Journal of Applied Biological Sciences 8 (2): 01-08, 2014
ISSN: 1307-1130, E-ISSN: 2146-0108, www.nobel.gen.tr

Genomics and Transcriptomics Analysis of Cu Accumulator Plant *Brassica nigra* L.

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Received: March 31, 2014
Accepted: May 01, 2014

Abstract

The genus *Brassica* contains a wide range of diploid and amphipolyploid species including some of the economically high valuable vegetables and oilseed crops used worldwide. The major industrial and food crops in Brassica are the closest relatives to the model plant *Arabidopsis thaliana*, and hence are major beneficiaries from the vast data of genomics and molecular genetics available in the database of *Arabidopsis thaliana*. Extensive genetic and molecular analyses have been undertaken for the six cultivated *Brassica* species. The four closely related crop species *B. rapa* (AA, 2n=20), *B. juncea* (AABB, 2n=36), *B. napus* (AACC, 2n=38), and *B. carinata* (BBCC, 2n=34) provide about 12% of the worldwide edible oil supply. The other two species *B. nigra* (BB, 2n=16) and *B. oleracea* (CC, 2n=18) provide many vegetables for healthy human diet having a valuable source of dietary fiber, vitamin C and other anticancer compounds. The comparative mapping between *Arabidopsis thaliana* and *Brassica* crop species, coupled with the base knowledge of mutation based functional analysis in *Arabidopsis thaliana* and QTL mapping in crop Brassicas, could greatly contribute towards a better understanding of the genetic architecture for the conserved as well as the evolved traits of agronomic value in the *Brassicaceae*. *Brassica nigra* has the second smallest genome size (~ 632 Mbp) among the six cultivated species of *Brassica*. *Brassica* species are well known as metal accumulators and some of them are being used for phytoremediation in contaminated soils. Approximately 25% of the documented metal hyper accumulating species are, like *A. thaliana*, members of the *Brassicaceae*. The super metal accumulating capacity of *Arabidopsis halleri* and *Noccaea caerulescens* (previously *Thlaspi caerulescens*) have been well documented. Because of their slow growth and low biomass, other fast-growing and high biomass brassica crop plants, for example *Brassica juncea* and *Brassica nigra* have been evaluated for their ability to hyper accumulate metals from contaminated soils.

The Diyabeker ecotype of *B. nigra* collected from southeastern part of Turkey was found to be hyperaccumulator of Cu. We carried out the comparative transcriptome analysis in order to find out the expression level of metal induced genes and transcriptome changes both in low and high Cu treated plants. Microarray analysis showed that some of the genes were highly expressed (several hundred fold) with Cu treated plants compared to control.

Our microarray data using Affymetrix GeneChip Arabidopsis Genome Array (ATH1-121501 Genechip) indicate that possibly several genes including the genes in glutathione pathway, metal ATPase and ABC transporters are involved in metal tolerances in this ecotype. In this communication the use of molecular tools and the exploitation of Arabidopsis knowledge will be presented in detail.

Keywords: *Brassica nigra*, Genomics, transcriptomics, microRNAs, Cu accumulator, phytoremediation

INTRODUCTION

The *Brasscicaceae* family (formerly *Cruciferae*) consists of approximately 375 genera and 3200 species of plants, commonly known as the mustard family. *Brassica* contains about 100 species, including rapeseed, cabbage, cauliflower, broccoli, Brussels sprouts, turnip, various mustards and weeds [1]. The cultivated *Brassica* species are the group of crops most closely related to *Arabidopsis thaliana*. Chromosome numbers in the *Brassicaceae* vary from 2n = 8 to 2n = 256 [2] *A. thaliana*, with 2n = 10, has one of the smallest chromosome numbers, an advanced character representing reduction from its ancestors in the clade including *A. lyrata* and *Capsella rubella* (both 2n = 16).

