Development and validation of a high density sunflower microarray for functional studies on biotic and abiotic stresses

Paula Fernandez[1, 3], Julio Di Rienzo[2], Marcelo Soria[6], Lucila Peluffo[1, 3], Sebastián Moschen[1, 3], Sergio González [5], Bernardo Clavijo[1], Máximo Rivarola[1, 3], Verónica Lia[1, 3], Federico Ehrenbolger[1, 3], Ana Conesa[4], Francisco García[4], David Blesa[4], H. Esteban Hopp[1], Joaquín Dopazo[4], Norma Paniego[1,3], Ruth A. Heinz[1,3]

[1]Instituto de Biotecnología, CICVyA, INTA Castelar, N. Repetto y Los Reseros S/N (1686) Hurlingham, Buenos Aires, Argentina, rheinz@cnia.inta.gov.ar; [2]Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina, [3] Comisión Nacional de Investigaciones Científicas y Técnicas – CONICET, Argentina; [4]Centro de Investigación Príncipe Felipe, Valencia, España; [5]Facultad de Ingeniería, Universidad Nacional de Buenos Aires, Argentina; [6]Facultad de Agronomía, Universidad de Buenos Aires.

- The identification of candidate genes underlying agronomically important traits represents a key strategy for molecular breeding applications. Different transcriptional strategies can be addressed, taking into consideration the target species and molecular tools available. In the case of sunflower, the lack of availability of a commercial oligonucleotide-based chip has limited transcriptional studies. Besides, transcriptional analyses based on RNA-seq technology are still limited in species lacking full genome sequences as sunflower. The goals of this study were the development of comprehensive unigene collection of *H. annuus*, its functional annotation and the design and validation of a custom sunflower oligonucleotide-based microarray for identification of concerted transcriptional responses associated to leaf senescence and fungal pathogen infections.
- A large scale EST (> 130,000 ESTs) cleaning, assembly and sequence annotation was done using Blast2GO (www.blast2go.de). The resulting unigene collection was used to design the first custom sunflower oligonucleotide-based microarray under Agilent technology. Pre-processing and differential expression analysis of Agilent microarrays was performed using functions implemented in the *limma* package, available from the open source Bioconductor platform (http://www.bioconductor.org/). Gene set analysis was based on Gene Ontology information using FatiScan software integrated in Babelomics suite (http://babelomics.bioinfo. The microarray performance was evaluated under two experimental assays to study the response of sunflower to water deficit, as a physiological event that induces senescence, and the response to the necrotrophic fungal pathogen *Sclerotinia sclerotiorum*. Factorial experiment designs were applied and statistical analysis based on mixed-models was followed. The selected differential genes were further validated by qPCR, using reference genes previously characterized for sunflower.
- The final EST assembly comprises 41,013 putative transcripts (12,924 contigs and 28,089 singletons). The resulting microarray includes a total number of 42,326 features: 1,417 Agilent controls, 74 control probes for sunflower 10 times replicated (740 controls) and 40,169 different non-control probes representing the Sunflower Unigen Resource (SUR version 1.0). The evaluation assays allowed the detection of 558 differentially expressed genes between water stress and control conditions from which ten genes were further validates by qPCR. Regarding the response to *S. sclerotiorum,* 33 genes were identified to be involved in the response to *S. sclerotiorum* ($p<0.001$). Thirteen of them were directly related to the response to the infection regardless of the sunflower time course and 20 were differentially expressed depending on the time course and/or showed interaction effect.
- The developed unigene collection used to generate the oligonucleotide microarray, gathers nearly all of the known functional sequences from cultivated sunflower. The global analysis of gene expression, validated under two stress conditions showed that the *H. annuus* microarray is suitable for a wide range of functional genomics analyses, showing a precise and accurate level of trustability along different gene expression profiles.

This work generated a curated and trustable sunflower unigene collection which resulted in the first custom sunflower oligonucleotide-based microarray under Agilent technology. The work presented here gives the sunflower community a trustable microarray to use under different transcriptional applications.

Key words: microarray – transcriptomics – bioinformatics – unigene – sunflower.

INTRODUCTION:

.

