Genome-wide Identification and Characterization of Heat Shock Protein Family Reveals Role in Development and Stress Conditions in *Triticum aestivum* L.

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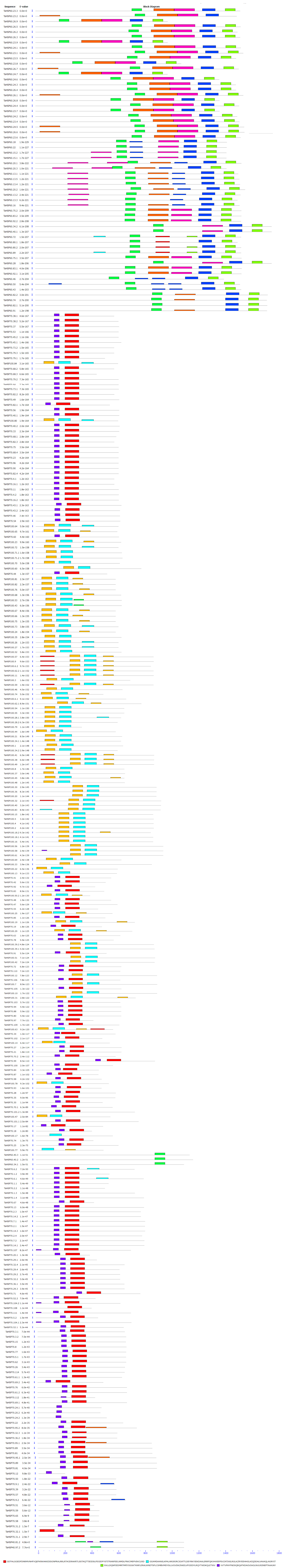

SFig. 1: Conserved Motifs of TaHSPs elucidated by MEME. Colored boxes representing different conserved motifs with different sequences and size.


