

THE GROWTH OF MAIZE SEEDLINGS AS FUNCTION OF FREE ENERGY AND REDOX POTENTIAL

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Abstract: The difference in the growth of maize seedlings originating from seeds injured by accelerated ageing, as well as those altered by restoring with low 2,4-D (2,4-dichlorophenoxyacetic acid) concentrations was examined, from the point of view of free energy and redox potential. The ageing decreased germination ability, the seedling growth and free energy, with no remarkable influence on the redox capacity. Meanwhile, the 2,4-D treatment increased the germination percentage and the seedling growth, by better energy utilisation, with shifting of the redox balance to a reducing environment. From this point of view, the free energy and the redox potential are useful tools for the determination of biological vitality.

Key words: maize seed, seedlings, accelerated ageing, 2,4-D, free energy, redox capacity.

Introduction

The loss of vitality during long-term storage is the consequence of many spontaneous, gradual and damaging processes (McDonald, 1999), owing to production of reactive oxygen species (ROS). During oxidative stress, ROS damage the mitochondria and then other cell parts, leading to a slacking of respiration and membrane disintegration (Leprince et al., 1994). Accelerated ageing treatment induces changes in the naturally occurring antioxidants of seed, such as glutathione (McDonald, 1999), which are integrated into the cellular redox status. Glutathione is well-known as one of the most important antioxidants in plants having the role of increasing the resistance of plants to stress factors (Noctor et al., 2002). Seed ageing mainly induces glutathione oxidation (Torres et al., 1997), which in some cases could lead to necrosis (Willekens et al., 1997). Schafer and Buettner (2001), for this reason suggested a model based on relations of the GSSG/GSH couple for the quantification of

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physiological states, defining the border between proliferation, differentiation, apoptosis and necrosis.

It is characteristic that seeds during imbibition and germination absorb high quantities of water over a relative short period, which activates a range of chemical reactions, with increasing level of free energy (Sun, 2002). The theoretical basis of the energy concept, i.e., thermodynamics enables the quantification of biological vitality (Davies, 1961; Boyer, 1969).

Low 2,4-D concentrations (2,4-dichlorophenoxyacetic acid) have stimulatory influence, from the stimulation of membrane redox activity, respiration intensity, metabolism of antioxidants (Moore et al., 1988). Low 2,4-D concentrations induce better viability of *Trigonella foenum-graceum* seeds in the second generation (Hariharan and Unnikrishnan, 1983). Regarding the fact that hormesis presents the stimulatory effects on growth, longevity, metabolism and physiological mechanisms of low concentrations of some toxic substances (Calabrese and Baldwin, 1998, 2000), it could be assumed that low concentrations of 2,4-D display a hormetic effect.

The objective of this study was to investigate the difference in growth of maize seedlings originating from seeds injured by accelerated ageing treatment, as well as those altered by the stimulatory (restoring) influence of low concentrations 2,4-D in terms of the thermodynamic parameter of free energy and the redox potential.

Materials and Methods

The seeds of four maize inbreds, having different abilities to germinate, i.e., ZP PL 175 (L1) and ZP PL 188 (L2) as dent inbreds, and ZP PL 51(L3) and ZP PL 67 (L4) as sugary inbreds, were subjected to accelerated ageing treatment (Waltz and TeKrony, 2001) at a temperature of 42°C and a relative air humidity of 100% for 3, 6 or 9 days (down to a percent germination drop < 50%). This moment was attained after 9 days for L1 (germination was reduced from the initial 92.5% to 41.0%), after 6 days for L2 (germination was reduced from the initial 89.0% to 15.2%), while for L3 and L4, it was after 3 days when the L3 germination was reduced from the initial 28.7% to 13.5%, while for L4, germination was reduced from 88.5% to 77.7% and further ageing induced a decrease under 2%.

