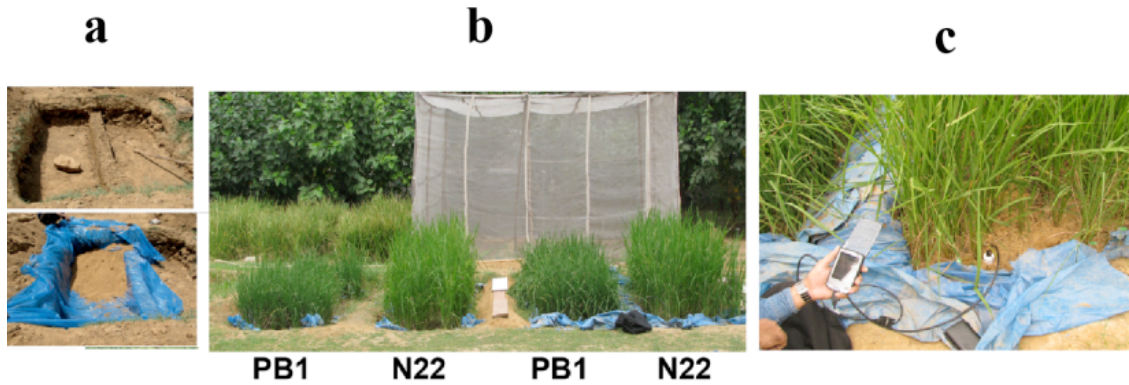


Supplementary Information

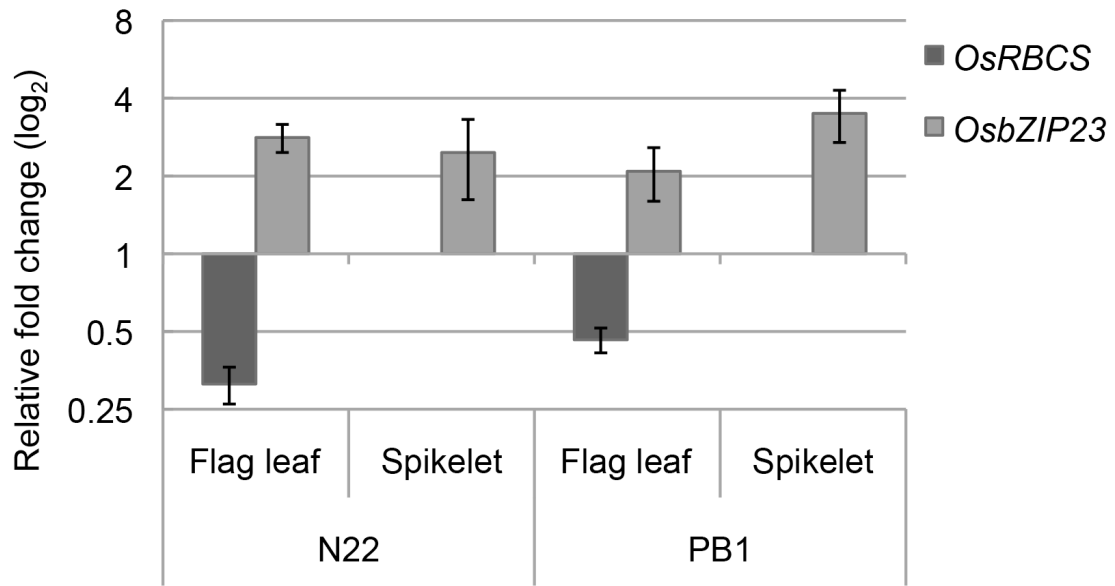
Identification of miRNA-mediated drought responsive multi-tiered regulatory network in drought tolerant rice, Nagina 22

Sonia Balyan¹, Mukesh Kumar¹, Roseeta Devi Mutum¹, Utkarsh Raghuvanshi¹, Priyanka Agarwal¹, Saloni Mathur², Saurabh Raghuvanshi^{1*}

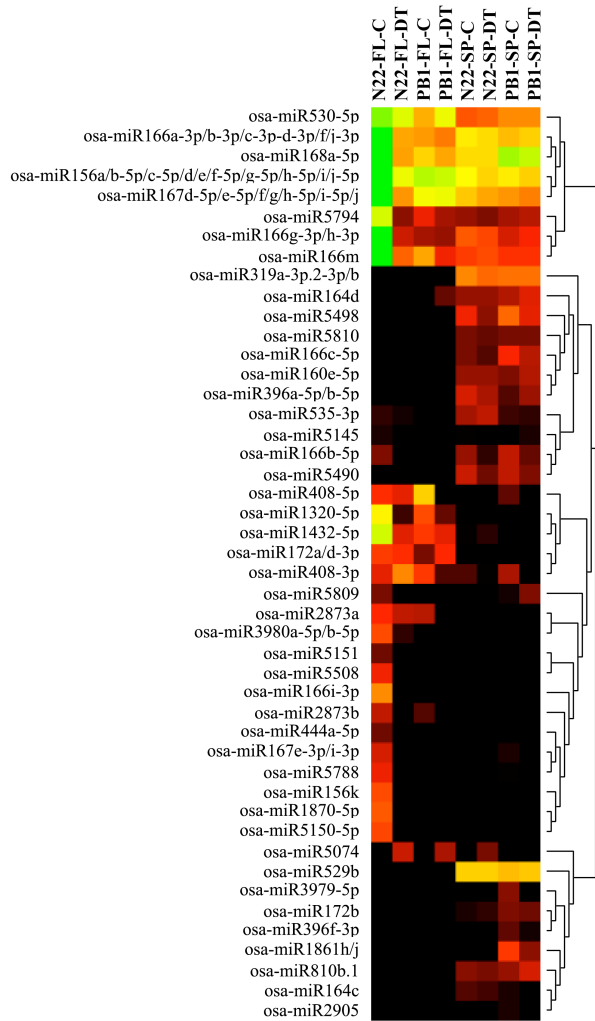
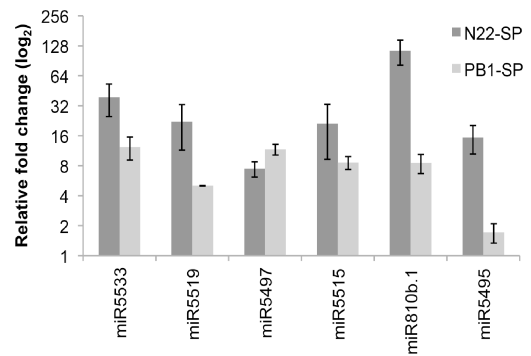
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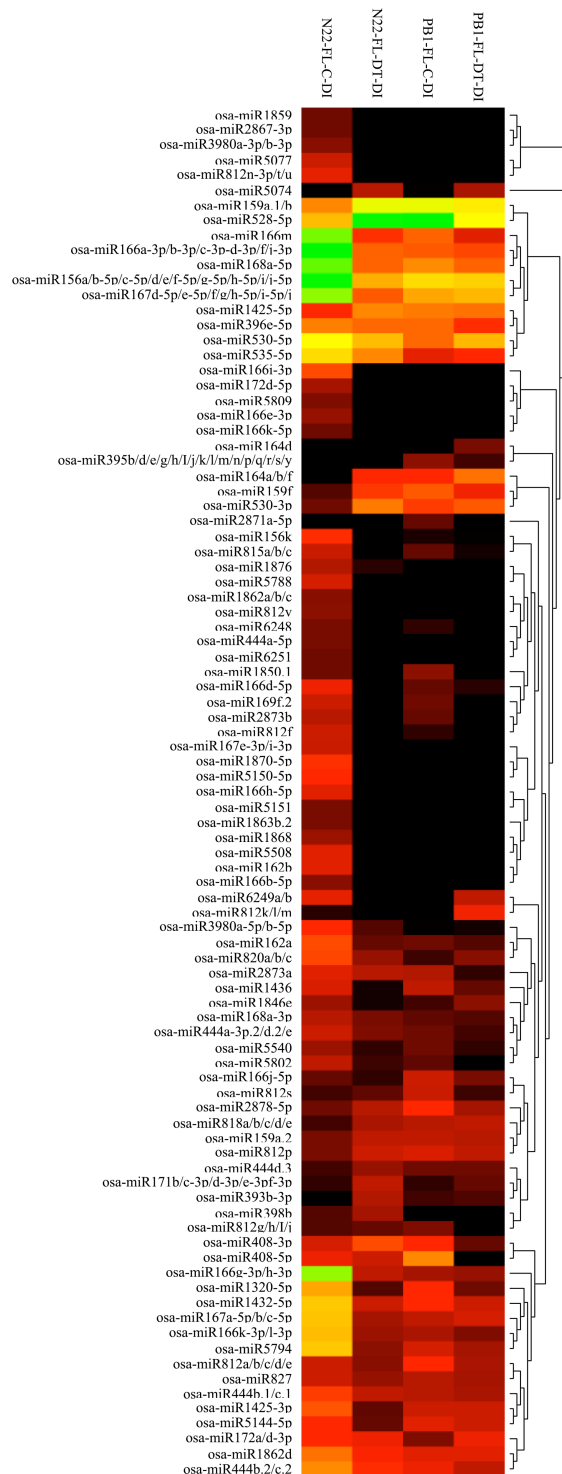
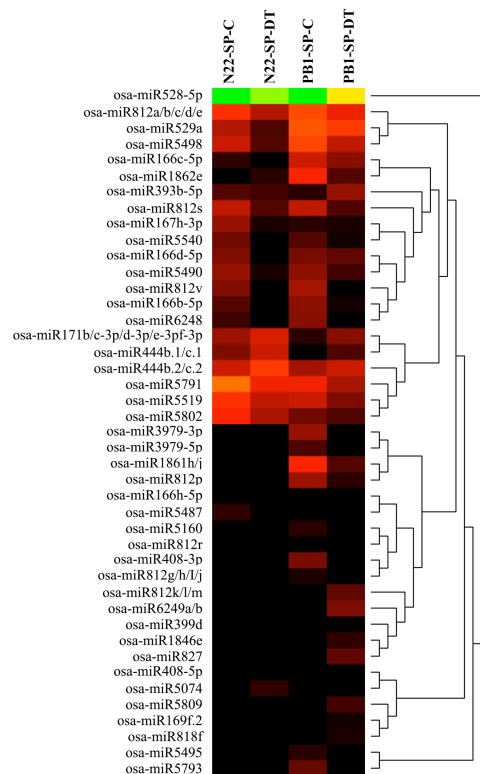
Supplementary Fig. S1. Field set up for implicating drought stress on rice cultivars. (a) Three feet deep pits were lined with polyethene were prepared to regulate water supply and avoid the water leakage from outside. (b) N22 and PB1 plants growing in the specially designed fields. (c) Picture showing the measurement of soil moisture content in the fields.



