INFLUENCE OF NITROGEN AND PHOSPHORUS RATES ON ACID PHOSPHATASE ACTIVITY

Gabriel R. Zglimbea, Vasile. Mihăilă

ABSTRACT

Maize plants from long-term experiments, cultivated under different quantities of fertilizers: 5 rates of nitrogen (0, 50, 100, 150, 200 kg N/ha) and 3 rates of phosphorus (0, 40, 80 kg P_2O_5/ha) were used in this study. Leaves from each variant were harvested at three different developmental stages (6-8 leaves, tasseling and milk-dough) in three replications and analyzed for total N content, P content and acid phosphatase activity. The results showed that acid phosphatase activity increased along with nitrogen rate increasing at low levels of phosphorus, and a good correlation between acid phosphatase activity and yield was registered for small rates of fertilizers.

Key words: Maize, long-term experiments, nitrogen and phosphorus fertilization, and phosphatase activity.

INTRODUCTION

One of the most important aspect of agricultural research consists of field experiments, both-short and long-term.

Army and Kemper (1991) showed that agricultural research, as well as those from other topics, are based on short-term sudies, but a "sustainable agriculture" needs long-term experiments and laboratories able to determine the complex interactions soil x plant x climate x management.

Long-term experiments reprezent a valuable source for agronomic science advance (Frye and Thomas, 1991).

Traditionally, long-term experiments were focused towards the study of soil agrochemical properties changes (Mihăilă and Hera, 1994) and their influence on yield (Hera and al., 1986), and less towards their influence on plant physiological processes.

It is well known that phosphorus is an essential element for plants. It is a constituent of many organic substances which play an important role in plant biolgy: nucleic acids, phospholipids (different types of cellular and subcellular membranes), electrogenic compounds (AMP, ADP, ATP), coenzymes (Zamfirescu, 1977). From this point of view one can say that phosphorus takes part to all important physiological processes (photosynthesis, respiration, carbohydrates synthesis protein synthesis, etc.).

Plants take their phosphorus mainly as ions, which are included to a large extend in different organic compounds even in the root.

Phosphatases catalyze the hydrolization of various orthophosphoric acid monoesters. Phosphoric esters of some primary and secondary alcohols, phosphoric esters of some cyclic alcohols, of some phenols, amines (phosphorus amides and creatin phosphate), orthophosphoric esters of saccharides and mononucleotides can act as substrates. Depending on the optimum pH, phosphatases can be divided as follows: alkaline – orthophosphoric – monoester phosphohydrolaze (alkaline optimum pH) EC 3.1.3.1 and acid – orthophosphoric – monoester phosphohydrolaze (acid optimum pH) EC 3.1.3.2.

Acid phosphatase hydrolyzes the phosphate from a large variety of phosphomonoesters and phosphoproteins. Various biological sources have also acid phosphatases which catalyze transphosphorilation reactions (Dumitru and Iordăchescu, 1981).

The general reaction catalyzed by phosphatases is: orthophosphoric monoester + $H_2O \xrightarrow{phosphatase}$ alcohol + orthophosphate.

Acid phosphatase is one of the most studied plant enzymes in very different physiological processes and conditions: nutritional deficiencies, (Kummerova and Buresova, 1990), pollution (Malhotra and Khan, 1980), sexual differentiation (Jaiswal et al., 1984) resistance to unfavourable environment conditions (Hagima, 1984) etc.

The purpose of this study was to estimate the influence of fertilizer rates on acid phosphatase activity in maize.

MATERIALS AND METHODS

Plant material:

Plant material was harvested from a long-term experiment with fertilizers, conducted in the autumn of 1966 on Fundulea cambic chernozem. Fundulea 376, a semilate maize hybrid was grown in this experiment. Physical and chemical characteristics of this soil are listed in Table 1.

Table 1. Physical and chemical properties of Fundulea cambic chernozem

Sand	-
Fine sand	31.2 - 34.6
pH	6.0 - 6.4
SB (m.e./100 g sol)	21.5 - 25.4
Ah (m.e./100 g sol)	5.1 - 6.4
T (m.e./100 g sol	27.8 - 31.5
V (%)	77 - 83
Humus (%)	3.0 - 3.2
N (%)	0.16 - 0.18
P_{AL}(p.p.m.)	23 - 49
K_{AL} (p.p.m.)	192 - 157

A split plot design was used, with phosphorus rates (0, 40, 80, 120 and 160 kg P_2O_5/ha) as main plots and five nitrogen rates (0 50, 100, 150, 200 kg/N/ha) splited into phosphorus variants. Plant material only from 0, 40 and 80 kg P_2O_5/ha was used in this experiment, in three replicates. Samples were harvested on 13.06.1995 (6-8 leaves stage); 22.07.1995 (tasseling stage) and 04.08.1985 (milk-dough stage). At first two sampling dates, the last completely developed leaf was harvested and at the third date the leaf opposite to ear from three plants, making an average sample. One gram of fresh substance was used to determine acid phosphatase activity, and the rest was dried at 105°C, prior to determine nitrogen and phosphorus content.

Enzyme activity assay:

Acid phosphatase activity was measured using β-glycerophosphate as substrate, accordingly to the procedure described by Iordăchescu and Dumitru (1980): 1g fresh plant tissue was mixed in a mortar with 10 ml 0.3% NaCl solution, then extracted for one hour at 4°C and centrifuged at 6,000 rpm and 4°C for 15 min. The reaction mixture consisting of 1 ml β-glycerophosphate, 3.5 ml 0.1 M acetic acid - sodium acetate buffer pH 5.5 and 0.5 ml supernatant was incubated for 15 min. at 37°C. The reaction was stopped with 2 ml 10% trichloroacetic acid solution. Precipitated proteins were filtered, and the released phosphorus in the filtrate was colorimetrically measured. For each sample a control was made by adding trichloroacetic acid solution before enzyme solution.

