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Introduction of the interdependence between the glutathione half-cell reduction potential and thermodynamic parameters during accelerated aging of maize seeds

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Abstract: Two maize hybrids with a different ability to maintain seed germination were examined during the course of accelerated aging (AA). Initially, the similar seed reduction potential of the GSSG/2GSH half-cell increased in H1 (dent hybrid) without influencing the seed germination ability up to the 6th day of AA, while in H2 (sweet corn hybrid), it was not changed up to the 6th day of AA but with a significant later loss of seed germination ability. During the AA course, the amount of free thiol decreased in H1 and increased in H2. Irrespective of the continual increase of the differential Gibbs energy during AA, the characteristics of the examined hybrids are possibly connected to the different metabolic pathways of the seeds: H1 is characterised by higher entropy and positive enthalpy values, while H2 had negative entropy values and a decreasing trend of enthalpy, indicating a shift of the system from a relatively ordered to a disordered state. The different types of nanomolecular switches, resulting in a faster decrease of GSH in the H2 than in the H1 hybrids, indicate that a combination of the GSSG/2GSH half-cell potential and thermodynamics could be a useful tool to quantify plant stress.

Keywords: aging; glutathione; maize seeds; free thiols; seed germination ability; thermodynamics.

INTRODUCTION

Despite the fact that the germination percentage is still the most important parameter that describes and integrates germination ability[1], the seed germination process, by itself, has many different aspects. The trend of loosing viability during long-term storage has two phases: the first is slower and longer lasting and the second, faster and shorter lasting. [2,3] Kittock and Law [4] and Anderson [5] ascertained that seed respiration, as the main producer of reactive oxygen species (ROS), could increase during storage, due to exogenous and endogenous factors:

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temperature, air humidity, *etc*.[6] During oxidative stress, ROS damage first the mitochondria and then the other cell components, leading to respiration reduction and membrane disintegration,[7] which could be assumed as the moment of irreversible injuring. Accelerated aging induces changes in the naturally occurring seed antioxidants, such as glutathione,[2] which are integrated into the cellular redox status. Some ROS species and NO [8] are capable of modulating transmembrane receptors and cytoplasmic signal transduction routes.[9] Molecular sensors with free thiols mainly react *via* their oxidation, forming disulphides,[10,11] having different redox and transcriptional signals. Although seeds represent relatively dry systems, the relations are even more complex, including the facts that most of the endosperm and a smaller part of the embryo represent dead cells, made through programmed cell death during seed formation.[12,13]

The theoretical basis of the energy concept, *i.e*., thermodynamics, gives the possibility of quantification of biological vitality,[14,15] considering that a change in the internal energy of a system represents the maximal work available for achievement. The vitality of seeds is maintained by the formation of a glass structure, which is thermodynamically unstable, while aging induces structural changes,[2] as a consequence of metabolic unbalance, originating from gradual desiccation and high oxidative activity.[16] Subsequently, the observed equilibrium shift, induced by oxidative activity during long-term desiccation or ageing, also leads to the breakdown of the antioxidants, *i.e*., when major parts of the GSH pool are converted into GSSG, the half cell potential increases and becomes a signal that initiates programmed cell death.[17]

The objective of study was to investigate the changes in seed germination ability during accelerated aging of two maize hybrids (H1 – dent hybrid and H2 – – sugary hybrid) consequently through alterations of the half-cell redox potential of the GSSG/2GSH couple, the amount of free thiols and the thermodynamic parameters of differential Gibbs energy, entropy and enthalpy.

EXPERIMENTAL

The seeds of two maize hybrids with different abilities of germination (ZP SC 580 – H1, and ZP SC 504_{su} – H2, originating from the same location and year, stored at 4 °C), were subjected to accelerated ageing treatment[18] at a temperature of 42 °C and a relative air humidity of 100 % for 3, 6 or 9 days (down to an economical limit of 80 %). Subsequently, the germination capacity was determined by ISTA Rules in four replications of 100 uniform seeds[1] after 7 days, on filter paper towels, used as the medium.

