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Supplementary Material

Molecular insights into the functional role of nitric oxide (NO) as a signal for plant responses in chickpea

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Table S1. List of few reports used 0.1mM sodium nitroprusside (SNP) as nitric oxide (NO) donor in plants

Plant species	Source of NO	Study	Response	References
Solanum lycopersicum, Zea mays, Cucumis sativus, Poncirus trifoliate, Phaseolus vulgaris, Vigna unguiculata	100 μM SNP	Drought	Enhanced capacity of antioxidants, induced Glycine-betaine accumulation, stomatal closure, reduced ion leakage and cell injury index, enhanced stomatal conductance, increased carbonic anhydrase activity and photosynthesis	Hayat et al. 2011;Zhang et al.,2012; Arasimowicz-jelonek et al. 2009; Fan et al. 2012; Zimmer-Prados et al. 2014
Triticum aestivum	100 μM SNP	Heat stress	Alleviates oxidative damage	Bavita et al. 2012
Musa Spp	100 μM SNP	Cold stress	Maintained the stability of cell membrane, up-regulate the antioxidant enzymes activities, recover process of photosystem II (PSII) and induce the expression of cold-responsive genes.	Wang et al. 2013
Zea mays	100 μM SNP	Salt stress	Increases dry matter of roots and shoots under salinity stress	Zhang et al. 2006
Solanum tuberosum	100 μM SNP	Herbicide	Decrease ion leakage and oxidative stress	Beligni and Lamttina 1999
Zea mays & Arabidopsis thaliana	100 μM SNP	UV-B	Scavenging ROS and upregulating phenylpropanoid biosynthetic pathway	Tossi et al. 2011
Lactuca sativa	100 μM SNP	Arsenic toxicity	Alleviating arsenic-induced oxidative damage	Silveira et al. 2015
Triticum aestivum	100 μM SNP	Lead toxicity	Detoxify reactive oxygen species (ROS)	Kaur et al. 2015
Lolium perenne	100 μM SNP	Copper toxicity	Increased chlorophyll content and photosynthesis, improved antioxidant enzyme activities, kept intracellular ion equilibrium	Dong et al. 2014
Boehmeria nivea	100 μM SNP	Cadmium toxicity	Increased SNO content, enhanced the activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), and glutathione reductase (GR) and reduced the accumulation of ROS	Wang et al. 2015
Zea mays	100 μM SNP	Boron toxicity	Improved the antioxidant capacity against born toxicity	Esim et al. 2013
Lactuca sativa, Arabidopsis thaliana	100 μM SNP	Development	Inhibited hypocotyl growth and stimulated de-etiolation and an increase in chlorophyll	Beligni and Lamattina 2000
Solanum lycopersicon, Cucumis sativus	100 μM SNP	Development	Decreased primary root growth, induced adventitious root development	Negi et al. 2010
Pisum sativum	100 μM SNP	Physiology	Anti-senescence in leaves	Leshem and Haramaty 1996
Zea mays	100 μM SNP	Physiology	Improved iron availability and inhibited chlorosis.	Graziao et al. 2002
Hordeum vulgare	100 μM SNP	Physiology	Delays programmed cell death in aleurone layer	Beligni et al. 2002

Medicago truncatula	100 μM SNP	Metabolism	Polyamines and proline metabolism, modification of nitrate metabolism	Filippou et al. 2013

Table S2. Comprehensive list of primers used for quantitative real time PCR. FP: Forward Primer, RP: Reverse Primer.

