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Calibration of Rapid Test and Sampling for Measurement of Phosphorus Nutrition Status in Maize

Jérôme Silvestre,[1,*] Philippe Morard,[1] Guillaume Piva,[2] Claire Jouany,[2] and Patrick Petitbon[2]

[1]Laboratoire d'Ingénierie Agronomique, E.N.S.A.T., Castanet, Tolosan, France
[2]Unité d'Agronomie, INRA Toulouse-Auzeville, Castanet, Tolosan, France

ABSTRACT

Fertilization management of intensive crops requires control methods to adjust the mineral fertilizer input to the actual needs of the crop. This paper outlines a method to estimate phosphorus (P) nutrient status of maize (*Zea mays* hybrid *c.v. Volga*) by analyzing the plant sap extracted from vascular tissue of the stem base. Phosphate concentration is analyzed with phosphomolybdic complex reaction, using test strip with a small autonomous pocket reflectometer. The experimental conditions for maize diagnosis P nutrition are: stem base harvested between 10 and 12 a.m., phosphate concentration measured in sap squeezed by a hand hydraulic press. The critical level of P nutrition should be between 20 and

*Correspondence: Jérôme Silvestre, Laboratoire d'Ingénierie Agronomique, E.N.S.A.T., BP 107, 31326 Castanet Tolosan, France; E-mail: silvestr@ensat.fr.

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25 ppm. The validation of this method by comparison of colorimetry and chromatography measurement on different plant P status indicates a reverse correlation between weight production and P lower stem sap concentration.

INTRODUCTION

Fertilization of intensive crops such as maize within the European Union requires more and more elaborate control methods, which have to meet two objectives. First, these control methods have to reduce the quantities of mineral fertilizers and adjust them to the actual needs of the plants to limit production costs. Secondly, these methods must result in a more environment friendly method of farming by limiting the potential for nutrient movement from the farm. Phosphorus is often a factor that limits crop yield and quality. For crops such as cereals, P fertilization is based on soil testing and the balance between harvest consumption and nutrient removal for a particular crop. However, the different methods of chemical extraction of phosphates available in the soil do not measure the exact quantities of phosphate ion actually available to the plants roots. Similarly, adjusted management of phosphate fertilization to P harvest consumption leads to a relatively empirical prediction.[1]

Techniques for controlling nutrition and fertilization based on the mineral content of the whole plant or the leaves are useful for diagnosing the nutritional P status of maize. However this method is hard to apply to P in maize as P content of leaf, at a given growth stage, is not related to maize yield.[2]

A new methodological approach could be to identify a nutrient flow indicator in the whole plant. For example, analyses may be conducted directly on plant sap extracted from vascular tissue. This technique was applied to tomato, cucumber,[3] lettuce,[4] wheat,[5] and potato.[6]

The first objective of this work is to determine the optimum conditions for measurement of P concentration in maize using test strips. The second objective is a validation of this rapid test method in controlled P nutrition and in maize field trials.

MATERIALS AND METHODS

Plant Culture

The variety of maize used in this study was a hybrid *c.v. Volga*, from Pioneer France Maïs.

Table 1. Composition of the nutrient solutions.

mmole L^{-1}	NO_3^-	$H_2PO_4^-$	SO_4^-	K^+	Ca^{++}	Mg^{++}
P-free	15.0	0	1.0	5.0	5.0	1.0
Standard P level	15.0	2.0	1.0	7.0	5.0	1.0

Controlled Tests in Soilless Culture

After seed germination in darkness (5 d at 30°C and 100% relative humidity) and after the seedlings had turned green (2 d at 25°C), the young plants of maize were grown until the 8–10 leaf stage with two rates of phosphorus concentration (0 and 2 mM), in hydroponics conditions. The composition of the nutrient solutions using KNO_3, $Ca(NO_3)_2\,4H_2O$, KH_2PO_4, $MgSO_4\,7H_2O$, was reported in Table 1. For all treatment micronutrients were added (mg L^{-1}): Fe 15; Mn 0.49; B 0.26; Zn 0.11; Cu 0.25; Mo 0.01.

