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Identifying differential expression of human genes under physical, chemical and biotic stresses

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Abstract

Human, a well-developed multicellular organism might experience substantial health damages caused by a variety of stresses including physical, chemical and biotic. These stresses are responsible for the modification in their gene expression. Many studies on gene expression physical, chemical and biotic stresses have been conducted with the help of DNA microarray technology. The current study reported the co-shared stress-responsive genes under these stresses by utilizing the existing microarray data. Total 22283 genes were studied and 241 genes are linked to being co-shared stress-responsive. Among these stress-responsive genes, 207 were identified as up-regulated and 34 as down-regulated. All co-shared stress-responsive genes were further characterized with respect to their GO functions and chromosome number.

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Introduction

The DNA content of humans is about 0.006 nanograms with a variation in genes pattern that are translated and expressed into proteins during the life cycle of the cell. The variation in gene expression is possibly due to any external or internal stress that might lead to disease (Aerssens *et al.*, 2001). These stresses such as physical, chemical and biotic impart significant damaging effects on human health (Hishikawa *et al.*, 1995; Goyer *et al.*, 2004; Liu, 2011). Among biotic stresses, an infectious disease caused by *Candida albican,* belongs to the genus *Candida a* form of dimorphic fungus (Brogden and Guthmiller, 2002). In humans, under different environmental conditions, they can be colonized most particularly in the vagina, rectum and oral cavity (Cottier & Mühlschlegel, 2009). They can also be found in the gastrointestinal tract and bloodstream and leave substantial damages heart, brain, heart and other organs (;Brogden & Guthmiller, 2002 Mader, 2004; Liu, 2011).

These stresses can also cause due to certain viruses. One such virus is Adenoviruses spread through feces and respiratory droplets (Mader, 2004). These infections are typically associated with the respiratory system (Rubin, 1993). Generally, the clinical symptoms associated with adenovirus are slight fever, diarrhea, shivering and local pain. Other infections including severe headache, relapsing seizures, and alteration of mental status (Vorburger and Hunt, 2002).

The chemical stresses are caused due to certain metals or their respective compounds. The adverse impact of certain metals is so high that they are considered human carcinogens including Arsenic, Cadmium, Chromium and Nickel (Goyer *et al.*, 2004). Nickel is one of the potential carcinogens which may enter the human body through skin contact, inhalation, or ingestion. Nickel inhalation is attributed to stomach and throat cancers. Other Nickels related adverse health effects are nickel allergy, respiratory tract cancer, lung fibrosis, and cardiovascular and kidney-related diseases (Duda-Chodak & Blaskzczyk, 2008).

Physical stresses might cause biomechanical forces such as intraluminal pressure (Carlström, 2007). In cultured human umbilical vein endothelial cell (HUVEC) the elevation in pressure is reported to increase the release of endothelin-1 (Hishikawa *et al.*, 1995). Endothelin is involved in many heart and brain-related diseases (Agapitov, 2002). The other effect of elevation in pressure is reported to involve a decrease in endothelium antithrombotic ability (Sjögren *et al.*, 2000).

When similar cells experienced different stresses, they show differential gene expression as these genes get constantly up and down-regulated. These expressions of Gene can be studied by Microarray expression analysis, a widely used technique for mRNA expressions profiling (Hedge *et al.*, 2000). A large amount of data has been created through microarray analysis and other technologies. A very useful database that serves as the largest resource of gene expression data in Gene Expression Omnibus (GEO). The available gene expression data with identical formats are used to identify the expression of common genes which further utilize to predict functions of uncharacterized genes (Barret *et al.*, 2007).

All the identified common genes are further characterized by Gene Ontology (GO) (Ashburner *et al.*, 2000). In GO various terminologies are used for the annotation of gene-gene product which is based on three main features named cellular components, biological processes and molecular function (Bada *et al.*, 2004). The present research aims to identify co-shared physical, chemical and biotic co-shared stress responding genes in humans. These findings would be helpful for the formulation of drugs against these co-shared stresses.

