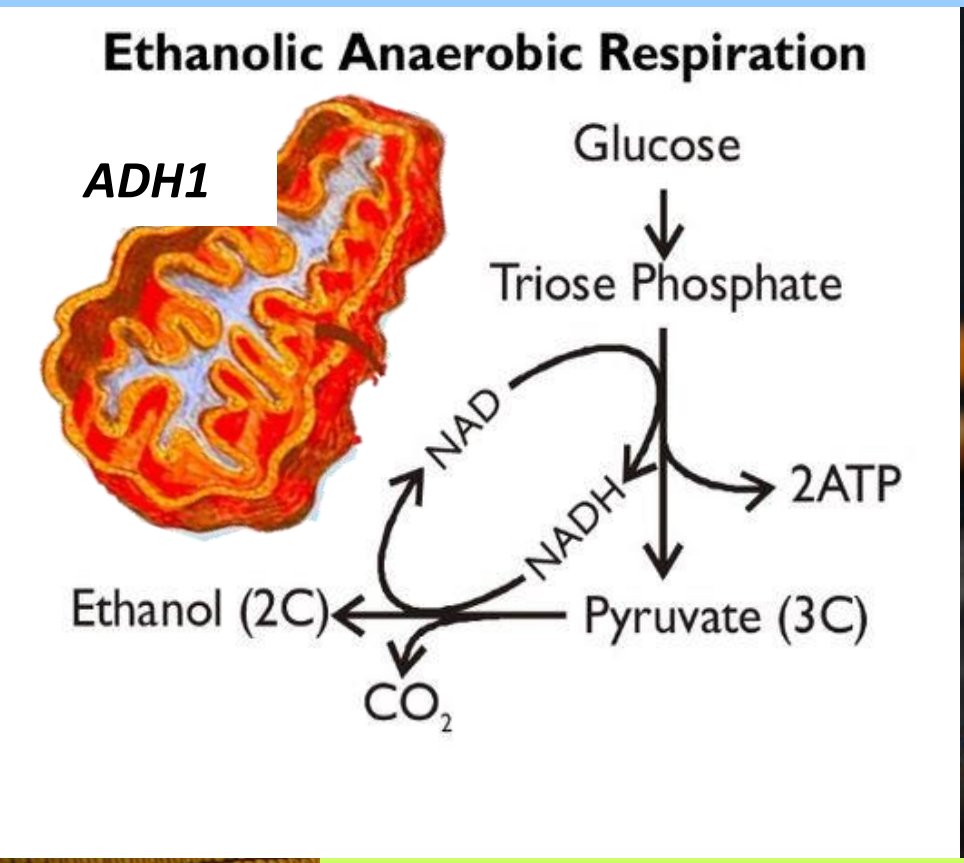
Ethanol Production and Mitochondria-Related Gene Expression of Maize Seed after Artificial Aging



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Introduction

Maize production of the world

- Maize production was 1,069.34 million tons in 2017/18 but the demand of maize has continuously increased to 1,091.77 million tons in 2018/2019 (Office of Agricultural Economics, 2018).

Maize production of Thailand

- Maize production was 4.93 million tons in 2017/18 tons but the demand of maize has continuously increased to 6.6 million tons in 2019 (Office of Agricultural Economics, 2018; Thai Feed Mill Association, 2018).

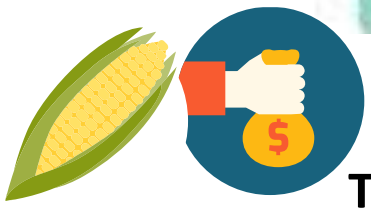
Asia

1. Japan
2. China
3. Thailand

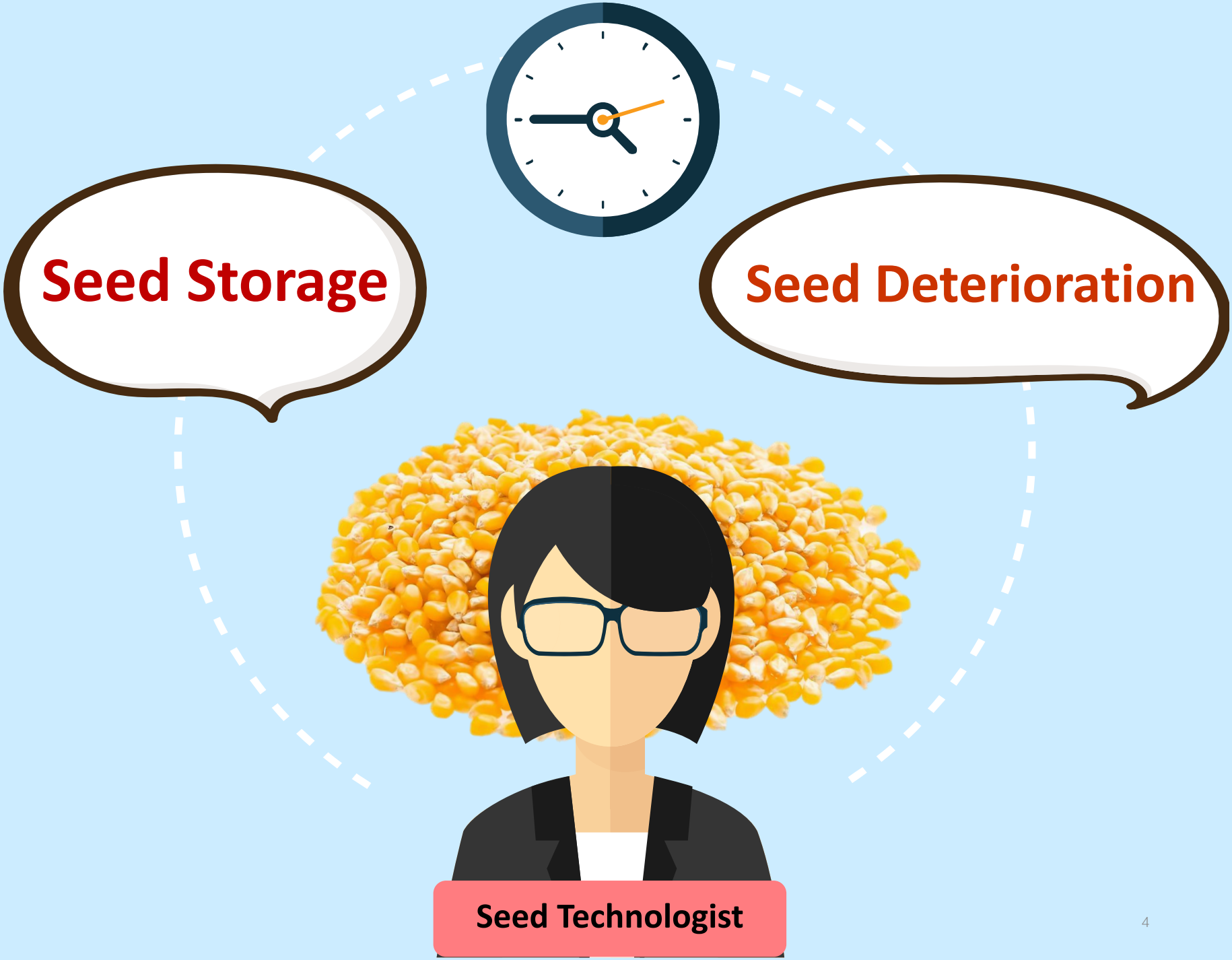
Thailand is the 24th largest seed exporter in the world

(Napasintuwong, 2015)

(Napasintuwong, 2015)

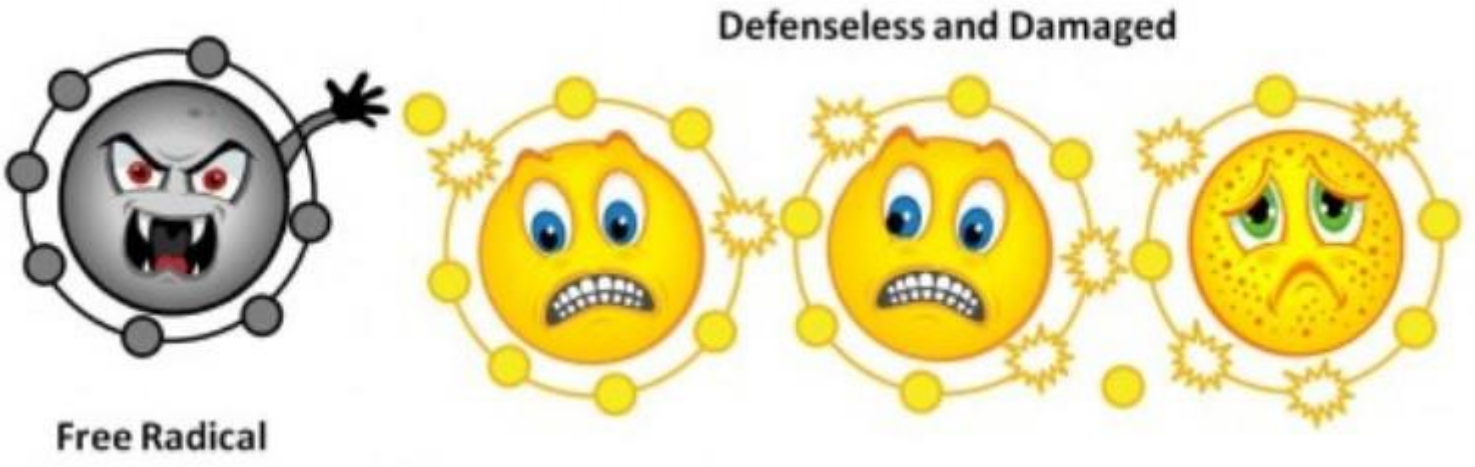


The export of value of maize seed form Thailand was estimated at about 1.72 billion bath in 2016 and 2.12 billion bath in 2017 (Thai Seed Trade Association, 2018).