Sand Alumina Mixture

An alumina–sand mixture (1.5% with acid washed sand) was used in a greenhouse experiment to simulate phosphate behavior in soil–plant relationships. Hydroxyalumina can adsorb a significant quantity of phosphate by exposing the mixture with different $H_2PO_4^-$ concentration solutions. On contact with a phosphate deficient nutrient solution, it can desorb a small amount of these phosphates; so, phosphate concentration in the nutrient solution remains constant.[7] Five P concentrations were tested ranging for three growth-limiting concentrations (4, 6.5, and 10 μM of P) and two non growth-limiting concentrations (75 and 133 μM of P). In a preliminary test with phosphorus free nutrient solution, P reserves of maize seedling were depleted 1 wk after germination, displayed by leaf visual symptoms. The efficiency of the treatment was followed by two growth indicators per pot: measures of leaf area and leaf dry weight at the harvest. Pots are randomized and each treatment was replicated eight times. Data are submitted to ANOVA variance analysis.

Field Trials

The efficiency of this method was also assessed in field trials on sandy loam soil (loam 50%, sand 37%, clay 12%, organic matter 1%). Four doses of

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superphosphate fertilizer were applied to maize plots: 0, 13, 26, 44 P kg ha^{-1} $year^{-1}$ (or 0, 30, 60, 100 P_2O_5 kg ha^{-1} $year^{-1}$). Maize plots were randomized complete block and each treatment was replicated four times. Ten maize plants were sampled at the 8–10 leaf stage between 10 and 12 a.m. On each plot, soil P concentration was analyzed by the Olsen method.[8] For plant samples, P extracted from stem base sap was analyzed with strip tests. Dry weight of maize samples was measured.

Preparation of Plant and Soil Sample

Plant Sample

The harvested organs were cut up in 1-cm-long pieces and then squeezed by means of a hand hydraulic press (HACH, Loveland, CO). The sap extracted from the various samples was collected then filtered at 10 μm on a cellulose filter. Filtration removed cellular aggregates that could interfere with measurements. The resulting sap samples were frozen to avoid hydrolysis of organic P, and allow simultaneous analysis of all samples. P measurements were not altered by frozen stage (ten sap samples, three replications). Press residues were collected to determine dry weight of each sample. Total P mineral was analyzed in maize leaves ashes with, molybdenum blue method (MBA) with ascorbic acid.[9]

Selection of Most Sensitive Plant Part for P Test

The optimum condition for determination of maize P uptake depends on the organ choice. P concentration was analyzed by reflectometer in extract from different organs: young leaves (YL), mature leaves (ML), base of stem (BS), and root system (RS). Each extract was replicated three times. These organ tissues were only taken on maize plants grown in soilless culture with basic nutritive solution (2 mM of P) or P free solution (0 mM of P). Data are submitted to ANOVA variance analysis.

Plant Test for Sampling Time Choice

Phosphate concentration in sap extracted from vascular tissue was measured with strip tests every 2 h throughout 24-h period on maize plant at 8–9 leaves stage. Two experiments were conducted: the first one with plants

grown in the greenhouse with a complete nutrient solution (2 mM of P; 4 replications), the other one in the field (9 kg P ha^{-1} $year^{-1}$; 9 replications). Data are submitted to ANOVA variance analysis.

Soil Samples

Soil samples were collected at state depth range, e.g., 0–30 cm close to harvested maize plant. Phosphorus was extracted in soil sample with 0.5 M sodium bicarbonate at pH 8.5.[8] After filtration (Whatman no. 40), phosphate was determined by the molybdenum blue method (MBM) with malachite green.[10]

Phosphorus Measurements

Reflectometer (RF)

The reflectometer (Rqflex, Merck Germany) is a small autonomous pocket colorimeter which allows measuring the $H_2PO_4^-$ concentration. A few drops of sulfomolybdic reactant were added to the sample, then a strip impregnated with reducer was dipped in a sample. A colored reaction was obtained with the phosphomolybdic complex. The reflectometer gives the average of the 2 measurements from the 2 reagent zones of the strip. Each sample was measured twice.