Materials and methods

Microarray data comparative analysis under physical, chemical and biotic stresses is conducted. Various bioinformatics tools and databases of gene expression

are used during analysis. The first step in this study was microarray data mining.

Microarray data mining

A huge microarray data on humans is publically available at NCBI GEO (www.ncbi.nih.nlm.gov/geo) as a human is one the most important organisms. Plate form 96 (GPL-96) contain a large number of data on human, therefore, it is selected to study the impact of physical, chemical and biotic stress-responsive genes. GPL-96 contains a total of 22283 genes comprised of 951 series with 32932 samples. Four series were selected, including genes under severe and non-severe effects and twenty-four samples were chosen from these series in this study.

Experimental design

The selected series contained four varying stresses named high intraluminal pressure, nickel chloride, adenovirus, and *candida albicans*. Three replicates were selected for each stress and the severe (under stress), non-severe (without stress) and combined effects were studied (Table.1).

Formation of tab-delimited file

All data under four varying stresses were imported into an excel sheet from NCBI GEO and their separate excel sheets were generated. All data under severe, non-severe and combine effects were aligned and saved as a tab-delimited file (Liu *et al.*, 2008).

Gene expression data analysis

All the saved data on tab-delimited file is further run on MeV (multi experiment viewer) which is a very useful bioinformatics tool for analyzing integrative data (Howe *et al.*, 2010). This software is accessible free of charge at (www.tm4.org/mev/).

Identification of genes

Response of genes under all the three stresses (physical, chemical and biotic) that showed microarray suite 5 (MAS5) showing about 10 folds signal intensity are identified and saved. Furthermore, the genes identified in all three replicates are saved as common genes.

Co-shared genes

For co-shared genes, the average signal intensity of the identified common genes for severe, non-severe and combine conditions was saved. The genes responsive under at least two conditions out of three showed MAS5 signal intensities ≥/≤10 folds is selected and saved as co-shared genes.

Characterization

The co-shared genes are further characterized on the basis of their chromosome number, cellular components, biological processes and molecular functions by using various bioinformatics tools including UCSC Genome Bioinformatics (genome.ucsc.edu) and European Molecular Biology Laboratory-European Bioinformatics Institute (EMBL-EBI) (www.ebi.ac.uk)

Results and discussion

The study aims to identify and characterize human genes that had varied expression levels upon exposure to physical, chemical and biotic stresses. To identify co-shared genes, a total of 22283 genes were analyzed under stressed and non-stressed conditions.

Table 1. Experimental design.

GSE	Severe effects	Non-severe effects	Combined effects
1518	High pressure	Normal-pressure	High and normal pressure
2299	Adenovirus	Control	Adenovirus infected and control
4852	Nickel chloride	Control	Nickel chloride infected and control
7355	*Candida albicans*	Control	Candida infected and control

Common genes

The genes showing MAS5 signal intensity ≥/≤10 folds under physical, chemical and biotic stresses are 1074. These 1074 genes are identified as common genes. Among these 1074 common genes, up-regulated and down-regulated are 639 and 435 respectively (Fig. 1).

Table 2. Key differentially expressed Co-shared genes under biotic, chemical and physical stresses.