Total nitrogen and phosphorus assay:

Total nitrogen content was measured by Kjeldahl method.

Phosphorus content was measured spectrophotometrically at 420 nm, by ammonium vanado-molibdate method.

RESULTS AND DISCUSSIONS

The total nitrogen content in leaves increased generally along with nitrogen rate increasing up to the highest rates. It is also obvious the decreasing of nitrogen content along with the plant development (Figure 1).

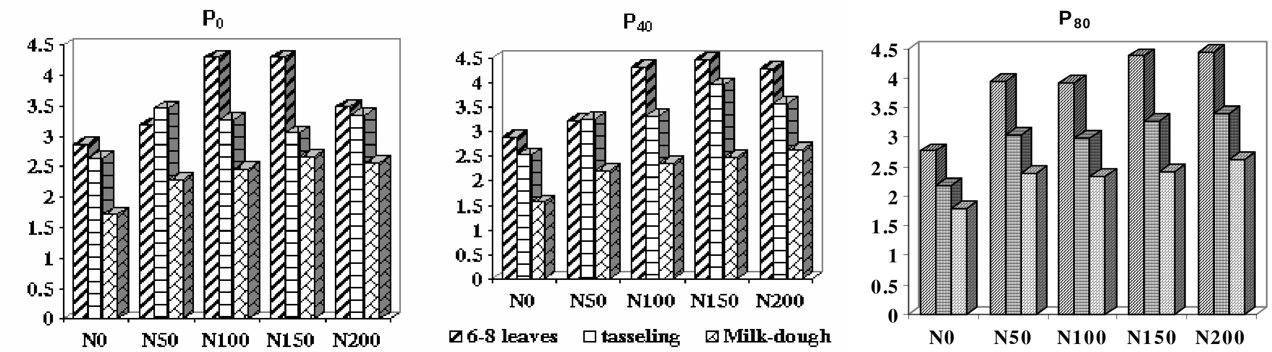

Figure 1. Total nitrogen content in leaves

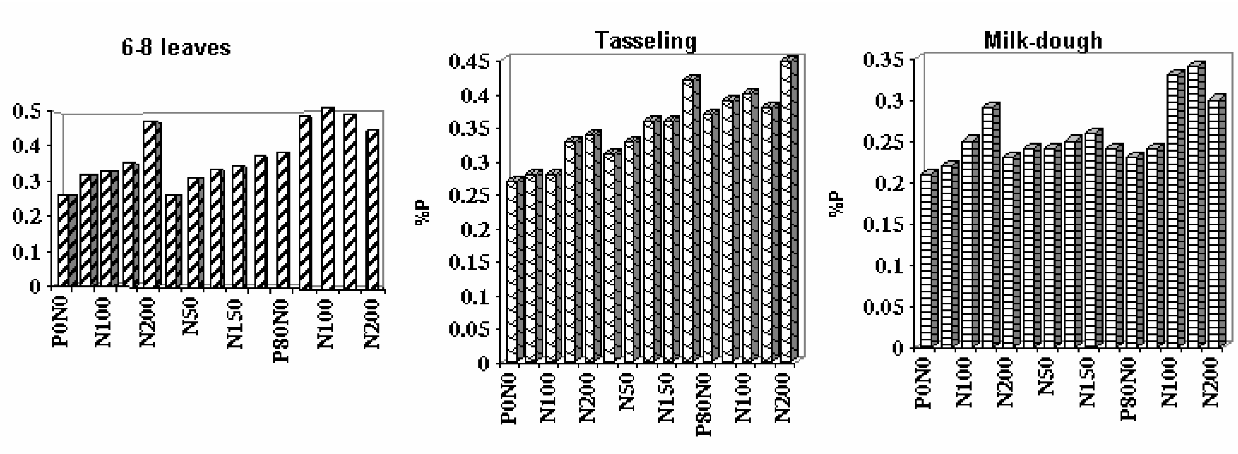

Figure 2. Phosphorus content in leaves

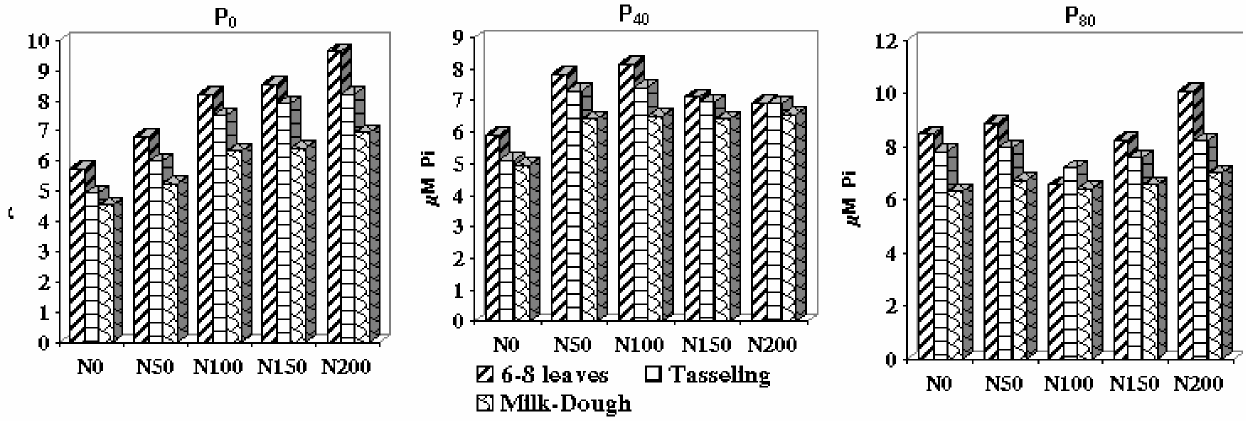

Figure 3. Acid phosphatase activity depending on nitrogen rate

It is noticeable the favourable effect of phosphorus at P_{40} level, where plants total nitrogen content is higher than that of the plants cultivated without phosphorus. A rate of 100 or 150 kg N/ha in the presence of 40 kg P/ha had the best effect on plant nitrogen content. Higher rates of phosphorus 80 kg P/ha had favourable effect al larger rates of nitrogen (200 kg N/ha).

