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Analysis of chickpea gene co-expression networks and pathways during heavy metal stress

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Crop productivity and yield are adversely affected by abiotic and biotic stresses. Therefore, finding out the genes responsible for stress tolerance is a significant stride towards crop improvement. A gene co-expression network is a powerful tool to detect the most connected genes during heavy metal (HM) stress in plants. The most connected genes may be responsible for HM tolerance by altering the different metabolic pathways during the biotic and abiotic stress. In the same line we have performed the GSE86807 microarray analysis of chickpea during exposure to chromium, cadmium and arsenic and analyzed the data. Common differentially expressed genes (DEGs) during exposure to chromium, cadmium and arsenic were identified and a co-expression network study was carried out. Hub and bottleneck genes were explored on the basis of degree and betweenness centrality, respectively. A gene set enrichment analysis study revealed that genes like haloacid dehydrogenase, cinnamoyl CoA reductase, F-box protein, GDSL esterase lipase, cellulose synthase, β-glucosidase 13 and isoflavone hydroxylase are significantly enriched and regulate the different pathways like riboflavin metabolism, phenyl propanoid biosynthesis, amino acid biosynthesis, isoflavonoid biosynthesis and indole alkaloid biosynthesis.

Keywords. Biological network; gene expression; metabolic pathway; microarray

1. Introduction

Chickpea is an economically important crop and a nitrogen fixer. Recently, a genome of chickpea was sequenced which has given access to use available genomics tools for crop improvement and genomics-based breeding. Here it becomes necessary to investigate the molecular insights of genes involved in the stress response. In this study we investigated the effect of heavy metal (HM) exposure-related stress and identified the responsive genes in chickpea.

Industrial efflux of cadmium causes accumulation of cadmium in soil which results in the alteration of physiological conditions of plants (Barceló and Poschenrieder 1990; Das *et al.* 1997). Root growth, length and dry mass are reduced in chickpea during exposure to a higher concentration of cadmium through roots (Salt *et al.* 1995). Mining, industrial and geochemical processes increased the level of arsenic (As) in the atmosphere. Anthropogenic activity leads to major contribution in As contamination in soil in developing countries like India and Bangladesh (Meharg 2004; Krämer 2005; Flora *et al.* 2007). Death of plants and inhibition of growth occurred on exposure to arsenate (Stoeva and Bineva 2003) and its interaction with different metabolic pathways resulted in toxicity (Meharg and Hartley-Whitaker 2002). Hexavalent chromium (Cr(VI)) is generated by industrial and chemical processes that become toxic for plants on enhanced concentration in soil. Not only the plants but also other forms of life are also affected by Cr(VI) at higher concentrations (Chatterjee *et al.* 2009; Dhal *et al.* 2010). Photosynthesis and respiration pathways are generally affected by Cr(VI) (Clijsters and Van Assche 1985).

It is through four different methods that HMs enter into plants namely (a) competitive absorption by essential elements, (b) interaction of HMs with thiol groups for the disruption of the structure, (c) showing more binding affinity toward binding sites rather than essential ions and (d) generation of reactive oxygen species which cause damage of macromolecules responsible for different functions for the survival of plants (Sharma and Dietz 2009; DalCorso *et al.*

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2013). Physiological and biochemical pathways involved in seed germination, pigment synthesis, photosynthesis and inactivation and denaturation of enzymes are altered during long exposure to HMs (Nagajyoti *et al.* 2010; Yadav 2010; Keunen *et al.* 2011; He *et al.* 2012; Silva 2012; Singh *et al.* 2013; Zaidi *et al.* 2012). Shunted root and stem growth and chlorosis in younger leaves are also seen during exposure of HMs to plants (Israr *et al.* 2006; Puckette *et al.* 2007; Warne *et al.* 2008).

More than one type of HM exposure to plants does not always give a cumulative negative effect. Plants may act in a unique way during exposure to different abiotic stresses at a time. Interaction of different abiotic stresses decides the combined positive and negative effects (Mittler 2006; Atkinson *et al.* 2013; Prasch and Sonnewald 2013; Suzuki *et al.* 2014; Pandey *et al.* 2015; Choudhary *et al.* 2016; Ramu *et al.* 2016). Puckette *et al.* (2007) reported that a combination of drought and ozone stress does not lead to the negative effect in *Medicago truncatula* but rather tolerance is increased during the combination of stress. De Abreu *et al.* (2015) performed meta-analysis of 36 different abiotic and biotic stresses in rice and reported alteration in their photosynthesis and they narrowed down common differentially expressed genes (DEGs) to a small significant number that can be investigated in detail. Jia *et al.* (2017) performed RNA sequencing of *P. simonii* under high temperature and drought conditions simultaneously and reported shared DEGs that play an important role in the regulation of transcriptome reprogramming. They also revealed that NCED3, ABF3 and PPC2 were up-regulated and GH3.9, GH3.10 and JAR1 were down-regulated during the combined exposure to stresses. Amrine *et al.* (2015) integrated 272 microarray experiments of *Arabidopsis* exposed to bacterial and fungal pathogens, and reported disease-associated genes and transcriptional regulators using the genomics and systems biology approach. Liang *et al.* (2014) performed gene-expression analysis of Grapevine under different abiotic and biotic stresses and used 374 publically available microarray data. Furthermore they performed gene ontology (GO) followed by quantitative reverse transcription polymerase chain reaction (qRT PCR) for checking the expression of key genes involved in various modules.

