEEUCl003319_Contig11     267
OLEEUCl003319_Contig10     584
OLEEUCl003319_Contig13     144

                                    2660       2670       2680       2690       2700
                                ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1638
cDNA CAROLEA               1462 gtcgttgttc gacaattttg aatgggctgc tggattcagt gttagatttg
cDNA KORONEIKI             1494 gtcgttgttc gacaattttg aatgggctgc tggattcagt gttagatttg
OLEEUCl003319_Contig1      317  TTAGT--TTG TT-GTCAAAA TTAATTA--- TTATGAAT-- -GTATTT-AC
OLEEUCl003319_Contig2 minus1032
OLEEUCl003319_Contig5      196  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      171  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      256
OLEEUCl003319_Contig12     261
OLEEUCl003319_Contig3      306
OLEEUCl003319_Contig11     267
OLEEUCl003319_Contig10     584
OLEEUCl003319_Contig13     144

                                    2710       2720       2730       2740       2750
                                ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1638
cDNA CAROLEA               1512 gcgttatgta tgtagactat gctaatggtc gttacacaag gttgccaaaa
cDNA KORONEIKI             1544 gcgttatgta tgtagactat gctaatggtc gttacacaag gttgccaaaa
OLEEUCl003319_Contig1      357  TTCACAGCAT TTACTCAATT CGTGCTAAAG ATT-TTGCTT TCCTTTTTCA
OLEEUCl003319_Contig2 min  1032
OLEEUCl003319_Contig5      196  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      171  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      256
OLEEUCl003319_Contig12     261
OLEEUCl003319_Contig3      306
OLEEUCl003319_Contig11     267
OLEEUCl003319_Contig10     584
OLEEUCl003319_Contig13     144

                                    2760       2770       2780       2790       2800
                                ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1638
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cDNA CAROLEA                1562 cgttcagctg tatggtggag gaacttcctc accaagccta cagcagttcc
cDNA KORONEIKI              1594 cgttcagctg tatggtggag gaacttcctc accaagccta cagcagttcc
OLEEUCl003319_Contig1       405  ----GCCAAG GTGTAAATTT GAAGGGTTAC TTCA------ -TATGGTCGT
OLEEUCl003319_Contig2 min   1032
OLEEUCl003319_Contig5       196  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4       171  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9       256
OLEEUCl003319_Contig12      261
OLEEUCl003319_Contig3       306
OLEEUCl003319_Contig11      267
OLEEUCl003319_Contig10      584
OLEEUCl003319_Contig13      144

                                    2810       2820       2830       2840       2850
                                ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA            1638
cDNA CAROLEA                1612 attgaagaat gaacctgaaa aatctgaaga tcgtcgtaaa aggcttagag
cDNA KORONEIKI              1644 attgaagaat gaacctgaaa aatctgaaga tcgtcgtaaa aggcttagag
OLEEUCl003319_Contig1       445  T--GTTCGAC AAT---TTTG AATGGGCTGC TGGATTCAGT GTTAGATTT-
OLEEUCl003319_Contig2 min   1032
OLEEUCl003319_Contig5       196  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4       171  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9       256
OLEEUCl003319_Contig12      261
OLEEUCl003319_Contig3       306
OLEEUCl003319_Contig11      267
OLEEUCl003319_Contig10      584
OLEEUCl003319_Contig13      144

                                    2860       2870       2880       2890       2900
                                ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA            1638
cDNA CAROLEA                1662 gcagcaccta gtttagctta tgaaactagt gcttccttga ttttatgttg
cDNA KORONEIKI              1694 gcagcaccta gtttagctta tgaaactagt gcttccttga ttttatgttg
OLEEUCl003319_Contig1       488  --GGCGTTAT GTA-TGTAGA CTATGCTAAT GGTCGTTACA CAAGGTTGCC
OLEEUCl003319_Contig2 min   1032
OLEEUCl003319_Contig5       196  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4       171  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9       256
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OLEEUCl003319_Contig12     261
OLEEUCl003319_Contig3      306
OLEEUCl003319_Contig11     267
OLEEUCl003319_Contig10     584
OLEEUCl003319_Contig13     144

                                    2910       2920       2930       2940       2950
                                ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1638
cDNA CAROLEA               1712 aataatgcac ggtggaggat gttgctttaa atttttgtt gtcgttgttg
cDNA KORONEIKI             1744 aataatgcac ggtggaggat gttgctttaa atttttgtt gtcgttgttg
OLEEUCl003319_Contig1      536  AAAAACCGT- TACAGCTG-- TATGGTGGAG GAACTTCCTC ACCAAG----
OLEEUCl003319_Contig2 min  1032
OLEEUCl003319_Contig5      196  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      171  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      256
OLEEUCl003319_Contig12     261
OLEEUCl003319_Contig3      306
OLEEUCl003319_Contig11     267
OLEEUCl003319_Contig10     584
OLEEUCl003319_Contig13     144

                                    2960       2970       2980       2990       3000
                                ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA           1638
cDNA CAROLEA               1762 ttggttgaaa agtcatgtca ttgttgttga atga
cDNA KORONEIKI             1794 ttggttgaaa agtcatgtca ttgttgttga atgacgcatg gttcaacttg
OLEEUCl003319_Contig1      578  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min  1032
OLEEUCl003319_Contig5      196  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4      171  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9      256
OLEEUCl003319_Contig12     261
OLEEUCl003319_Contig3      306
OLEEUCl003319_Contig11     267
OLEEUCl003319_Contig10     584
OLEEUCl003319_Contig13     144

                                    3010       3020       3030       3040       3050
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                                 ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA                 1638
cDNA CAROLEA                     1795
cDNA KORONEIKI                   1844 tactcgttat gttccaataa aggagttggg ctccatgtca aaaaaaaaaa
OLEEUCl003319_Contig1            578  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig2 min        1032
OLEEUCl003319_Contig5            196  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig4            171  ---------- ---------- ---------- ---------- ----------
OLEEUCl003319_Contig9            256
OLEEUCl003319_Contig12           261
OLEEUCl003319_Contig3            306
OLEEUCl003319_Contig11           267
OLEEUCl003319_Contig10           584
OLEEUCl003319_Contig13           144

                                         3060       3070       3080       3090       3100
                                 ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA                 1638
cDNA CAROLEA                     1795
cDNA KORONEIKI                   1894 aaaaaaaaaa
OLEEUCl003319_Contig1            578  ---------- ---
OLEEUCl003319_Contig2 min        1032
OLEEUCl003319_Contig5            196  ---------- ------GATT CGCGCACTAA GACTTCACAT GAACGCAATA
OLEEUCl003319_Contig4            171  ---
OLEEUCl003319_Contig9            256
OLEEUCl003319_Contig12           261
OLEEUCl003319_Contig3            306
OLEEUCl003319_Contig11           267
OLEEUCl003319_Contig10           584
OLEEUCl003319_Contig13           144

