UDC 632.5
*Original scientific paper*

# THE APPLICATION OF STANDARD SI UNITS AND TERMODYNAMICS IN DETERMINATION OF HERBICIDES' INFLUENCE TO MAIZE INBREDS
## 2. The free energy status

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Sredojević Slobodanka, Lidija Stefanović, Vesna Dragičević, Mirjana Srebrić, Petar Piper and Miroslav Vrvić (2006): *The application of standard si units and termodynamics in determination of herbicides' influence to maize inbreds. 2. The free energy status.*- Acta herbologica, Vol. 15, No. 2, 83-90, Beograd.

The two herbicides and their combined treatment influenced the change of water input in each of 15 investigated maize inbreds. The water volume (ml) fluctuated between 11 to 110% in shoot and 23 to 275% in root, what introduced the change of free energy (G) from -254.6 to -151.6 KJ in shoot and from -248.0 to -172.3 KJ in root. The difference in free, potential energy between non- and treatment ($\circ\Delta_{treat}G$) was in shoot from 11.1 to 90.2 KJ and in root from 16.8 to 75.7 KJ. Further, the lower magnitude of $\circ\Delta_{treat}G$ amplitude between root and shoot implicated the tolerance of genotypes to applied herbicides. The energy, spent in biosynthesis of 1mg dry substance was amounted from 75.6 to 16.8 KJ mg-1; the applied herbicide treatments assembled the group of genotypes with lowered necessity of this energy, what has the positive implications in the sense of thermodynamics.

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*Key words*: alachlor, atrazine, biosynthesis, differential free energy, free (potential, Gibbs) energy, maize inbreds, water volume

## INTRODUCTION

The free energy of seedling has the two origins: it can be inputted by water input in plant () and liberated by biosynthesis (). The metabolic cycles with short and quick reactions, introduced by incorporated herbicide into maize plant, makes disorder in energy balance, while partial biosynthetic reactions are quicker than the sum of dominant biosynthesis, what engaged energy spending (). Further, disordered biosynthesis means obstructed growth, which encircled reduced energy input by water, too (). Namely, the water proceeding from outer environment to inner space of plant system through protein channels of aquaporines () has two-phase changes: the first one is a liquid inverting into gas state by absorbing of energy from the environment; then, the second one is the liberation of the same quantity of energy by condensing of gaseous $H_2O$ molecules to liquid water in plant system. Thus, the second phase change means the energy input to seedling system.

This essay intends to discus the change of free energy, liberated by biosynthesis and inputted by water in 15 maize inbred lines treated by two herbicides and their combination.

## MATERIAL AND METHODS

In this essay was used the same trial and parameters as in previous paper.

Water content as volume (ml) was used in calculation of thermodynamics' parameter free or Gibbs energy (SUN, 2002; SREDOJEVIĆ *et al.* 2005) into root, shoot and seedling:

$$G = -RT \ln Vw \quad [1]$$

The free energy (G) of plant biosynthesis (ΣBs) was determined over constant (k) of reaction (DAVIES, 1967; SREDOJEVIC *et a*l. 2005):

$$\Sigma Bs = rtBs + shtBs \quad [2]$$

$$k_{Bs} = [rtBs] \times [shtBs] / [\Sigma Bs] \quad [3]$$

$$G_{Bs} = -RT \ln k\Sigma_{Bs} \quad [4]$$

where is R universal gas constant, T is temperature in Kelvin's, ΣBs sum of root and shoot DW.

## RESULTS AND DISCUSSION

The results presented in previous paper underlined that the main disorder in the essay of 15 inbreds' reaction to alachlor and atrazine was in water content and biosynthesis of seedlings dry substance (SREDOJEVIC *et al,* 2006, in press). The observed disorder in water accumulation implicated also the disordered free (potential, GIBBS) energy input in shoot and root fragments of seedling. The new side mechanisms started in plant, concerned to herbicides' inactivation, shifted the energy balance of the dry substance biosynthesis per weight unit.