Various studies have been carried out on plants with abiotic and biotic stresses. But very few studies are available to find out the common DEGs, pathways involved and altered metabolic production during exposure to different HMs at a time using the systems biology approach. Another similar approach is used to find out the important role of DEGs during different abiotic stresses in chickpea, which is also performed (Yadav and Mani 2019). We have taken microarray data of chickpea treated with Cr, As and Cd at 150 μM concentration and shared DEGs are identified and validated with qRT PCR. On the basis of shared genes, a biological network was constructed to find out the hub and bottleneck genes. GO and gene set enrichment analysis (GSEA) were also performed on hub and bottleneck genes to see the role of molecular functions, cellular localization and biological processes for those genes. The GSEA studies enabled us to see the different roles of hub and bottleneck genes in different places. Finally, the pathways are also identified which are regulated by hub and bottleneck genes.

2. Materials and methods

2.1 *Identification of shared DEGs*

The DEGs under As, Cr and Cd stress of 150 μM were identified from GSE86807 (Yadav *et al.* 2019) data using the limma package of R-bioconductor (Robinson *et al.* 2010) and common DEGs were explored and shown graphically using a Venny online tool (Oliveros 2007). The DEGs having a fold change (FC) outlying + 2 and − 2 with a *p*-value less than 0.02 were up- and down-regulated, respectively. A total of 111 genes were significantly and commonly up-regulated and 40 genes were significantly commonly down-regulated at the 2% level of significance. Table 1 shows the no. of genes which are up- and down-regulated during treatment of chromium, cadmium and arsenic in chickpea.

2.2 *RT-PCR and data analysis*

A measure of 1 μg of total RNA was used for the synthesis of cDNA using the cDNA RT kit. Primers of specific genes were provided as given in supplementary table 1 and were designed by using a NCBI primer tool (Ye *et al.* 2012) by selecting the amplicon size of 150 nt. The default parameter of RT-PCR was used and verified by melting-curve analysis using Rotor gene Q software. The level of each transcript was normalized by using control genes of elongation factor $1 - \alpha$ and the $2^{-\Delta\Delta ct}$ method (Rao *et al.* 2013) was used for the calculation of FC.

2.3 *Biological network construction*

Gene expression similarity is measured by using the gene co-expression network. We use the Pearson coefficient

Table 1. No. of over- and under-expressed genes during treatment of chromium, cadmium and arsenic having *p*-value less than 0.02 and FC greater than ±2

Treatment	No. of genes up-regulated	No. of genes down-regulated
Cr treated	238	236
Cd treated	1123	608
As treated	490	646