                                         3110       3120       3130       3140       3150
                                 ....|....| ....|....| ....|....| ....|....| ....|....|
GENOMICO CAROLEA                 1638
cDNA CAROLEA                     1795
cDNA KORONEIKI                   1903
OLEEUCl003319_Contig1            578
OLEEUCl003319_Contig2            1032
OLEEUCl003319_Contig5            231  AGGTGCCTAT TGGTGCACAG GTATGACAAA ATCTTGCCCC TTCCCTACGA
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OLEEUCl003319_Contig4      171
OLEEUCl003319_Contig9      256
OLEEUCl003319_Contig12     261
OLEEUCl003319_Contig3      306
OLEEUCl003319_Contig11     267
OLEEUCl003319_Contig10     584
OLEEUCl003319_Contig13     144

                                    3160       3170
                               ....|....| ....|....| ....|...
GENOMICO CAROLEA           1638
cDNA CAROLEA               1795
cDNA KORONEIKI             1903
OLEEUCl003319_Contig1      578
OLEEUCl003319_Contig2 min  1032
OLEEUCl003319_Contig5      281  AAGAAGATAT ATTGGATTAT ATTCTAAC
OLEEUCl003319_Contig4      171
OLEEUCl003319_Contig9      256
OLEEUCl003319_Contig12     261
OLEEUCl003319_Contig3      306
OLEEUCl003319_Contig11     267
OLEEUCl003319_Contig10     584
OLEEUCl003319_Contig13     144
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CONTIG LIST OF SSH LIBRARY

TUG name	Shallow	SWISS-PROT	hit	eValue	EST Dr. Zompo Hit
Contig205	190	Proteasome subunit alpha type-2 putative			Yes
Contig132	43	Chlorophyll a-b binding protein 16, chloroplastic (Nicotiana tabacum)	P27492	8.61E-87	Yes
Contig18	24	Proteasome subunit alpha type-2 putative			Yes
Contig444	10	F-box protein At5g67140 (Arabidopsis thaliana)	Q9FH99		No
Contig209	8	No Swissprot-Hit			No
Contig95	7	Heat shock cognate 70 kDa protein 1 (Arabidopsis thaliana)	P22953	2.84E-10	Yes
Contig248	6	Cytochrome c oxidase polypeptide VIb (Schizosaccharomyces pombe)	O94581	2.52E-07	Yes
Contig284	6	Photosystem II 10 kDa polypeptide, chloroplastic (Nicotiana tabacum)	Q40519	1.67E-35	Yes
Contig341	6	No Swissprot-Hit			Yes
Contig250	6	Ketol-acid reductoisomerase, chloroplastic (Arabidopsis thaliana)	Q05758	1.36E-60	No
Contig449	6	No Swissprot-Hit			No
Contig386	5	Chlorophyll a-b binding protein 37, chloroplastic (Petunia sp.)	P12062	1.38E-77	Yes
Contig142	5	No Swissprot-Hit			Yes
Contig365	5	Cytochrome b6-f complex iron-sulfur subunit, chloroplastic (Fritillaria agrestis)	O49078	1.43E-37	Yes
Contig130	5	No Swissprot-Hit			No
Contig198	5	No Swissprot-Hit			No
Contig36	5	ATP-dependent Clp protease proteolytic subunit (Azoarcus sp.)	Q5P161	1.80E-22	No
Contig434	5	ETO1-like protein 1 (Arabidopsis thaliana)	Q9ZQX6	2.43E-88	No
Contig162	4	Regulator of ribonuclease activity A (Ralstonia eutropha)	Q0K9J4	1.80E-42	Yes
Contig287	4	Protein TIFY 3B putative			Yes
Contig382	4	No Swissprot-Hit			Yes
Contig391	4	Probable ATP synthase 24 kDa subunit, mitochondrial (Arabidopsis thaliana)	Q9SJ12	3.71E-27	Yes
Contig7	4	Acyl-CoA-binding protein (Brassica napus)	Q39315	1.25E-14	Yes

Contig116	4	No Swissprot-Hit			No
Contig168	4	No Swissprot-Hit			No
Contig208	4	No Swissprot-Hit			No
Contig362	4	No Swissprot-Hit			No
Contig415	4	No Swissprot-Hit			No
Contig422	4	No Swissprot-Hit			No
Contig44	4	Putative U-box domain-containing protein 53 (Arabidopsis thaliana)	Q9LU47	1.27E-33	No
Contig97	3	Aquaporin PIP2-5 (Zea mays)	Q9XF58	2.56E-52	Yes
Contig229	3	Light-regulated protein putative			Yes
Contig149	3	Proteasome subunit alpha type-2 putative			Yes
Contig327	3	Ammonium transporter 1 member 1 (Arabidopsis thaliana)	P54144	3.16E-16	Yes
Contig337	3	Ethylene-responsive transcription factor 6 (Arabidopsis thaliana)	Q8VZ91	3.45E-17	Yes
Contig384	3	Phytosulfokines putative			Yes
Contig61	3	Acyl-CoA-binding protein (Fritillaria agrestis)	O22643	2.16E-06	Yes
Contig110	3	No Swissprot-Hit			No
Contig118	3	Translocase of chloroplast 159, chloroplastic (Arabidopsis thaliana)	O81283	4.76E-27	No
Contig146	3	No Swissprot-Hit			No
Contig163	3	No Swissprot-Hit			No
Contig215	3	No Swissprot-Hit			No
Contig230	3	No Swissprot-Hit			No
Contig298	3	Chalcone synthase (Arabis alpina)	Q9SEP4	1.37E-33	No
Contig314	3	No Swissprot-Hit			No
Contig315	3	No Swissprot-Hit			No
Contig331	3	No Swissprot-Hit			No
Contig370	3	Cytochrome P450 76A1 (Fragment) (Solanum melongena)	P37121	1.16E-35	No
Contig417	3	No Swissprot-Hit			No
Contig428	3	Cysteine-rich receptor-like protein kinase 2 (Arabidopsis thaliana)	Q9CAL3	2.19E-07	No