Gene id	Gene symbol	Gene name	Expression
200003_s_at	RPL28	ribosomal protein L28	Up
200016_x_at	HNRNPA1	heterogeneous nuclear ribonucleoprotein A1	Up
200021_at	CFL1	cofilin 1 (non-muscle)	Up
200025_s_at	RPL27	ribosomal protein L27	Up
200030_s_at	SLC25A3	solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 3	Up
200031_s_at	RPS11	ribosomal protein S11	Up
200064_at	HSP90AB1	heat shock protein 90kDa alpha (cytosolic), class B member 1	Up
200081_s_at	RPS6	ribosomal protein S6	Up
200633_at	RPS27A	ribosomal protein S27a	Up
200715_x_at	RPL13A	ribosomal protein L13a	Up
200741_s_at	RPS27	ribosomal protein S27	Up
201254_x_at	RPS6	ribosomal protein S6	Up
207243_s_at	CALM1	calmodulin 1 (phosphorylase kinase, delta)	Up
208768_x_at	RPL22	ribosomal protein L22	Up
208929_x_at	RPL13	ribosomal protein L13	Up
209134_s_at	RPS6	ribosomal protein S6	Up
210338_s_at	HSPA8	heat shock 70kDa protein 8	Up
210646_x_at	RPL13A	ribosomal protein L13a	Up
211296_x_at	RPS27A	ribosomal protein S27a	Up
211940_x_at	H3F3A	H3 histone, family 3A	Up
211942_x_at	RPL13A	ribosomal protein L13a	Up
212581_x_at	GAPDH	glyceraldehyde-3-phosphate dehydrogenase	Up
212734_x_at	RPL13	ribosomal protein L13	Up
212790_x_at	RPL13A	ribosomal protein L13a	Up
213356_x_at	HNRNPA1	heterogeneous nuclear ribonucleoprotein A1	Up
213453_x_at	GAPDH	glyceraldehyde-3-phosphate dehydrogenase	Up
213828_x_at	H3F3A	H3 histone, family 3A	Up
214328_s_at	HSP90AA1	heat shock protein 90kDa alpha (cytosolic), class A member 1	Up
217398_x_at	GAPDH	glyceraldehyde-3-phosphate dehydrogenase	Up
220960_x_at	RPL22	ribosomal protein L22	Up
221775_x_at	RPL22	ribosomal protein L22	Up
AFFX-HUMGAPDH/M33197_3_at	GAPDH	glyceraldehyde-3-phosphate dehydrogenase	Up
AFFX-HUMGAPDH/M33197_5_at	GAPDH	glyceraldehyde-3-phosphate dehydrogenase	Up
AFFX-HUMGAPDH/M33197_M_at	GAPDH	glyceraldehyde-3-phosphate dehydrogenase	Up
205358_at	GRIA2	glutamate receptor, ionotropic, AMPA 2	Down
207397_s_at	HOXD13	homeobox D13	Down
211083_s_at	MAP3K13	mitogen-activated protein kinase kinase kinase 13	Down
220821_at	GALR1	galanin receptor 1	Down

Co-shared genes

241 genes out of 1074 genes were identified as co-shared genes under physical, chemical and biotic stresses. Out of these 241 co-shared genes, 207 genes were up-regulated while only 34 genes were down-regulated with MAS5 signal intensity ≥/≤10 folds under physical, chemical and biotic stresses (Fig. 2). The genes over-expressed under these co-shared

stresses are RPL28, RPL27, RPS11, RPS6, RPS27, RPL22, RPL13, GAPDH, HNRNP A1, CFL1, HSP90, CALM1 and SLC25A3 (Table 1). The RPL and RPS human genes switched on their expression underexposure to chemical stresses. The RPL27, RPS6, RPS11, and RPS27A are linked to non-small lung carcinoma (Yim *et al.*, 2011). Previous studies have shown the involvement of over-expressed HNRNP A1 in human colorectal cancers (Ushigome *et al.*, 2005).

