Characterization of genes relevant to the biological functionality and transcriptional regulation of the ZmNADP-ME3 malic enzyme

Poster N° 239

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Introduction

ZmNADP-ME3 is a maize NADP-dependent malic enzyme that catalyses the oxidative decarboxylation of malate and is expressed almost exclusively during kernel development and in response to ABA hormone-mediated stresses. Although the *ZmNADP-ME3* transcript is found at high levels in embryo and endosperm, the enzyme activity was significantly higher in embryos. In addition, it could be verified that the activity is maximal 20 days after pollination. The presence of *ZmNADP-ME3* was also studied by Western blots. Although the reaction it catalyses is well characterized, its biological role is still unknown. To further study the function of *ZmNADP-ME3*, a meta-analysis of genes co-expressed in different tissues and stages of maize kernel maturation was performed. As a result, genes strongly linked to *ZmNADP-ME3* were identified that could provide clues to the processes in which this malic enzyme participates. In addition, we searched for conserved motifs in the promoters of the co-expressed genes to determine whether they respond to a common signalling pathway. These results, together with those previously obtained on transcription factors that bind to the *ZmNADP-ME3* promoter, provide relevant information on the expression networks in which the *ZmNADP-ME3* protein is involved.

Aims

Analyse the malic activity at different stages of seed development in endosperm and embryo. Analyse genes co-expressed. Elucidate possible Transcription Factors.

Results

1. *ZmNADP-ME3* is upregulated in final stages of embryo maturation

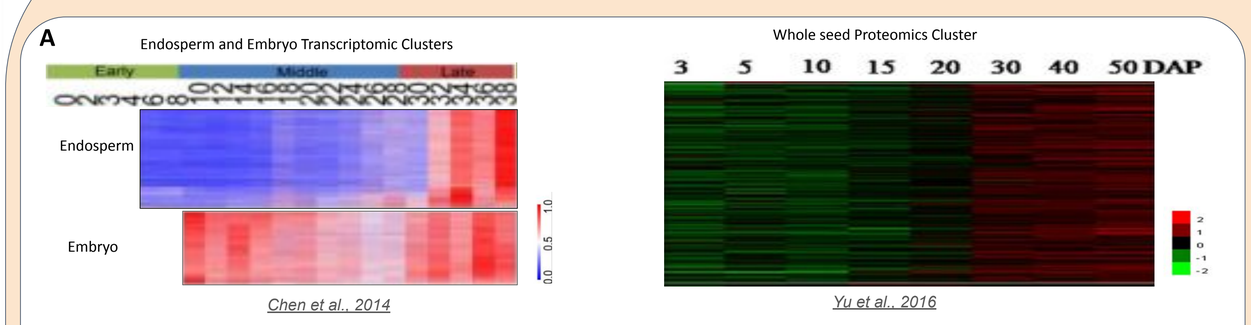
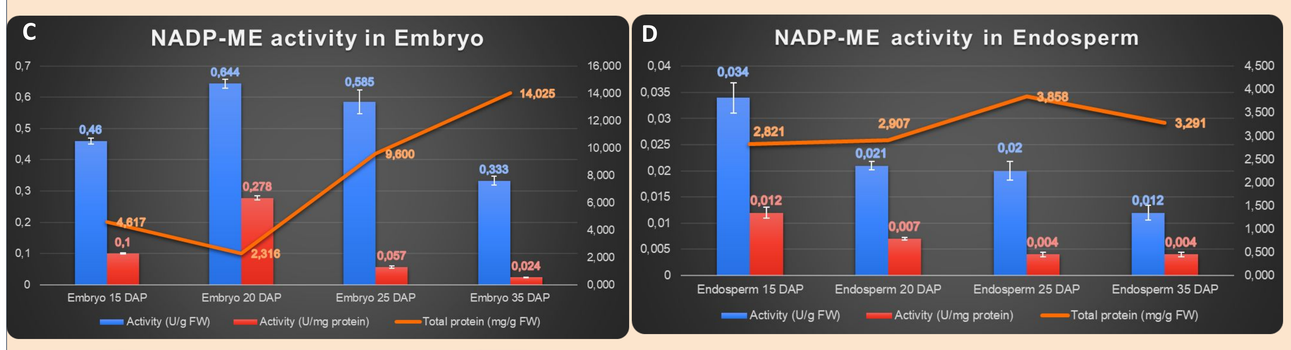


Figure 1A: Expression patterns of *ZmNADP-ME3*

Figure 1B: Samples collected in this work. AX882 maize plants were manually pollinated. Embryos and endosperms were sampled at 15, 20, 25 and 35 DAP.