Molybdenum Blue Method with Ascorbic Acid (MBA)

Ascorbic acid was used as reducer of the phosphomolybdic complex obtained from $H_2PO_4^-$ ions and sulfomolybdic reactant. The blue reaction thus obtained was measured at 660 nm.[9] Each sample was measured twice.

Molybdenum Blue Method with Malachite Green (MBM)

In this method, phosphomolybdic complex was complexed by malachite green oxalate. The coloration produced by this reaction was not stable, so polyvinyl alcohol stabilizer (PVA) was required. The colorimetric reaction was measured at 610 nm. This method is very sensitive since it measures scale ranges from 0.155 to 1.24 mg L^{-1} of P.[10] Each sample was measured twice.

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Liquid Ionic Chromatography Method (IC)

Phosphate concentrations were analyzed by HPLC (Dionex® DX-100). The measurement was carried out with conductimetric detector after crossing an AS4A ion exchanger cationic column[11] using carbonate/dicarbonate eluent (2 mL mn^{-1}). Each sample was measured twice.

RESULTS AND DISCUSSION

Conditions of Using Test Strips for Measurement of Phosphorus Nutrition Status

Comparison of Inorganic Phosphate Measurement Methods

The reliability of Merck's reflectometer was compared to various techniques of P measurement such as colorimetry and ionic chromatography (Fig. 1) on all organs of maize plant grown only in hydroponics conditions. The results given by the reflectometer are systematically about 10% below those given by the two other methods. These higher values of the two other methods are due to the different conditions of each dosage (reactant acidity, different dilution assays). These conditions should be more or less favorable to release inorganic phosphate from organic molecules, such as carbohydrates. Nevertheless, whatever the referring method, the strip reliability is acceptable since the regression coefficients range from 0.83 to 0.87.[12]

Choice of Plant Organ

Choice of the most representative organ for maize P nutrition and the easiest one to collect in the field is important (Fig. 2). Young leaves do not reveal any significant difference of P concentration, so they cannot be considered as indicative organs. Roots are a good indicator of P nutrition status, but they are not easily harvested, and moreover soil fragments adhering to the roots are difficult to remove. Mature leaves are easy to sample, but their low water content does not allow a sufficient amount of expressed sap. Thus, the stem base, which is easily harvested, allows the extraction of a sufficient sap volume and gives good information on maize P nutrition. The stem base should be the organ of choice and was used in the following discussion.