Phosphorus content variation in plants had the same characteristics as nitrogen (increasing as the phosphorus fertilizer quantity increases, and decreasing as the plants advance in vegetation). Phosphorus content increased along with the nitrogen rates accordingly to the well known nitrogen-phosphorus sinergism (Figure 2).

In early vegetation stages (6-8 leaves) the highest phosphorus content was recorded in plants grown in the presence of 200 kg N/ha and no phosphorus, and in the presence of 50, 100, 150 kg N/ha and 80 kg P_2O_5. At tasseling, the favourable sinergetic effect of nitrogen and phosphorus was found for P_0N_{150}, $P_0N_{200,}$ $P_{40}N_{200}$ and $P_{80}N_{200}$ variants. Increasing phosphorus quantity over 40 kg/ha seemed to have no noticeable effect on plants at this vegetation stage. At milk-dough stage high rates of nitrogen and phosphorus induced a longer plant metabolic activity and led to an increased accumulation of nutritional elements.

Acid phosphatase activity increased concomitantly with nitrogen rates on P_0 background up to N_{100}, followed by a slightly decrease on P_{40} (Figure 3). Increased phosphorus quantity produced an increase of phosphatase activity only for N_0 and N_{50} variants, the activity being approximately constant for N_{100} (Figure 4). It is obvious that for N_{200}, at all three data of sampling (but especially at 6-8 leaves stage) the highest enzymatic activity was found in the plants that recieved no phosphorus. A possible explanation could be that in the case of very high nitrogen rates the lack of phosphorus becomes so important that determines the increase of acid phosphatase activity in order to release all the available phosphorus. These data are consistent with