Supplementary Fig. S2. qRT-PCR analysis of Rubisco small sub-unit (*RBCS*) in flag leaf and *OsbZIP23* in flag leaf and spikelet of N22 and PB1 under drought stress at heading stage. Four biological replicate were performed and the standard error is represented as error bar on the top of the bar.

a**b**

Supplementary Fig. S3. Flag leaf and spikelet preferential miRNAs in N22 and PB1. (a) Hierarchical clustering of \log_2 transformed expression levels (TPM) of flag leaf and spikelet enriched miRNAs (having 10 fold higher expression in one tissue w.r.t to the other) in N22 and PB1. The clustering was performed using the Euclidean distance algorithm with average linkage. (b) qRT-PCR analysis of spikelets enriched miRNAs in spikelets relative to the expression in flag leaf of N22 and PB1, respectively. The expression represents the data of 3 biological replicates with three technical repeats. The error bar represents the standard error. FL- flag leaf; SP- spikelet; C- control; DT- drought.

a**b**

Supplementary Fig. S4. Drought regulated miRNAs in flag leaf and spikelet. Hierarchical clustering of \log_2 transformed expression levels (TPM) of drought responsive miRNAs in flag leaf (**a**) and spikelet (**b**) of N22 and PB1. miRNAs with ≥ 2 fold up/down regulation in flag leaf of spikelet drought samples w.r.t. respective control in N22 and PB1. The clustering was performed using the Euclidean distance algorithm with average linkage.

Biological Process

Adaxial/abaxial axis specification	2
adaxial/abaxial pattern specification	2
Adventitious root development	3
Anatomical structure development	38
ATP synthesis coupled proton transport	3
Auxin metabolic process	3
Auxin polar transport	4
auxin-activated signaling pathway	9
Biological process	299
Biosynthetic process	118
Catabolic process	43
Cellular amino acid metabolic process	18
Cellular carbohydrate metabolic process	3
Cellular component assembly	19
Cellular nitrogen compound metabolic process	85
Chlorophyll metabolic process	3
Cinnamic acid biosynthetic process	2
Cofactor metabolic process	13
Defense response to bacterium	10
Determination of bilateral symmetry	5
Electron transport chain	3
embryo development	15
embryo development ending in seed dormancy	11
embryonic pattern specification	4
Endonucleolytic cleavage in ITS1 to separate SSU-rna from 5.8S rna and LSU-rna from tricistronic rna transcript	2
SSU-rna from	2
Flower development	8
Gene silencing by miRNA	3
Gene silencing by RNA	5
Generation of precursor metabolites and energy	16
glycolytic process	6
Hydrogen peroxide biosynthetic process	3
Innate immune response	3
Integument development	2
Leaf morphogenesis	7
L-phenylalanine catabolic process	2
Macromolecular complex assembly	16
Malate metabolic process	3
Meristem initiation	5
modulation by virus of host morphology or physiology	2
Mrna transport	2
Photorespiration	7
Phylome development	2
Polarity specification of adaxial/abaxial axis	4
Posttranscriptional gene silencing	3
Primary cell wall biogenesis	2
Primary shoot apical meristem specification	4
Proteasome core complex assembly	4
Protein complex assembly	12
Protein folding	12
Proteolysis involved in cellular protein catabolic process	5
Proton transport	4
Purine nucleotide transport	2
Pyridoxine biosynthetic process	2
Radial pattern formation	2
Regulation of cell cycle process	3
Regulation of lipid metabolic process	2
Regulation of photomorphogenesis	2
response to auxin	12
Response to cadmium ion	35
Response to cold	18
Response to far red light	7
Response to fructose	4
response to hormone	4
Response to light stimulus	10
Response to misfolded protein	5
Response to salt stress	18
Response to stress	58
Response to sucrose	6
Ribosomal small subunit biogenesis	2
RNA interference	4
shoot system development	4
Small molecule metabolic process	51
Spermidine biosynthetic process	2
Sphingolipid biosynthetic process	2
Stomatal lineage progression	3
Symbiosis, encompassing mutualism through parasitism	7
transcription, DNA-templated	31
Translation	27
Translational elongation	5
Tricarboxylic acid cycle	5
Ubiquitin-dependent protein catabolic process	11
Virus induced gene silencing	5
Vitamin B6 biosynthetic process	2

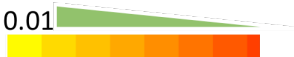
Cellular Component

Apoplast	22
Cell	237
Cell wall	26
Cellular component	262
Chloroplast	69
Chloroplast envelope	25
Chloroplast stroma	29
Cytoplasm	168
Cytoskeleton	10
Cytosol	60
Cytosolic ribosome	17
Cytosolic small ribosomal subunit	8
Eukaryotic translation elongation factor 1 complex	2
External encapsulating structure	21
Extracellular region	21
Golgi apparatus	19
Intracellular	223
Membrane	75
Mitochondrial proton-transporting ATP synthase complex, catalytic core F(1)	2
Mitochondrial respiratory chain complex I	7
Mitochondrion	38
Nucleolus	19
Nucleus	99
Organelle	198
Phragmoplast	5
Plasma membrane	69
Plasmodesma	21
Plastid	69
Proteasome complex	6
Proteasome core complex	5
Proteasome core complex, alpha-subunit complex	3
Protein complex	39
Ribonucleoprotein complex	13
Ribosome	25
Stromule	6
Vacuole	33

Molecular Function

2 iron, 2 sulfur cluster binding	5
Ammonia-lyase activity	2
ATP:ADP antiporter activity	2
Copper ion binding	14
Endopeptidase activity	5
Endoribonuclease activity	4
Hydrogen-translocating pyrophosphatase activity	2
Inositol phosphoceramide synthase activity	2
Iron-sulfur cluster binding	6
Lipid binding	11
L-malate dehydrogenase activity	3
Malate dehydrogenase activity	3
Mirna binding	3
Molecular function	344
Oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor	6
Phenylalanine ammonia-lyase activity	2
Phospholipid binding	5
Protein domain specific binding	2
RNA binding	27
Sirna binding	3
Structural constituent of ribosome	20
Structural molecule activity	25
Threonine-type endopeptidase activity	5
transcription factor binding	3

P-value 0.01



Supplementary Fig. S5. Gene Ontology enrichment analysis of genes targeted by miRNAs showing drought mediated downregulation in N22 flag leaf. The different GO terms with an enrichment pvalue of ≤ 0.01 belonging to biological process, molecular function and cellular component were shown with the number of genes and heat map representing the p-value.

Biological Process


Cellular response to UV-B	2
Cysteine biosynthetic process	3
Multicellular organismal development	3
Removal of superoxide radicals	3
Response to copper ion	2
Response to iron ion	2
Response to ozone	2
Response to salt stress	8
Stomatal lineage progression	2
Superoxide metabolic process	3
Ubiquitin-dependent protein catabolic process	4
Vegetative phase change	2
Virus induced gene silencing	2

Cellular Component

Cell	46
Chloroplast	14
Chloroplast envelope	7
Cytoplasm	32
Cytosol	12
Cytosolic large ribosomal subunit	3
Cytosolic ribosome	6
Intracellular	41
Membrane	20
Mitochondrial inner membrane	3
Nucleolus	6
Organelle	35
Plastid	19
Ribonucleoprotein complex	5
Ribosome	8

Molecular Function

Copper ion binding	14
Superoxide dismutase activity	3
Electron carrier activity	9
Antioxidant activity	2
Protein homodimerization activity	3
Phosphoprotein phosphatase activity	3
Structural constituent of ribosome	5

P-value 0.01 

Supplementary Fig. S6. Gene Ontology enrichment analysis of genes targeted by miRNAs showing drought mediated up-regulation in N22 flag leaf. The different GO terms with an enrichment pvalue of ≤ 0.01 belonging to biological process, molecular function and cellular component were shown with the number of genes and heat map representing the p-value .

a

Biological Process

Adaxial/abaxial axis specification	2
adaxial/abaxial pattern specification	2
Biosynthetic process	35
Chlorophyll metabolic process	2
Determination of bilateral symmetry	4
Embryonic pattern specification	2
Integument development	2
Lignin biosynthetic process	2
Malate metabolic process	2
Meristem initiation	4
Polarity specification of adaxial/abaxial axis	4
Primary shoot apical meristem specification	2
Proteolysis involved in cellular protein catabolic process	2
Radial pattern formation	2
regulation of transcription, DNA-templated	14
transcription, DNA-templated	13
Translation	8
Xylem and phloem pattern formation	2

Cellular Component

Cell	62
Cellular_component	73
Chloroplast	17
Cytoplasm	39
Cytosol	20
Intracellular	54
Membrane	24
Nucleolus	7
Nucleus	30
Organelle	52
Plasma membrane	25
Plasmodesma	11
Plastid	18
Proteasome core complex	2
Ribonucleoprotein complex	7
Ribosome	7
Vacuolar membrane	7
Vacuole	10

Molecular Function

Copper ion binding	9
Electron carrier activity	8
Endopeptidase activity	2
Hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides	8
Lipid binding	5
Structural constituent of ribosome	7
Structural molecule activity	7
Threonine-type endopeptidase activity	2

b

Biological Process

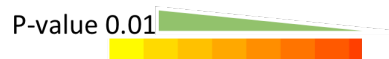
Aging	4
Anatomical structure development	10
Biosynthetic process	18
Leaf senescence	2
Methionine biosynthetic process	2
regulation of transcription, DNA-templated	13
Response to cold	4
Response to salt stress	6
transcription, DNA-templated	7
Water transport	2

Cellular Component

Cell	34
Chloroplast envelope	6
Intracellular	31
Mitochondrial respiratory chain complex I	2
Mitochondrion	8
Organelle	27
Plastid	12