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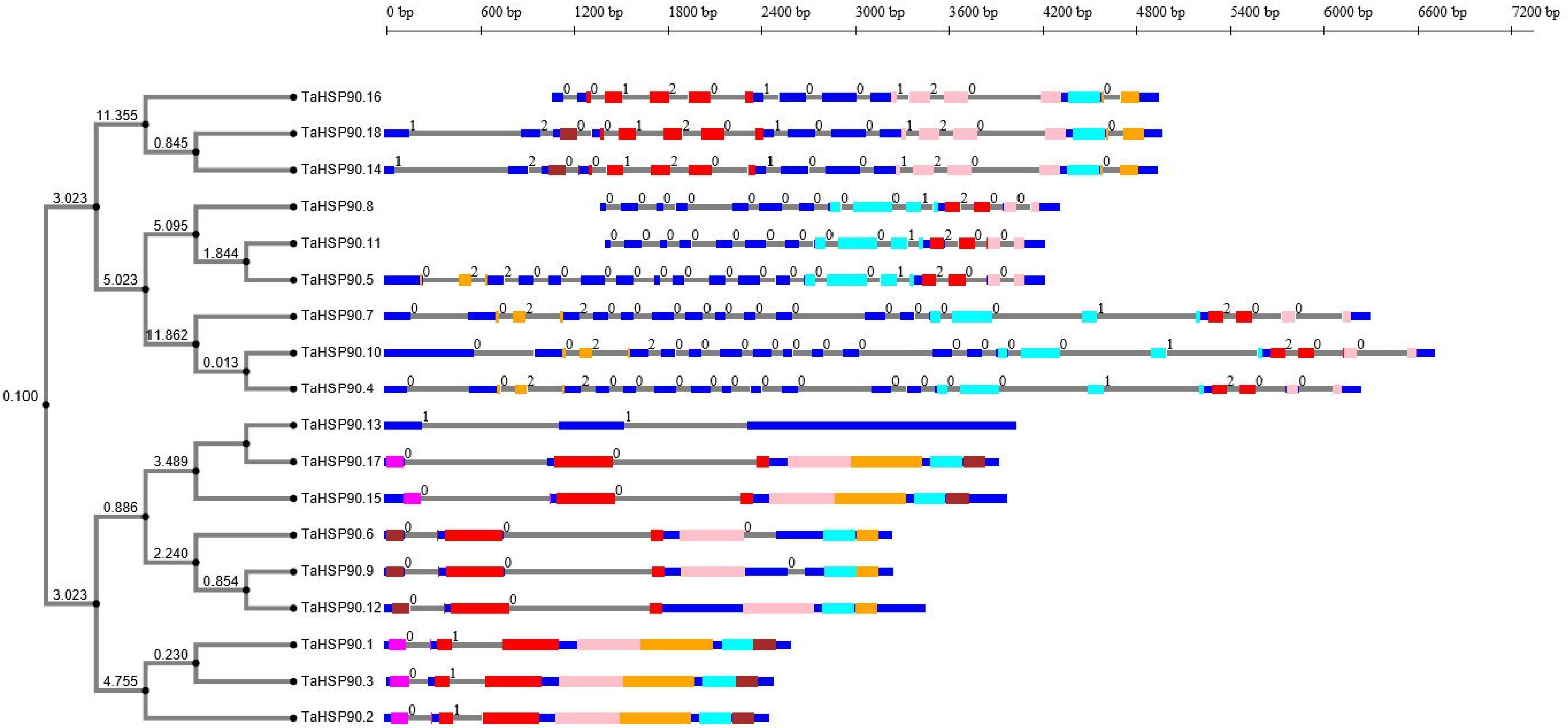
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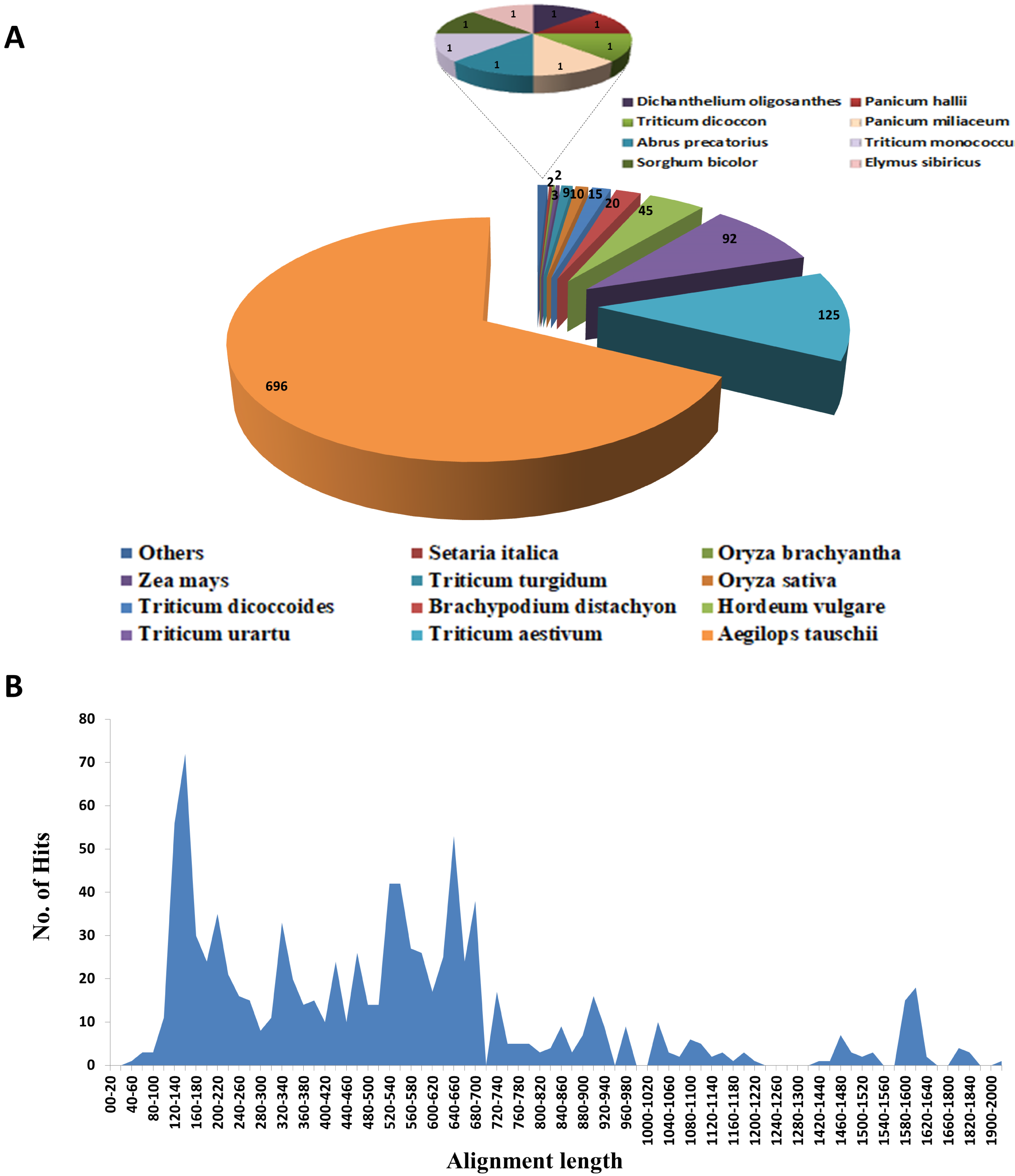
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Elymus sibiricus
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Zea mays
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Oryza sativa
Triticum dicoccoides
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Hordeum vulgare
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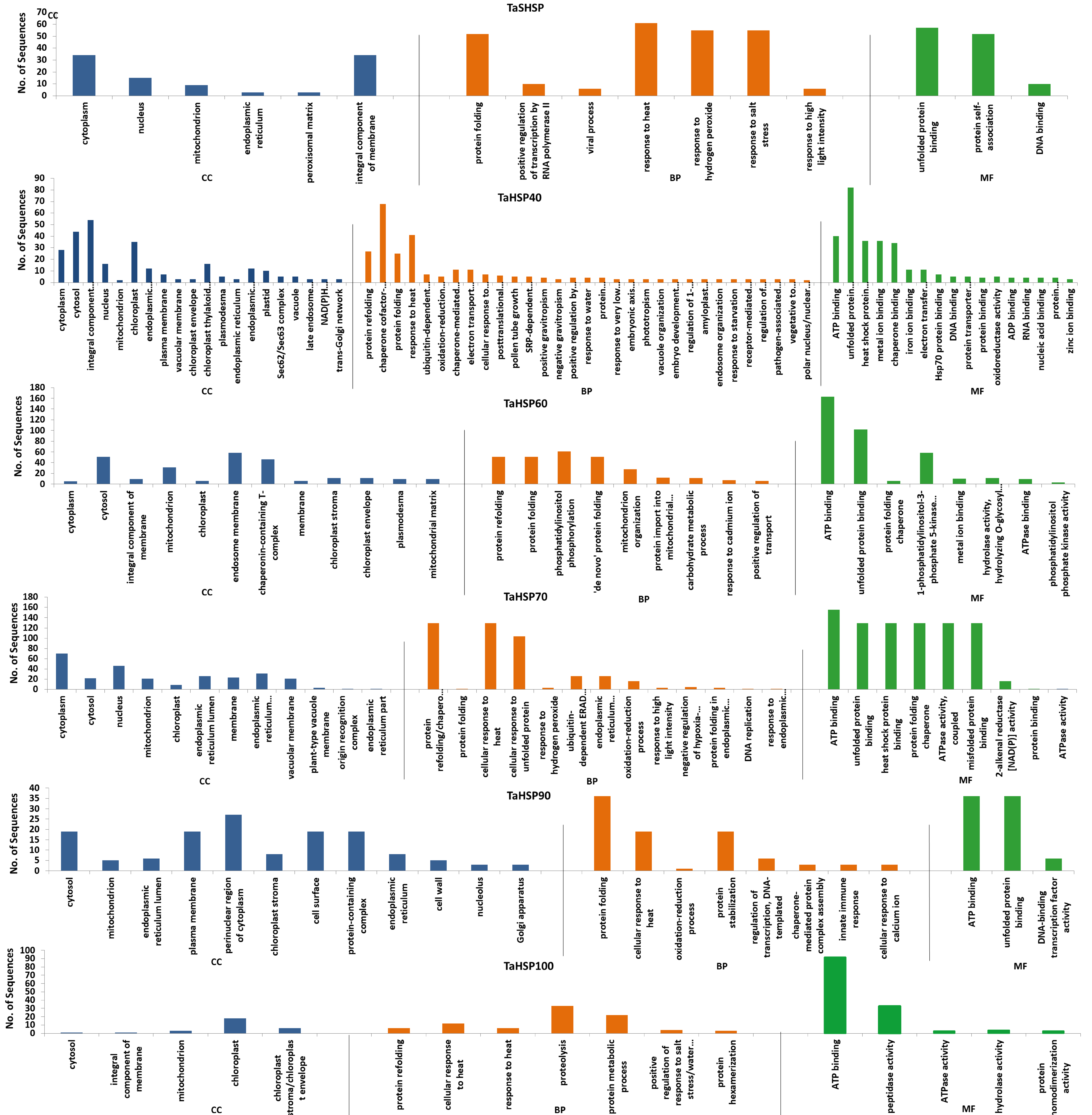TaSHSP
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integral component of membrane
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viral process
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response to hydrogen peroxide
response to salt stress
response to high light intensity
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endoplasmic…
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vacuole
late endosome…
NAD(P)H…
trans-Golgi network
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chaperone cofactor-…
protein folding
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ubiquitin-dependent…
oxidation-reduction…
chaperone-mediated…
electron transport…
cellular response to…
posttranslational…
pollen tube growth
SRP-dependent…
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negative gravitropism
positive regulation by…
response to water
protein…
response to very low…
embryonic axis…
phototropism
vacuole organization
embryo development…
regulation of 1-…
amyloplast…
endosome organization
response to starvation
receptor-mediated…
regulation of…
pathogen-associated…
vegetative to…
polar nucleus/nuclear…
ATP binding
unfolded protein…
heat shock protein…
metal ion binding
chaperone binding
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DNA binding
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ATP binding
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response to endoplasmic…
ATP binding
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protein binding
ATPase activity
CC
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cell surface
protein-containing complex
endoplasmic reticulum
cell wall
nucleolus
Golgi apparatus
protein folding
cellular response to heat
oxidation-reduction process
protein stabilization
regulation of transcription, DNA-templated
chaperone-mediated protein complex assembly
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cellular response to calcium ion
ATP binding
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chloroplast
chloroplast stroma/chloroplast envelope
protein refolding
cellular response to heat
response to heat
proteolysis
protein metabolic process
positive regulation of response to salt stress/water…
protein hexamerization
ATP binding
peptidase activity
ATPase activity
hydrolase activity
protein homodimerization activity
CC
BP
MF