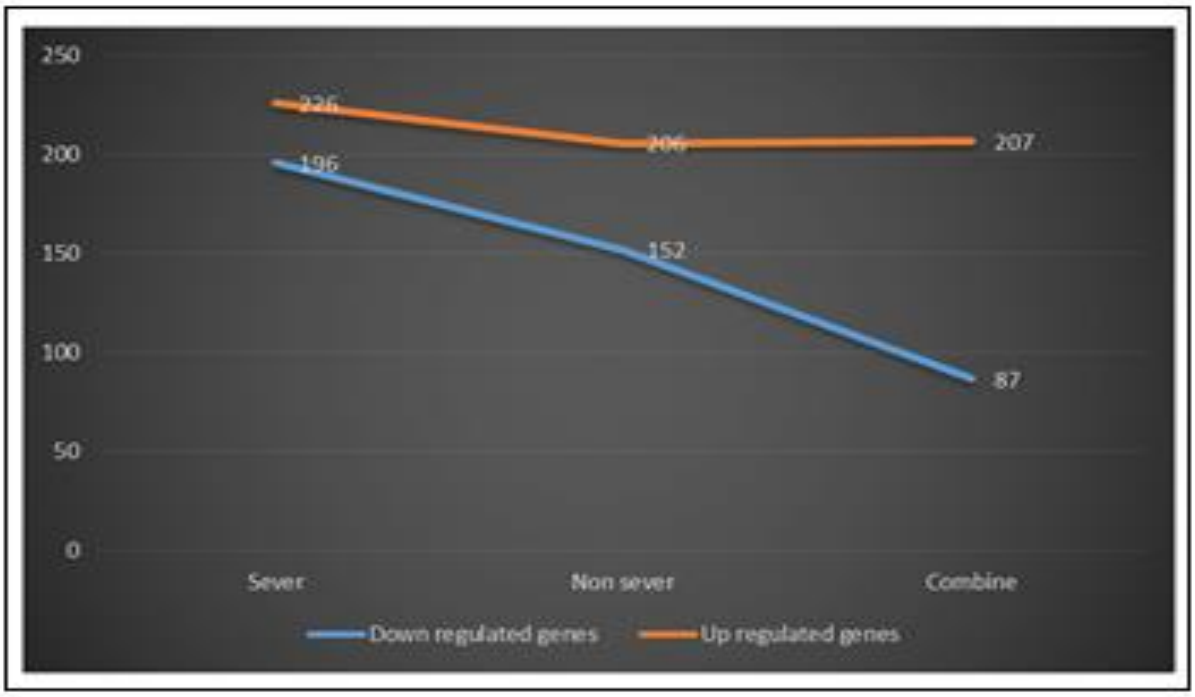

Fig. 1. Identified common up and down-regulated genes in severe, non-severe and combine effects under biotic, chemical and physical stresses.