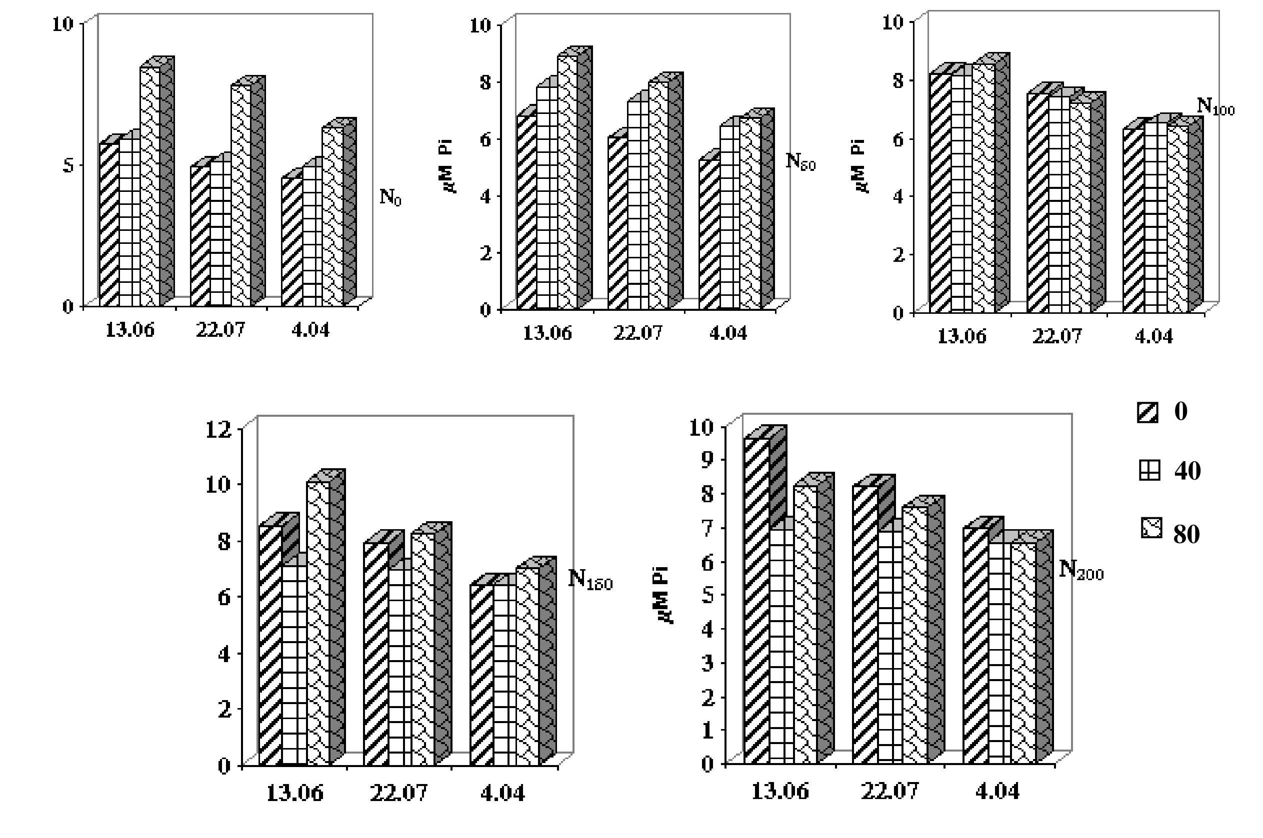

Figure 4. Acid phosphatase activity depending on phosphorus rates

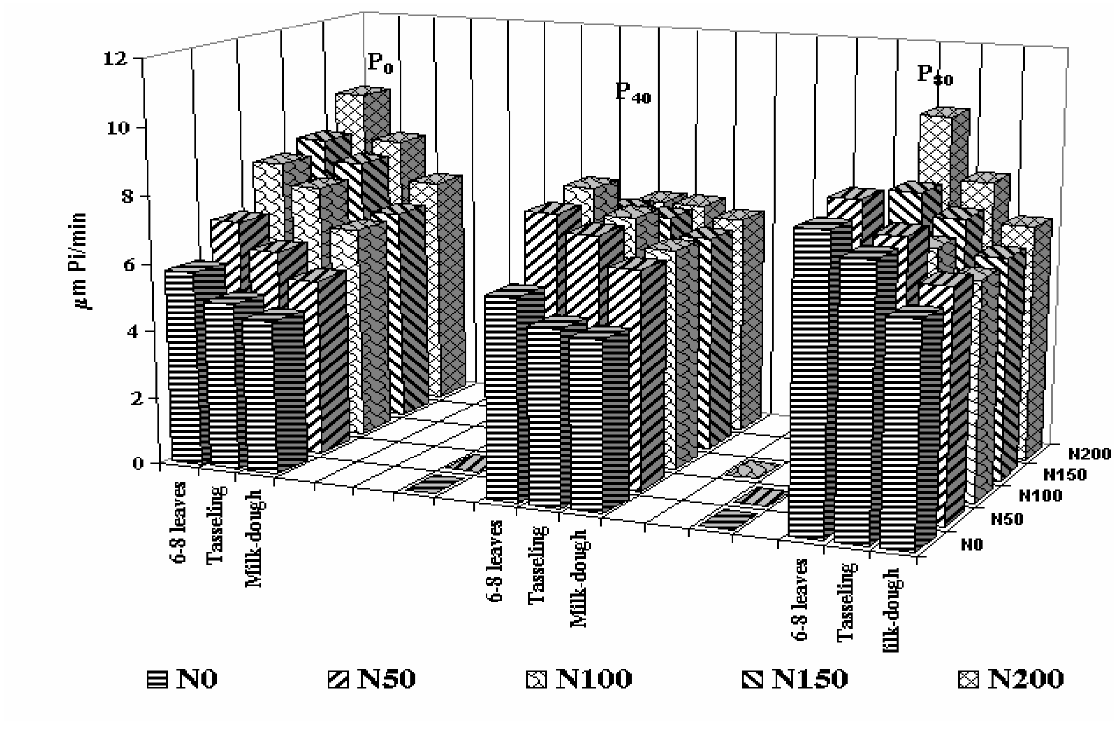

Figure 5. Acid phosphatase activity depending on fertilizers rates

other results (Kummerova and Burešova, 1990; McLachlan et al., 1987). According to Strother (1980) increasing of acid phosphatase activity is a possible response of favouring phosphate homeostasis. These data corroborated with studies regarding the isozymes responsible for the increasing of phosphatase activity could lead to establish a biochemical method of phosphorus status evaluation, before the occurrence of any visible symptom.

At the same time, high quantities of phosphorus (80 kg/ha), in the presence of high rates of nitrogen (200 kg/ha) have a favourable effect on acid phosphatase activity. When phosphorus was lacking, the need for this element induced a higher metabolic activity and by that a higher activity of acid phosphatase in order to release the existing phosphorus in the cells (Figure 5).

From the same figure one can notice that while plants advance in vegetation, acid phosphatase activity decreases. This behaviour can be explained by metabolic activity decrease during later developmental stages, when the main processes point assimilates partitioning.

Table 2. Maize yield obtained at Fundulea 1995 (kg grains/ha)

	P_0	P_{40}	P_{80}
N_0	6418	6933	7291
N_{50}	7316	7756	7555
N_{100}	7366	7618	8020
N_{150}	7505	7769	8076
N_{200}	7825	7837	7687

LSD for P< 0.05 –238; P≤0.05 –288; 0.001 -503

Table 2 presents the maize yield obtained at Fundulea in 1995. The highest grain yields were obtained for $P_{80}N_{100}$ and $P_{80}N_{150}$ variants, in which the highest phosphorus content in leaves was recorded. This fact suggests a better filling due to longer metabolic activity determined by sinergetic effect of nitrogen and phosphorus. Significant correlation coefficients between yield and enzymatic activity at each of the three sampling stage were recorded only for P_0 and P_{40} (Table 3 and Figures 6).

Table 3. Relationship of maize yield (Y) to acid phosphatase activity (A) . Fundulea 1995

Phosphorus (P_2O_5 kg/ha)	Vegetation stage	Equation	Correlation coefficients
0	6-8 leaves	y = 4819 + 316.47A	0.918*
	tasseling	y = 4927 + 340.75A	0.910*
	milk-dough	y = 4411 + 487.05A	0.914*
40	6-8 leaves	y = 5492 + 270.71A	0.687
	tasseling	y = 5137 + 363.12A	0.908*
	milk-dough	y = 4322 + 588.27A	0.975**
80	6-8 leaves	y = 5530 + 247.86A	0.553
	tasseling	y = 8323 - 77.03A	-0.09
	milk-dough	y = 3665 + 614.84A	0.523

