

A *Osnac2* Tag 399-421 bp (-strand)

Tag	G	G	A	G	T	A	A	C	G	A	G	A	T	C	A	G	C
Wt	C	C	T	C	A	T	T	G	C	T	C	T	A	G	T	C	G
mut	C	C	T	C	-	-	-	-	-	-	-	T	A	G	T	C	G

Osnac2 Tag 1350-1360 bp

Wt	C	A	G	A	T	G	G	A	A	T	G	T	C	C	A	A	A	C
mut	C	A	G	A	T	G	G	C	A	T	G	T	C	C	A	T	-	C

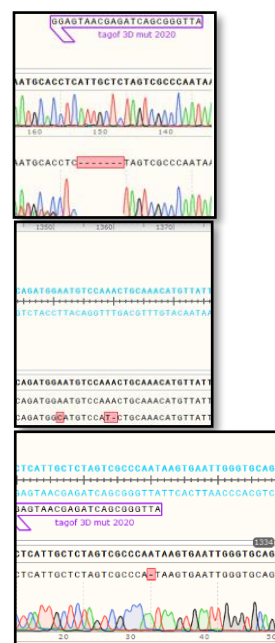
B *Osnac2* Tag 399- 421 bp (-strand)

Tag	G	G	A	G	T	A	A	C	G	A	G	A	T	C	A	G	C	G	G	G	T	T	A
Wt	C	C	T	C	A	T	T	G	C	T	C	T	A	G	T	C	G	C	C	C	A	T	T
mut	C	C	T	C	A	T	T	G	C	T	C	T	A	G	T	C	G	C	C	C	A	-	T

Osnac6 Tag 1256-1278 bp (-strand)

tag	C	C	T	C	A	T	C	G	C	A	A	G	A	T	C	C	G	-	A	A	A	T	G	G
Wt	G	G	A	G	T	A	G	C	G	T	T	C	T	A	G	G	C	-	T	T	T	A	C	C
mut1	G	G	A	G	T	A	G	C	G	T	T	C	T	A	G	G	C	T	T	T	T	A	C	C
mut2	C	C	T	C	A	T	C	G	C	A	A	G	A	T	-	-	-	-	T	T	T	A	C	C

C

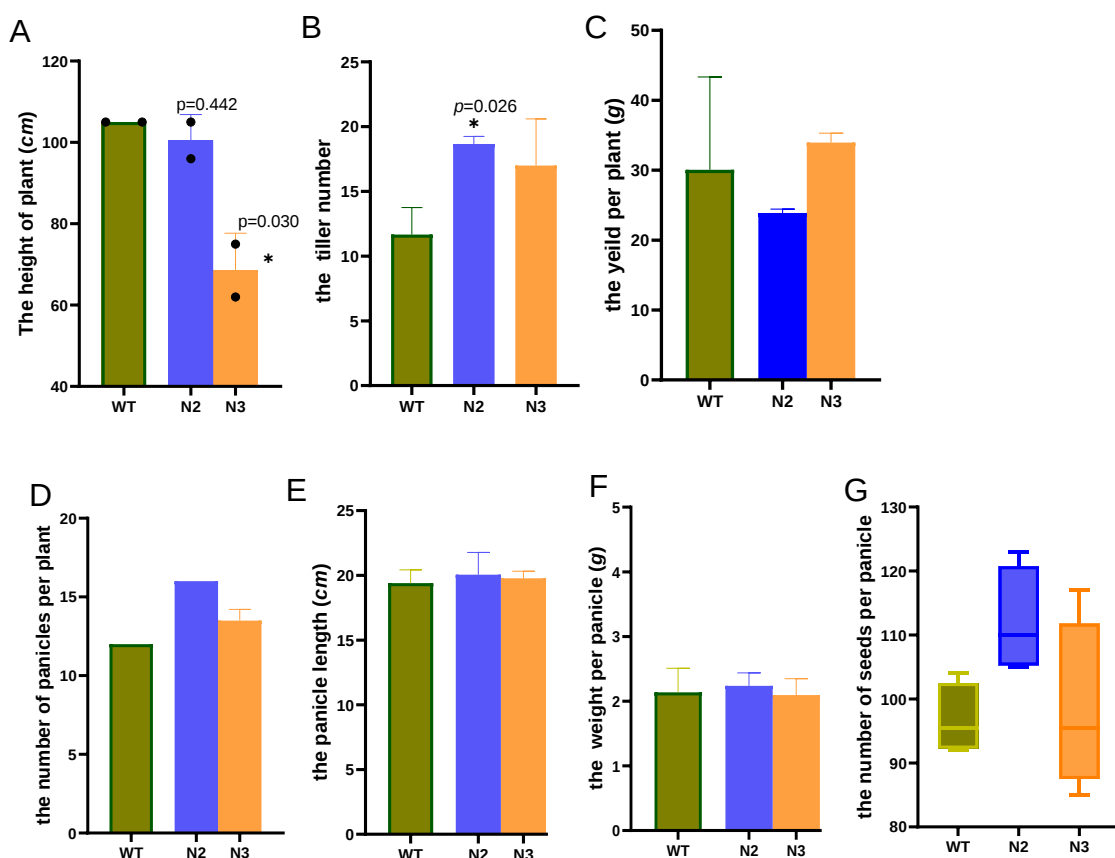


S1 Fig. The mutants detection by PCR sequencing

(A) Ko-*Osnac02* *mut* construction with two mutant sites in *Osnac02* CDS. The genetic type of mutants are labeled in red and the PAM “GGA” is highlighted in bold and underline. The mutant with “ATTGCTC” 7 bp-deletion in N2 *mut* located at 399-421 bp of *Osnac02* sequence, and the mutant with “A→C” at 1350 bp, “A→T” / “A→x” transversion at 1360 bp of *Osnac06* sequence.

(B) N3 *mut* constructed the mutant sites in *Osnac02* / *Osnac06* CDS with PAM “GGA” / “TGG” respectively. The Crispr-Cas9 induced “T” deletion in N2 and “T” insertion and 3 bp “GGC” deletion in N3 at 1256-1278bp region respectively.

(C) As these figures shows, the mutant sites of N2/N3 *mut* in *Osnac02* CDS detected by the alignment with DNA sequence of WT (NIP).

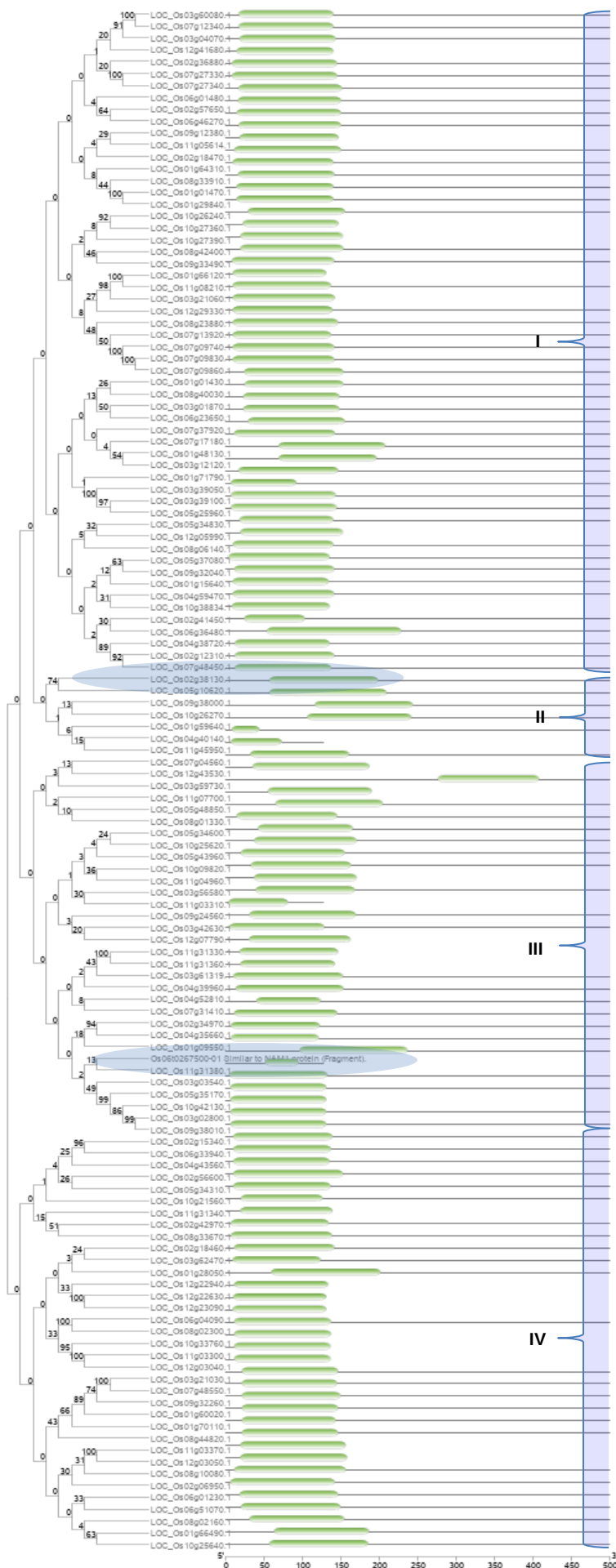


S2 Fig. The yield characters of N2 / N3 *mut* in field (95DAG in 2021, 125DAG in 2020)