Molecular Function

2 iron, 2 sulfur cluster binding	2
DNA binding	14
Transporter activity	5



Supplementary Fig. S7. Gene Ontology enrichment analysis of genes targeted by miRNAs showing drought mediated (a) down and (b) up-regulation in PB1 flag leaf. The different GO terms with an enrichment pvalue of ≤ 0.01 belonging to biological process, molecular function and cellular component were shown with the number of genes and heat map representing the p-value.

a

Biological Process	
Biosynthetic process	25
Catabolic process	18
Cellular amino acid biosynthetic process	3
Cellular amino acid metabolic process	7
Cellular nitrogen compound metabolic process	23
endonucleolytic cleavage in ITS1 to separate SSU-rRNA from 5.8S rRNA and LSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	2
Endonucleolytic cleavage to generate mature 3'-end of SSU-rRNA from (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	2
Nitrogen compound metabolic process	3
N-terminal protein myristoylation	2
Pentose-phosphate shunt	4
Peptidyl-proline modification	2
Photorespiration	3
Response to cadmium ion	7
Ribosomal small subunit biogenesis	2
Small molecule metabolic process	20
sulfur compound metabolic process	5
Translation	8
Translational elongation	4
Ubiquitin-dependent protein catabolic process	5

Cellular Component	
Cell	57
Cellular component	63
Chloroplast	18
Chloroplast envelope	7
Chloroplast thylakoid membrane	5
Cytoplasm	41
Cytoplasmic vesicle	2
Cytosol	17
Intracellular	52
Membrane	22
Organelle	44
Plasma membrane	18
Plastid	21
Protein complex	13
Thylakoid	6

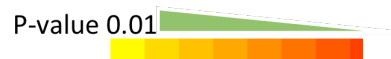
Molecular Function	
Coenzyme binding	3
Copper ion binding	6
FK506 binding	2
Hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds	3
Isomerase activity	5
Ligase activity	6
Racemase and epimerase activity, acting on carbohydrates and derivatives	2
Structural molecule activity	6
Translation elongation factor activity	2

b

Biological Process	
Biosynthetic process	9
Transcription, DNA-templated	5
Stomatal lineage progression	2

Cellular Component	
Cytosol	5
Intracellular	13

Molecular Function	
Transcription factor binding	2
DNA binding	7
Protein dimerization activity	3
ATP binding	7
Helicase activity	3



Supplementary Fig. S8. Gene Ontology enrichment analysis of genes targeted by miRNAs showing drought mediated (a) down and (b) up-regulation in N22 spikelet. The different GO terms with an enrichment pvalue of ≤ 0.01 belonging to biological process, molecular function and cellular component were shown with the number of genes and heat map representing the p-value.

a

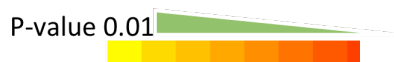
Biological Process	
Anthocyanin-containing compound metabolic process	2
ATP biosynthetic process	2
Biosynthetic process	35
Catabolic process	17
Cellular amino acid metabolic process	9
Cysteine biosynthetic process	3
Defense response to bacterium	5
Endonucleolytic cleavage in ITS1 to separate SSU-rRNA from 5.8S rRNA and LSU-rRNA from tricistronic rRNA transcript	2
Endonucleolytic cleavage to generate mature 3'-end of SSU-rRNA from (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	2
Regulation of ARF GTPase activity	2
Ribosomal small subunit biogenesis	2
Salicylic acid biosynthetic process	2
Small molecule metabolic process	22
Translation	11
Translational elongation	5
Ubiquitin-dependent protein catabolic process	6

Cellular Component

Cell	63
Cellular component	71
Chloroplast	24
Chloroplast envelope	9
Cytoplasm	43
Cytosol	18
Cytosolic ribosome	6
Cytosolic small ribosomal subunit	3
Intracellular	56
Organelle	48
Plastid	25
Ribonucleoprotein complex	6
Ribosome	8

Molecular Function

Acid-amino acid ligase activity	4
ARF GTPase activator activity	2
Copper ion binding	10
Electron carrier activity	9
Ligase activity	8
Phosphotransferase activity, alcohol group as acceptor	3
Structural constituent of ribosome	8
Structural molecule activity	8



b

Biological Process	
ATP synthesis coupled proton transport	2
auxin-activated signaling pathway	3
Biosynthetic process	25
Calcium-mediated signaling	2
Cellular component assembly	6
Cellular protein metabolic process	2
Cellular response to nitrate	2
Cellular response to phosphate starvation	3
Iron ion homeostasis	2
Lateral root development	2
Lateral root formation	2
Macromolecular complex assembly	6
Pollen maturation	2
Protein complex assembly	5
Protein folding	4
Protein targeting to chloroplast	2
Regulation of photomorphogenesis	2
Response to cadmium ion	10
Response to molecule of bacterial origin	2
Response to salt stress	7
Stamen development	2

Cellular Component

Apoplast	7
Cell	54
Cell wall	8
Cellular component	60
Chloroplast stroma	7
Cytoplasm	39
Cytosol	17
Extracellular region	7
Intracellular	49
Membrane	20
Mitochondrial respiratory chain complex I	3
Mitochondrion	11
Nucleolus	6
Nucleus	19
Organelle	43
Plasma membrane	22
Plasmodesma	8
Protein complex	12
SCF ubiquitin ligase complex	2
Vacuolar membrane	6
Vacuole	8

Molecular Function

Auxin binding	2
Inositol hexakisphosphate binding	2
Transcription factor binding	2

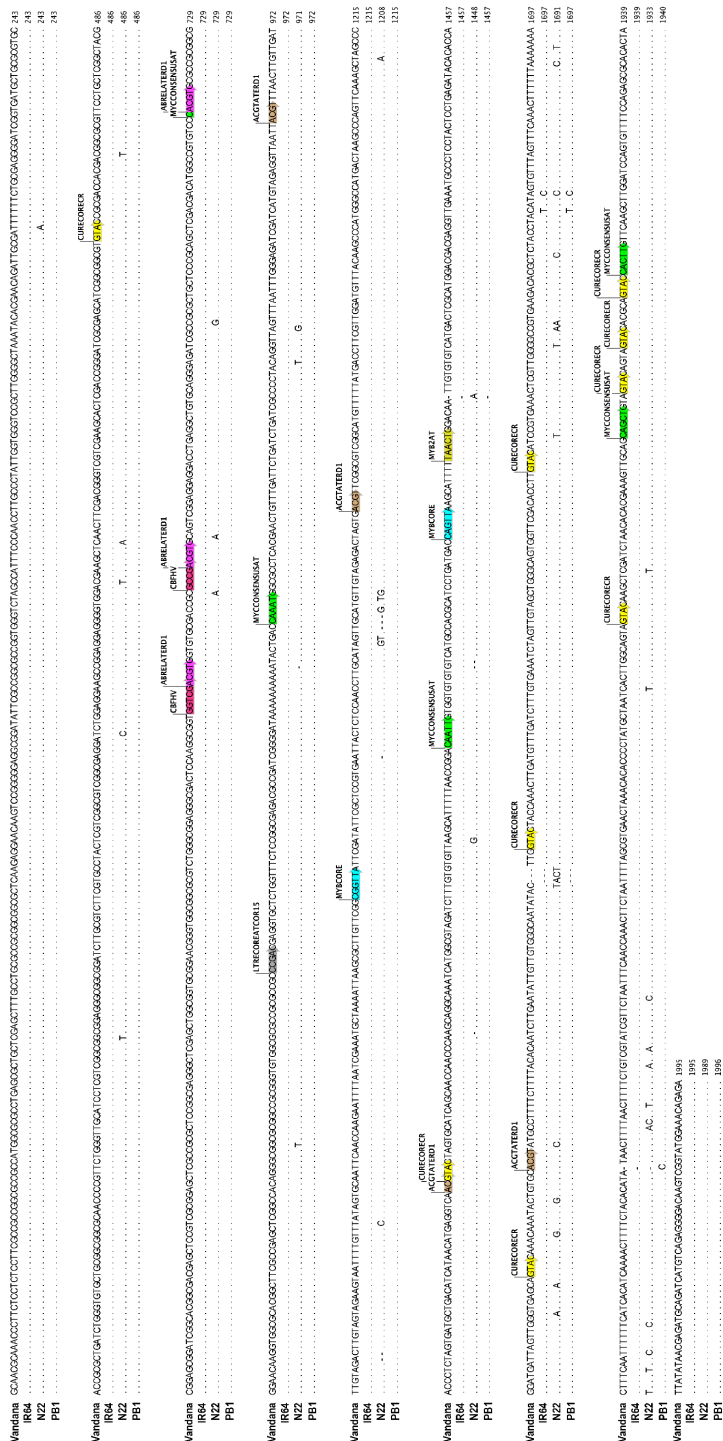
Supplementary Fig. S9. Gene Ontology enrichment analysis of genes targeted by miRNAs showing drought mediated (a) down and (b) up-regulation in PB1 spikelet. The different GO terms with an enrichment pvalue of ≤ 0.01 belonging to biological process, molecular function and cellular component were shown with the number of genes and heat map representing the p-value.

a.