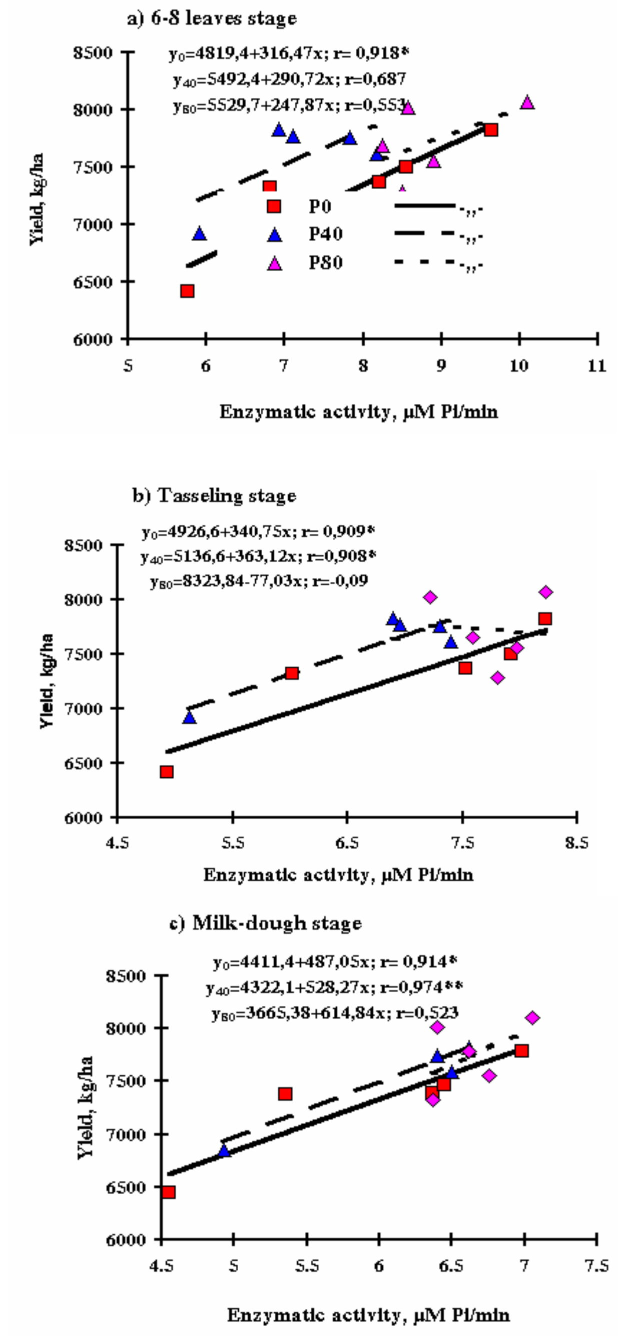

Figure 6. Correlation between yield and acid phosphatase activity

The significant correlations between yield and acid phosphatase activity only for P_0 or small quantities of phosphorus emphasize the importance of this enzyme. When high phosphorus quantities are present in the soil (80 kg/ha), yield increasing is a consequence of general metabolism intesification, acid phosphatase playing no longer the same important role.

CONCLUSIONS

These results can contribute to the theoretical explanation of yields obtained in long term experiments, showing the physiological status of the plants.

Continuing these studies in order to find out the possible differences between genotypes during early vegetation stages, could improve the efficiency of fertilizing systems by a new method of diagnosis before any visible simptom appears.

REFERENCES

Army, J.T., Kemper, D.W., 1991. Support for long-term agricultural research. Agronomy J., 83 (1): 62-65.

Dumitru, I.F., Dana Iordăchescu, 1981. Enzimologie, București, Edit. Medicală, 44.

Frye, W.W., Thomas, W.G., 1991. Management of long-term field experiment. Agronomy J., 83 (1) 38-44.

Hagima, Ioana, 1984. Studiul activității unor enzime în relație cu rezistența la ger a plantelor. Probl. genet. teor. aplic. XIV (2): 111-121.

Hera, Cr., Idriceanu, Alina, Dobrescu, Ecaterina, Mihăilă, V., Rusu, Paraschiva, Chardas, G., Pattakou, Vassiliki, 1986. Interacțiuni nutritive în procesul fertilizării minerale și influența lor asupra calității recoltei de porumb. Probl. Aagrofit. Teor. Aplic. VIII (2): 171-181.

Iordăchescu, Dana, Dumitru, I.F., 1980. Biochimie practică. Partea I, Proteine și enzime, Univ. București: 131-132.

Jaiswal, V.S., Pratap, Narayan, Madan, Lal, 1984. Acid and alkaline phosphatase activities related to sexual differentiation in *Carica papaya*. Biochemie und Physiologie der Pflanzen, 179: 799-801.

Kummerova, Marie, Ivana, Buresova, 1990. The Effect of exogenous phosphate deficiency on the activity of acid phosphatase of the root of the two maize genotypes. Biologia Plantarum, 32 (1): 1-7.

Malhotra, S.S, Khan, A.A., 1980. Effects od SO_2 and other air polluants on acid phosphatase in pine seedlings. Biochemie und Physiologie der Pflanzen, 175 (3): 228-236.

McLac Chlan, K.D., Elliott, D.E., DeMarco, D.G., Garran, J.H., 1987. Leaf acid phosphatase isozymes in the diagnosis of phosphorus status in field-grown wheat. Austr. J. Agr. Res., 38: 1-15.

Mihăila, V., Hera, Cr., 1994. Some recent results from the long-term experiment at Fundulea, Romania. In: R.A. Leigh and A.E. Johnson (eds.): Long-term experiments in agricutural and ecological sciences: 220-227.

Strother, S., 1980. Homeostastis in germinating seed. Ann. Bot., 45: 217-218.

Zamfirescu, N., 1977. Bazele biologice ale producției vegetale, Edit. Ceres, București: 172.

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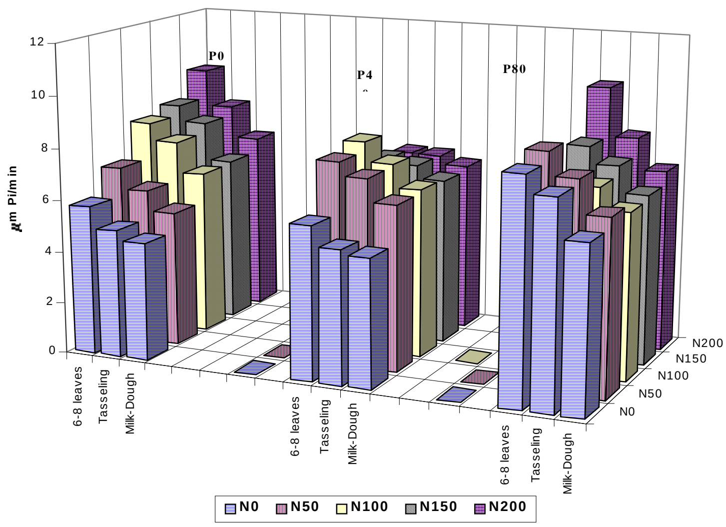