The species typically termed the "diploid" *Brassica* species, *B. rapa* (*n* = 10), *B. nigra* (*n* = 8) and *B. oleracea* (*n* = 9) contain the A, B and C genomes, respectively. Each pairwise combination has hybridized spontaneously to form the three allotetraploid species [3,4], *B. napus* (*n* = 19, comprising A and C genomes), *B. juncea* (*n* = 18, comprising A and B genomes) and *B. carinata* (*n* = 17, comprising B and C genomes). The genome of *B. rapa* is the smallest, at *ca*. 500 Mb [5], and annotation and analysis of the draft genome sequence of *Brassica rapa* accession Chiifu-401-42, a Chinese cabbage has been reported [6] and see http:// brassica.bbsrc.ac.uk/.

The relationships among the cultivated species as presented in Fig. 1 were first clarified by Morinaga (1934) and verified by U (1935) (Fig. 1). The four most widely cultivated species *B. juncea*, *B. napus*, *B. oleracea*, and *B. rapa* are all highly polymorphic and include oilseed crops, root crops, and vegetables crops such as Chinese cabbage, broccoli, and Brussels sprouts [7].

Because the *Brassica* amphidiploids species can be generated synthetically with the help of embryo rescue techniques, this complex of three diploid species and their corresponding polyploids (Fig 1) is today one of the most useful model systems for investigations of polyploidy in crop plants [8-10].

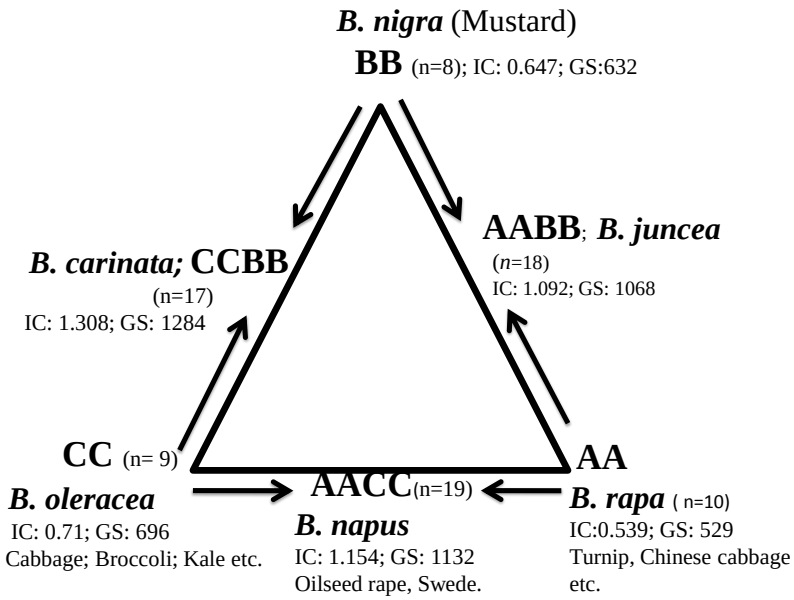

Fig.1. The "Triangle of U" representing the genomic relationships among different diploid and amphidiploids Brassica species. 1C, 1C nuclear DNA content (pg); GS, genome size (Mbp) (N. U. 1935; Johnston et al. 2005)

Comparative Genome Studies

Genomic-assisted breeding approaches have considerably advanced with increasing availability of genome and transcriptome sequence data for several model plants and crop species. Complete and/or draft genome sequences have become available for many plant species such as *Arabidopsis thaliana*, rice, poplar, grape, papaya, sorghum, *Medicago truncatula* and soybean [11]. Whole genome sequencing is either sequenced but is not yet publicly available or is in progress for several other crops such as maize, wheat, rapeseed, Chinese cabbage and some other crops [11].

The genome of *A. thaliana* was the first of any plant to be sequenced (Arabidopsis Genome Initiative 2000) and it is one of the smallest known nuclear genomes in higher plants. This complete sequence of *Arabidopsis* genome has developed into the most important resource for gene isolation and characterization in *Brassica* crops and has also served as an important reference genome for other members of the *Brassicaceae*. The data available from the Arabidopsis Information Resource (TAIR) includes the complete genome sequence along with gene structure, gene product information, metabolism, gene expression, DNA and seed stocks, genome maps, genetic and physical markers, and information about the Arabidopsis research community. Gene product function data is updated every two weeks from the latest published research literature and community data submissions. Gene structures are updated 1-2 times per year using computational and manual methods as well as community submissions of new and updated genes [12](http://www.arabidopsis.org/).

