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Original scientific paper

THE APPLICATION OF STANDARD SI UNITS AND TERMODYNAMICS IN DETERMINATION OF HERBICIDES' INFLUENCE TO MAIZE INBREDS
3. The low temperature effect

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The five maize inbred lines were subjected to concomitant treatments of herbicides: EPTC, alachlor and S-metolachlor and extended low temperature (22^{o}C during 6 days and 10^{o}C during 15 days). The fresh and dry substance and water content were determined. The calculated parameters fluctuated: concentration between 72 and 219 g L^{-1}; then, pseudo-specific density between 47 and 51 µmol mg $^{-1}$. The free energy, spend for 1 mg of dry substance biosynthesis was higher in low temperature conditions and in alachlor treatment at both temperature conditions. Meanwhile, the changes of differential free energy, enthalpy and entropy, as thermodynamical parameters, followed the changes of fresh, dry substance and water volume in root and shoot of examined inbreds, therefore the inbreds with lower values of differential free

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energy, enthalpy and entropy had the higher tolerance to herbicide treatments.

Key words: alachlor, atrazine, biosynthesis

INTRODUCTION

The application of herbicides is required in contemporary cropping, what could be a problem in maize inbreds production, from the reason of their sensitiveness. There are present many formulations on the market (JANJIĆ, 2004), what requests their testing in certain inbred crop. Very often, the young, germinating plants could be in the field conditions exposed to a concomitant stress of herbicides (metabolically active substances) and extended low temperature (ZARIĆ *et al* 1983; STEFANOVIC and ZARIĆ, 1991; STEFANOVIĆ *et al,* 1996; SREDOJEVIĆ *et al*, 2005). This essay was undertaken to test the tolerance or susceptibility of maize inbreds to the herbicides in artificial, controlled conditions of germinating room, simulating environmental extended (in days) low temperature (in degrees) in early spring.

MATERIAL AND MEHODS

The essay with 5 maize older generation inbreds, which were subjected to pre-emergence treatment with herbicides: EPTC $2.5x10^{-4}$ M (S-ethyl dipropil thiocarbamte), alachlor (Al) 10^{-4} M (2-chlor-2',6'-diethyl-N-metoximethylac-etanilid) and S-metolachlor (M) $3x10^{-5}$ M ((S)-2-chlor-N-(2-ethyl-6-methylfenyl-N-(2-metxy-methylethyl)-acetamide) in conditions of extended low temperature. Those conditions mean: 6 days of 22°C temperature and 15 days of 10°C (T_1); with photoperiod of 12 h, relative humidity of 75 % and illumination of 1250 lux. Moreover, essay comprehends the optimal growing conditions of 22°C, during period of 20 days (T_2). The planting (3x50 seeds) was done in plastic boxes, with 3.7 kg of sterilised soil in each and water added to 60% of water-retaining capacity; in germinating chamber. After observed period of each temperature treatment, there was measured weight: fresh and dry (60°C; until constant weight) of root and shoot. All collected parameters were expressed per plant or plant sequence (root and shoot).

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

$$\Sigma Bs = rtBs + shtBs \qquad [1]$$
$$k_{Bs} = [rtBs] \times [shtBs] / [\Sigma Bs] \qquad [2]$$
$$G_{Bs} = -RT\, lnk\Sigma_{Bs} \qquad [3]$$

where is R universal gas constant, T is sum of average daily temperatures in Kelvin, ΣBs sum of root and shoot DW.

Gravimetrically determined fresh (FW) and dry (DW) weights were used to calculate water content as volume (Vw), while g = ml:

$$Vw\ (cW) = FW – DW \qquad [4]$$

The two forms of weights (F and D) and water content was used to calculate the concentration of dry substance (conc, g L^{-1}) and pseudo specific density (d^{-1}, *d*, µmol H_2O mg $^{-1}$ FW).