Subsequently, the germination capacity was determined by ISTA Rules in four replications of 100 uniform seeds (ISTA, 2007) after 7 days (with four additional replicates, necessary to produce an adequate amount of seedlings). The seeds were weighed before germination. Filter paper towels, used as the germination medium, were soaked in distilled water (Ø), or low concentrations, 5×10^{-9} mol L⁻¹ (D1) and 10^{-6} mol L⁻¹ (D2), of 2,4-D as recovery treatment.

The following conditions were maintained in the germination chamber: a temperature of 25°C, with an 8 h light regime of 1250 lux (simulation of daylight) and a relative humidity of 97%.

The uniformly grown seedlings were separated into four replications of 25 plants, the length of each root and shoot was measured and then they were fractioned into the root, the shoot and the seed rest. The roots and the shoots were weighed (fresh weight determination) and dried in the ventilation drier at 60°C to the constant mass (dry weight determination). The content of reduced (GSH) and oxidised glutathione (GSSG) in the parts of the seedlings was analysed according to the method of de Kok et al. (1981).

Based on the data obtained from fresh and dry weight determinations, the water content was calculated:

$$Wc = FW - DW \quad (1)$$

where Wc is the water content (whereby 1 g = 1 ml), FW is the fresh weight and DW is the dry weight. The thermodynamic parameter of free energy based on water content was calculated by the model proposed of Davies (1961) and Sun (2002):

$$G_{wc} = -RT \ln (Wc) \quad (2)$$

where G_{wc} is free energy based on the water content, R is the universal gas constant (8,314 J K⁻¹ mol⁻¹), T is the sum of average daily temperatures (K).

Moreover, the free energy of biosynthesis was calculated based on the dry weight:

$$G_{Bs} = -RT \ln (k_{Bs}) \quad (3)$$

$$k_{Bs} = (DW_{root} + DW_{shoot}) \times ((SdW - DW_{seed\ rest} - (DW_{root} + DW_{shoot})) / (SdW - DW_{seed\ rest})) \quad (4)$$

where G_{wc} is the free energy of biosynthesis, Bs is biosynthesis (mg) and k_{Bs} is the constant of biosynthesis, DW_{root} , DW_{shoot} and $DW_{seed\ rest}$ are the dry weights of the root, shoot and seed rest, respectively, and SdW is the seed weight.

The redox capacity of the GSSG/2GSH couple was estimated by the method of Schafer and Buettner (2001):

$$E_{hc} = -240 - (59.1/2) \log ([GSH]^2 / [GSSG]) \quad (5)$$

where E_{hc} is redox capacity (mV), $[GSH]$ is the concentration of reduced glutathione, while $[GSSG]$ is the concentration of oxidised glutathione.

The results of the germination test, fresh weight of root and shoot, length of root and shoot were calculated with SD values.

Results and Discussion

It is well known that seeds lose their ability to germinate during long storage. Thus, the percent of germination decreased to 5% and 74% for L1 and L2, respectively, while for L3 and L4, it was 15% and 11% (Table 1). Reduced ability to germinate is connected to the decrease of the fresh weight of the roots and the shoots, which depended on the genotype and the duration of the accelerated ageing. According to Ajayi and Fakorede (2000), the growth of seedlings correlates with the germination percent. The decrease in the fresh weight was followed by a reduction in the length of the root and the shoot (Đukanović, 2003), which was proportional to the duration of the ageing. Hence, the L1 root length was reduced by 9% and the shoot length by 10% (Table 1).

Table 1. Influence of the accelerated ageing (C-control; AA-accelerated ageing) and two 2,4-D concentrations (\emptyset -without 2,4-D influence; D1- 5×10^{-9} mol L⁻¹; D2- 10^{-6} mol L⁻¹) on germination and seedling growth: length and fresh weight of root and shoot of four maize inbred lines.