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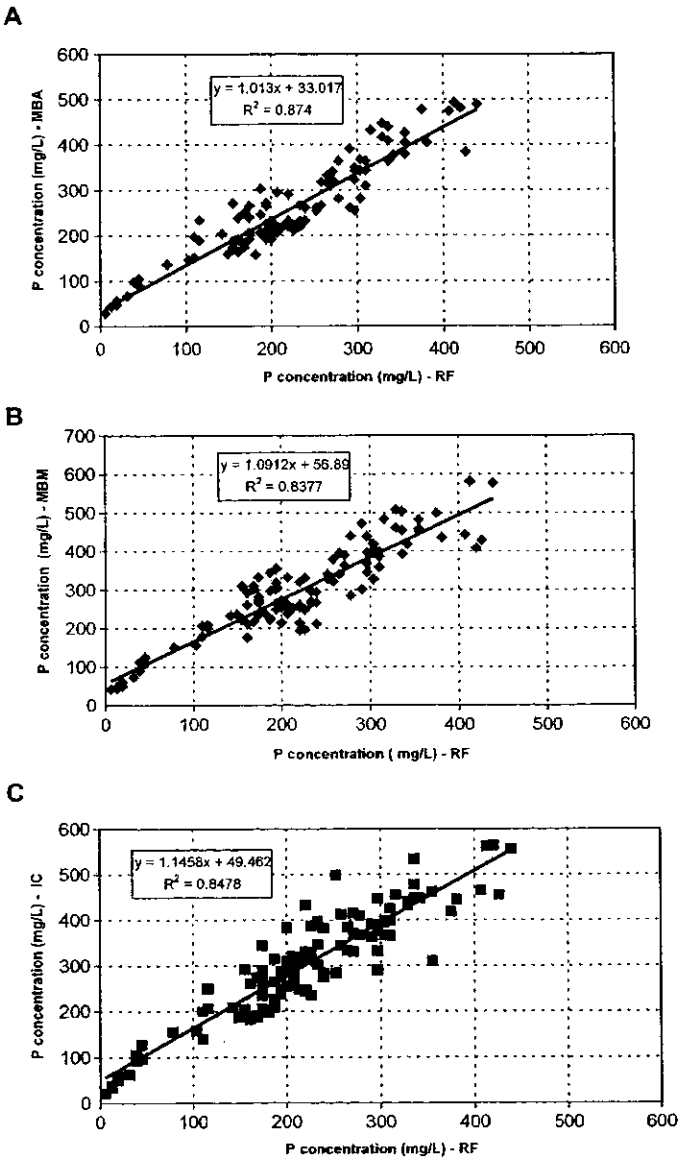

Figure 1. A-B-C: Comparison of the P concentration in vascular sap extract measured by Marck's reflectometer (RF) versus molybdenum blue method with ascorbic acid (MBA), molybdenum blue method with malachite green (MBM) and liquid ionic chromatography method (IC).

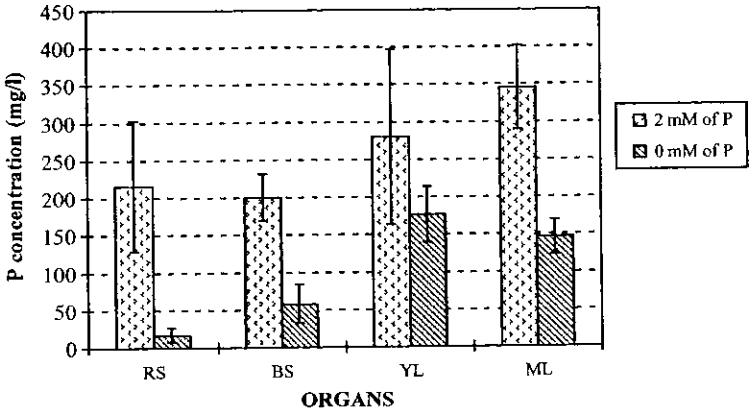

Figure 2. P concentration measured in sap extracted from different maize organs with two level of P: root system (RS), base of stem (BS), mature leaves (ML), and young leaves (YL).

Choice of Sampling Time During Day

Mineral uptake is related to the plant photosynthetic activity and to hydromineral flux. As a result, it depends on the intercepted luminous energy that varies according to the time of the day. P concentration in sap extracted from hydroponic plants was 6 times higher than from field plants (respectively $220 \pm 25\,mg\,L^{-1}$ and $40 \pm 10\,mg\,L^{-1}$). In both experiments, P concentration in tissue extracts are stable between 10.00 AM and 12.00 AM (data not shown). This most adapted sampling period will be used for the next assays.

Validation of Maize P Nutrition Status with Rapid Rests

Controlled P Nutrition on Sand Alumina Mixture

The five treatments were differentiated and ranked by a significant effect on plant growth indicators (Table 2): leaf area and dry weight were higher for plants grown with non-limiting P conditions (75, 133 μM of P) in comparison with P limiting concentration (4, 6.5, and 10 μM of P). Moreover, visual symptoms of P deficiency appeared on basal leaves for 4, 6.5, and 10 μM P levels. With P analysis and test strips of sap extract, the same group of treatments was also differentiated. Phosphorus concentration on leaves was lower in P deficient plants (4, 6.5, and 10 μM of P). On the contrary, sap

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Table 2. Indicators of maize growth in sand alumina experiment.