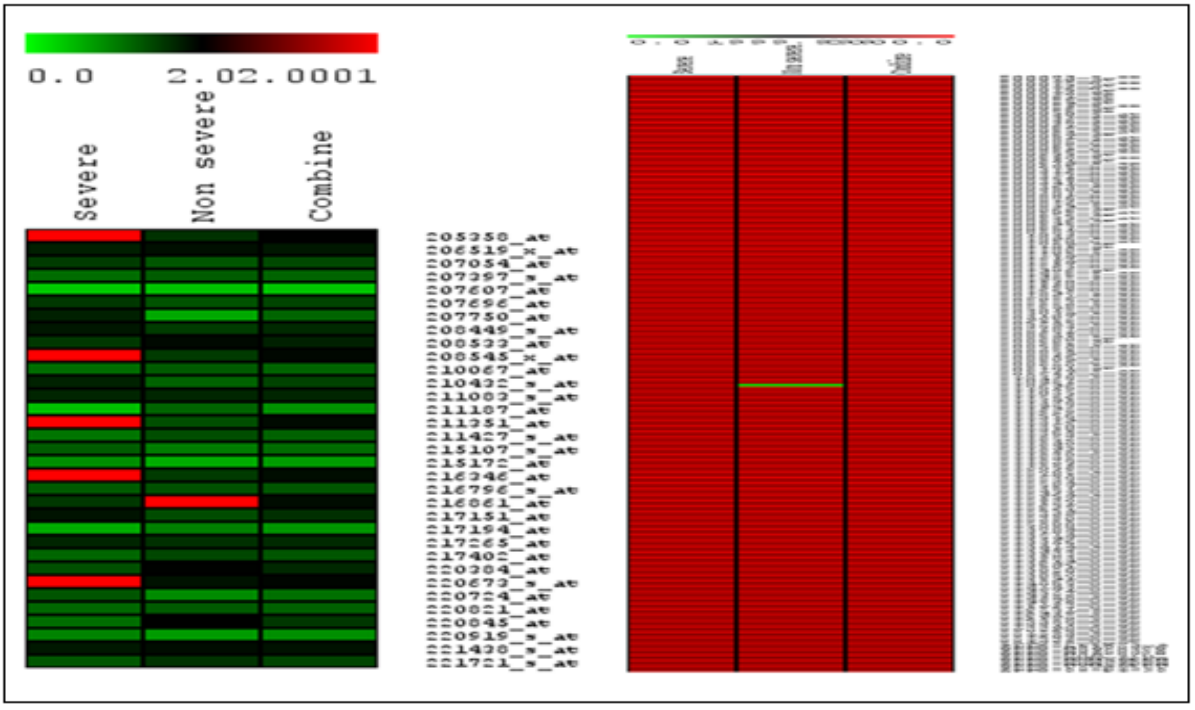

Fig. 2. MeV analysis of down-regulated (left) and up-regulated (right) Co-shared biotic, chemical and physical stress responding genes in severe, non-severe and combine conditions.

The high activity of CFL1 can be related to breast cancer and its metastasis. Similarly, the increased expression of HSP 90 can be related to human breast cancer propagation (Yano *et al.*, 1999), which might result in a patient's death (Cheng *et al.*, 2012).

Likewise, the under-expressed co-shared stress-responsive genes are GRIA2, GalR1, SIGLEC 6, MAP3K13 and HOXD13 (Table 1). Among these, GRIA2 is expressed differentially in the chemo-sensitive tumor.