Maize lines		L1	L2	L3	L4
Germination (%)	Control	92.5 ± 8.8	89.0 ± 3.5	28.7 ± 1.7	88.5 ± 0.1
	\emptyset	41.0 ± 6.4	15.2 ± 3.3	13.5 ± 4.1	77.0 ± 0.8
	AA D1	44.2 ± 4.2	17.0 ± 3.6	15.0 ± 4.5	79.5 ± 0.1
	D2	40.0 ± 5.1	19.7 ± 0.9	19.2 ± 2.9	77.7 ± 0.1
Fresh weight (g) Root	Control	280 ± 5.1	173 ± 12.9	72 ± 6.6	112 ± 5.0
	\emptyset	132 ± 20.8	81 ± 19.1	34 ± 11.5	83 ± 18.2
	AA D1	193 ± 9.6	179 ± 9.2	184 ± 8.3	139 ± 12.6
	D2	154 ± 8.2	138 ± 10.1	110 ± 6.2	122 ± 20.0
Fresh weight (g) Shoot	Control	489 ± 39.2	303 ± 17.1	192 ± 33.2	250 ± 31.1
	\emptyset	408 ± 17.3	311 ± 46.9	129 ± 26.5	312 ± 72.3
	AA D1	490 ± 25.5	338 ± 41.1	164 ± 29.8	353 ± 53.4
	D2	441 ± 24.3	330 ± 31.0	192 ± 26.3	379 ± 45.1
Length (cm) Root	Control	10.5 ± 1.4	5.6 ± 0.7	4.0 ± 0.8	9.9 ± 0.8
	\emptyset	9.5 ± 1.2	6.7 ± 1.1	5.8 ± 0.9	9.1 ± 0.4
	AA D1	9.8 ± 0.6	9.8 ± 0.9	6.9 ± 0.6	12.1 ± 0.4
	D2	12.9 ± 0.7	9.5 ± 0.3	7.7 ± 0.9	10.8 ± 1.5
Length (cm) Shoot	Control	9.9 ± 2.2	5.5 ± 0.3	4.4 ± 0.6	9.9 ± 0.5
	\emptyset	8.9 ± 0.7	6.4 ± 0.4	6.4 ± 1.0	10.5 ± 0.8
	AA D1	10.0 ± 0.4	10.1 ± 0.9	6.2 ± 0.5	13.1 ± 1.0
	D2	10.5 ± 0.5	9.2 ± 0.5	8.0 ± 0.4	11.8 ± 0.8

C: control; AA; accelerated ageing; 2,4-D treatment: \emptyset -without, D1- 5×10^{-9} mol L⁻¹, D2- 10^{-6} mol L⁻¹; Values are mean ± SD.

Water, penetrating into seeds yields a distinct energetic potential, which initiates a whole range of biochemical reactions, increasing the energetic capacity for the growth. Regarding the fact that detached parts of seedlings grow unequally (especially at the start of the growth phases) their free energy is also disposed unequally, i.e., a higher energetic potential is obtained on the shoot level than on the root level: in the control seedlings of all the examined genotypes it is in the range from -12 to -15 J, while at the root level, the values were in the range from -10 to -13.5 J. (Figure 1). The free energy of biosynthesis has the lowest values (Figure 2), while the variations, induced by accelerated ageing had a descending trend (to 1.5 J for L1). The general influence of accelerated ageing on the growth process leads to a decrease of the energetic potential, primarily at the root and biosynthesis levels, having as a consequence root elongation, based on increased water absorption, which Boyer (2001) defined as a negative state of the system.