Contig486	3	Cytochrome b (Coracias caudata)	Q9ZZD4	1.51E-27	No
Contig310	2	Chlorophyll a-b binding protein of LHCII type III, chloroplastic (Hordeum vulgare)	P27523	1.00E-32	Yes
Contig122	2	ATP-citrate synthase (Rattus norvegicus)	P16638	1.51E-12	No
Contig207	2	No Swissprot-Hit			Yes
Contig126	2	F-box/LRR-repeat protein 2 (Mus musculus)	Q8BH16	1.06E-08	No
Contig8	2	No Swissprot-Hit			No
Contig112	2	Chlorophyll a-b binding protein CP24, chloroplastic (Spinacia oleracea)	P36494	1.22E-14	Yes
Contig159	2	Proteasome subunit alpha type-2 putative			Yes
Contig172	2	Proteasome subunit alpha type-2 putative			Yes
Contig184	2	Chalcone--flavonone isomerase putative			Yes
Contig20	2	Uncharacterized protein At4g01150,putative			Yes
Contig343	2	Magnesium-protoporphyrin IX monomethyl ester [oxidative] cyclase, chloroplastic (Euphorbia esula)	Q945B7	1.20E-65	Yes
Contig366	2	Non-specific lipid-transfer protein putativae			Yes
Contig426	2	Catalase-1/2 (Glycine max)	P29756	1.25E-22	Yes
Contig43	2	No Swissprot-Hit			Yes
Contig468	2	Zinc transporter 7 (Arabidopsis thaliana)	Q8W246	1.50E-28	Yes
Contig473	2	Calmodulin (Capsicum annuum)	P93087	1.36E-13	Yes
Contig485	2	No Swissprot-Hit			Yes
Contig91	2	Proteasome subunit alpha putative			Yes
Contig103	2	No Swissprot-Hit			No
Contig133	2	No Swissprot-Hit			No
Contig134	2	No Swissprot-Hit			No
Contig136	2	No Swissprot-Hit			No
Contig171	2	No Swissprot-Hit			No
Contig174	2	No Swissprot-Hit			No
Contig211	2	No Swissprot-Hit			No

Contig214	2	No Swissprot-Hit			No
Contig228	2	No Swissprot-Hit			No
Contig247	2	No Swissprot-Hit			No
Contig249	2	No Swissprot-Hit			No
Contig255	2	Argininosuccinate synthase (Carboxydothermus hydrogenoformans)	Q3A9W5	4.99E-52	No
Contig260	2	Uncharacterized TPR repeat-containing protein At2g32450 (A.thaliana)	Q8S8L9	1.43E-37	No
Contig301	2	No Swissprot-Hit			No
Contig305	2	Cytochrome c oxidase subunit 2 (Ctenocephalides felis)	P29872	2.83E-21	No
Contig306	2	No Swissprot-Hit			No
Contig324	2	No Swissprot-Hit			No
Contig325	2	Chaperone protein clpB (Gloeobacter violaceus)	Q7NFE9	1.04E-28	No
Contig378	2	No Swissprot-Hit			No
Contig4	2	No Swissprot-Hit			No
Contig414	2	No Swissprot-Hit			No
Contig439	2	No Swissprot-Hit			No
Contig45	2	Cytochrome P450 98A2 (Glycine max)	O48922	1.11E-15	No
Contig454	2	No Swissprot-Hit			No
Contig475	2	No Swissprot-Hit			No
Contig481	2	No Swissprot-Hit			No
Contig54	2	No Swissprot-Hit			No
Contig58	2	Methylisocitrate lyase (Aeropyrum pernix)	Q9YFM7	1.35E-15	No
Contig421	1	S-norcoclaurine synthase (Thalictrum flavum subsp. glaucum)			Yes
Contig262	1	Caffeoyl-CoA O-methyltransferase (Populus tremuloides)	Q43095	1.11E-10	Yes
Contig447	1	Metallothionein-like protein type 3 (Malus domestica)	O24059	1.31E-12	Yes
Contig271	1	Photosystem I reaction center subunit III, chloroplastic (Arabidopsis thaliana)	Q9SHE8	3.55E-06	Yes
Contig213	1	Alcohol dehydrogenase 1 putative			Yes

Contig319	1	Metallothionein-like protein type 3 (Malus domestica)	O24059	3.53E-13	Yes
Contig360	1	Chlorophyll a-b binding protein, chloroplastic (Fragment) (Silene pratensis)	P12332	1.20E-48	Yes
Contig201	1	Fructose-bisphosphate aldolase 1, chloroplastic (Fragment) (Pisum sativum)	Q01516	1.47E-47	Yes
Contig293	1	Chlorophyll a-b binding protein 1, chloroplastic (Oryza sativa subsp. japonica)	P12330	2.85E-14	Yes
Contig307	1	Zeaxanthin epoxidase, chloroplastic (Solanum lycopersicum)	P93236	7.61E-07	Yes
Contig462	1	Chlorophyll a-b binding protein CP26, chloroplastic (Arabidopsis thaliana)	Q9XF89	4.00E-44	Yes
Contig143	1	No Swissprot-Hit			No
Contig339	1	No Swissprot-Hit			No
Contig418	1	No Swissprot-Hit			No
Contig5	1	No Swissprot-Hit			No
Contig138	1	No Swissprot-Hit			Yes
Contig158	1	No Swissprot-Hit			Yes
Contig235	1	50S ribosomal protein L35 (Anabaena sp. (strain PCC 7120))	Q8YRL9	2.00E-08	Yes
Contig267	1	RING-box protein 1a (Arabidopsis thaliana)	Q940X7	2.48E-54	Yes
Contig27	1	No Swissprot-Hit			Yes
Contig317	1	No Swissprot-Hit			Yes
Contig333	1	Auxin-induced in root cultures protein 12 putative			Yes
Contig334	1	Glutaredoxin-C3 (Arabidopsis thaliana)	Q9FVX1	1.24E-06	Yes
Contig340	1	Proteasome subunit alpha type-2 (Oryza sativa subsp. japonica)			Yes
Contig346	1	No Swissprot-Hit			Yes
Contig347	1	Translationally-controlled tumor protein homolog (Fragaria ananassa)	O03992	4.36E-43	Yes
Contig350	1	Autophagy-related protein 8c (Arabidopsis thaliana)	Q8S927	1.37E-29	Yes
Contig357	1	Proteasome subunit alpha type-2 putative			Yes
Contig37	1	No Swissprot-Hit			Yes

