Figure S1 Diagrammatic illustration for analysis of key carbohydrate metabolism enzymes activity (A), antioxidant enzymes activity (B) and 4D-proteomics (C).

Figure S2 SDS-PAGE of total proteins extracted from the last fully expanded leaf of wild type (WT) and *Az34* barley under optimum and low temperature.

Figure S3 Subcellular localization of the differentially expressed proteins for the comparisons of WT-LT vs WT-OT, *Az34*-LT vs WT-LT, *Az34*-OT vs WT-OT, and *Az34*-LT vs *Az34*-OT. (A) Subcellular localization of the differentially expressed proteins in the comparison of WT-LT vs WT-OT; (B) Subcellular localization of the differentially expressed proteins in the comparison of *Az34*-LT vs WT-LT; (C) Subcellular localization of the differentially expressed proteins in the comparison of *Az34*-OT vs WT-OT; (D) Subcellular localization of the differentially expressed proteins in the comparison of *Az34*-LT vs *Az34*-OT. OT, optimum temperature; LT, low temperature.

Figure S4 GO terms of the differentially expressed proteins for the comparisons of WT-LT vs WT-OT, *Az34*-OT vs WT-OT, *Az34*-LT vs WT-LT and *Az34*-LT vs *Az34*-OT. (A) GO terms of the differentially expressed proteins for the comparison of WT-LT vs WT-OT; (B) GO terms of the differentially expressed proteins for the comparison of *Az34*-OT vs WT-OT; (C) GO terms of the differentially expressed proteins for the comparison of *Az34*-LT vs WT-LT; (D) GO terms of the differentially expressed proteins for the comparison of *Az34*-LT vs *Az34*-OT. OT, optimum temperature; LT, low temperature.

Figure S5 Functional enrichment cluster of the differentially expressed proteins for the comparisons of WT-LT vs WT-OT, *Az34*-LT vs WT-LT, *Az34*-OT vs WT-OT, and *Az34*-LT vs *Az34*-OT. OT, optimum temperature; LT, low temperature.

Figure S6 Activities of key carbohydrate metabolism enzymes in the last fully expanded leaf of wild

type (WT) and *Az34* barley under optimum and low temperature. Ald, aldolase; UGPase, UDP-glucose pyrophosphorylase; Susy, sucrose synthase; AGPase, ADP-glucose pyrophosphorylase; HXK, hexokinase; PGI, phosphoglucomutase; G6PDH, glucose-6-phosphate dehydrogenase; PFK, phosphofructokinase; PGM, phosphoglucomutase; FK, fructokinase; vacInv, vacuolar invertase; cytInv, cytoplasmic invertase; cwInv, cell wall invertase. Different small letters indicate significant differences at $P<0.05$ level. Data are expressed as means ± SEM (n=3).

Figure S7 Activities of antioxidant enzymes in the last fully expanded leaf of wild type (WT) and *Az34* barley under optimum and low temperature. SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase; POX, cytoplasmic peroxidase; cwPOX, apoplastic peroxidase; GST, glutathione S-transferase; MDHAR, monodehydroascorbate reducate; GR, glutathione reductase; DHAR, dehydroascorbate reductase. Different small letters indicate significant differences at $P<0.05$ level. Data are expressed as means ± SEM (n=3).

Figure S8 Concentrations of soluble sugar (A), soluble protein (B), proline (C) and H_2O_2 (D) in the last fully expand leaf of wild type and *Az34* barley under optimum temperature and low temperature. OT: optimum temperature, LT: low temperature. Different small letters indicate significant differences at $P<0.05$ level. Data are expressed as means ± SEM (n=3).

Figure S9 Biplot of principal component analysis (PCA) of ABA with physiological traits and ABA with enzyme activities performed separately in WT and *Az34* plants under optimum temperature and low temperature. (A) Biplot of PCA of ABA with physiological traits in WT and *Az34* plants under optimum temperature and low temperature; (B) Biplot of PCA of ABA with enzyme activities performed separately in WT and *Az34* plants under optimum temperature and low temperature. ABA, abscisic acid; *An*, net photosynthetic rate; g_s, stomatal conductance; φP_O, maximum quantum yield

for primary photochemistry; ψE_O, probability that an electron moves further than Q_A; φE_O, quantum yield for electron transport; φR_O, quantum yield for reduction of end electron acceptors at the PSI acceptor side. F_V/F_M, maximum quantum efficiency of photosystem II; AGPase, ADP-glucose pyrophosphorylase; UGPase, UDP-glucose pyrophosphorylase; FK, fructokinase; vacInv, vacuolar invertase; cytInv, cytoplasmic invertase; cwInv, cell wall invertase; SOD, superoxide dismutase; MDHAR, monodehydroascorbate reductase.

