Transcriptome and Co-expression Network Analyses Identify Key Genes Regulating Nitrogen Use Efficiency in *Brassica juncea* L.

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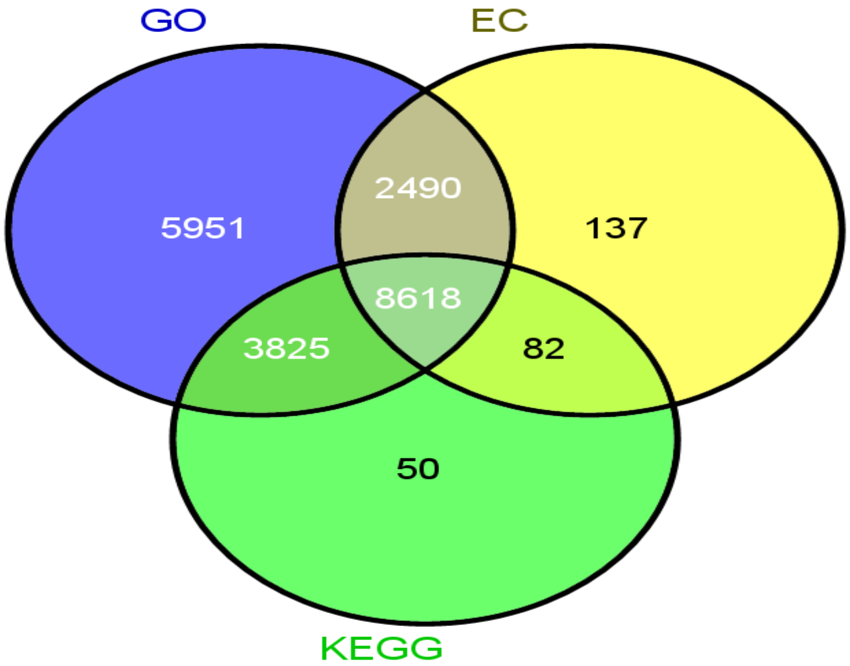
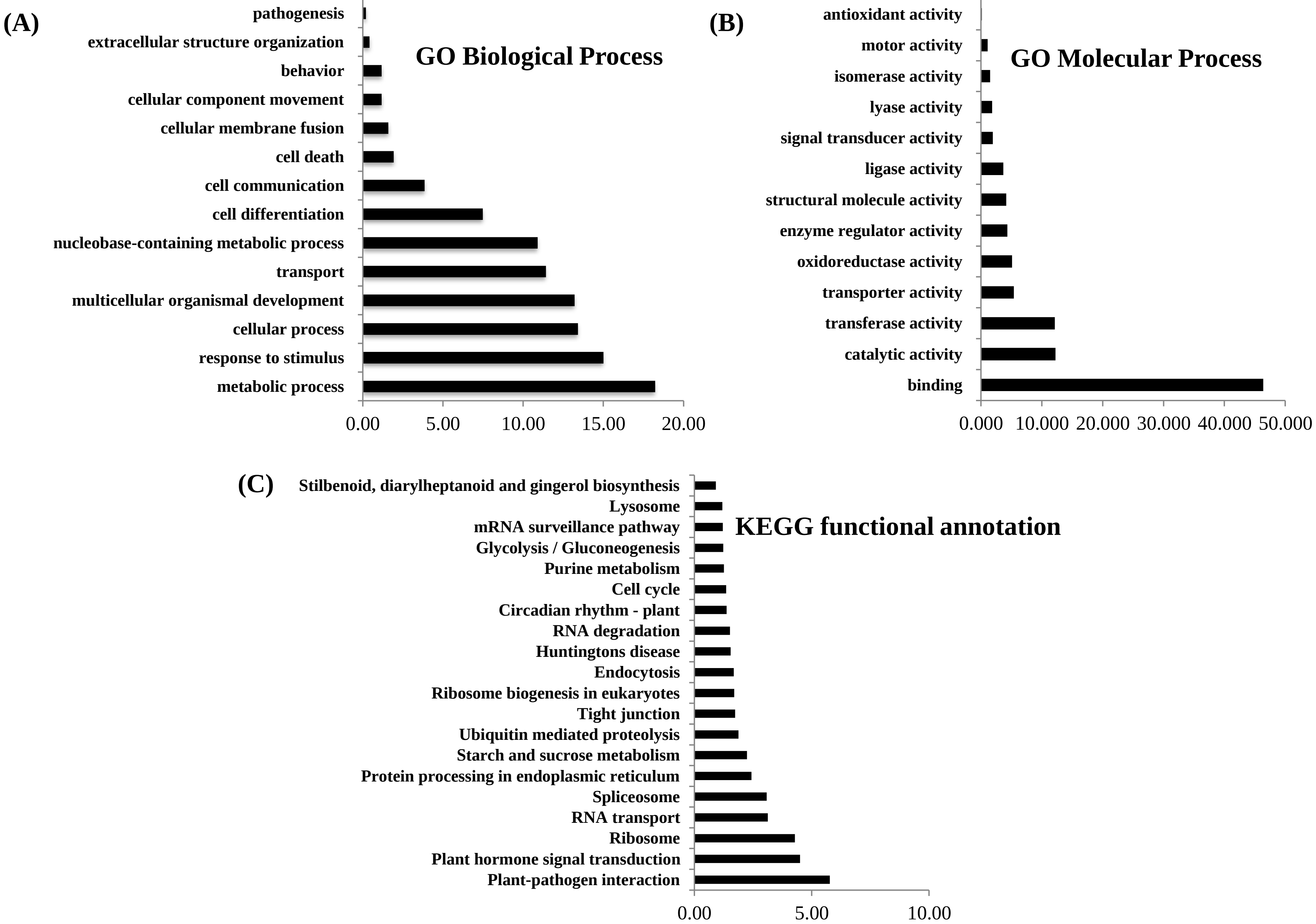
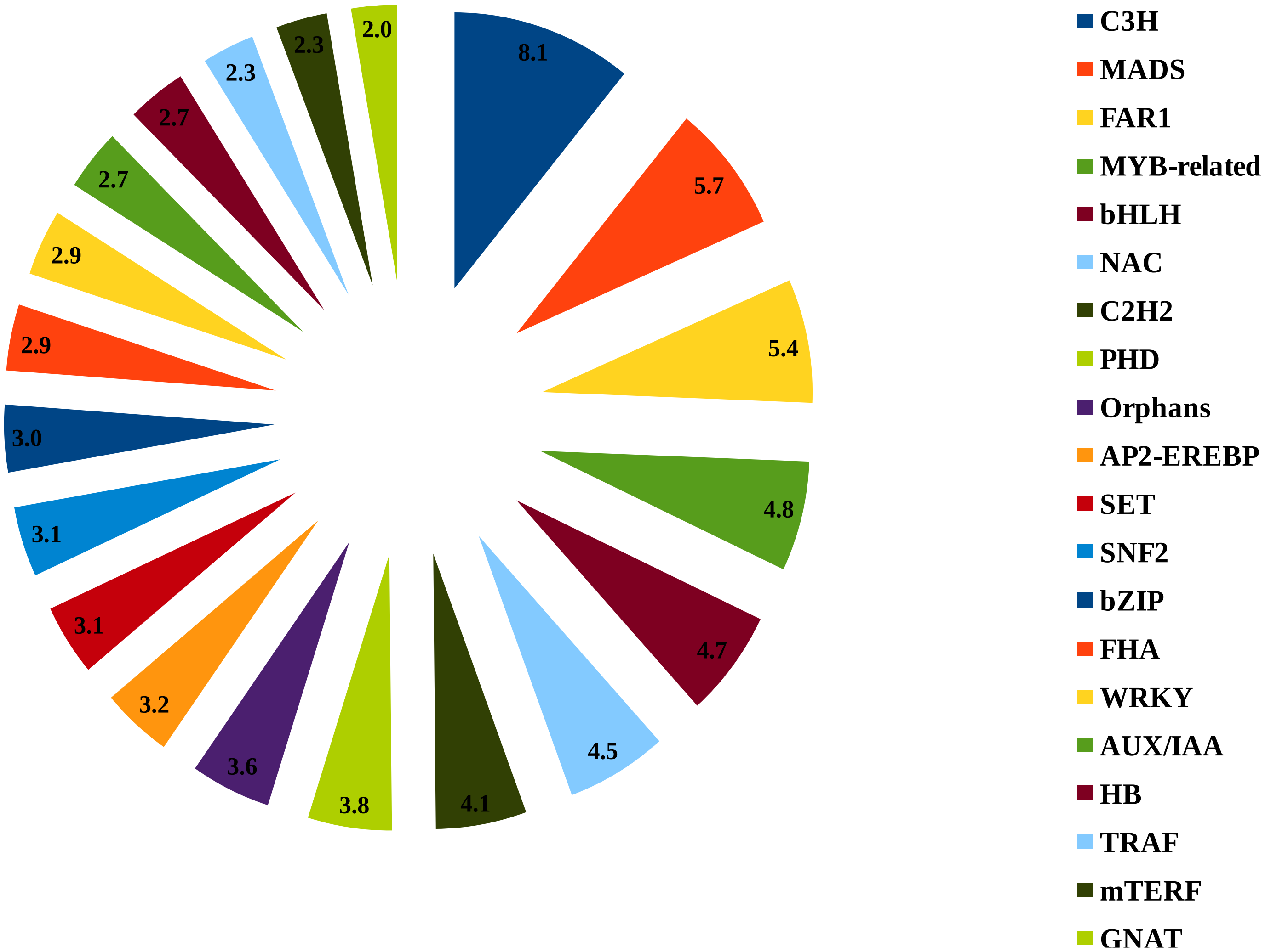