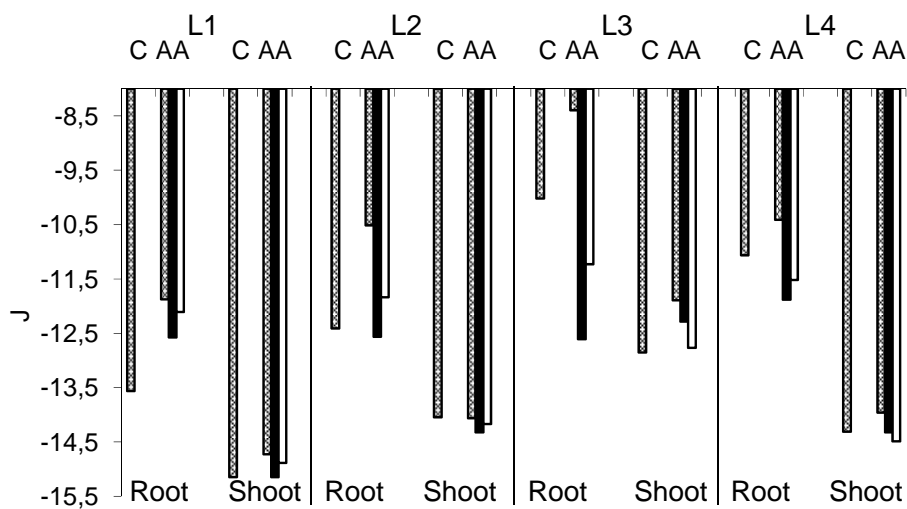


Figure 1. Influence of accelerated ageing (C-control; AA-accelerated ageing) and two 2,4-D concentrations (⊠-without 2,4-D influence; ■-D1, 5x10⁻⁹ mol L⁻¹; □-D2, 10⁻⁶ mol L⁻¹) on free energy of seedling parts: root and shoot, of four maize inbred lines.

The redox capacity of the root and the shoot, as calculated parameter (Figure 3) has developed into a theoretical scale of physiological states (<-240 mV is proliferation, from -240 to -200 mV is differentiation, from -200 to -170 mV is apoptosis and >-170 mV is necrosis; Schafer and Buettner, 2001), confirming whether the root and the shoot are in phase of intensive growth. However,

alterations of the redox capacity, induced by accelerated ageing are unable to determine a poorer growth of seedling originating from aged seeds in a correct manner, as its free energy of biosynthesis (Figure 2).

It is well known that the germination percentage could be elevated by the application of different treatments (McDonald, 1999), particularly when seeds have poor vitality. Whereas 2,4-D is a morphogenic substance, its usage in low concentrations, as hormetic (Calabrese and Baldwin, 1998, 2000; Raghavan et al., 2006), had the consequence of increasing the number of vigorous seeds. The largest stimulation was observed for L1 and L4, 3.2% and 2.5%, respectively with D1, while for L2 and L3, it was 4.5% and 5.7%, respectively, with D2 (Table 1). Similar results were obtained by Hariharan and Unnikrishnan (1983), although they observed increased germination percent of *Trigonella foenum-graceum* seeds in the following generation. The 2,4-D treatment mainly caused an increase of the fresh weight and root elongation, whereby D1 was much more effective with L1 and L2, while D2 display better effects with L3 (Table 1). From this point of view, morphogenic influence of 2,4-D has been confirmed, dependently on the applied concentration (Raghavan et al., 2006).

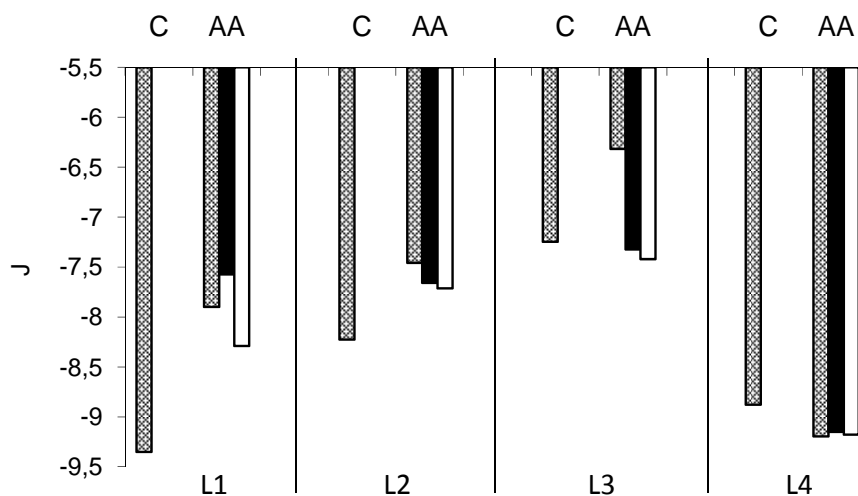


Figure 2. Influence of accelerated ageing (C-control; AA-accelerated ageing) and two 2,4-D concentrations (⊠-without 2,4-D influence; ■-D1, 5×10^{-9} mol L⁻¹; □-D2, 10^{-6} mol L⁻¹) on free energy of biosynthesis of four maize inbred lines.