Figure S1

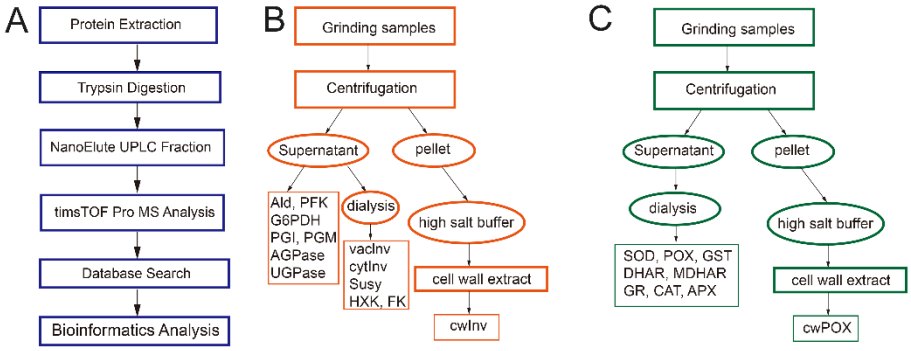

A
Protein Extraction
Trypsin Digestion
NanoElute UPLC Fraction
timsTOF Pro MS Analysis
Database Search
Bioinformatics Analysis
B
Grinding samples
Centrifugation
Supernatant
pellet
Ald, PFK
G6PDH
PGI, PGM
AGPase
UGPase
dialysis
vacInv
cytInv
Susy
HXK, FK
high salt buffer
cell wall extract
cwInv
C
Grinding samples
Centrifugation
Supernatant
pellet
dialysis
SOD, POX, GST
DHAR, MDHAR
GR, CAT, APX
high salt buffer
cell wall extract
cwPOX