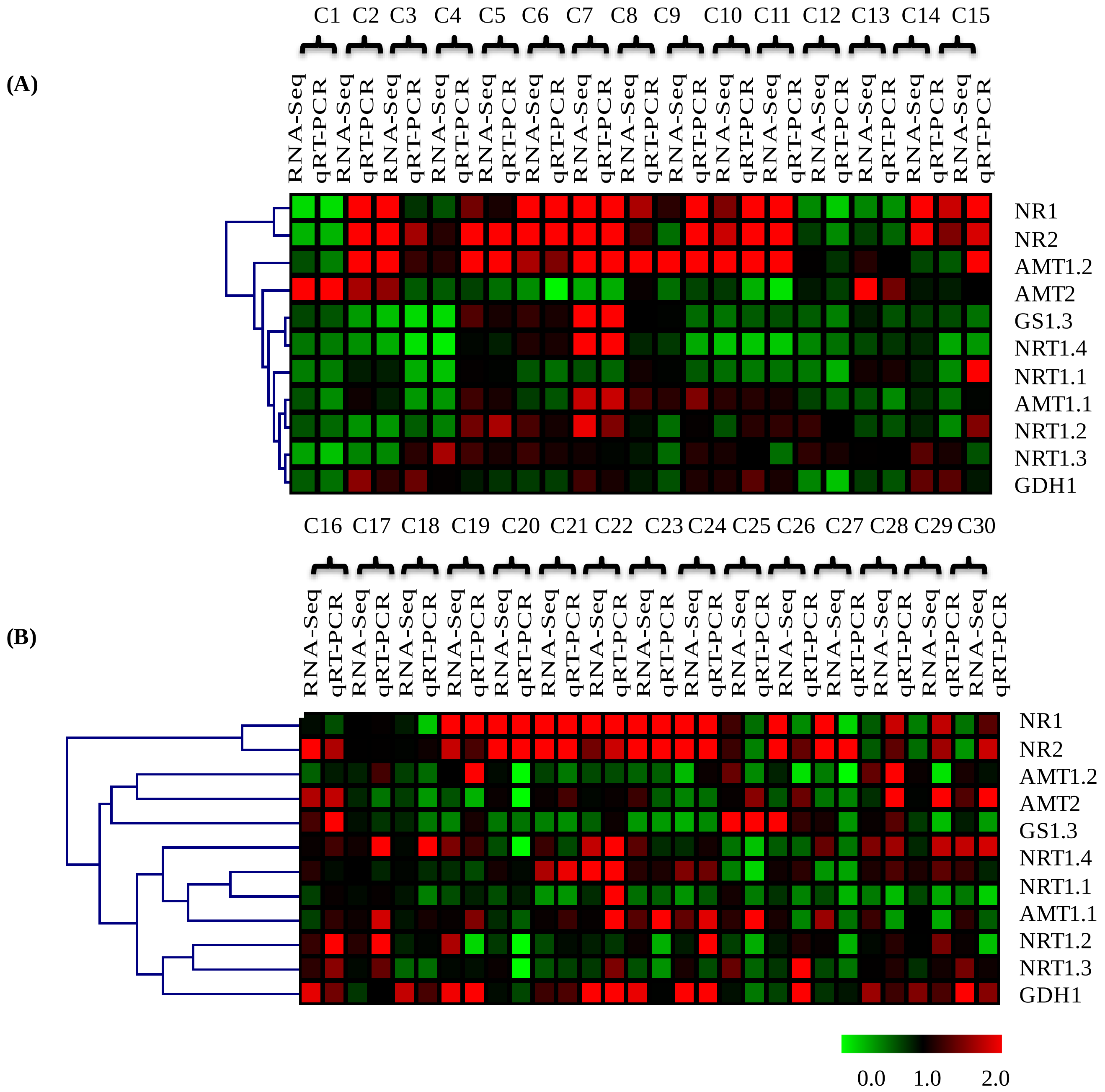
Supplementary Fig S1: Venn diagram showing transcripts annotated under GO, EC and KEGG