Upstream regulatory region of MIR1871

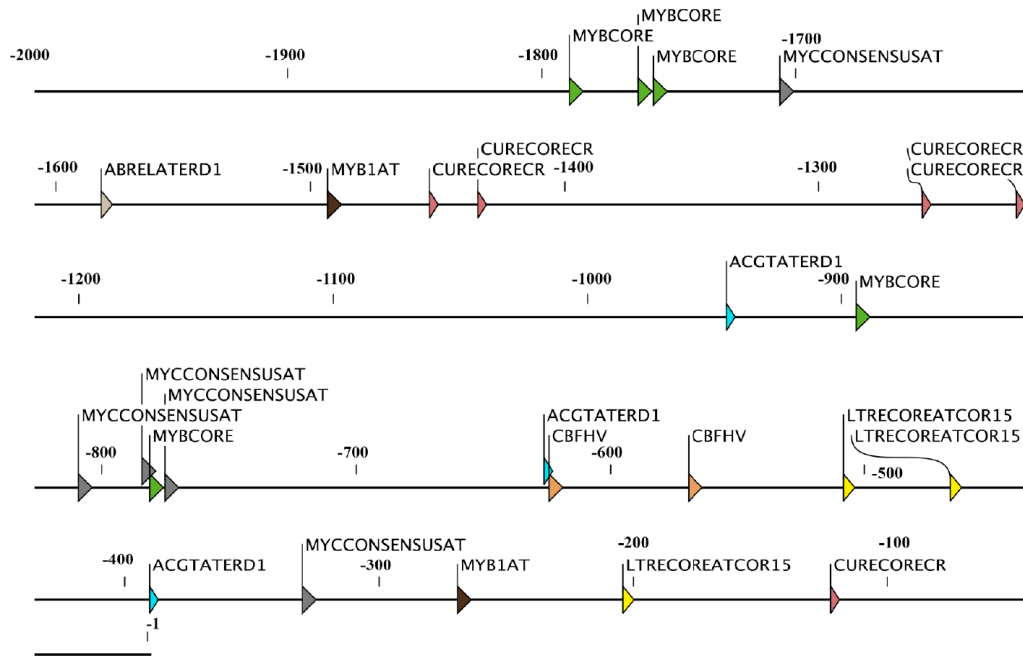
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36840 36870 36900 36930 36960 36990 37020 37050 37080 37110 37140 37170 37200 37230 37260 37290 37320 37350 37380 37410 37440 37470 37500 37530 37560 37590 37620 37650 37680 37710 37740 37770 37800 37830 37860 37890 37920 37950 37980 38010 38040 38070 38100 38130 38160 38190 38220 38250 38280 38310 38340 38370 38400 38430 38460 38490 38520 38550 38580 38610 38640 38670 38700 38730 38760 38790 38820 38850 38880 38910 38940 38970 39000 39030 39060 39090 39120 39150 39180 39210 39240 39270 39300 39330 39360 39390 39420 39450 39480 39510 39540 39570 39600 39630 39660 39690 39720 39750 39780 39810 39840 39870 39900 39930 39960 39990 40020 40050 40080 40110 40140 40170 40200 40230 40260 40290 40320 40350 40380 40410 40440 40470 40500 40530 40560 40590 40620 40650 40680 40710 40740 40770 40800 40830 40860 40890 40920 40950 40980 41010 41040 41070 41100 41130 41160 41190 41220 41250 41280 41310 41340 41370 41400 41430 41460 41490 41520 41550 41580 41610 41640 41670 41700 41730 41760 41790 41820 41850 41880 41910 41940 41970 42000 42030 42060 42090 42120 42150 42180 42210 42240 42270 42300 42330 42360 42390 42420 42450 42480 42510 42540 42570 42600 42630 42660 42690 42720 42750 42780 42810 42840 42870 42900 42930 42960 42990 43020 43050 43080 43110 43140 43170 43200 43230 43260 43290 43320 43350 43380 43410 43440 43470 43500 43530 43560 43590 43620 43650 43680 43710 43740 43770 43800 43830 43860 43890 43920 43950 43980 44010 44040 44070 44100 44130 44160 44190 44220 44250 44280 44310 44340 44370 44400 44430 44460 44490 44520 44550 44580 44610 44640 44670 44700 44730 44760 44790 44820 44850 44880 44910 44940 44970 45000 45030 45060 45090 45120 45150 45180 45210 45240 45270 45300 45330 45360 45390 45420 45450 45480 45510 45540 45570 45600 45630 45660 45690 45720 45750 45780 45810 45840 45870 45900 45930 45960 45990 46020 46050 46080 46110 46140 46170 46200 46230 46260 46290 46320 46350 46380 46410 46440 46470 46500 46530 46560 46590 46620 46650 46680 46710 46740 46770 46800 46830 46860 46890 46920 46950 46980 47010 47040 47070 47100 47130 47160 47190 47220 47250 47280 47310 47340 47370 47400 47430 47460 47490 47520 47550 47580 47610 47640 47670 47700 47730 47760 47790 47820 47850 47880 47910 47940 47970 48000 48030 48060 48090 48120 48150 48180 48210 48240 48270 48300 48330 48360 48390 48420 48450 48480 48510 48540 48570 48600 48630 48660 48690 48720 48750 48780 48810 48840 48870 48900 48930 48960 48990 49020 49050 49080 49110 49140 49170 49200 49230 49260 49290 49320 49350 49380 49410 49440 49470 49500 49530 49560 49590 49620 49650 49680 49710 49740 49770 49800 49830 49860 49890 49920 49950 49980 50010 50040 50070 50100 50130 50160 50190 50220 50250 50280 50310 50340 50370 50400 50430 50460 50490 50520 50550 50580 50610 50640 50670 50700 50730 50760 50790 50820 50850 50880 50910 50940 50970 51000 51030 51060 51090 51120 51150 51180 51210 51240 51270 51300 51330 51360 51390 51420 51450 51480 51510 51540 51570 51600 51630 51660 51690 51720 51750 51780 51810 51840 51870 51900 51930 51960 51990 52020 52050 52080 52110 52140 52170 52200 52230 52260 52290 52320 52350 52380 52410 52440 52470 52500 52530 52560 52590 52620 52650 52680 52710 52740 52770 52800 52830 52860 52890 52920 52950 52980 53010 53040 53070 53100 53130 53160 53190 53220 53250 53280 53310 53340 53370 53400 53430 53460 53490 53520 53550 53580 53610 53640 53670 53700 53730 53760 53790 53820 53850 53880 53910 53940 53970 54000 54030 54060 54090 54120 54150 54180 54210 54240 54270 54300 54330 54360 54390 54420 54450 54480 54510 54540 54570 54600 54630 54660 54690 54720 54750 54780 54810 54840 54870 54900 54930 54960 54990 55020 55050 55080 55110 55140 55170 55200 55230 55260 55290 55320 55350 55380 55410 55440 55470 55500 55530 55560 55590 55620 55650 55680 55710 55740 55770 55800 55830 55860 55890 55920 55950 55980 56010 56040 56070 56100 56130 56160 56190 56220 56250 56280 56310 56340 56370 56400 56430 56460 56490 56520 56550 56580 56610 56640 56670 56700 56730 56760 56790 56820 56850 56880 56910 56940 56970 57000 57030 57060 57090 57120 57150 57180 57210 57240 57270 57300 57330 57360 57390 57420 57450 57480 57510 57540 57570 57600 57630 57660 57690 57720 57750 57780 57810 57840 57870 57900 57930 57960 57990 58020 58050 58080 58110 58140 58170 58200 58230 58260 58290 58320 58350 58380 58410 58440 58470 58500 58530 58560 58590 58620 58650 58680 58710 58740 58770 58800 58830 58860 58890 58920 58950 58980 59010 59040 59070 59100 59130 59160 59190 59220 59250 59280 59310 59340 59370 59400 59430 59460 59490 59520 59550 59580 59610 59640 59670 59700 59730 59760 59790 59820 59850 59880 59910 59940 59970 60000 60030 60060 60090 60120 60150 60180 60210 60240 60270 60300 60330 60360 60390 60420 60450 60480 60510 60540 60570 60600 60630 60660 60690 60720 60750 60780 60810 60840 60870 60900 60930 60960 60990 61020 61050 61080 61110 61140 61170 61200 61230 61260 61290 61320 61350 61380 61410 61440 61470 61500 61530 61560 61590 61620 61650 61680 61710 61740 61770 61800 61830 61860 61890 61920 61950 61980 62010 62040 62070 62100 62130 62160 62190 62220 62250 62280 62310 62340 62370 62400 62430 62460 62490 62520 62550 62580 62610 62640 62670 62700 62730 62760 62790 62820 62850 62880 62910 62940 62970 63000 63030 63060 63090 63120 63150 63180 63210 63240 63270

Upstream regulatory region of MIR397a

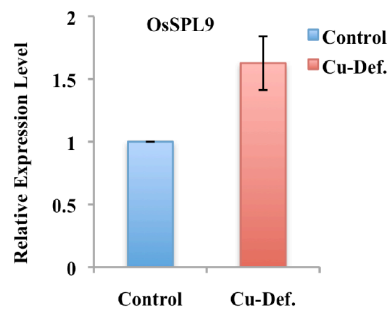


Supplementary Fig. S11. Sequence alignment of DTA-miRNA promoter sequences in N22, Vandana, PB1 and IR64. Sequence alignment of 2kb upstream region of (a) MIR1871, (b) MIR159f 2kb, (c) MIR2878, (d) MIR528, (e) MIR408, (f) MIR398b, (g) MIR397a in four cultivars namely N22, Vandana, PB1 and IR64. Dots (.) represents the identical sequence, dashes (-) represents gaps and the cis-acting elements associated with stress were highlighted.