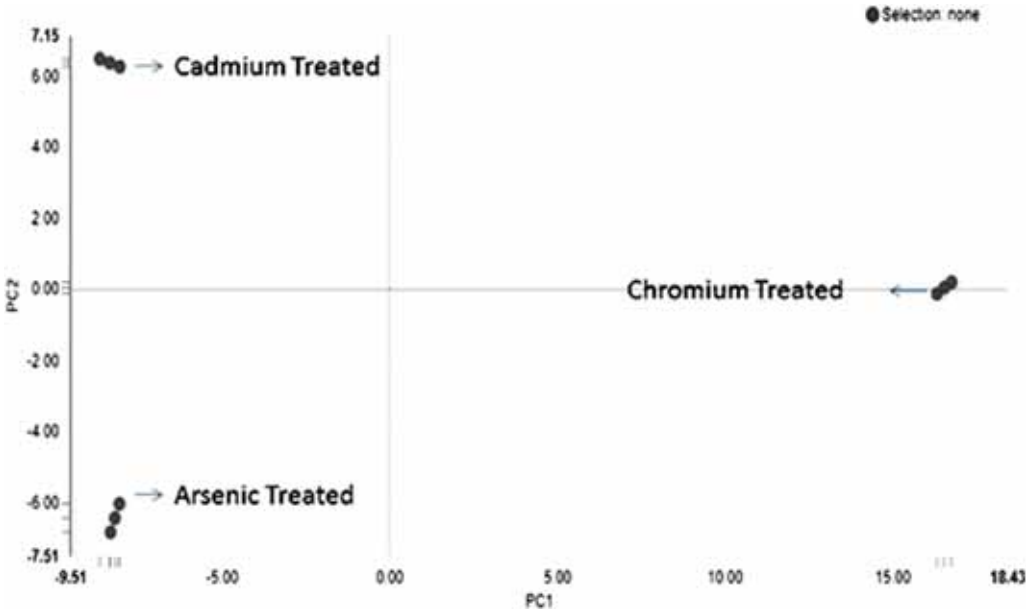

Figure 1. PCA of microarray data of chickpea during different HM treatment.

correlation (PCC) matrix between two genes for seeing the similarity using Cytoscape software (Shannon *et al.* 2003). The network analyst tool of cytoscape was used to check the properties of the biological network. A PCC cut off of 0.95 was set and a co-expression network was constructed for those genes which cross the threshold limit.

Table 2. List of top ten significant common up-regulated genes during exposure to cadmium, chromium and arsenic along with their FC FC_Cd, FC_Cr and FC_As respectively and putative functions

S. No.	Probe Id	FC_Cd	*p*-Value	FC_Cr	*p*-Value	FC_As	*p*-Value	Putative function
1.	TC00178	2.47	0.0008	5.06	0.0000	3.84	0.0006	Nucleic acid binding
2.	TC00951	3.13	0.0004	2.61	0.0006	3.00	0.0006	Cysteine-rich receptor-like protein; putative protein kinase
3.	TC04791	6.32	0.0042	4.97	0.0055	6.22	0.0035	Expressed protein
4.	TC02897	2.68	0.0022	2.74	0.0024	3.00	0.0047	Putative transcription factor
5.	TC03196	2.66	0.0013	4.79	0.0005	4.41	0.0014	Histone superfamily protein
6.	TC03231	2.09	0.0488	5.59	0.0008	2.49	0.0046	Cyclin D6
7.	TC03374	2.57	0.0008	2.81	0.0007	2.85	0.0007	HVA22-like protein F (HVA22F)
8.	TC03504	2.12	0.0183	5.62	0.0027	3.25	0.0081	Homeobox protein 31 (HB31)
9.	TC04595	2.67	0.0007	2.11	0.0007	2.24	0.0028	Putative β-expansion/allergen protein
10.	TC04760	2.32	0.0115	3.30	0.0075	4.33	0.0033	TBL (trichome birefringence-like) gene family protein

Table 3. List of top ten significant common down-regulated genes during exposure to cadmium, chromium and arsenic along with their FC FC_Cd, FC_Cr and FC_As respectively and putative functions

S. No.	Probe Id	FC_Cd	*p*-Value	FC_Cr	*p*-Value	FC_As	*p*-Value	Putative function
1.	TC00658	− 2.51	0.0001	− 2.12	0.0024	− 3.06	0.0012	Sulphate transporter. Contains STAS domain
2.	TC02221	− 2.59	0.0012	− 5.45	0.0002	− 2.28	0.0052	Expressed protein
3.	TC02222	− 2.69	0.0002	− 4.93	0.0002	− 2.33	0.0010	FASCICLIN-like arabinogalactan-protein 12 (FLA12)
4.	TC03180	− 2.20	0.0055	− 4.89	0.0012	− 2.63	0.0037	Xylem-specific cellulose synthase
5.	TC04195	− 2.29	0.0031	− 2.88	0.0030	− 2.10	0.0179	SKU5 similar 11 (sks11)
6.	TC07091	− 4.24	0.0006	− 4.06	0.0002	− 2.32	0.0353	TCP family transcription factor
7.	TC07929	− 2.35	0.0444	− 2.17	0.0454	− 2.58	0.0371	Catalyzes hydrolysis of FMN to riboflavin, and phosphorylation of riboflavin to FMN
8.	TC08356	− 2.73	0.0021	− 2.63	0.0951	− 2.71	0.0034	Expressed protein
9.	TC08769	− 2.97	0.0009	− 2.19	0.1095	− 3.11	0.0001	PIN5, an atypical the PIN family
10.	TC09816	− 2.18	0.0020	− 3.33	0.0003	− 2.30	0.0001	Expressed protein