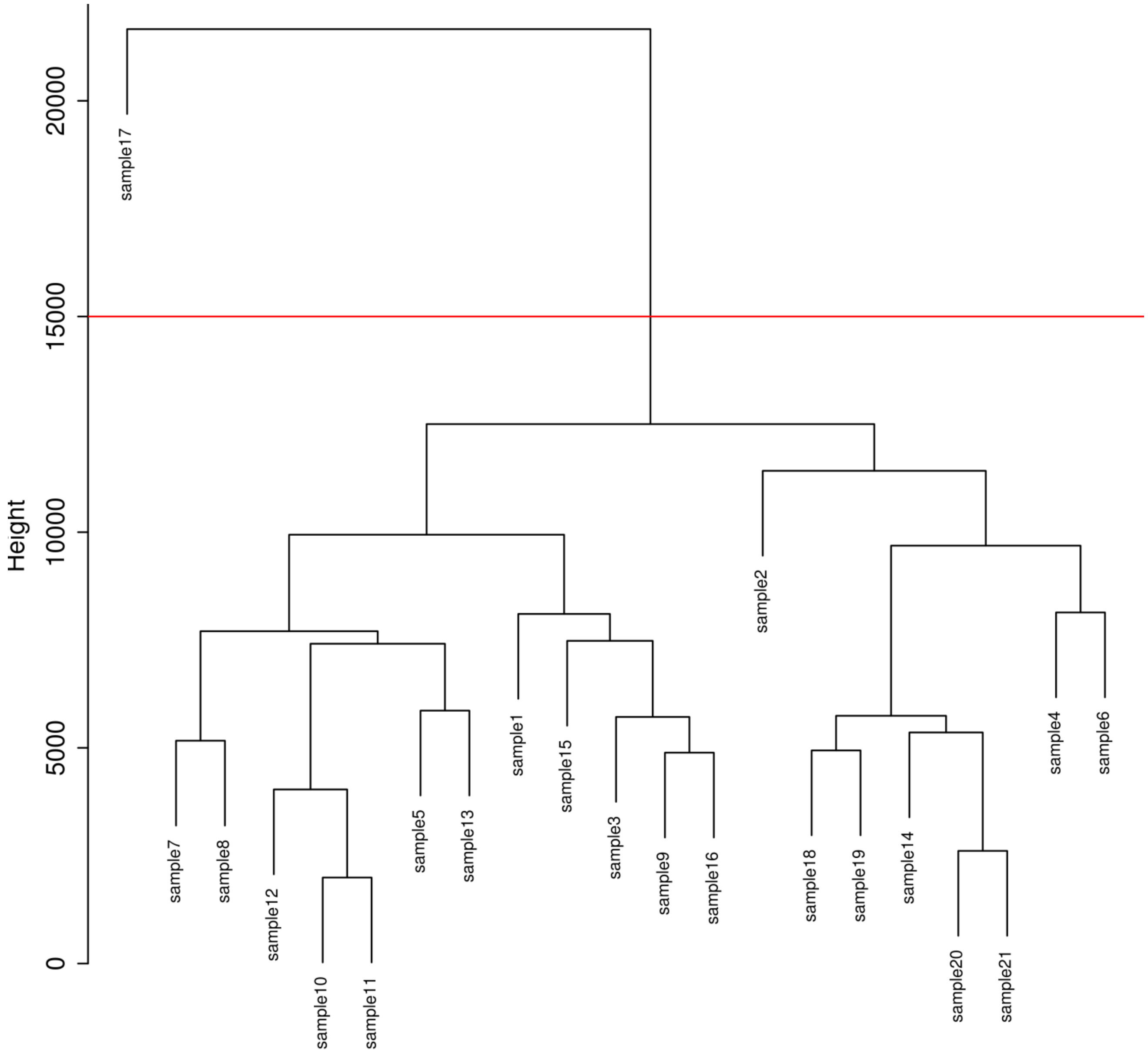
Supplementary Fig S2: Functional Annotation detail of transcriptome data: (A): Occurrence of different biological processes and molecular processes (B) based on GO slim categories with their corresponding percentage, (C): Top-20 KEGG pathways on the basis of their transcript abundance.



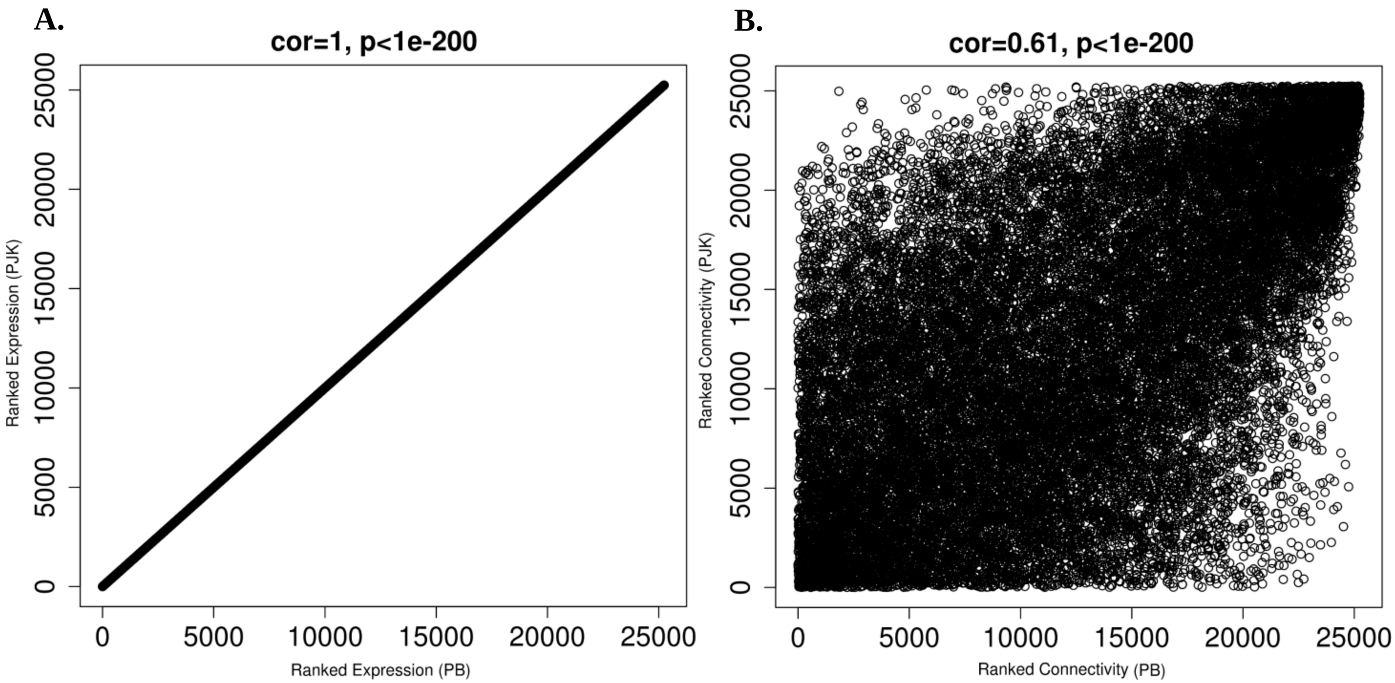
Supplementary Fig S3: Top-20 transcription factor (TF) families on the basis of transcript abundance.



