

IMPROVED METHOD TO ESTIMATE SACCHARASIC POTENTIAL AS AN INDICATOR FOR SOIL FERTILITY

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ABSTRACT

This paper aims at discussing the introduction of a small quantity of aluminium ions to prevent the interference of released fructose + glucose combination to the reaction mixture during saccharasic process obtained by Hofmann and Seegerer's method (1950) and phosphate ions released in the same reaction mixture by spontaneous phosphatasic processes as ^atefanic et al. (1965) and Irimescu and ^atefanic (1998) proved. In this way, phosphate ions are adsorbed by aluminium hydroxide physico-chemically and the real saccharase potential of soil can be obtained correctly.

Key words: epistatic deviation, maize, observed yield, predicted yield.

INTRODUCTION

Saccharasic activity (beta-h-fructosidasic) was proved by Willstätten et al. (1925) using some yeast cultures.

Soon after that the saccharasic activity was tested and studied in vegetal extracts and organs or animal tissues as well as with purified enzymatic products.

The first demonstration of saccharasic activity in soil and its recommendation as an indicator for soil fertility was done by Hofmann and Seegerer (1950), using as an enzymatic source the soil itself, biotically inactivated with toluen, adding saccharose as a specific enzymatic substratum and measuring the enzymatic activity by the quantity of monoses (glucose and fructose) found out at the end of enzymatic reaction period, using Fehling reagent.

Kiss (1958) proved the saccharasic activity in soil samples treated with toluen and saccharose, quantifying its level by polarimetry, as difference of rotation $\Delta\alpha^\circ$ measured before and after saccharasic reaction.

^atefanic (1972) suggested the spectrophotometric method for testing the quantity of monoses resulting from saccharasic activity.

The necessity to test the level of saccharasic activity, using the soil itself instead of a soil extract in enzymatic mixture, was explained by

Kiss and Balint (1959) considering that saccharase does not accumulate in soil in the form released from cells and then adsorbed on soil particles, but it exists in desintegrated cells, in the dead ones (but not desintegrated) and in the living ones (but nonprolific), all these cells being in the adsorbtion state. For this reason, all pedo-enzymatic activities are tested, having the soil as an unique source of enzymes in the reaction mixture.

In case of saccharasic activity analysis, corresponding to Hofmann and Seegerer's method (1950) other enzymatic processes also take place at the same time but less extensive (because they do not have large quantities of enzyme substrate or these processes take place with a reduced speed). On the contrary, because phosphatasic activities and phosphorus compounds are in large quantities, they represent an exception in soil as Pavlovschi and Ionescu (1940) affirmed and ^atefanic et al. (1965) proved experimentally. This method was accepted by Speir and Rose (1978) and transformed into an efficient testing method for the total phosphatasic potential of soil by Irimescu and ^atefanic (1998).

Pavlovschi and Ionescu (1940) proved the combination of ions of o-phosphate with manite in soil and ^atefanic (1993) and Irimescu and ^atefanic (1998) the combination with glucose.

Correlating enzymatic activities (of saccharase and phosphatases) in the same reaction mixture of the soil sample, ^atefanic and Irimescu Orzan formulated the hypothesis that o-phosphoric acid (phosphatasic product) and glucose + fructose (saccharasic products) could have an interaction, diminishing apparently the original saccharasic potential of soil.

Considering the hypothesis shown above we suggested an improved method to determine the saccharasic potential for preventing the recombination glucose + fructose with o-phosphoric acid (released during simultaneous

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phosphatasic activities) in the same reaction mixture of soil.

MATERIALS AND METHODS

In order to hinder the reaction between phosphoric acid and glucose – fructose, it is necessary to block o-phosphate ions (released during simultaneous enzymatic reactions or pre-existing in soil) with aluminium ions. A small quantity of potash alumen is introduced into the mixture of enzymatic reaction. Aluminium hydroxide is able to make insoluble the o-phosphate ions by a physical-chemical adsorption. The quantity of $\text{AlKSO}_4 \cdot 12 \text{H}_2\text{O}$ depends on the level of P ions released enzymatically into the reaction mixture. At least 15 mg of potash alumen are necessary for 1 mg P. For this correction it is necessary to test with anticipation the total phosphatasic potential of soil.

Two types of soil: cambic chernozem and reddish-brown soil were used to control the improvement of saccharasic method.

The analysis development:

100 ml plastic flasks with 5 g of fresh soil, passed through a 2.5 mm sieve were divided into two groups for each type of soil. In each group, some flasks have a reaction mixture according to the principle of the conventional method (Hofmann and Seegerer, 1950): 10 ml solution of sodium azide (0.015%) containing 1 g saccharose; other flasks have the reaction mixture improved: 10 ml solution of sodium azide (0.015%) containing 1 g saccharose + 20 mg potash alumen ($\text{AlK}(\text{SO}_4 \cdot 12 \text{H}_2\text{O})$). The plastic flasks were tightly closed and kept for 24 hours in thermostat at 28°C, and after that, they were supplemented with 40 ml of watery solution of 0.3% potash alumen (for a better

filtration) stirred for 15 minutes and then filtered.