Additionally, based on the water volume there were calculated the thermodynamical parameters of differential free energy (ΔG), differential enthalpy (ΔH) and differential entropy (ΔS):

$$\Delta G = R\,(T_2 - T_1)\ \ln\,(cW_1 / cW_2) \qquad [5]$$

$$\Delta H = R\ T_1T_2 / T_2\text{-}T_1\ \ln\,(cW_1 / cW_2) \qquad [6]$$

$$\Delta S = (\Delta H - \Delta G) / \Delta T \qquad [7]$$

where is R universal gas constant, T is sum of average daily temperatures in Kelvin (T_1 – for low temperature conditions and T_2 – for optimal temperature conditions), cW_1 is water content in low temperature conditions and cW_2 is water content in optimal temperature conditions (VERTUCCI *et al*, 1984; SUN, 2002; SREDOJEVIC *et al*, 2005).

RESULTS AND DISCUSSION

Table 1. - The influence of herbicides treatment on fresh, dry weight and water content at diferent temperature conditions

	Fresh weight (mg)															
	T1								T2							
	EPTC		Alachlor		Metolachlor		Control		EPTC		Alachlor		Metolachlor		Control	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
L1	267	103	231	80	274	125	293	111	1583	768	1348	556	1506	663	1955	856
L2	376	228	290	208	396	257	439	297	2033	870	1028	585	1541	625	2121	796
L3	196	100	196	80	245	89	245	101	2330	2012	2085	1172	2115	1415	2425	1555
L4	153	69	188	86	241	92	193	76	2350	1437	1690	975	1732	940	2297	1615
L5	241	113	271	95	241	87	231	96	1709	413	1351	340	1367	378	1636	338
	Dry weight (mg)															
	T1								T2							
	EPTC		Alachlor		Metolachlor		Control		EPTC		Alachlor		Metolachlor		Control	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
L1	27	12	24	9	42	14	32	13	141	96	124	67	141	76	159	96
L2	37	22	33	26	38	34	42	35	154	106	121	89	149	112	202	137
L3	18	11	18	9	21	11	20	9	195	198	168	95	165	127	180	128
L4	15	7	24	10	21	11	19	8	182	125	130	73	130	70	154	128
L5	22	12	25	15	22	11	23	15	128	59	126	63	128	66	126	54
	Water content (mg)															
	T1								T2							
	EPTC		Alachlor		Metolachlor		Control		EPTC		Alachlor		Metolachlor		Control	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
L1	240	91	207	71	232	111	262	98	1442	672	1224	489	1366	587	1796	761
L2	338	206	256	182	358	223	397	262	1879	764	908	495	1392	512	1919	659
L3	179	89	178	71	224	78	226	92	2135	1814	1917	1077	1950	1288	2245	1427
L4	138	62	164	76	219	81	175	67	2168	1312	1560	902	1602	870	2143	1487
L5	219	100	246	80	219	76	208	81	1581	354	1226	277	1239	312	1510	283

The extended low temperature, applied after sprouting, reduced the fresh and dry substance and water content in all inbreds (Tab. 1, 2 and 3). The observed parameters at low temperature (T_1) had in L3 and L4 root values below and in their shoots above 90% of optimal temperature conditions (T_2). Meanwhile, in L2 root, fresh weight and water volume was below 60% and dry weight was below 70%; in shoot values of all three parameters were above or below 80%, what was similar in L5.

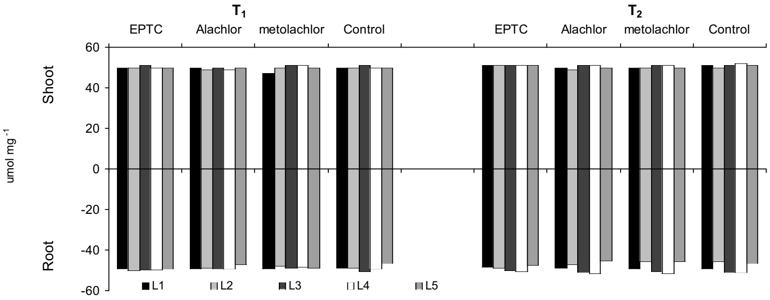

Figure 1. - The influence of herbicides treatment on pseudo-specific density of shoot and root at diferent temperature conditions