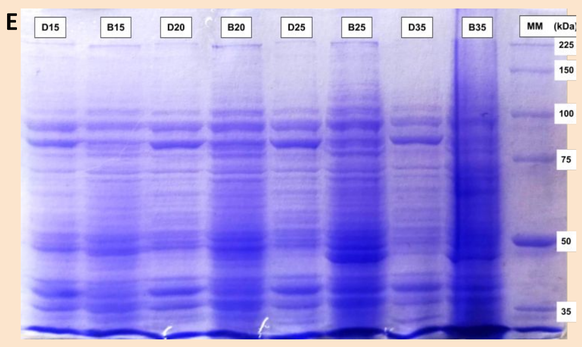


Figure 1C-1D. Protein extracts from endosperm and embryo at different development stages were analyzed to determine NADP-ME activity.

Figure 1E. The SDS-PAGE assay of these samples allowed to observe the quality of the preparations and the variation of protein content.
Lines 1-8 'D' refers to extract from endosperm and 'B' refers to embryo, the number refers to DAP. 10 uL of each extract were loaded in each well (corresponds to 3mg FW).
Line 9 MM: Molecular mass markers.

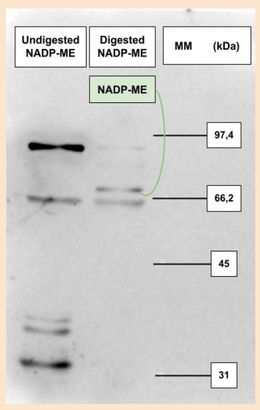


Figure 1F. Preparation of recombinant ZmNADP-ME3
The samples were analyzed by SDS-PAGE coupled to Western blot.
Lane 1: Purified recombinant ZmNADP-ME3 (8 uL)
Lane 2: Purified Recombinant ZmNADP-ME3 after enterokinase digestion (8uL);
Lane 3: Molecular mass markers

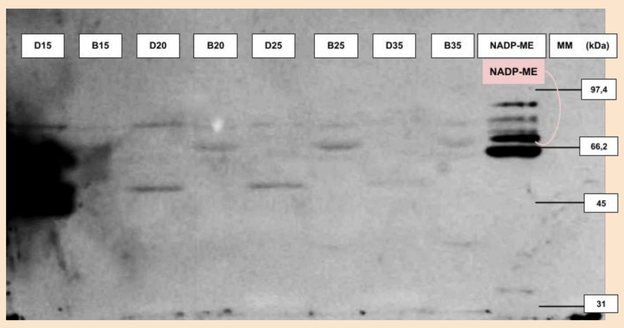


Figure 1G. Detection of ZmNADP-ME3 in protein extracts from endosperm and embryo of 15, 20, 25 1nd 35 DAP. The samples were analyzed by SDS-PAGE coupled to Western blot.
Lanes 1-8: 'D' refers to endosperm and 'B' refers to embryo, the number refers to DAP (20 uL of each extract, 7mg of FW)
Lane 9: Purified Recombinant ZmNADP-ME3 after enterokinase digestion (15 uL)
Lane 10: MM: Molecular mass markers

2. ¿Which genes are coexpressed with ZmNADP-ME3?