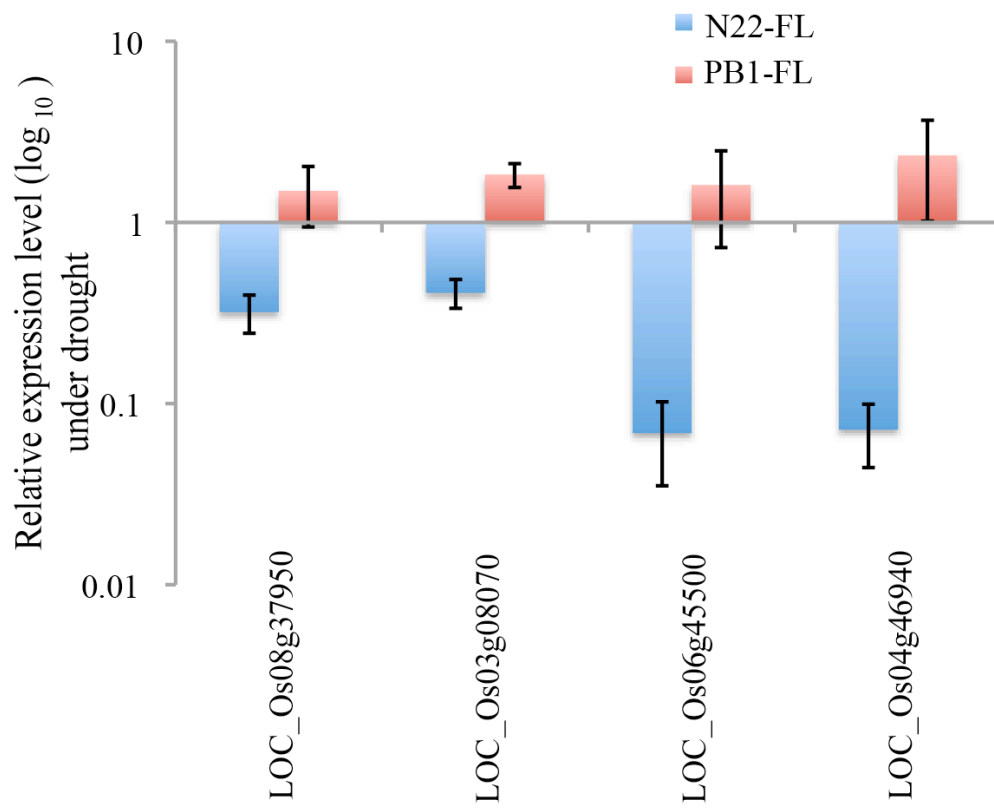
a



b

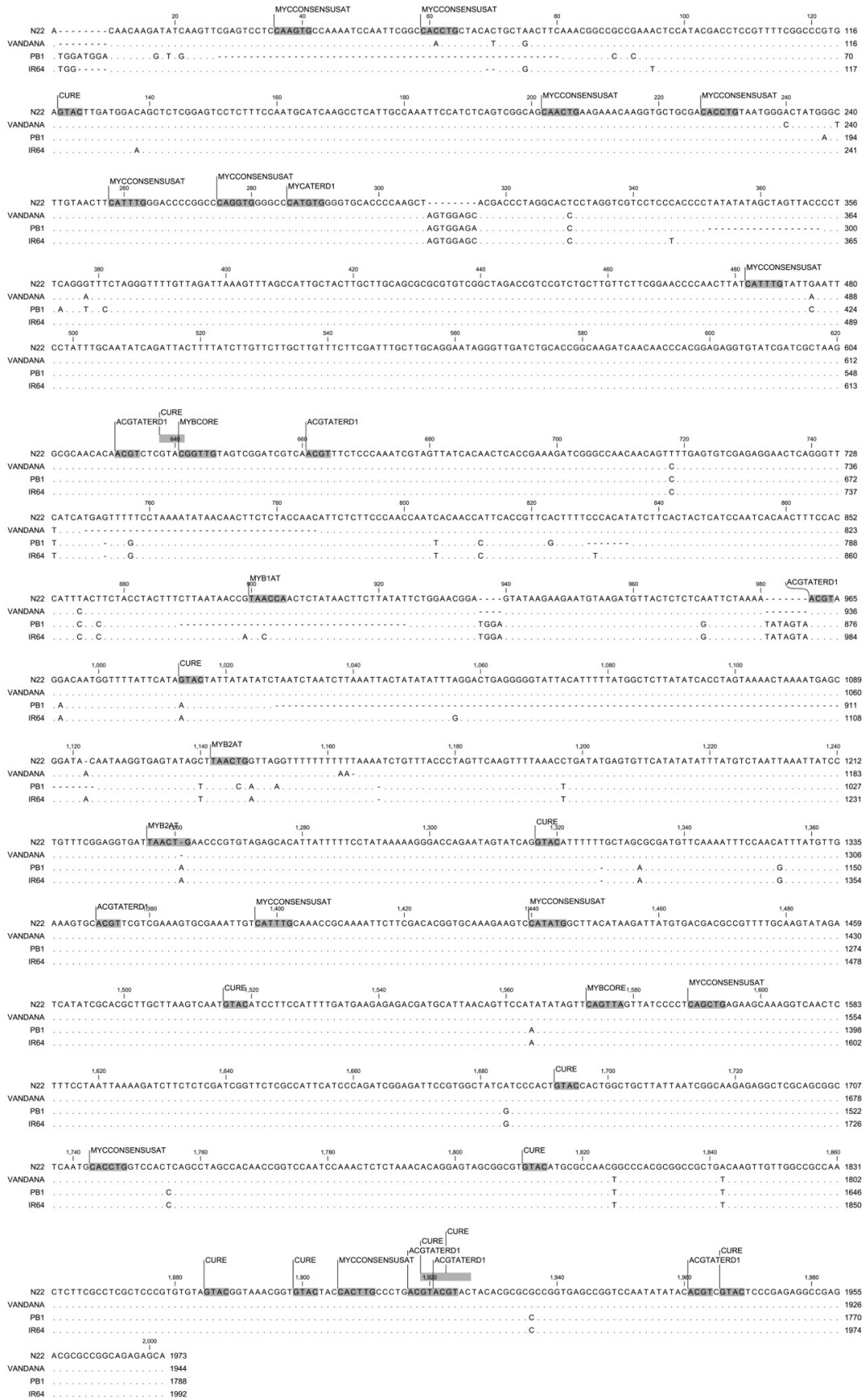


Supplementary Fig. S12. *OsSPL9* promoter analysis (a) Cis-regulatory elements in the 2kb upstream promoter of *OsSPL9*. (b) RT-PCR of *OsSPL9* in response to copper starvation in N22 seedlings. Data represents the mean value of three biological replicates and three technical repeats.

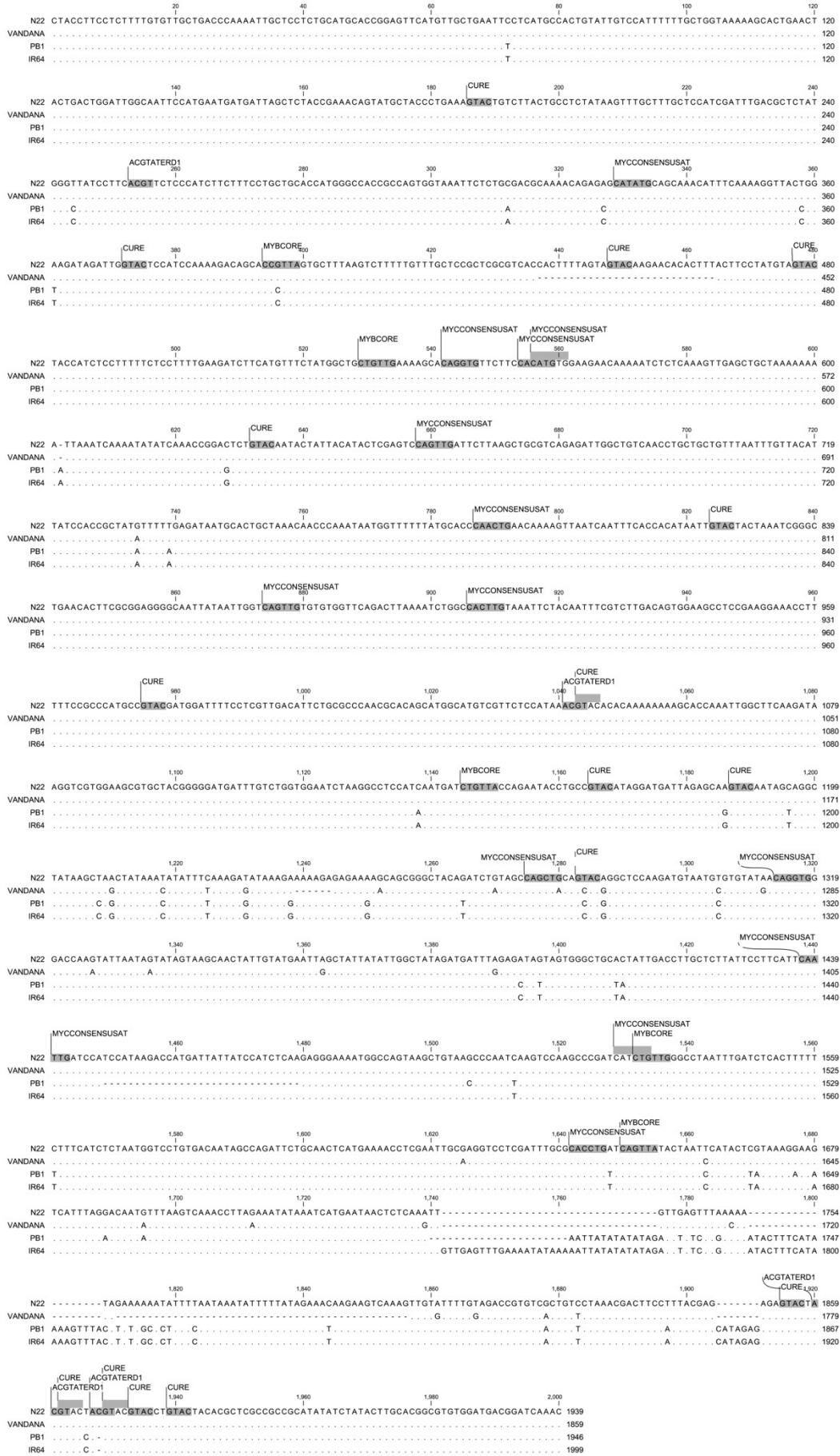


Supplementary Fig. S13. qRT-PCR of copper ATPases in N22 and PB1 flag leaf in response to drought. Three biological replicates were analyzed with three technical repeats. Error bars represent the standard error.