- (A) The plant height of N2/N3 mutant (T1) shows obvious difference in 2021, and both of them were dwarf compared with the WT. The height of N3 is higher than N2 mutant.
- (B) The number of tillers in N2 and N3 mutant are more compared with WT in 2021.
- (C) The N2/N3 mutant (T0) harvested in Hainan are detected for the yield per plant. All the seeds of T1 generation (125 DAG) are pre-treated with drying in the green house for two months. The yield per plant in N3 *mut* was heaviest and N2 *mut* was the lightest as a result.
- (D) The number of panicles per plant (95DAG) in N2/N3 mutant, and the plant height, the tiller number per plant are detected simultaneously in 2020.
- (E) The panicle length per plant (95DAG) in N2/N3 mutant was detected in 2021.
- (F) The weight per panicles (95DAG) of N2/N3 mutant are detected, and there is no significant difference between mutants and WT. Five panicles from each plant are selected randomly for detection respectively.
- (G) The number of seeds per panicle in T1 generation (95DAG) was detected, and N2 *mut* has most seeds per plant. This phenotype together was detected with the number of panicles together in the same materials.

All the mutants planted in field under the normal conditions (Fuyang, Hangzhou in 2021), the statistic analysis are running by the **student t-test**, *, $p < 0.05$ and **, $p < 0.01$. The T0 generation of transplants was planted in Lingshui (Hainan Province Island, 18.5°110'E) in the winter of 2020, and we obtained the T1 generation. Following the T1 generation was planted in Fuyang (Hangzhou in Zhejiang Province, 30°120'E) during the summer in 2021, and T2 generation were harvested in the autumn of 2022.

As the yield characters shown in **Figure 1**, the shape phenotype in seeds (125 DAG) of N2/N3 mutant are no difference compared with the control (nip).



S3 Fig.
The homologous clusters of OsNAC family genes by phylogenetic analysis

The *OsNAC02* (*Os02g0594800*, *LOC_Os02g38130*) and *OsNAC06* (*Os06g0267500*) are labeled by blue circle. The two genes are belonged to subgroup III and IV respectively.

The phylogenetic tree of OsNAC family was constructed using the MEGA 7.0. and the NAM regions in all genes are highlighted by TBtools11.2. The green boxes in the phylogenetic tree are NAC domains of genes.

A

Os06g0267500	
ARATH_At1g26390	MAGR S W L IDS N RIAT K IMS A SASSDPRQVVWKS N SPSRH
ARATH_ARATH_NAC_85	MKT H RR T W I VDG P WIS R NV K NASL S SALQ T KDCG A YIN .
ARATH_NAC_44	MARAW I VDGR G IA A K V KNASL S SALQ T QDCG A HI K ..
Os02g0594800	MAR S W L ITGRG V AK K IR N APHCS S SRPT S E L SGAE A Q M E
MAIZE_NAC	MD S WIM N GL G LA E K I RSAT Q S T LR L IGEL F AK P RT K
MAIZE_ANAC044	MAR S W L ITCRG I AK K IR Y AN F SANRQ I S Q LIA E AR R E
MAIZE_NAC1	MAR S W L ITCRG I AK K IR Y T N F G ASH H SEL V AEAR R E
MAIZE_NAC2	MTG T TT W IL D SQ R FA T K I KN S G S SDPSR Q K V IG N FS P SK E
Consensus	

Os06g0267500	
ARATH_At1g26390	C PK C QH V ID N SD V VD D W P GL F R G V K F D PS D PE I W H L L AK
ARATH_ARATH_NAC_85	C FNCSY R ID N SN V L T PP W PGL F PK G V K FE P TDE E VIE H LE A K
ARATH_NAC_44	C PNCT Y RID N SN V L I P W PGL F PK G V K FE P TDE D IE F LE A K
Os02g0594800	C PNCK H VID N SD V AI Q W P GL F PA G V K F D PS D LE L LE H LE Q K
MAIZE_NAC	C TYCD C V I DI S NS V SL V W P AL F PA G V K F D PT D LE L LO H L Q E K
MAIZE_ANAC044	C PNCSY V VD N SD V AM Q W P GL F PA G V K F D PS D LE L LE L LO H L Q E K
MAIZE_NAC1	C PNCSY I ID N SD V AM Q W P GL F PA G V K F D PS D LE L LE H LE Q K
MAIZE_NAC2	C PK C SH V ID N SD V V H Q W PGL F L K V G V K F D PS D Q E L I W H L MA K
Consensus	

Os06g0267500	
ARATH_At1g26390	S GL S GL S SH F FI D E F I P T V N Q DD G IC Y TH F KN L PG V K S D G
ARATH_ARATH_NAC_85	C GID G L K PH L L I Q D FI C SV T Q D VG I NY T HE Q N L PG V K S D G
ARATH_NAC_44	C GIG S SE P PH V LIE F FI R P V TE D VG I NY T HE Q N L PG A KN D G
Os02g0594800	I SL G SS K ET F DI E FI T ID N DE G IC Y SH F EN L PG K M K D G
MAIZE_NAC	S SL E NS V SH L ID S FI T IK E RE G IC Y TH F KN P FG I K M D G
MAIZE_ANAC044	V CL G DS R PH V LIE F FI T ID N DE G IC Y SH F EN L PG K M K T D G
MAIZE_NAC1	V CL G DS R PH V LIE F FI T ID N DE G IC Y SH F EN L PG K M K T D G
MAIZE_NAC2	H SK S G I K H PH F FI D E F I P T V EE D E G IC Y TH F Q K L P GV K Q N G
Consensus	

Os06g0267500	
ARATH_At1g26390	T VS H FF H KA I K A Y S T G TR K RR K THDD D FG..... D VR W
ARATH_ARATH_NAC_85	T SVFF F FN K TA H AY Q NG Q R K RR R IT P T..... S L K DD T VR W
ARATH_NAC_44	T SVFF F FN K TA H AY Q NG Q R K RR R IT P T..... L V N DE P VR W
Os02g0594800	T SG H FF H RV S NA Y CG Q R K RR K IS N C..... D H V SV E H V VR W
MAIZE_NAC	S YL H FF F NR V L N AY D T G TR K RR R II A T G DI C DC G V V NG S SR W
MAIZE_ANAC044	S NA H FF H RV S K A Y G Y G Q R RR R II N CS..... D HT V PD D RV W
MAIZE_NAC1	S NA H FF F RV S K A Y G CG Q R K RR R IT N CS..... D HT V SD E H V VR W
MAIZE_NAC2	S VS H FF H RT F K A Y N T G TR K RR R INT D DA..... D VR W
Consensus	

Os06g0267500	
ARATH_At1g26390	H K T GR T EP V LD E V Q R G CK K IM V LY S T MR K GR P E K T N
ARATH_ARATH_NAC_85	H K T GR T EP V LD E V Q R G CK K IM V LY S T MR K GR P E K T N
ARATH_NAC_44	H K T GR T EP V LD E V Q R G CK K IM V LY S T MR K GR P E K T N
Os02g0594800	H K T GR T EP V LD E V Q R G CK K IM V LY S T MR K GR P E K T N
MAIZE_NAC	H K T GR S KA I V..... E KG V TK G WK K IM V LY S S QR A K P DK A N
MAIZE_ANAC044	H K T GR S KA I V..... E KG V TK G WK K IM V LY S S QR A K P DK A N
MAIZE_NAC1	H K T GR S KA I V..... E KG V TK G WK K IM V LY S S QR A K P DK A N
MAIZE_NAC2	H K T GR S KA I V..... E KG V TK G WK K IM V LY S S QR A K P DK A N
Consensus	