Clusters	1	2	3	4	5
Genes	6508	1074	117	8	2

Name	Data	Clusters
ZmNADP-ME3	Malic enzyme. Embryo specific	5
Inositol-3P Kinasa	Myo-inositol hexakisphosphate biosynthetic process. Phosphoprotein. Embryo specific. P-loop NTPase domain-containing protein LPA1 homolog	5
ELMO	Involved in intracellular signaling mediated by protein-protein interactions.Reproductive organ specific	4
Fes1B, Hsp70	PROTEIN FOLDING REGULATOR, integral component of membrane (In ATH Mutants showed increased heat-sensitive phenotype suggesting the involvement of Fes1A in acquired thermotolerance)	4
Glycerol-3-phosphate permease	In ATH, Encodes a member of the phosphate starvation-induced glycerol-3-phosphate permease gene family.	4
Beta-glucosidase 11	X-Glc -> Glc+ X . X =DIMBOA o Scopolina. Integral component of membrane. Also coexpresses at proteomics clusters.	4
Protein ROOT HAIR DEFECTIVE 3	Endoplasmic reticulum membrane fusion, endoplasmic reticulum, integral component of membrane, GTPase activity	4
Sodium pump	K-stimulated pyrophosphate-energized sodium pump protein . Phosphoprotein. Whole Seed specific.	4

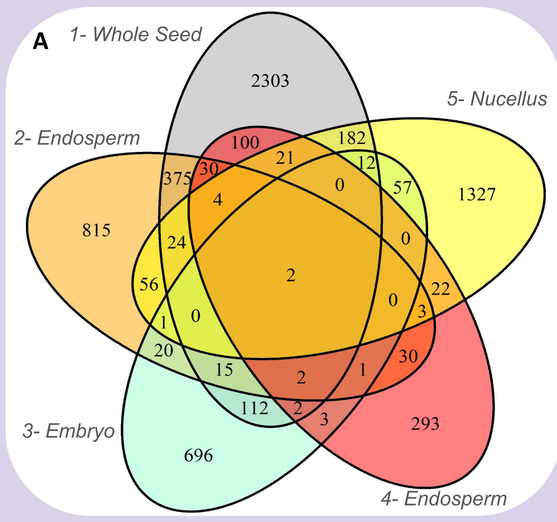


Figure 2A. Venn diagram of a meta-analysis of maize transcriptomics. ZmNADPME3 transcript is found coexpressed with IP3 kinase in 5 transcriptomics. Six transcripts are found coexpressed with ZmNADPM3 in 4 transcriptomics. 1-3(Chen *et al.*, 2014), 4 (Qu *et al.*, 2016), 5 (Yi *et al.*, 2019)

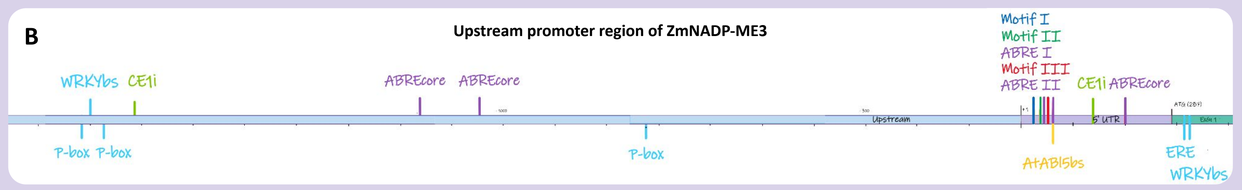


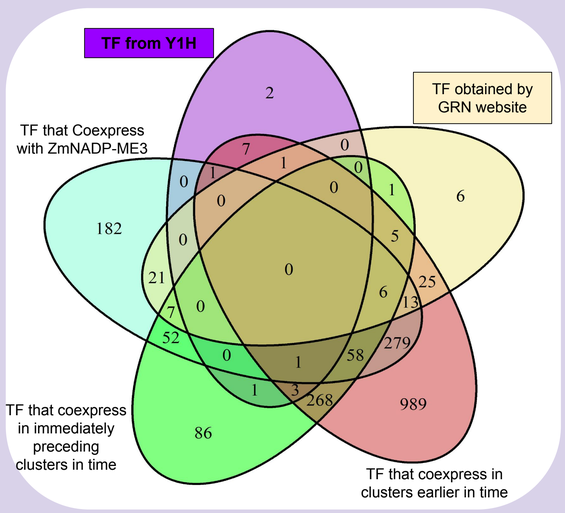
Figure 2B. The upstream promoter of ZmNADPME3 is highly enriched in Abscisic acid (ABA) response elements (ABRE).

Table 2A. Analysis of the upstream promoter region of the transcripts based on motif composition similarity with ZmNADP-ME3.