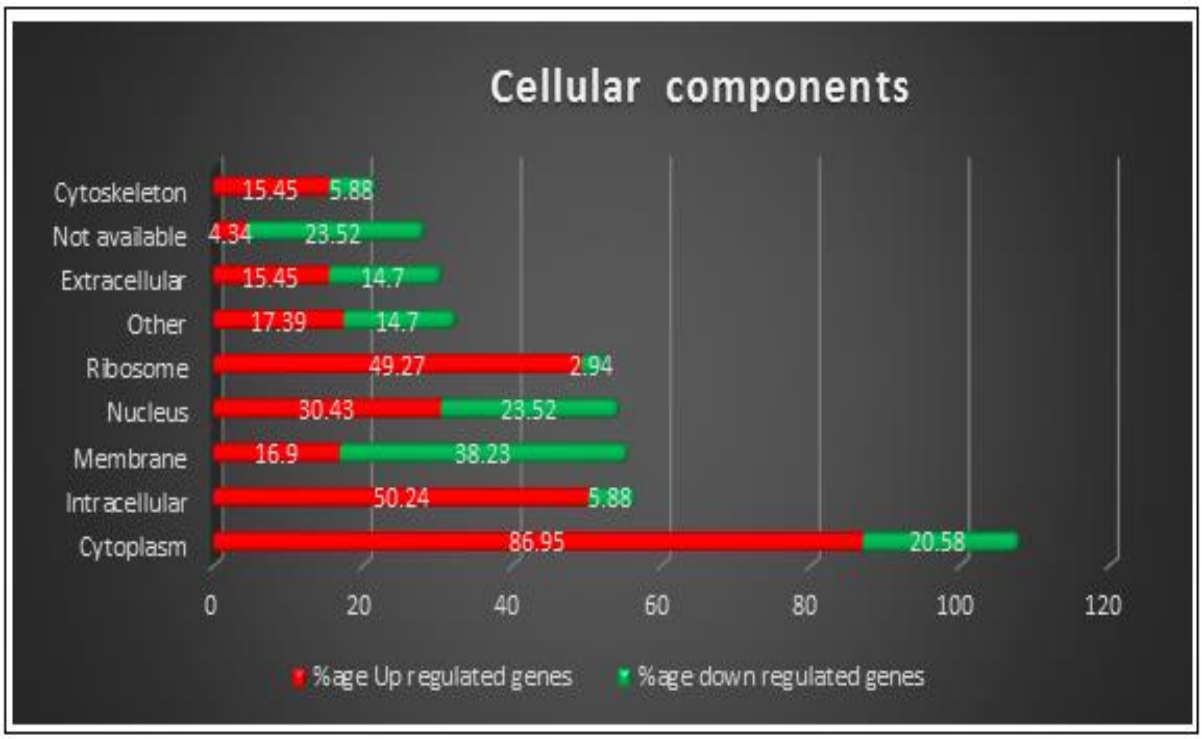

Fig. 3. Up and down-regulated Co-shared genes participating in the functions of various cellular components under biotic, chemical and physical stresses.

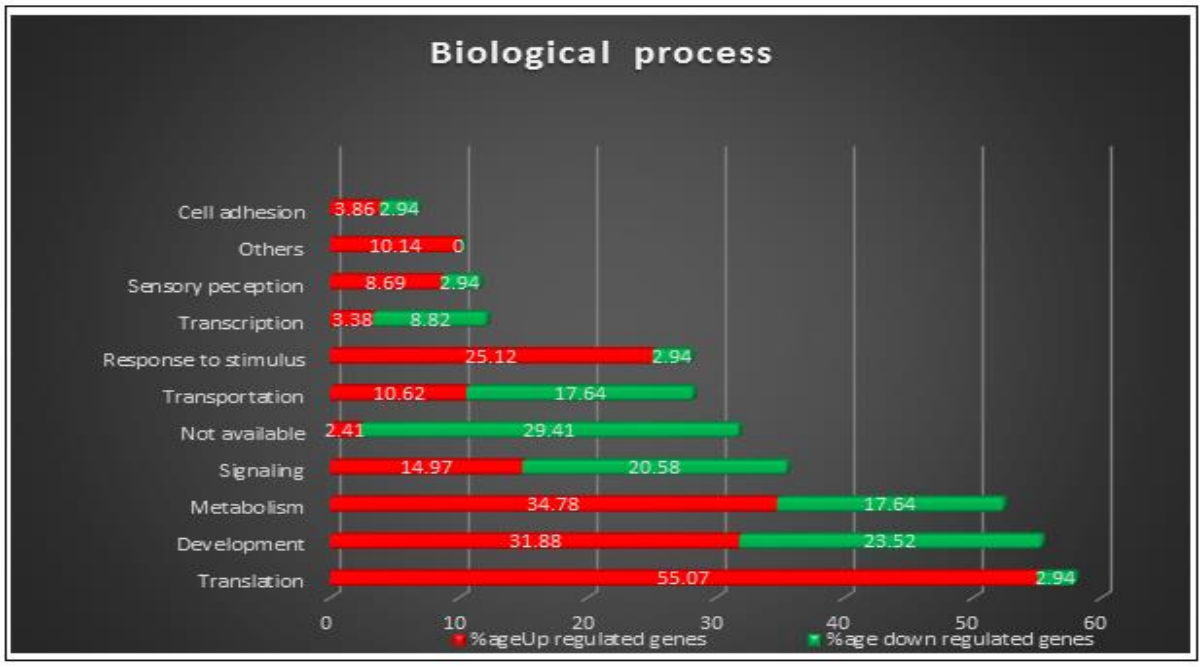

Fig. 4. Up and down-regulated Co-shared genes involved in different biological processes under biotic, chemical and physical stresse.

The differential expression of this gene helps in the diagnosis of patients with ovarian adenocarcinoma (Choi *et al.*, 2012). Another under-expressed gene is MAP3K13 which was reported to be involved in the survival and propagation of various cancer cells (Stanislaus *et al.*, 2012). Similarly, HOXD that is under-expressed (HOXD13) in this study is critical for the normal growth of limbs along the anterior-posterior axis and its alterations in humans HOXD13 is reported to lead with synpolydactyly (Campo *et al.*, 1999).