Os06g0267500	
ARATH_At1g26390	V WM H Q Y HL G TE D ER D CE V Y V VS K LF Q Q..... F K P E K N
ARATH_ARATH_NAC_85	V WM H Q Y HL G TE D ER D CE V Y V VS K LF Q Q..... F K P E K N
ARATH_NAC_44	V WM H Q Y HL G TE D ER D CE V Y V VS K LF Q Q..... F K P E K N
Os02g0594800	V WM H Q Y HL G TE D ER D CE V Y V VS K LF Q Q..... F K P E K N
MAIZE_NAC	V WM H Q Y HL G TE D ER D CE V Y V VS K LF Q Q..... F K P E K N
MAIZE_ANAC044	V WM H Q Y HL G TE D ER D CE V Y V VS K LF Q Q..... F K P E K N
MAIZE_NAC1	V WM H Q Y HL G TE D ER D CE V Y V VS K LF Q Q..... F K P E K N
MAIZE_NAC2	V WM H Q Y HL G TE D ER D CE V Y V VS K LF Q Q..... F K P E K N
Consensus	

Os06g0267500	
ARATH_At1g26390	A Q D LT S A D A LES I VA E D..LP N IP L FL E EH V
ARATH_ARATH_NAC_85	B Q E VS E D I FAAV T PT A DEV T PK L AT P E R NAVR I CS D SH I
ARATH_NAC_44	.. E K T IES..... E SS G VR G GF T PK T ST I TQVR P V I
Os02g0594800	.. E NP D EG E S SS G VR G GF T PK T NT T PP R SL V D
MAIZE_NAC	E T G NA D ES..... D AF A VR G GF T PK T NT T PP R CL N
MAIZE_ANAC044	K TD V V V ES..... D AS V AK I NP R TP M TD P Q R RL N S
MAIZE_NAC1	E T E T A CE P D AP S V I GP R TP K IT I TP Q RR H PK S
MAIZE_NAC2	E T E T A Y E P..... G AP S V I GP R TP K IT I TP Q RR H PK N
Consensus	

Os06g0267500	
ARATH_At1g26390	F T N .. Q E L EV L E K SE T IT D Q G KE T SE I NN D NA V ED V A H M
ARATH_ARATH_NAC_85	A SD V Y T PS D Y S A H EV S LA E T S EV M C M ED V Q S I Q P N H R
ARATH_NAC_44	S .. V DE D E I AF D DD S K M V L
Os02g0594800	G .. V AG D E E AF D D..... L K M F
MAIZE_NAC	S PC E T E NY D PI E LD Q D E E E
MAIZE_ANAC044	S SC T NG Q HT P T Q ED G EE C GT S K M K T E A AE C S A C P AE S S
MAIZE_NAC1	S PC T Q N I S IV Q DL..... L L D SE G E
MAIZE_NAC2	S PC T E Q SI F L Q DL..... L L D GE E
Consensus	

Os06g0267500	
ARATH_At1g26390	A TE K P..... E D G DN P SS Q DP K
ARATH_ARATH_NAC_85	P SS G PE L EH G LE N AK E ML D D K EE Q E K DR D N E N Q GE D PT
ARATH_NAC_44	D SY A E G LE N I Q E
Os02g0594800	D PF F E E LD S I PE
MAIZE_NAC	S N F V TS K DD A GN P A WC A G
MAIZE_ANAC044	P AI P TS D A P V TS P MD P Q R RL N SR C NT R
MAIZE_NAC1	P TM P I VS L ED D AM N P..... A WC A VA
MAIZE_NAC2	P TM P I VS L AD D AM N P..... A WC A VA
Consensus	

Os06g0267500	
ARATH_At1g26390	W W E GES Q F..LL D S Q LA E N L AI C DE F L Q S Q ST S CGGG D
ARATH_ARATH_NAC_85	W FD S GS Q F..IL N S Q LV E AL S LD D LL G S Q DR E ENT N SG
ARATH_NAC_44	A SS G ST S DK A K V CG N .. V SV I ED N LL S .. K K I BA S S.....
Os02g0594800	A AL K ME V SK K AR M DE V UN L SE D EN L CP D ME A SS L W E N
MAIZE_NAC	.. E T Q AA R .. E AV Q AC P N L DES L R H E V LD S PY H ET L LP S DR
MAIZE_ANAC044	R CT P I Q SD Q GA E EC G TS K V K IE A EC S S A C I AR L SP A IP T
MAIZE_NAC1	E ER Q V V G.. E AS R A Q SN S VE T LL C RED F NS L NY E ALL P WD Y
MAIZE_NAC2	E EE Q V V G.. E AS R A Q SN S DE P LL C RED F NS L ND E ALL P WD Y
Consensus	

Os06g0267500	
ARATH_At1g26390	E TD K IK P RL A Y A VA Q LP V ED L K D LE C Q R LD P SD G T N LE T
ARATH_ARATH_NAC_85	S L K DK Q EC I AD Y A H LG P ED F K R DL E EC Q K I VL D PS N IE L
ARATH_NAC_44	I EN H GN V D V G.. S C N .. I VS S DL E N..... A E L
Os02g0594800	Q VL N PS L GT V G.. D ED G .. F SS I DL E N..... A D L
MAIZE_NAC	P IL S Q G GN E IL D R N IN A V.. Y GL P DL Y N..... V D L
MAIZE_ANAC044	S A E PM Q PT D AL D AS L PV D DL F H G LL D LD N T L P.. F TE T
MAIZE_NAC1	P IL S Q C R N EML D GN L NG.. Y GL P DL H N..... V D L
MAIZE_NAC2	P IL S Q C R S E I FD R N L NG.. Y GL P DL H N..... V D L
Consensus	

Os06g0267500	
ARATH_At1g26390	E NA S E F RL S Q I E F .. S Q S ET T A R AG G K V ID
ARATH_ARATH_NAC_85	D TP F E F RL S Q I E F GS Q DS FL A.. G T G K T D
ARATH_NAC_44	G T L P.. D LL S FA S ED S LM N .. L GW F
Os02g0594800	G TF P .. D FL T IAS Q ES L LN.. L GW L
MAIZE_NAC	G TF P FD L AD L Q F GS Q ES I GN.. L DS I
MAIZE_ANAC044	P SG G GIS L DD I .. F GL D SL G A.. P D G FL
MAIZE_NAC1	G TF P DL Q AD F Q F GS Q ES L GS.. L D R I
MAIZE_NAC2	G TF P DL Q AD F Q F GS Q ES L GS.. L D R I
Consensus	

B

Name	Pro-aa	Gene ID	Protein entry
OsNAC02	393	Os02g0594800	Q6Z165_ORYSJ
Zem-NAC	438	Zm00014a_036995	A0A3L6FR16_MAIZE
ANAC044	399	Zm00001e022870	B6TBS0_MAIZE
NAC1	399	NAC1	C0PGC9_MAIZE
NAC2	412	ZEAMMB73_Zm00001d037315	A0A1D6LWF7

Name	Pro-aa	Gene ID	Protein entry
OsNAC06	246	Os06g0267500	Q5Z9V2_ORYSJ
AtSOG1	499	At1g26390	A0A178WL11
ARATH_NAC085	361	At5g13980	A0A178UA43
NAC_44	366	At3g00730	A0A178VDA1

S4 Fig. The homologous analysis of NAC in plant

(A) The homologous analysis of NAC proteins with amino acid sequence alignment in the rice, maize and Arabidopsis by using DNAMAN 7.0.