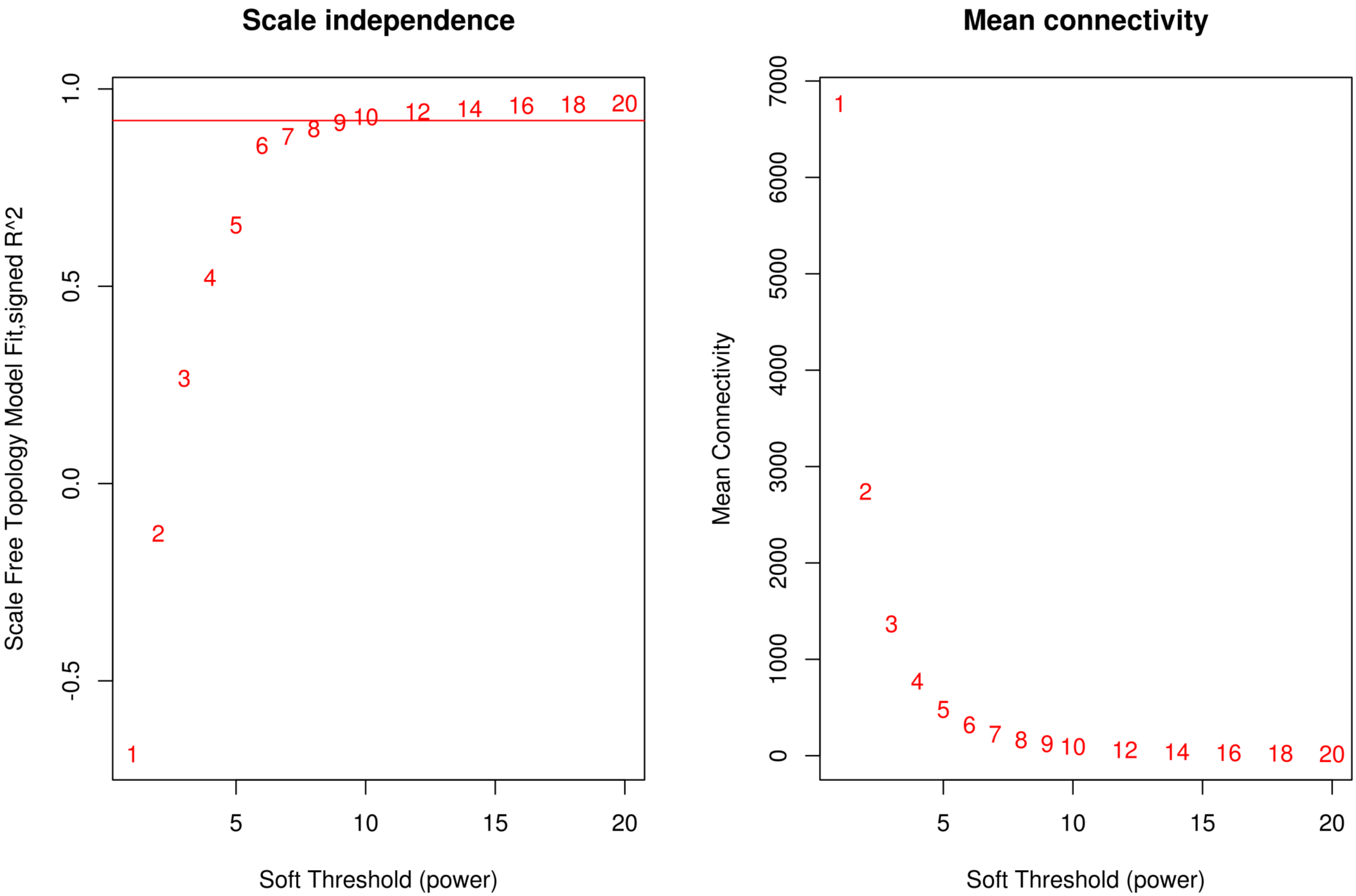
Supplementary Fig S4: Validation of the RNA-seq data from qRT-PCR: The expression level of eleven genes is measured through qRT-PCR in all 30 comparative conditions of (A) Pusa Bold and (B) Pusa Jaikisan. *Ubq9* was used as endogenous reference. The qRT-PCR data was significantly correlated with RNA-seq data ($r=0.76$, $p<0.0001$)



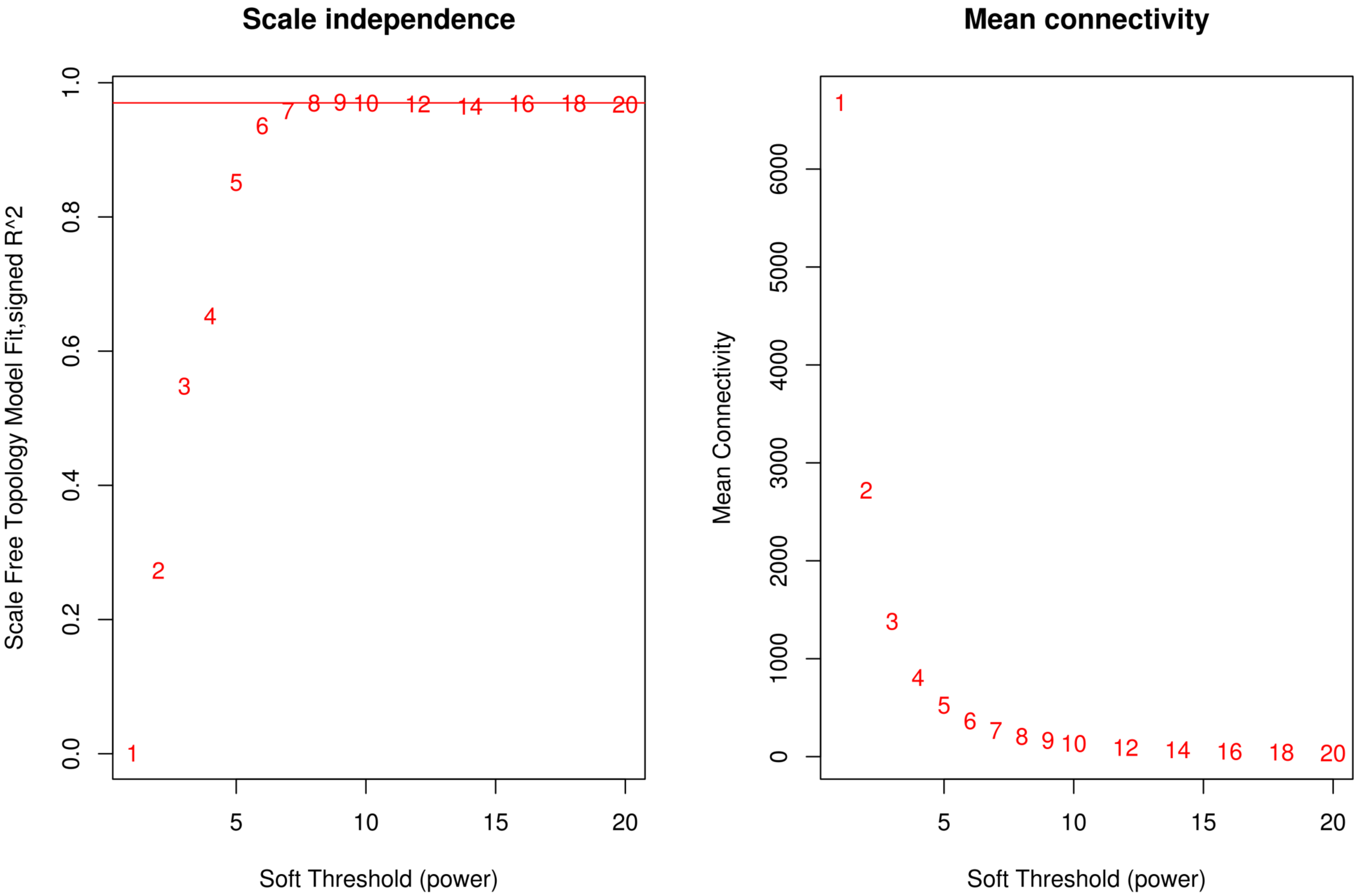
Supplementary Fig S5: Filtering and pre-processing of data: Sample 17 was eliminated as outlier from Pusa Bold.