GO:0008150 biological_process
GO:0050896 (6.21e-10) response to stimulus 12/30 | 1985/52381
GO:0006950 (1.68e-10) response to stress 12/30 | 1665/52381
TaSHSP
GO:0008150 biological_process
GO:0009987 cellular process
GO:0008152 metabolic process
GO:0044237 cellular metabolic process
GO:0043170 macromolecule metabolic process
GO:0044238 primary metabolic process
GO:0044260 cellular macromolecule metabolic process
GO:0019538 protein metabolic process
GO:0044267 cellular protein metabolic process
GO:0006457 (1.27e-13) protein folding 13/86 | 294/52381
TaHSP40
GO:0008150 biological_process
GO:0032502 (1.33e-09) developmental process 6/49 | 78/52381
GO:0032501 (2.64e-06) multicellular organismal process 6/49 | 317/52381
GO:0016043 cellular component organization
GO:0009987 (0.00016) cellular process 35/49 | 22228/52381
GO:0008152 (0.0186) metabolic process 35/49 | 27447/52381
GO:0007275 (6e-10) multicellular organismal development 6/49 | 66/52381
GO:0006996 (0.00984) organelle organization 5/49 | 1013/52381
GO:0044237 (2.29e-07) cellular metabolic process 35/49 | 17255/52381
GO:0043170 (1.69e-09) macromolecule metabolic process 35/49 | 14522/52381
GO:0044238 (1.42e-06) primary metabolic process 35/49 | 18481/52381
GO:0009790 (1.37e-12) embryonic development 6/49 | 20/52381
GO:0044260 (8.11e-11) cellular macromolecule metabolic process 35/49 | 12930/52381
GO:0019538 (8.37e-15) protein metabolic process 35/49 | 9488/52381
GO:0044267 (7.05e-17) cellular protein metabolic process 35/49 | 8078/52381
GO:0006457 (1.05e-36) protein folding 23/49 | 294/52381
TaHSP60
GO:0008150 biological_process
GO:0050896 (5.73e-14) response to stimulus 18/41 | 1985/52381
GO:0009987 cellular process
GO:0008152 metabolic process
GO:0042221 (0.00644) response to chemical stimulus 5/41 | 987/52381
GO:0006950 (5.73e-14) response to stress 17/41 | 1665/52381
GO:0009628 (6.61e-17) response to abiotic stimulus 10/41 | 99/52381
GO:0044237 cellular metabolic process
GO:0043170 macromolecule metabolic process
GO:0044238 primary metabolic process
GO:0010035 (5.69e-12) response to inorganic substance 5/41 | 9/52381
GO:0006979 (0.00115) response to oxidative stress 5/41 | 659/52381
GO:0009314 (1.46e-11) response to radiation 5/41 | 12/52381
GO:0009266 (2.08e-08) response to temperature stimulus 5/41 | 65/52381
GO:0044260 cellular macromolecule metabolic process
GO:0019538 protein metabolic process
GO:0009416 (5.69e-12) response to light stimulus 5/41 | 9/52381
GO:0044267 cellular protein metabolic process
GO:0006457 (1.71e-11) protein folding 9/41 | 294/52381
TaHSP70
GO:0008150 biological_process
GO:0050896 (5.12e-06) response to stimulus 7/14 | 1985/52381
GO:0009987 cellular process
GO:0008152 metabolic process
GO:0006950 (2.78e-05) response to stress 6/14 | 1665/52381
GO:0044237 cellular metabolic process
GO:0043170 (0.0222) macromolecule metabolic process 9/14 | 14522/52381
GO:0044238 primary metabolic process
GO:0044260 (0.0109) cellular macromolecule metabolic process 9/14 | 12930/52381
GO:0019538 (0.00115) protein metabolic process 9/14 | 9488/52381
GO:0044267 (0.000391) cellular protein metabolic process 9/14 | 8078/52381
GO:0006457 (4.16e-16) protein folding 9/14 | 294/52381
TaHSP90
GO:0008150 biological_process
GO:0050896 (1.21e-14) response to stimulus 14/20 | 1985/52381
GO:0009987 cellular process
GO:0008152 metabolic process
GO:0006950 (2.16e-15) response to stress 14/20 | 1665/52381
GO:0051716 (2.25e-07) cellular response to stimulus 6/20 | 499/52381
GO:0044237 cellular metabolic process
GO:0006807 nitrogen compound metabolic process
GO:0044238 (0.00201) primary metabolic process 15/20 | 18481/52381
GO:0043170 (9.92e-05) macromolecule metabolic process 15/20 | 14522/52381
GO:0033554 (2.25e-07) cellular response to stress 6/20 | 496/52381
GO:0006139 nucleobase, nucleoside, nucleotide and nucleic acid metabolic process
GO:0044260 cellular macromolecule metabolic process
GO:0019538 (0.0271) protein metabolic process 9/20 | 9488/52381
GO:0006974 (2.25e-07) response to DNA damage stimulus 6/20 | 492/52381
GO:0006259 (3.21e-05) DNA metabolic process 6/20 | 1204/52381
GO:0006281 (2.25e-07) DNA repair 6/20 | 489/52381
GO:0006289 (5.97e-13) nucleotide-excision repair 6/20 | 47/52381
TaHSP100
Level9
Level8
Level7
Level6
Level5
Level4
Level3
Level2
Level1
is_a
part_of
positive_regulate
regulate
negative_regulate
two significant nodes
one significant node


TaSHSP
TaHSP40
TaHSP60
TaHSP70
TaHSP90
TaHSP100
Level9
Level8
Level7
Level6
Level5
Level4
Level3
Level2
Level1
is_a
part_of
positive_regulate
regulate
negative_regulate
two significant nodes
one significant node


TaSHSP
Number of TaSHSP proteins
0
20
40
60
80
100
120
anchored component of plasma membrane
chloroplast
chloroplast outer membrane
endomembrane system
extracellular space
mitochondrion
nucleus
organelle membrane
TaHSP40
Number of TaHSP40 proteins
0
50
100
150
200
250
chloroplast
chloroplast outer membrane
cytoplasm
endomembrane system
extracellular space
mitochondrion
nucleus
plasma membrane
TaHSP60
Number of TaHSP60 proteins
0
10
20
30
40
50
60
70
80
chloroplast
chloroplast outer membrane
chloroplast stroma
chloroplast thylakoid membrane
cytoplasm
endomembrane system
mitochondrion
nucleus
plasma membrane
TaHSP70
Number of TaHSP70 proteins
0
10
20
30
40
50
60
70
80
90
100
chloroplast
chloroplast outer membrane
cytoplasm
extracellular space
mitochondrion
nucleus
TaHSP90
Number of TaHSP90 proteins
0
5
10
15
20
25
chloroplast
chloroplast outer membrane
chloroplast thylakoid membrane
extracellular space
mitochondrion
nucleus
TaHSP100
Number of TaHSP100
0
5
10
15
20
25
30
chloroplast
chloroplast outer membrane
cytoplasm
extracellular space
mitochondrion
nucleus
organelle membrane