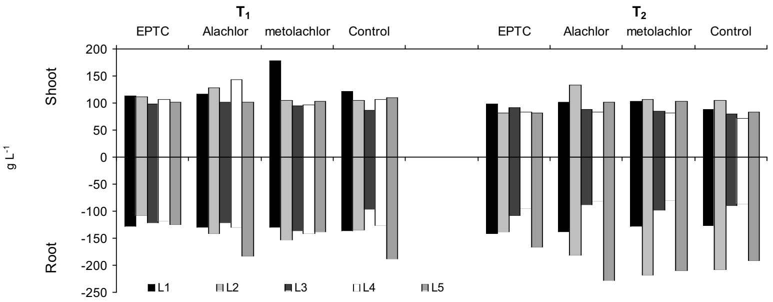

Figure 2. - The influence of herbicides treatment on Concentration of shoot and root at diferent temperature conditions

The concomitant action of herbicides and temperature segregated genotypes, as well as the seedling parts: the fresh weight of root and shoot was strongly reduced in Alachlor at both temperatures (particularly at T_2), the reduction was expressed in lesser degree at T_1 than at T_2 in Metolachlor treatment, the lowest digression level was expressed in EPTC treatment, with positive effect of T_2. It is important to underline that all applied herbicides elevated water accumulation in shoot, with only one exception in L2 (Al treatment at both temperatures). The Metolachlor decreased water amassing in root of all inbreds at both temperatures, while the Alachlor was reduced it, also, but at T_1.

The physical parameter of pseudo-specific density (*d*, μmol mg^{-1}) in control, at both temperatures (Fig. 1) was between 46.51 (in root) and 50.52 μmol mg^{-1} (in shoot). This parameter was little bit lower in root and shoot, at both temperatures, while the shoots' values oscillated in lesser extent. It could be noticed, that treatments in shoot, at both temperatures, as well as, in root at T_1, equalized density in all inbreds. This was discerned in essay with 15 maize genotypes, submitted to a herbicides in different dilution level (SREDOJEVIĆ *et al.*, 2006 a).

According to expected, T_1 elevated concentration up to 150% in L4 root and shoot, but only in the root of L2 it was lowered at 65% of T_2. The herbicide treatments rearrange the concentration in root and shoot: at T_1 from 66 to 148% and at T_2 from 66 to 127% (Fig. 2) (SREDOJEVIĆ *et al.*, 2005 b,c).

Thermodynamics

The energy of dry substance biosynthesis, in chemical sense (through the relation of inputted and outputted reactional components) is a part of inner status of dynamic integrity, as plant system is, in circumstances of time and temperature. The result of such simultaneous and/or concomitant processes of biosynthesis is the plant, as physical body. During growth and development the plant change the volume in function of time [f(t)]; although, the volume is changed in function of temperature [f(T)], in conditions of unchangeable other environmental parameters (light, humidity, medium). Hence, each inbred will change the volume (regarding to genetic dependency) with temperature per time [f(dV/dT) / t]. The usage of thermodynamics in analytical purpose requires the necessity to make application of reactional constant (Equ. 2) and extrapolation of water weight into water volume (g = ml). The change of water density with temperature could be approximated as neglected, compared to change of volume in plant system by temperature and time.

Free energy of biosynthesis

The free energy of biosynthesis, as it was expected, was higher at T_1 (147-191 KJ mg^{-1}), then at T_2 (93 – 122 KJ mg^{-1}), with widely ranged: 44 and 29 KJ mg^{-1}, respectively. The combined treatment of herbicide and temperature elevated energy from 150 KJ mg^{-1} (L2, Metolachlor) to 198 KJ mg^{-1} (L4, EPTC) with range at T_1 of 34 KJ mg^{-1} (Alachlor, Metolachlor). The lowering of energy of biosynthesis at T_2 was expected, also: from 86 KJ mg^{-1} in L3, EPTC to 119 KJ mg^{-1} in L5, EPTC. The range lowered to 14 KJ mg^{-1} (Alachlor) and 19 KJ mg^{-1} (Metolachlor) and increased to 33 KJ mg^{-1} (EPTC) (Fig. 3). In the essay with 15 maize inbreds and two herbicides in two diluted forms, there were noticed that herbicide treatment narrows the range of biosynthesis energy, compared to control (SREDOJEVIĆ *et al*., 2006 b).