One ml from this filtrated product was treated with 3 ml of 3.5 dinitrosalicilic acid in a tube and then kept for 5 minutes in boiling water, then cooled in cold water and 25 ml of distilled water was added. The content of the tube was homogenized and measured spectrophotometrically at an extinction of 540 nm. The result was calculated as glucose mg/100 g soil d.w.

The reactive 3.5 dinitrosalicilic acid (Sumner and Howell, 1935) has the following composition:

I. 10 g fenol cryst. + 22 ml NaOH solution 10% + 10 g NaHSO_3 (we used with good results dry Na_2SO_3) + distilled water to fill up to 100 ml.

II. 880 ml of 3.5 dinitrosalicilic acid (1% solution in water) + 300 ml NaOH sol. 4.5% + 255 g $\text{KN}_4\text{H}_4\text{O}_6 \cdot \text{H}_2\text{O}$.

The second solution is mixed with 69 ml from the first solution. The reactive is immediately ready and can be kept for a long time in full bottle, tightly closed with a cork.

RESULTS AND DISCUSSIONS

We notice from tables 1 and 2 the same good influence of aluminium ions regarding the quantity of glucose released into the soil by saccharasic activity. The factorial analysis shows that within the framework of factor A, in the reaction mixture without aluminium ions (a_1), the average of all variants is 3341 mg (for chernozem), 1530 mg (for brown-reddish soil), 4312 mg (10% larger for chernozem) and 1681 mg (29% larger for brown-reddish soil).

Table 1. Influence of aluminum ions, in reaction mixture, on the level of saccharasic activity in a reddish-brown soil fertilized with compost with different quantities (glucose+fructose mg/100 g soil d.w./24 hours)

A- Factor	B Factor: Compost (t/ha)								Average (A)	%
	b ₁ -0	%	b ₂ -30	%	b ₃ -50	%	b ₄ -70	%		
a ₁ -without Al ³⁺	b 3287	100	b 4028	100	b 2579	100	b 3472	100	b 3341	100
a ₂ -with Al ³⁺	a 4228	129	a 5019	125	a 3472	135	a 4528	130	a 4312	129
Average (B)	3757 c		4523 a		3025 d		4000 b			
LSD (P)	A	B	AB	BA						
5%	291	101	302	144*						
1%	673*	142	628*	201						
0.1%	2143	201*	1799	284						

Table 2. Influence of aluminum ions, in reaction mixture, on the level of saccharasic activity in a chernozem soil fertilized chemicaly or manured with farmyard compost (glucose+fructose-mg/100 g soil d.w./24 hours)

A Factor	B Factor: Fertilized with:										Average (A)	
	b ₁ -0	%	b ₂ -N ₃₂ P ₃₂	%	b ₃ -N ₉₄ P ₉₆	%	b ₄ -N ₁₂₈ P ₁₂₈	%	b ₅ -compost	%	b	%
a ₁ -without Al ³⁺	b 1564	100	b 1496	100	b 1459	100	b 1401	100	b 1732	100	b 1530	100
a ₂ - with Al ³⁺	a 1686	108	a 1581	106	a 1684	115	a 1589	113	a 1864	108	a 1681	110
Average (B)	1625 b		1538 d		1571 c		1495 e		1798 a			
LSD P	A	B	AB	BA								
5%	7	14	19	20*								
1%	17	20	28	28								
0.1%	54*	27*	45*	39								

Within the frame of factor B, in both tables, a comparison between each b₁-b₄ (a₂) shows that the quantities of glucose are larger in case of variant a₂. The fact that the differences are not the same (in percentage) proves that there is not a systematic error, there is the effect of cancelling the aluminium ions in the mixture of enzymatic reaction.

CONCLUSIONS

There is a chemical combination between the ions o-phosphate and glucose + fructose (all released in the same reaction mixture during saccharasic activities and spontaneous phosphatasic activities).

This chemical combination diminishes the quantity of monoses (glucose + fructose) released into the reaction mixture from saccharose, giving a false image of soil saccharasic potential.

By introducing aluminium ions in the reaction mixture for saccharasic activity, it is possible to prevent the combination of monoses with phosphate ions by physical-chemical adsorbtion of phosphate ions on aluminium hydroxide. In

this way, the Hofmann and Seegerer method could be improved

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A- Factor	B – Factor – COMPOST (t/ha)								Average (A)	
	b1-0	%	b2-0	%	b3-0	%	b4-0	%		%
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a2- with Al ³⁺	a 4228	129	a 5019	125	a 3472	135	a 4528	130	a 4312	129
Average (B)	3757 c		4523 a		3025 d		4000 b			
LD P	5%	1%	0,1%							
A	291	673*	2143							
B	101	142	201*							
AB	302	628*	1799							
BA	144*	201	284							

Table 2. Influence of aluminum ions, in reaction mixture, on the level of saccharasic activity in a chernozem mineral fertilized or manured with farmyard compost (glucose+fructose-mg/100 g soil dw/24 hours)

A- Factor	B – Factor – COMPOST (t/ha)										Average (A)	
	b1-0	%	b2-N ₃₂ P ₃₂	%	b3-N ₉₄ P ₉₆	%	b4-N ₁₂₈ P ₁₂₈	%	b5 com-post	%		%
a1–without Al ³⁺	b 1564	100	b 1496	100	b 1459	100	b 1401	100	b 1732	100	b 1530	100
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