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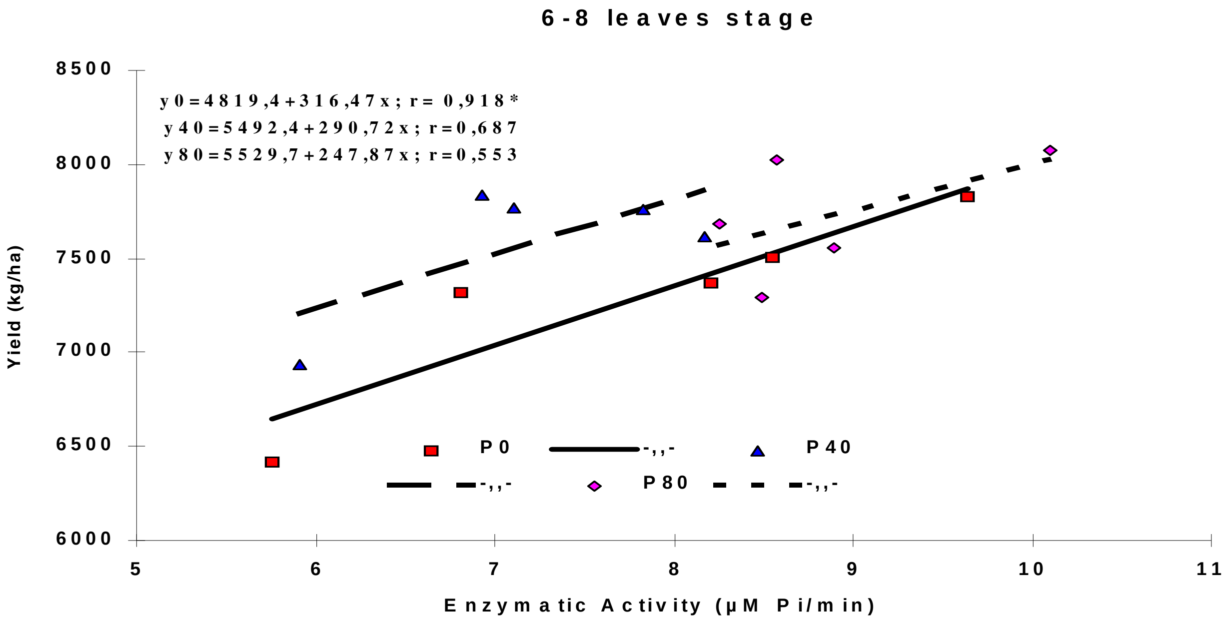
6-8 leaves stage
y0=4819,4+316,47x; r= 0,918*
y40=5492,4+290,72x; r=0,687
y80=5529,7+247,87x; r=0,553
8500
8000
7500
7000
6500
6000
Yield (kg/ha)
P0
-,,-
P40
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P80
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10
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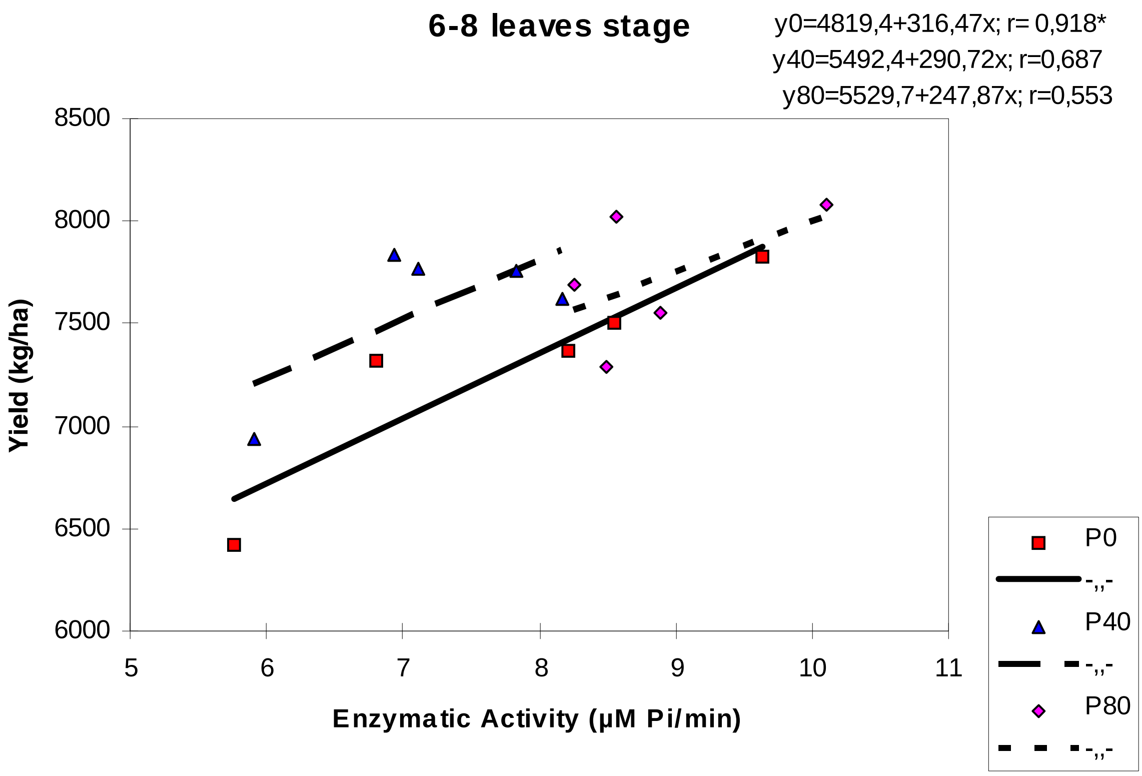
Enzymatic Activity (µM Pi/min)

Figure 6.