S.No.	Accession No.	Gene name	Primer sequence(5'-3')	Amplicon length (bp)
1.	AJ131050	*Ribulose 1,5-bisphosphate carboxylase (RuBisCo)*	FP: TCCACCATTGACTGAAGAGC RP: GGTGAGTTGTTGTGCTCACG	123
2.	X85252	*SAM-synthetase(SAMS)*	FP: ACCGAGGTTAGGAAGGATGG RP: CGTGTTGTGTGGAAATGAGG	136
3.	AJ012822	*Chalcone synthase(CHS)*	FP: TGCAGTCACATTTCGTGGTC RP: TGTGCAGTCCAAACCATCTC	150
4.	AJ549384	*1-aminocyclopropane-1-carboxylic acid Oxidase (ACO)*	FP: TCATGGCATTCCAGAGGAAC RP: TCCTCCCAATCCACATTCTC	165
5.	KC464462	*1-pyrroline-5-carboxylate synthetase (P5CS)*	FP: CCTCCAAATGACCCAAACTC RP: AGCATGAGCAGCACAAACAG	147
6.	JN561786	*Bowman-Birk-type protease inhibitor (BBI)*	FP: AGCGATGAGCCTTCTGAGTC RP: TCATCAGTGTCAAGGCAACG	176
7.	XM_004494254	*Peroxidase*	FP: TGGGAAGTGCTTACGGGTAG RP: TCCAAGAGTGTGTGCTCCTG	168
8.	XM_004513380	*Eukaryotic initiation factor 4A (IF4A)*	FP: GTCTCAGCAACTCAT GAGACA RP: CACGTCAATACCACGAGCTA GA	120
9.	AJ010224	*Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)*	FP: AGTTGTACCACCAACTGCCTTG RP: GCCCTCAACAGTCTTCTGAGT	120

