## ABSTRACT

Rocket (*Eruca sativa*) is a salad vegetable classified in Brassicaceae family. It derives its origin from the Mediterranean region but it is widely used in human diet all over the world. It is rich in glucosinolates, amino acid-derived compounds belonging to the general category of plant secondary metabolites.

Glucosinolates can be found mainly in the order Caparrales and especially in the Brassicaceae family. Being rich in nitrogen and sulfur, their molecule consists of a thioglucose unit (the core), a sulfonated oxime unit, and a variable side chain. Their division in three major groups (aliphatic, aromatic and indolyl glucosinolates) depends on the amino acid they derive from, which may be methionine, phenylalanine and tryptophan respectively.

As intact molecules, glucosinolates are inactive and have no known function, but after cell disruption they react with a thioglucosidase known as myrosinase, and hydrolyse into various other metabolites (isothiocyanates, nitriles, thiocyanates, epithionitriles and oxazolidine-2-thiones). These products are bioactive and have some interesting properties, ranging from antimicrobial and cancer-preventing to inflammatory activities.

Their biosynthetic pathway has been studied in *Arabidopsis thaliana*, which also belongs to the Brassicaceae family. The procedure begins with a precursor amino acid, which can be one of the three mentioned above, and contains three stages: (i) side-chain elongation of amino acids, (ii) development of the core structure, and (iii) secondary side-chain modifications. Most of the enzymes and genes involved are known in *A.thaliana* and comparative genomics studies have also been performed. As a result, a lot of sequences of glucosinolate pathway genes from other Brassicaceae plants hane been identified and can be obtained from databases such as GenBank.

Neither the genome of *Eruca sativa* nor the sequences of genes participating in glucosinolate biosynthesis are known. The aim of this study is to make a start in

understanding the biosynthetic process of glucosinolates in *Eruca sativa*. Sequences related with biosynthetic genes and transcriptional factors were isolated. The influence of N and S fertilization in the biosynthetic genes expression was also examined.

CDSs of glucosinolate biosynthesis and regulatory genes obtained from NCBI database were used to design specific and degenerated primers in order to amplify one part of the sequence of each gene we were interesting in. Primers were designed based on conserved areas of genes of other Brassicaceae plants such as *Arabidopsis thaliana* and *Brassica rapa*. A total of 15 genes were isolated, containing a full-length (12 of them) or a partial (3 of them) coding sequence. All genes are highly related with glucosinolate biosynthetic and regulator genes of *Arabidopsis thaliana* and they share a similarity ranging from 82 – 90 %. When they are compared to orthologs of other Brassica species such as *Brassica rapa* similarity is higher and ranges from 85 to 95 %. Three of the full-length genes were cloned in expression vectors in order to isolate the corresponding protein.

It was also interesting to find whether the expression levels of each gene are decreased or increased when plants grow in different nitrogen and sulfur nutrition conditions. Two experimental approaches were designed. Plants of the first experimental approach were separated into 4 groups-treatments, grown in a different nutrient solution each. The first group consisted of plants grown in full strength Hoagland nutrient solution, whereas plants of the other groups were watered with modified Hoagland nutrient solutions; low nitrogen for the second, low nitrogen and sulfur for the third and low sulfur for the fourth group. In the second approach, the condition of a short-term sulfur starvation was investigated. Tissues from leaves and roots were harvested for total RNA extraction and cDNA synthesis. Relative expression of genes was studied by real-time PCR. The applied nutritional conditions affected the expression of the biosynthetic genes in both experimental approaches. It is also known that the concentration of the glucosinolates is affected by such conditions as well. Transcriptional and metabolic profiles from each treatment were statistically analyzed in order to find if they are correlated and in most cases there was a correlation between them.

Extracts from the tissues of the plants growing in different nutrition conditions were tested towards the growth of HeLa, MCF7 and HepG2 cancer cell lines, in order to study their biological activity. Differention in their biological activity was noted, depending on the fertilization levels.