Comapartive genomics is a powerful tool for genome analysis and annotation. The biology of *Arabidopsis* and *Brassica* are very similar and the comparison of genetic mapping between species of *Brassicaceae* revealed collinear blocks even though the species differed with respect to genome size, base chromosome number, and ploidy. The *Arabidopsis* genome may act as an anchor genome, and markers positioned on it can be utilized for reciprocal localization of markers in *Brassica* species [13-15]. Knowledge of the position of genes controlling qualitative traits as well as quantitative trait loci (QTLs) in *A. thaliana* can be used to predict the location of homologous genes in *Brassica* species.

Physical genome maps and sequence data from *A. thaliana* together with comparative analysis of its syntenic relationships to *Brassica* genomes provide potentially powerful tools for genome analysis and gene discovery in rapeseed (*Brassica napus* L.), the closest major crop relative to the model plant. Generally 80 to 90% homology is found between the exons of putative orthologous genes in *Arabidopsis* and *Brassica* [16], meaning that knowledge from *Arabidopsis* is highly relevant for gene isolation and characterization in *Brassica* crops.

An effective method to use functional PCR markers for physical mapping of *A. thaliana* gene loci in *B. napus* has been described [17,18]. Several genetic linkage maps based on a range of marker types, including Restriction Fragment Length Polymorphism (RFLPs), Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSRs) and Amplified Fragment Length Polymorphisms (AFLPs), have been produced for *B. rapa* [19,20]. PCR based markers have been widely used in developing genetic linkage maps for *B. oleracea* [21], *B. nigra* [22], *B. juncea* [23] and *B. napus* [24].

Because of the high economic value of *Brassica* species throughout the world and their potential to be models for the study of polyploidization, genome sequencing projects for *Brassica* species, especially *B. rapa* and *B. oleracea*, have been initiated (http://www.brassica.info)[25]. The genome of *B. rapa* is expected to be completely sequenced within the near future [6] (for progress see http://www.brassica.info), and current technological developments in the field of ultra-fast DNA sequencing are beginning to revolutionize the fields of polymorphism discovery, genome analysis and molecular breeding. The number of expressed sequence tag (EST) sequences available for *Brassica* species has skyrocketed in the past few years as sequencing costs have diminished, enabling DNA sequence mining to become extremely useful for the identification and development of single nucleotide polymorphism (SNP) markers in oilseed rape. In the near

future it can be expected that high-density *B. napus* SNP arrays will play an important role in development of dense genetic maps for oilseed rape. Next-generation sequencing technologies are also set to rapidly accelerate SNP discovery, so that ultra-high density SNP maps will probably become available in the relatively near future. High throughout SNP screening methods will also be a valuable resource for whole-genome allele-trait association studies, which can potentially play a major role in the identification of genes contributing to complex traits.

Microsatellites or simple sequence repeat (SSR) markers are an important genomic resource and have gained increasing importance for determining genetic relationship among closely related species. The SSRs are present and distributed in the genomes of all eukaryotes. Because of the abundance and specificity of SSRs, these are considered as important DNA markers for genetic mapping and population studies. The important features of SSR markers coupled with their ease of detection have made them useful molecular marker in different crops [10]. Therefore, detection of SSRs in the unigenes and ESTs of *Brassicaceae* species may help in designing a new set of DNA markers and may provide more insight in the genetic diversity among these species. This will also provide an excellent opportunity to breeders to find some agriculturally important genes, to clone and use them in Brassica breeding programs. In one study, 131,286 ESTs of five *Brassicaceae* species were assembled into unigene contigs and compared with *Arabidopsis* gene indices. Almost all the unigenes of *Brassicaceae* species showed high similarities with *Arabidopsis* genes except those of *B. napus*, where 90% of unigenes were found similar. A total of 9,699 SSRs were identified in the unigenes. Functional annotation of unigenes showed that the majority of the genes are present in metabolism and energy functional classes. The DNA markers developed in this study can be used for mapping, tagging, and cloning of important genes in *Brassicaceae* [26].