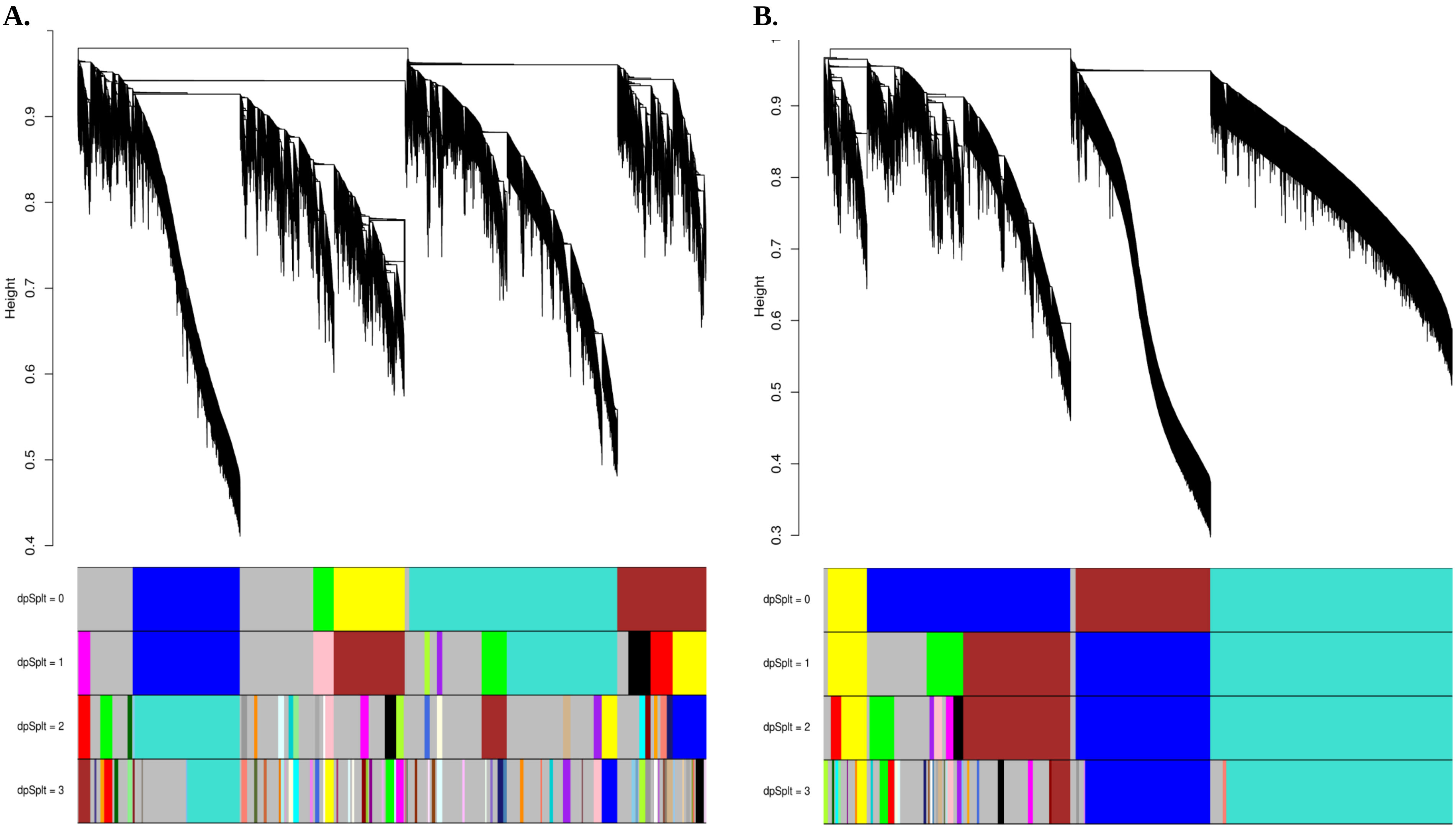
Supplementary Fig S6: Positive correlation and significant p-values of average gene expression (cor=1, p ≤ 1e-200) (a) and overall connectivity (cor=0.61, p ≤ 1e-200) (b).