Seed Storage
Seed Deterioration
Seed Technologist

Introduction

A model of seed deterioration is forwarded arguing that free radical assault on mitochondrial membranes may be a primary cause of seed deterioration (McDonald, 2006).

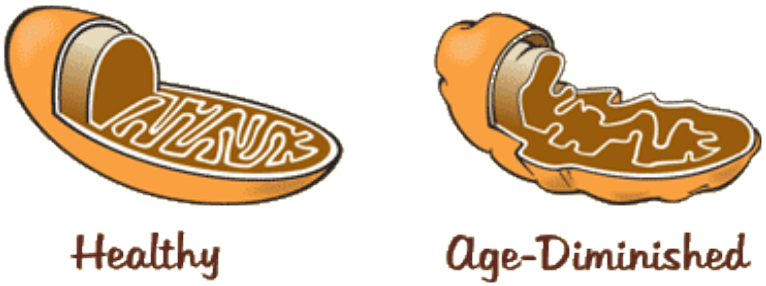


goo.gl/mtisqp

Introduction

Damage of inner mitochondrial membrane results in impairment of aerobic energy metabolism and reduced oxidative phosphorylation (Benamar *et al.*, 2003).

Seed produces metabolic energy by anaerobic fermentation, of which the end products are ethanol and lactic acid (Kennedy *et al.*, 1992).



Introduction

One alternative towards assessing the quality of seed is to monitor the concentration of certain volatiles (acting as biological markers) evolved from the seeds; examples are ethanol (EtOH), acetaldehyde (Bicanic *et al*., 2003.)

Kodde *et al*. (2012) succeeded in test with cabbage seed (*Brassica oleracea* L.) by modified breath analyser in "Agri" version and called "fast ethanol assay".

https://goo.gl/OQ7zdi

Introduction

Pathogens borne in maize seed

Aflatoxin

Aspergillus flavus

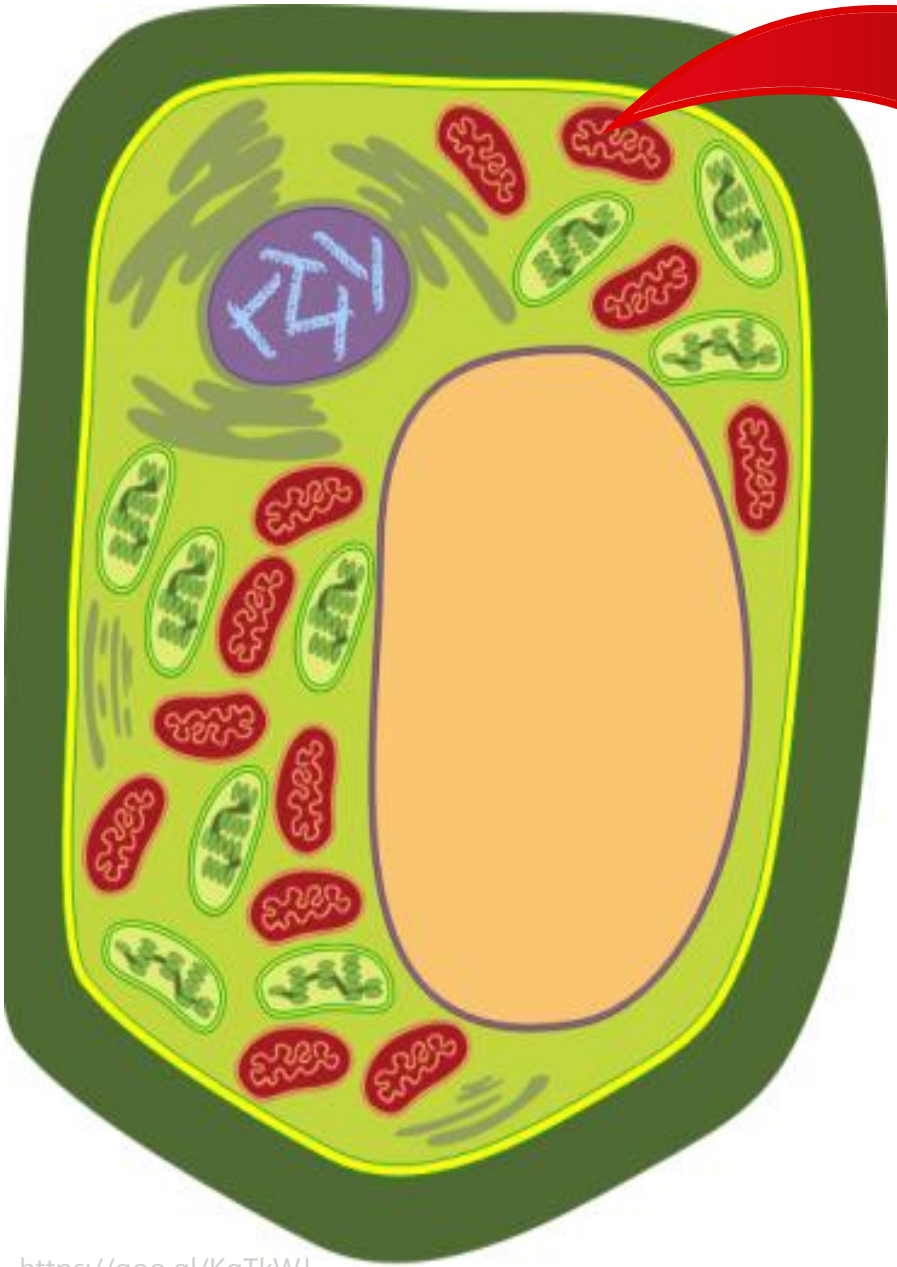
Aflatoxins are primarily a problem associated with maize, as it is infested with *Aspergillus flavus* and *Aspergillus parasiticus* in the field as well as storage (Nasir & Jolley, 2002).

Fungi separate from maize can produced aflatoxin such as *Aspergillus flavus, Aspergillus parasiticus and Aspergillus nomius* (Saito and Tsuruta, 1993).

Introduction

Pathogens borne in maize seed

Thailand found *Aspergillus flavus* more than *Aspergillus parasiticus* (Moss,1998)



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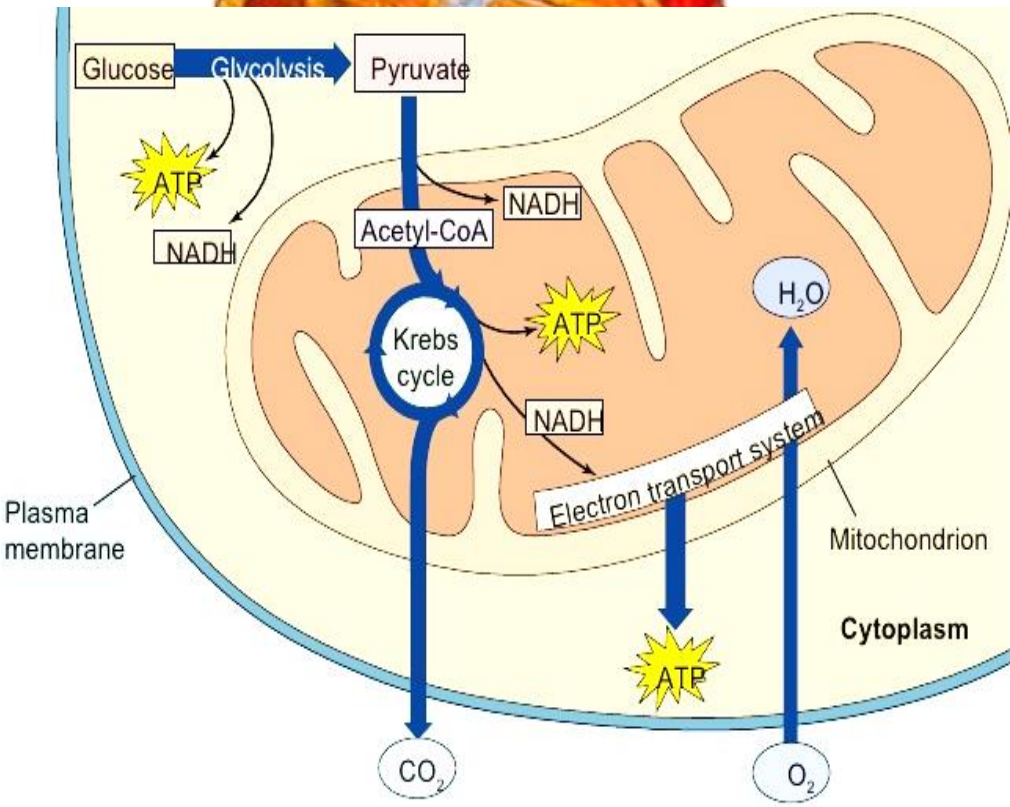
Plant cell