Advances in sunflower (*Helianthus annuus L.*) genomics accomplished since 1995 have greatly enhanced the development and application of new tools for crop improvement (Jan and Seiler, 2007; Paniego et al., 2007; Seiler and Jan, 2010),. Regarding concerted transcriptional studies, during the last decade, cDNA macro and microarrays were developed to study sunflower seed development (Hewezi et al., 2006) and the response to biotic (Alignan et al., 2006), and abiotic stresses (Hewezi et al., 2006; Roche et al., 2007; Fernandez et al., 2008). In this context, functional tools as high density oligonucleotide microarrays strongly support the discovery and characterization of novel genes. Oligonucleotide-based chips are considered more accurate than cDNA-based chips due to the reduction of manipulation steps (Larkin et al., 2005). The possibility to implement this technology on any custom array system like Agilent, Nimblegen, and others, has the potential to create a highly useful tool for gene discovery in non-model crops (Nazar et al., 2010; Ophir et al., 2010). Recently, an Affymetrix chip for the *Helianthus* genus has been designed based on raw EST public data (Bazin et al., 2011). On the other hand, it was claimed that the use of a longer probe format represents an advantage of Agilent oligonucleotide microarrays over others technologies based on a higher hybridization stability in cases of sequence mismatches, being consequently, more suitable for the analysis of highly polymorphic regions. In this work, we present the development of a comprehensive Sunflower Unigene Resource of *H. annuus L.*, its functional annotation and the design and validation of a custom sunflower oligonucleotide-based microarray for identification of concerted transcriptional changes associated to leaf senescence and response to *Sclerotinia sclerotiorum* infection. This development represents an initiative of the Sunflower Argentinean Consortium, working in collaboration with the Príncipe Felipe Research Center (CIPF, Valencia, Spain), within the frame of a public research project.

MATERIALS AND METHODS

EST assembly and annotation

A total of 133,682 EST sequences of *H. annuus L.* were downloaded from Genbank (http://www.ncbi.nlm.nih.gov/nucest) in December 2008. These sequences were screened for the presence of remnants of cloning or sequencing vectors by running BLASTN (Altschul et al., 1990) optimized for short matches against the UniVec database (ftp://ftp.ncbi.nih.gov/pub/UniVec/). An additional subset of 120 new EST sequences derived from local SSH-identified transcripts that had not been deposited in Genbank at the moment of downloading was compiled into the former assembly. Sequence annotation was performed using different tools (Blast2GO and InterproScan) under default parameters (Conesa et al., 2005), using a local database generated from GO database (2011-08 update). This annotation procedure resulted in 49.6% of total sequences with GO annotation.

Microarray design and synthesis

For the custom Gene Expression chip design, Agilent Technologies eArray® web application was used. Probe sequences were obtained using GE Probe Design considering 3' end biased 60mer oligonucleotide, one probe per target, vector sequence and masking function on probe sense orientation. Two probe sets were designed: one including non-control specific probes for the Sunflower Unigene Resource (SUR v 1.0) and a second control probe set consisting of 74 probes derived from 80 differentially expressed sunflower genes identified in a previous work (Fernandez et al., 2008). This design was then used to manufacture microarrays using Agilent SurePrint™ Technology in the 4 x 44 format. Agilent's microarrays include the Spike-In Kit that consists of a set of 10 positive control transcripts optimized to anneal to complementary probes on the microarray, minimizing self-hybridization or cross-hybridization.

RNA isolation and quality controls

Total RNA isolation was performed from leaf and florets of control and stressed plants. Samples were immediately frozen in liquid nitrogen and conserved at -80°C until their processing. High quality total RNA was isolated from 100 mg of frozen tissue using Trizol® (Invitrogen, Argentina) or RNAqueos (Ambion, U.S.A) following manufacturer´s instructions. Genomic DNA was eliminated after treatment with Dnase I for 20 min at RT using DNAse I® (Invitrogen, Argentina). RNA concentration was measured using a Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware USA). Purity and integrity of total RNA was determined by 260/280 nm ratio and the integrity was checked by electrophoresis in 1%

agarose gel and quality confirmed by RNA 6000 Nano Bioanalyzer (Agilent Technologies, Palo Alto, California USA) assay.

cRNA synthesis, labeling and microarray hybridization

An amount of 200 ng of total RNA was used to produce Cyanine 3-CTP-labeled cRNA using the Low Input Quick Amp Labelling Kit, One-Color (Agilent Technologies) according to the manufacturer's instructions. Following 'One-Color Microarray-Based Gene Expression Analysis' protocol version 6.0 (Agilent Technologies), 2 µg of labeled cRNA was hybridized with a Sunflower Custom Oligo Microarray (Agilent Technologies) containing 42,326 probes derived from SUR v 1.0. Agilent's recommended protocol for microarray workflow quality control was implemented using the Agilent's Spike-In Kit.