GO:0003674 molecular_function
GO:0005488 (1.61e-11) binding 75/86 | 27509/52381
GO:0005515 (1.47e-47) protein binding 73/86 | 7461/52381
GO:0051082 (3.74e-28) unfolded protein binding 18/86 | 137/52381
TaHSP40
GO:0003674 molecular_function
GO:0003824 catalytic activity
GO:0005488 binding
GO:0016740 transferase activity
GO:0043167 ion binding
GO:0001882 (7.88e-06) nucleoside binding 22/49 | 8054/52381
GO:0000166 (4.57e-06) nucleotide binding 24/49 | 9089/52381
GO:0005515 (1.45e-07) protein binding 24/49 | 7461/52381
GO:0016772 transferase activity, transferring phosphorus-containing groups
GO:0043169 cation binding
GO:0001883 (6.67e-06) purine nucleoside binding 22/49 | 7934/52381
GO:0017076 (2.11e-05) purine nucleotide binding 22/49 | 8650/52381
GO:0032553 (8.03e-06) ribonucleotide binding 22/49 | 8141/52381
GO:0051082 (9.98e-39) unfolded protein binding 21/49 | 137/52381
GO:0016301 kinase activity
GO:0046872 metal ion binding
GO:0030554 (6.67e-06) adenyl nucleotide binding 22/49 | 7934/52381
GO:0032555 (8.03e-06) purine ribonucleotide binding 22/49 | 8141/52381
GO:0001727 (6.16e-13) lipid kinase activity 8/49 | 80/52381
GO:0004428 (2.69e-12) inositol or phosphatidylinositol kinase activity 8/49 | 101/52381
GO:0016773 phosphotransferase activity, alcohol group as acceptor
GO:0046914 transition metal ion binding
GO:0032559 (3.07e-06) adenyl ribonucleotide binding 22/49 | 7440/52381
GO:0016307 (5.14e-13) phosphatidylinositol phosphate kinase activity 8/49 | 74/52381
GO:0008270 (0.00182) zinc ion binding 8/49 | 1834/52381
GO:0005524 (1.45e-07) ATP binding 22/49 | 6235/52381
TaHSP60
GO:0003674 molecular_function
GO:0005488 binding
GO:0000166 (2.34e-09) nucleotide binding 26/41 | 9089/52381
GO:0001882 (2.69e-06) nucleoside binding 20/41 | 8054/52381
GO:0032553 (2.69e-06) ribonucleotide binding 20/41 | 8141/52381
GO:0017076 (6.41e-06) purine nucleotide binding 20/41 | 8650/52381
GO:0001883 (2.69e-06) purine nucleoside binding 20/41 | 7934/52381
GO:0032555 (2.69e-06) purine ribonucleotide binding 20/41 | 8141/52381
GO:0030554 (2.69e-06) adenyl nucleotide binding 20/41 | 7934/52381
GO:0032559 (1.62e-06) adenyl ribonucleotide binding 20/41 | 7440/52381
GO:0005524 (1.2e-07) ATP binding 20/41 | 6235/52381
TaHSP70
GO:0003674 molecular_function
GO:0005488 binding
GO:0000166 (0.0176) nucleotide binding 7/14 | 9089/52381
GO:0005515 (0.00424) protein binding 8/14 | 7461/52381
GO:0001882 (0.0113) nucleoside binding 7/14 | 8054/52381
GO:0032553 (0.0113) ribonucleotide binding 7/14 | 8141/52381
GO:0017076 (0.0145) purine nucleotide binding 7/14 | 8650/52381
GO:0051082 (3.12e-16) unfolded protein binding 8/14 | 137/52381
GO:0001883 (0.0113) purine nucleoside binding 7/14 | 7934/52381
GO:0032555 (0.0113) purine ribonucleotide binding 7/14 | 8141/52381
GO:0030554 (0.0113) adenyl nucleotide binding 7/14 | 7934/52381
GO:0032559 (0.0113) adenyl ribonucleotide binding 7/14 | 7440/52381
GO:0005524 (0.00673) ATP binding 7/14 | 6235/52381
TaHSP90
GO:0003674 molecular_function
GO:0005488 (0.0108) binding 17/20 | 27509/52381
GO:0003824 catalytic activity
GO:0003676 nucleic acid binding
GO:0001882 (4.57e-07) nucleoside binding 14/20 | 8054/52381
GO:0000166 (2.29e-07) nucleotide binding 15/20 | 9089/52381
GO:0005515 (2.29e-07) protein binding 14/20 | 7461/52381
GO:0016787 (1.46e-08) hydrolase activity 15/20 | 7246/52381
GO:0003677 (0.00574) DNA binding 6/20 | 3401/52381
GO:0001883 (4.05e-07) purine nucleoside binding 14/20 | 7934/52381
GO:0017076 (9.49e-07) purine nucleotide binding 14/20 | 8650/52381
GO:0032553 (4.6e-07) ribonucleotide binding 14/20 | 8141/52381
GO:0008233 (9.98e-05) peptidase activity 6/20 | 1599/52381
GO:0016788 (3.2e-06) hydrolase activity, acting on ester bonds 7/20 | 1415/52381
GO:0016817 (3.82e-14) hydrolase activity, acting on acid anhydrides 14/20 | 2219/52381
GO:0030554 (4.05e-07) adenyl nucleotide binding 14/20 | 7934/52381
GO:0032555 (4.6e-07) purine ribonucleotide binding 14/20 | 8141/52381
GO:0004518 (1.59e-10) nuclease activity 7/20 | 278/52381
GO:0016818 (2.39e-14) hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides 14/20 | 2110/52381
GO:0032559 (2.29e-07) adenyl ribonucleotide binding 14/20 | 7440/52381
GO:0016462 (2.39e-14) pyrophosphatase activity 14/20 | 2076/52381
GO:0005524 (2.79e-08) ATP binding 14/20 | 6235/52381
GO:0017111 (2.39e-14) nucleoside-triphosphatase activity 14/20 | 2032/52381
GO:0070011 (8.68e-05) peptidase activity, acting on L-amino acid peptides 6/20 | 1547/52381
GO:0016887 (1.62e-05) ATPase activity 6/20 | 1141/52381
GO:0042623 (2.22e-06) ATPase activity, coupled 6/20 | 793/52381
GO:0004176 (2.39e-14) ATP-dependent peptidase activity 6/20 | 24/52381
TaHSP100
Level9
Level8
Level7
Level6
Level5
Level4
Level3
Level2
Level1
is_a
part_of
positive_regulate
regulate
negative_regulate
two significant nodes
one significant node


stress_type
3
2
1
0
−1
−2
stress_type
BGT
Drought
Heat
Heat_and_Drought
PST
Septoria
HSP_family
TaHSP100
TaHSP40
TaHSP60
TaHSP70
TaHSP90
TaSHSP
HSP_family
BGT_Leaf_24dpi
BGT_Leaf_72dpi
BGT_Leaf_48dpi
Septoria_10dai
Septoria_13dai
PST_Leaf_72dpi
PST_Leaf_48dpi
PST_Leaf_24_dpi
Septoria_4dai
DS_6h
DS_1h
HS_6h
HS+DS_6h
HS_1h
HS+DS_1h


stage
3
2
1
0
−1
−2
stage
Flowering
Grain Development
Seedling
Vegetative
HSP_family
TaHSP100
TaHSP40
TaHSP60
TaHSP70
TaHSP90
TaSHSP
HSP_family
Root_z10 (S)
Root_z13 (V)
Root_z39 (V)
Spike_z32 (V)
Stem_z30 (V)
Spike_z39 (V)
Leaf_z10 (S)
Leaf_z23 (V)
Leaf_z71 (G)
Stem_z32 (V)
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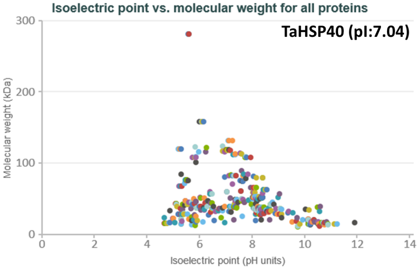
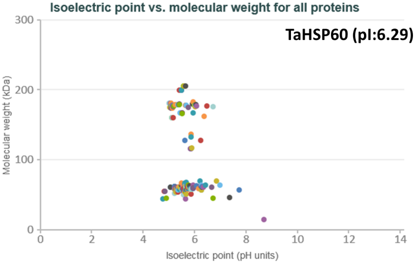
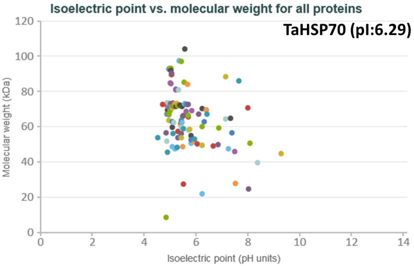
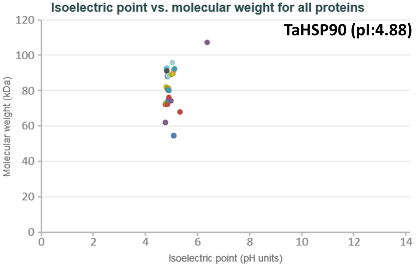
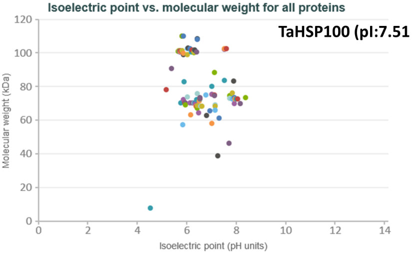
Grain_z85 (G)