*Tab. 1 - Water cont. (mg) IN non-treated (control) and treated seedlings by herbicides (mg plant$^{-1}$)*

| Genotype | Shoot | | | | | Root | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Contol | Al $10^{-5}$ M | Al $10^{-4}$ M | A $10^{-4}$ M | Al+A $10^{-4}$ M | Contol | Al $10^{-5}$ M | Al $10^{-4}$ M | A $10^{-4}$ M | Al+A $10^{-4}$ M |
| L1 | 1207 | 403 | 362 | 601 | 940 | 408 | 306 | 110 | 607 | 566 |
| L2 | 716 | 499 | 332 | 600 | 552 | 873 | 648 | 298 | 697 | 657 |
| L3 | 469 | 422 | 384 | 502 | 372 | 824 | 701 | 614 | 781 | 561 |
| L4 | 462 | 304 | 90 | 413 | 478 | 546 | 392 | 237 | 684 | 448 |
| L5 | 460 | 237 | 89 | 262 | 296 | 480 | 328 | 108 | 388 | 322 |
| L6 | 435 | 442 | 184 | 520 | 400 | 483 | 480 | 210 | 590 | 330 |
| L7 | 558 | 667 | 59 | 553 | 483 | 287 | 577 | 103 | 788 | 406 |
| L8 | 335 | 285 | 300 | 437 | 467 | 504 | 377 | 360 | 501 | 449 |
| L9 | 308 | 341 | 215 | 346 | 320 | 500 | 392 | 300 | 476 | 355 |
| L10 | 427 | 258 | 201 | 511 | 478 | 384 | 280 | 176 | 687 | 512 |
| L11 | 323 | 387 | 281 | 449 | 340 | 453 | 464 | 374 | 589 | 389 |
| L12 | 234 | 218 | 178 | 392 | 302 | 345 | 212 | 174 | 299 | 312 |
| L13 | 269 | 278 | 193 | 531 | 378 | 294 | 262 | 197 | 582 | 360 |
| L14 | 202 | 316 | 169 | 424 | 320 | 318 | 335 | 153 | 419 | 364 |
| L15 | 251 | 337 | 141 | 441 | 438 | 237 | 295 | 216 | 614 | 393 |

Control - non-treatment; Al - Alachlor; A- Atrazine; Al+A - Alachlor + Atrazine

The water status, according to the results presented in Tab. 1 showed that all applied herbicides induced lowering or elevating of water content in shoot and root of all 15 investigated maize inbreds, compared to non-treatment; thus, compared to the non-treatment, in shoot was occurred the highest disorder of water accumulation caused by pure and concentrated forms ($10^{-4}$ M) of 11- 87% in alachlor and 50-210% in atrazine, ranged 8 and 4 times from lowest to highest, respectively; then, in root 23-91% and 80-275%, ranged about 3,5 times from lowest to highest for both herbicides. Further, the diluted and combined forms of herbicides (Al $10^{-5}$ M and Al + A, 2:1, $10^{-4}$ M) shortened the range at 64-175% and 34-156%, respectively, according to non-treatment meaning 2,5 and 3,3 times range from the lowest to the highest value. The noted, changed status of water volume in treated seedling's parts: shoot and root could be attributed.

The observed, changed status of water volume in treated seedling's parts: shoot and root could be assigned to disorder of proteins at two levels; at first, to proteins which form water passing canals - aquaporines (MAUREL *et al*, 1997; Schaeffner, 1998) and other one which are polar and ionic water binding centres (VERTUCCI, 1984; RUSI, 197-; INDUSI, 200-). The reaction of herbicides with fatty acids from cell membranes could disorder the water holding in cells (JANJIC, 2004; NETTING, 2000; STEUDEL E. and FRANSCH J. 1998; STEUDEL and PETERSON, 1998).