Microarray scanning and data analysis

Slides were scanned in an Agilent Microarray Scanner (G2565BA) according to the manufacturer's protocol. Signal data were collected with dedicated Agilent Feature Extraction Software (v9.5.1) following the Agilent protocol GE1_107_Sep 09 and the QC Metric Set GE1_QCMT_Sep09.

Agilent Processed Signals (generated by the Agilent Feature Extraction software) were pre-processing with functions implemented in limma package (Smyth, 2004), available from open source Bioconductor platform (http://www.bioconductor.org/). The background correction was done with the backgroundCorrect () function, using normexp method and offset=1 to avoid negative values after log-transformation. Normalization was achieved using the normalizeBetweenArrays () function, applying a quantile method (Bolstad et al., 2003). The raw data are available from the GEO repository, accession number GSE29390.

Differential gene expression analysis was also carried out using the limma package. Multiple testing adjustments of p-values were done according to Benjamini and Hochberg (Benjamini and Hochberg, 1995) methodology. Gene set analysis was carried out according to the Gene Ontology terms using FatiScan (Al-Shahrour et al., 2007) integrated in Babelomics suite (Al-Shahrour et al., 2005).

Quantitative RT-PCR analysis

Ten genes were selected according to their highest level of expression in the water deficit treatment compared to the control condition for further qPCR validation analysis. Specific primers for qPCR were designed from each target sequence using Primer 3 (Rozen and Skaletsky, 2000) under default parameters. The expression profiles of these genes were estimated in relation to reference genes using fgStatistic software, which uses previously published algorithms (Pfaffl, 2001).

RESULTS

Assembly and annotation of sunflower unigenes

Clustering and assembling of 133,682 ESTs was done using CAP3 (Huang and Madan, 1999) with parameters set accordingly to the most relevant and recently published microarray designs (p=95, f=45, h=25, o=80) (Close et al., 2004; Martinez-Godoy et al., 2008; Trick et al., 2009; Booman et al., 2010; Privat et al., 2011). After cleaning and removal of low quality and short (<100 bp) sequences, the dataset was reduced to 132,479 reads. Also, additional processed ESTs or gene sequences of special interest for relevant traits were added to the initial dataset. The final assembly resulted in 41,013 putative transcripts (12,924 contigs and 28,089 singletons) The outcome SUR v 1.0 allowed the generation of a microarray platform which is available at the NCBI Gene Expression Omnibus (GEO) according to MIAME guidelines (Brazma et al., 2001), under the Accession Number GPL13610, whereas raw data corresponding to chip validation are deposited under Accession Number GSE29390.

Microarray validation

In order to confirm that the sunflower microarray do generate biologically-useful information, we used this platform to analyze global changes in gene expression profiles in response to water deficit as a physiological event which induces senescence and in response to the necrotrophic pathogen *Sclerotinia sclerotiorum.* Senescence marker genes have been previously identified and validated by our group (Fernandez et al., 2011). Hence, the 44K Sunflower Chip was tested for two field experiments 1) leaves from plants growing under control and mild WD conditions and 2) florets from a moderate resistant cultivar (RHA 801) mock inoculated or pathogen inoculated at two different days after inoculation (2, 4 DAI). RNA was isolated for each treatment and replicate, making a total of six independent samples for the first experiment and 12 for the second one. The gene expression profile for each sample was generated by labeling and hybridizing each sample to one microarray field..