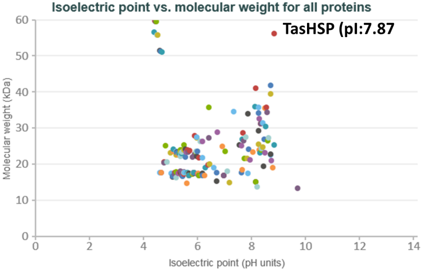

Isoelectric point vs. molecular weight for all proteins

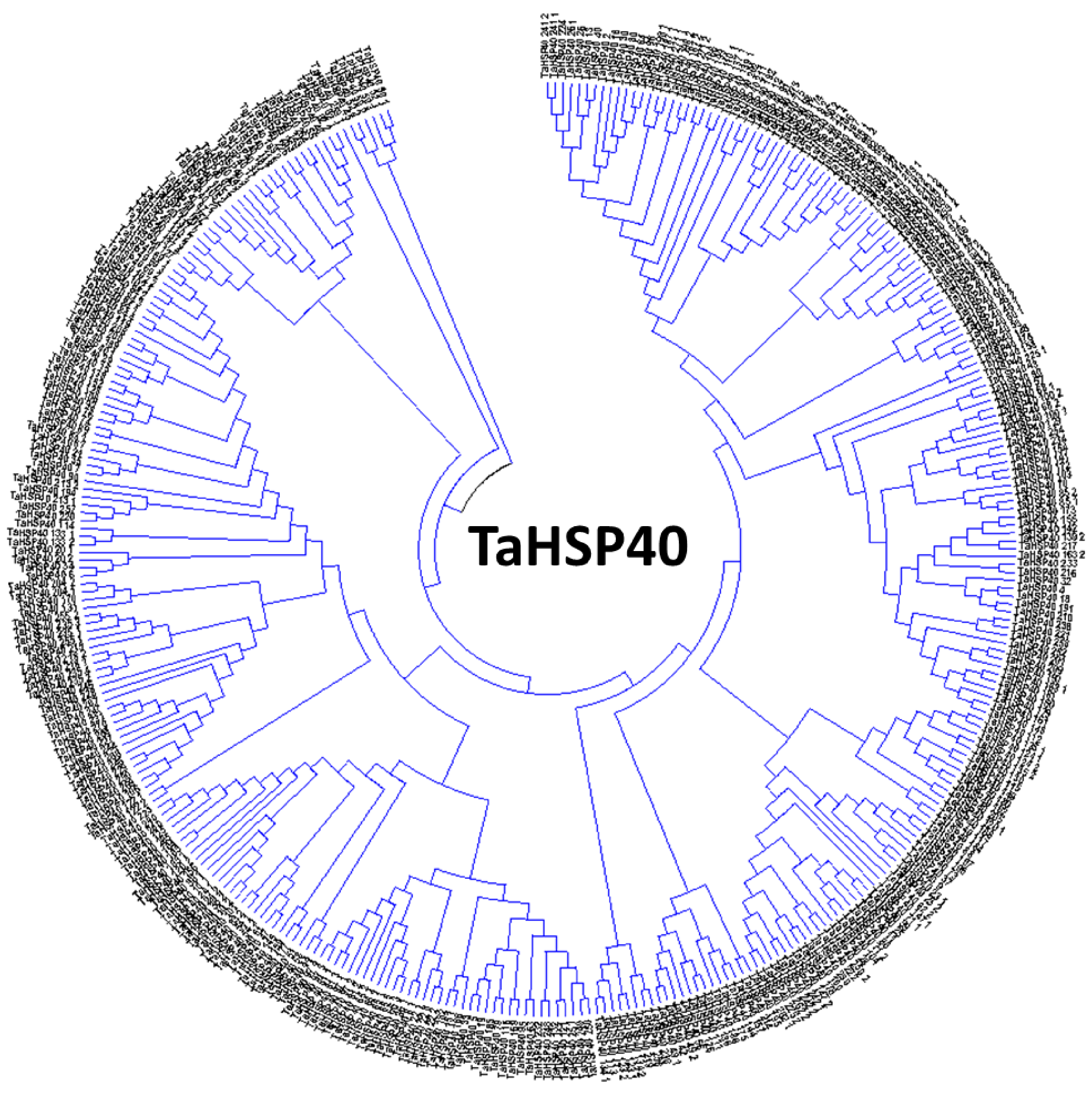

Isoelectric point vs. molecular weight for all proteins


Isoelectric point vs. molecular weight for all proteins


Isoelectric point vs. molecular weight for all proteins


Isoelectric point vs. molecular weight for all proteins