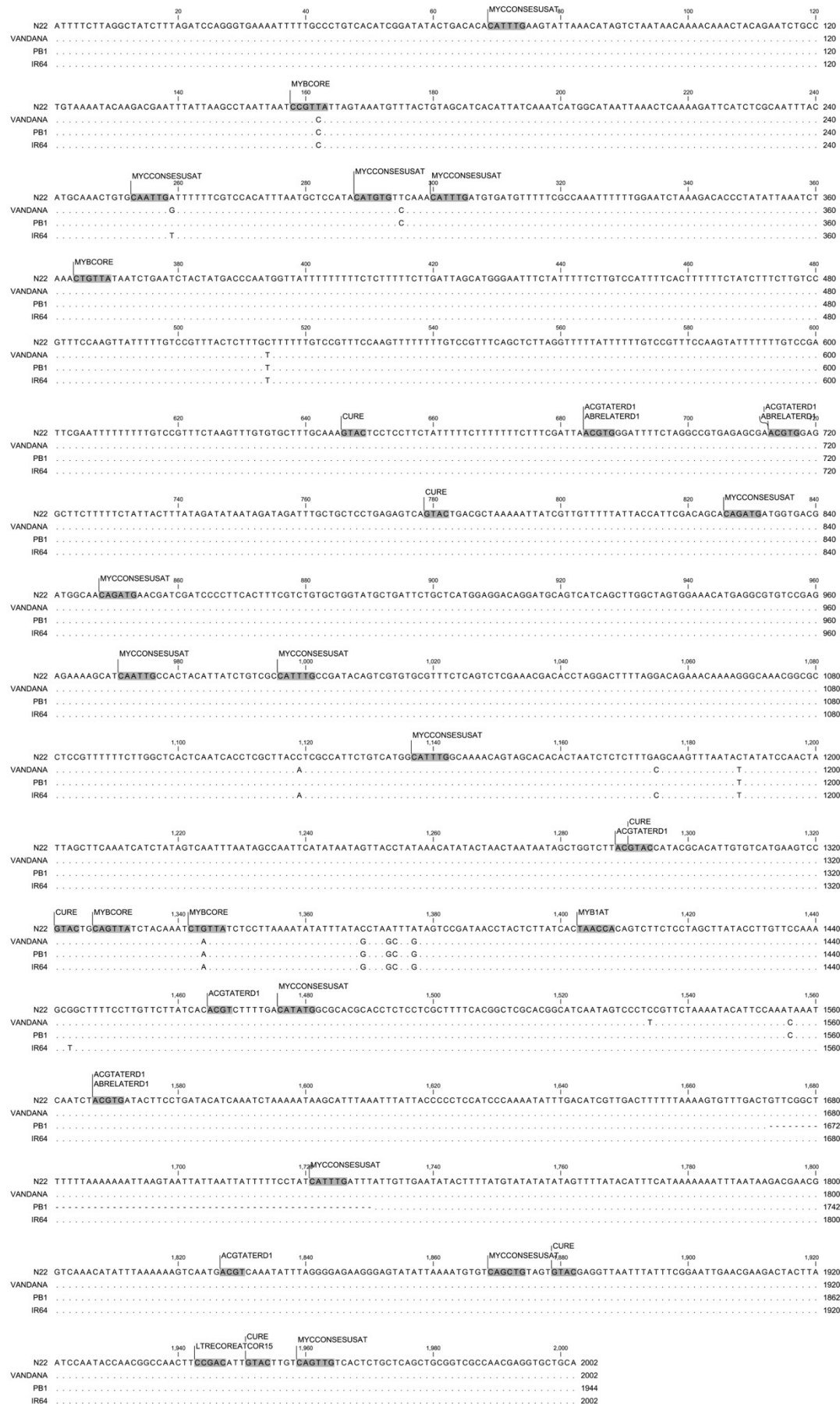
a COPT1



b COPT2



c COPT3



d COPT4

ACGTATERD1

CURE

N22 CTGGTACTAATAACCTTAGGATTTAATTTGTCAAATCTTTTACTTATGTTATGGTGGGTTTACACTCGTCCCTTGCTAATATCTTCCATAGTTGGCTGGTGGTTTACAACCTAGAAAT 120
VANDANA G 120
PB1 120
IR64 120

140 160 180 200 220

CURE MYCCONSENSUSAT

N22 AAATGTTAACTTTTATAGTGGCCGTAGCTTTGTTGGACGGTAAGACTTTGTAGTAAATGATATAATCTCAATGGCTTAACTACTAACCTCCCTCTTTGTAGTAATTTCCAGTGAA 240
VANDANA 240
PB1 240
IR64 240

260 280 300 320 340 360

MYCCONSENSUSAT

N22 CCCACTGCGCTAGTGGCTATTGCTTAAACGAGGAAATAGCGATGTAGTAAACACATGCACAAAGTTACCGGAGGCTAGAGTTGGGATTTCTTTCCAGATTGCGTGAACCTTGAG 360
VANDANA G 360
PB1 A 360
IR64 A 360

380 400 420 440 460 480

MYBCORE CURE

N22 TAAGCGTTGGAGACCTAGTATCCACCTTTGTCATCTTTTGTAGTGGCTCTATTACGAGACGACTTCGTTGTGAGGTGTTAGTCTAACTACCTCTGTTTATTGGTCGGTGGT 480
VANDANA 480
PB1 T 480
IR64 T 480

500 520 540 560 580 600

CURE ACGTATERD1

N22 GTTGTCTTATGCAAGGTGGCTGCTAACTACTATTGACAATGTATGTTGGCTATGACCTATAGTTTGGATGTAGAGGATAGGTTTTAATCATTTTTTAAAAAATAATTGACATTT 600
VANDANA T 599
PB1 G 599
IR64 G 599

620 640 660 680 700 720

CURE ACGTATERD1

N22 TCTCCCTACATGTTTATCTATGTTTGTAGTATTGTTGCTCTCACTAAGCCTAGAGTCTCGCTAGATATAATGATTTTTTGTCTACTTGTACTCCCTCCCTACCTTCAAAT 720
VANDANA 719
PB1 719
IR64 719

740 760 780 800 820 840

N22 TTTTATGAGACAGACACAACTATATACAATAGGATGTTCCATCCAATCGAACAAATATATTTTGGAAATGGGAAAGTGAAGAATTA--GTTGCTAGCATATCTGCACCTTT 837
VANDANA A . C . AG . T 839
PB1 A . C . G 836
IR64 A . C . G 836

860 880 900 920 940 960

MYBIAT DRE1COREZMRAB17

N22 CCCTCCATCCAAAATATAAAAAGGAAACTTTTGAACCACTAAACAGAGTAAACCGGACCGGAGACGAGAATAAGCCAGCGGACACCGGCAACAAGACAATAATTTTTGTTGGCA 957
VANDANA A . . C . . A . . A . . T 959
PB1 A . . A . . A . . T 956
IR64 A . . A . . A . . T 956

980 1,000 1,020 1,040 1,060 1,080

MYBIAT

N22 TTCATCAAAAATATCCCAACATAGCATAGCTTCTAGTGGAAACTGTGAACCACTGAACCAACCACATAGACGATAGGCTTGTAGCTGCTGCAACGGACAAACCAATAAATACCC 1077
VANDANA 1079
PB1 1076
IR64 G 1076

1,100 1,120 1,140 1,160 1,180 1,200

ACGTATERD1

N22 CCTCCGTAAACCGGAGATTTCAAGTTTCAACGAAGGCTGATTTTATTCACCTCAAATATAAAGTTTGAAGAAATAGAAATGATGTACAGAAAGTTAGAAAGTTGTGTGT - AGA 1195
VANDANA G 1197
PB1 A . . A . . A . . A . . GT . A . 1196
IR64 A . . A . . A . . A . . 1194

1,220 1,240 1,260 1,280 1,300 1,320

MYCCONSENSUSAT CURE

N22 AAAGTTCGATATGACGAAAAGTTAAAAGTTAAAGAAAAGTTAGAATCTAAATAGGCCAAAGCGAGAAGCTTCCTTCCAAATCCTGGCTGTGTGATATGTA---CTAGTACT 1311
VANDANA G 1313
PB1 G 1286
IR64 G 1314

1,340 1,360 1,380 1,400 1,420 1,440

N22 CCTTCGCTCAGTAAGTTAATTTTTTATT - TTTTCTATTTATCCAAAATAAATTTATTTTTAAGTAATAATTTGATCAAAATGATGAAAGTAGAAAATAATATATTGGAATAG 1430
VANDANA T . A . . T 1430
PB1 T . A . . T 1400
IR64 G 1431

1,460 1,480 1,500 1,520 1,540 1,560

MYBCORE

N22 AATAATGAGAAATAGTTGCATTGCATTTGATAAAGCAAAAGTATTTAGTTTTTATGATAAATGTAAGAAAGATACTACTCCTCCGTTAAAAAATAAATCTAGAACAGGATA 1550
VANDANA A 1546
PB1 A 1520
IR64 A 1551

1,580 1,600 1,620 1,640 1,660 1,680

CURE

N22 TGACACATTCTACTAAT A GCACACCACACACACCAGCAACGGGGATTAGAAGTTGACACGA 1615
VANDANA AAATATGAACAAAGATATCTAGCACACTGC CACGACACACACACCAGCAACGGGG G 1665
PB1 A . T . T TATG CTGCACACTGC CACGACACACACACCAGCAACGGGG G 1626
IR64 A . T . T AATATAACAAAGATATATGTTTAG G 1641

1,700 1,720 1,740 1,760 1,780 1,800

MYCCONSENSUSAT MYBCORE

N22 CTGTCTACTGGAATCCTAGCTGTGCTGCGGTAGCACTGTCTCAGATGCTGATACTCCTGATAGCCATAGCCACGACGGATGGGTCCCATCCATCCTCCCTCCTGTCCGTTG 1735
VANDANA 1785
PB1 1746
IR64 1761