Differential gene expression
Genes having differential expression among treatments were identified using the lmFit and contrasts.fit functions of limma package (Smyth, 2004). For the senescence experiment, an empirical Bayes procedure (eBayes) was used to improve the estimation and augment the degrees of freedom for the individual variances. No multiple testing adjustments of p-values were done. Although these methods control the false discovery rate, they also increase the rate of false negatives. Differentially expressed genes were identified according to their p-values after eBayes correction using a significance level of 0.01. These results are in line to what it is expected from controls and non-control genes included in the oligo-based chip. A total of 558 differentially expressed genes between control and WD conditions were obtained. For the *S. sclerotiorum* experiment, a factorial design was applied and statistical analysis based on mixed-models was followed. Thirty tree genes were identified to be involved in the response to the pathogen in a resistant genotype ($p<0.001$). Thirteen of them were directly related to the response to the infection regardless of the sunflower time course and twenty were differentially expressed depending on the time course.
qPCR analysis for differentially expressed genes
In order to validate candidate genes outcome from the microarray analysis, quantitative real time PCRs (qPCR) were performed on the same mRNA samples used for the microarray experiments. Based on their statistics and e-Bayes corrected p-value ranking and Fatiscan output results, ten genes analysis from the 558 differentially expressed genes of the senescence experiment and 10 out of 33 genes involved in the response to *S. sclerotiorum* were selected for qPCR. These results showed statistically significant differences (p-value $\leq$ 0.05) for nine of ten genes assessed for the senescence process and one of the ten genes selected for the response to *S. sclerotiorum* infection with a ratio of expression consistent with the statistical analysis of microarray.

DISCUSSION

Microarray technology has first-opened a new era approximately fifteen years ago (Schena et al., 1995; Chee et al., 1996) for transcriptome high-throughput analysis. Although new generation sequencing technologies are open wide to explore and analyze large genomes, the lack of a reference genome becomes a main constrain to provide with significant biological information. In the particular case of sunflower even though a genome sequence initiative is in progress (Kane et al., 2011), and massive functional studies have recently been published base on a proprietary Affymetrix Sunflower Array over *Helianthus sp* dbEST (Bazin et al., 2011), still a shortage of genomic tools has limited the research opportunities to non-profit organizations. At present, more than 130,000 ESTs are publicly available (http://ncbi.nlm.nih.gov/dbEST/dbEST_summary.html) for cultivated sunflower (*Helianthus annuus L.*), but it is worth noting that these databases tend to be significantly contaminated with vector sequences and chimeras, and have relatively low quality DNA information derived from the library sequencing strategy which prioritizes obtaining a large number of single pass sequences, thus leading to the concomitant decrease in the quality of the deposited sequences (Boguski et al., 1993). Considering this particular situation for a relevant crop like sunflower, a 60-mer oligonucleotide microarray was successfully developed using a curated unigene database. *H. annuus L.* 44K Agilent oligonucleotide microarray consisted on 28,089 singletons and 12,924 contigs, could address potential variants stemming from CAP3 assembly parameters settings (p=95, f=45, h=25, o=80), that may explain the genetic diversity due to sequencing errors, gene duplication and allelic variation from the different EST libraries. However, biological interpretation of data generated by microarray analysis for non-model species might be fully enriched with further validations, since the poor understanding of plant genomes and the low quantity of GO terms defined for these organisms (Rensink and Buell, 2005). Considering that whole sequences were selected from public web deposited ESTs, which are based on non-normalized, normalized and SSH cDNA libraries from different developmental stages and tissues, and taking in consideration that sunflower is a non-model crop with no genome sequence available, this microarray represents a key tool offering high coverage of genes involved in diverse biochemical pathways, according to metabolic annotations. Moreover, GO term mapping was carefully done running Blast2GO (Conesa et al., 2005) against a local GO database (2011-08 update). In order to store, visualize, analyze and share this information, plus the probes associated to each unigene represented in the microarray, we created a sunflower microarray database (SUR v1.0) available at http://bioinformatica.inta.gov.ar/ATGC/). The validation of the microarray for analysis of transcriptional profiles was performed using sunflower leaves and florets derived

from plants grown under two growth conditions, including biological and technical replicas. Our results show that the *H. annuus L.* microarray is suitable for functional genomics analysis. Nowadays, this microarray is already being used for other experiments showing a precise and accurate level of trustability along different gene expression profiles. In the future, this transcriptome tool added to the full sunflower genome public sequence data will facilitate comparative and functional analysis of *Asteracea*, one of the most diverse and agronomically relevant families of flowering plants, currently with scarce genomic information available. This work generated a curated and trustable sunflower unigene collection which resulted in the first custom sunflower oligonucleotide-based microarray under Agilent technology. The work presented here gives to the sunflower community a trustable microarray to use under different transcriptional applications.