Isoelectric point vs. molecular weight for all proteins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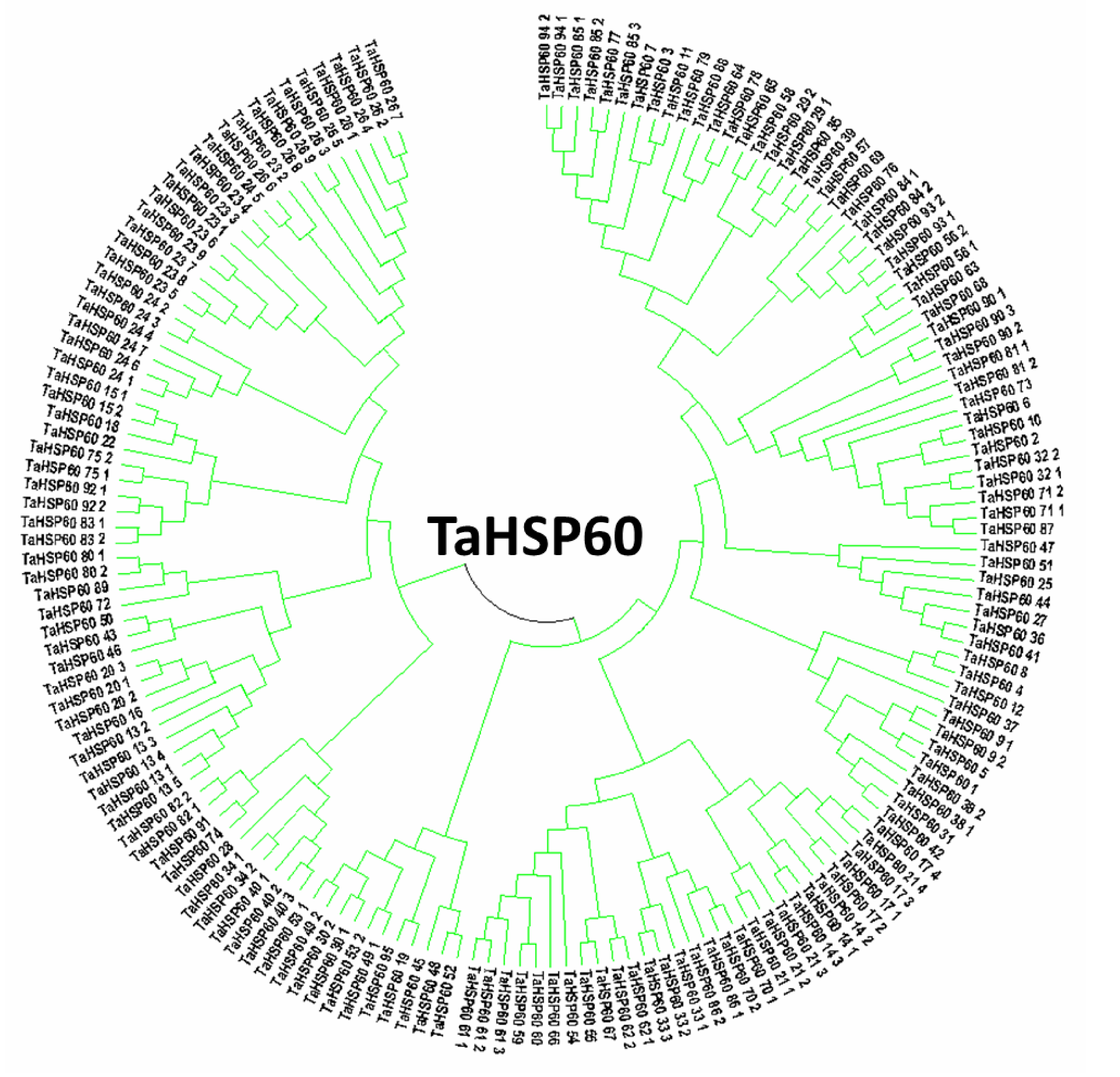
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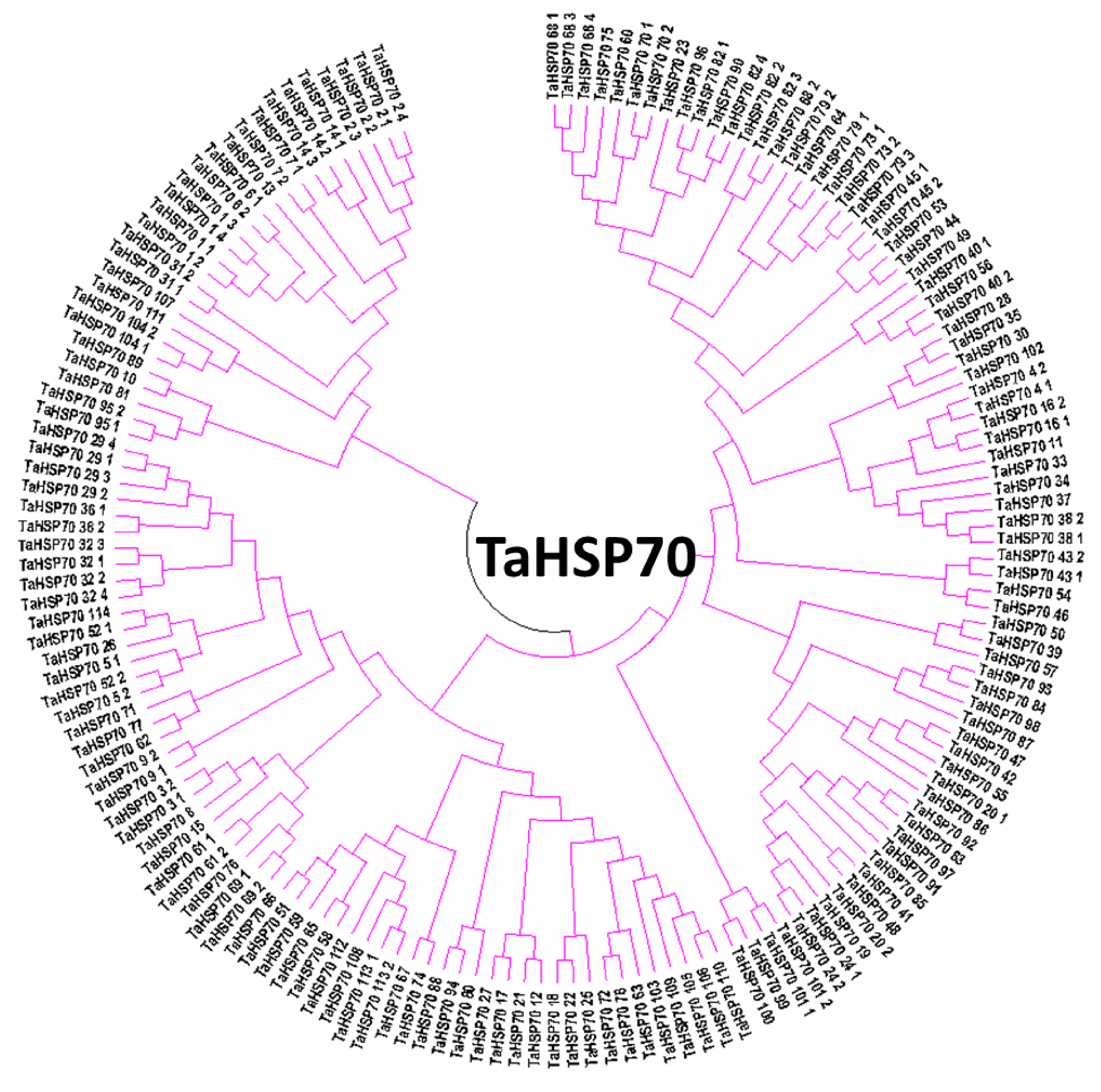
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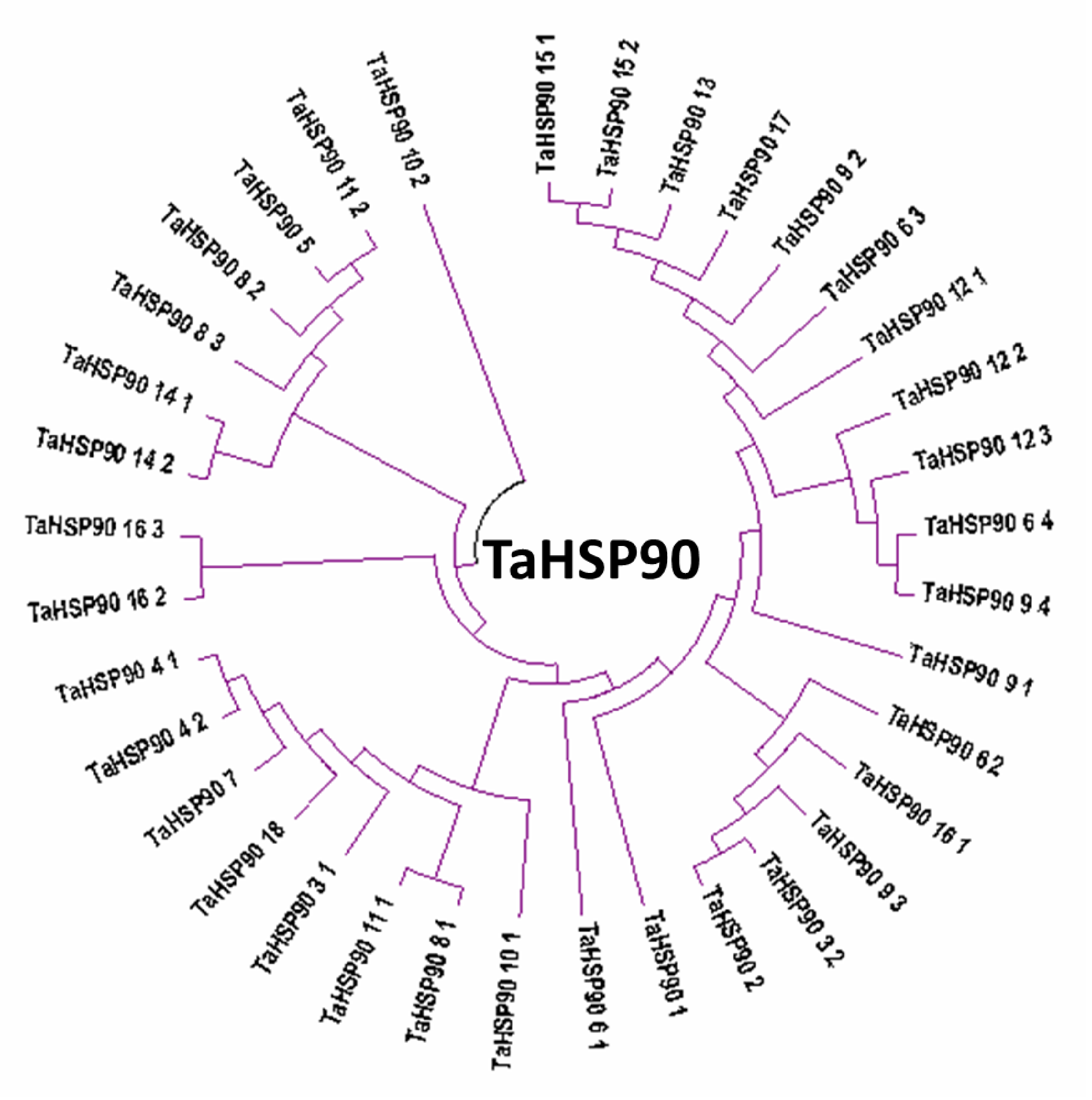
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TaHSP90 11 2
TaHSP90 5
TaHSP90 8 2
TaHSP90 8 3
TaHSP90 14 1
TaHSP90 14 2
TaHSP90 16 3
TaHSP90 16 2