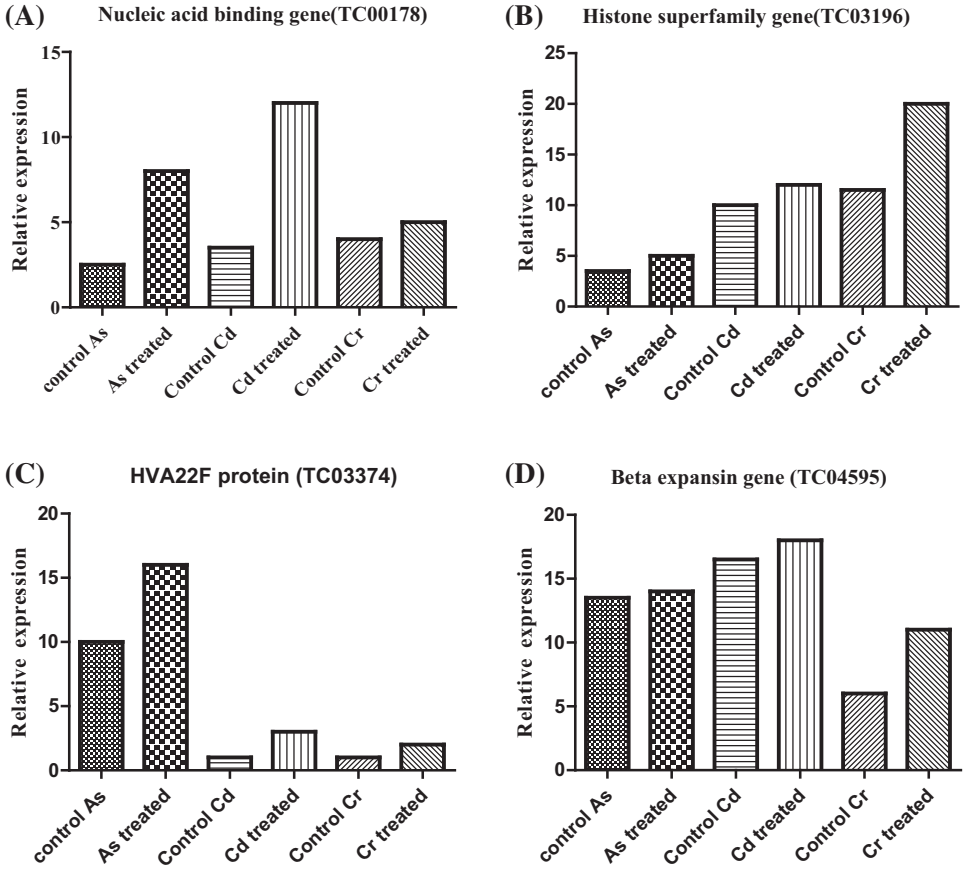

Figure 2. (**A**) Relative expression of nucleic acid binding protein under control, arsenic treated (As), cadmium treated (Cd) and chromium treated (Cr). (**B**) Relative expression of histone superfamily gene under control, arsenic treated (As), cadmium treated (Cd) and chromium treated (Cr). (**C**) Relative expression of HAV22F protein under control, arsenic treated (As), cadmium treated (Cd) and chromium treated (Cr). (**D**) Relative expression of beta expansin under control, arsenic treated (As), cadmium treated (Cd) and chromium treated (Cr).

2.4 *Principle component analysis*

The MeV tool (Howe *et al.* 2010) was used to perform the principle component analysis (PCA) to observe the variations in microarray data. PCA was performed on FC of common DEGs and it was seen in three different clusters and dimensions for cadmium treated, chromium treated and arsenic treated and is shown in figure 1.

2.5 *Gene ontology and gene set enrichment analysis*

Hub and bottleneck genes were identified in common DEGs using cytohubba (Chin *et al.* 2014) plugin of cytoscape. GSEA was performed using BiNGO (Maere *et al.* 2005). The hypergeometric test was performed for checking the statistical significance of significant genes. The corrected *p*-value having less than 0.05 was selected as a significant gene. GO of commonly DEGs was performed using tool GO (*http://www.nipgr.res.in/ctdb.html*).

2.6 *Pathway identification*

The Hub and bottleneck genes having an adjusted *p*-value less than 0.05 were used for searching the role of particular genes in the Kyoto encyclopedia of genes and genome (KEGG) (Ogata *et al.* 1999; Kanehisa *et al.* 2016, 2017) pathway which is an online database used for the identification of pathways associated with hub genes. The chickpea KEGG pathway database was selected for finding out the role of differentially expressed hub and bottleneck genes. Few hub and bottleneck genes were found to be regulating pathways by up and down regulation and these were involved in the biosynthesis of secondary metabolites.

2.7 *Comparison of role of genes in different plants*

Significant hub and bottleneck genes were searched in different plants using orthologous search (*http://www.nipgr.res.in/ctdb.html*). It helped in finding out similar genes in different plants which were individually searched in different

KEGG plant metabolic databases. The role of different genes in different metabolic pathways was compared.