REFERENCES

Al-Shahrour F, Arbiza L, Dopazo H, Huerta-Cepas J, Minguez P, Montaner D, Dopazo J (2007) From genes to functional classes in the study of biological systems. BMC Bioinformatics **8:** 114

Al-Shahrour F, Minguez P, Vaquerizas JM, Conde L, Dopazo J (2005) BABELOMICS: a suite of web tools for functional annotation and analysis of groups of genes in high-throughput experiments. Nucleic Acids Res **33:** W460-464

Alignan M, Hewezi T, Petitprez M, Dechamp-Guillaume G, Gentzbittel L (2006) A cDNA microarray approach to decipher sunflower (*Helianthus annuus*) responses to the necrotrophic fungus *Phoma macdonaldii.* New Phytol **170:** 523-536

Altschul S, Gish W, Miller W, Myers E, Lipman D (1990) Basic local alignment search tool. Journal of Molecular Biology **215:** 403-410

Bazin J, Langlade N, Vincourt P, Arribat S, Balzergue S, El-Maarouf-Bouteau H, Bailly C (2011) Targeted mRNA oxidation regulates sunflower seed dormancy alleviation during dry after-ripening. Plant Cell **23:** 2196-2208

Benjamini Y, Hochberg Y (1995) Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society **57:** 289-300

Boguski MS, Lowe TM, Tolstoshev CM (1993) dbEST--database for "expressed sequence tags". Nat Genet **4:** 332-333

Bolstad BM, Irizarry RA, Astrand M, Speed TP (2003) A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics **19:** 185-193

Booman M, Borza T, Feng CY, Hori TS, Higgins B, Culf A, Leger D, Chute IC, Belkaid A, Rise M, Kurt Gamperl A, Hubert S, Kimball J, Ouelelette RJ, Johnson SC, Bowman S, Rise ML (2010) Development and Experimental Validation of a 20K Atlantic Cod (*Gadus morhua*) Oligonucleotide Microarray Based on a Collection of over 150,000 ESTs. Mar Biotechnol **DOI: 10.1007/s10126-010-9335-6**

Brazma A, Hingamp P, Quackenbush J, Sherlock G, Spellman P, Stoeckert C, Aach J, Ansorge W, Ball CA, Causton HC, Gaasterland T, Glenisson P, Holstege FC, Kim IF, Markowitz V, Matese JC, Parkinson H, Robinson A, Sarkans U, Schulze-Kremer S, Stewart J, Taylor R, Vilo J, Vingron M (2001) Minimum information about a microarray experiment (MIAME)-toward standards for microarray data. Nat Genet **29:** 365-371

Close TJ, Wanamaker SI, Caldo RA, Turner SM, Ashlock DA, Dickerson JA, Wing RA, Muehlbauer GJ, Kleinhofs A, Wise RP (2004) A New Resource for Cereal Genomics: 22K Barley GeneChip Comes of Age. Plant Physiology **134:** 960-968

Conesa A, Gotz S, Garcia-Gomez JM, Terol J, Talon M, Robles M (2005) Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics **21:** 3674-3676

Chee M, Yang R, Hubbell E, Berno A, Huang XC, Stern D, Winkler J, Lockhart DJ, Morris MS, Fodor SP (1996) Accessing genetic information with high-density DNA arrays. Science **274:** 610-614

Fernandez P, Di Rienzo JA, Fernandez L, Hopp H, Paniego N, R.A. H (2008) Transcriptomic identification of candidate genes involved in sunflower responses to chilling and salt stresses based on cDNA microarray analysis. BMC Plant Biology **8**

Fernandez P, Di Rienzo JA, Moschen S, Dosio GAA, Aguirrezabal LAN, Hopp HE, Paniego N, Heinz RA (2011) Comparison of predictive methods and biological validation for qPCR reference genes in sunflower leaf senescence transcript analysis. Plant Cell Report **30:** 63-74