Supplementary Figure 1

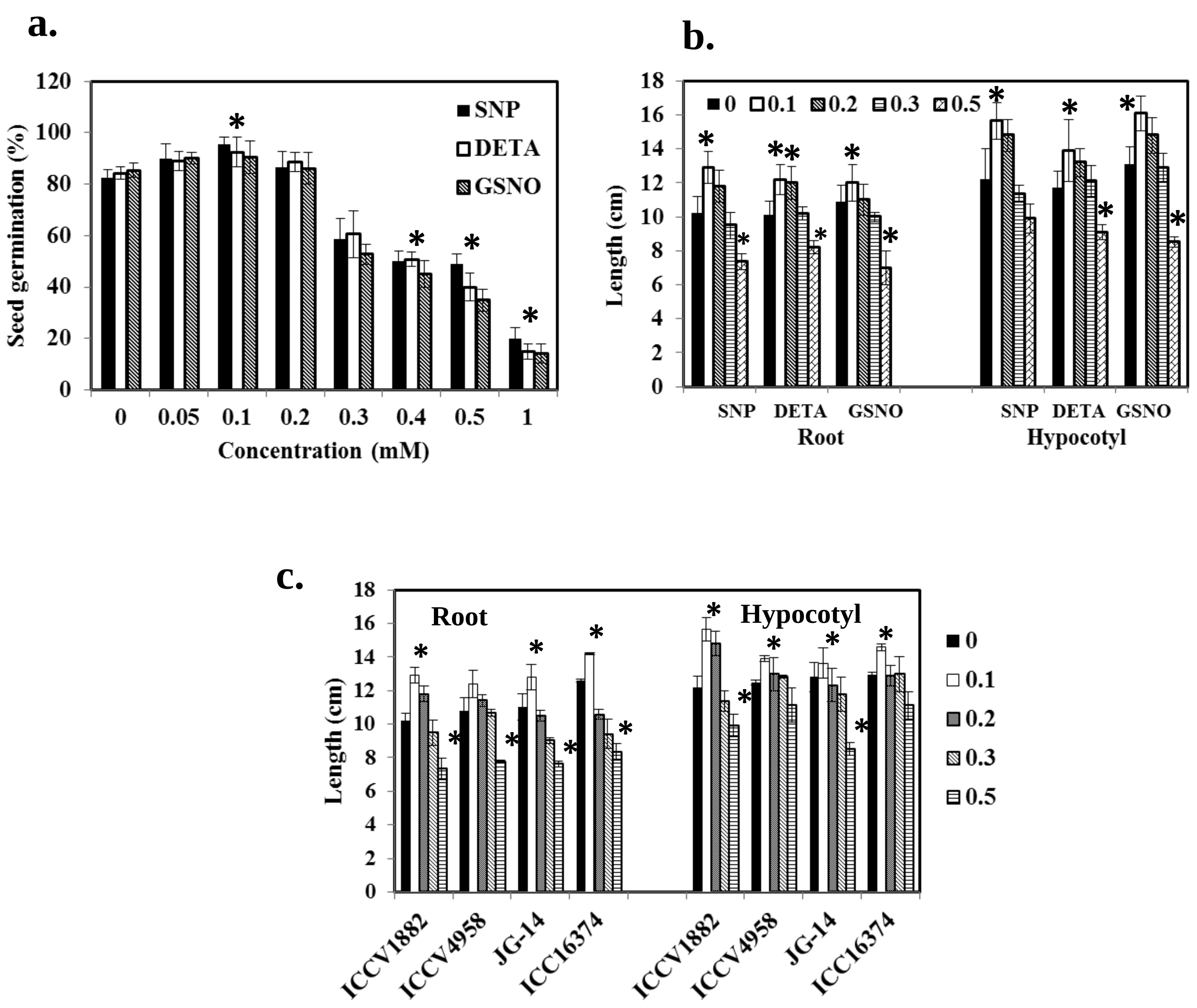

Fig. S1. Effects of NO donors on seed germination and growth of chickpea seedlings (a) Comparative effect of increasing concentrations (0.05 to 1 mM) of various NO donors, SNP, DETA and GSNO on seed germination. Seeds were germinated on 0.8% water agar medium under controlled conditions (26/16ºC temperature and 60% RH, 12 h light) and % of germination was scored after 48 hours. (b) Dose response of chickpea seedlings in presence of various NO donors. Germinated seeds of ICCV1882 genotype were sown on soil wetted with increasing concentrations (0.1- 0.5 mM) of SNP, DETA and GSNO in growth chamber. Root and hypocotyl lengths were measured after ten days. (c) SNP dose response using four chickpea genotypes. Seedlings of four chickpea genotypes (ICCV1882, ICCV4958, JG-14, ICC16374) grown on soil in presence of varying doses of SNP(0.1-0.5 mM) and root and hypocotyl lengths were measured after ten days of growth. *-indicates the statistically significant differences compared to the respective control ($n=30\pm SD$; $p\leq 0.05$)