Contig40	1	60S acidic ribosomal protein P2B (Zea mays)	O24415	1.53E-18	Yes
Contig437	1	No Swissprot-Hit			Yes
Contig483	1	Cytochrome b-c1 complex subunit Rieske-1, mitochondrial (Fragment) (Nicotiana tabacum)	P49729	2.35E-32	Yes
Contig50	1	Olee1-like protein (Betula verrucosa)	O49813	9.24E-06	Yes
Contig87	1	60S ribosomal protein L3 (Bos taurus)	P39872	8.93E-13	Yes
Contig89	1	Transcription factor CPC (Arabidopsis thaliana)	O22059	7.10E-10	Yes
Contig1	1	No Swissprot-Hit			No
Contig10	1	Putative lipoxygenase 5 (Oryza sativa subsp. japonica)	Q7XV13	1.53E-41	No
Contig104	1	No Swissprot-Hit			No
Contig109	1	No Swissprot-Hit			No
Contig111	1	Cell division cycle protein 48 homolog (Capsicum annuum)	Q96372	1.03E-08	No
Contig115	1	Two-component response regulator-like PRR37 putative			No
Contig127	1	No Swissprot-Hit			No
Contig137	1	No Swissprot-Hit			No
Contig14	1	No Swissprot-Hit			No
Contig148	1	No Swissprot-Hit			No
Contig15	1	No Swissprot-Hit			No
Contig150	1	Pantoate--beta-alanine ligase (Arabidopsis thaliana)	Q9FKB3	1.5981E-09	No
Contig155	1	No Swissprot-Hit			No
Contig165	1	No Swissprot-Hit			No
Contig166	1	No Swissprot-Hit			No
Contig176	1	No Swissprot-Hit			No
Contig179	1	No Swissprot-Hit			No
Contig196	1	No Swissprot-Hit			No
Contig203	1	No Swissprot-Hit			No
Contig204	1	No Swissprot-Hit			No
Contig22	1	No Swissprot-Hit			No

Contig222	1	No Swissprot-Hit			No
Contig227	1	TIP41-like protein (Homo sapiens)	O75663	1.63E-06	No
Contig232	1	No Swissprot-Hit			No
Contig24	1	Ethylene receptor (Cucumis melo var. reticulatus)	O82436	1.27E-07	No
Contig25	1	No Swissprot-Hit			No
Contig254	1	Nitrate reductase [NADH] 2 (Phaseolus vulgaris)	P39866	3.10E-29	No
Contig256	1	No Swissprot-Hit			No
Contig270	1	Coatomer subunit alpha-1 (Arabidopsis thaliana)	Q94A40	1.91E-55	No
Contig273	1	No Swissprot-Hit			No
Contig276	1	No Swissprot-Hit			No
Contig289	1	No Swissprot-Hit			No
Contig29	1	No Swissprot-Hit			No
Contig294	1	No Swissprot-Hit			No
Contig295	1	No Swissprot-Hit			No
Contig299	1	No Swissprot-Hit			No
Contig300	1	No Swissprot-Hit			No
Contig302	1	Gypsy retrotransposon integrase-like protein 1 (Mus musculus)	Q8K259	2.76E-06	No
Contig326	1	DAG protein, chloroplastic (Antirrhinum majus)	Q38732	2.36E-13	No
Contig329	1	No Swissprot-Hit			No
Contig345	1	Probable dolichyl pyrophosphate Glc1Man9GlcNAc2 alpha-1,3-glucosyltransferase (Mus musculus)	Q6P8H8	2.84E-35	No
Contig369	1	No Swissprot-Hit			No
Contig375	1	No Swissprot-Hit			No
Contig389	1	Probable E3 ubiquitin-protein ligase ARI9 (Arabidopsis thaliana)	Q9SKC3	1.26E-06	No
Contig394	1	No Swissprot-Hit			No
Contig398	1	Apoptosis 1 inhibitor (Drosophila melanogaster)	Q24306	3.37E-06	No
Contig400	1	Nucleobase-ascorbate transporter 4 (Arabidopsis thaliana)	P93039	3.21E-31	No
Contig402	1	No Swissprot-Hit			No

Contig404	1	No Swissprot-Hit			No
Contig416	1	No Swissprot-Hit			No
Contig425	1	50S ribosomal protein L29, chloroplastic (Zea mays)	Q9SWI6	1.49E-23	No
Contig427	1	No Swissprot-Hit			No
Contig429	1	No Swissprot-Hit			No
Contig443	1	No Swissprot-Hit			No
Contig446	1	No Swissprot-Hit			No
Contig455	1	Transcription factor BIM2 (Arabidopsis thaliana)	Q9CAA4	2.60E-13	No
Contig461	1	Cell division protease ftsH homolog 1, chloroplastic (Oryza sativa subsp. japonica)	Q5Z974	1.49E-52	No
Contig474	1	No Swissprot-Hit			No
Contig480	1	PP2A regulatory subunit TAP46 (Arabidopsis thaliana)	Q8LDQ4	2.62E-37	No
Contig482	1	SCO2-like protein RC0895 (Rickettsia conorii)	Q92H76	2.80E-16	No
Contig484	1	No Swissprot-Hit			No
Contig69	1	No Swissprot-Hit			No
Contig71	1	No Swissprot-Hit			No
Contig72	1	No Swissprot-Hit			No
Contig73	1	No Swissprot-Hit			No
Contig80	1	Putative 40S ribosomal protein S4-like (Fragment) (Pneumocystis carinii)	Q01688	3.22E-15	No
Contig85	1	No Swissprot-Hit			No
Contig9	1	Trans-cinnamate 4-monooxygenase (Catharanthus roseus)	P48522	5.51E-23	No
Contig93	1	Probable eukaryotic translation initiation factor 5-1 (Arabidopsis thaliana)	Q9C8F1	3.87E-16	No
Contig99	1	No Swissprot-Hit			No

TUG	# clones	SWISS-PROT	hit	eValue	EST Dr.

					Zompo Hit
Contig132	44	Chlorophyll a-b binding protein 16, chloroplastic (Nicotiana tabacum)	P27492	8.61E-87	Yes
Contig205	10	Proteasome subunit alpha type-2 putative			Yes
Contig386	10	Chlorophyll a-b binding protein 37, chloroplastic (Petunia sp.)	P12062	1.38E-77	Yes
Contig217	10	No Swissprot-Hit			No
Contig142	9	No Swissprot-Hit			Yes
Contig42	8	Cytochrome c oxidase subunit 1 (Petromyzon marinus)	Q35536	1.61E-11	No
Contig421	7	S-norcoclaurine synthase (Thalictrum flavum subsp. glaucum)			Yes
Contig97	6	Aquaporin PIP2-5 (Zea mays)	Q9XF58	2.56E-52	Yes
Contig182	6	Protochlorophyllide reductase, chloroplastic (Cucumis sativus)	Q41249	1.16E-20	Yes
Contig376	6	Universal stress protein A-like protein (Arabidopsis thaliana)	Q8LGG8	2.98E-07	Yes
Contig246	6	No Swissprot-Hit			No
Contig259	6	No Swissprot-Hit			No
Contig262	5	Caffeoyl-CoA O-methyltransferase (Populus tremuloides)	Q43095	1.11E-10	Yes
Contig447	5	Metallothionein-like protein type 3 (Malus domestica)	O24059	1.31E-12	Yes
Contig231	5	Centrin-2 putative			Yes
Contig304	5	No Swissprot-Hit			Yes
Contig275	5	No Swissprot-Hit			No
Contig271	4	Photosystem I reaction center subunit III, chloroplastic (Arabidopsis thaliana)	Q9SHE8	3.55E-06	Yes
Contig274	4	Protein sym1 (Aspergillus fumigatus)			Yes
Contig286	4	No Swissprot-Hit			Yes
Contig31	4	Ferredoxin (Sambucus nigra)	P00226	1.13E-14	Yes
Contig81	4	Oxygen-evolving enhancer protein 3, chloroplastic (Oryza sativa subsp. indica)	P83646	3.91E-36	Yes
Contig438	4	29 kDa ribonucleoprotein A, chloroplastic (Nicotiana sylvestris)	Q08935	1.51E-34	No
Contig442	4	Soluble hydrogenase 42 kDa subunit (Anabaena cylindrica)	P16421	2.77E-11	No
Contig469	4	Signal peptide peptidase-like 2B (Mus musculus)	Q3TD49	2.62E-13	No