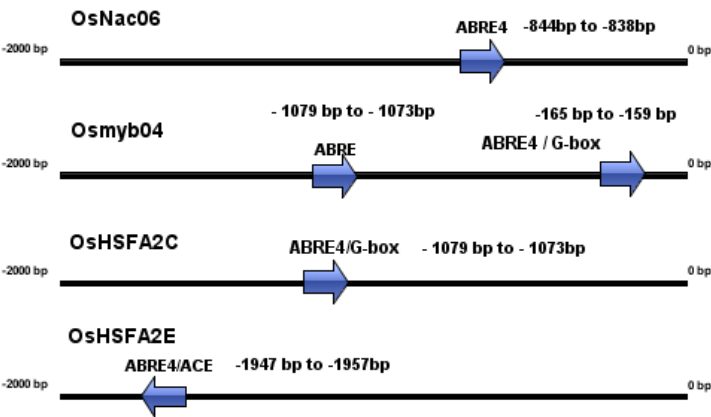
(B) The Uniprot accession of 9 genes in homologous analysis. (<https://www.uniprot.org/>). OsNAC06 (Os06g0267500) has simple protein constructure and contains only 246 aa, which was highly homologous to AtSOG1. Its N-terminal region with 0 - 45aa was a strictly conserved region of NAM domain.

A

Table the motif analysis of genes regulated by OsNAC02 in Co-network

Gene	ID	Motif	Motif seq	start	end
OsNAC02	LOC_Os02g38130	---	---	---	---
OsNAC06	Os06g0267500	ABRE4	CACGTA	838	844
OsMYB04	LOC_Os04g43680	ABRE	AACCCGG	166	171
		ABRE	ACGTG	1559	1552
		G-Box	CACGTT	165	159
OsHSFA2C	LOC_Os10g28340	ABRE/G-box	CACGTG	1079	1073
OsHSFA2E	LOC_Os03g58160	ABRE	GACACGTATG	1947	1939
		ACE	GACACGTATG	1947	1957

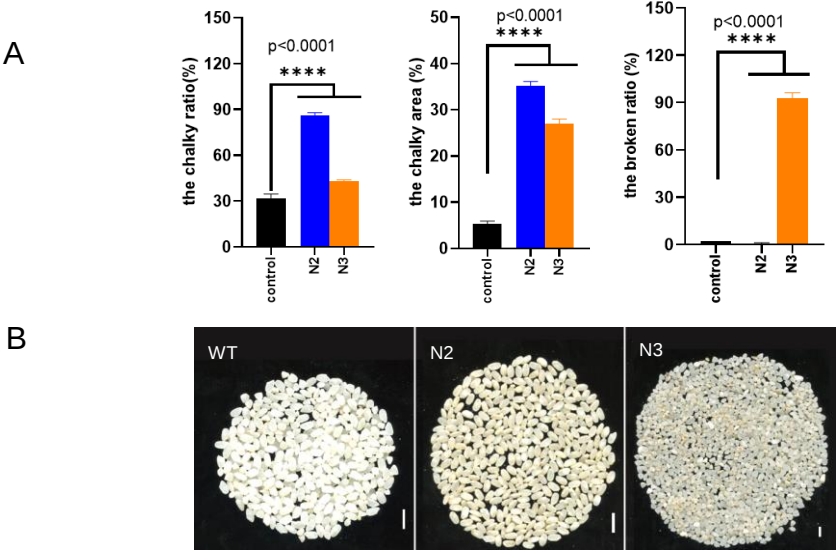
B



S5 Fig. The ABRE/ G-box motif locations on the promoter regions of OsNAC02 regulated genes

(A) The motif analysis of genes regulated by OsNAC02 in co-network, all the location are detected by the PlantCARE. (<http://bioinformatics.psb.ugent.be/webtools/plantcare>).

(B) The model of the motif analysis in the promoter region (-2000bp-'ATG') of the OsNAC02 effective genes, as the arrow shows the direction of motifs with (+/-) on the promoter and the fig construction was completed by the software IBS1.0.

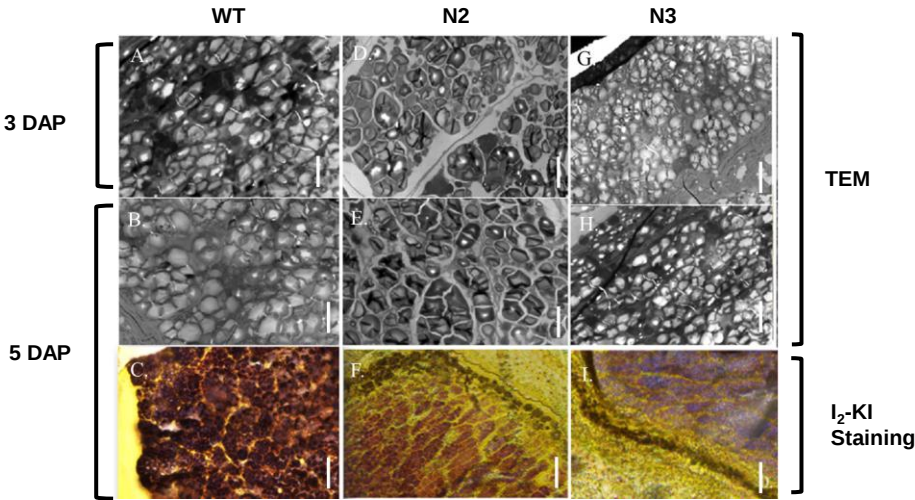


C **Table** Chalky phenotype of mutants by the statistics analysis (%)

Characters	WT	N2 (<i>Osnac02</i>)	N3 (<i>Osnac02/Osnac06</i>)
Chalky ratio (Number)	12%	75%	12.6%
Chalky ratio (area)	5%	30%	10%
rice broken ratio	2%	0%	90%

S6 Fig. The chalky phenotype of N2/N3 *mut* seeds (T2) in 2021

- (A) The chalky phenotypes of the milled rice are calculated by chalky ratio and the chalky area . The transparent are low in both mutant and WT, but the chalky ratio in mutants are obvious higher than that in WT. The rice broken ratio of N3 *mut* is highest of all. All the statistic analysis were performed by running a two-way ANOVA on the mixed model, *, $p < 0.05$, **, $p < 0.01$ and ***, $p < 0.0001$.
- (B) The sample names are WT, N2 and N3 in the figure respectively. As the chalky phenotypes of milled rice and broken ratio of N2 and N3 *mut* per plant shown in this figure, both N2 and N3 are core-white in milled rice, but only N2 has core and belly white in its floury endosperm. As these figures show the yield per plant simultaneously, only N3 has highest yield and broken ratio in the milled rice per plant. $n=10$ mm.
- (C) The three phenotypes of transparent in N2 and N3 mutant by statistics analysis.



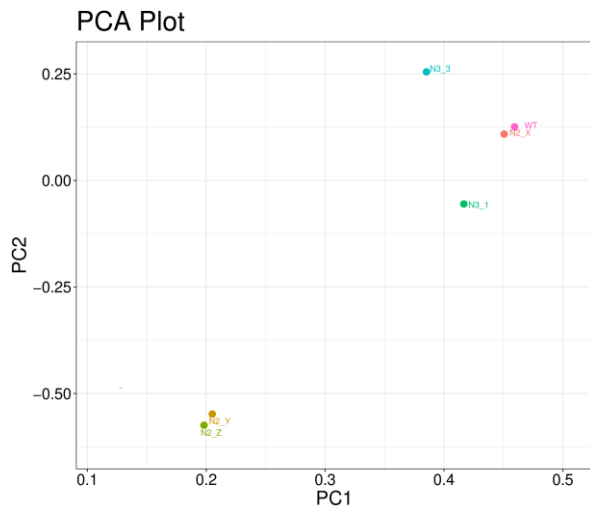
S7 Fig. The histological analysis during endosperm development

Histological analysis of endosperm for maturation detection. As above shows the semi-thin sections of the opaque endosperm scanning in TEM and KI-I₂ stains. $n=100$ μ m.

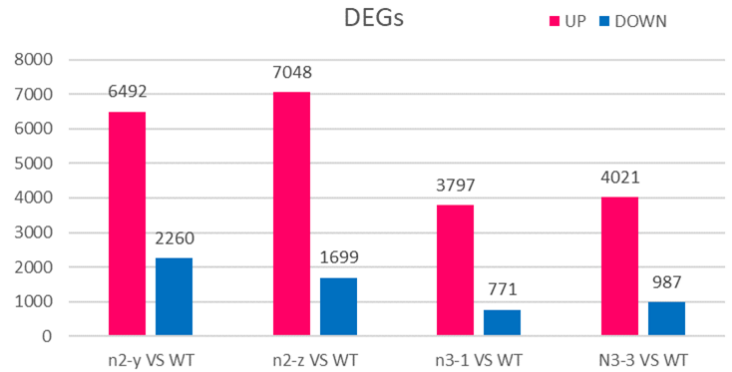
The processes in endosperm development of WT/N2/N3 are shown in this figure respectively. As the **fig C**, **fig F**, **fig L** show the semi-thin sections stained of opaque endosperms at 5 DAP by the I₂-KI. The iodine-staining(I₂-KI) semi-sections implied the distinct starch content between N3 *mut* and N2 *mut*. For a darker blue in N3 *mut* (5 DAP) implies there are higher Amylase content in N3 *mut* (**fig L**) than that in N2 *mut* (**fig F**).