COXc

Aerobic Respiration



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ADH1

Ethanol

AOX1

AOX1 **were expression when include abiotic stresses such as low temperature, drought, and nutrient deficiency, as well as biotic stresses such as bacterial infection (Vanlerberghe, 2013).**

ATPase

Yin *et al.* (2016) reported the assembly of mitochondrial ATP synthase was inhibited in aged seed, causing ATP production to decrease.

Objective

To evaluate mitochondria degradation *via* gene expression analysis including fast ethanol assay for detecting ethanol production from seed after artificial aging

Materials and Method

Maize F1 hybrid seed

Sterilization

Sterilization

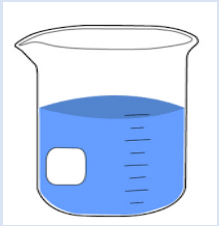
Unsterilization

Data collection

- **Germination test (ISTA, 2015)**
- **Vigor test (ISTA, 2015)**
- **Fast ethanol assay**
- **Gene expression analysis (qPCR)**

Sterilization

Maize seed



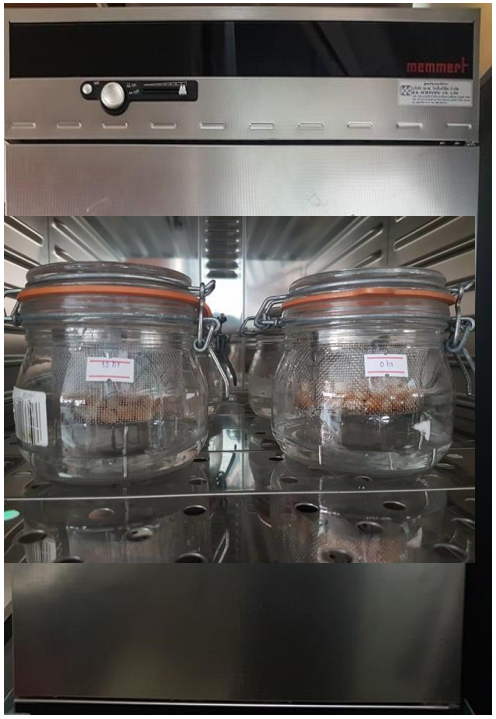
1.0% sodium hypochlorite
10 minute
(Sauer and Burroughs, 1986)

Sterilized seed

Artificial Aging by Hot Humid Treatment

Experimental design: CRD

Maize seed



Hot air oven 42˚C
0, 12, 24, 48 hours

Data Collection

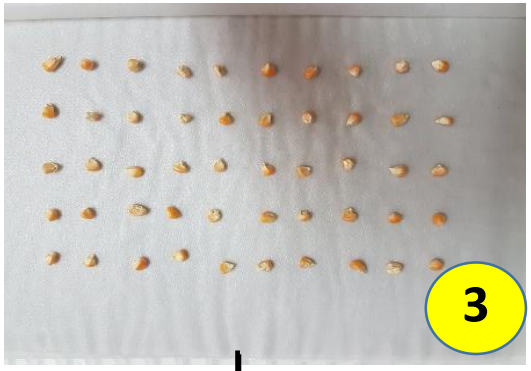
- Germination test (ISTA, 2015)
- Vigor test (ISTA, 2015)
- Fast ethanol assay
- Gene expression analysis (qPCR)

Germination test (ISTA, 2015)

Between paper-BP- techniques

Maize seed

Paper towel



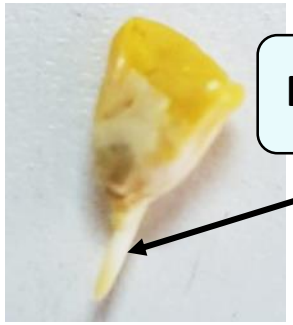
50 seed
4 replicates

Germinator 25°C

Vigor tests (ISTA, 2015)

Single count of radicle emergence
(modified by ISTA, 2015)

After germination
50 hours 25°C,
(modified by ISTA, 2015)



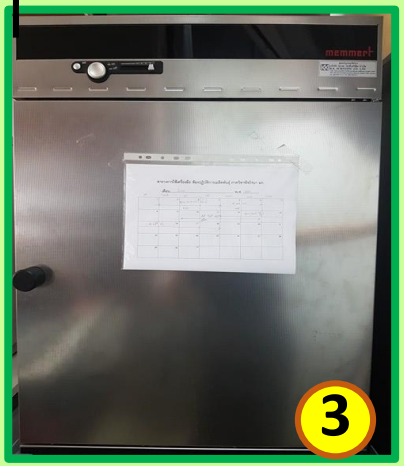
Radicle 2 mm.

Accelerated aging test (ISTA, 2015)

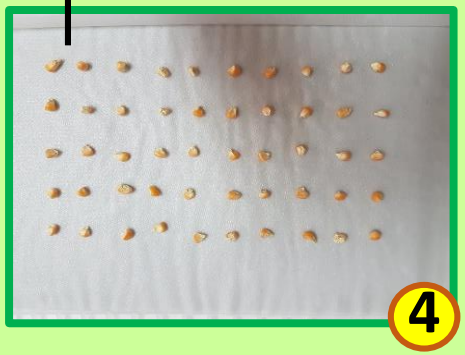
Maize seed

AA Jar

Hot air oven
42°C 72 hours



Seed Germination Test



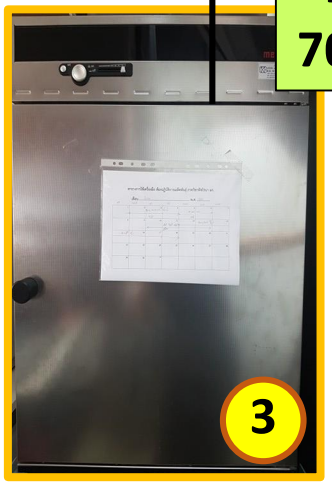
Vigor test (ISTA, 1995)

Fast ethanol assay

Maize seed

10 seed and add water for 20% SMC

Hot air oven 70˚C 1.5 Hours



Modified breath analyser “Agri” version

Gene Expression Analysis (qPCR)

1 **Maize seed**

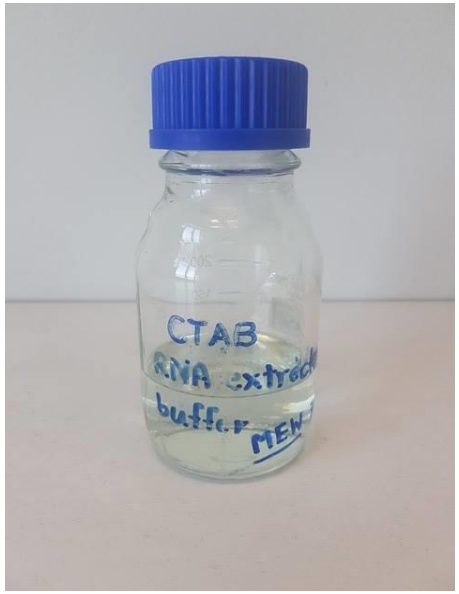
2 **Seed grinder**
(MICRO HAMMER CUTTER MILLLABORATORY GRINDER 601 MHCM)

https://goo.gl/0R1hCG

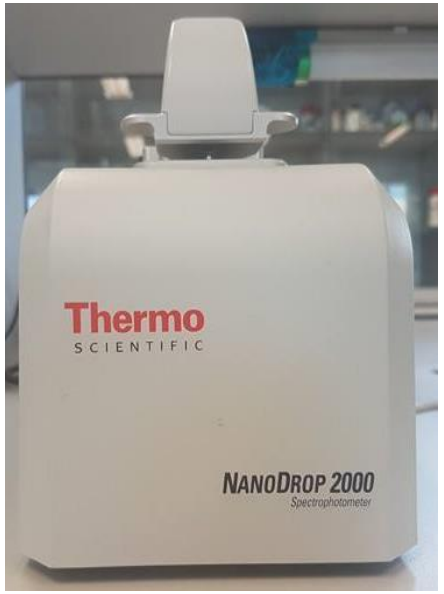
Gene Expression Analysis (qPCR)