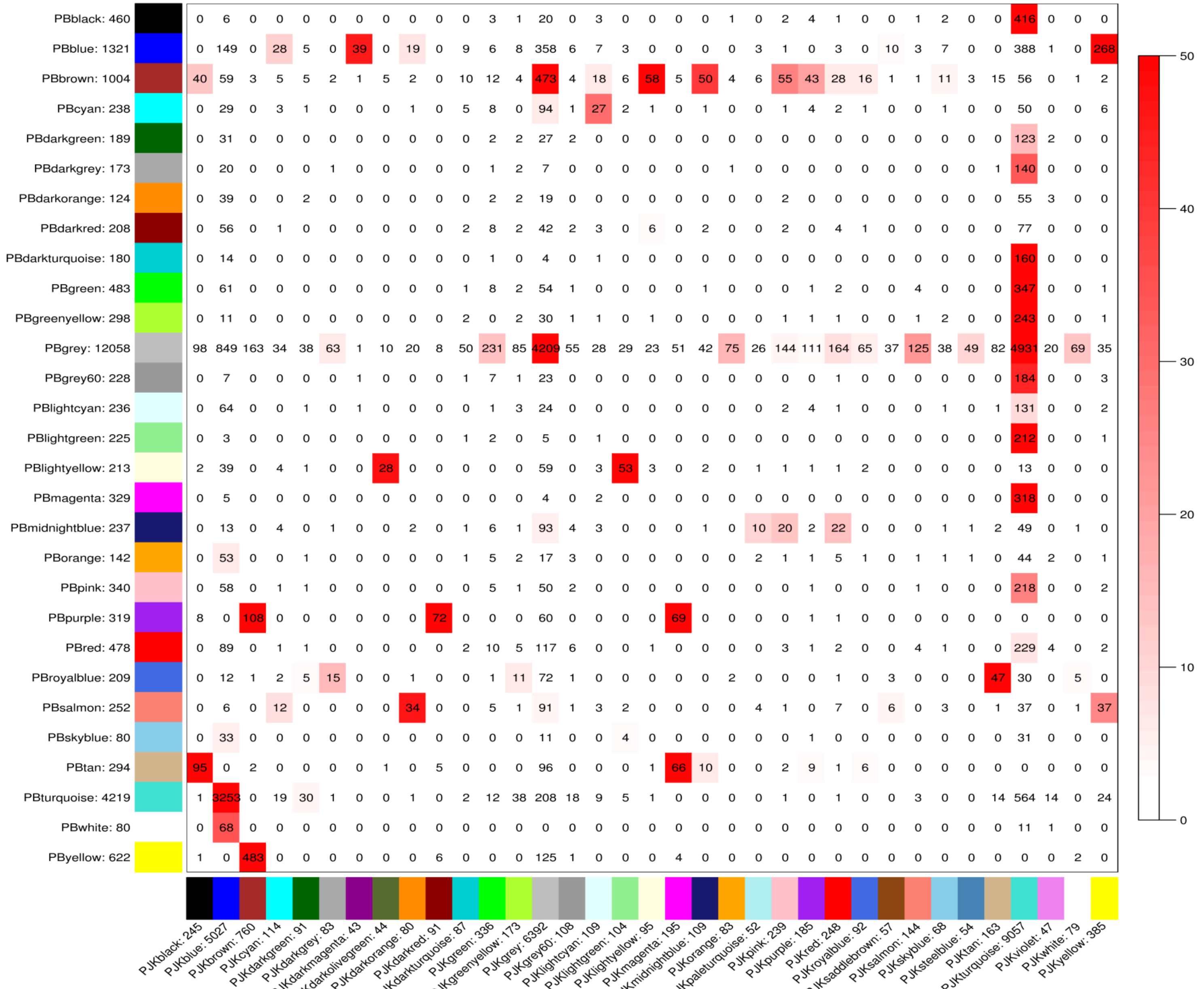
Supplementary Fig S7: Evaluation of network topology of Pusa Bold for various soft thresholding powers using pickSoftThreshold function and appropriate power.



Supplementary Fig S8: Evaluation of network topology of Pusa Jaikisan for various soft thresholding powers using pickSoftThreshold function and appropriate power.

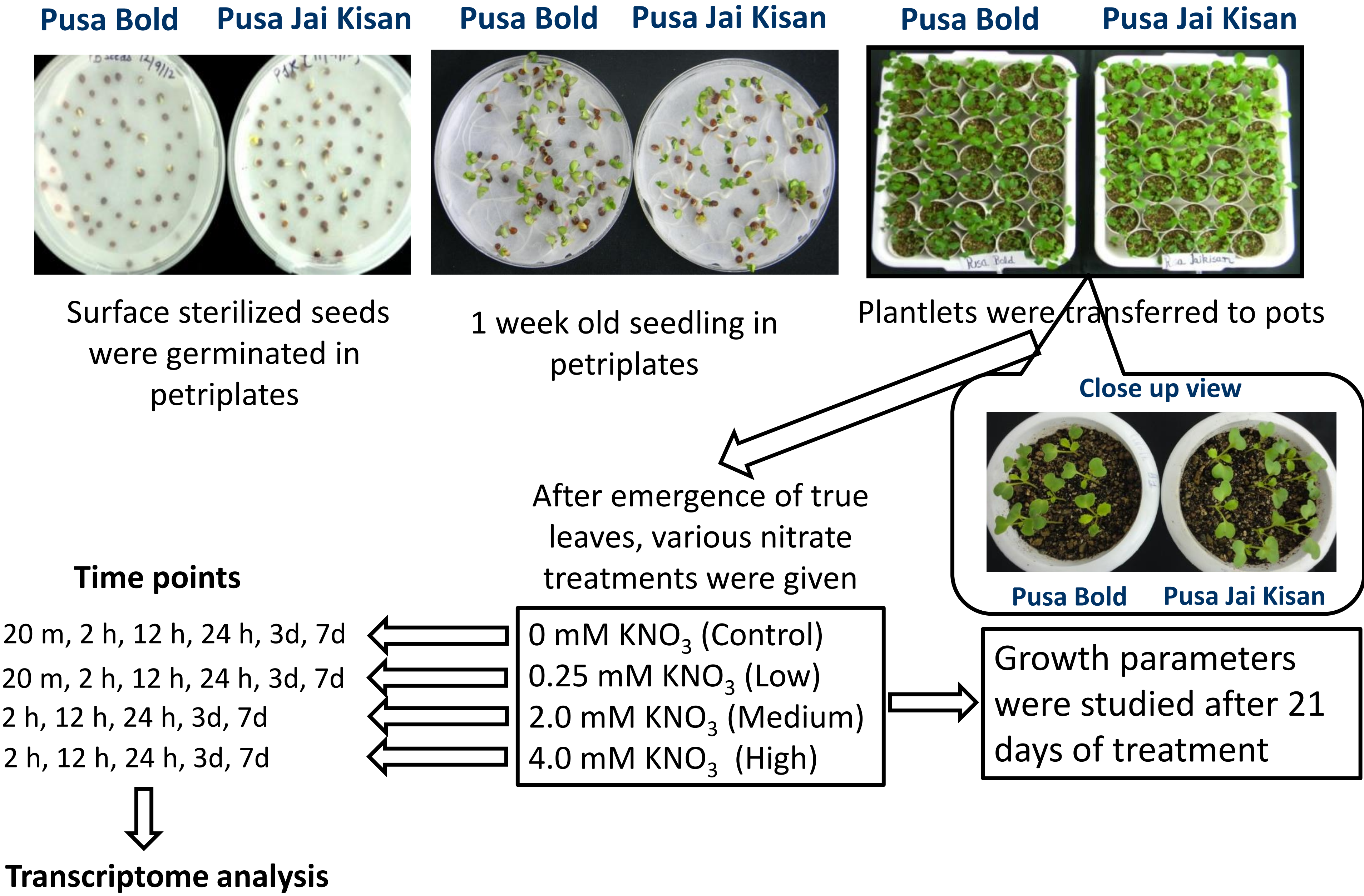


Supplementary Fig S9: Hierarchical clustering based on DisTOM: 29 modules in (A) Pusa Bold and 35 modules in (B) Pusa Jaikisan were obtained by deepslit 2 and 3.