Treatments P conc. (μM)	Leaf area (cm^2 per plant)	Dry weight (g per plant)	P leaf content (% dw)	P in stem base sap ($mg\,L^{-1}$)
4	483 ± 77 *b*	2.3 ± 0.4 *a*	0.10 ± 0.02 *b*	47 ± 6 *a*
6.5	900 ± 150 *a*	5.9 ± 0.7 *b*	0.14 ± 0.02 *b*	31 ± 9 *b*
10	1102 ± 209 *b*	7.1 ± 1.2 *b*	0.13 ± 0.02 *b*	24 ± 5 *bc*
75	2292 ± 254 *a*	14.8 ± 1.8 *a*	0.23 ± 0.05 *a*	18 ± 4 *c*
133	2005 ± 345 *ab*	133 ± 0.6 *a*	0.28 ± 0.03 *a*	22 ± 2 *bc*

Values followed by the same letter are not statistically different at $P < 0.05$.

extract measurement with test strips indicated that the highest P levels were obtained in the most deficient plants. The reason was a limiting effect of P deficiency for leaf growth, and phosphate accumulated in stem base sap. Conversely, treatments without P limiting nutrition (75 and 133 μM of P) expressed rapid growth, as well as a higher P need, which induced low amounts in stem base sap ("dilution effect").

The test strip on lower stem base saps is an indicator of P nutritional status of maize plants. The data of this experiment indicate that the optimal level of the medium is between 10 and 75 μM of P. P critical level for a maize crop is about 20–25 $ppm\,L^{-1}$ in stem base saps.

Field Trials

Phosphorus extracted from soils (Olsen) and dry weights of leaves of maize were increased with fertilizer rate (Table 3): a maximum was reached with 44 kg of $P\,ha^{-1}\,year^{-1}$. The four treatments could not be statistically

Table 3. Field trials with four P doses.

Treatment P manure (kg/ha/year)	P Olsen in soil (ppm)	Dry weight (g per plant)	P leaf content (% dw)	P in stem base sap ($mg\,L^{-1}$)
0	2.03 ± 1.34 *b*	4.1 ± 0.7 *c*	0.23 ± 0.30 *a*	39.5 ± 5.7 *a*
13	2.43 ± 0.58 *b*	8.3 ± 1.3 *b*	0.29 ± 0.05 *a*	30.3 ± 4.8 *ab*
26	3.60 ± 1.15 *b*	8.9 ± 1.3 *b*	0.31 ± 0.05 *a*	33.1 ± 4.8 *ab*
44	7.70 ± 1.68 *a*	12.2 ± 1.6 *a*	0.29 ± 0.03 *a*	26.4 ± 6.8 *c*

Values followed by the same letter are not statistically different at $P < 0.05$.

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differentiated by P-leaf concentrations. The lower P concentrations in stem base sap were related to the higher biomass production and to the higher fertilizer doses supplied. The field results confirmed the sand alumina mixer findings. Namely, there is a reverse relation between P concentrations in lower stem base sap and leaf dry weight in properly fertilized, actively growing maize.

CONCLUSIONS

The results of this work present the experimental conditions to evaluate the P nutrition status of maize at an early stage (8–10 leaves). The optimum sampling conditions deduced from our experiments are:

- sections of one centimeter of leaf sheaths at the base of maize stem,
- sampling time between 10 and 12 a.m.,
- stem base sap with a hand hydraulic press,
- P dosage, without dilution, with strip tests and a pocket colorimeter.

This method was calibrated with two experiments: one on a sand–alumina mixture with controlled conditions, the other one on experimental plots. Phosphorus dosages of extracts from base of stems permit differentiation of the P nutritional status. The critical level corresponding to non-limiting P nutrition is about 20–25 ppm phosphates in stem base sap. Future research should address the relationships between P stem base sap and yield or quality of maize grain.

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