The mechanism of water absorption in plant's root is double *phase change* (from liquid to gas and *vice versa*). The aquaporines are single $H_2O$ molecule diameter canals and there is request for energy to separate the molecules to pass through, and than to condense inside the system, liberating, i.e. inputting energy as the second phase change (SCHAEFFNER, 1998; MAUREL *et al*, 1997; MOORE, 1966). So, the changed water volume by herbicide treatment introduced the change of energy input in shoot and root. The initial state of free energy in non-treated inbreds was in shoot from –263,9 to –197,4 KJ sh$^{-1}$ and in root from –251,6 to –203,3 KJ r$^{-1}$ (Fig. 1). Following the occurred water volume by Al $10^{-4}$ M-treatment the energy input was from –221,3 to –151,6 KJ sh$^{-1}$ for shoot and from –251,8 to –203,3 KJ r$^{-1}$ for root. All other applied treatments (Al $10^{-5}$, A$10^{-4}$ and Al+A $10^{-4}$ M) accomplished that the elevated values were over non-treatment, then, they downed between non- and Al$^{-4}$-treatment, where they were more closed to non-treatment (Fig.1).

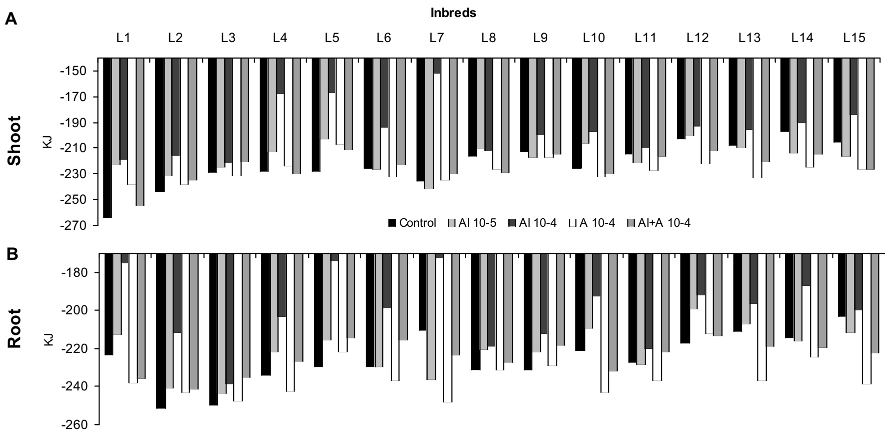


Figure 1 - The free energy in shoot (A) and root (B);
Control - non-treatment; Al - Alachlor; A- Atrazine; Al+A - Alachlor + Atrazine

Despite, the inbred L7 owned lowest energy in shoot (-151,5 KJ sh$^{-1}$; Al $10^{-4}$ M), in this inbred also was occurred the highest oscillation in relation to non-treatment ($\circ\Delta_{treat}G$) of 90,2 KJ sh$^{-1}$ (83,6 KJ sh$^{-1}$in Al $10^{-4}$ M; 6,6 KJ sh$^{-1}$in Al+A $10^{-4}$ M). On the other hand, the lowest $\circ\Delta_{treat}G$ of 11,1 KJ shl$^{-1}$was occurred in L3 (8,6 KJ sh$^{-1}$ in Al $10^{-4}$ M; 1,3 KJ sh$^{-1}$ in Al $10^{-5}$ M). Also, the root of L7, with the lowest free energy input (-172,3 KJ r$^{-1}$ in Al $10^{-4}$ M) occurred, had the highest $\circ\Delta_{treat}G$ of 75,7 KJ r$^{-1}$ (38,1 KJ r$^{-1}$ in Al $10^{-4}$ M; 37,6 KJ r$^{-1}$ in Al $10^{-5}$ M), and the lowest had in L8 of 12,5 in Al $10^{-4}$ M.

Hence, the inbreds: L3, L8, L9 and L11 according to $\circ\Delta_{treat}G$ values in shoot of: 11,1; 18,3; 17,7 and 17,4 KJ sh$^{-1}$, respectively; then, in root of inbreds: L8, L9 and L11 with values: 12,5; 19,0 and 16,8 KJ r$^{-1}$, respectively, could be separated as partial (shoot or root) and in whole (plant) tolerant systems (Fig. 1).