Correspodence of PJK and PB consensus modules

	PJKblack: 245	PJKblue: 5027	PJKbrown: 760	PJKcyan: 114	PJKdarkgreen: 91	PJKdarkgrey: 83	PJKdarkmagenta: 43	PJKdarkolivegreen: 44	PJKdarkorange: 80	PJKdarkred: 91	PJKdarkturquoise: 87	PJKgreen: 336	PJKgreenyellow: 173	PJKgrey: 6392	PJKgrey60: 108	PJKlightcyan: 109	PJKlightgreen: 104	PJKlightyellow: 95	PJKmagenta: 195	PJKmidnightblue: 109	PJKorange: 83	PJKpaleturquoise: 52	PJKpink: 239	PJKpurple: 185	PJKred: 248	PJKroyalblue: 92	PJKsaddlebrown: 57	PJKsalmon: 144	PJKskyblue: 68	PJKsteelblue: 54	PJKtan: 163	PJKturquoise: 9057	PJKviolet: 47	PJKwhite: 79	PJKyellow: 385
PBblack: 460	0	6	0	0	0	0	0	0	0	0	0	3	1	20	0	3	0	0	0	0	1	0	2	4	1	0	0	1	2	0	0	416	0	0	0
PBblue: 1321	0	149	0	28	5	0	39	0	19	0	9	6	8	358	6	7	3	0	0	0	0	3	1	0	3	0	10	3	7	0	0	388	1	0	268
PBbrown: 1004	40	59	3	5	5	2	1	5	2	0	10	12	4	473	4	18	6	58	5	50	4	6	55	43	28	16	1	1	11	3	15	56	0	1	2
PBcyan: 238	0	29	0	3	1	0	0	0	1	0	5	8	0	94	1	27	2	1	0	1	0	0	1	4	2	1	0	0	1	0	0	50	0	0	6
PBdarkgreen: 189	0	31	0	0	0	0	0	0	0	0	0	2	2	27	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	123	2	0	0
PBdarkgrey: 173	0	20	0	0	0	1	0	0	0	0	0	1	2	7	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	140	0	0	0
PBdarkorange: 124	0	39	0	0	2	0	0	0	0	0	0	2	2	19	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	55	3	0	0
PBdarkred: 208	0	56	0	1	0	0	0	0	0	0	2	8	2	42	2	3	0	6	0	2	0	0	2	0	4	1	0	0	0	0	0	77	0	0	0
PBdarkturquoise: 180	0	14	0	0	0	0	0	0	0	0	0	1	0	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	160	0	0	0
PBgreen: 483	0	61	0	0	0	0	0	0	0	0	1	8	2	54	1	0	0	0	0	1	0	0	0	1	2	0	0	4	0	0	0	347	0	0	1
PBgreenyellow: 298	0	11	0	0	0	0	0	0	0	0	2	0	2	30	1	1	0	1	0	0	0	0	1	1	1	0	0	1	2	0	0	243	0	0	1
PBgrey: 12058	98	849	163	34	38	63	1	10	20	8	50	231	85	4209	55	28	29	23	51	42	75	26	144	111	164	65	37	125	38	49	82	4931	20	69	35
PBgrey60: 228	0	7	0	0	0	0	1	0	0	0	1	7	1	23	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	184	0	0	3
PBlightcyan: 236	0	64	0	0	1	0	1	0	0	0	0	1	3	24	0	0	0	0	0	0	0	0	2	4	1	0	0	0	1	0	1	131	0	0	2
PBlightgreen: 225	0	3	0	0	0	0	0	0	0	0	1	2	0	5	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	212	0	0	1
PBlightyellow: 213	2	39	0	4	1	0	0	28	0	0	0	0	0	59	0	3	53	3	0	2	0	1	1	1	1	2	0	0	0	0	0	13	0	0	0
PBmagenta: 329	0	5	0	0	0	0	0	0	0	0	0	0	0	4	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	318	0	0	0
PBmidnightblue: 237	0	13	0	4	0	1	0	0	2	0	1	6	1	93	4	3	0	0	0	1	0	10	20	2	22	0	0	0	1	1	2	49	0	1	0
PBorange: 142	0	53	0	0	1	0	0	0	0	0	1	5	2	17	3	0	0	0	0	0	0	2	1	1	5	1	0	1	1	1	0	44	2	0	1
PBpink: 340	0	58	0	1	1	0	0	0	0	0	0	5	1	50	2	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	218	0	0	2
PBpurple: 319	8	0	108	0	0	0	0	0	0	72	0	0	0	60	0	0	0	0	69	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
PBred: 478	0	89	0	1	1	0	0	0	0	0	2	10	5	117	6	0	0	1	0	0	0	0	3	1	2	0	0	4	1	0	0	229	4	0	2
PBroyalblue: 209	0	12	1	2	5	15	0	0	1	0	0	1	11	72	1	0	0	0	0	0	2	0	0	0	1	0	3	0	0	0	47	30	0	5	0
PBsalmon: 252	0	6	0	12	0	0	0	0	34	0	0	5	1	91	1	3	2	0	0	0	0	4	1	0	7	0	6	0	3	0	1	37	0	1	37
PBskyblue: 80	0	33	0	0	0	0	0	0	0	0	0	0	0	11	0	0	4	0	0	0	0	0	0	1	0	0	0	0	0	0	0	31	0	0	0
PBtan: 294	95	0	2	0	0	0	0	1	0	5	0	0	0	96	0	0	0	1	66	10	0	0	2	9	1	6	0	0	0	0	0	0	0	0	0
PBturquoise: 4219	1	3253	0	19	30	1	0	0	1	0	2	12	38	208	18	9	5	1	0	0	0	0	1	0	1	0	0	3	0	0	14	564	14	0	24
PBwhite: 80	0	68	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	1	0	0
PByellow: 622	1	0	483	0	0	0	0	0	0	6	0	0	0	125	1	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0

Supplementary Fig S10: Consensus match of Pusa Bold and Pusa Jaikisan modules: Modules identifier in the Pusa Bold were compared with most similar module in the Pusa Jaikisan based on significant overlap genes using overlap Table function of WGCNA



Pusa Bold **Pusa Jai Kisan**

Surface sterilized seeds were germinated in petriplates

Pusa Bold **Pusa Jai Kisan**

1 week old seedling in petriplates

Pusa Bold **Pusa Jai Kisan**

Plantlets were transferred to pots

Close up view

Pusa Bold **Pusa Jai Kisan**

After emergence of true leaves, various nitrate treatments were given

0 mM KNO_3 (Control)
0.25 mM KNO_3 (Low)
2.0 mM KNO_3 (Medium)
4.0 mM KNO_3 (High)