The B genome of *B. nigra* is considered to be an important source of useful genes in *Brassica* breeding, including drought tolerance, disease resistance, and oil seed quality [27]. Desirable traits can be transferred or combined through interspecific hybridization. The B genome of *B. nigra* is a major donor as a source of resistance to several blackleg diseases (*Leptosphaeria maculans*, *Phoma lingam*) [28]. This trait has been successfully transferred to *B. napus*, a major oilseed crop in the Europe and North America [29,30].

PCR based IP (Intron polymorphism) markers were used to analyze genome wide synteny between *Brassica juncea* (AABB genome) and *Arabidopsis thaliana*. The arrangement of 24 genomic block segments in the A, B and C *Brassica* genomes were analyzed in order to understand the karyotypic variations in three diploid *Brassica* genomes. Comparative genomics between the three *Brassica* lineages established the major rearrangements, translocations and fusions pivotal to karyotype diversification between the A, B and C genomes of *Brassica* species [31]. This inter-relationship between the *Brassica* lineages vis-à-vis *Arabidopsis* would facilitate the identification and isolation of candidate genes contributing to traits of agronomic value in crop Brassicas and the development of unified tools for *Brassica* genomics.

The genomes of *Brassica* species, although 4 to 10 times larger than that of *A. thaliana*, are still of a tractable size for genomic technologies. Physical maps are being constructed for the *Brassica* A genome in Korea and for both the A and C genomes in the UK. Partial physical mapping of the genome of *B. napus* is being conducted in Canada and in the EU. Although such physical maps will be of great value for the identification of specific regions of the genomes of these important crops, they will not permit the detailed analysis of the entire *Brassica* genome, the preparation of microarrays to analyse the transcriptome, or the efficient design of markers associated with the sequences of specific genes for use in breeding programmes. To achieve these things, the complete sequence of at least one of the *Brassica* genomes will be required. It is necessary to sequence only one *Brassica* genome initially at both the macrostructure (chromosome level) and the microstructure (gene-by-gene level) level. The international Brassica research community is working together to establish communal genomic resources (see also at brassica.info). A steering group has been formed for the Multinational *Brassica* Genome Project. This steering group represents the international *Brassica* research community and has the roles of promoting international cooperation and helping to defining the strategic goals of the community in the area of *Brassica* genomics. The steering group recently agreed that these goals should include the genetic anchoring of the BAC-based physical maps being constructed for *Brassica* genomes, and that the500Mb *Brassica* A genome should be sequenced by an international consortium, with a some target completion date (http://www.brassica/resource/dna-sequences).

Microarray resource for transcriptome profiling in Brassica species

Complementary to genome sequencing is the wide spread application of transcriptome sampling strategies, which has resulted in large collections of expressed sequences tags (ESTs) for nearly all economically important plant species (see in above section) (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html). Additionally, advances in sequencing technologies (the next generation sequencing technologies) have reduced the sequencing cost and increased the sequence capacity at an unprecedented rate, making whole –genome sequencing possible for each important crop species. As a result, genomics-assisted breeding have gained momentum, with the capacity for significant improvements in the accuracy and efficiency for predicting phenotypes from genotypes.

A genome wide view of gene expression programs is required to understand the underlying mechanisms of the coordination of genes at the molecular level. As described above, the completion of the full genome sequence for *Arabidopsis* has led to the development of several genomics resources for high throughput gene expression studies, including cDNA and oligonucleotide-based microarrays [32,33]. DNA microarray technology has made a revolutionary transformation in studying differential gene expression and can be used to study the collaboration of multigene for one trait and meanwhile discovering and locating the target genes. This technology has been widely used to study different traits [34,35] since its appearance, including transcriptional regulation in response to drought, salinity and heavy metal treatments [36-38].

A microarray resource for use by the Brassica research community has been recently developed [39]. A 60-mer oligo microarray comprising 94,558 probes was developed using the unigene sequences. Gene expression was analyzed in reciprocal resynthesised *B. napus* lines and the *B. oleracea* and *B. rapa* lines used to produce them. The analysis showed that significant expression could

consistently be detected in leaf tissue for 35,386 unigenes. Expression was detected across all four genotypes for 27,355 unigenes, genome-specific expression patterns were observed for 7,851 unigenes and 180 unigenes displayed other classes of expression pattern. Principal component analysis (PCA) clearly resolved the individual microarray datasets for *B. rapa*, *B. oleracea* and resynthesised *B. napus*. Quantitative differences in expression were observed between the resynthesised *B. napus* lines for 98 unigenes, most of which could be classified into non-additive expression patterns, including 17 that showed cytoplasm-specific patterns.