Abbreviation: TEM, transmission electron microscope; DAP, days after pollination.

A



B



S8 Fig. PCA clusters and DEGs of each samples based on RNA-seq analysis

(A) The trend of PCA analysis are based on the RNA-seq analysis of seed (3 DAP), and four samples are clustered into 3 PCA groups: The red spot was PCA1 cluster with WT and N2-1 (N2-x); The yellow spot was PCA2 cluster with N2-2 (N2-y), which used as duplicate sample of N2-x for the KO-OsNAC02 *mut*; The blue spot was PCA3 cluster with N3_1 and N3_3.

(B) The DEGs in PCA2 cluster with N2-x and N2-z as one replicate, PCA3 cluster with N3_1 and N3_3 as one replicate. The similar number of DEGs in repeat samples of N2 and N3 proven the PCA analysis in Fig A .

S9 Fig. The GO enrichment of DEGs in N2/ N3 mutant vs WT

The gene enrichments of the GO analysis including 3 indexes: the top 9 GO pathways of the biological process (purple), the cellular component (green), the molecular function (yellow).

(A) The top 9 pathways of GO enrichment in **N2 vs WT**.

(B) The top 9 pathways of GO enrichment in **N3 vs WT**.

(C) The GO bubble Statistics includes biological process (blue), cellular component (red), molecular function (green), which were in accordance with results in **fig A** and **fig E**.

In the cellular component (red), the two GO pathways :GO :0009507- the chloroplast pathway and GO:0009535- the chloroplast thylakoid membrane pathway were significantly enriched in **N2 vs WT**.

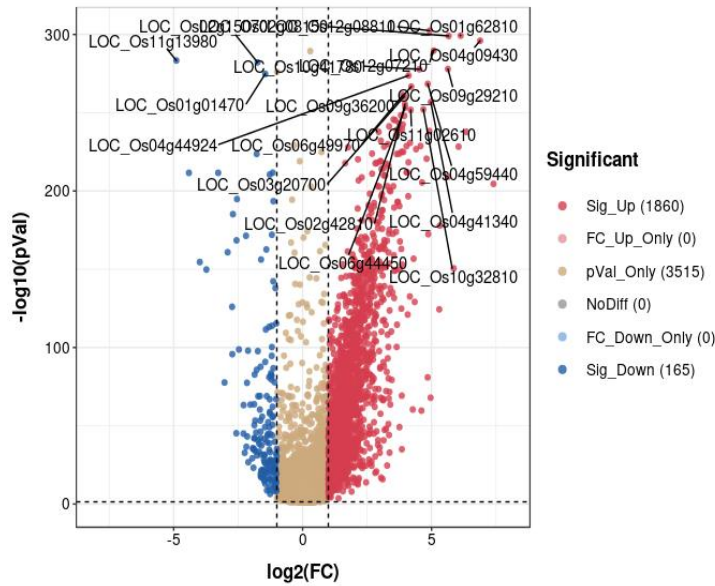
(D) The bubble analysis of Go pathways in the **N3 vs WT** . In molecular function (green), the GO:0005618- cell wall pathway is the most significantly enriched pathway. While less genes are enriched in the other cellular component (red) GO:0005739- mitochondrion pathway.

(E) The results represents the top 20 GO pathways of seed transcriptome including the GO significantly enriched pathways as appearing in **fig C** and **fig D**.

A

Advanced Volcano Plot

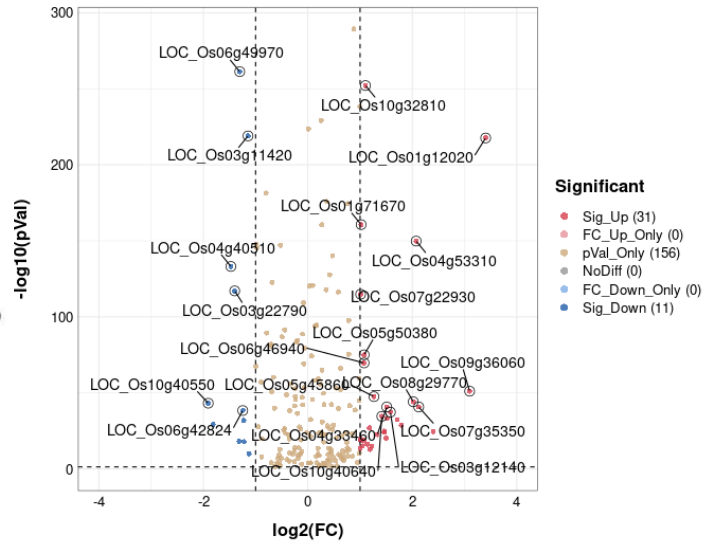
Total Genes: 5540



B

Advanced Volcano Plot

Total Genes: 198

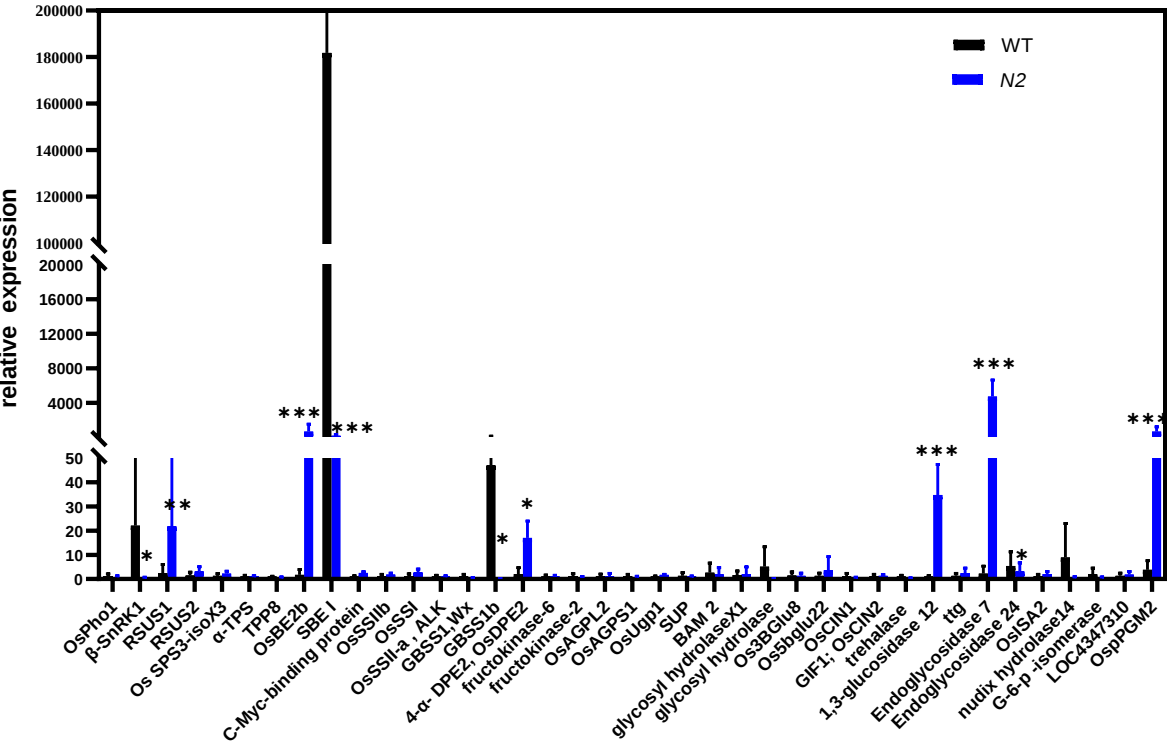


S10 Fig. The DEGs clusters in the Volcano plots of RNA-seq in seeds (3DAP)

The top 20 genes in DEGs are labeled with loc_IDs in the Volcano plots.