Contig68	4	No Swissprot-Hit			No
Contig70	4	No Swissprot-Hit			No
Contig84	4	1-deoxy-D-xylulose-5-phosphate synthase (Rhizobium meliloti)	Q92RJ1	6.09E-46	No
Contig310	3	Chlorophyll a-b binding protein of LHCII type III, chloroplastic (Hordeum vulgare)	P27523	1.00E-32	Yes
Contig144	3	No Swissprot-Hit			Yes
Contig169	3	BEL1-like homeodomain protein 6 putative			Yes
Contig17	3	Ferredoxin-thioredoxin reductase catalytic chain putative			Yes
Contig194	3	BI1-like protein (Arabidopsis thaliana)	Q94A20	9.53E-12	Yes
Contig219	3	Magnesium-protoporphyrin IX monomethyl ester [oxidative] cyclase 2, chloroplastic (Chlamydomonas reinhardtii)	Q9AR22	1.78E-21	Yes
Contig226	3	NADH-ubiquinone oxidoreductase subunit 8 (Reclinomonas americana)	O21233	1.81E-24	Yes
Contig279	3	Putative syntaxin-24 (Arabidopsis thaliana)	Q9C615	1.25E-06	Yes
Contig312	3	Ferredoxin-thioredoxin reductase, catalytic chain (Porphyra yezoensis)	Q1XDA1	2.94E-38	Yes
Contig313	3	40S ribosomal protein S15 (Picea mariana)	O65059	1.04E-60	Yes
Contig332	3	No Swissprot-Hit			Yes
Contig349	3	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 7 putative			Yes
Contig363	3	Putative mitochondrial import receptor subunit TOM40 homolog 2 (Arabidopsis thaliana)	Q9SX55	2.17E-56	Yes
Contig385	3	60S acidic ribosomal protein P2 putative			Yes
Contig441	3	S-norcoclaurine synthase putative			Yes
Contig452	3	Glyceraldehyde-3-phosphate dehydrogenase A, putative			Yes
Contig108	3	No Swissprot-Hit			No
Contig190	3	No Swissprot-Hit			No
Contig216	3	Desiccation protectant protein Lea14 homolog (Glycine max)	P46519	2.53E-30	No
Contig239	3	No Swissprot-Hit			No
Contig282	3	No Swissprot-Hit			No

Contig290	3	No Swissprot-Hit			No
Contig33	3	Putative metal tolerance protein C3 putative			No
Contig356	3	No Swissprot-Hit			No
Contig373	3	No Swissprot-Hit			No
Contig395	3	No Swissprot-Hit			No
Contig409	3	Probable 6-phosphogluconolactonase 2 (Oryza sativa subsp. japonica)	Q6Z4H0	8.79E-14	No
Contig423	3	No Swissprot-Hit			No
Contig472	3	Probable serine/threonine-protein kinase WNK4 (Arabidopsis thaliana)	Q9LVL5	4.13E-09	No
Contig229	2	Light-regulated protein putative			Yes
Contig122	2	ATP-citrate synthase (Rattus norvegicus)	P16638	1.51E-12	No
Contig213	2	Alcohol dehydrogenase 1 putative			Yes
Contig319	2	Metallothionein-like protein type 3 (Malus domestica)	O24059	3.53E-13	Yes
Contig360	2	Chlorophyll a-b binding protein, chloroplastic (Fragment) (Silene pratensis)	P12332	1.20E-48	Yes
Contig101	2	No Swissprot-Hit			Yes
Contig107	2	Thioredoxin M-type, chloroplastic (Zea mays)	Q41864	1.01E-34	Yes
Contig113	2	No Swissprot-Hit			Yes
Contig120	2	Chlorophyll a-b binding protein 1B, putative			Yes
Contig173	2	GTP-binding protein SAR1A putative			Yes
Contig177	2	Phytosulfokines 4 (Oryza sativa subsp. japonica)	Q9AR88	3.18E-09	Yes
Contig180	2	Protochlorophyllide reductase, chloroplastic (Daucus carota)	Q9SDT1	1.27E-73	Yes
Contig185	2	PGR5-like protein 1A, chloroplastic putative			Yes
Contig189	2	Uncharacterized protein ART3 (Saccharomyces cerevisiae)	Q8TGM5	3.30E-07	Yes
Contig2	2	Chlorophyll a-b binding protein 4, putative			Yes
Contig220	2	Proteasome subunit alpha type-3 (Fragment) (Acanthamoeba castellanii)	P90513	2.65E-25	Yes
Contig225	2	Isocitrate dehydrogenase [NADP] cytoplasmic (Dictyostelium	Q75JR3	1.31E-14	Yes