Additionally, the Affymetrix GeneChip® Brassica Exon 1.0 ST Array has been developed and it is a 5 µM 49-7875 format array, containing 2.4 million 25-base oligonucleotide probes representing 135,201 gene models, with 15 probes per gene distributed among exons. Discrimination of the gene models was based on an E-value cut-off of $1E^{-5}$, with <98% sequence identity. The 135 k Brassica Exon Array was validated by quantifying transcriptome differences between leaf and root tissue from a reference Brassica rapa line (R-o-18), and categorization by Gene ontologies (GO) based on gene orthology with *Arabidopsis thaliana*. Technical validation involved comparison of the exon array with a 60-mer array platform using the same starting RNA samples. The 135 k Brassica Exon Array is a robust platform and is accessible as a track within the public BrassEnsembl genome browser at http://www.brassica.info/BrassEnsembl/index.html [40].

Transcriptional regulation in response to Cu treatments to *Brassica nigra*

Arabidopsis thaliana has become a model molecular genetics system because of its extensive genetic characterization, compact genome, known genomic sequence and compact growth habit, and the availability of a wide variety of tools for its molecular genetic manipulation. However, it does not accumulate metal. Interestingly, approx. 25% of the documented metal hyperaccumulating species are the members of the *Brassicaceae* [41].

The heavy metal accumulating species *Brassica nigra* and *B. juncea* have received attention due to its possible use for phytoremediation of heavy metal-polluted soils [46, 48]. A strong Cd accumulation has been demonstrated for the trichomes covering the leaf surface of *Brassica juncea* [42]. The ability to efficiently translocate heavy metal ions from the root to the shoot has led to the proposal that *B. juncea* could be used to decontaminate heavy metal polluted soils [42]. Though *B. juncea* is not reported to be a hyper accumulator of metals but its relative high biomass, rapid growth, high economic value as industrial crop and relatedness to metal hyperaccumulating plants makes it promising plant for both use in phytoremediation and for generation of biofuel.

Tolerance to metals is based on multiple mechanisms such as cell wall binding, active transport of ions into the vacuole and formation of complexes with organic acids or peptides [43]. Here, one of the most important mechanisms for metal detoxification in plants appears to be chelation of metals by low molecular weight proteins such as metallothioneins and a family of peptide ligands, the phytochelatins. For example, glutathione (GSH), a precursor of phytochelatin synthesis, plays a key role not only in metal detoxification but also in protecting plant cells from other environmental stresses including oxidative stress. The enzymes in this pathway have been well characterized in *Brassica juncea* [44].

In the last decade, the tremendous developments in molecular biology and the success of genomics have highly encouraged studies in molecular genetics, mainly transcriptomics, for the identification of the functional genes implied in metal tolerance in plants [45,46]. These studies have already succeeded in the identification of hundreds of genes that largely belong to the metal-homeostasis network [43]. To understand the genetics of metal accumulation and adaptation, the vast arsenal of resources developed in *A. thaliana* could be extended to one of its closest relatives that display the highest level of adaptation to high metal environments such as *A. halleri* and *N. caerulescens.* Further studies could also be carried out with fast growing, high biomass producing and economically important *Brassica* sps which can be used for both for phytoremediation and for biofuel and/or industrial oil production.

In this section we will describe recent advances in understanding the genetic and molecular basis of the metal induced gene expression in plants including the gene expression work which is being carried out in our laboratory on some metal accumulating plant species in *Brassicaceae* family.

While surveying the flora of Cu mining areas of Southeastern Anatolia, we discovered several endemic metal accumulator plants and interestingly a *Brassica nigra* ecoptype found from Diyarbakir site contained a very high amount of Cu in their shoots (around 700 ppm Cu in their leaves) (see Fig 2) [47]. When plants from this ecotype were regenerated from callus culture and grown in soil culture containing 200 ppm Cu, the shoots accumulated x3 more Cu (700 µg/g D.W.) than roots (Yildizhan and Memon unpublished data).