TaHSP90 4 1
TaHSP90 4 2
TaHSP90 7
TaHSP90 18
TaHSP90 3 1
TaHSP90 11 1
TaHSP90 8 1
TaHSP90 10 1
TaHSP90 6 1
TaHSP90 1
TaHSP90 2
TaHSP90 3 2
TaHSP90 9 3
TaHSP90 16 1
TaHSP90 6 2
TaHSP90 9 1
TaHSP90 9 4
TaHSP90 6 4
TaHSP90 12 3
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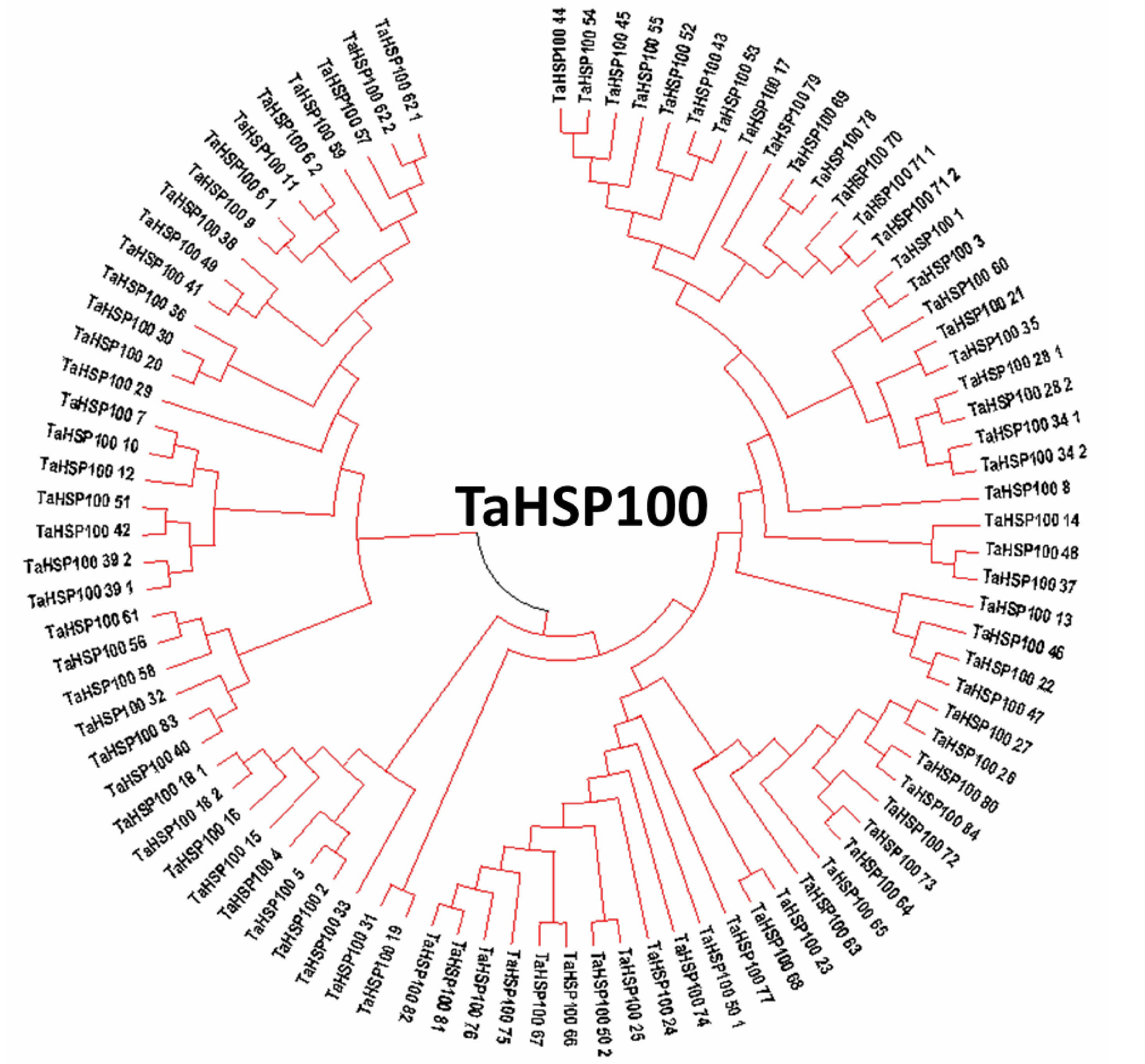
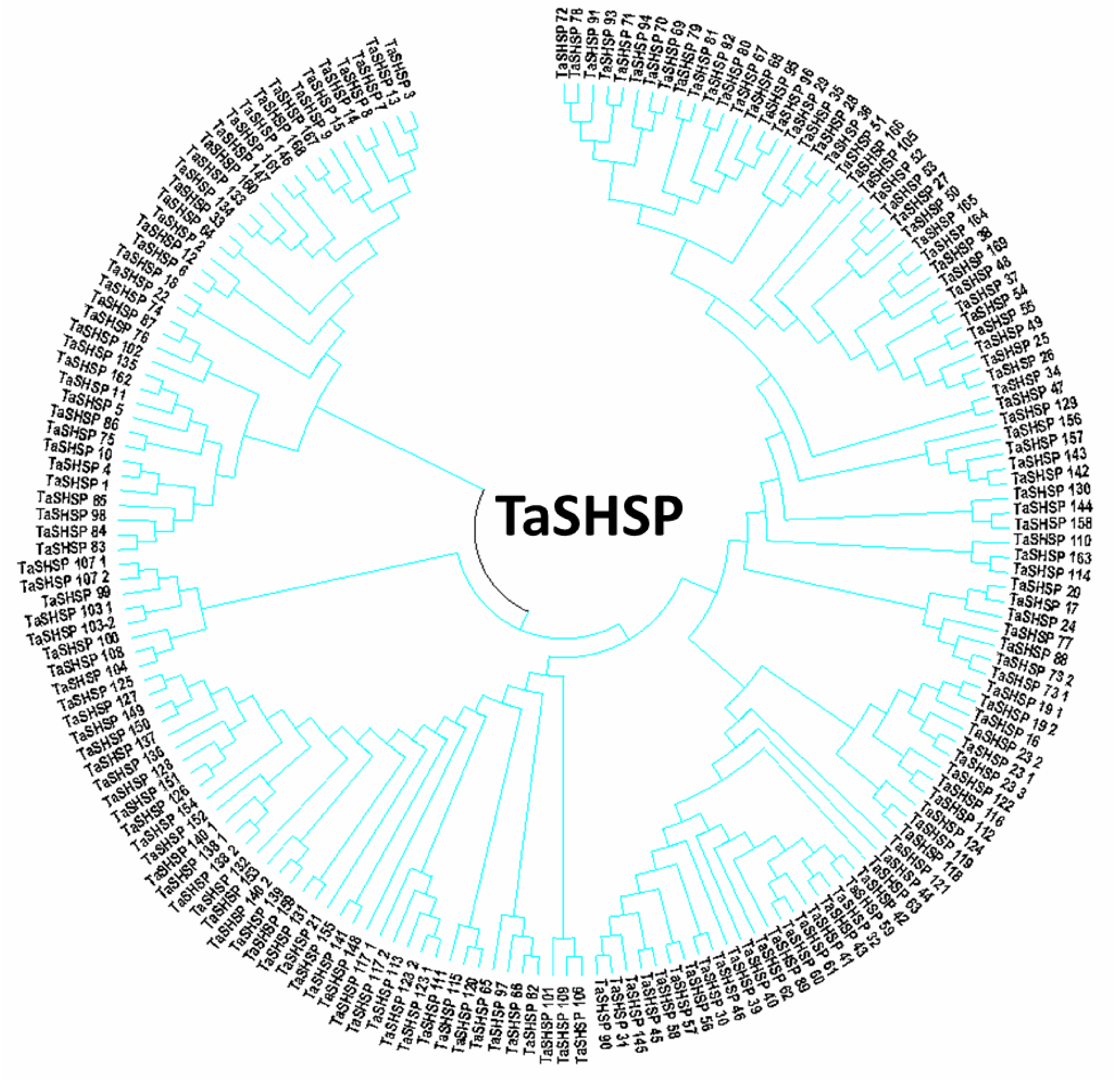
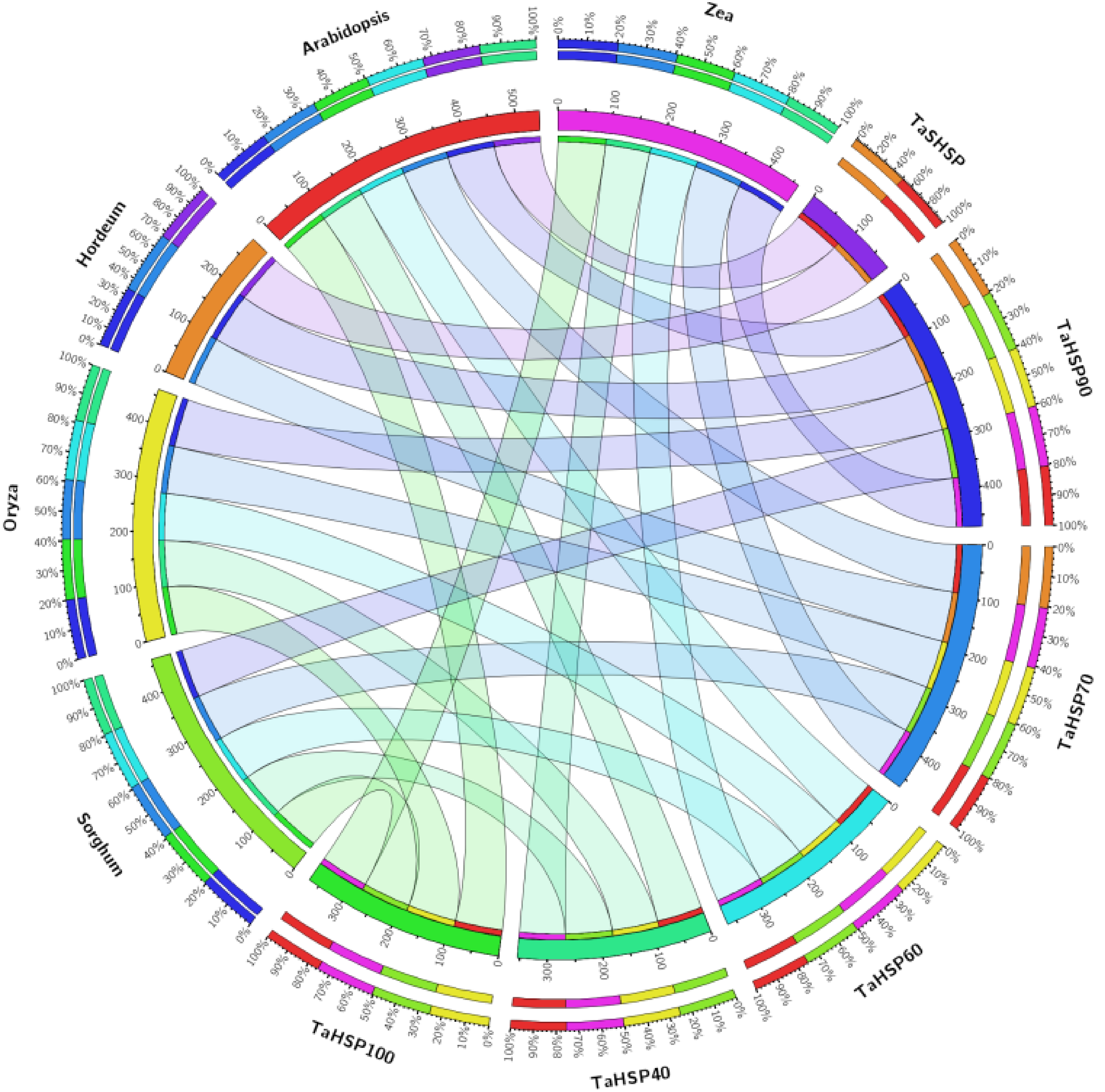
TaHSP90 15 2
TaHSP90 15 1