3

RNA Extraction
(Modified by Lodhi *et al*., 1994)



CTAB and PVP Buffer

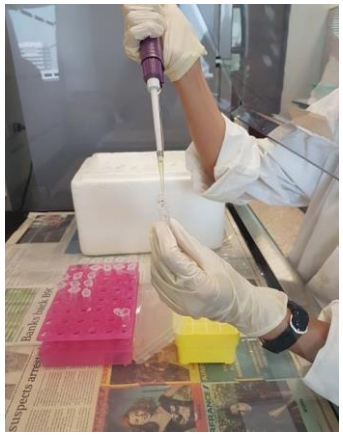


NanoDrop®(scientific)

Gene Expression Analysis (qPCR)

4 **cDNA**

(Thermo Scientific Revert Aid First Strand cDNA Synthesis Kit)



5 **qPCR**

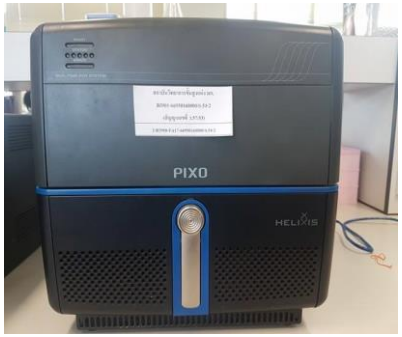


Table. 1 Primer for gene expression analysis (qPCR)

Gene	Accession	Types	Sequence (5'→3')	Annealing temperature
Alcohol dehydrogenase (Adh1-S)	L23548.1	Forward primer	GGTTCGACAGTGGCTGTTTT	58°C
		Reverse primer	TGTGGTCTTTTGGGTTCACA	
ATP synthase	NM_001158454.1	Forward primer	TGAAGATAAGCAGCGTGGTG	60°C
		Reverse primer	TAGGAGCAACAGGAGCGACT	
Alternative oxidase	AY059646.1	Forward primer	AAGAAAATGCCTGGCTGCTA	58°C
		Reverse primer	CCTTCGTTGCTCCTTTTCAG	
Cytochrome c oxidase	EU976023.1	Forward primer	ACCTTGGCACAAGAGACGAT	59°C
		Reverse primer	TGGGCCCATCACTTAAAAAG	
Elongation factor 1-alpha (EF1-A)	U76259.1	Forward primer	AGGTCCACCAACCTTGACTG	58°C
		Reverse primer	ACGTCCAACAGGGACAGTTC	

Results and Discussions

● Seed qualities after artificial aging

Table. 2 Germination and seed vigour of maize seed cv. A after subjected in different periods of time of hot-humid treatment.

Hours after hot-humid treatment	Germination (%)	Accelerated aging test (%)	Single count of radicle emergence (%)
0 (control)	74.5 a	60 a	47.5 a
12	61.5 b	59 a	40.5 ab
24	61 b	56 a	35 b
48	52.5 b	44 b	29.5 b
F-test	*	*	*
C.V. (%)	11.91	9.61	20.72

* Significantly different at P ≤ 0.05

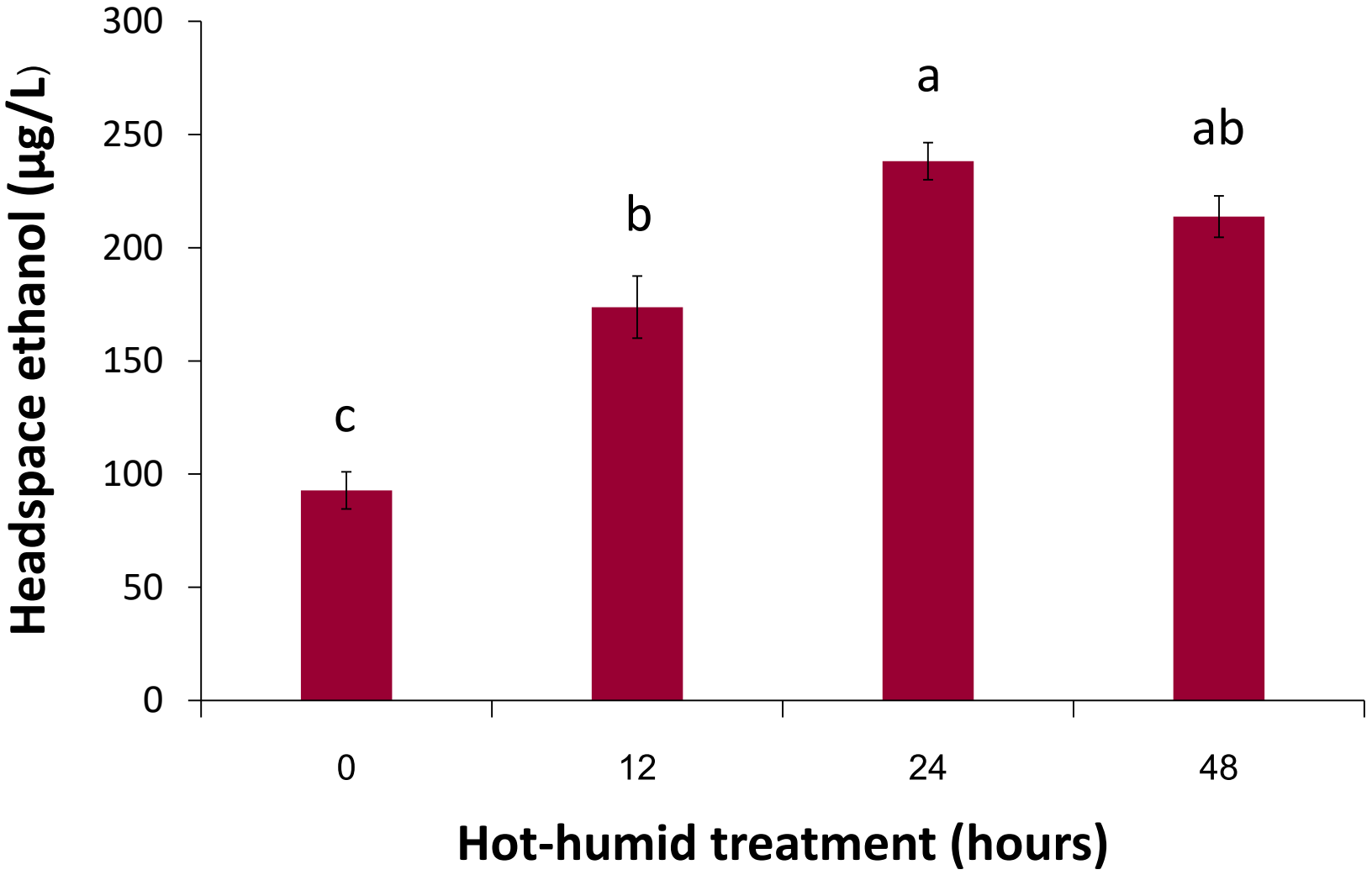
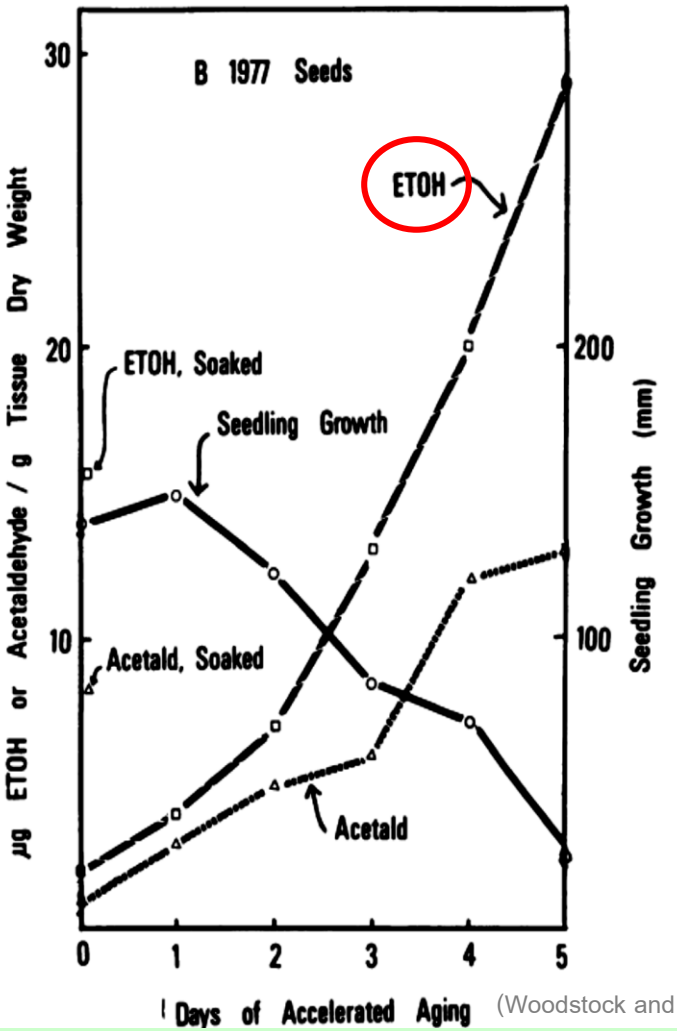