Supplementary Figure. 2

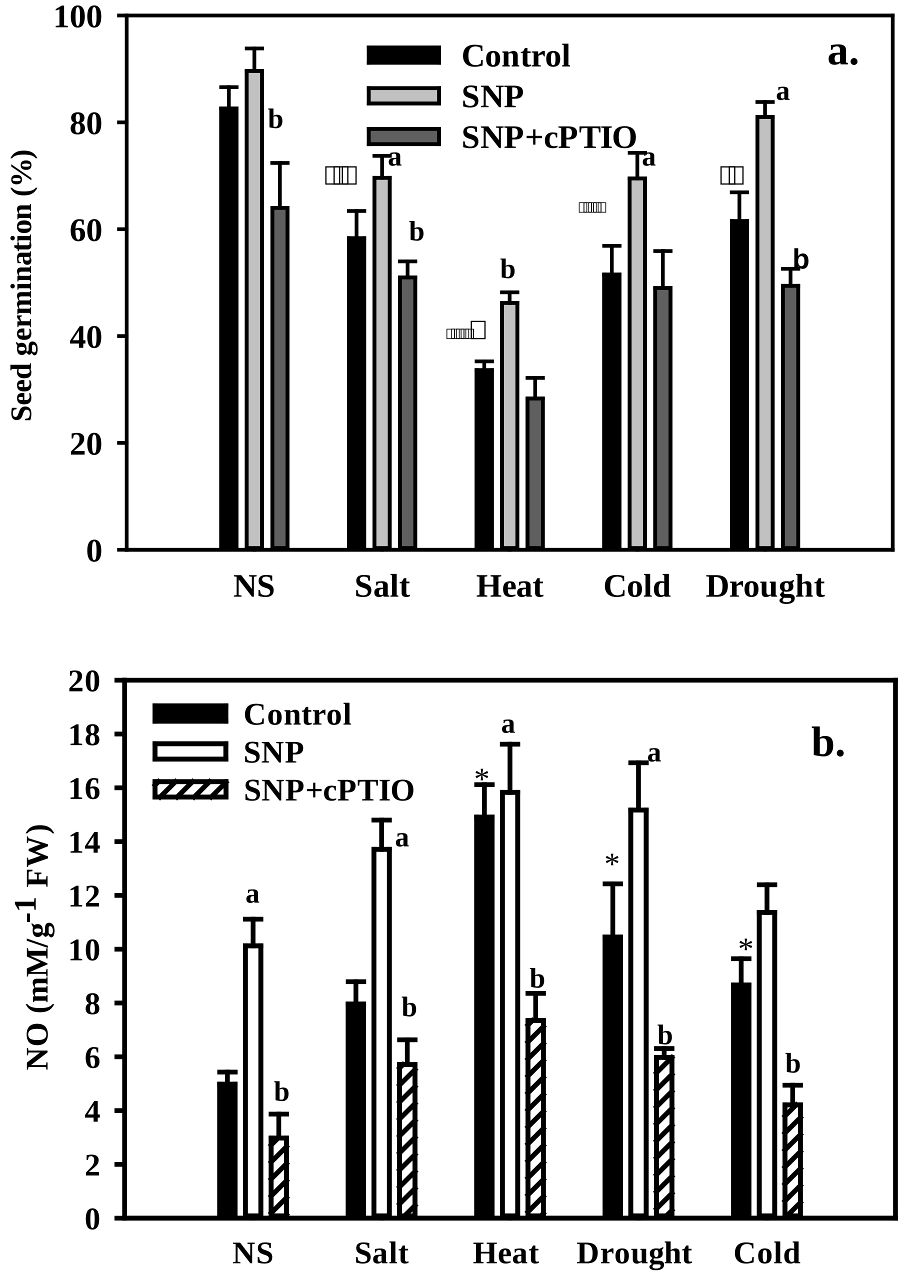

Fig. S2. Exogenous nitric oxide donor enhances abiotic stress tolerance and NO levels in chickpea. (a) 0.1 mM SNP reduced stress-induced inhibition of seed germination under various abiotic stress conditions mention in the figure (n=35 ±SD). (b) Endogenous NO levels in chickpea seedlings in response to the applied stress conditions before treating with 0.1 mM

SNP ($n=30 \pm SD$; $p \leq 0.05$). 1 mM cPTIO was used along with 0.1mM SNP to reverse the NO mediated effects in both the experiments. * indicates statistically significant differences in response to stress treatment compared to control, a- denotes statistically significant differences between control and SNP treatment and b-denotes statistically significant differences between SNP and SNP+cPTIO treatments.

Supplementary Figure. 3.