Figure S2

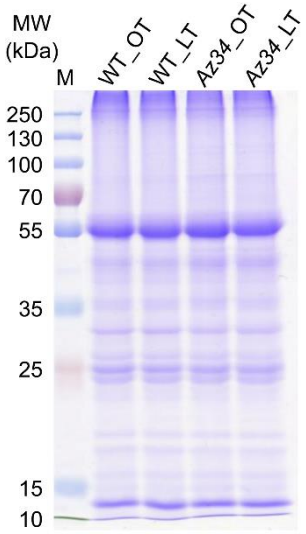


Figure S3

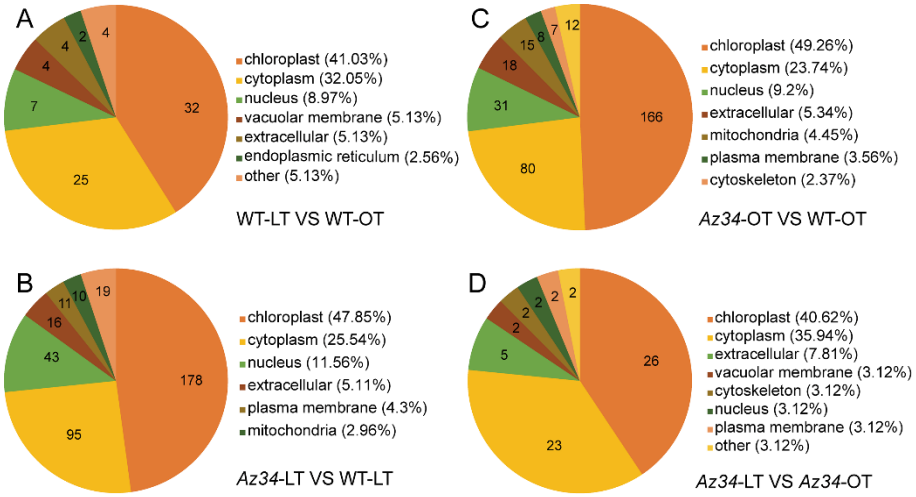

A
32
25
7
4
4
2
4
chloroplast (41.03%)
cytoplasm (32.05%)
nucleus (8.97%)
vacuolar membrane (5.13%)
extracellular (5.13%)
endoplasmic reticulum (2.56%)
other (5.13%)
WT-LT VS WT-OT
C
166
80
31
18
15
8
7
12
chloroplast (49.26%)
cytoplasm (23.74%)
nucleus (9.2%)
extracellular (5.34%)
mitochondria (4.45%)
plasma membrane (3.56%)
cytoskeleton (2.37%)
Az34-OT VS WT-OT
B
178
95
43
16
11
10
19
chloroplast (47.85%)
cytoplasm (25.54%)
nucleus (11.56%)
extracellular (5.11%)
plasma membrane (4.3%)
mitochondria (2.96%)
Az34-LT VS WT-LT
D
26
23
5
2
2
2
2
2
chloroplast (40.62%)
cytoplasm (35.94%)
extracellular (7.81%)
vacuolar membrane (3.12%)
cytoskeleton (3.12%)
nucleus (3.12%)
plasma membrane (3.12%)
other (3.12%)
Az34-LT VS Az34-OT