Fig. 1 Effect of time period during hot-humid treatment on ethanol production of maize seeds (cv. A). Headspace ethanol was measured above 1,200 mg seed samples at 20% moisture in 20 mL vials after 1.5h incubation. Error bars denote the S.E. (n = 4).

Results and Discussion
Maize seed after artificial aging

- **Fast ethanol assay of maize seed after artificial aging**



(Woodstock and Taylorson, 1980)

Fig. 2 Effect of accelerated aging on ethanol

High-vigor seed accumulated little or no ethanol while deteriorated seed accumulated substantial ethanol (Buckley and Huang ,2011)

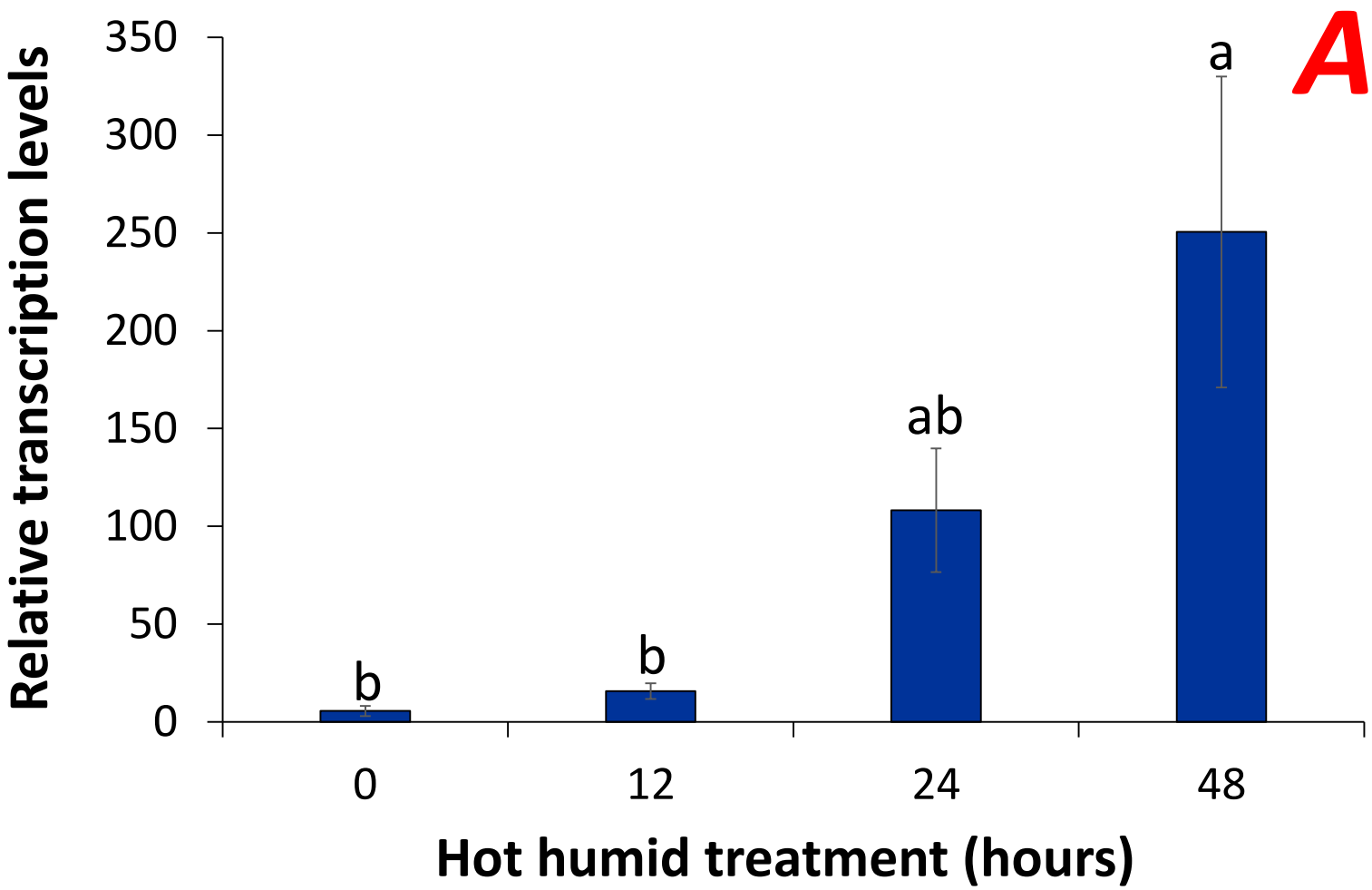
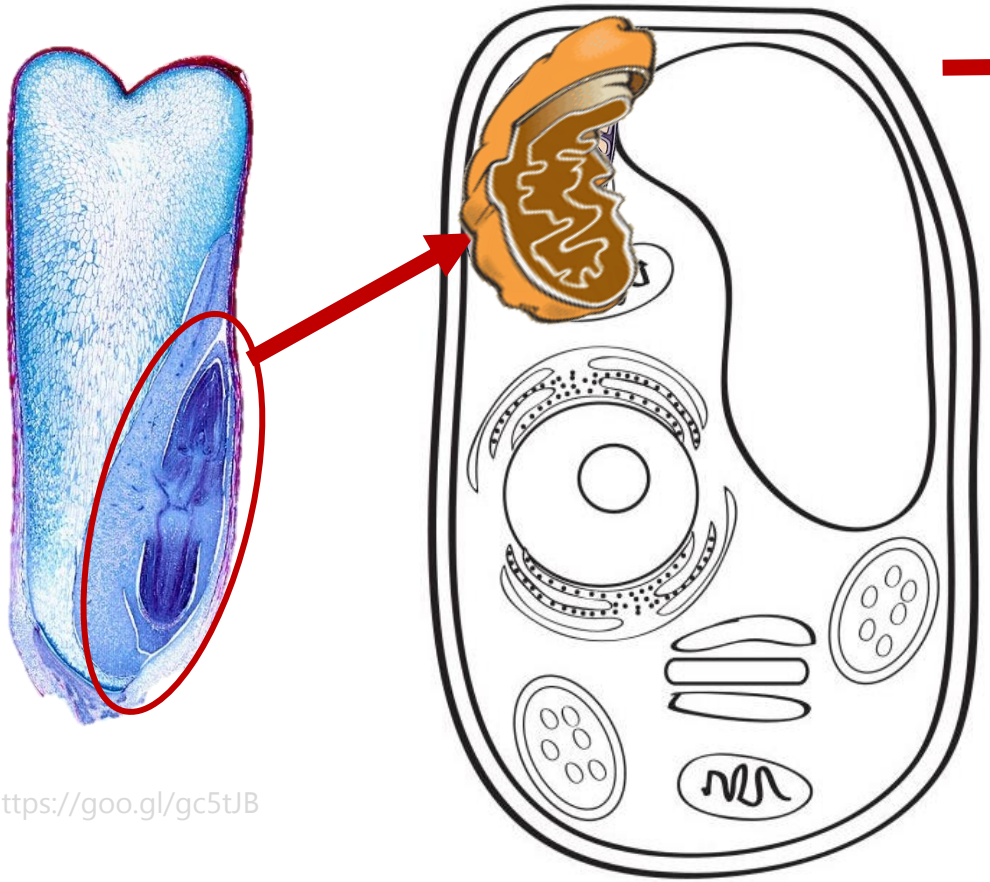
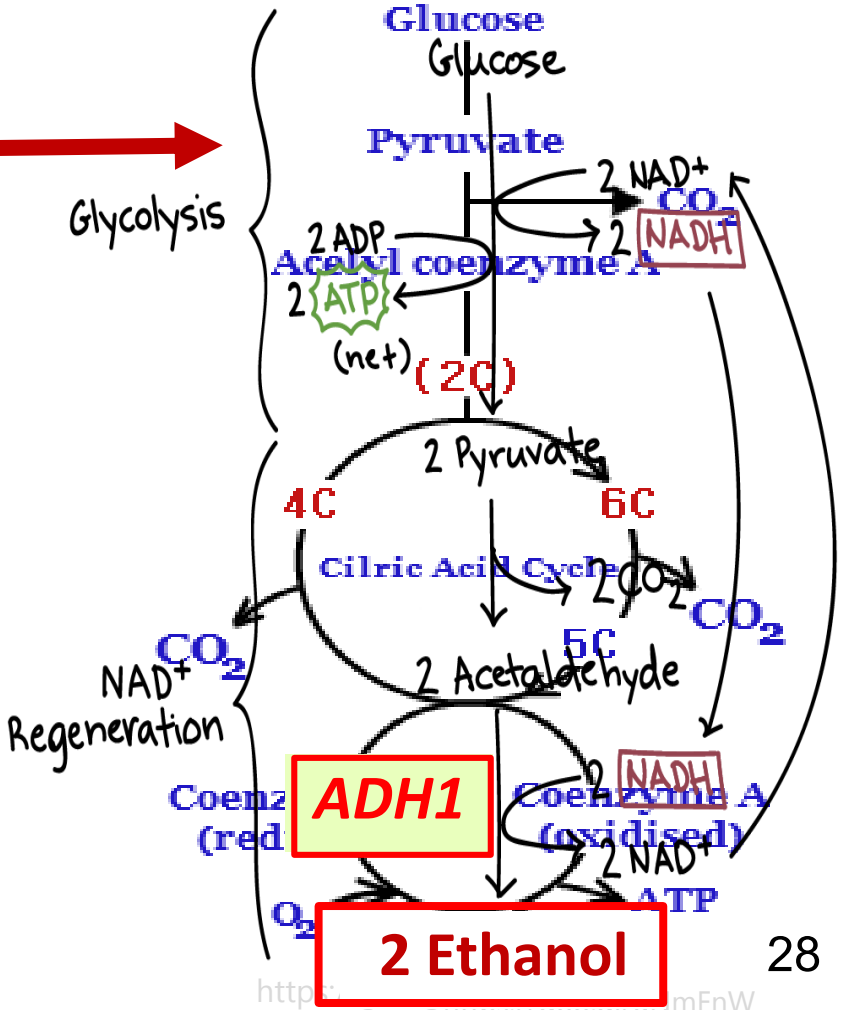