3. Results

3.1 *Identification of DEGs during HM stress*

Among 161 common DEGs during As, Cr and Cd treatment, 111 genes were up-regulated and 40 genes were down-regulated at the 2% level of significance. Top 10 up- and down-regulated genes are shown in tables 2 and 3 with FC values and putative functions during As, Cr and Cd treatment. Most of the up-regulated genes are nucleic acid binding proteins, cysteine-rich receptors, histone superfamily proteins and home box proteins. Down-regulated genes are transporter proteins, cellulose synthase and TCP family transcription factors. Eight genes were selected and RT-PCR was performed and the results are shown in table. RT-PCR results strongly recommend the results obtained from the microarray data as shown in figures 2 and 3.

3.2 *Biological network analysis*

A total of 161 common DEGs were used for the construction of biological networks using cytoscape software with default parameters. Only 141 genes have a PCC value in the range of 0.95 to − 0.95. Therefore, in the biological co-expression network, there were 141 nodes and 14% were shortest paths among the all edges formed between the nodes shown in supplementary figure 1. There were six connected components and the network density of 0.127 was very low, which is the indication of a biological network and 83% have the shortest path length.

3.3 *Gene ontology and gene set enrichment analysis*

Twenty-five top most hub and bottleneck genes on the basis of degree and betweenness centrality respectively were investigated. Tables 4 and 5 show the gene identifier along with their degree and betweenness centrality. A total of 24 hub genes were selected to search homolog genes in different organisms. Eighteen genes were found to be similar to *Arabidopsis* genes while 16 genes were also similar to *Arabidopsis* from bottleneck genes. At the 10% level of significance only 10 genes were involved in the biological process and molecular function from hub and bottleneck genes. Enrichment analysis was performed on the basis of genes having important roles in molecular functions and biological processes in *Arabidopsis*. Enriched genes were found to be involved in several metabolic pathways as shown in supplementary figure 2. The results of GO including the molecular functions, biological processes and cellular localization of DEGs are shown in supplementary figure 3a and b, in which 50% of genes are involved in other molecular functions.

Table 4. List of bottleneck genes along with gene identifier, betweenness centrality and putative function obtained from the cytoscape software using cytohubba plugin

S. No.	Identifier	Betweenness centrality	Putative function
1.	TC00658	36	Sulphate transporter. Contains STAS domain
2.	TC04939	29	Polyketide cyclase/dehydrase and lipid transport superfamily protein
3.	TC09594	29	Lipid acyl hydrolase specificity
4.	TC12268	28	Pectate lyase family protein
5.	TC13800	26	Expressed protein
6.	TC14162	25	Expressed protein
7.	TC16080	25	Lipoxygenase
8.	TC16253	24	Eukaryotic aspartyl protease family protein
9.	TC16462	17	GDSL-like lipase/ acylhydrolase superfamily protein
10.	TC17290	16	Histone superfamily protein
11.	TC18742	16	HTA3
12.	TC21928	15	Expressed protein
13.	TC24564	14	β-Glucosidase 13 (BGLU13)
14.	TC25128	14	CYP81F
15.	TC25892	12	Bacteriocin class II with double-glycine leader peptide
16.	TC28224	11	The cellulose synthase family
17.	TC29018	7	TLP family
18.	TC29173	7	Expressed protein
19.	TC30213	7	Expressed protein
20.	TC31020	7	Eukaryotic aspartyl protease family protein
21.	TC31413	6	Expressed protein
22.	TC32418	5	Expressed protein
23.	TC32480	5	Expressed protein
24.	TC33807	5	Expressed protein
25.	TC34237	5	Expressed protein

3.4 *Pathway identification and analysis*

Hub and bottleneck genes were found to play important roles in different metabolic pathways and secondary metabolite production, as shown in table 6. NAD(P)-binding Rossmann fold superfamily protein is up-regulated and plays an important role in phenylpropanoid biosynthesis. F-box family protein is also up-regulated and regulates the histidine metabolism, biosynthesis of secondary metabolites and biosynthesis of amino acid. GDSL like lipase super family protein is also over expressed and plays an important role in indole alkaloid biosynthesis and biosynthesis of secondary metabolites. β-Glucosidase 13 is up-regulated and regulates the starch and glucose metabolism pathway, phenylpropanoid biosynthesis and biosynthesis of secondary metabolites. In the isoflavonoid biosynthesis pathway and

Table 5. List of hub genes identified from Cytoscape software using microarray data chickpea under different HMs along with total degree associated with hubs and their respective name/function