Figure S4

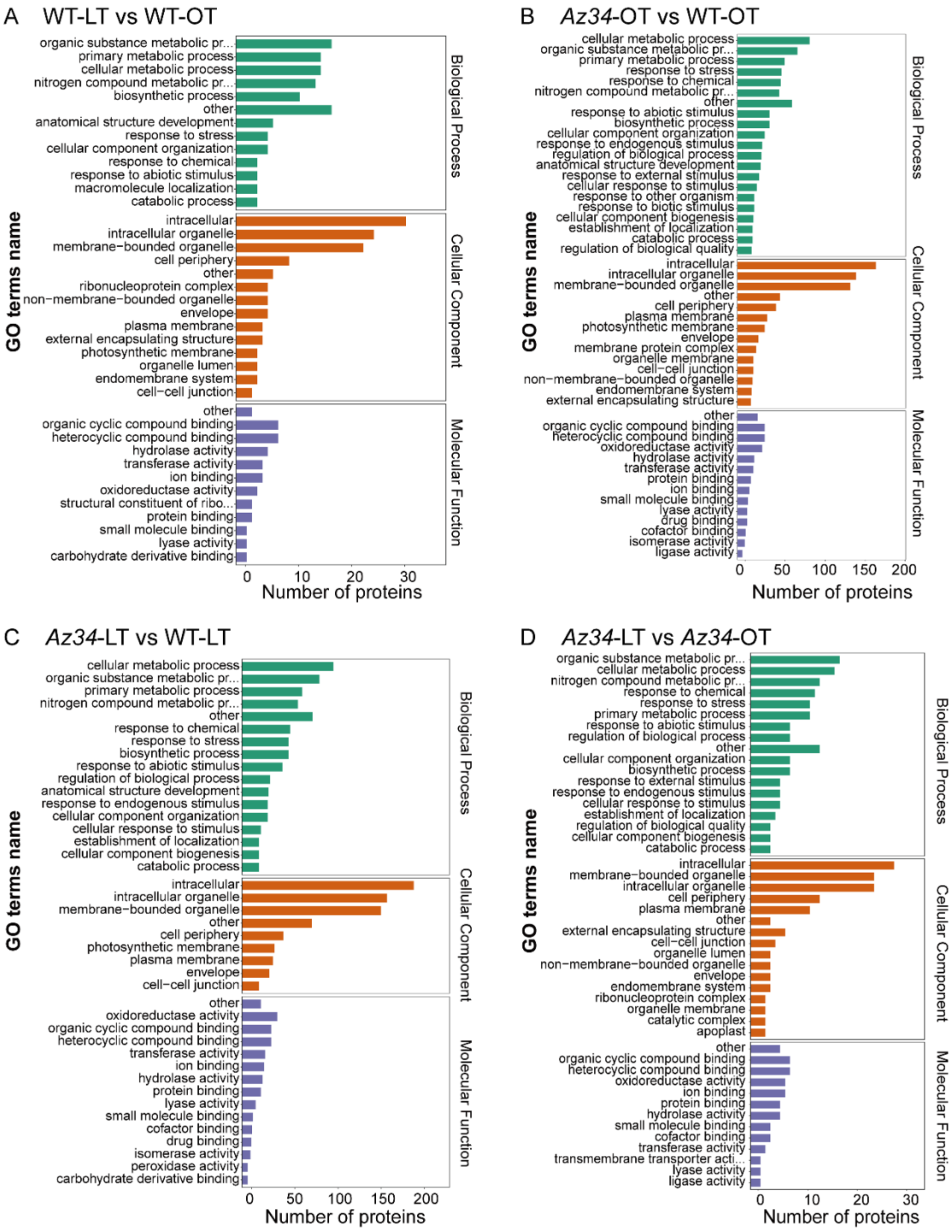

A WT-LT vs WT-OT
organic substance metabolic pr...
primary metabolic process
cellular metabolic process
nitrogen compound metabolic pr...
biosynthetic process
other
anatomical structure development
response to stress
cellular component organization
response to chemical
response to abiotic stimulus
macromolecule localization
catabolic process
intracellular
intracellular organelle
membrane−bounded organelle
cell periphery
other
ribonucleoprotein complex
non−membrane−bounded organelle
envelope
plasma membrane
external encapsulating structure
photosynthetic membrane
organelle lumen
endomembrane system
cell−cell junction
other
organic cyclic compound binding
heterocyclic compound binding
hydrolase activity
transferase activity
ion binding
oxidoreductase activity
structural constituent of ribo...
protein binding
small molecule binding
lyase activity
carbohydrate derivative binding
Biological Process
Cellular Component
Molecular Function
GO terms name
0 10 20 30
Number of proteins
B Az34-OT vs WT-OT
cellular metabolic process
organic substance metabolic pr...
primary metabolic process
response to stress
response to chemical
nitrogen compound metabolic pr...
other
response to abiotic stimulus
biosynthetic process
cellular component organization
response to endogenous stimulus
regulation of biological process
anatomical structure development
response to external stimulus
cellular response to stimulus
response to other organism
response to biotic stimulus
cellular component biogenesis
establishment of localization
catabolic process
regulation of biological quality
intracellular
intracellular organelle
membrane−bounded organelle
other
cell periphery
plasma membrane
photosynthetic membrane
envelope
membrane protein complex
organelle membrane
cell−cell junction
non−membrane−bounded organelle
endomembrane system
external encapsulating structure
other
organic cyclic compound binding
heterocyclic compound binding
oxidoreductase activity
hydrolase activity
transferase activity
protein binding
ion binding
small molecule binding
lyase activity
drug binding
cofactor binding
isomerase activity
ligase activity
Biological Process
Cellular Component
Molecular Function
GO terms name
0 50 100 150 200
Number of proteins
C Az34-LT vs WT-LT
cellular metabolic process
organic substance metabolic pr...
primary metabolic process
nitrogen compound metabolic pr...
other
response to chemical
response to stress
biosynthetic process
response to abiotic stimulus
regulation of biological process
anatomical structure development
response to endogenous stimulus
cellular component organization
cellular response to stimulus
establishment of localization
cellular component biogenesis
catabolic process
intracellular
intracellular organelle
membrane−bounded organelle
other
cell periphery
photosynthetic membrane
plasma membrane
envelope
cell−cell junction
other
oxidoreductase activity
organic cyclic compound binding
heterocyclic compound binding
transferase activity
ion binding
hydrolase activity
protein binding
lyase activity
small molecule binding
cofactor binding
drug binding
isomerase activity
peroxidase activity
carbohydrate derivative binding
Biological Process
Cellular Component
Molecular Function
GO terms name
0 50 100 150 200
Number of proteins
D Az34-LT vs Az34-OT
organic substance metabolic pr...
cellular metabolic process
nitrogen compound metabolic pr...
response to chemical
response to stress
primary metabolic process
response to abiotic stimulus
regulation of biological process
other
cellular component organization
biosynthetic process
response to external stimulus
response to endogenous stimulus
cellular response to stimulus
establishment of localization
regulation of biological quality
cellular component biogenesis
catabolic process
intracellular
membrane−bounded organelle
intracellular organelle
cell periphery
plasma membrane
other
external encapsulating structure
cell−cell junction
organelle lumen
non−membrane−bounded organelle
envelope
endomembrane system
ribonucleoprotein complex
organelle membrane
catalytic complex
apoplast
other
organic cyclic compound binding
heterocyclic compound binding
oxidoreductase activity
ion binding
protein binding
hydrolase activity
small molecule binding
cofactor binding
transferase activity
transmembrane transporter acti...
lyase activity
ligase activity
Biological Process
Cellular Component
Molecular Function
GO terms name
0 10 20 30
Number of proteins