1,820 1,840 1,860 1,880 1,900 1,920

ACGTATERD1

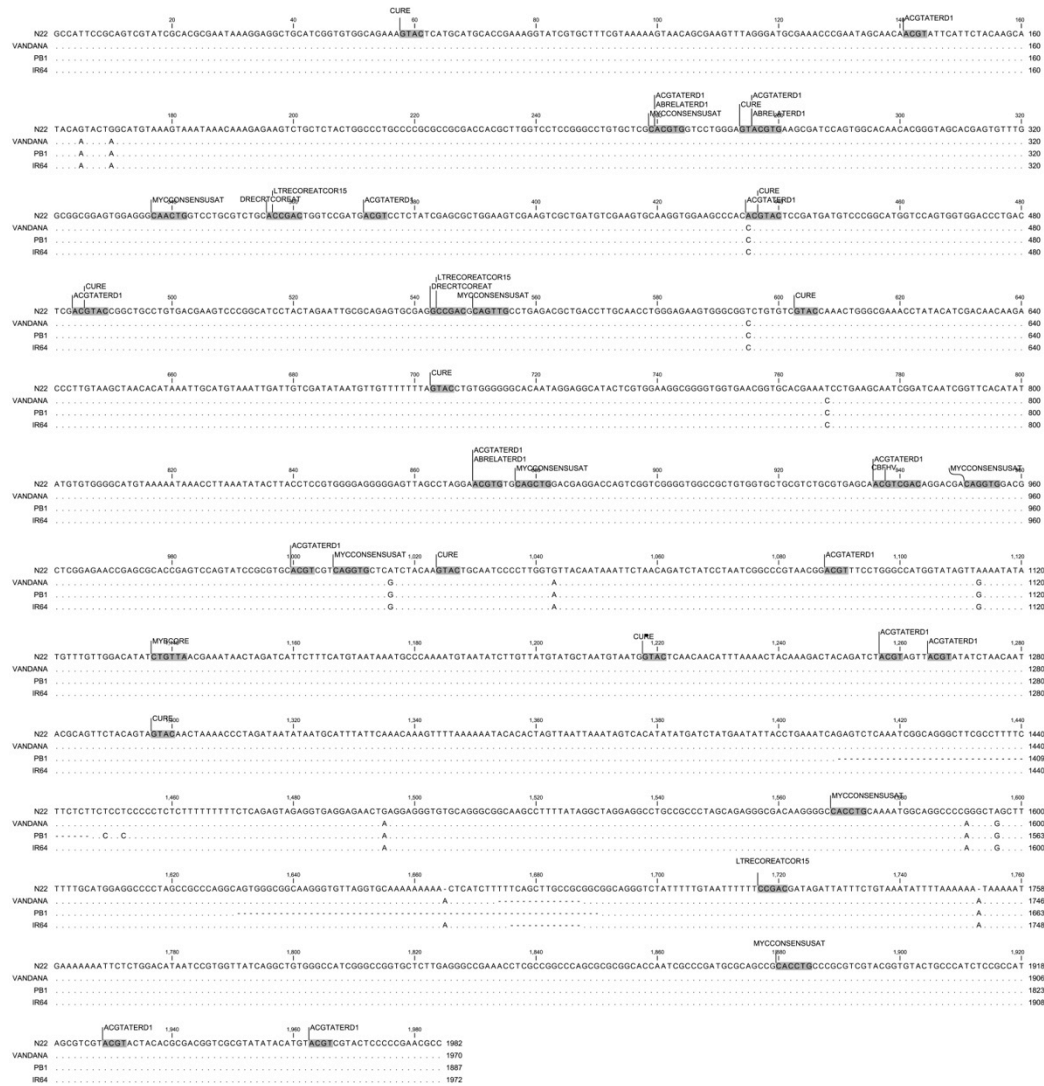
N22 TTTTGGCTCAGGTTCAAGTTCAGTGCAGTACAGGCGGGACGCCCAAAAACTGCCACGCTGTGTGCCGCGCGCCACCGGATCCCCTGATCCCATTAACGTTTACCCGCGCGCC 1855
VANDANA 1905
PB1 1866
IR64 1881

1,940 1,960 1,980

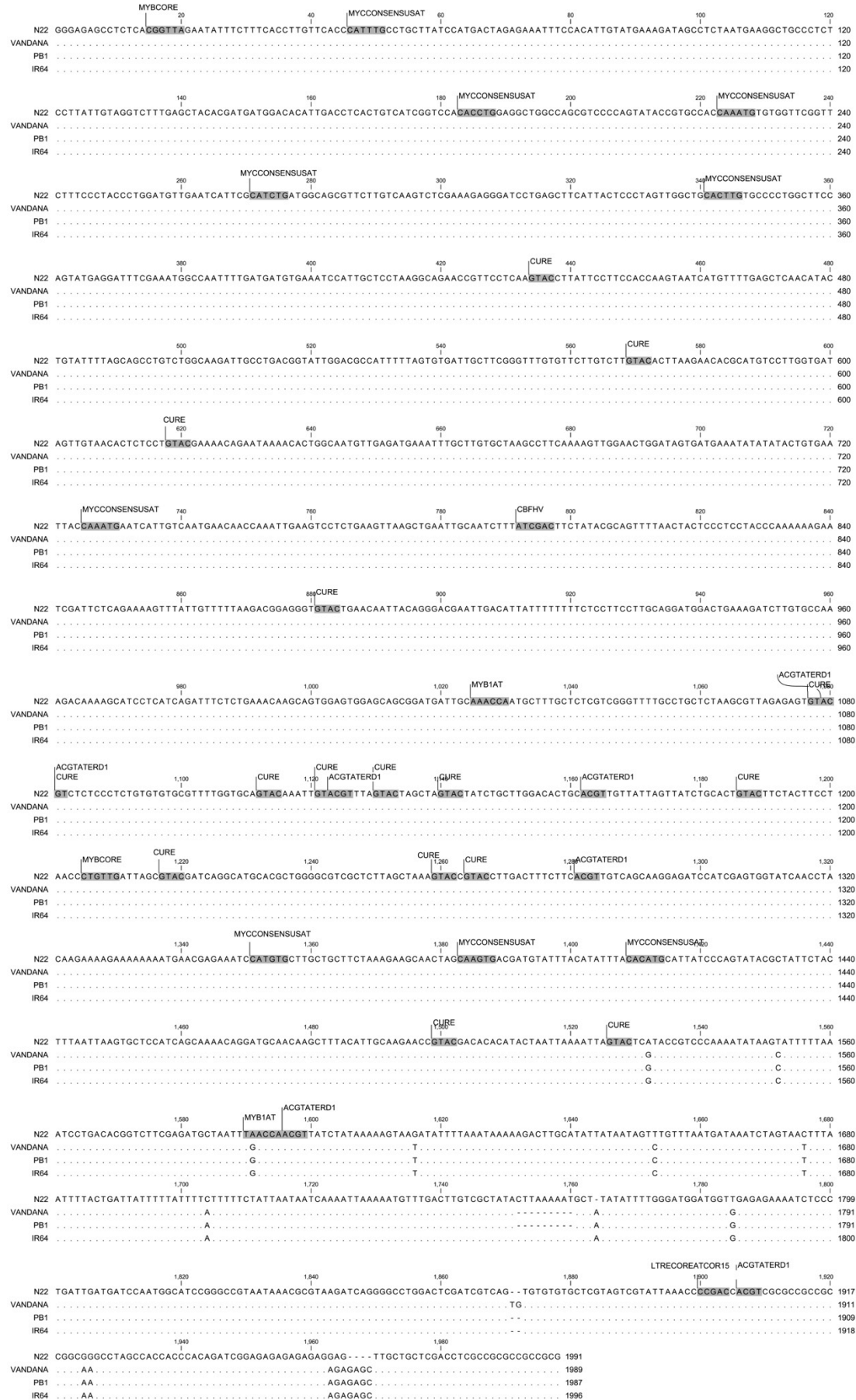
MYCCONSENSUSAT

N22 CACCTGCCGCTGCAATATTTTATTT - - CGCCGGCC - - CGCGCCACGCCACCTTGCCGCGCGCCGACA 1922
VANDANA CGCCGGC . C . . ACG . A G . . 1978
PB1 CGCCGGC . C G . . 1936
IR64 G . . 1948