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Contig240	2	Photosystem II reaction center W protein putative			Yes
Contig266	2	40S ribosomal protein S28 putative			Yes
Contig268	2	Chlorophyll a-b binding protein 7, chloroplastic (Solanum lycopersicum)	P10708	4.19E-10	Yes
Contig28	2	Ribulose bisphosphate carboxylase small chain SSU5B, chloroplastic (Lemna gibba)			DrZompo-Hit
Contig288	2	Remorin putative			Yes
Contig3	2	No Swissprot-Hit			Yes
Contig320	2	Chlorophyll a-b binding protein CP24, chloroplastic (Spinacia oleracea)	P36494	1.44E-51	Yes
Contig348	2	Defensin Tk-AMP-D1.1 (Triticum kiharae)	P84965	3.65E-06	Yes
Contig351	2	No Swissprot-Hit			Yes
Contig352	2	Fasciclin-like arabinogalactan protein 16 (Arabidopsis thaliana)	Q8RWC5	1.47E-23	Yes
Contig361	2	Photosystem I reaction center subunit V, putative			Yes
Contig364	2	Chlorophyll a-b binding protein 6A, chloroplastic (Solanum lycopersicum)	P12360	1.71E-08	Yes
Contig367	2	60S ribosomal protein L10 (Vitis riparia)			Yes
Contig374	2	Calmodulin (Helianthus annuus)	P93171	3.98E-63	Yes
Contig388	2	Blue copper protein putative			Yes
Contig420	2	Glutamine synthetase PR-2 (Phaseolus vulgaris)	P04771	1.49E-23	Yes
Contig430	2	No Swissprot-Hit			Yes
Contig436	2	No Swissprot-Hit			Yes
Contig465	2	No Swissprot-Hit			Yes
Contig47	2	Chlorophyll a-b binding protein 2, chloroplastic (Oryza sativa subsp. japonica)	P12331	8.83E-21	Yes
Contig471	2	No Swissprot-Hit			Yes
Contig478	2	Cytochrome b6-f complex iron-sulfur subunit, putative			Yes
Contig55	2	Universal stress protein A-like protein (Arabidopsis thaliana)	Q8LGG8	5.70E-10	Yes

Contig56	2	No Swissprot-Hit			Yes
Contig83	2	Oxygen-evolving enhancer protein 2, chloroplastic (Fritillaria agrestis)	O49080	1.60E-66	Yes
Contig98	2	Naringenin,2-oxoglutarate 3-dioxygenase (Dianthus caryophyllus)	Q05964	1.04E-21	Yes
Contig11	2	No Swissprot-Hit			No
Contig117	2	No Swissprot-Hit			No
Contig119	2	No Swissprot-Hit			No
Contig12	2	Probable beta-1,3-galactosyltransferase 19 (Arabidopsis thaliana)	Q9LV16	1.13E-07	No
Contig129	2	No Swissprot-Hit			No
Contig13	2	No Swissprot-Hit			No
Contig151	2	No Swissprot-Hit			No
Contig153	2	No Swissprot-Hit			No
Contig156	2	No Swissprot-Hit			No
Contig157	2	No Swissprot-Hit			No
Contig164	2	No Swissprot-Hit			No
Contig187	2	No Swissprot-Hit			No
Contig191	2	Dual specificity protein phosphatase 12 (Mus musculus)	Q9D0T2	1.72E-13	No
Contig192	2	No Swissprot-Hit			No
Contig206	2	Fructose-1,6-bisphosphatase, chloroplastic (Spinacia oleracea)	P22418	1.15E-28	No
Contig218	2	No Swissprot-Hit			No
Contig221	2	No Swissprot-Hit			No
Contig223	2	No Swissprot-Hit			No
Contig23	2	Probable palmitoyltransferase ZDHHC14 (Homo sapiens)	Q8IZN3	2.105E-17	No
Contig236	2	No Swissprot-Hit			No
Contig237	2	No Swissprot-Hit			No

Contig252	2	No Swissprot-Hit			No
Contig26	2	No Swissprot-Hit			No
Contig263	2	No Swissprot-Hit			No
Contig264	2	No Swissprot-Hit			No
Contig269	2	No Swissprot-Hit			No
Contig278	2	No Swissprot-Hit			No
Contig280	2	No Swissprot-Hit			No
Contig292	2	Clavaminate synthase-like protein At3g21360 (Arabidopsis thaliana)	Q9LIG0	2.79E-07	No
Contig30	2	Cytochrome P450 86A1 (Arabidopsis thaliana)	P48422	2.09E-18	No
Contig311	2	No Swissprot-Hit			No
Contig321	2	No Swissprot-Hit			No
Contig35	2	No Swissprot-Hit			No
Contig358	2	Coatomer subunit beta'-2 (Oryza sativa subsp. japonica)	Q6H8D5	2.76E-65	No
Contig359	2	No Swissprot-Hit			No
Contig380	2	No Swissprot-Hit			No
Contig393	2	No Swissprot-Hit			No
Contig399	2	No Swissprot-Hit			No
Contig401	2	No Swissprot-Hit			No
Contig403	2	No Swissprot-Hit			No
Contig41	2	Chalcone synthase (Petroselinum crispum)	P16107	2.78E-22	No
Contig432	2	No Swissprot-Hit			No
Contig440	2	14 kDa proline-rich protein DC2.15 (Daucus carota)	P14009	2.70E-09	No
Contig457	2	Peroxisomal membrane protein PMP47A (Candida boidinii)	P21245	1.35E-08	No
Contig458	2	No Swissprot-Hit			No
Contig459	2	No Swissprot-Hit			No
Contig460	2	No Swissprot-Hit			No
Contig477	2	No Swissprot-Hit			No

Contig479	2	No Swissprot-Hit			No
Contig51	2	Alanyl-tRNA synthetase, cytoplasmic (Homo sapiens)	P49588	1.72E-12	No
Contig57	2	No Swissprot-Hit			No
Contig76	2	Hydrophobic protein LTI6A (Oryza sativa subsp. japonica)	Q8H5T6	1.39E-13	No
Contig79	2	No Swissprot-Hit			No
Contig82	2	Zinc finger A20 and AN1 domain-containing stress-associated protein 1 (Oryza sativa subsp. japonica)	A3C039	1.45E-07	No
Contig90	2	Uncharacterized GMC-type oxidoreductase y4nJ (Rhizobium sp. (strain NGR234))	P55582	8.17E-06	No
Contig95	1	Heat shock cognate 70 kDa protein 1 (Arabidopsis thaliana)	P22953	2.84E-10	Yes
Contig365	1	Cytochrome b6-f complex iron-sulfur subunit, chloroplastic (Fritillaria agrestis)	O49078	1.43E-37	Yes
Contig207	1	No Swissprot-Hit			Yes
Contig126	1	F-box/LRR-repeat protein 2 (Mus musculus)	Q8BH16	1.06E-08	No
Contig8	1	No Swissprot-Hit			No
Contig201	1	Fructose-bisphosphate aldolase 1, chloroplastic (Fragment) (Pisum sativum)	Q01516	1.47E-47	Yes
Contig293	1	Chlorophyll a-b binding protein 1, chloroplastic (Oryza sativa subsp. japonica)	P12330	2.85E-14	Yes
Contig307	1	Zeaxanthin epoxidase, chloroplastic (Solanum lycopersicum)	P93236	7.61E-07	Yes
Contig462	1	Chlorophyll a-b binding protein CP26, chloroplastic (Arabidopsis thaliana)	Q9XF89	4.00E-44	Yes
Contig143	1	No Swissprot-Hit			No
Contig339	1	No Swissprot-Hit			No
Contig418	1	No Swissprot-Hit			No
Contig5	1	No Swissprot-Hit			No
Contig106	1	No Swissprot-Hit			Yes
Contig124	1	Chlorophyll a-b binding protein, chloroplastic (Petunia hybrida)	P13869	1.774E-16	Yes
Contig128	1	Hydrophobic protein OSR8 putative			Yes