Figure S5


A Biological Process
tRNA aminoacylation for protein translation
protein localization to vacuole
inorganic anion transport
response to far red light
indolalkylamine biosynthetic process
serine family amino acid biosynthetic process
tryptophan biosynthetic process
regulation of TOR signaling
protein targeting to lysosome
positive regulation of TOR signaling
negative regulation of autophagy
protein localization to lysosome
leucyl−tRNA aminoacylation
intra−Golgi vesicle−mediated transport
glutaminyl−tRNA aminoacylation
cellular response to leucine starvation
cellular response to leucine
valyl−tRNA aminoacylation
NADH dehydrogenase complex assembly
regulation of TORC1 signaling
negative regulation of chromosome organization
cysteine biosynthetic process from serine
retrograde vesicle−mediated transport, Golgi to ER
positive regulation of GTPase activity
glutamine metabolic process
negative regulation of cellular catabolic process
jasmonic acid metabolic process
jasmonic acid biosynthetic process
regulation of mRNA metabolic process
photosystem II repair
pectin metabolic process
regulation of translational initiation
cell wall pectin metabolic process
regulation of mRNA catabolic process
glycosaminoglycan biosynthetic process
valine biosynthetic process
dicarboxylic acid biosynthetic process
chorismate metabolic process
chorismate biosynthetic process
B Cellular Component
large ribosomal subunit
mediator complex
ribonucleoprotein complex
cell periphery
plasma membrane
plant−type vacuole
cell wall
external encapsulating structure
aminoacyl−tRNA synthetase multienzyme complex
NAD(P)H dehydrogenase complex (plastoquinone)
Golgi−associated vesicle
COPI−coated vesicle
COPI vesicle coat
membrane protein complex
COPI−coated vesicle membrane
Golgi−associated vesicle membrane
coated membrane
membrane coat
plastoglobule
thylakoid lumen
chloroplast thylakoid membrane protein complex
thylakoid part
thylakoid
stromule
chloroplast thylakoid membrane
plastid thylakoid membrane
chloroplast thylakoid
plastid thylakoid
organelle subcompartment
thylakoid membrane
photosynthetic membrane
C Molecular Function
1.5
1
0.5
0
−0.5
−1
−1.5
Regulated.Type
all
Sample
Az34_LTvsAz34_OT
Az34_LTvsWT_LT
Az34_OTvsWT_OT
WT_LTvsWT_OT
methyltransferase activity
S−methyltransferase activity
nuclease activity
single−stranded RNA binding
poly(U) RNA binding
poly−pyrimidine tract binding
molybdopterin cofactor binding
flavin adenine dinucleotide binding
inorganic anion transmembrane transporter activity
coenzyme binding
transferase activity, transferring hexosyl groups
galactosyltransferase activity
GTPase regulator activity
cysteine synthase activity
GTPase activator activity
sequence−specific DNA binding
glutamine−tRNA ligase activity
valine−tRNA ligase activity
leucine−tRNA ligase activity
drug binding
vitamin binding
vitamin B6 binding
pyridoxal phosphate binding
transferase activity, transferring glycosyl groups
galactosidase activity
UDP−glucose 6−dehydrogenase activity
porphobilinogen synthase activity
acetolactate synthase activity
carboxy−lyase activity
tubulin binding
cytochrome−c peroxidase activity
peroxidase activity
peptidyl−prolyl cis−trans isomerase activity
linoleate 13S−lipoxygenase activity
glutamate synthase activity
carbonate dehydratase activity
glutamate synthase (NADH) activity
glutamate synthase activity, NAD(P)H as acceptor
alpha−galactosidase activity
phosphoenolpyruvate carboxylase activity
phosphoenolpyruvate carboxykinase activity
oxidoreductase activity
lyase activity
monooxygenase activity
cis−trans isomerase activity
carbon−oxygen lyase activity
hydro−lyase activity
D KEGG pathway
Cyanoamino acid metabolism
Ribosome biogenesis in eukaryotes
Phenylpropanoid biosynthesis
Starch and sucrose metabolism
Peroxisome
Caffeine metabolism
beta−Alanine metabolism
Thiamine metabolism
Ascorbate and aldarate metabolism
Photosynthesis
Phenylalanine, tyrosine and tryptophan biosynthesis
Nitrogen metabolism
Linoleic acid metabolism
Glutathione metabolism
Photosynthesis − antenna proteins
Glycerolipid metabolism
alpha−Linolenic acid metabolism
Sulfur metabolism