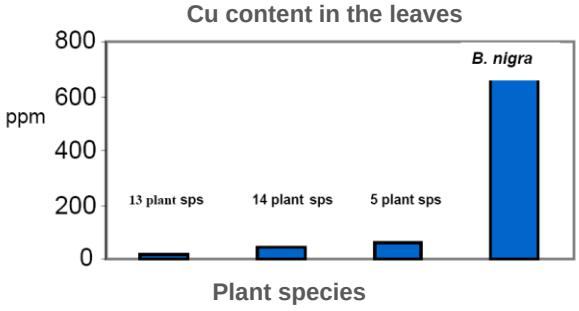

Fig. 2. A histogram showing the Cu content in the leaves of plant species collected from Southeastern part of Turkey [48].

This ecotype could be considered as a good candidate for Cu phytoremdiation [49]. Our data showed that ~ 20000 µg Cu g-1 DW was accumulated in the shoots of *B. nigra* when grown at 500 µM Cu. The expression γ-ECs and PCS was also increased several times in shoots when plants were subjected to high Cu concentration [50]. Specially the expression of key regulatory enzyme in glutathione pathway (γ-EC) was increased around 9 fold in the leaves when plants were subjected to 200µM Cu treatment (Fig 3).

Comparative transcriptome analysis was carried in order to find out the expression level of metal induced genes and transcriptome changes both in low and high Cu treated plants. Microarray analysis showed that some of the genes were up regulated (several hundred folds) and some were down regulated when plants were exposed to high Cu [50]. The results of up regulated genes are shown in Table 1.The Cu accumulation capacity of Diyarbekir ecotype was

Table1. Summary of microarray on *A. thaliana* Genechips (Arabidopsis ATH 1-14501 Genome array) hybridized with cDNA from *Brassica nigra* grown at 0 (control) and 500 µM Cu. The results show the fold (x) expression of genes in 500 µM Cu treated plants compared to control [50].

Enterez Gene	Uni Gene ID	Annotation	Fold Expression
814671	At4290	Homeobox-leucine zippi protein 17 (HB-17)	542.66
841606	At37745	Leucine-rich repeat protein kinase	305.303
839766	At18928	Lipase	222.9
826933	At51233	Protein kinase family protein	988.867
843799	At34864	Glutathione 5- transferase	4453.22
817206	At68413	AKT1 potassium channel protein	268.304
828055	At54106	Small lipase related protein	944.145
832383	At8800	Cytochrome P450 family protein	853.668
826797	At63693	S-locus Lectin Protein kinase family protein	257.197
840050	At62375	MHD-box family protein	627.242
829490	At48932	Metal transporting P-type ATPase (PAA1)	298.124
824253	At35444	Zinc finger homeobox protein ZF-HD homeobox	274.521
843519	At23741	Sucrose transporter/ sucrose- proton symporter (SUC1)	131.859
823644	At1043	2-phosphoglycerate kinase related	747.391
835115	At62438	CCAAT-box binding transcription factor Itap5a putative	542.568
829483	At31581	ABC transporter family protein	374.546
835775	At27567	Histone acetyl transferase family protein	2464.59
821403	At38505	F-box family protein	392.733
839706	At23008	Auxin responsive GH3 family protein	636.692
831284	At10905	myb family transcription factor (MYB40)	240.056
838207	At41885	transporter-related	4437.08
832081	At54918	Sec7 domain- containing protein	409.803
835989	At50537	wound-responsive protein related	349.045
821739	At70382	E3 ubiquitin ligase SCF complex submit SKP1 / ASK1 (At9)	360.018
827187	At33200	ABC transporter family protein	767.817
821369	At53370	No apical meristem (NAM) family protein	64755.1
840117	At51872	Calmodulin (putative)	395.849
818315	At37430	Heavy metal associated domain containing protein	962.659
829867	At31273	Hydroxyproline-rich glycoprotein family protein	167.632
818856	At36994	LEH domain containing protein	957.017
840311	At10999	Inositol phospholi 5-phosphatase I IP5PI	60.167
816596	At39660	Glycosylhydrolase family 5 protein/ cellulase family protein	266678
839166	At17257	proline-rich extensin, putative	14276.4
843088	At52423	lysine and histidine specific transporter putative	755.624
839416	At51465	epsin N terminal homology (ENTH) domain-containing protein/ Clathrin assembly protein	53631.3
828430	At54490	Receptor - like protein kinase putative	108.975
828480	At2572	High mobility group (HMG1/2) family protein	280.771
838269	At41859	α-Trehalose- phosphate synthase, UDP-forming putative/ Trehalose-6-phosphate	452.523
830681	At54772	SNAP 25 like	169.613
818520	At70170	ABC transporter	907.673
833815	At55216	Plant defensin- fusion protein putative	845.084
821552	At50206	Cytochrome P450 family protein	1546.55
834610	At43916	Heatshock transcription factor	223.442
827857	At32663	UV-damaged DNA binding protein putative	1472.9
841241	At20838	serine/threonine protein kinase putative	835.259
816759	At39463	Oxidoreductase 20G-Fe(II) oxygenase family protein	366027
829309	At31713	WRKY family transcription factor	169.063