The contents of free thiol (PSH), reduced (GSH) and oxidised glutathione (GSSG) in the seeds were determined according to the method of de Kok *et al*.[19] After homogenisation of 1 g of a sample with 10 mL of 0.15 % Na-ascorbate, the sample was centrifuged at 20,000 *g* for 20 min. Then, the free thiol content was determined: 1.5 ml of 0.20 M potassium phosphate buffer (pH 8.0) and 0.20 ml of 10 mM DTNB reagent (5,5'-dithio(2-nitrobenzoic acid) solved into 0.020 M potassium phosphate buffer (pH 7.0)) were added to 1.5 ml of the extract. The absorbance was measured at 415 nm. Then, the supernatant was deproteinised in the water

bath at 95 ° C for 3 min. After repeated ce ntrifugation at 150 00 *g* for 15 min, the content o f total glutathione from the supernatant was analysed. After repeated c entrifugation at 150 00 g for 15 min, th e content of to tal glutathione in the sup ernatant wa s analy zed as d escribed above: to 1.5 ml of supernatant , 1.5 m L of 0.20 M potassiu m phosphate buffer (pH 8.0) and 0.20 mL of 10 m M DTNB reagent (pH 7.0) were added. The absorba nce was read at 415 n m. In the other 1.5 mL of superna tant, 0.5 m L of 0.25 M potassi um pho sphate buffer (pH 6.8), 0.3 m L of albu min, 0.020 m L of glyoxalase I (Sigma grade I V) and 0.08 mL of 0.10 M methylglyoxal are added. After incubation at 30 °C for 15 min, the conte nt of reduced glutathione (GHS) was determined in the abov e described manner. GSH (Sig ma Ultra 98–100 %) was used a s th e stan dard. The content of oxid ised glutathione (GSSG) wa s calculated as the difference between the total and reduced glutathione.

The redox capa city of the GSSG/2GSH c ouple was estimated by the method of Schafer and Buettner:[20]

$$E_{hc} = -240 - \frac{59.1}{2} \log \frac{[GSH]^2}{[GSSG]} \quad (1)$$

The ther modynamic para meters were c alculated fro m the water content, whi ch was determined after drying at 130 °C,[21] by a modified model proposed by Davies[14] and Sun:[22,23]

$$G^0 = -RT \ln Wc \quad (2)$$

$$\Delta G = -G^0 + RT \ln \frac{Wc_1}{Wc_2} \quad (3)$$

$$\Delta H = \frac{RT_1T_2}{T_2 - T_1} \ln \frac{Wc_1}{Wc_2} \quad (4)$$

$$\Delta S = \frac{\Delta H - \Delta G}{T} \quad (5)$$

where G^0 is the starting Gibbs energy, R is the universal gas constant (8.314 J K^{-1} mol^{-1}), T is the sum of the average daily temperatures, in K, Wc is the water content (where by, 1 g = 1 mL), ΔG is the Gibbs energy change, ΔH is the enthalpy change and ΔS is the entropy change.

It is important to stress that the time factor is insignificant in thermodynamics, while it is important for li ving systems; this paradox w as surpassed by the introduction of daily te mperature su ms (ob tained on the d ays of AA). The result s of g ermination te st, t he GSH, G SSG and PSH cont ents were statistically calculat ed with th e Anov a T-test (LSD = 5 %); th e E_{hc}, ΔG, ΔH and ΔS values were calculated with SD value; the depende nce bet ween the ger mination percentage, E_{hc}, ΔG, ΔH and ΔS were expressed by multiple regressions and correlation coefficients, calculated with Statistica 7.0 software (StatSoft Inc.).

RESULTS AND DISCUSSION

Redox signal s are key regulators of va rious plant m etabolic processes, including morphogenesis and growth. Glutathione is the major redox regulating substance in seeds. More tolerant genotypes have higher quantities of total glutathione, with different relations between reduced and oxidised glutathione (2GSH/GSSG).[24,25] During the AA treatment, similar and significant decreases of GSH were found in the seeds of both hybrids, down to 55 and 56 % in H1 and H2, respectively (Table I), while the percentage ger mination was significantly decreased by 11 %

in H1 and by 19 % in H2. The decreased GSH amount correlates with the significant increase in GSSG amount, which is in agreement with the results of De Paula *et al.*[26] and Torres *et al.*,[27] although their ratio was shifted to a greater extent in H2. Generally, the relationship between seed viability and GSH decrease does not coincide with a GSSG increase, with the values increasing by only to 28 % in H1 and over 3.6 times in H2 (Table I), signifying an irreversible loss of GSH from the system.[28] This is an indication of its reaction with other seed biomolecules, which could, moreover, be linked to a reaction shift to necrotic processes[29] *i.e.*, to programmed cell death.[13,17] It seems that the protective role of GSSG,[30] was emphasized in H2, having a 3.5 times higher GSSG content after 9 days of AA, with only a 7.6 % loss of total glutathione.

