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The Quantitative Determination of Seed Mass Defect during Germination. 1. The Daily Dynamics of Net Supplemental Free Energy

- Original scientific paper -

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Abstract: A dynamics of seed mass defect and a previous process of the water input during germination was determined by a trial with five soybean cultivars and four sunflower hybrids, following the ISTA Rules. It was noticed that leaching, which led to the mass defect (smDef), was permanent during 8 and 10 days of soybean and sunflower germination, respectively. The two confronted processes, the liquid input and the solid output, could be operable at the level of a thermodynamic parameter of Gibbs free energy (G), i.e. *net* supplemental free energy (*net*Gspl). Hence, this type of free energy - *net* supplement, differentiates in two examined plant species (between the first and the last day of germination): soybean in a range from -53.23 to -69.82 J mol^{-1} seedling^{-1} and sunflower from -46.35 to -91.94 J mol^{-1} seedling^{-1}. Furthermore, the daily change of *net* supplemental energy underlined the three points ingenotypes of both species: after the first, before the last day of germination and in the maximum (-67.50 and -75.25 J mol^{-1} seedling^{-1} in soybean, then, -94.97 and -103.79 J mol^{-1} seedling^{-1} in sunflower) between the 4th and the 7th day of germination depending on a genotype.

Key words: Energy input by water, energy output by seed mass defect, *net* energy supplement.

Introduction

It has been known for a long time that the process of leaching starts immediately after the beginning of progression into the seed and endures during the whole period of the seed germination and growth, ***McKersie*** and ***Stinson***, 1980, ***Murphy*** and ***Noland***, 1982, ***Duke et al***., 1983. The origin of leaching phenomenon is attributed to an injury of cell membranes during seed filling and desiccation, osmotic shock, etc., ***Seewaldt et al***., 1981, ***O'Neill*** and ***Leopold***, 1982, ***Opra***, 2002. The problem is discussed partially, depending on the type of damage, hence, that exudation hinged on injury degree. During the time, the total seed leached mixture - exudates become a subject of seed viability research. Namely, when it was found that this exuded mixture had ability to conduct current, an apparatus was developed with the aim to determine the percentage of seed viability, i.e. seed ageing, ***Ratkovic et al.,*** 1992, ***Ratkovic et al***., 1994. Also, the quantitative method was developed with the purpose to detect the seed germination ability through a content of total phenolics in exudates after seed imbibition in distilled water, ***Sredojevic et al***., 2000, ***Srebric et al***., 2000, ***Simic et al***., 2005.

However, there were minor evidences about the quantitative determination of a sum (total) **seed weight (substance)** losses during germination, ***Opra***, 2002. There)fore, a trial was set up with five soybean cultivars and four sunflower hybrids under standard germination conditions, ***ISTA Rules***, 2007, with the purpose to give an answer to the first question: how much of seed mass was lost in soybean, i.e. sunflower during the period of eight, i.e. 10 days, respectively?

Materials and Methods

Materials. - Twelve replications of 100 uniform seeds of five soybean varieties (015, Nena, Lana, Lidija and Laura) and four sunflower hybrids (Altesse, Alvaro, Allium and Leila) were weighed and placed on the filter paper towels (as a germination medium) in the germination cabinet at the temperature of 25°C and 8-h light regime of 1250 lux (simulation of the daylight) and a relative humidity of 97%, ***ISTA Rules***, 2007. Seeds and, after while, uniformly formed seedlings were divided into four replications of 25 seedlings, very 24 hours up to 8^{th} day for soybean and 10^{th} day for sunflower.

Method. - The gravimetry, as a standard chemical analytical method, was used for the measurement of samples: seeds and seedlings. The analytical balance calibrated to four decimal places was used to measure: seed weight, fresh and dry weight of seedlings. The seedlings were dried at 60°C in the calibrated ventilation dryer until the constant weight. The weights are presented in Table 1.

Theory - The data of the first order. - The measurements presented in Table 1 subjected to an operation of subtraction resulted in the data of the first order: the water content of the seed-seedling system and the seed mass defect:

$$Wc = FW - DW \quad (1)$$
$$smDef = Sd - DW \quad (2)$$

where:
Wc is water content
FW is fresh weight

DW is dry weight
smDef is seed mass deficit
Sd is seed mass.