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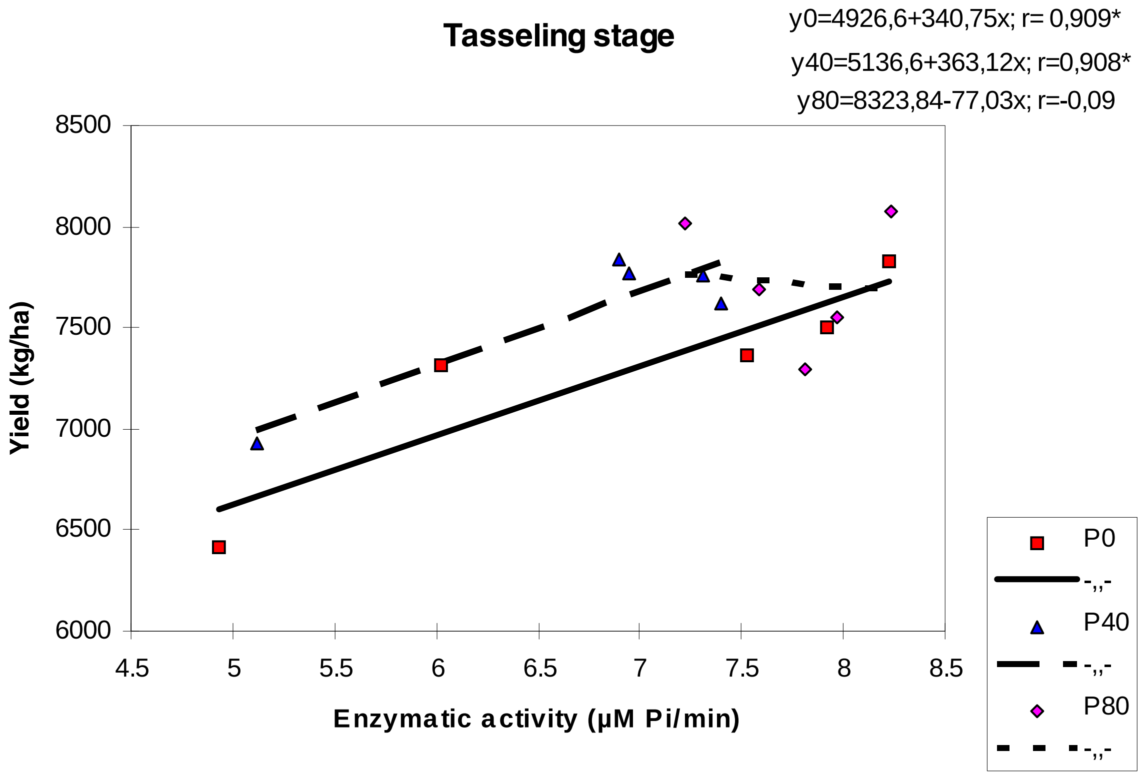