Figure S6

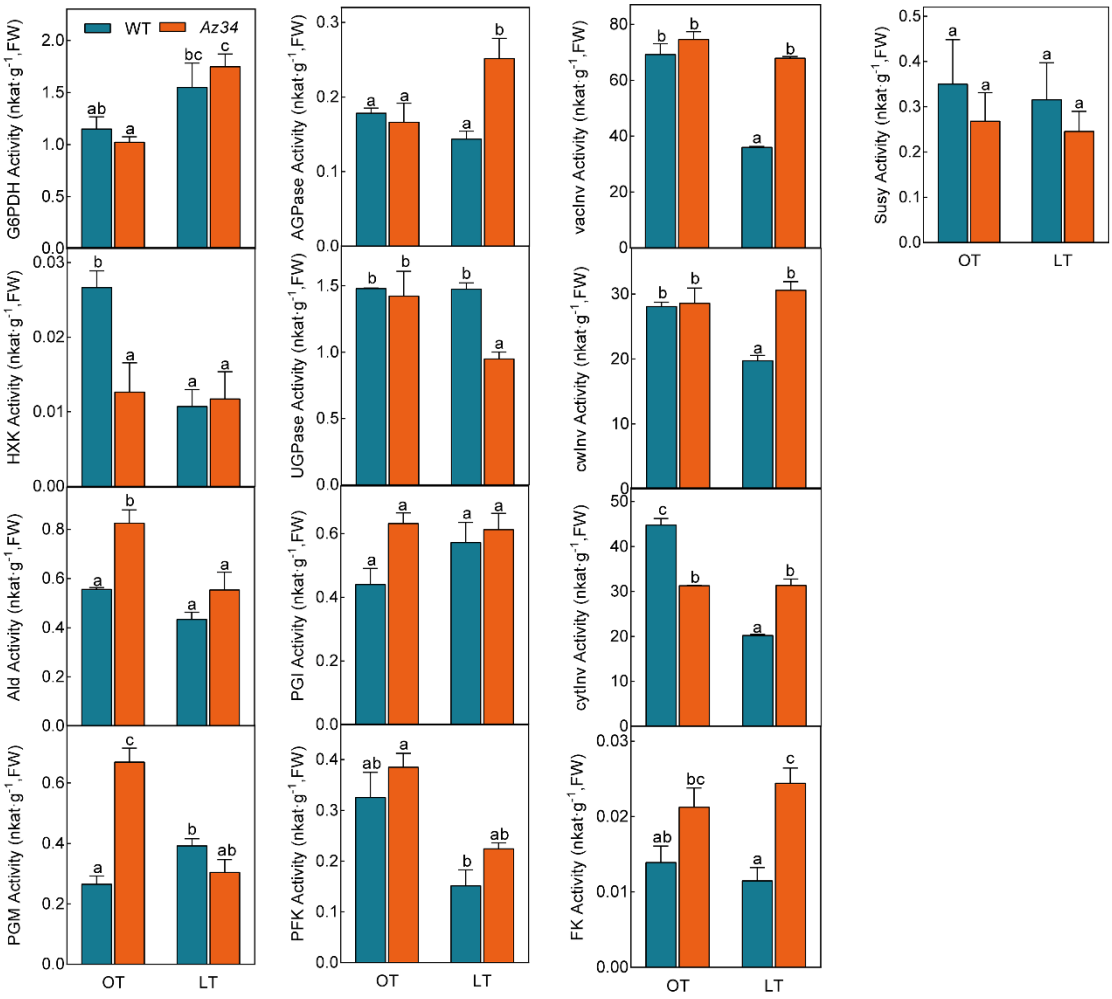

WT
Az34
G6PDH Activity (nkat·g-1,FW)
AGPase Activity (nkat·g-1,FW)
vacInv Activity (nkat·g-1,FW)
Susy Activity (nkat·g-1,FW)
HXK Activity (nkat·g-1,FW)
UGPase Activity (nkat·g-1,FW)
cwInv Activity (nkat·g-1,FW)
Ald Activity (nkat·g-1,FW)
PGI Activity (nkat·g-1,FW)
cytInv Activity (nkat·g-1,FW)
PGM Activity (nkat·g-1,FW)
PFK Activity (nkat·g-1,FW)
FK Activity (nkat·g-1,FW)
OT
LT