According to previous observations, it could be noted that there is emphasised an amplitude between $\circ\Delta_{treat}G$ of shoot and root ($_{sh}\Delta_{r}GA$). A magnitude of the amplitude $m_{sh}\Delta_r GA$, underlines genotypic difference. The treatments perform the differentiation of investigated inbreds. In a frame of single genotype the value of $m_{sh}\Delta_r GA$ could intend the stability of plant system, i.e. as great the value is, the greatest energy is spend to maintain the balance between shoot and root. Thus, the genotypic range of $m_{sh}\Delta_r GA$ presented at Fig.2; a, b, c, d in treatments was suited between L7 with values: 45,4 KJ (AL$10^{-4}$ M); 37,9 KJ (A $10^{-4}$ M); 19,3 KJ (Al $10^{-5}$ M); 18,3 KJ (Al+A $10^{-4}$ M) and L6 with values: 11,0 KJ (Al+A $10^{-4}$ M); 1,0 (Al $10^{-4}$ M); 0,82 KJ (Al $10^{-5}$ M) and 0,80 KJ (A $10^{-4}$ M).

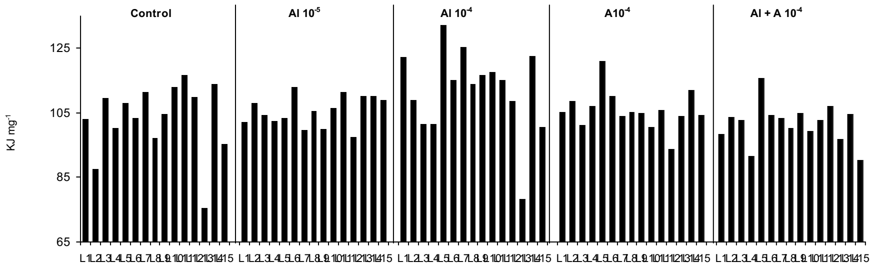


Figure 2 - The free energy spent for 1 mg of dry subastance biosyntheis; Control - non-treatment; Al - Alachlor; A- Atrazine; Al+A - Alachlor + Atrazine

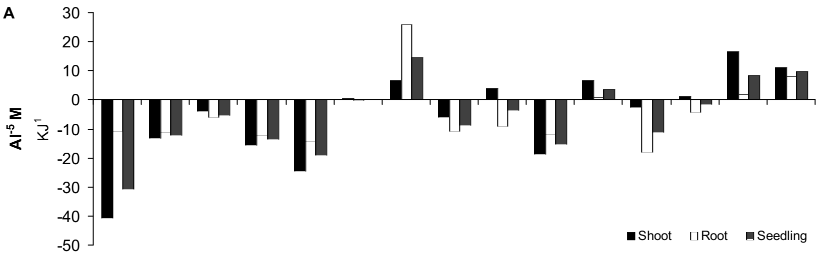


Figure 3 - The differential free energy represented as difference between control (non-treatment) and herbicide treatment; Al - Alachlor; A- Atrazine; Al+A - Alachlor + Atrazine

It could be recognized that less diluted alachlor (AL$10^{-4}$ M) gives highest values of: $\circ\Delta_{treat}G$ G (-83,6 KJ) and $m_{sh}\Delta_r GA$ (45,4 KJ); then, more diluted (Al $10^{-5}$ M) converges the values towered 0, lowering at the same time the both: $m_{sh}\Delta_r GA$ (19,3 KJ) and $m_{sh}\Delta_r GA$ (0,82 KJ). Despite, the reactions of genotypes to atrazine, neither concentrated (A $10^{-4}$ M), nor diluted (Al+A $10^{-4}$ M) were not resulted uniformly (Fig. 1; a, b; Fig.2; a, b, c, d).