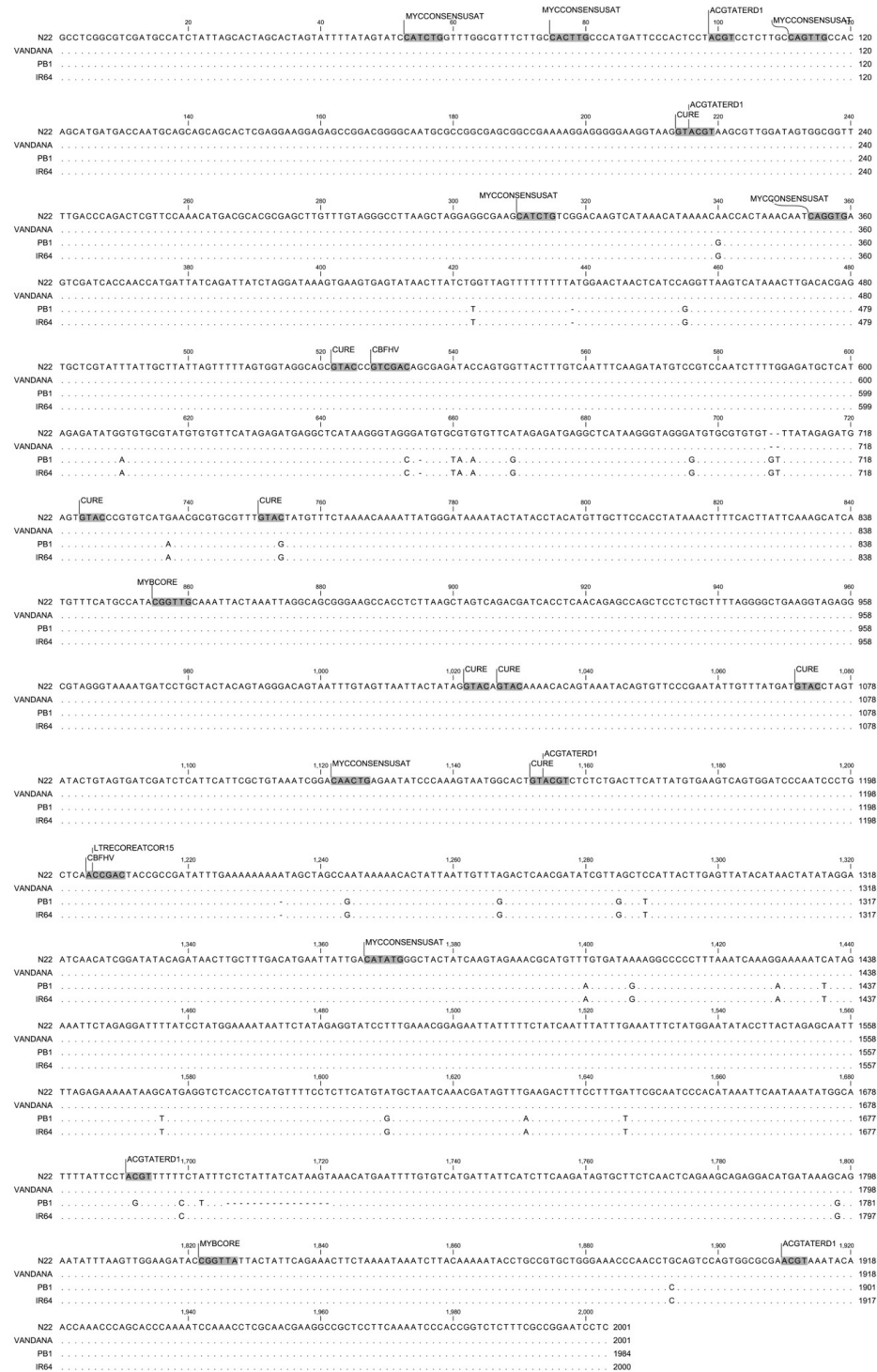
e COPT5



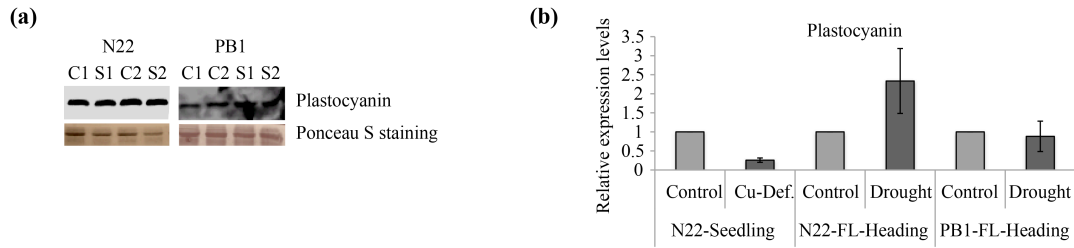
f COPT6



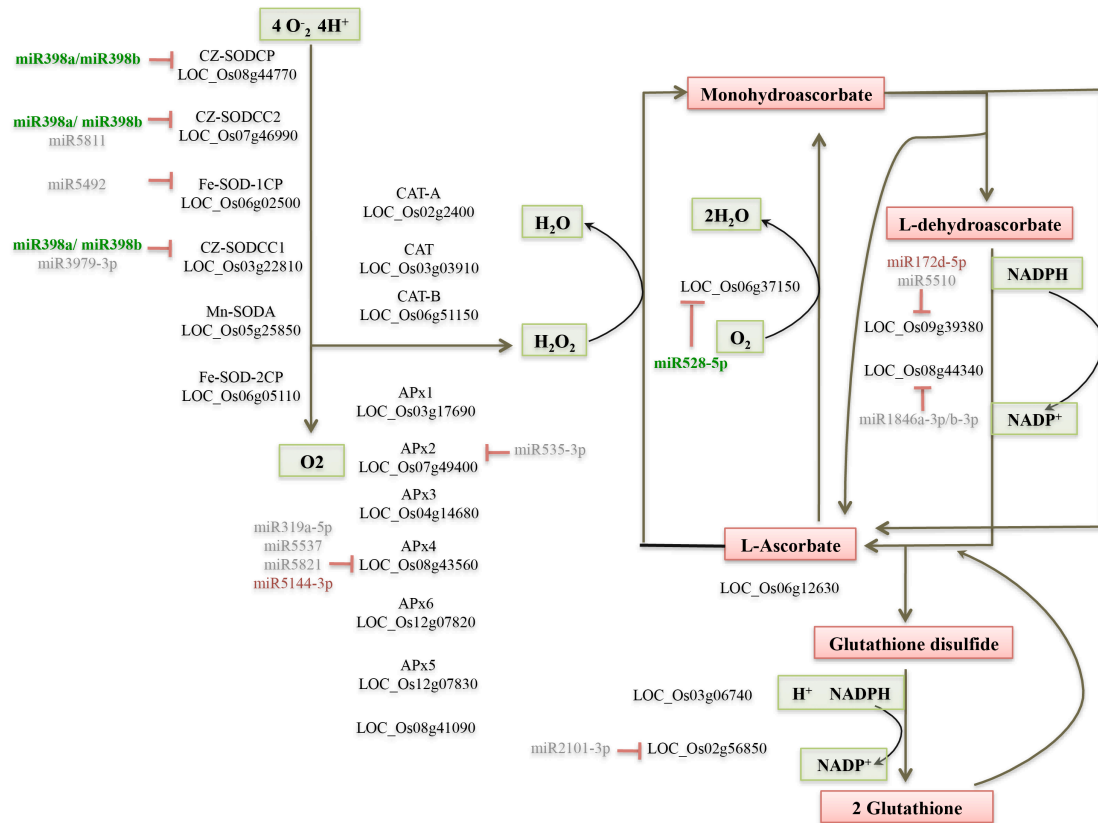
g COPT7



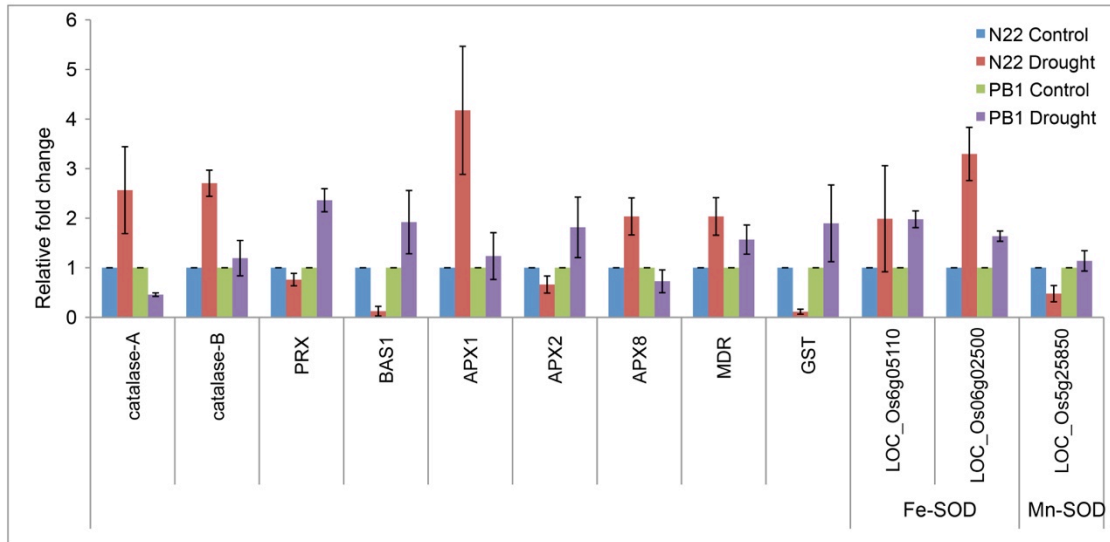
Supplementary Fig. S14. Comparative sequence alignment of upstream regulatory region of the seven rice copper transporters. Sequence alignment of 2kb upstream region of (a) COPT1, (b) COPT2, (c) COPT3, (d) COPT4, (e) COPT5, (f) COPT6, (g) COPT7 in four cultivars namely N22, Vandana, PB1 and IR64. Dots (.) represents the identical sequence, dashes (-) represents gaps and the cis-acting elements associated with stress were highlighted



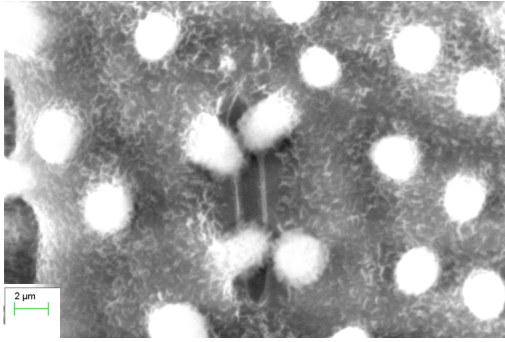
Supplementary Fig. 15. Differential copper homeostasis does not modulate plastocyanin levels but affects ROS homeostasis. (a) Quantitative immunoblot showing the plastocyanin protein levels in N22 and PB1 flag leaf under control (C) and drought stress (S) conditions. Ponceau S staining was used to show the equal loading of samples. (b) Relative expression analysis of plastocyanin in N22 seedlings under copper starvation and in N22 and PB1 flag leaf under drought. Three biological with three technical replicates were analyzed.



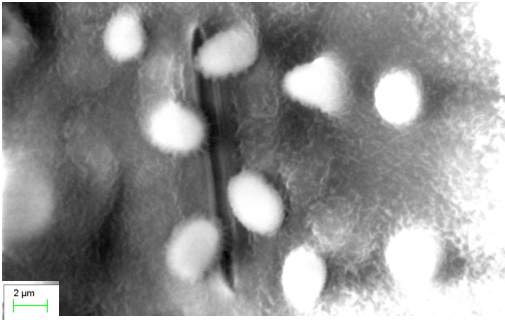
Supplementary Fig. S16. miRNA-mediated regulation of the pathways involved in the acquisition of ROS detoxification of ROS through removal of superoxide ions and ascorbate glutathione cycle. All genes were searched for putative targeting miRNA in the PARE data and the miRNA in green are significantly upregulated in N22 flag leaf while downregulated in PB1 flag leaf. miRNA in red are low in abundance but downregulated in N22 flag leaf and upregulated in PB1 flag leaf.



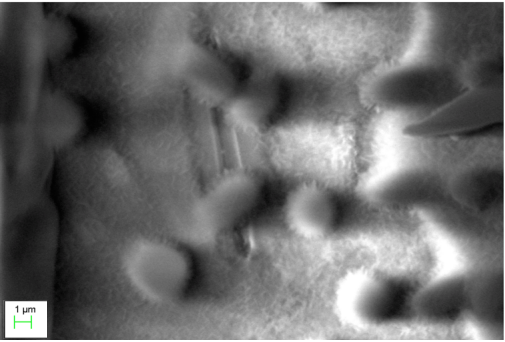
Supplementary Fig. S17. Expression analysis of genes involved in ROS homeostasis. qRT-PCR analysis of genes involved in the maintenance of ROS homeostasis in the flag leaf of N22 and PB1 under drought. Data represents the analyzed value of at least three biological and technical replicate. Error bars represents the standard error.



Open stomata



Partially-open stomata



Completely closed stomata

Supplementary Fig. S18. Electron micrographs showing the completely open, partially open and closed stomata in PB1 flag leaf.