S. No.	Identifier	Degree	Putative function
1.	TC03180	45	Xylem-specific cellulose synthase
2.	TC03231	41	Cyclin D6
3.	TC04262	47	β-Glucosidase 15 (BGLU15)
4.	TC04834	42	TPX2 (targeting protein for Xklp2) protein family
5.	TC05322	45	HAD-like hydrolase superfamily protein
6.	TC09594	49	Lipid acyl hydrolase specificity
7.	TC09816	45	Expressed protein
8.	TC10565	44	NAD(P)-binding Rossmann-fold superfamily protein
9.	TC12926	43	Disease resistance-responsive (dirigent-like protein) family protein
10.	TC13248	43	Expressed protein
11.	TC15312	46	α/β-Hydrolases superfamily protein
12.	TC16431	42	Cell surface immobilization antigen SerH
13.	TC18295	41	SYP11 syntaxin gene family
14.	TC18296	44	FASCICLIN-like arabinogalactan-protein 12 (FLA12)
15.	TC21907	40	FASCICLIN-like arabinogalactan-protein 12 (FLA12)
16.	TC24036	42	Plant protein of unknown function (DUF936)
17.	TC25128	47	CYP81F
18.	TC25525	46	Expressed protein
19.	TC25976	40	Expressed protein
20.	TC27873	42	F-box family protein
21.	TC29931	47	Expressed protein
22.	TC30157	41	GDSL-like lipase/acylhydrolase superfamily protein
23.	TC32480	41	Expressed protein
24.	TC32736	45	The COBRA family

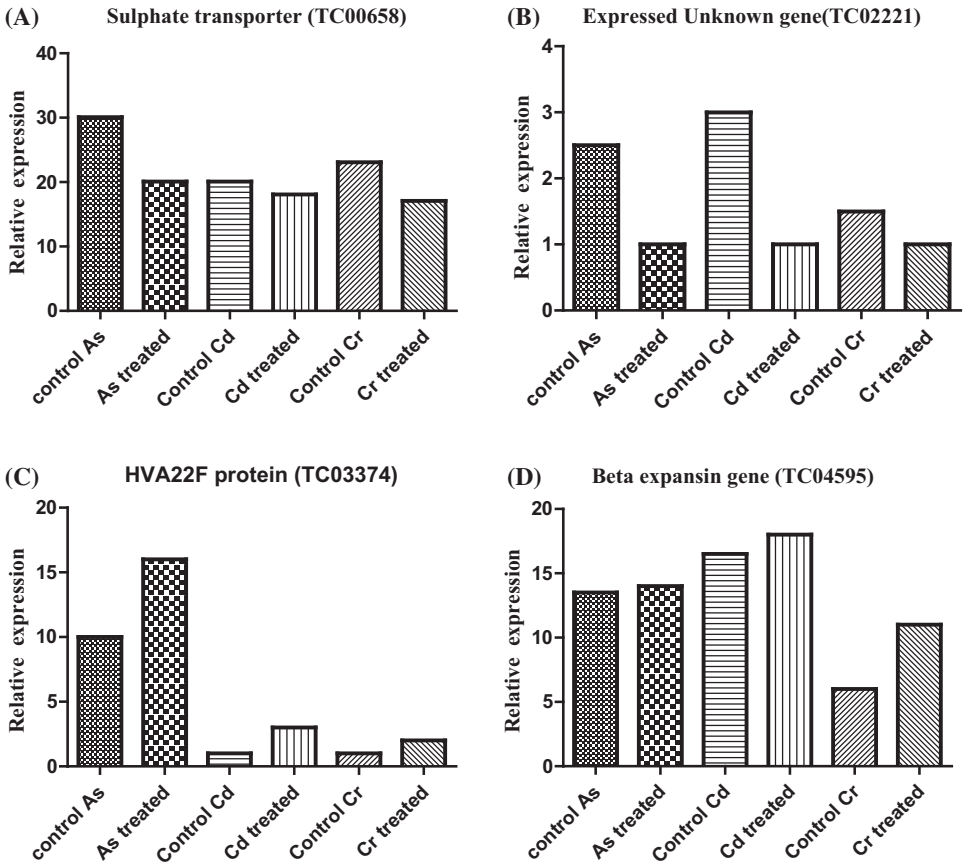

Figure 3. (**A**) Relative expression of sulphate transporter under control, arsenic treated (As), and cadmium treated (Cd) and chromium treated (Cr). (**B**) Relative expression uncharacterized gene under control, arsenic treated (As), cadmium treated (Cd) and chromium treated (Cr). (**C**) Relative expression of cellulose synthase under control, arsenic treated (As), and cadmium treated (Cd) and chromium treated (Cr). (**D**) Relative expression of sks11 gene under control, arsenic treated (As), and cadmium treated (Cd) and chromium treated (Cr).