Differential free energy

The differential free energy (ΔG) presents the possibility of system to perform its function, according to environmental conditions. If the value inclines to 0 ($\Delta G \rightarrow 0$), the system is more ready to perform work in both temperature conditions. Hence, ΔG has the lowest value in L5 root (-5.23 KJ, Fig. 4) in control and treatments: (-5.53 KJ) in EPTC to (-4.96 KJ) in Metolachlor; then the shoot values of -6.31 to -6.02 KJ in herbicide treatments, L5 was closed with L1 and L2. In early stage of growth L3 and L4, which ΔG values in root, between -6.52 and -

5.87 KJ, were unfit, almost as their shoots. The closed was occurred in the essay with application of merchant pesticides to family triplet (mother and father inbreds and their progeny), the free energy distributed inside the seedlings, signified certain genotypes as tolerant or susceptible (SREDOJEVIĆ *et al.*, 2005a).

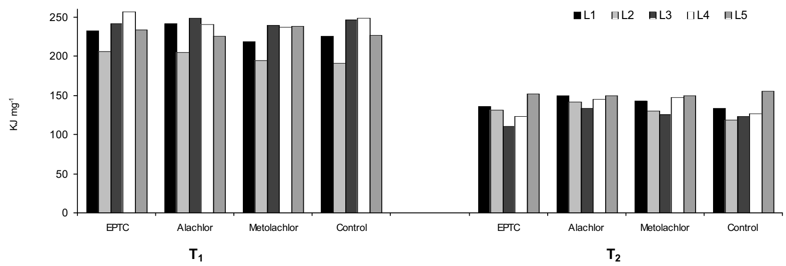

Figure 3. - The influence of herbicides treatment on the free energy spent for 1 mg of dry subastance biosyntheis at diferent temperature conditions

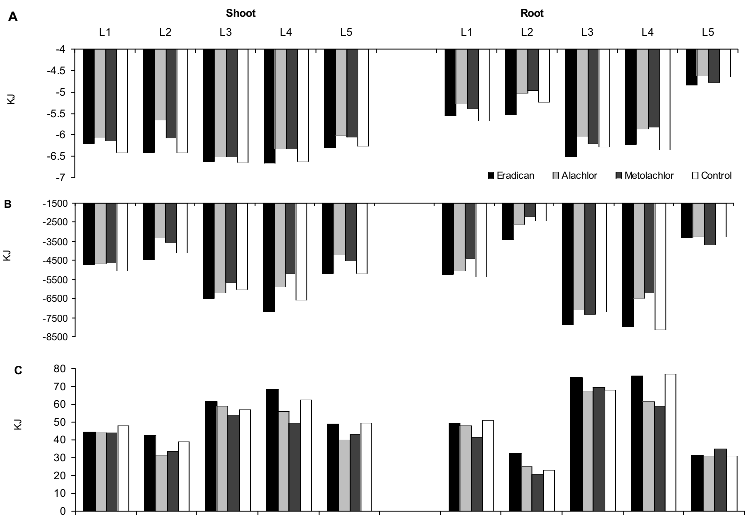

Figure 4. - The influence of herbicides treatment at the differential free energy (A), enthalpy (B) and entropy (C) at diferent temperature conditions

Differential enthalpy

The differential enthalpy (ΔH), a thermal capacity of system was the best in L2, i.e. in control better in root (-2416 KJ) than in shoot (-4126 KJ), since this was the lowest difference between values at T_1 and T_2 (Fig. 4). Additionally, L5 could be considered close to L2, according to ΔH. The influence of herbicides' treatments was more expressed to the shoots of L2 and L5. On the other side, L3 and L4 had the ΔH distant of 0, in root (-7170 KJ, L3 in control and -7893 KJ L3 EPTC; -8109 KJ; L4 in control and -7989 KJ, L4 in EPTC), also in shoot (-6016

KJ, L3 in control and -6499KJ, L3 in EPTC; -6567 KJ, L4 in control and -7216 KJ L4 in EPTC). Hence, L2 and L5 could be separated as tolerant to extended low temperature, i.e. with the best thermal capacity between investigated inbreds, but L3 and L4 as very susceptible.