Fig. 3 Effect of time period during hot-humid treatment on energy metabolism gene (ethanol metabolomics gene) expression. Maize seeds (cv. A) were subjected in 100%RH at 42 °C for 0, 12, 24 and 48 hours. The quantitative RT–PCR was measured the change of expression of *Alcohol* dehydrogenase (*ADH1*)-Maize. Error bars denote the S.E. (n = 4).

Results and Discussion
Maize Seed after Artificial Aging

● Mitochondria-related gene expression





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(Boris *et al.*, 2002; Corinne *et al.*, 2008; Judith, 2010; Ying *et al.*, 2017)

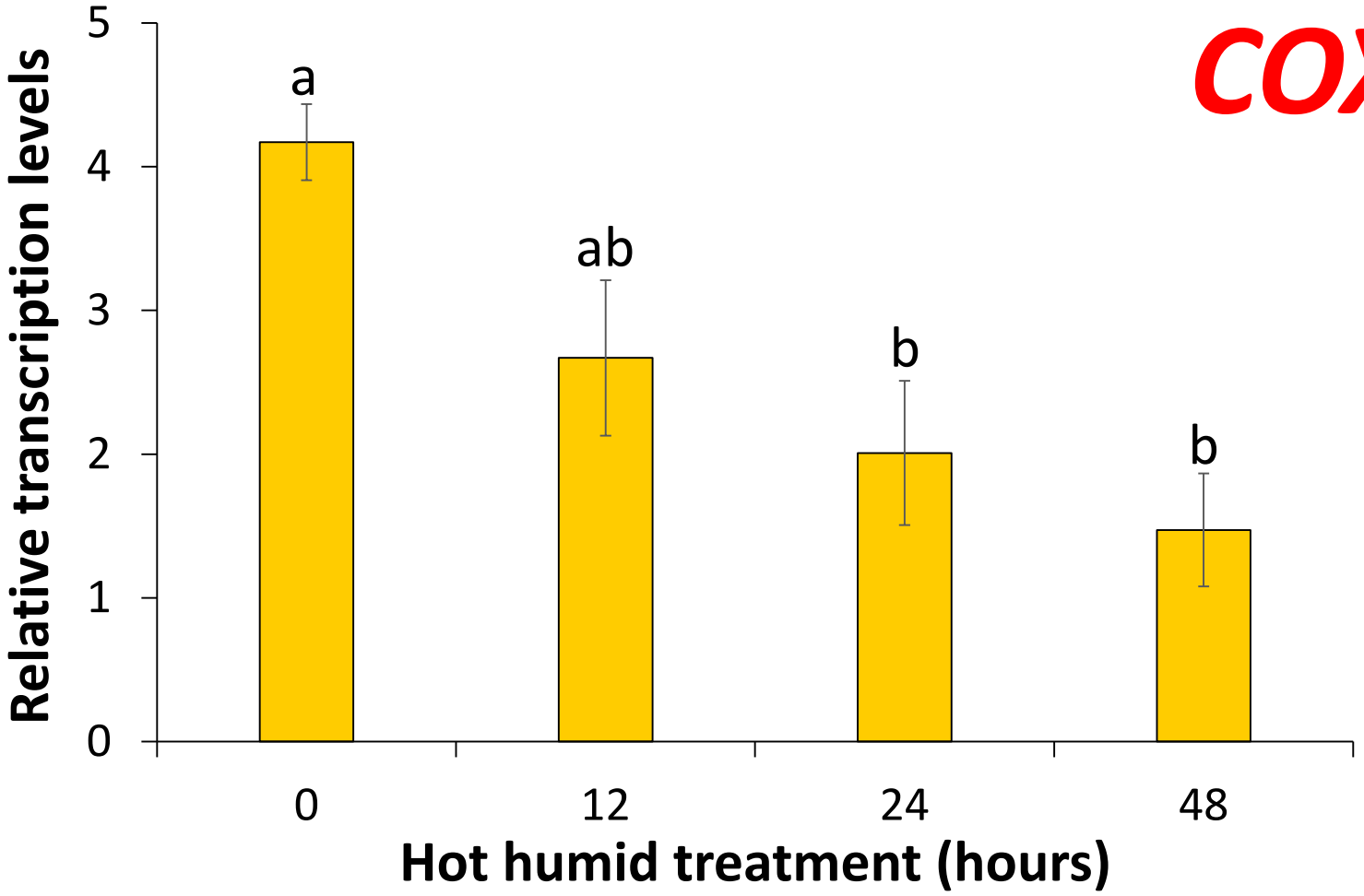


Fig. 4 Effect of time period during hot-humid treatment on energy metabolism gene(ethanol metabolomics gene) expression. Maize seeds (cv. A) were subjected in 100%RH at 42 °C for 0, 12, 24 and 48 hours. The quantitative RT–PCR was measured the change of expression of cytochrome c oxidase (*COXc*)-*Maize*. Error bars denote the S.E. (n = 4).