Figure 7. Correlation between yield and acid phosphatase activity

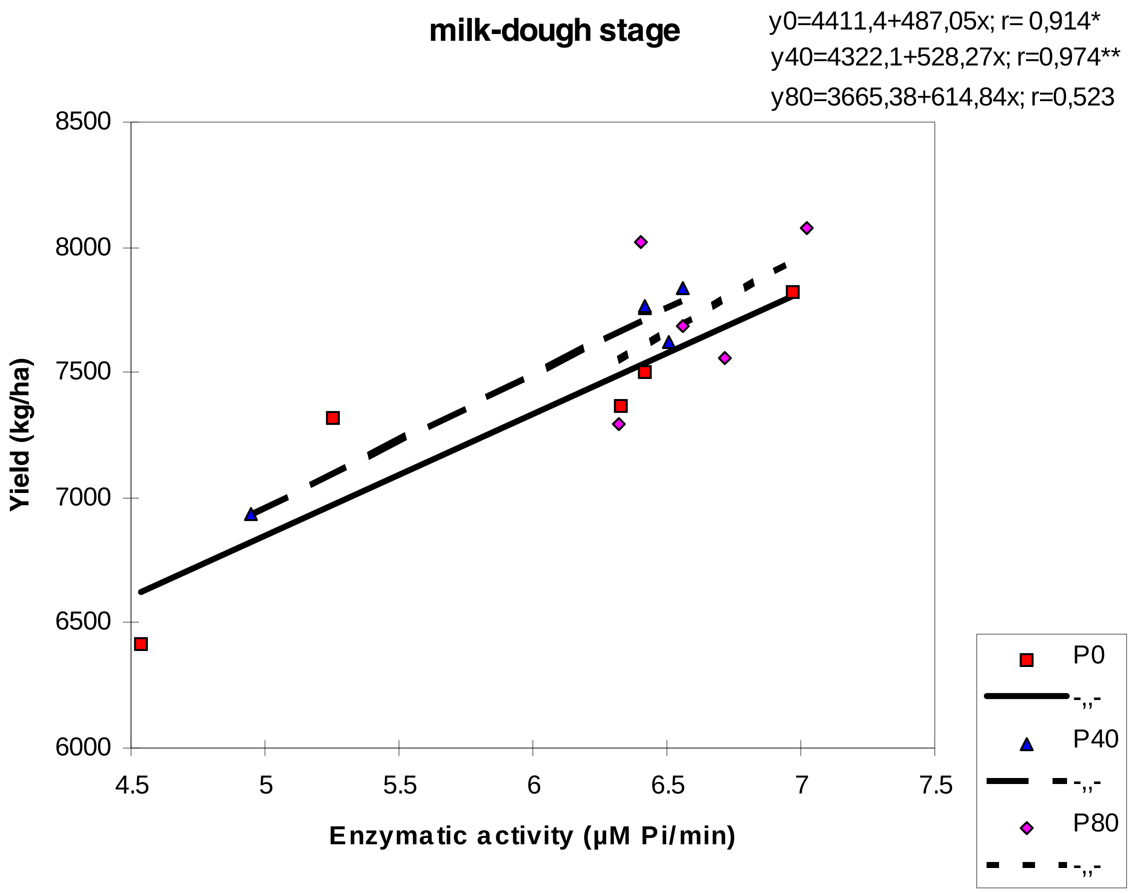


Figure 8. Correlation between yield and acid phosphatase activity

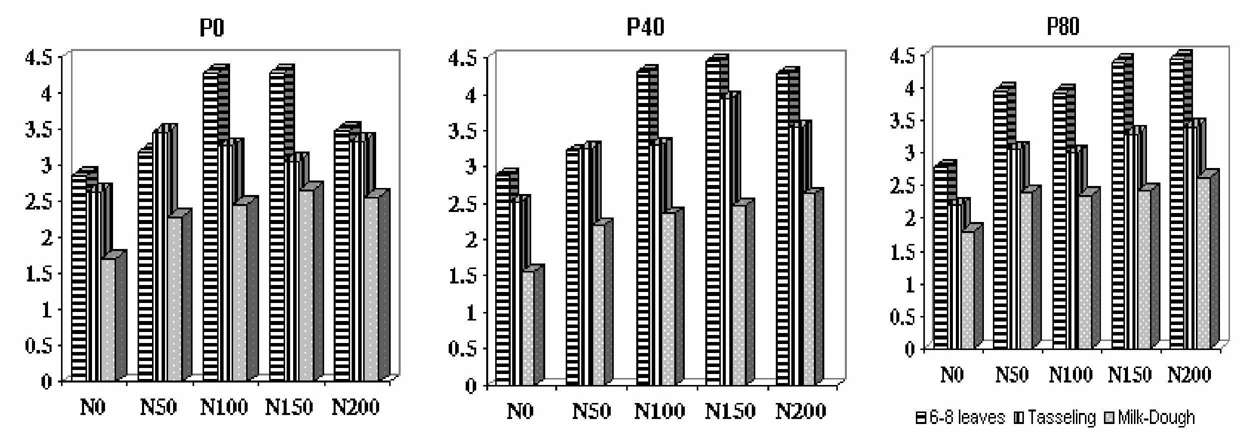


Figure 1. Total nitrogen content in leaves

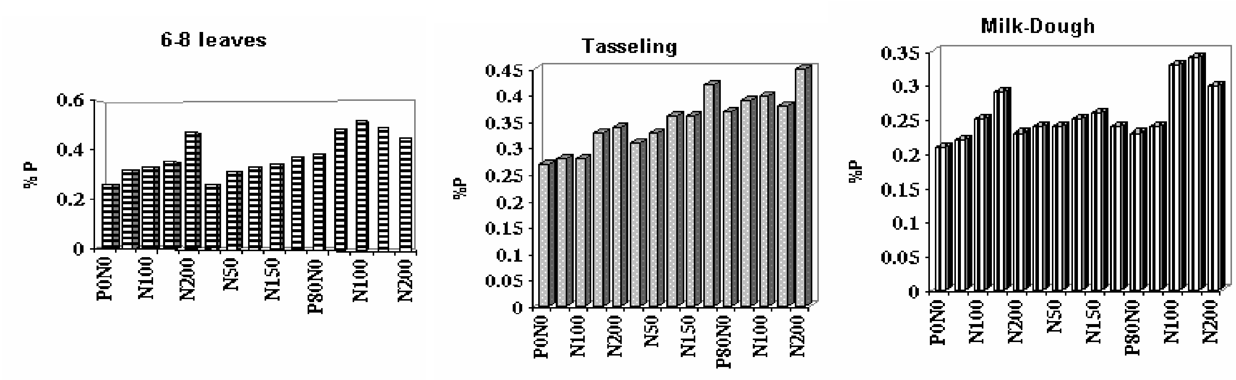
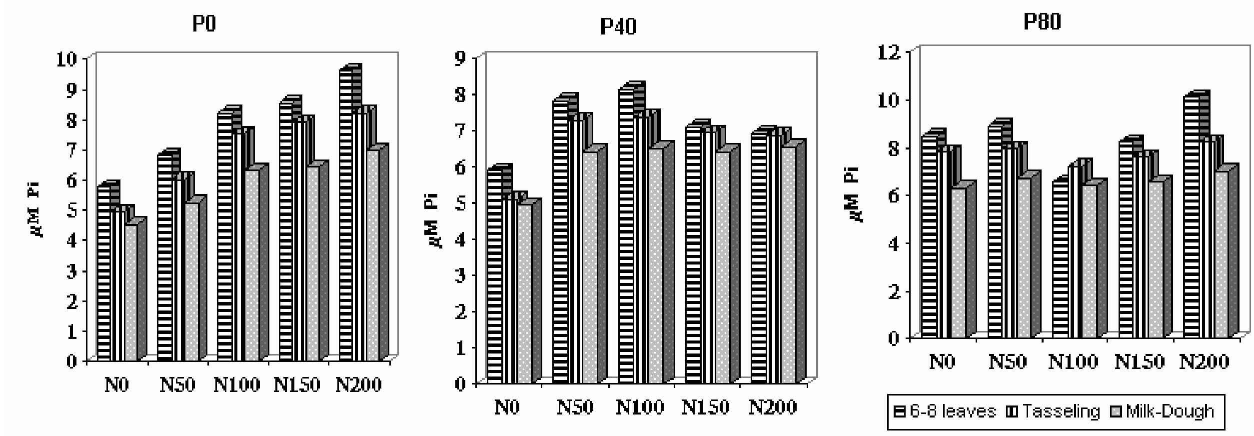


Figure 2. Phosphorus content in leaves



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