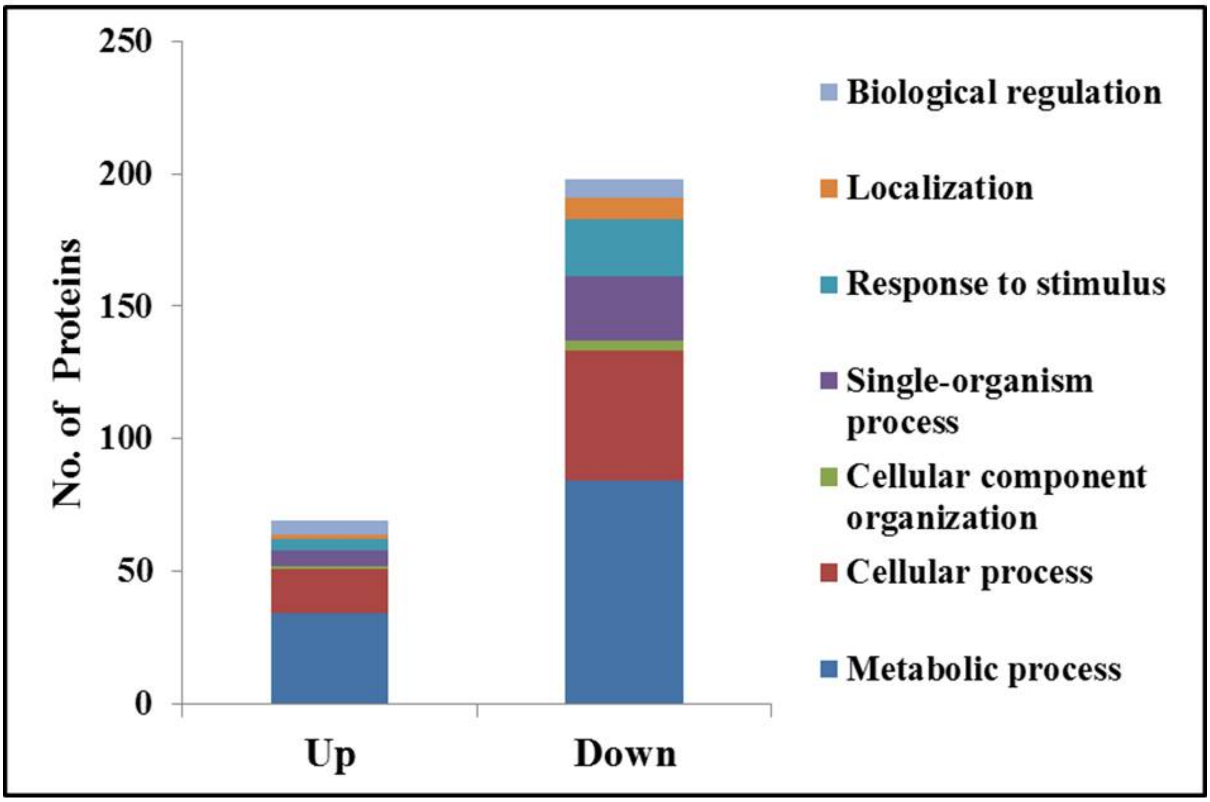

Fig. S3. Comprehensive view of the significantly ($p \leq 0.05$) up- and down- regulated proteins by SNP treatment based on biological process. The list of these proteins is given in supplementary data sheet 1.

Supplementary Figure 4.

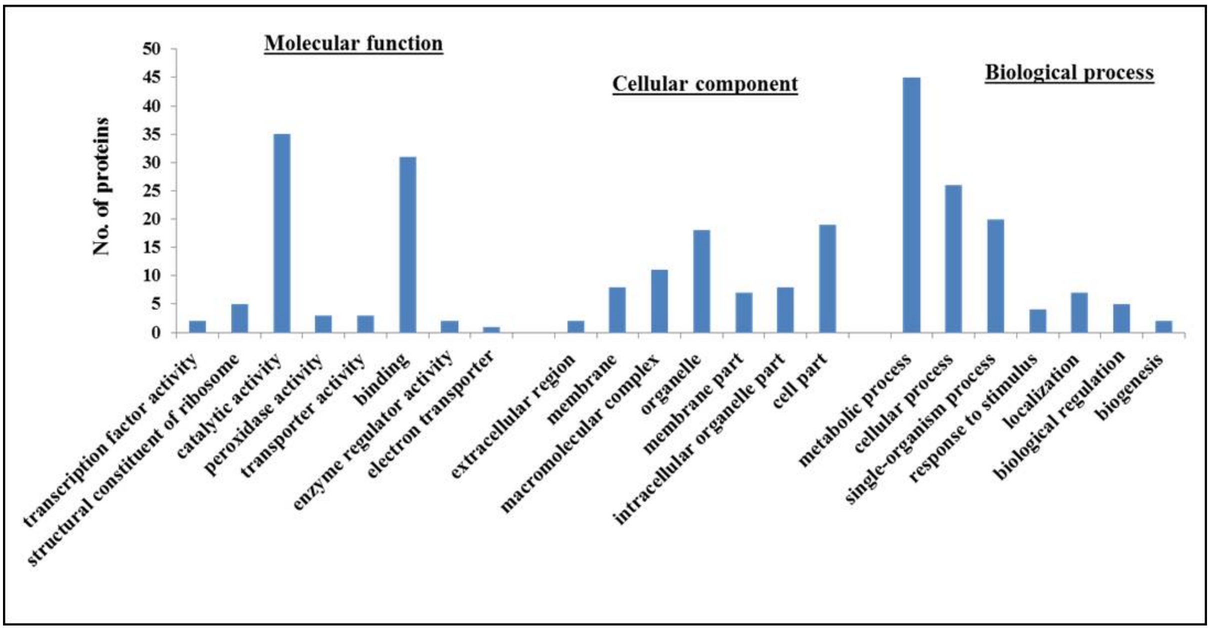

Fig. S4. Functional categorization of SNP- responsive proteins altered at least by 2 folds (Table 1&2), based on molecular function, cellular location and biological process using Uniprot ontology function.