TABLE I. Changes in the germination percentage, GSH, GSSG and PSH in maize seeds (H1 – – dent hybrid, H2 – sugary hybrid) during accelerated (AA) aging treatment for 3, 6 and 9 days

Property	Hybrid	*AA* / days				*LSD* 0.05
		0	3	6	9[a]	
Germination, %	H1	98.0	97.7	96.0	87.7	5.74
	H2	95.5	95.0	83.0[a]	77.5	1.97
GSH / nmol	H1	296.8	277.1	232.4	132.4	73.37
	H2	265.4	260.9	246.5	116.0	71.45
GSSG / nmol	H1	154.9	159.6	165.2	216.0	36.22
	H2	49.0	50.9	57.3	174.1	60.67
PSH / nmol	H1	91.3	87.8	80.4	25.9	30.61
	H2	78.4	88.9	98.1	133.2	23.88

[a]Least significant difference, Student's *T*-test, $P = 0.05$

Seeds contain most of the thiols and disulphides in proteins,[31,32] which are liable to aging changes owing to their role in the regulation of the cell redox environment. Pukacka[33] found a decrease in PSH during the aging of *Acer platanoides* seeds, which is in agreement with the changes in the H1 seeds (decrease of 72 %, Table I). The significant increase in the PSH content was accompanied by a considerable decrease in GSH and germination ability during AA treatment in H2, compared to H1, which may develop from proteins undergoing dethiolation, although the similar changes were already established by Seres and co-workers.[34] The observed mechanism was suggested by Grant *et al.*,[35] as the last defence in the irreversible oxidation of cysteine residues, the occurrence of which would otherwise lead to polypeptide aggregation. It is also possible that two different types of nanomolecular switches:[20]

$$GSSG + PSH \rightleftharpoons PSSG + GSH \text{ (Type I)} \quad (6)$$

$$GSSG + P(SH)_2 \rightleftharpoons PSS + 2GSH \text{ (Type II)} \quad (7)$$

are present in H1 and H2, resulting in different alterations of the GSH/PSH ratio, which had a sigmoid trend in H1, decreasing from 3.2 to 2.9 (up to 6th day of

AA) and the n increasing, up to 5.1, underlining intensive PSH consumption to GSH, while in H2, the ratio had a more continual decrease to the 6 th day of AA (from 3.4 to 2.5) and then a steeper reduction to 0.9, emphasising intensive GSH expenditure.

However, Schafer and Buettner[20] used the GSSG/2GSH half-cell potential (E_{hc}), which gave the opportunity to quantify the physiological status influencing plant growth. The changes in the E_{hc} of the maize seeds shown in Fig. 1 clearly illustrate the changes developed during the course of AA. In the seeds of both hybrids, the GSSG increased due to GSH oxidation, as was also evidenced in other seeds.[26,27] In the initial maize hybrid seeds, the value of E_{hc} was similar, *i.e.*, –158.2 and –157.8 mV in H2 and H1, respectively, with negligible changes after 9 days of AA, *i.e.*, –119.1 and –118.5 mV, respectively. The slight variation in E_{hc} accompanied with intensive changes in GSH and GSSG (Table I), as well as a decrease in the total glutathione to 55 and 56 % in H1 and H2, respectively, were similar to the results obtained by De Vos *et al.*,[28] indicating that the glutathione redox system is not a closed one and that GSH reacts with other macromolecules in the seeds during aging. Dean and Devarenne[36] suggested conjugation of GSH with soluble phenolic compounds as the mechanism of its elimination from cells, while the significant amount of GSH in H2 may serve for disulphide dethiolation.[34,35]

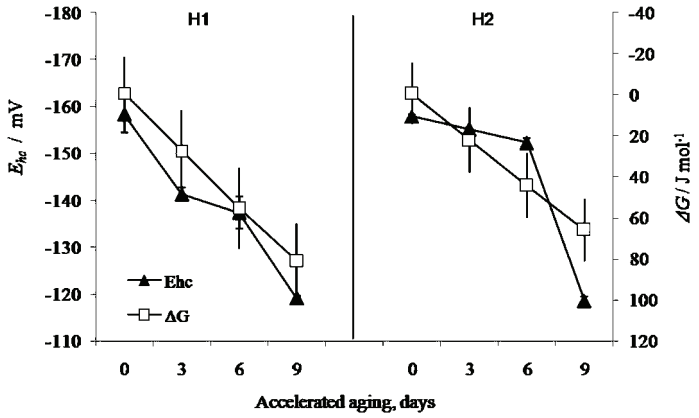


Fig. 1. Changes of the GSSG/2GSH half cell potential (E_{hc}) and Gibbs energy change (ΔG), as a result of accelerated aging for 3, 6 or 9 days (H1 – dent hybrid, H2 – sugary hybrid); vertical bars represent the *SD* values.