Figure S7

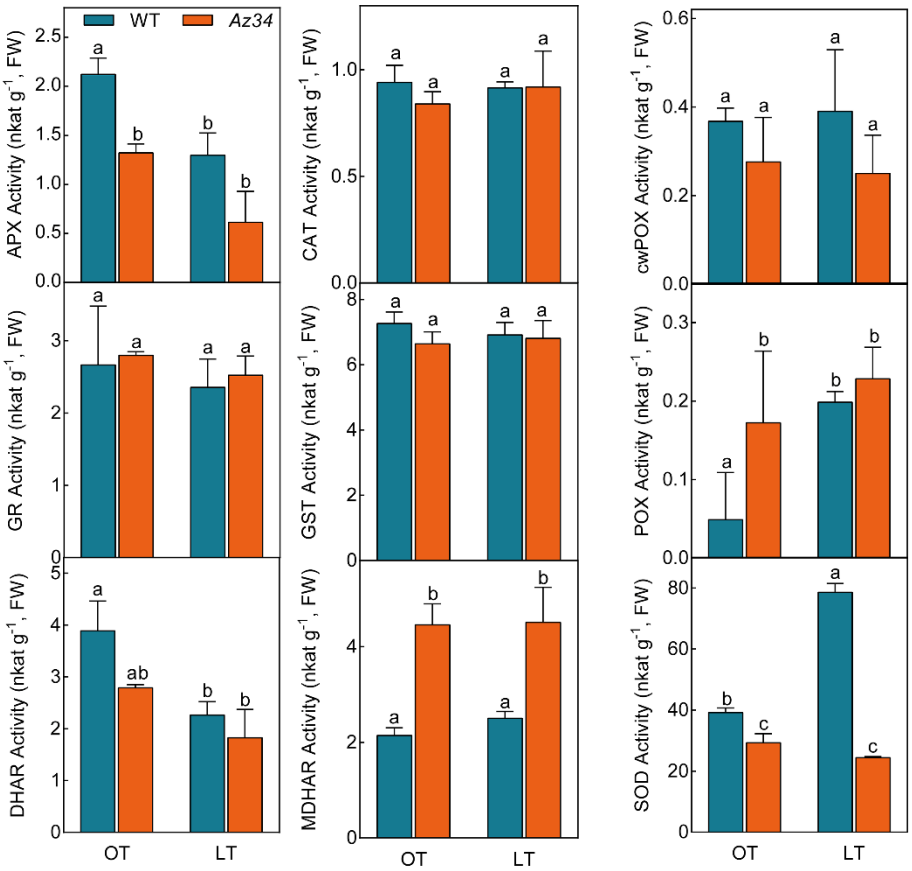

WT
Az34
APX Activity (nkat g-1, FW)
CAT Activity (nkat g-1, FW)
cwPOX Activity (nkat g-1, FW)
GR Activity (nkat g-1, FW)
GST Activity (nkat g-1, FW)
POX Activity (nkat g-1, FW)
DHAR Activity (nkat g-1, FW)
MDHAR Activity (nkat g-1, FW)
SOD Activity (nkat g-1, FW)
OT
LT