Results and Discussion
Maize Seed after Artificial Aging

- **Mitochondria-related gene expression**

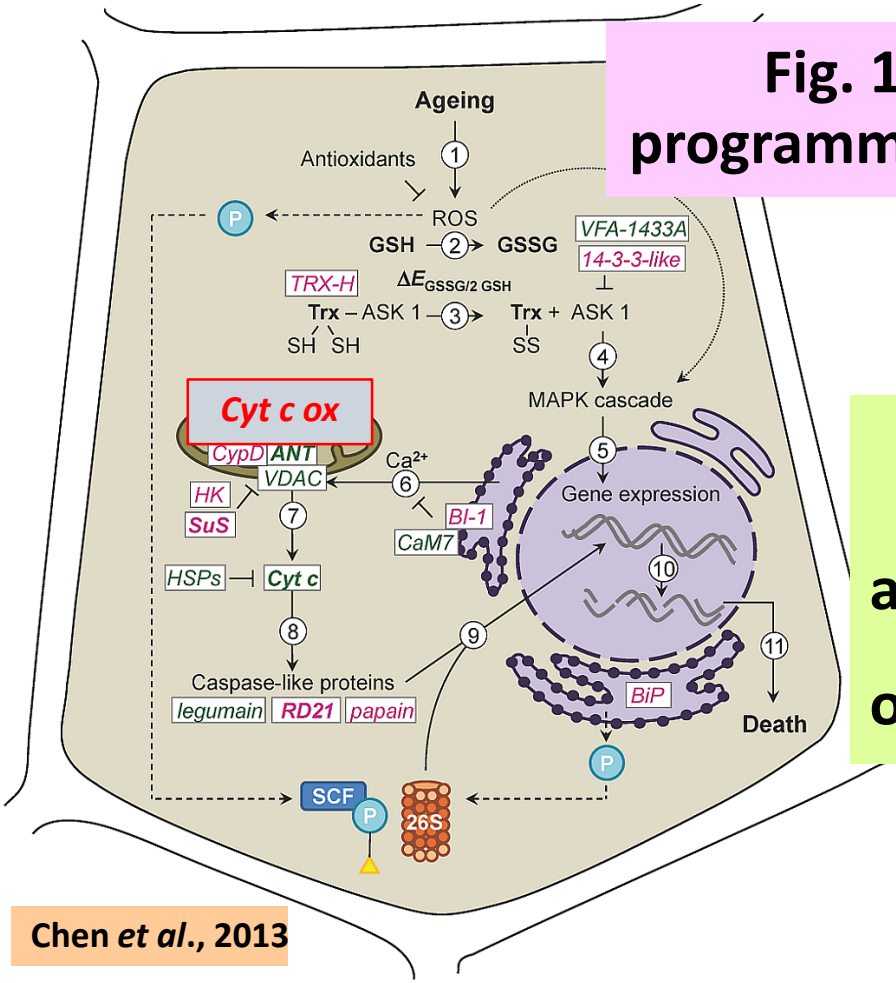


Fig. 13 A model of events leading to programmed cell death during Pea seed ageing

Cytochrome *c* oxidase reduce activity may be important in the loss of viability (Sowa *et al.*, 1992)

Chen *et al.*, 2013

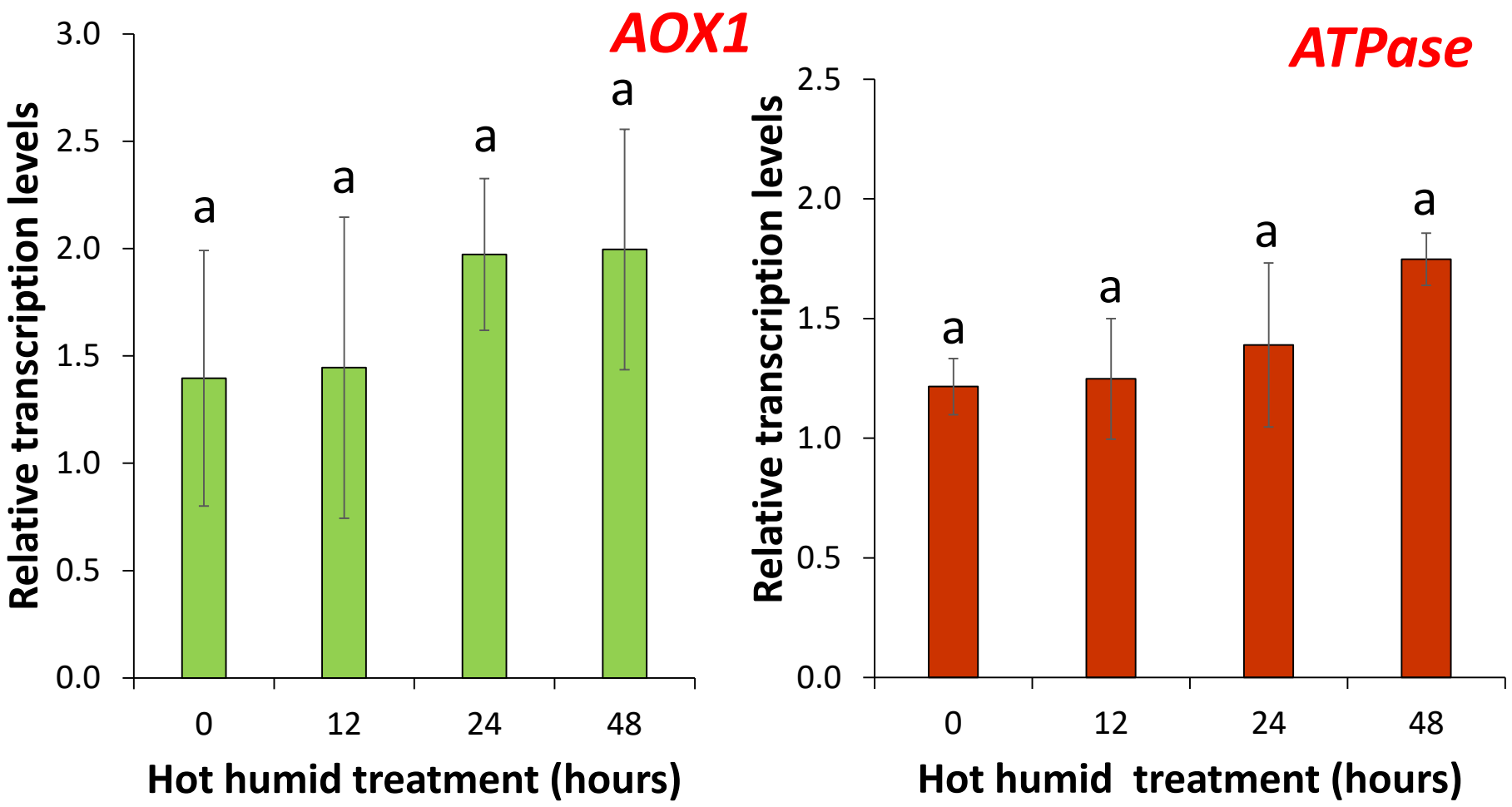


Fig. 7 Effect of time period during hot-humid treatment on energy metabolism gene (ethanol metabolomics gene) expression. Maize seeds (cv. A) were subjected in 100%RH at 42 °C for 0, 12, 24 and 48 hours. The quantitative RT–PCR was measured the change of expression of Alternative oxidase (AOX1) and ATP synthase (*ATPase*)-*Maize*. Error bars denote the S.E. (n = 4).