The found E_{hc} values were higher than the results obtained by Kranner *et al.*,[17] which could be connected to the level of the Gibbs energy change (ΔG, Fig. 1). Thus, ΔG negatively correlates with the germination decrease ($R = –0.66$) to a higher degree than the E_{hc} increase ($R = –0.59$). The thermal treatment (aging

mode)[6] elevated the Gibbs energy, but to a higher degree in H1 (81 J mol^{-1}) than in H2 (66 J mol^{-1}). The relatively parallel changes in the ΔG and E_{hc} of the seeds (R = 0.90) are indicative of an intensification of endergonic reactions and a larger energy expenditure.[14,23] Furthermore, the ΔG increase does not correspond with the ΔS change, which was decreased by a maximum of 0.06 J mol^{-1} in H1 (Fig. 2). Considering that entropy presents capacity, *i.e.*, the presence of energy unavailable for work, the system is under conditions of restricted energetic capacity (thermodynamic equilibrium, $\Delta S \approx 0$) and lower molecular mobility,[23,37] from the 3rd day of AA. It is necessary to emphasise that the lower entropy values of the H1 seeds indicate an enhanced capacity to tolerate change with respect to H2 seeds. Only for the ΔH, *i.e.*, measurement of the total energy change, were significant alterations observed, which had a sigmoid shape with the H1 seeds and a maximum ($\Delta H > 0$) on the 3–6th day of accelerated aging, while for H2, ΔH had general decreasing trend, with the negative values indicating a shift of the system from a relatively ordered to a disordered state.[14,23]

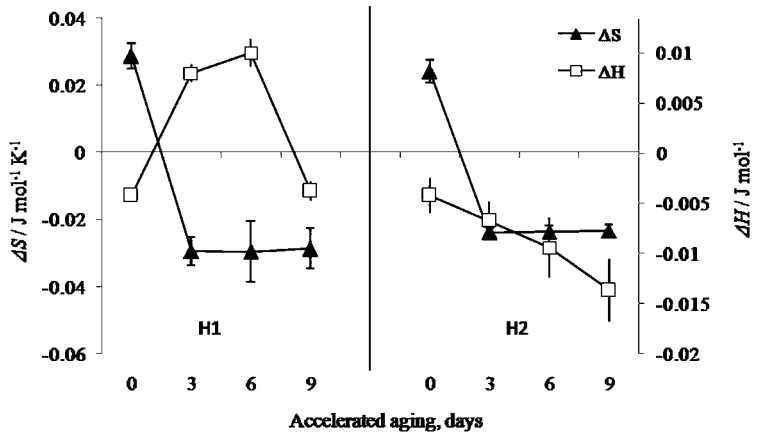
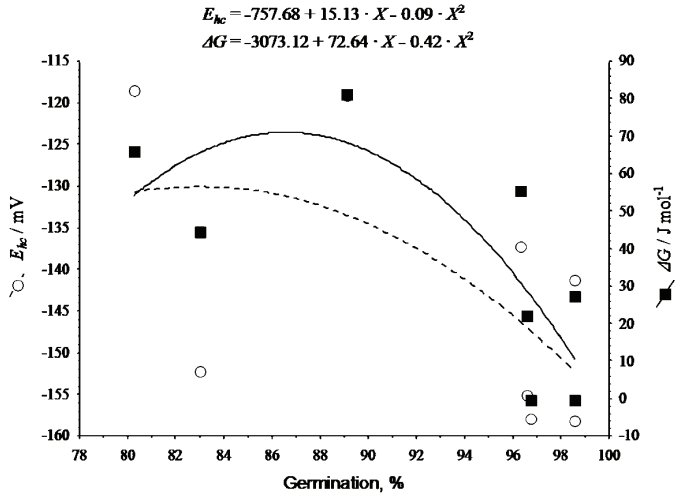