Table 6. List of genes along with their gene identifier and putative function and the corresponding pathway of genes

Identifier	Putative function/name	Pathway
TC24564	β-Glucosidase 13 (BGLU13)	Starch and sucrose Metabolism, phenyl propanoid biosynthesis, cyanoamino acid metabolism, biosynthesis of metabolites
TC25128	CYP81F	Isoflavonoid biosynthesis, biosynthesis of secondary metabolites
TC10565	NAD(P)-binding Rossmann-fold superfamily protein	Phenyl propanoid biosynthesis
TC27873	F-box family protein	Histidine metabolism
TC30157	GDSL-like lipase/acylhydrolase superfamily protein	Indole alkaloid biosynthesis
TC03180	Xylem-specific cellulose synthase	Starch and sucrose metabolism
TC05322	HAD-like hydrolase superfamily protein	Riboflavin metabolism
TC32736	The COBRA family	Involved in secondary cell wall biosynthesis

Table 7. Homologous genes in different plants that are involved in the metabolic pathway in chickpea during exposure to chromium, cadmium and arsenic

S. No.	Chickpea	Name of gene/protein	*Glycine max*	*Medicago truncatula*	*Arabidopsis thaliana*
1.	TC10565	Cinnamoyl-CoA reductase 2-like (LOC101489401)	Glyma07g02990.1	Medtr4g009690.1	AT2G33590.1
2.	TC15312	Carboxylesterase 15 (LOC101514764)	Glyma03g36380.1	AC233660_26.1	AT5G06570.2
3.	TC24564	Non-cyanogenic β-glucosidase-like (LOC101491699)	Glyma12g05770.1	Medtr2g098540.1	AT5G44640.1
4.	TC25128	Isoflavone 2′-hydroxylase-like (LOC101502156)	Glyma09g05390.1	Medtr4g035320.1	AT4G37410.1
5.	TC27873	Probable F-box protein (LOC101514556)	Glyma19g38290.1	Medtr2g086920.1	AT3G44326.1
6.	TC30157	GDSL esterase/lipase (LOC101501677)	Glyma06g20900.1	–	AT1G74460.1
7.	TC32736	COBRA-like protein 4 (LOC101512579)	Glyma04g32120.2	Medtr3g117690.1	AT5G15630.1
8.	TC05322	HAD superfamily (LOC101492748)	Glyma10g33470.1	Medtr1g073140.1	AT2G33255.1
9.	TC03180	Cellulose synthase (LOC101500798)	Glyma12g17730.1	Medtr8g063270.1	AT5G17420.1

biosynthesis of secondary metabolites, the CYP81F gene plays an important role which is also over-expressed during the exposure to Cr, Cd and As.

3.5 *Comparative study of genes involved in metabolic pathways in different plants*

Table 7 represents the different homologs of significant hub genes in different plants. A comparison of genes in different plants is shown in table 8 showing homologs of significant genes involved in various pathways in chickpea during HM exposure to other plants. Haloacid dehydrogenase (HAD) superfamily protein is up-regulated in chickpea but its homologous gene does not have a role in *Glycine max*, *Medicago* except *Arabidopsis*. Xylem-specific cellulose synthase is under expressed and regulates the starch and sucrose metabolism. GDSL esterase lipase is over expressed and is involved in indole alkaloid biosynthesis in chickpea and *Arabidopsis* while homologous of GDSL was searched against the KEGG pathway of *Medicago* and *G. max* but no results were obtained. F-box protein is up-regulated and regulates the histidine metabolism chickpea while the homologs of it in *Arabidopsis* are involved in the regulation of steroid metabolic pathways. The COBRA family gene is under-expressed and involved in the secondary cell wall biogenesis in chickpea, *Medicago*, *G. max* and *Arabidopsis*.

4. Discussion

The growth, development and adaptation in plants are individually very complicated processes though they are interconnected. A gene co-expression network can be drawn for understanding the complicated process and the function of genes can be predicted on the basis of the co-expression network (Mao *et al.* 2009).

In this study we integrated three-different expression data of chickpea treated with Cr, Cd and As. Common DEGs were determined and there were 161 genes; among them 141 were over-expressed and 40 were under-expressed at the 2% level of significance. Top eight DEGs were selected for qRT PCR validation and the results suggested no difference in microarray data and in qRT PCR results. DEGs at 1% level of significance played an important role in different metabolic pathways as well as in secondary metabolite production. Their expression data were used to construct the PCC matrix, which is further used for the construction of the co-expression network. The threshold of PCC was 0.95 and − 0.95 and data below this were discarded for the construction of the network. A total of 141 genes have the above-mentioned value of PCC cut-off and act as node in the networks. A total of 24 hub and 25 bottleneck genes were identified and enriched genes at 10% level of significance were selected.