Gibbs free energy. - Further operations with a liquid (volume) and a solid state (mass) permit only mathematical calculations (g = ml), if they are in the unique, equal form. Gibbs free energy as a thermodynamic parameter has two aspects: physical by a volume and chemical by a constant of a reaction balance, ***Davies***, 1961, ***Philip***, 1966, ***Sredojevic et al***., 2005 **a, d.** Therefore, the transfer of the volume and the mass into free energy enables new options in mathematical operations of a primary data.

$$[Sd] = [Sdl] + [smDef] \quad (3)$$
$$k = [Sdl] \times [smDef] / [Sd] \quad (4)$$
$$G = - RT \ln V \quad (5a)$$
$$G = - RT \ln k \quad (5b)$$

where:
k is constant of reaction,
G is Gibbs free energy,
R is universal gas constant (8.314 J K^{-1} mol^{-1}),
T is temperature in kelvins.

Theory - The data of the higher order. - The energies of the seed-seedling water content (G_{Wc}) and the seed mass defect (smGdef) in the form of Gibbs free energy (G) give *net* energy (*net*G):

$$G_{net} = G_{Wc} - G_{smDef} \quad (6)$$

A partial daily change of any above stated data is given by n Δn+1 X:

where:
n is the day of the treatment, and
X is water, seed mass defect and net energy.

Results and Discussion

Water and seed mass defect. - The data of the first order, in this examination, the water accumulation and the seed mass defect (smDef) were obtained by the equations (1) and (2) according to Table 1. The water and mass defect elevation in soybean and sunflower, from the first to the last day of germination (8^{th} and 10^{th}, respectively) was not linear. Namely, this non-linearity in raise was expressed by maximums and minimums of a daily change (n Δn+1 W/smDef).

Figure 1a shows that the maximums of the water accumulation were in soybean between the 3rd and 4th, then, the 5th and the 6th (Lidia and Laura) or the 6th and the 7th day (015, Nena and Lana). Hence, it is interesting to observe that one or both maximums of smDef precede maximums of the water accumulation, except in cultivars Lidia and Laura (Figure 1a). On the other hand, the minimums of the water content are dispersed. The smDef minimums mainly overlap water maximums as expected. This could be explained by a higher amount of water that dilutes a hydrolysed substance in the seed-seedling system and supports leaching.

The analogous behaviour could not be yet noticed in sunflower, ***Boyer***, 1971. Thus, the maximums of the daily water accumulation were between the 5th and the 6th and the 7th and the 8th in Alvaro and Altesse; then, between the 4th and the 5th in Allium and between the 6th and the 7th in Leila (Figure 1b). On the other hand, the maximums of smDef were dispersed, presenting genotypic properties, starting with the 2nd and the 3rd and 3rd day (Alvaro and Altesse, respectively) until the 9th and the 10th day (Alvaro and Allium, respectively). So, it was difficult to recognise the compatibility with minimums of the daily water accumulation.

Energy: input and output. - The mechanism of the water absorption introduces Gibbs free energy in the seed-seedling system by a double phase transition, ***Parrish*** and ***Leopold***, 1977, ***O'Neill*** and ***Leopold***, 1982, thus, the elevation of the water content (Wc) during germination raises the input of free energy in seedlings (see theory, equation 5a). After the first day of imbibition the free energy income in soybean cultivars was: 95.98; 92.02; 95.41; 94.89; 96.28 J mol^{-1} $seedling^{-1}$ (015, Nena, Lidija, Lana and Laura, respectively), and in sunflower hybrids: 78.82; 73.95; 68.10; 68.93 J mol^{-1} $seedling^{-1}$ (Alvaro, Altesse, Allium and Leila, respectively (Figure 2). Meanwhile, at the examined end point of germination, the 8th day in soybean, the free energy was: -125.12; -125.63; -125.25; -125.56; -122.34 J mol^{-1} $seedling^{-1}$ (in above stated cultivars) and the 10th day in above stated sunflower hybrids was: -118.03; -122.62; -118.95; -116.50 J mol^{-1} $seedling^{-1}$. On the other hand, permanent leaching of a hydrolysed substance from the seed-seedling system, starting with imbibition, means the permanent lost of energy (see theory, equation 3 and 5b). So, after the first day the loss of energy was: 30.00; 30.87; 40.31; 41.66 and 38.97 J mol^{-1} $seedling^{-1}$ in the above stated soybean cultivars, and 16.38; 20.10; 21.75; 14.02 J mol^{-1} $seedling^{-1}$ in above stated sunflower hybrids (Figure 2). Thus, at the end point, the energy loss was: 55.30; 55.87; 57.32; 57.63; 59.63 J mol^{-1} $seedling^{-1}$ in the above stated soybean cultivars and 37.39; 30.68; 32.87; 35.16 J mol^{-1} $seedling^{-1}$ in above stated sunflower hybrids.