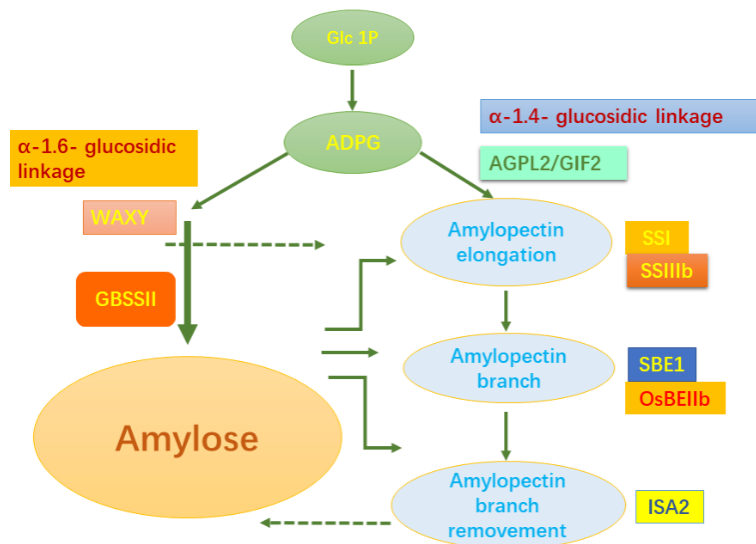
(A) Volcano analysis showed the distinctive expressed genes (DEGs) in seeds of mutants vs WT, and the most genes of DEGs are up-regulated.

(B) Volcano analysis showed the DEGs of all the relevant genes in the Starch and Source metabolism pathway in the seeds of mutants vs WT.



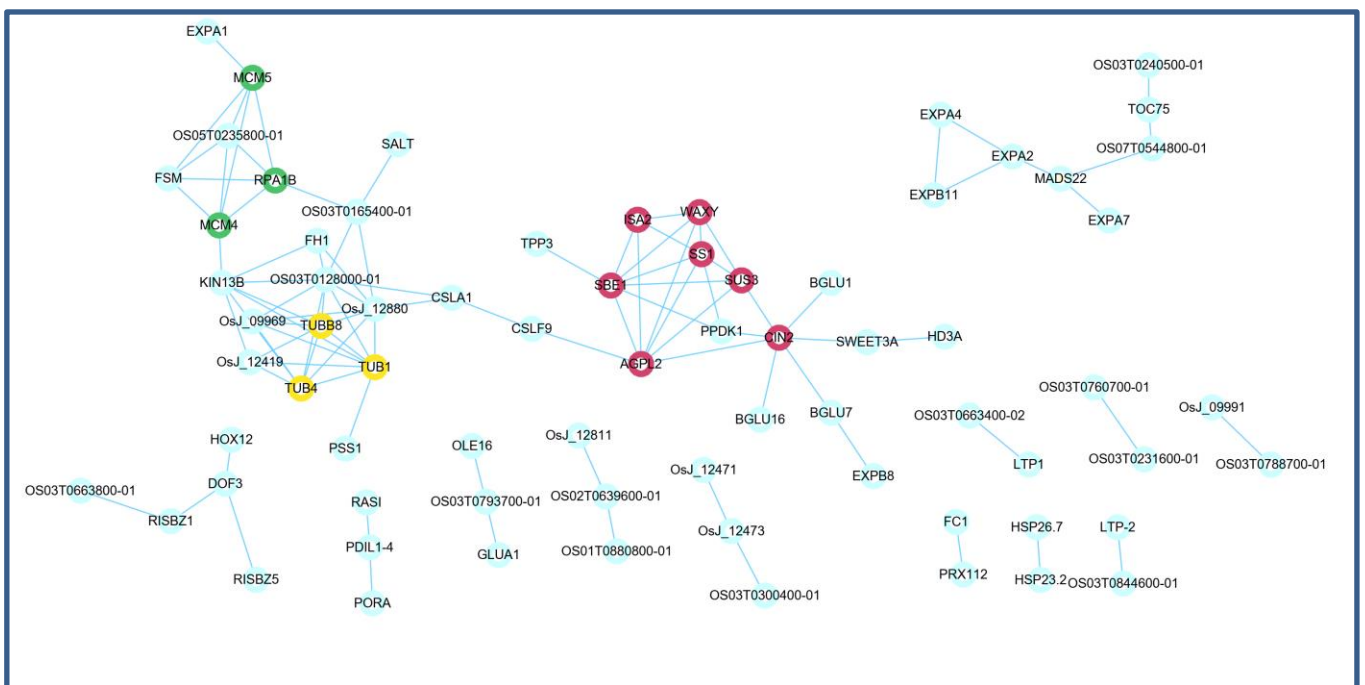
S11 Fig. The genes of 39 ECs in Sucrose and Starch Metabolism pathway in Mapman of N2 vs WT seeds (3DAP) are verified by qRT-PCR

The qRT-PCR verification in seeds (3DAP) displayed the expressions of *OsNAC02* relative genes in its regulatory co-network. All the statistical analyses are performed by student *t*-test, * *p* < 0.05, ** *p* < 0.01 and *** *p* < 0.001.



S12 Fig. The model of starch synthesis in amyloplast/chloroplast of *N2* *mut*

The pro-pro co-network during the early endosperm development in *N2* *mut*, the crucial genes in starch synthesis are screened ,which functions has been displayed clearly in the previous study.



S13 Fig. The Pro-pro interaction analysis by Cytoscape network

The three groups of proteins in co-network were screened on the Score_threshold >900, which are significantly co-expressed ;

S10 Table. The data of the pro-pro notation of WTVSN2_xVSN2_y_DEGs for heatmap

Gene name	FPKM.N2 1	FPKM.N2 2	FPKM.WT	Gene ID
RPA1B	15.27	0.12	12.27	LOC_Os03g11540
MCM4	12.22	0.03	9.13	LOC_Os01g36390
MCM5	13.44	0	9.62	LOC_Os02g55410
MCM7	5.93	0	4.33	LOC_Os12g37400
TUB1	73.45	0.48	54.00	LOC_Os01g18050
TUB4	383.03	2.48	297.69	LOC_Os01g59150
TUBB8	2.88	0	7.82	LOC_Os03g45920

Gene name	FPKM.N2 X	FPKM.N2 Y	FPKM.WT	Gene ID
CIN2	20.68	0.08	8.35	LOC_Os04g33740
ISA2	138.01	4.08	156.74	LOC_Os05g32710
AGPL2	1098.16	22.48	835.73	LOC_Os01g44220
WAXY	2169.58	1.41	1142.76	LOC_Os06g04200
SBE1	1215.05	20.30	1117.17	LOC_Os06g51084
SUS3	2146.29	0.09	1261.04	LOC_Os07g42490

Gene name	FPKM.N2-2	FPKM.N2-Z	FPKM.WT	Gene ID
TPP3	1.03861154	0.577313	2.357583	LOC_Os07g43160

S1 Table. The H-1-Y vectors construction of genes in OsNAC02 co-network

<i>EcoR I</i>	<i>pB42AD</i> vector-CDS	Forward Primer (5'-3')	Reverse Primer (5'-3')
LOC_Os02g38130	OsNAC02	AATTCGAGCTCGGGCACATTACCAATAT ACTACTACGT	CATGGTCAAGAGACTGCATAGCTGAAG TCGTG
<i>Xho I</i>	<i>pLacZi</i> vector-pro	Forward Primer (5'-3')	Reverse Primer (5'-3')
LOC_Os04g43680	<i>pOsMYB04</i>	GAGCACATGCCTCGAGTGCTCCTTCCC TGCTCTGA	ATCTGTGACCTCGAGGACGGGCGAC AGATACGATA
LOC_Os10g28340	<i>pOsHSFA2C</i>	GAGCACATGCCTCGAGCCCAGTTTGGC AGCTTTTG	ATCTGTGACCTCGAGTAGCACAGTG GCTATGGTCG
LOC_Os03g58160	<i>pOsHSFA2E</i>	ATCTGTGACCTCGAGGCACATAAAAA CGAGGCAGACA	GAGCACATGCCTCGAGGACTGGATCA CCCGATTCT

S4 Table.