TaHSP100


TaSHSP


Zea
TaSHSP
TaHSP90
TaHSP70
TaHSP60
TaHSP40
TaHSP100
Sorghum
Oryza
Hordeum
Arabidopsis

Supplementary Data

Figure S1: Conserved Motifs of *TaHSP*s elucidated by MEME. Colored boxes representing different conserved motifs having different sequences and sizes.

Figure S2: Gene structure of all the 753 *TaHSP* genes, prepared using GSDraw

Figure S3: Blast2GO statistics based on BLAST search against the NR database

A: BLAST top-hit species distribution, **B**: Sequence similarity distribution

Figure S4: Bar graph representing the number of sequences of different subfamilies of *TaHSPs* distributed among various Cellular component (CC) Biological Process (BP) and Molecular Function (MF) depicted by blue, orange and green color.

Figure S5: Gene ontology term distribution in different HSP sub-families predicted using BLAST2GO in Biological Process.

Figure S6: Gene ontology term distribution in different HSP sub-families predicted using BLAST2GO in Cellular component

Figure S7: Sub-cellular localization predicted using BUSCO

Figure S8: Gene ontology term distribution in different HSP sub-families predicted using BLAST2GO in Molecular function.

Figure S9: Heatmaps representing the expression of all 753 *TaHSP* genes in various developmental stages. FPKM values were directly used to create the heatmaps.

Figure S10: Heatmaps representing the expression of all the 753 *TaHSP* genes in various stress conditions. FPKM values were directly used to create the heatmaps. Pst: *Puccinia striiformis*, Bgt: *Blumeria graminis*, dpi: days post inoculation

Figure S11: Isoelectric point vs. Molecular weight (kDa) plots of different *TaHSPs* subfamily.

Figure S12: Phylogenetic relationship within members of the different HSP sub-families in wheat, inferred using maximum likelihood method. A: *TaHSP40*; B: *TaHSP60*; C: *TaHSP70*; D: *TaHSP90*; E: *TaHSP100*, F: *TaSHSP*

Figure S13: Syntenic relationship of *TaHSP*s orthologous with its different monocots and dicots viz., *O. sativa, H. vulgure, S. bicolor, Z. mays* and *A. thaliana.*