The fluctuations of the water input and the smDef output of seeds and seedlings in the form of free energy allowed the calculation of an actual, *net* status of energy (data of higher order), ***Sredojevic et al.***, 2005 a, b, c, which is supplemental to energy, released from exothermal biochemical reactions, minimised by endothermic reactions in a given system, ***Davis***, 1961. In such a way, both energies, inputted and released, are a result of processes and reactions, which minimise energies of a given system. Consequently, the absolute values supplemental, *net* energy shown in Figure 2, which after the first day were -65.97; 61.15; -55.11; -53.23; -57.31 J mol^{-1}

seedling^{-1} in soybeans raised up to -75.25; -72.80; -70.62; -67.50; -67.92 J mol^{-1} seedling^{-1} (015, Nena, Lidija, Lana and Laura, respectively) until the 5th, 5th, 5th, 4th and the 6th day of germination depending on a genotype, and then, it was lowered to: -69.82; -69.75; -67.93; -67.92 and -62.98 J mol^{-1} seedling^{-1}. Furthermore, in sunflower the flow of *net* energy went as follows: after the first day values of -54.44; -53.85; -46.35; -54.90 J mol^{-1} seedling^{-1} (Alvaro, Altesse, Allium and Leila, respectively), raised to -103.79; -94.97; -95.28; -96.07 J mol^{-1} seedling^{-1} until the 5th, 4th, 5th and the 7th day of germination depending on genotype, then, they lowered to: -80.64; -91.94; -86.08; -81.34 J mol^{-1} seedling^{-1}.

According to the ascend and drop of *net* energy, shown in Figure 2 the daily change of supplemental free energy, nΔn+1G*net*, in the seed-seedling system oscillated about the steady state, i.e. near 0 J mol^{-1} seedling^{-1} (see Figure 3) with higher amplitudes that elevate *net* energy. The raise of this energetic type in soybeans was between -10,61(Laura) and -17,59 (Nena) J mol^{-1} seedling^{-1}, then, with a drop from 2,53 (Lana) to 11,17 (015) J mol^{-1} seedling^{-1}, giving the raise of supplemental *net*G on the 8th day of the trial from -3.85 (015) to -14.53 (Lana) J mol^{-1} seedling^{-1} to a starting value in the first day of the trial (Figure 3). Then, the increase in sunflowers was between -43.07 (Altesse) and -58.00 (Alvaro) J mol^{-1} seedling^{-1}, followed by a drop between 13.98 (Altesse) and 23.37 (Alvaro) J mol^{-1} seedling^{-1}, giving the raise of supplemental *net*G on the 10th day of trial from -26.44 (Leila) to -39.73 (Allium) J mol^{-1} seedling^{-1}.

Conclusion

The elaboration of successive processes: the water absorption and the seed mass losses, by expressing their values in the form of Gibbs free energy, enables the creation of a new parameter - *net* absorbed energy, which is determined as a supplement to released energy (generated by biochemical reactions, also net), after enclosing exo- and endothermic reactions. These two confrontedprocesses, the liquid input and the solid output, operable at the level of *net* supplemental free energy, makes a difference between two examined plant species. Furthermore, the daily change of *net* supplemental energy differentiates the genotypes of both spaces in three points: after the first, last and the maximum, between the 4th and the 7th day of germination. The appearance of daily *net* supplemental free energy comes right in time, when, according to ***ISTA Rules***, 2007, the first count in determination of seed quality is made.

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Kvantitativno određivanje masenog defekta semana tokom klijanja. 1. Dnevna dinamika neto dodatne slobodne energije

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I z v o d

U ovom radu izneta su kvantitativna određivanja dinamike gubitka mase semena tokom klijanja kod pet sorti soje i četiri hibrida suncokreta po ISTA pravilima. Kako je metoda određivanja masenog defekta semena davala i dinamiku usvajanja vode, ta dva podatka prevedena u Gibsovu slobodnu energiju omogućila su operaciju sa dva suprotstavljena procesa: usvajanja tečne i ispuštanja čvrste supstance, koje su oduzimanjem dale neto energiju unetu u sistem seme-klijanac. Pokazalo se da neto, dopunska slobodna energija u opsegu od -53.23 do -69.82 J mol^{-1} $klijanac^{-1}$ za soju (od prvog do osmog dana) i od -46.35 do -91.94 J mol^{-1} $klijanac^{-1}$ za suncokret (od prvog do desetog dana), daje razliku između dve ispitivane vrste. Dalje, dnevna promena neto, dodatne energije razdvaja genotipove kod obe biljne vrste u tri tačke: posle prvog dana, pre zadnjeg dana i maksimum (-67.50 i -75.25 J mol^{-1} $klijanac^{-1}$ za soju, a -94.97 i –103.79 J J mol^{-1} $klijanac^{-1}$ za suncokret), između četvrtog i sedmog dana, što zavisi od genotipa.

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