The GO enrichment analysis of N2 vs WT

GO_ID	GO_Term	GO_Category	Significant ID Number :	Total Significant ID Number	Rich.Factor	P.value
GO:0015979	photosynthesis	Biological Process	62	13278	0.8052	4.87E-09
GO:0009658	chloroplast organization	Biological Process	78	13278	0.7429	4.31E-08
GO:0046777	protein autophosphorylation	Biological Process	115	13278	0.6647	8.52E-07
GO:0006457	protein folding	Biological Process	156	13278	0.7393	1.85E-14
GO:0006412	translation	Biological Process	196	13278	0.6405	1.38E-08
GO:0046686	response to cadmium ion	Biological Process	172	13278	0.6394	1.23E-07
GO:0009414	response to water deprivation	Biological Process	161	13278	0.5812	0.000535
GO:0009651	response to salt stress	Biological Process	228	13278	0.5846	2.6E-05
GO:0008150	biological_process	Biological Process	1572	13278	0.5497	9.73E-15
GO:0009534	chloroplast thylakoid	Cellular Component	110	13278	0.8148	1.12E-15
GO:0009535	chloroplast thylakoid membrane	Cellular Component	215	13278	0.7288	3.78E-18
GO:0009941	chloroplast envelope	Cellular Component	291	13278	0.7442	1.58E-26
GO:0009570	chloroplast stroma	Cellular Component	340	13278	0.7173	7.94E-26
GO:0005773	vacuole	Cellular Component	277	13278	0.6564	2.45E-13
GO:0009507	chloroplast	Cellular Component	1628	13278	0.6604	2.67E-78
GO:0005829	cytosol	Cellular Component	1219	13278	0.629	1.08E-41
GO:0005739	mitochondrion	Cellular Component	1013	13278	0.5917	3.08E-21
GO:0016021	integral component of membrane	Cellular Component	1705	13278	0.5601	2.72E-20
GO:0005737	cytoplasm	Cellular Component	1947	13278	0.5392	7.45E-14
GO:0003735	structural constituent of ribosome	Molecular Function	248	13278	0.6408	1.66E-10
GO:0003729	mRNA binding	Molecular Function	205	13278	0.6699	1.87E-11
GO:0003824	catalytic activity	Molecular Function	148	13278	0.6218	8.9E-06
GO:0004672	protein kinase activity	Molecular Function	156	13278	0.629	1.97E-06
GO:0046872	metal ion binding	Molecular Function	701	13278	0.6379	1.8E-26
GO:0016787	hydrolase activity	Molecular Function	257	13278	0.6134	3.4E-08
GO:0003674	molecular_function	Molecular Function	1414	13278	0.5515	6.96E-14
GO:0005524	ATP binding	Molecular Function	838	13278	0.5348	8.65E-06
GO:0005515	protein binding	Molecular Function	1473	13278	0.5302	3.94E-08

S4 Table.

The GO enrichment analysis of N3 vs WT

GO_ID	GO_Term	GO_Category	Significant ID Number :	Total Significant ID Number	Rich.Factor	P.value
GO:0006096	glycolytic process	Biological Process	72	14165	0.809	6.88E-09
GO:0009658	chloroplast organization	Biological Process	83	14165	0.7905	4.09E-09
GO:0006470	protein dephosphorylation	Biological Process	83	14165	0.7545	1.85E-07
GO:0006457	protein folding	Biological Process	157	14165	0.7441	5.35E-12
GO:0006412	translation	Biological Process	224	14165	0.732	3.75E-15
GO:0016192	vesicle-mediated transport	Biological Process	102	14165	0.7286	1.64E-07
GO:0006397	mRNA processing	Biological Process	117	14165	0.7267	2.65E-08
GO:0009793	embryo development ending in seed dormancy	Biological Process	234	14165	0.7006	2.33E-12
GO:0008150	biological_process	Biological Process	1748	14165	0.6112	1.54E-28
GO:0022625	cytosolic large ribosomal subunit	Cellular Component	134	14165	0.8121	1.3E-15
GO:0005730	nucleolus	Cellular Component	220	14165	0.8088	2.85E-24
GO:0009941	chloroplast envelope	Cellular Component	305	14165	0.7801	6.94E-28
GO:0009570	chloroplast stroma	Cellular Component	359	14165	0.7574	4.26E-28
GO:0009507	chloroplast	Cellular Component	1715	14165	0.6957	3.67E-82
GO:0005739	mitochondrion	Cellular Component	1171	14165	0.684	3.59E-49
GO:0005829	cytosol	Cellular Component	1318	14165	0.6801	2.3E-53
GO:0005737	cytoplasm	Cellular Component	2103	14165	0.5824	5.23E-19
GO:0016021	integral component of membrane	Cellular Component	1766	14165	0.5802	4.67E-15
GO:0005634	nucleus	Cellular Component	3951	14165	0.5595	3.63E-19
GO:0051082	unfolded protein binding	Molecular Function	60	14165	0.8333	1.46E-08
GO:0003729	mRNA binding	Molecular Function	241	14165	0.7876	2.28E-23
GO:0003735	structural constituent of ribosome	Molecular Function	292	14165	0.7545	1.17E-22
GO:0005525	GTP binding	Molecular Function	112	14165	0.7368	1.46E-08
GO:0003924	GTPase activity	Molecular Function	111	14165	0.7303	3.86E-08
GO:0003723	RNA binding	Molecular Function	496	14165	0.7116	4.17E-27
GO:0003676	nucleic acid binding	Molecular Function	190	14165	0.6738	3.38E-08
GO:0046872	metal ion binding	Molecular Function	715	14165	0.6506	7.19E-21
GO:0003674	molecular_function	Molecular Function	1570	14165	0.6123	4.68E-26
GO:0005618	cell wall	Cellular Component	218	14165	0.3444	1

S5 Table. the genes co-expressed in the down-regulated Venn diagram (N2 vs N3 vs WT , Fig 4B)

Co-Venn clusters	Gene ID	Function	Reference
N2-x\N2-z\N3-1	LOC_Os10g11889	The pathogenous stress such as the (<i>Magnaporthe</i> L.) induced	(Drogue et al., 2014)
N2-x\N2-2\N3-1	LOC_Os11g37270	Ar-AMP have the broad-spectrum potent activity to the multi-drug resistant , which similar to Embryo-specific protein	(Khamis et al., 2015)
N2-2\N2-Z\N3-3	LOC_Os07g43160	OsTPP3 as the enzyme in ABA pathways induced by the abiotic stress and expressed high in endosperm	(Yang et al, 2018)
N2-1\N3-3	LOC_Os04g36750	α-protein crystallography hsp20 is protein chaperones	(Wang et al., 2014)
N2-1\N2-2	LOC_Os01g03390	BBT17 - Bowman-Birk type bran trypsin inhibitor precursor	(Sonsungsan et al., 2021)
N2-1\N2-2	LOC_Os08g03410	Glutelin as storage in endosperm	(Gan et al., 2021)
N3-1\N3-3	LOC_Os03g63330	aspartokinase, chloroplast which mainly response to the biotic stress	(Shaar-Moshe et al., 2015)

S8 Table. the volcano diagram of genes hub in DEGs of the seeds (N2 vs N3 vs WT, Fig 4C)

Up-regulated	All DEGs in top 5	Function
1	LOC_Os02g08150	CCT/B-box zinc finger protein, putative, expressed
2	LOC_Os12g08810	VTC2, putative
3	LOC_Os01g62810	regulator of chromosome condensation,
4	LOC_Os04g09430	cytochrome P450,
5	LOC_Os09g29210	OsPUP2
Down-regulated	LOC_Os01g01470	OsNAC20; ONAC020
2	LOC_Os02g15070	glutelin, putative, expressed
3	LOC_Os11g13980	hsp20/alpha crystallin family protein, putative, expressed

S9 Table. the volcano plot of labeled genes in Sucrose and Starch pathway of the seeds (N2 vs N3 vs WT, Fig 4D)

Up-regulated	DEGs in the top 5	Gene name	Sucrose and Starch pathway
1	LOC_Os10g32810	OsBAM2	(Kim et al., 2017)
2	LOC_Os01g12020	LTPL18 - Protease	(Zhang et al., 2010)
3	LOC_Os01g71670	OsGLN2	(Akiyama et al., 2004)
4	LOC_Os04g53310	OsSSIIIb	(Wang et al., 2015a)
5	LOC_Os07g22930	GBSSII	(Maung et al., 2021)
Down-regulated	LOC_Os06g49970	alpha-amylase	(Damaris et al., 2019)
2	LOC_Os03g11420	Os3BGlu6, glycosyl hydrolase family 1	(Sun et al., 2021)
3	LOC_Os04g40510	glycosyl hydrolase family 5	(Xiang et al., 2019)
4	LOC_Os03g22790	beta-amylase,	(Wang et al., 2022a)
5	LOC_Os10g40550	CPuORF23	(Shaar-Moshe et al., 2015)