determined and compared with other *Brassica nigra* ecotypes 6619, 6620 and 6630 obtained from different sites of Western Europe. In these comparative studies *Brassica nigra* Diyarbekir ecotype was found to be a super accumulator of Cu compare to other European ecotypes [50].

Microarray analysis showed several hundred folds up regulation of metal related genes including the genes involved in glutathione pathway, metal ATPase and ABC transporters in *B. nigra* when treated with 500 µM Cu. Currently we are carring out metabolomic studies with metal treated accumulator and non accumulator ecotypes of *B. nigra* by using HPLC-MS-MS inorder to identify metabolomic pattern in accumulator and non-accumulator ecotypes. Our aim is to identify specific metabolites which are upregulated and/or down regulated with Cu treatment in both ecotypes.

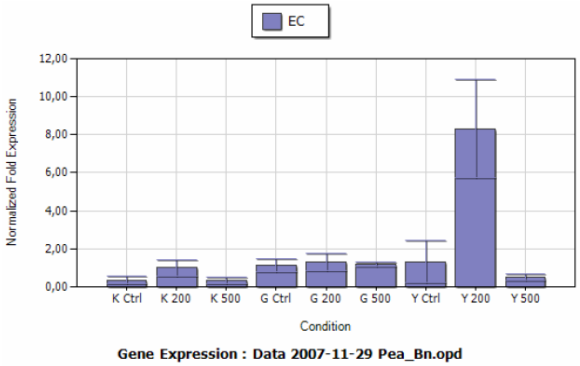

Fig. 3. R-T(real time) PCR experiments showing γ-EC expression in roots (K), stems (G) and leaves (Y) of Brassica nigra grown in 0 (control), 200, and 500 µM Cu [48].

CONCLUSION

To understand fully the genetics of metal accumulation, the vast genetic resources developed in *A. thaliana* must be extended to other metal accumulator species that display traits absent in this model species. *A. thaliana* microarray chips could be used to identify differentially expressed genes in metal accumulator plants in *Brassicaceae*. The integration of resources obtained from model and wild species of the *Brassicaceae* family will be of utmost importance, bringing most of the diverse fields of plant biology together such as functional genomics, population genetics, phylogenetics, and ecology. Like in present work with *Brassica nigra*, *Arabidopsis* array have been previously used to compare *Arabidopsis* transcriptome to other related *Brassicaceae* species such as *Arabidopsis halleri* [51,52], *Thlaspi caerulescens* [45], *Thellungiella halophila* [53,54], *Brassica oleracea* [40] and *B. napus* [55]. It will be interesting to use recently developed Affymetric GeneChip® Brassica Exon 1.0 ST Array to identify gene expression profile in *Brassica nigra* and other *Brassica* sps subjected to high and low metal concentrations.

Further development of phytoremediation technology requires an integrated multidisciplinary research effort that combines plant biology, genetic engineering, soil chemistry, soil microbiology, as well as agricultural and environmental engineering.

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