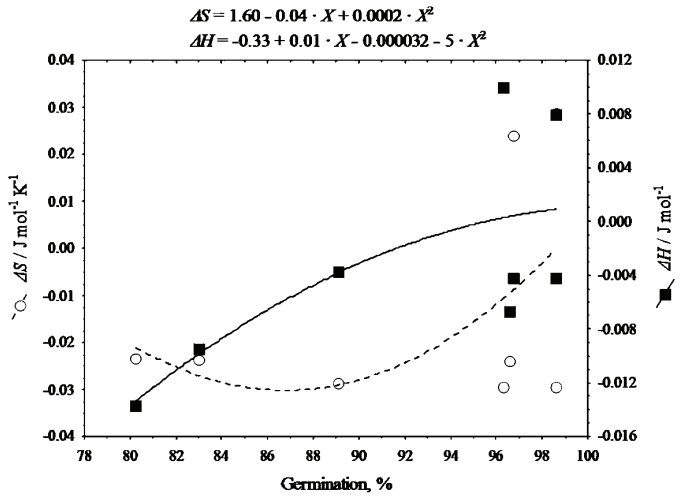
Fig. 2. Changes of entropy (ΔS) and enthalpy (ΔH) due to accelerated aging for 3, 6 or 9 days (H1 – dent hybrid, H2 – sugary hybrid); vertical bars represent the *SD* values.

The observed dynamics could be connected to a possible melting of the glass matrix and irreversible metabolic changes, attributed to desiccation.[16,22,38] Additionally, the trends of the ΔH and ΔS changes indicate a different organisation structure of the dent and sugary hybrids, irrespective of their positive correlation with germination reduction (Fig. 3) and the observed correlation between them (R = 0.71 for ΔH; R = 0.38 for ΔS). Namely, the rapid increase in enthalpy, followed by a negligible increase in entropy for the H1 seeds could be related to glass relaxation,[39] followed by a later domination of stronger bonds, as consequence of desiccation[23] and furthermore impairment of metabolism by free radicals.[16] Moreover, the H2 seeds could be characterised by weaker molecule move-

ments and stronger bonds, hence the system is above the edge of enthalpy equilibrium, with a relatively weak relaxation, characteristic for seeds with a lower germination ability.[39] It could be assumed that desiccation tolerance and prolonged seed longevity in the desiccated state depend on the ability of the system to scavenge free radicals. The failure of the antioxidant system during long-term desiccation appears to trigger programmed cell death, causing ageing and eventual death of the organism.[13,16]



(a)



(b)

Fig. 3. Correlation between the maintenance of germination (percentage) ability; a) changes of the redox potential, E_{hc}, and Gibbs energy change, ΔG, and b) changes in enthalpy, ΔH, and entropy ΔS.

CONCLUSIONS

The genotypic characteristics of the two examined hybrids indicated possible different metabolic pathways of the seeds, which is connected to different trends of the changes in the thermodynamic parameters (ΔG, ΔH and ΔS) and different types of nanomolecular switches, resulting in relative faster decrease of GSH in the H2 hybrid than in the H1 hybrid, emphasising that the GSSG/2GSH half cell potential is a useful tool for quantifying plant stress, but it cannot be alone a measure for seed germination ability. The observed changes in the seeds could be described from the viewpoint of thermodynamics by the enthalpy: $\Delta H = 0$ J mol^{-1} might be considered as the border between reversible and irreversible injuries in seeds. The combination of the GSSG/2GSH half-cell potential with thermodynamic parameters may possibly be a more effective tool for determination of seed germination ability and the physiological status of seeds.

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И З В О Д

УВОД У МЕЂУСОБНУ ЗАВИСНОСТ ЋЕЛИЈСКОГ РЕДОКС ПОТЕНЦИЈАЛА ГЛУТАТИОНА И ТЕРМОДИНАМИКЕ ТОКОМ УБРЗАНОГ СТАРЕЊА СЕМЕНА КУКУРУЗА

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Проучавано је убрзано старење семена два хибрида кукруза која имају другачију способност очувања клијавости. Сличан почетни редукциони потенцијал GSSG/2GSH пара је код H1(зубан) растао без утицаја на способност клијања до шестог дана убрзаног старења, док се код H2 (шећерац) није мењао до шестог дана старења, уз каснији значајан пад клијавости. Количина PSH се смањивала код H1, док је расла код H2 током третмана старења. Без обзира на континуиран пад слободне енергије током убрзаног старења, особине семена испитиваних хибрида су можда биле везане за другачије метаболичке путеве: H1 карактерише висока ентропија и позитивне вредности енталпије, док је за H2 карактеристична релативно ниска ентропија, уз негативне вредности енталпије, указујући на померање система из релативно уређеног у неуређено стање. Другачији типови наномолекулских прекидача, утичући на бржи пад GSH код H2 у односу на H1, истичу да се комбинација GSSG/2GSH ћелијског потенцијала и термодинамике могу користити при квантификацији биљног стреса.

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