Name	Coexpresses in:	Motifs
AP2-like ethylene-responsive transcription factor	3 clusters	ABREcore, MotifI, MotifII, MotifIII, P-box, CE1i, WRKYbs
Adenosinetriphosphatase / Triphosphatase // Microtubule-severing ATPase	3 clusters	ABRE, MotifI, MotifII, MotifIII
Protein ABA DEFICIENT 4	2 clusters	ABREcore ,ABRE, ABREI, ABREII, AtABI5bs, P-box, CE1i, WRKYbs
Glycosyltransferases; Involved in the synthesis of glucuronoxylan hemicellulose	2 clusters	ABREcore, ABRE, ABREI, ABREII, AtABI5bs, CE1i, WRKYbs

Table 2A: The coexpressed genes that share the highest number of regulatory motif with ZmNADPME3 are shown in the table.

3. Transcription Factors possibly involved in ZmNADP-ME3 expression regulation



Transcription Factors obtained from Y1H experiments	Specific Tissue/Organ	mRNA-Enriched
bHLH74	Root	Root Elongation Zone
TRAF38	-	-
bZIP90/LRS1*	-	-
GNAT3/HAG19	-	-
HMG4	-	-
bZIP37*	-	-
WRKY124	Root	-
FARL10	-	-
WOX9c/HB117	-	-
MYBR47	-	**Embryo 20 DAP**
MYB40	-	-
ARF27	-	-
KUA1/MYBR29	Endosperm	Endosperm 12 DAP
DOF41	-	-
MADS2/ZAG2	Seed	Female Spiklet
Orphan31/dbb5	Root	-

TF families derived from GRN	Count
MYB	11
bZIP	10
AP2	7
ERF	6
bHLH	6
Orphan	5
B3	4
NAC	4
ZF-HD	3
MIKC	3
C3H	3
WOX	3
EIL	2
GRF	2
HSF	2
HD-Zip	1
WRKY	1
Trihelix	1
SRS	1
G2-like	1
PLATZ9	1
Nin-like	1
ARF	1

Figure 3. Venn diagram that resumes data from Yeast one hybrid assay (Y1H), transcriptomics and Genes Regulatory Networks (GRN).
MYBR47 is a good candidate to regulate ZmNADPME3 expression in maize embryos.

Conclusions

- Malic activity is greater in the embryo than in the endosperm. In the embryo, the activity per gram of FW is maximum at 20 DAP.
- The expression of Inositol 3P Kinase is highly conserved at the transcriptional level along with *ZmNADP-ME3.*
- Several Transcription Factors could be regulating to *ZmNADP-ME3*, more studies of candidate TFs would help elucidate their importance.

Materials and methods

Analytical balance was used to weigh the grain tissues. Maize embryo and endosperm protein extracts were obtained by grinding in a buffer containing 100 mM Tris-HCl pH 7,5; 1 mM EDTA; 10 mM MgCl2; 20% (v/v) glycerol; 0,5 % (p/v) β-mercaptoethanol; 1mM protease inhibitors cocktail. Then the extracts were centrifuged and desalted in Sephadex G-25 columns. Total proteins were quantified by Bradford assay. NADP-ME activity was determined at 30°C in a Jasco spectrophotometer, following the production/ consumption of NADPH at 340nm, as detailed in Detarsio et al., 2008. NADP-ME reaction buffer contained 50 mM Hepes-NaOH pH 7; 5 mM $MgCl_2$; 20 mM malate and 0,5 mM $NADP^+$. Recombinant expression of ZmNADP-ME3 was performed in autoinduction medium and the purification was performed by affinity chromatography on Ni-NTA column with further desalting by Sephadex G-25 column (Calace et al., 2021). Western Blot assays were perform with anti-ZmNADPME3 (Detarsio et al., 2008) and chemiluminescence band detection (Bio lumina, Kallium technologies)
RStudio was used to work with transcriptome and proteomics clustering datasets.

References

Chen et al., 2014; Qu et al., 2016; Yi et all., 2019; Hoopes et al., 2018; Yu et al., 2016 ; Walley et al., 2016; Detarsio et al., 2008; Calace et al., 2021.