Contig135	1	2-Cys peroxiredoxin BAS1-like, chloroplastic (Arabidopsis thaliana)	Q9C5R8	1.499E-63	Yes
Contig139	1	Peroxidase 21 (Arabidopsis thaliana)	Q42580	5.69E-07	Yes
Contig147	1	Ferredoxin, chloroplastic (Triticum aestivum)	P00228	1.467E-15	Yes
Contig152	1	Peroxiredoxin Q, chloroplastic (Gentiana triflora)	Q75SY5	1.571E-50	Yes
Contig160	1	Probable phospholipid hydroperoxide glutathione peroxidase (Gossypium hirsutum)	O49069	1.204E-17	Yes
Contig167	1	No Swissprot-Hit			Yes
Contig178	1	Brain protein 44 (Homo sapiens)	O95563	1.87E-10	Yes
Contig183	1	S-adenosylmethionine synthetase (Medicago falcata)	A4ULF8	1.24E-22	Yes
Contig195	1	Oxygen-evolving enhancer protein 1, chloroplastic (Nicotiana tabacum)	Q40459	1.40E-42	Yes
Contig197	1	Uncharacterized protein C216.04c putative			Yes
Contig200	1	No Swissprot-Hit			Yes
Contig212	1	Photosystem II 22 kDa protein, chloroplastic (Solanum lycopersicum)	P54773	1.21E-09	Yes
Contig224	1	No Swissprot-Hit			Yes
Contig241	1	Glutaredoxin-C2 (Arabidopsis thaliana)	Q9FNE2	1.84E-38	Yes
Contig242	1	GATA transcription factor 16 (Arabidopsis thaliana)			Yes
Contig251	1	Probable lactoylglutathione lyase (Synechocystis sp. (strain PCC 6803))	Q55595	1.79E-37	Yes
Contig261	1	Zinc finger protein 1 putative			Yes
Contig265	1	GDSL esterase/lipase LTL1 putative			Yes
Contig272	1	Chlorophyll a-b binding protein CP29.3, chloroplastic (Arabidopsis thaliana)	Q9S7W1	3.23E-31	Yes
Contig281	1	Tubulin alpha-6 chain (Arabidopsis thaliana)	P29511	2.62E-41	Yes
Contig296	1	Oxygen-evolving enhancer protein 1, chloroplastic (Solanum lycopersicum)	P23322	1.47E-23	Yes

Contig297	1	No Swissprot-Hit			Yes
Contig316	1	Phospholipase D alpha 2 (Arabidopsis thaliana)	Q9SSQ9	2.80E-08	Yes
Contig322	1	Histone H3.3 (Nicotiana tabacum)	Q76N23	2.53E-31	Yes
Contig330	1	Putative metal tolerance protein C3 (Arabidopsis thaliana)	Q9M2P2	4.72E-61	Yes
Contig342	1	Magnesium-protoporphyrin IX monomethyl ester [oxidative] cyclase, chloroplastic (Hordeum vulgare)			DrZompo-Hit
Contig353	1	Fructose-bisphosphate aldolase 2, putative			Yes
Contig368	1	60S ribosomal protein L10 (Fragment) (Nicotiana tabacum)	Q40592	1.00E-42	Yes
Contig371	1	No Swissprot-Hit			Yes
Contig38	1	Phosphoglycerate kinase (Anabaena variabilis (strain ATCC 29413 / PCC 7937))	Q3MF40	1.89E-07	Yes
Contig383	1	40S ribosomal protein S25 putative			Yes
Contig390	1	Actin (Fragment) (Costaria costata)	P30161	1.02E-16	Yes
Contig392	1	No Swissprot-Hit			Yes
Contig408	1	Chlorophyll a-b binding protein 16, chloroplastic (Nicotiana tabacum)	P27492	2.81E-42	Yes
Contig410	1	60S ribosomal protein L29 (Mus musculus)	P47915	8.30E-11	Yes
Contig411	1	Putative mitochondrial carrier protein PET8 (Saccharomyces cerevisiae)			Yes
Contig419	1	GTP-binding nuclear protein Ran/TC4 (Vicia faba)	P38548	3.81E-56	Yes
Contig424	1	No Swissprot-Hit			Yes
Contig435	1	No Swissprot-Hit			Yes
Contig445	1	Chlorophyll a-b binding protein CP26, chloroplastic (Arabidopsis thaliana)	Q9XF89	3.90E-08	Yes
Contig453	1	UPF0414 transmembrane protein C20orf30 homolog putative			Yes
Contig46	1	No Swissprot-Hit			Yes
Contig466	1	Photosystem I reaction center subunit V, chloroplastic (Hordeum vulgare)	Q00327	1.10E-18	Yes
Contig467	1	No Swissprot-Hit			Yes
Contig476	1	No Swissprot-Hit			Yes

Contig60	1	No Swissprot-Hit			Yes
Contig63	1	40S ribosomal protein S3a (Brassica rapa)	P49396	1.20E-25	Yes
Contig64	1	Chlorophyll a-b binding protein 8, chloroplastic (Solanum lycopersicum)	P27522	1.27E-06	Yes
Contig65	1	Glyceraldehyde-3-phosphate dehydrogenase A, chloroplastic (Fragment) (Sinapis alba)	P09672	2.21E-35	Yes
Contig67	1	Chlorophyll a-b binding protein CP29.2 putative			Yes
Contig75	1	Probable pyridoxal biosynthesis protein PDX1.2 (Oryza sativa subsp. japonica)	Q8W3D0	1.88E-46	Yes
Contig88	1	Metallothionein-like protein 2 (Cicer arietinum)			Yes
Contig94	1	Photosystem I reaction center subunit VI-2, chloroplastic (Arabidopsis thaliana)	Q9SUI6	2.91E-32	Yes
Contig100	1	Fatty acid oxidation complex subunit alpha (Yersinia pestis (strain Pestoides F))	A4TR27	2.24E-15	No
Contig102	1	No Swissprot-Hit			No
Contig105	1	26S protease regulatory subunit S10B (Dictyostelium discoideum)	Q54PJ1	4.84E-53	No
Contig114	1	No Swissprot-Hit			No
Contig121	1	No Swissprot-Hit			No
Contig123	1	No Swissprot-Hit			No
Contig125	1	No Swissprot-Hit			No
Contig131	1	No Swissprot-Hit			No
Contig140	1	Alpha-1,4-glucan-protein synthase [UDP-forming] 1 (Solanum tuberosum)	Q9SC19	1.363E-58	No
Contig141	1	No Swissprot-Hit			No
Contig145	1	No Swissprot-Hit			No
Contig154	1	No Swissprot-Hit			No
Contig16	1	No Swissprot-Hit			No
Contig161	1	Selenium-binding protein 1 (Xenopus tropicalis)	Q569D5	1.56E-52	No
Contig170	1	Nodulation receptor kinase (Medicago truncatula)	Q8L4H4	1.01E-16	No