All the co-shared genes are functionally characterized in terms of their cellular components, biological processes, and molecular functions. All these three categories were further grouped based on the GO functions (Fig. 3a, b, c). Related GO categorization has also been done by other researchers (Andersson, 2005; Muller, 2007; Viemann, 2007; Yim, 2011). The study of cellular components and biological process of up-regulated co-shared genes shows the highest percentage is of cytoplasm (86.95%) and translation (55.07%). Literature shows that the up-regulated genes belonged to the RPL and RPS families are positioned in various regions of the cytoplasm (Yim, 2011). Another report shows the involvement of most up-regulated genes by nickel chloride in the process of translation (Viemann, 2007).

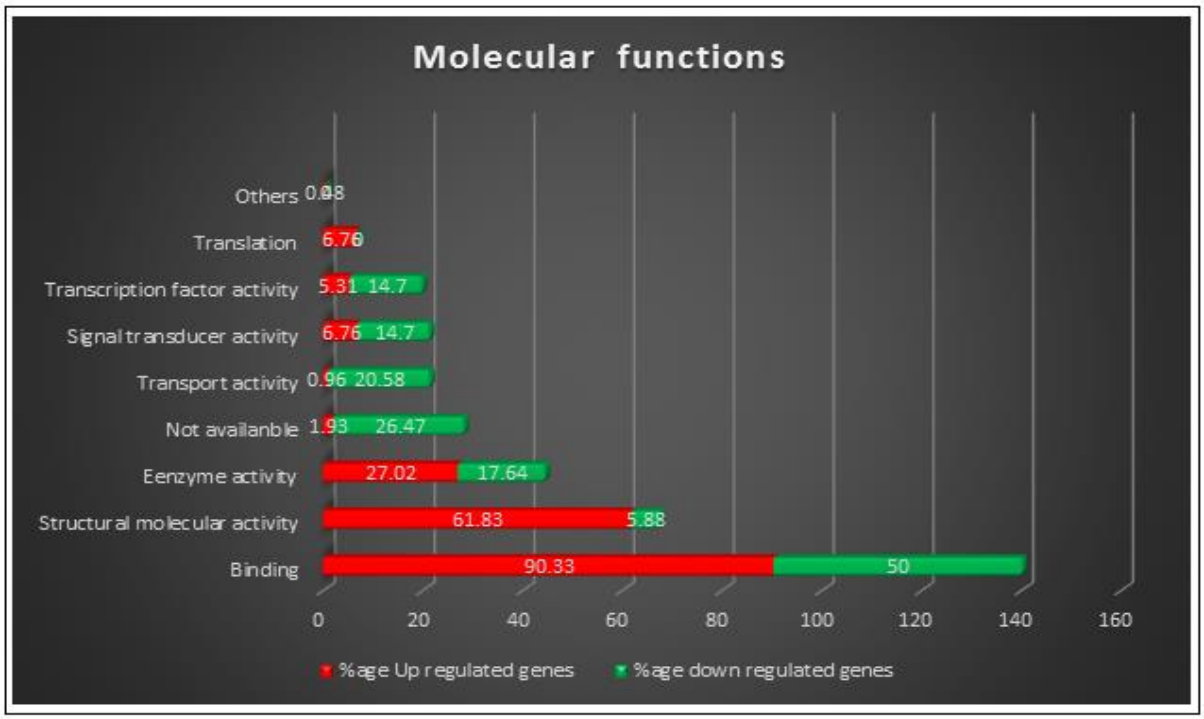

Fig. 5. Molecular functions of up and down-regulated Co-shared genes under biotic, chemical and physical stresses.

In the case of down-regulated genes, the cellular component with the highest percentage (38.23%) is the membrane. These findings were also reported by Muller (2007).

Conclusion

In the present study, Co-shared stress-responsive genes in humans under physical, chemical and biotic stresses are identified. These genes are deferentially expressed under these three stress conditions. Total 22283 genes were analyzed among which 207 genes were reported to switch on while 34 genes switched off their expression upon exposure to these stresses.

These results could help drug manufacturers in formulating drugs that are applicable to these multiple stresses.

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