The genes involved in phenylpropanoid biosynthesis, indole alkaloid biosynthesis and isoflavonoid biosynthesis were up-regulated in the responses of Cr, Cd and As

Table 8. Role of significant hub and bottleneck genes and their homologs in different plant metabolic pathways

Chickpea gene identifier	Putative function	*Glycine max*	*Medicago truncatula*	*Arabidopsis thaliana*	Chickpea
TC03180	Xylem-specific cellulose synthase	Starch and sucrose metabolism	Starch and sucrose metabolism	Starch and sucrose metabolism	Starch and sucrose metabolism
TC30157	GDSL esterase/lipase	NA	NA	Indole alkaloid biosynthesis	Indole alkaloid biosynthesis
TC05322	HAD-like hydrolase superfamily protein	Uncharacterized	Uncharacterized	Riboflavin metabolism	Uncharacterized
TC27873	Probable F-box protein	NA	NA	Involved in regulation of steroid metabolic process	Histidine metabolism
TC25128	Isoflavone 2′-hydroxylase-like	Isoflavonoid biosynthesis	Isoflavonoid biosynthesis	Biosynthesis of secondary metabolites	Isoflavonoid biosynthesis
TC15312	α/β-Hydrolases superfamily protein	Uncharacterized	Uncharacterized	Uncharacterized	Uncharacterized
TC24564	Non-cyanogenic β-glucosidase-like	Phenylpropanoid biosynthesis	Phenylpropanoid biosynthesis	Phenylpropanoid biosynthesis	Phenylpropanoid biosynthesis
TC32736	COBRA family protein	Involved in secondary cell wall biosynthesis	Involved in secondary cell wall biosynthesis	Involved in secondary cell wall biosynthesis	Involved in secondary cell wall biosynthesis
TC10565	Cinnamoyl-CoA reductase 2-like	No information	No information	Galactose metabolism	Phenylpropanoid biosynthesis

treatment in chickpea. The genes/proteins involved in the above-mentioned pathways belonging to hub and bottleneck genes were identified by using the systems biology approach. Few genes belonging to hub and bottleneck were down-regulated and involved in amino acid metabolism and secondary metabolite production.

Overall, the study suggested that some common DEGs are responsible for tolerance towards HMs. Few of them were experimentally verified by RT PCR. Systems biology tools were used to find out the hub and bottleneck genes that play important roles in different metabolic pathways. The homologs of hub and bottleneck genes of chickpea were identified and their role in corresponding plants were searched and justified.

Reportedly the expression of disease resistance gene in papaya (Fang *et al.* 2016), F-box protein in wheat (Zhang *et al.* 2018) and TCP protein in *Gossypium barbadense* (Zheng *et al.* 2018) changes during abiotic stress which attributes towards the tolerance against different stress types. In our study it was observed that the above-mentioned genes were highly connected and differentially expressed during stress conditions in chickpea. HAD superfamily proteins play important roles in riboflavin metabolism which act as coenzymes in many physiological processes and hence priming of riboflavin is carried out by up-regulation of HAD genes which increase the plant resistance towards HM exposure in chickpea. The molecular mechanism involved in the priming of riboflavin in defense mechanism is unknown (Dong and Beer 2000). Cellulose synthase is down-regulated and hence the production of cellulose had decreased, which resulted in the degradation of the plant cell wall. It is already reported that the thickness of the cell wall of the plant is increased due to the large amount of cellulose and hemicellulose while during HM exposure, the thickness is reduced. Mutant plants, which are cellulose deficient, are more sensitive to abiotic stress (Wang *et al.* 2016). During abiotic stress, the levels of polyamines are increased that enhanced the tolerance level of the plant towards abiotic stress (Groppa and Benavides 2008; Gupta *et al.* 2013). We report that chickpea's F-box protein and β-glucosidase 13 are up-regulated and play important roles in histidine metabolism, biosynthesis of amino acid and cyanoamino acid metabolism. GDSL esterase lipase plays an important role in indole alkaloid biosynthesis which over-expressed during HM exposure resembles the study which concludes that stress induces the over-expression of genes involved in indole alkaloid biosynthesis in the *Catharanthus roseus* plant (Zhu *et al.* 2015). Abiotic stress induces the phenyl propanoid biosynthesis by increasing the level of mRNAs playing important roles in the above-mentioned pathways (Dixon and Paiva 1995). The present study also reveals that cinnamoyl CoA reductase is involved in phenylpropanoid biosynthesis and its over expression provides the increased tolerance level to the plant during exposure to HMs.

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