Contig175	1	Putative flavin-containing monooxygenase 2 (Arabidopsis thaliana)	Q9FKE7	1.04E-13	No
Contig181	1	Cyclin-K (Homo sapiens)	O75909	5.14E-08	No
Contig186	1	No Swissprot-Hit			No
Contig188	1	Potassium channel AKT6 (Arabidopsis thaliana)	Q8GXE6	1.92E-07	No
Contig19	1	No Swissprot-Hit			No
Contig193	1	5'-adenylylsulfate reductase 3, chloroplastic (Arabidopsis thaliana)	P92980	1.76E-71	No
Contig199	1	26S proteasome non-ATPase regulatory subunit 14 (Dictyostelium discoideum)	Q86IJ1	1.05E-13	No
Contig202	1	No Swissprot-Hit			No
Contig21	1	No Swissprot-Hit			No
Contig210	1	No Swissprot-Hit			No
Contig233	1	Inorganic phosphate transporter 1-4 (Arabidopsis thaliana)	Q96303	1.87E-42	No
Contig234	1	Elongation factor 1-gamma 1 (Oryza sativa subsp. japonica)	Q9ZRI7	1.57E-09	No
Contig238	1	No Swissprot-Hit			No
Contig243	1	No Swissprot-Hit			No
Contig244	1	No Swissprot-Hit			No
Contig245	1	Transcription-associated protein 1 (Saccharomyces cerevisiae)	P38811	1.36E-06	No
Contig253	1	No Swissprot-Hit			No
Contig257	1	No Swissprot-Hit			No
Contig258	1	No Swissprot-Hit			No
Contig277	1	Glutamate decarboxylase (Solanum lycopersicum)	P54767	4.73E-22	No
Contig283	1	No Swissprot-Hit			No
Contig285	1	No Swissprot-Hit			No
Contig291	1	Peroxisome assembly factor 2 (Rattus norvegicus)	P54777	2.19E-06	No
Contig303	1	No Swissprot-Hit			No
Contig308	1	Mitogen-activated protein kinase kinase kinase dlk-1	O01700	2.19E-11	No

		(Caenorhabditis elegans)			
Contig309	1	No Swissprot-Hit			No
Contig318	1	Protein transport protein Sec61 subunit alpha-like 2 (Danio rerio)	Q90YL4	1.11E-55	No
Contig32	1	No Swissprot-Hit			No
Contig323	1	Nectarin-1 (Nicotiana plumbaginifolia)	Q9SPV5	1.27E-41	No
Contig328	1	No Swissprot-Hit			No
Contig335	1	No Swissprot-Hit			No
Contig336	1	No Swissprot-Hit			No
Contig338	1	No Swissprot-Hit			No
Contig34	1	No Swissprot-Hit			No
Contig344	1	Heat shock cognate 70 kDa protein 1 (Arabidopsis thaliana)	P22953	1.61E-38	No
Contig354	1	No Swissprot-Hit			No
Contig355	1	BEL1-like homeodomain protein 1 (Arabidopsis thaliana)	Q9SJ56	7.85E-12	No
Contig372	1	Ferrochelatase-1, chloroplastic/mitochondrial (Arabidopsis thaliana)	P42043	1.91E-31	No
Contig377	1	No Swissprot-Hit			No
Contig379	1	No Swissprot-Hit			No
Contig381	1	No Swissprot-Hit			No
Contig387	1	No Swissprot-Hit			No
Contig39	1	CRS2-like protein, chloroplastic (Oryza sativa subsp. japonica)	Q10LI6	1.40E-18	No
Contig396	1	No Swissprot-Hit			No
Contig397	1	Dihydroxy-acid dehydratase (Francisella tularensis subsp. novicida (strain U112))	A0Q6R5	1.47E-07	No
Contig405	1	No Swissprot-Hit			No
Contig406	1	No Swissprot-Hit			No
Contig407	1	Probable xyloglucan endotransglucosylase/hydrolase protein 28 (Arabidopsis thaliana)	Q38909	1.09E-22	No

Contig412	1	Acidic endochitinase SP2 (Beta vulgaris)	P42820	1.35E-40	No
Contig413	1	ADP-ribosylation factor 1 (Drosophila melanogaster)	P61209	2.70E-17	No
Contig431	1	No Swissprot-Hit			No
Contig433	1	No Swissprot-Hit			No
Contig448	1	Salt tolerance-like protein (Arabidopsis thaliana)	Q9SID1	1.79E-16	No
Contig450	1	Actin-related protein 5 (Arabidopsis thaliana)	Q940Z2	2.79E-14	No
Contig451	1	Probable proteasome inhibitor (Arabidopsis thaliana)	Q9M330	9.40E-07	No
Contig456	1	Serine/threonine-protein kinase CTR1 (Arabidopsis thaliana)	Q05609	2.75E-06	No
Contig463	1	No Swissprot-Hit			No
Contig464	1	No Swissprot-Hit			No
Contig470	1	No Swissprot-Hit			No
Contig48	1	3-ketoacyl-CoA synthase 1 (Arabidopsis thaliana)	Q9MAM3	8.47E-24	No
Contig49	1	Zinc finger CCCH domain-containing protein 66 (Oryza sativa subsp. japonica)	Q2QT65	4.91E-15	No
Contig52	1	60S ribosomal protein L19 (Pongo abelii)	Q5RB99	2.68E-09	No
Contig53	1	No Swissprot-Hit			No
Contig59	1	No Swissprot-Hit			No
Contig6	1	No Swissprot-Hit			No
Contig62	1	No Swissprot-Hit			No
Contig66	1	No Swissprot-Hit			No
Contig74	1	Cyanate hydratase (Arabidopsis thaliana)	O22683	5.42E-53	No
Contig77	1	Cytochrome P450 85A1 (Arabidopsis thaliana)	Q9FMA5	4.64E-43	No
Contig78	1	Katanin p60 ATPase-containing subunit (Arabidopsis thaliana)	Q9SEX2	1.67E-82	No
Contig86	1	No Swissprot-Hit			No
Contig92	1	No Swissprot-Hit			No
Contig96	1	No Swissprot-Hit			No

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