Figure S8

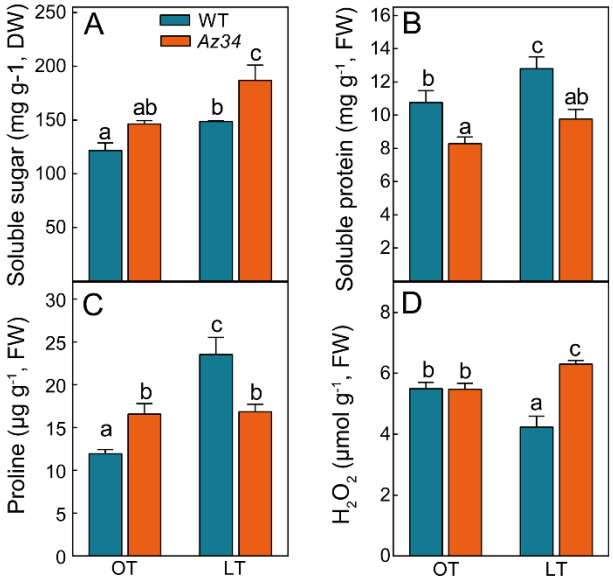
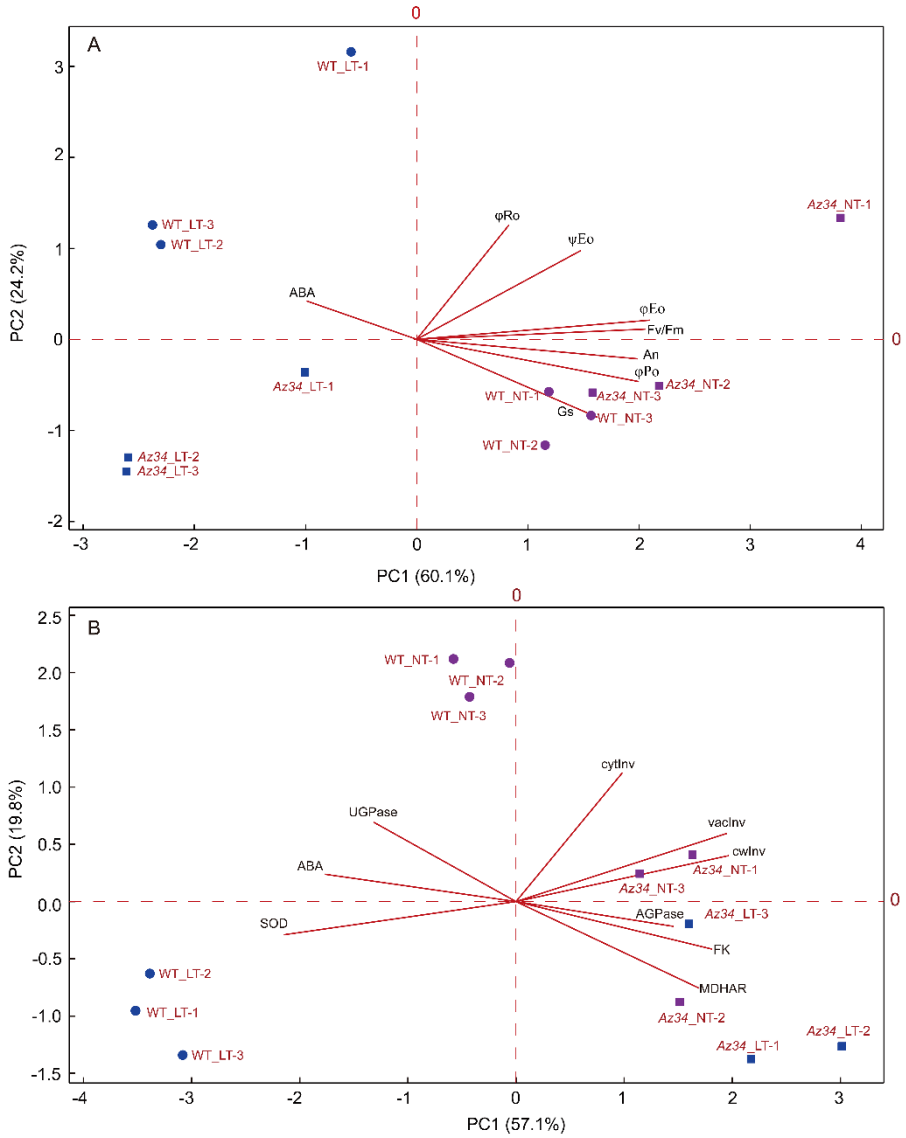

A
WT
Az34
Soluble sugar (mg g-1, DW)
250
200
150
100
50
a
ab
b
c
B
Soluble protein (mg g-1, FW)
16
14
12
10
8
6
4
2
b
a
c
ab
C
Proline (μg g-1, FW)
30
25
20
15
10
5
0
a
b
c
b
D
H2O2 (μmol g-1, FW)
8
6
4
2
0
b
b
a
c
OT
LT
OT
LT

Figure S9


A
WT_LT-1
WT_LT-3
WT_LT-2
Az34_NT-1
φRo
ψEo
ABA
φEo
Fv/Fm
An
φPo
Az34_NT-2
Az34_LT-1
WT_NT-1
Az34_NT-3
Gs
WT_NT-3
WT_NT-2
Az34_LT-2
Az34_LT-3
PC2 (24.2%)
PC1 (60.1%)
B
WT_NT-1
WT_NT-2
WT_NT-3
cytInv
UGPase
vacInv
cwInv
ABA
Az34_NT-1
Az34_NT-3
AGPase
Az34_LT-3
SOD
FK
MDHAR
WT_LT-2
WT_LT-1
Az34_NT-2
Az34_LT-2
Az34_LT-1
WT_LT-3
PC2 (19.8%)
PC1 (57.1%)