Differential entropy

The differential entropy (ΔS), as capacity of system to take out doubled stress: thermal and chemical, underlined again the L2 and L5 as tolerant, more in root, than in shoot, according to the lowest values of ΔS among inbreds (Fig. 4). Despite of the lower ΔS values of the L2 in root (20.70 KJ in Metolachlor) than in L5 root (30.81 KJ in Alachlor), the range was broadened from 11.92 KJ (L2) to 4.22 KJ (L5).

There is qualitative relation in capacity of potential energy (ΔG), then thermal capacity (ΔH) and the capacity to take over the stress (ΔS) to T_1 and T_2 yield's the percentage difference in examined inbreds (Tab. 1; Tab. 4). Namely, the tolerant inbreds L2 and L5 with ΔG range -4.64 and -5.23 KJ in root were related to the range of dry weight percentage of T_1 to T_2 (27 and 26%). The inbreds L2 and L5 had the better hold on of biosynthesis (dry weight) at T_1 in root, than in shoot, with their relation of 1.24 and 1.50. The root of L3 and L4 was below 10 % at T_1, compared to T_2. Further, the ranges of ΔH (Fig. 4) and water content percentage (Tab. 1) at T_1 to T_2 in the root were the supreme for L2 and L5, but the poorer in shoot. Again, L3 and L4 were with the deprived thermal capacity and water content percentage of root and shoot, with better values of shoot. Thus, as third, ?S and fresh weight percentage were in accordance, like previous: L2 and L5 were underlined as most effective and L3 and L4 as the poorest capacity.

CONCLUSION

The influence of different herbicides on five maize inbreds in conditions of extended low temperature was examined: the values of fresh and dry weight and water accumulation displayed that changes induced by low temperature (T_1) were more highlighted than herbicides' treatments, what was confirmed by changes of differential free energy, enthalpy and entropy (ΔG, ΔH, ΔS). The herbicides' treatments rearrange inbreds into groups: which reacts at whole plant level (L3 and L4), root level (L1) and tolerant inbreds, particularly on root level (L2 and L5). Moreover, the herbicides were rearranged into groups, with approved influence on root or shoot. It is important to underline that the EPTC was favourable, but alachlor unfavourable at the shoot and root level of the most inbreds. Generally, the inbreds, with better low temperature withstanding, i.e. improved thermodynamical stability, displayed the tolerance to the herbicide treatment, too.

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UPOTREBA STANDARDNIH SI JEDINICA I TERMODINAMIKE PRI ODREĐIVANJU UTICAJA HERBICIDA NA SAMOOPLODNE LINIJE KUKURUZA
3. Efekat niske temperature

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I z v o d

Pet samooplodnih linija kukurza bilo je izloženo kombiniovanom tretmanu herbicida: EPTC, alachlora i S-metolachlora u kombinaciji sa produženom niskom temperaturom (22^{o}C tokom 6 dana i 10^{o}C tokom 15 dana). Određivan je sadržaj sveže i suve supstence i sadržaj vode. Obračunski parametari su varirali: izmedju 72 i 219 g L^{-1}, zatim, pseudo-specific density izmedju 47 I 51 μmol mg^{-1}. Slobodna energija, utrošena za biosintezu 1 mg suve supstance imala je niže vrednosti pri nižoj temperature i u tretmanu sa alahlorom, u oba temperaturna režima. Takođe, promene diferencijalne slobodne energije, entalpije i entropije, kao termodinamičkih parametara, pratile su promene sveže i suve supstance i sadržaj vode u korenu i izdanku svih ispitivanih linija. Sa druge strane, linije koje su imale niže vrednosti diferencijalne slobodne energije, entalpije i entropije, imale su i veću tolerantnost prema upotrebljenim herbicidima.

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