The energy spend in substance biosynthesis has individual, genotypic characteristics in the range of 75,6 (L13) to 116,8 KJ $mg^{-1}$ (L11) in non-treatment, as it was expected. Also, it could be predicted that Al $10^{-4}$ M treatment was elevated energy of biosynthesis in range from 78,4 (L13) to 132,2 KJ $mg^{-1}$ (L5). Meanwhile, the other treatments leaded to more equalized energy; so, it was 97,5 - 113,1 KJ $mg^{-1}$ for Al $10^{-5}$ M in L12 and L6; 93,8 – 120,9 KJ $mg^{-1}$ for A $10^{-4}$ M in L12 and L6; then, 91,7 – 115,7 KJ $mg^{-1}$for Al+A $10^{-4}$ M in L4 and L5 (Fig.3). Nearby, the treatments: Al $10^{-5}$ M, A $10^{-4}$ M and Al+A $10^{-4}$ M influenced the reducing energy per mg of biosynthate in a few number of inbreds such as L3, L7, L10, L11 and L14 compared to non-treatment (Fig.3).

The energy of biosynthesis depends on convergence of endo- and egzo-thermal reactions and more rapid partial side related to the main reactions (Davies, 1961; Mayers, 1989). The energy, spent in biosynthesis of 1mg dry substance in non-treatment was amounted from 75.6 to 16.8 KJ mg-1; the applied herbicide treatments assembled the group of genotypes (L3, L7, L10, L11, L12 and L14) with lowered necessity of this energy. Hence, in thermodynamical sense those inbreds could be admired as tolerant.

## CONCLUSION

The universal, natural parameter of thermodynamics, free, i.e. potential energy could be applied to plant systems in two manners. Those systems were observed as physical bodies, maintained by continual running of biochemical reactions, so the free energy changes induced by treatment were examined through constant of biosynthesis. Additionally, the applied treatment changed input of free energy by water volume alteration, what rearranges the examined genotypes (whole seedlings and its fragments: root and shoot) as tolerant or sensitive. Meanwhile, if the outset is reactional constant of biosynthesis yielding, genotypes were arranged into groups, based on energy requirement for synthesis of 1 mg of its substance. The standardised, controlled conditions of essay (ISTA Rules) enable the comparability of cumulative results in the aim of quantitative, gravimetrically based set up of analytical standards, without any application of chemical reagents.

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Recieved Aprile 7, 2005
Accepted December 30, 2006

**UPOTREBA STANDARDNIH SI JEDINICA I TERMODINAMIKE PRI ODREĐIVANJU UTICAJA HERBICIDA NA SAMOOPLODNE LINIJE KUKURUZA**
**2. Status slobodne energije**

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I z v o d

Primena dva herbicida i njihove kombinacje kod klijanaca 15 samooplodnih linija kukuruza uticala je na iymenjenu apsorpciju vode. Zapremina vode (ml) je varirala između 11 i 100% u izdanku i 23 i 275% u korenu, što utiče na promenu sloboden energije (G) od 254.6 do -151.6 KJ u izdanku i od -248.0 to -172.3 KJ u korenu. Razlika u slobodnoj energiji između netretiranih i tretiranih biljaka ($\circ\Delta_{treat}G$) iznosila je u izdanku od 11.1 do 90.2 KJ i u korenu od 16.8 do 75.7 KJ. Takođe manja magnituda $\circ\Delta_{treat}G$ amplitude između korena i izdanka može da ukaže na tolerantnost genotipova prema primenjenom herbicidu. Energija, utrošena za sintezu 1 mg suve supstance je iznosila 75.6 do 16.8 KJ mg-1. Primenjeni herbicidi su istakli grupu genotipova koja je imala manje energetske potrebe za procese biosinteze, što predstavlja pozitivnu tendenciju, sa termodinamičke tačke gledišta.

Primljeno 7. aprila 2005.
Odobreno 30. decembra 2006.