Hewezi T, Leger M, El Kayal W, Gentzbittel L (2006) Transcriptional profiling of sunflower plants growing under low temperatures reveals an extensive down-regulation of gene expression associated with chilling sensitivity. J Exp Bot **57:** 3109-3122

Hewezi T, Petitprez M, Gentzbittel L (2006) Primary metabolic pathways and signal transduction in sunflower (*Helianthus annuus L.*): comparison of transcriptional profiling in leaves and immature embryos using cDNA microarrays. Planta **223:** 948-964

Huang X, Madan A (1999) CAP3: A DNA sequence assembly program. Genome Res **9:** 868-877

Jan CC, Seiler GJ (2007) Sunflower. *In* RJ Singh, ed, Genetic Resources, Chromosome Engineering, and Crop Improvement. Oilseed Crops, Vol 4. CRC Press, pp 103-165

Kane NC, Gill N, King MJ, Bowers JE, Berges H, Gouzy J, Bachlava E, Langlade NB, Lai Z, Stewart M, Burke JM, Vincourt P, Knapp SJ, Rieserberg LH (2011) Progress towards a reference genome for sunflower. Botany **89:** 429-437

Larkin JE, Franc BC, Gavras H, Sultana R, Quackenbush J (2005) Independence and reproducibility across microarray platforms. Nat. Methods **2:** 337-344

Martinez-Godoy MA, Mauri N, Juarez J, Carmen Marques M, Santiago J, Forment J, Gadea J (2008) A genome-wide 20K citrus microarray for gene expression analysis. BMC Genomics **9**

National Sunflower Association [http://www.sunflowernsa.com/stats/table.asp?contentID=109&htmlID=74&submit170=View&submit.x=57&submit.y=12)]. *In*,

Nazar NR, Chen P, Dean D, Robb J (2010) DNA Chip Analysis in Diverse Organisms with Unsequenced Genomes. Mol. Biotechnol **44:** 8-13

Ophir R, Eshed R, Harel-Beja R, Tzuri G, Portnoy V, Burger Y, Uliel S, Katzir N, Sherman A (2010) High-throughput marker discovery in melon using a self-designed oligo microarray. BMC Genomics **11:** 269

Paniego N, Heinz R, Fernandez P, Talia P, Nishinakamasu V, Hopp H (2007) **Sunflower.** *In* C Kole, ed, Genome Mapping and Molecular Breeding in Plants, Vol 2. Springer-Verlag, Berlin Heidelberg, pp 153-177

Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acid Research **29:** e45 2001

Privat I, Bardil A, Gomez AB, Severac D, Dantec C, Fuentes I, Mueller L, Joet T, Pot D, Foucrier S, Dussert S, Leroy T, Journot L, de Kochko A, Campa C, Combes MC, Lashermes P, Bertrand B (2011) The 'PUCE CAFE' Project: the first 15K coffee microarray, a new tool for discovering candidate genes correlated to agronomic and quality traits. BMC Genomics **12:** 5

Rensink WA, Buell R (2005) Microarray expression profiling resources for plant genomics. Trends in Plant Science **10:** 1360-1385

Roche J, Hewezi T, Bouniols A, Gentzbittel L (2007) Transcriptional profiles of primary metabolism and signal transduction-related genes in response to water stress in field-grown sunflower genotypes using a thematic cDNA microarray. Planta **DOI 10.1007/s00425-007-0508-0**

Rozen S, Skaletsky H (2000) **Primer3 on the WWW for general users and for biologist programmers**. Humana Press, Totowa, NJ

Schena M, Shalon D, Davis RW, Brown PO (1995) Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science **270:** 467-470

Seiler GJ, Jan CC (2010) Basic information. *In* J Hu, GJ Seiler, C Kole, eds, Genetics, Genomics, and Breeding of Sunflower. Science Publishers, Enfield, NH., pp 2-50

Smyth G (2004) Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Stat Appl Genet Mol Biol **3:** Article3

Sunflower Unigene Repository (SUR v1.0). http://bioinformatica.inta.gov.ar/sunflower/ATGC/

Trick M, Cheung F, Drou N, Fraser F, Lobenhofer EK, Hurban P, Magusin A, Town CD, Bancroft I (2009) A newly-developed community microarray resource for transcriptome profiling in *Brassica* species enables the confirmation of *Brassica*-specific expressed sequences. BMC Plant Biology **9**