Supplementary Figure. 5.

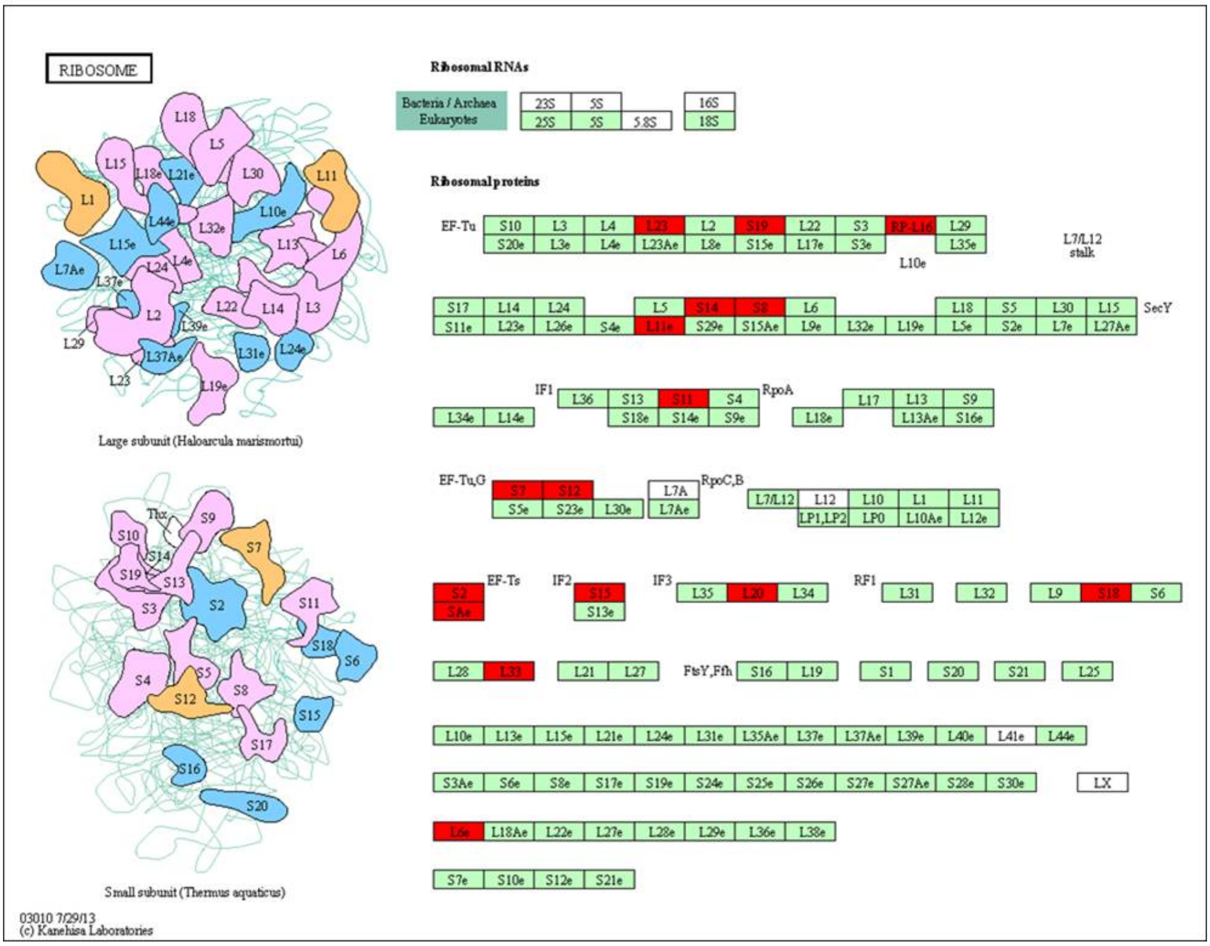

Fig. S5. KEGG pathway analysis of differentially regulated proteins (16) from SNP -treated chickpea leaves relevant to ribosome metabolism. Proteins indicated in red were found to be significantly upregulated or downregulated by SNP in our analysis.