Additionally, the thermodynamic parameter of free energy could clearly define the observed alterations. Namely, both the applied 2,4-D concentrations

induced an increase in the absolute values of the free energy of the root and the shoot (up to 33%, Figure 1), underlining better employment of the available energetic potential. The greatest energetic distinction was observed at the root level, whereby the D1 had better effects on all the examined genotypes, elevating the values up to 4.21 J (L3). However, at the shoot level of the sugary inbreds, better results were obtained with D2, increasing their energetic value to 0.87 J and 0.52 J (L3 and L4).

Nevertheless, biosynthesis responded to a high degree of 2,4-D stimulation, increasing its energetic potential, whereby D2 was a more effective concentration, but at a higher level in L1 and L2. In this manner, the hypothesis of Hicks and Morre (1998), Morre (2000), Raghavan et al. (2006) was upheld that higher concentrations of a hormetic toxogene, in this case 2,4-D, are decisive factor influencing some highly sensitive physiological reactions.

It could be assumed that the 2,4-D treatment generally increased the germination percent and growth, due to a better energy consumption, as well as that the observed processes are basically connected to redox signals (Pastori and Foyer, 2002; Noctor et al., 2002). Therefore, an increase of the absolute E_{hc} values (Schafer and Buetner, 2001), i.e., shifting of the redox balance to the reduced state, was observed in the roots and shoots of all the examined genotypes upon 2,4-D treatment, but to a higher extent in the seedling part which shrank under the damaging effect of accelerated ageing. Thus, the D2 concentration induced an increase of E_{hc} for the L2 root from -180.79 mV (bottom of the differentiation part of the scale) to -224.97 mV (proliferation part of the scale), while for the L4 shoot, the value of E_{hc} increased from -91.05 mV (necrosis part of the scale) to -173.72 mV (apoptosis part of the scale).

Conclusion

It could be concluded that low vitality, expressed through a lowering of the ability to germinate and the growth, induced by accelerated ageing is connected to a decrease of energetic potential, primarily at the root and biosynthesis levels. The ageing duration generally alters potential energy of biosynthesis, with no remarkable influence on the redox capacity. Meanwhile, 2,4-D treatment generally increased the germination percent and growth of the seedlings, based on an improved employment of the energy (especially at the biosynthesis level), with a parallel shifting of the redox balance to a reduced environment. From this point of view, the thermodynamic parameters of free energy and redox capacity are useful tools for the determination of biological vitality.

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RAST KLIJANACA KUKURUZA KAO FUNKCIJA SLOBODNE ENERGIJE I REDOKS POTENCIJALA

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R e z i m e

Ispitivana je razlika u rastu klijanaca poreklom iz semena koje je bilo izloženo tretmanu ubrzanog starenja, kao i semena koje je potom bilo izloženo regenerativnom uticaju niskih koncentracija 2,4-D (2,4-dihlorofenoksisirćetne kiseline) i to iz ugla slobodne energije i redoks potencijala. Starenje je smanjilo sposobnost klijanja semena, rast klijanaca i njihovu slobodnu energiju, bez znatnog uticaja na redoks potencijal. Sa druge strane, 2,4-D je uticao na bolje iskorišćenje energije i pomeranje redoks ravnoteže ka redukovanoj sredini, povećavajući klijavost i rast klijanca. Sa ove tačke gledišta, slobodna energija i redoks potencijal mogu biti korisni pri određivanju biološke vitalnosti.

Ključne reči: seme kukuruza, klijanci, ubrzano starenje, 2,4-D, slobodna energija, redoks kapacitet.

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