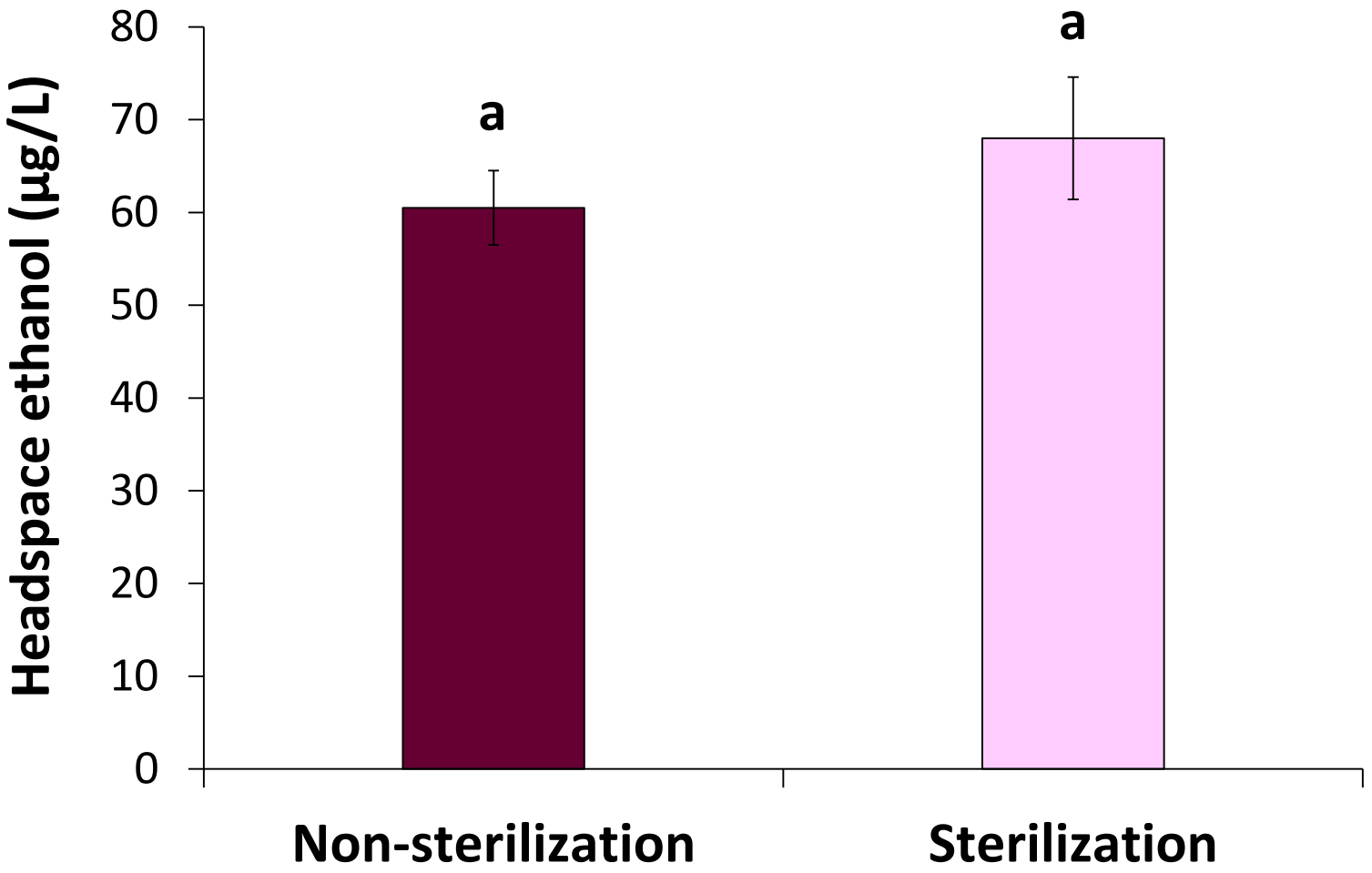


Fig. 8 Effect of seed surface sterilization on ethanol production. Maize seeds cv. B were surface sterilized in 1.0% sodium hypochlorite for 10 minutes, then subsequently washed with sterile water for 10 minutes. Non-sterilized seed and sterilized seed were ■ and □ , respectively. Headspace ethanol was measured above 1,200mg seed samples at 20% moisture in 100 mL vials after 6h incubation. Error bars denote the S.E. (n = 4).

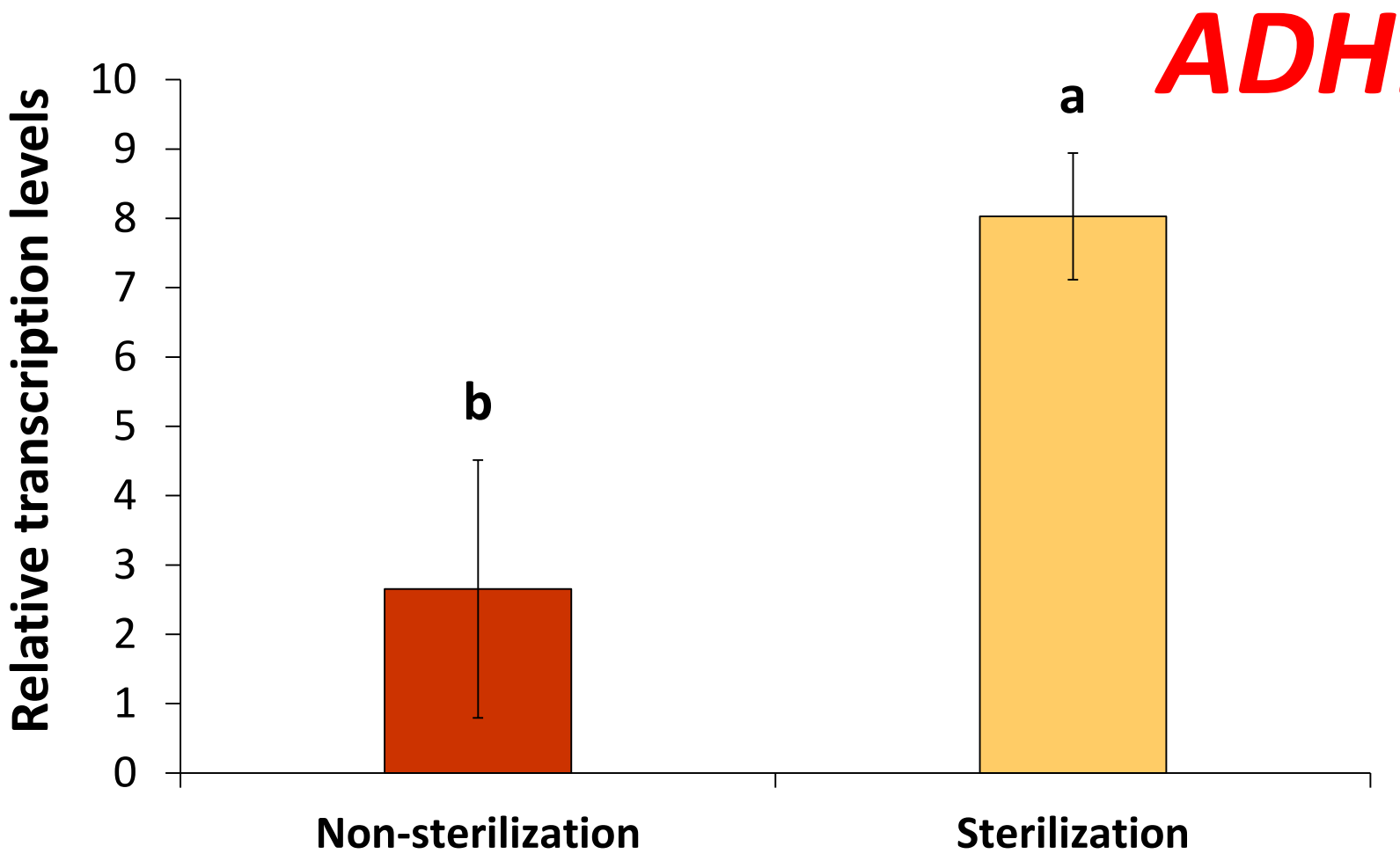
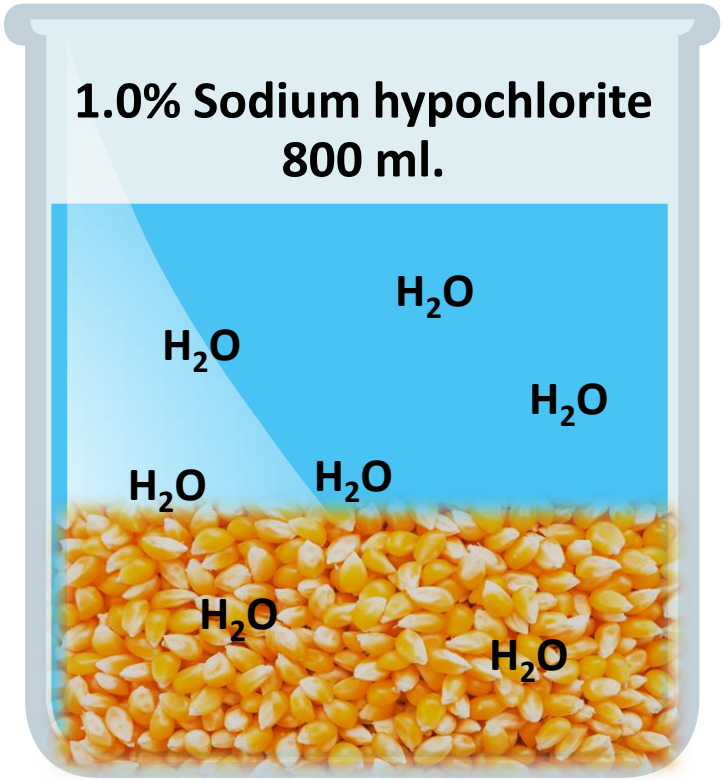


Fig. 9 Effect of seed surface sterilization on relative transcription levels gene expression of *alcohol dehydrogenase* (*ADH1*)-*Maize*. Maize seeds cv. B were surface sterilized in 1.0% sodium hypochlorite for 10 minutes, then subsequently washed with sterile water for 10 minutes. Non-sterilized seed and sterilized seed were ■ and □, respectively. Error bars denote the S.E. (n = 4). An independent-samples t-test was conducted to compare ambient condition and control condition.



The relative transcription levels of *ADH1* of maize seed sterilized has more than seed non-sterilized.

Kibinza (2006) seed moisture content is key factor of aging is discussed with regards to energy metabolism and may be including the transcription of cell.

Conclusion

Artificial aging of maize seed shown low vigor and germination including the transcription level of gene involving in mitochondria degradation especially the increase of transcription of *ADH1* gene according with ethanol that investigated by fast ethanol assay.

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Acknowledgements

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Thesis committees

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“ Thank you for your attention ”