Supplementary Figure. 6.

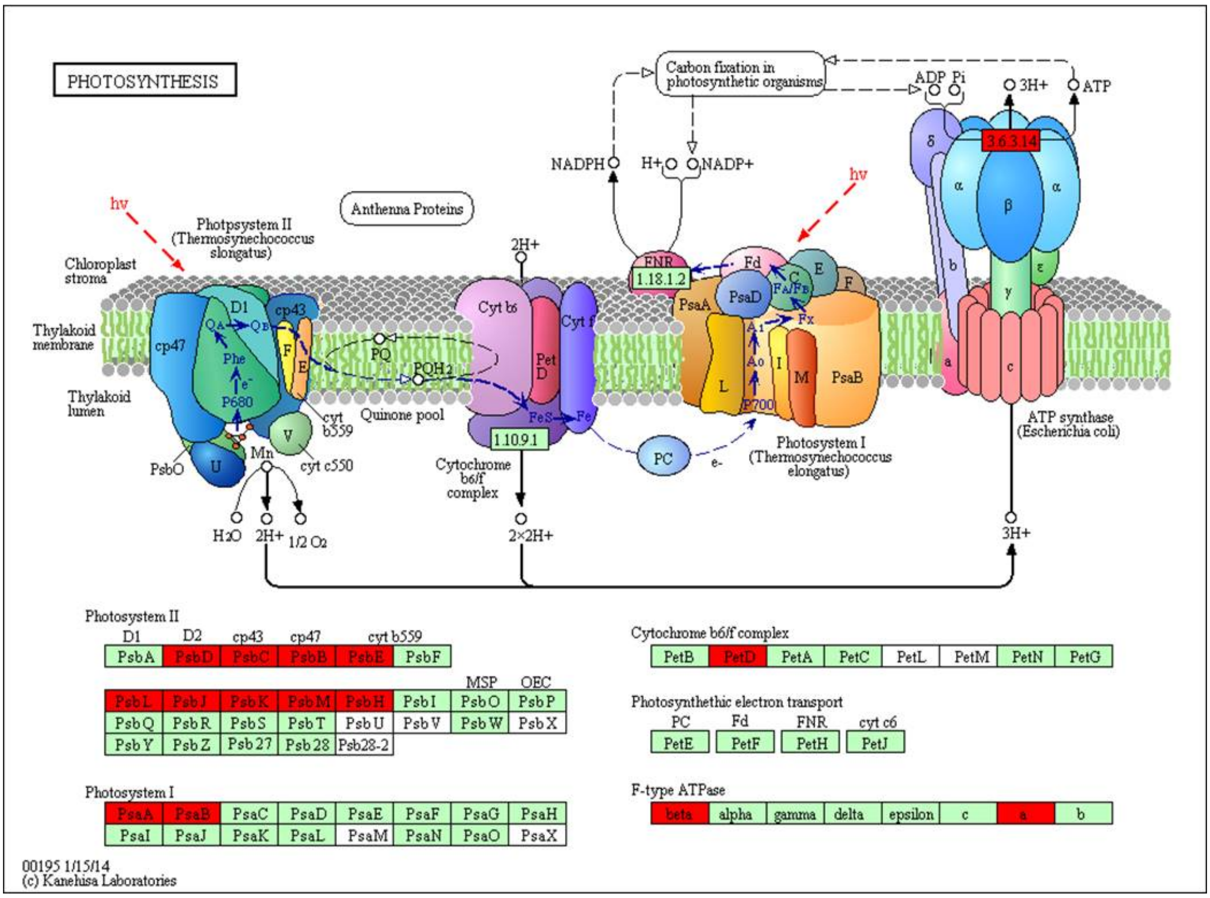

Fig. S6. Pathway analysis of differentially regulated proteins (14) in SNP-treated chickpea leaves. The photosynthesis pathway derived from KEGG. Proteins indicated in red were found to be significantly upregulated or downregulated by SNP in our analysis.

Data sheet S1. Complete list of proteins up-and down- regulated by SNP treatment in chickpea leaves. The highlighted proteins represent the significant variation ($P \leq 0.05$) in protein expression ratio. See separate Excel file.

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