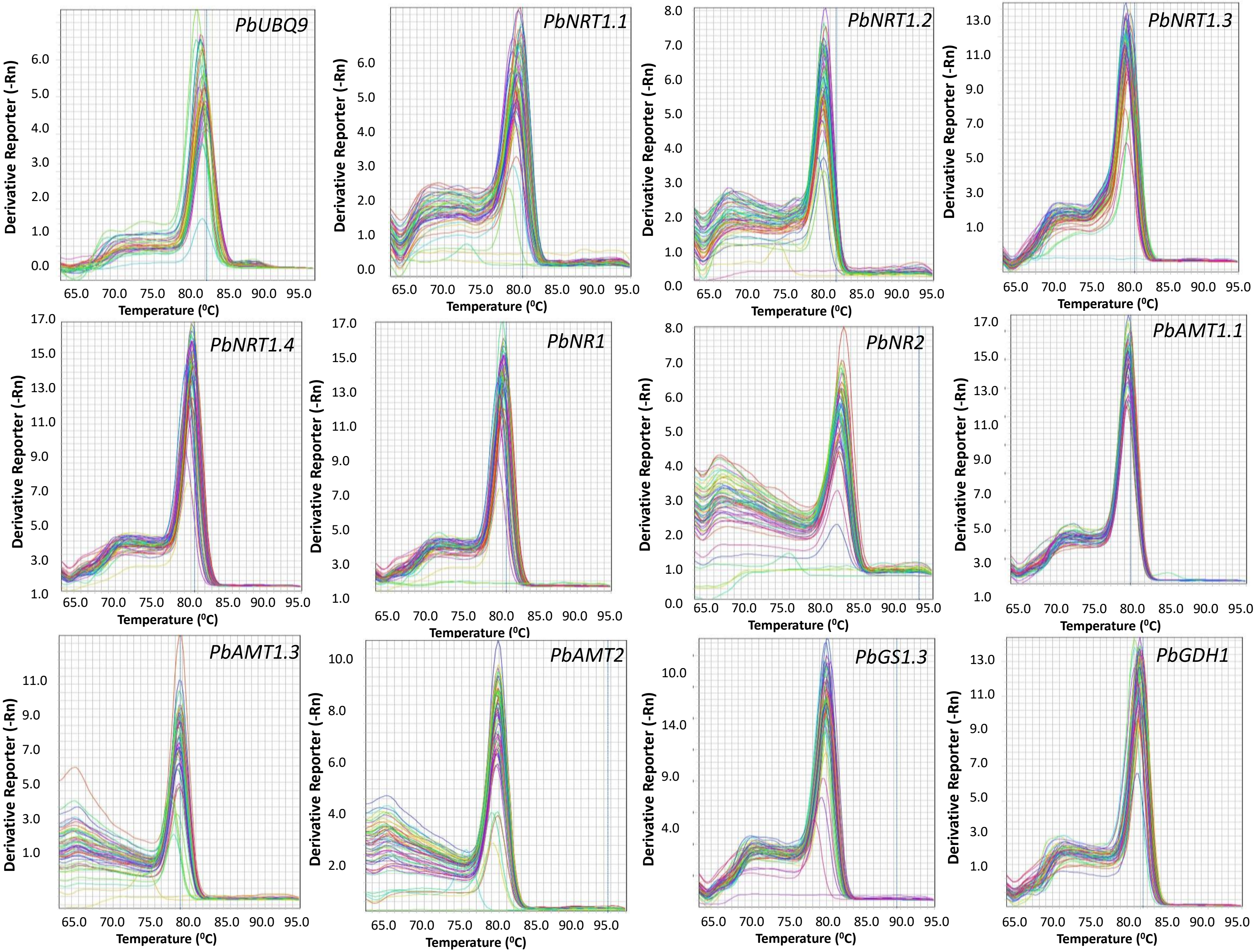
Growth parameters were studied after 21 days of treatment

Time points

20 m, 2 h, 12 h, 24 h, 3d, 7d
20 m, 2 h, 12 h, 24 h, 3d, 7d
2 h, 12 h, 24 h, 3d, 7d
2 h, 12 h, 24 h, 3d, 7d

Transcriptome analysis

Supplementary Fig S11: Experimental design to study growth parameters and transcriptome analysis of Pusa Bold and Pusa Jaikisan in response to various nitrate treatments.



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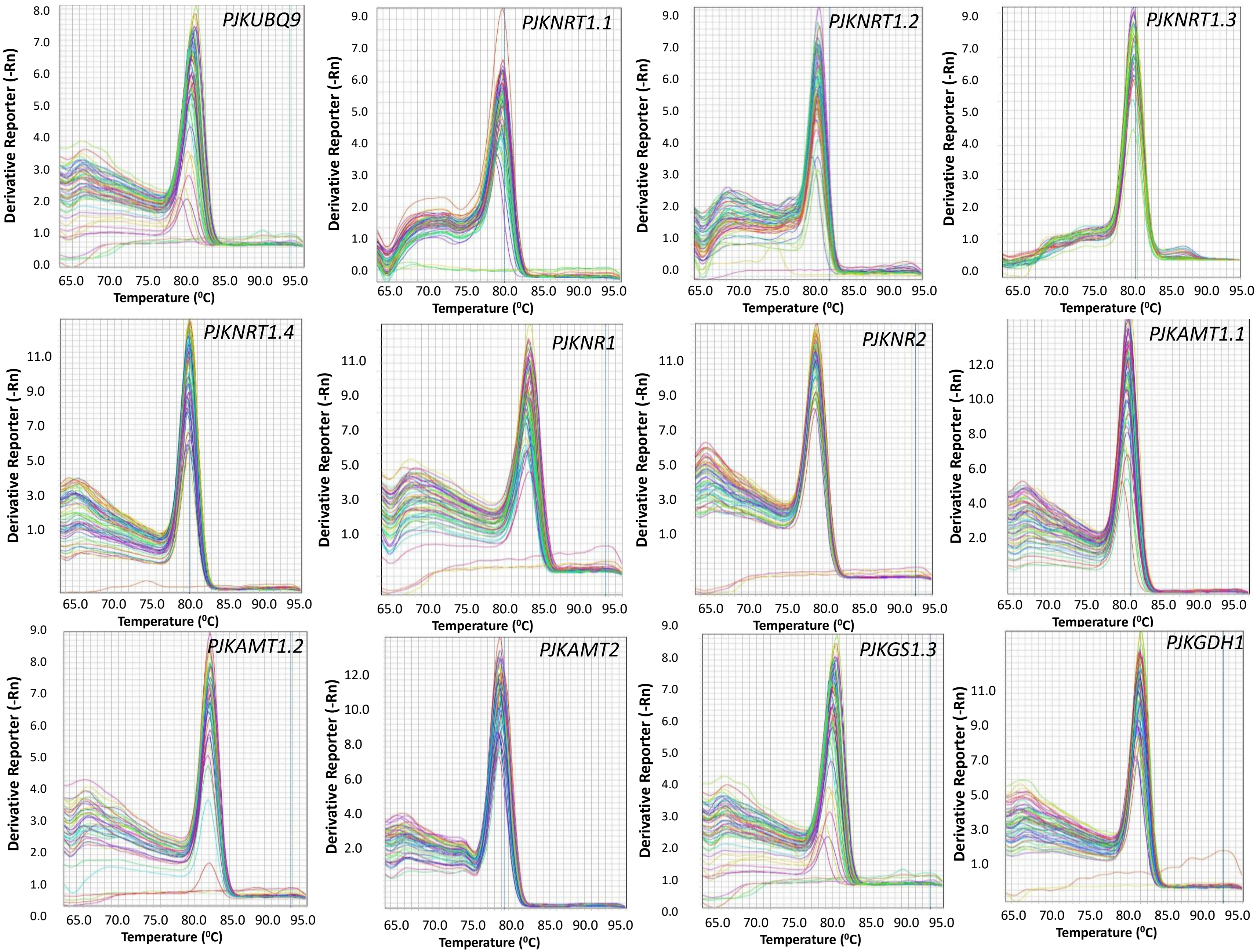
Supplementary Fig S12: Melt curves results of all the genes used for qRT-PCR analysis.



Supplementary Fig S12: Melt curves results of all the genes used for qRT-PCR analysis.