S12 Table. The qRT-PCR primers of DEGs in starch and sucrose pathway ECs for Mapman verification

EC in KEGG	Gene ID	Gene name	Forward Primer (5'-3')	Reverse Primer (5'-3')
EC:2.4.1.1	LOC_Os03g55090	starch phosphorylase	GGATGGTGCTAATGTGGAA	TGGCTGTGTTGAGGATTG
EC:2.4.1.12	LOC_Os05g41220	1,OsPho1	GAATCCACCAATAAACCATCC	AGTATCACCAAGAACAGACA
EC:2.4.1.13	LOC_Os03g28330	β-SnRK1	ACAACAACGAGCACAAGT	CACCACAGACAACCACAA
EC:2.4.1.13	LOC_Os06g09450	OsSuSy1,RSUS1	GAGTTGGCAGTGGAAGAG	GGAAGGAGGCATTGAAGG
EC:2.4.1.14	LOC_Os06g43630	OsSuSy2,RSUS2	CACGGTCTAAGGCTCTAAG	CGGCATCTCCATCAAGTAT
EC:2.4.1.15;	LOC_Os02g54820	OsSPS3-isoX3	CAAGGAGGCAACAGATGG	GAGGTGGTCAAGCAGTTC
EC:3.1.3.12	LOC_Os09g25890	α-TPP	GCTACTACCTCAACGACAA	GCAGCAATCAATCTTACTCA
EC:2.4.1.18	LOC_Os02g32660	TPP8	TTGCCACTGATGCTGATG	CACTTCTCTGACCACCTTC
EC:2.4.1.18	LOC_Os06g51084	OsBE2b	GAAGAAGTGAAGGAAGGT	CCACAAGAAGAAGAGGAAGA
EC:2.4.1.18	LOC_Os07g08820	SBEI	GTGGTGTGCTTGATACTCT	CATACGACTTGATGATGGATG
EC:2.4.1.21	LOC_Os02g51070	C-Myc-binding protein	TATACTCGCTCTGTTCTTGT	CACCGTCCATTGTCTTGA
EC:2.4.1.21	LOC_Os06g06560	OsSSIIb	ATTCTCGCCACTAACCATT	ATTGCTGCTTCTTCTCAA
EC:2.4.1.21	LOC_Os06g12450	OsSSI	GCGATGACGATGATGACT	CCAGCAGCCTTGTAGTATT
EC:2.4.1.242	LOC_Os06g04200	OsSSII-a , ALK	TGGTGAGGATGTTGTGTTTC	GGTAGGAGATGTTGTGGATG
EC:2.4.1.242	LOC_Os07g22930	GBSS1,wx; qGC-6	CAGCAGGAAGAAGAGAACAACA	G
EC:2.4.1.25	LOC_Os07g46790	GBSS1b	ACTTGGATTGATTGGATTGC	CCTTGATGGTTGCTTGGA
EC:2.7.1.4	LOC_Os06g12600	4-α- DPE2, OsDPE2	AACAAGCAGTTCTCCACAT	ATTCATCATCGCCAACCTT
EC:2.7.1.4	LOC_Os08g02120	fructokinase-6	CATCCTCTGCTCCTACGA	CCTTGAAGTCCTTGGTGAA
EC:2.7.7.27	LOC_Os01g44220	fructokinase-2	GGACAGCAGAGACGGACAT	GTGAAGCGGAGGACGAGAT
EC:2.7.7.27	LOC_Os09g12660	cytosolic-like,OsAGPL2	GCACATCAAGCAATCAACT	CGGACTTACAACACCTCTAA
EC:2.7.7.9	LOC_Os09g38030	amyloplastic,OsAGPS1	TTGAAGGTCTCTGGTGTGTCT	ACGGATGTGCCAAGATGATTAC
EC:3.1.3.24	LOC_Os02g05030	UTP-glucose-1,OsUgp1	AACAGCGTCCACATAAGG	TTACAACAGCATCCGTAGG
EC:3.2.1.2	LOC_Os03g04770	SUP,sucrose-phosphatase,	CGCAACTACGAATACCTCT	TGTCATAGCACTGGAATC
EC:3.2.1.20	LOC_Os06g46284	BAM2,OsBMY4	CCAGAAGTTCGTCGTCAT	CACCGTCTTGTTGTTGATG
EC:3.2.1.20	LOC_Os06g46340	glycosyl hydrolase X1	GACGATTCAAGTAGCATTAGG	AAGCAGAGACACAGCAATA
EC:3.2.1.21	LOC_Os03g49610	glycosyl hydrolase	TTCAGCCATCTCAAGTAGC	ATAGCGTAATCACCACAAGT
EC:3.2.1.21	LOC_Os05g30350	β-glucosidase 8, Os3BGlu8	CGTCTCCTCTTCTACCTCTA	GGCTCCTTCCATCTTCTATC
EC:3.2.1.26	LOC_Os02g33110	β-glucosidase 22, Os5bglu22	CAGGACAGGATACCACTTC	ATTGCGTTGCGTTTCATAC
EC:3.2.1.26	LOC_Os04g33740	β-FUS1, OsCIN1	TCTCCTCTTCTCGCTCTC	GTGGCTGGAAGTGATACC
EC:3.2.1.28	LOC_Os10g37660	β-FUS2, GIF1; OsCIN2	GAGTGCCTACCAGGCTAT	AGATGTTGTCTAGAGTTGTCA
EC:3.2.1.39	LOC_Os02g53200	trehalase	GAGGCAGAGATGTTGGAAT	CGATGGAGGAGGAAGAGT
EC:3.2.1.39	LOC_Os05g43690	endo-1,3-β-glucosidase 12	GCACCATTCTGTTCCAT	GCCACCACCAACTACTATT
EC:3.2.1.4	LOC_Os02g50490	plasmodesmata callose-binding proteins	CGACACGGATCACTACTG	GCGATACTGCTGTCATACT
EC:3.2.1.4	LOC_Os09g36350	endoglucanase 7	TTGGTGTGGATAAGTGGATGGA	GGTGCCGCTGTTGTAAGTG
EC:3.2.1.68	LOC_Os05g32710	endoglucanase 24	ACCGATGAACTTGATAGCA	TGGCAATGTAATACGAGGAA
EC:3.6.1.21	LOC_Os06g03910	isoamylase 2,OsISA2	AGTCCGTGAGGTTGAAGA	AGTAATTGGCTCGCTCTG
EC:5.3.1.9	LOC_Os06g14510	nudix hydrolase 14	CCATCTCAACCAACAGTAGT	CTCGGCATCATCCATCAG
EC:5.3.1.9	LOC_Os09g29060	G6-P-isomerase	CGGTGGTAACGATGATGA	GACGATATGTATGTGGCAAT
EC:5.4.2.2	LOC_Os03g50480	LOC4347310	GATTCTTCGTAACACCTTCAG	TCCAGCATCAACATTCTCAT
		phosphoglucumutase		
		2,OspPGM		

S13 Table. The qRT-PCR primers of DEGs in co-network of OsNAC02.

Gene ID	qRT-PCR	Forward primer	Reverse primer
LOC_Os03g13170.1	Ubiquitin	GCTCCGTGGCGGTATCAT	CGGCAGTTGACAGCCCTAG
LOC_Os03g13170.1	Os03g0234200		
LOC_Os02g38130	OsNAC02	CTTCTGACCGCCCAATTCTG	TCTGGAGGTGTTCCAAGGTC
LOC_Os04g43680	Osmby4	TTCTCCAAGGAGGAGGAGGA	GTGCCACACGTTCTTGATCT
LOC_Os10g28340	OsHSFA2C	GAGCAACTTGATTGCCGTA	TCTGCCCAGAAGTCATCTCC
LOC_Os09g35790	OsHSFB2C	AAACGATGGGACCTGGTGAATT	CCTTACGCCACTACCGCATT
LOC_Os03g58160	OsHSFA2E	GCCGATGGACGGGCTC	TACTGTGGCGATTGTTGGTCT
Os06g0267500	OsNAC06	ACCACCTAGGAACTGGGGAA	TCAATCGATCACCTTGCCAC