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SAMPLING FOR PLANT ANALYSIS OF COMMON BEANS, *Phaseolus vulgaris* L.

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Paper prepared at Cornell University 1975

Turrialba, Costa Rica
1977

ENRIQUEZ, G.A* Sampling for plant analysis of Common beans, *Phaseolus vulgaris* L**. Turrialba, Costa Rica, CATIE, 1977. 12 p. 23 ref.

RESUMEN

El trabajo es una revisión que resume la información obtenida del análisis químico de partes de la planta de frijol considerando especialmente el tiempo de muestreo, los tejidos más adecuados para representar el estado nutricional de la planta; sobre que plantas debe hacerse el análisis, su localización en el terreno y el tamaño de la muestra.

Trata sobre como se debe manejar la muestra una vez obtenida, como lavarla, como conservarla en caso necesario. Se recomienda la forma de molerla y los análisis que se deben hacer. Contiene 23 referencias bibliográficas.

SUMMARY

The paper is a literature review which sumarizes the information on chemical analysis of the bean plant, considering the time of sampling, the best tissue for the analysis, the selection of the plant and it type, and the size of the sample.

The way of washing the sample, the drying methods, the grinding and milling and some recommendation of the type of analysis done is pointed out.

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Content

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INTRODUCTION

Plant diagnosis is defined (18,19) as the composition of a tissue taken from a predetermined position i.e. of a definite physiological age, on the plant at the moment of sampling.

The foliar diagnosis during a season's growth cycle will then consist of a sequence of chemical states (composition) indicative of the nutrition during that cycle for the particular plant examined.

The composition is based on the dry matter of the tissue without taking into consideration the weight of the dry material at each sampling or the number of parts sampled from each plant.

The plant diagnosis is a more logical basis for fertilizer practice than is the traditional one which relates development and yield to the fertilizer applied, because the relation of yield to nutrition during the growth cycle is more direct than to the fertilizer applied (19).

Plant are not homegenous in their nutrient element make-up since leaves, stems, petioles, etc as well as similar plants parts at a different location on the plant, will differ in composition (9).

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The best analysis and interpretation is of no value without proper sampling (12). In sampling it is important that the tissue used always be present and identifiable at the designate-period; if the tissue is not readily identifiable, errors may be made in sampling. Also is advisable to use tissue that is easy to obtain and if possible, can be removed without any noticiable harmful effect to the plant.

Nutrient element concentration change with time. Large changes in concentration generally occur early in the initial stage and usually immediately following pollination (9). Some of this changes occur as the result of the dilution effect as the plant enters its rapid growth period.

The ideal sample at any selected sampling date would consists of parts of the same rank (physiological or metabolic age) on the stalk taken at the same time, for a specific plant part, from a sufficient number of plants.

The plant part and the time of smapling must correspond to the best relationship that exist between the nutrients elements concentration and the physical appearance of the plant or yield.

A question arise logically. What not to sample?

Young emerging leaves, old mature leaves and seeds are not usually suitable plant materials to be analized since they do not ordinarily reflect the nutrient elements status of the whole plant. Diseases or dead plant material. Plant or tissues damaged for insects or mechanical injury. Plant which has been stressed by cold, heat or moisture deficiency or excess. Plants which have been stressed for a period of time due to a nutrient element deficiency should not be sampled (10).

TIME OF SAMPLING

The time of sampling could be according:

a) The best time to test if only one determination is to be made, and;

b) That a series of sampling dates centering on the best single date is highly desirable (1).

Several authors agree that the best time of sampling for characterized the plant status for fertilizer problems is prior to or during (10%) initial flowering, (6,10,14).

Wade 1943 (22) studying the ascorbic acid content of strains of snap beans used a different sampling methods. Two picking of leaves were made, the first a few days after the second picking of pods, and the second a few days after the fourth and last picking of pods. Leaf harvest were begun at about 8:30 a.m. and were completed in two hours.

For seedling studies the samples are recommended from : 24 hours after the cordate leaves emerged from between the cotyledons, by this time these leaves were one-fifth to one-fourth of their final size and had already lost the crumpled appearance which is characteristic of them for the first few hours after emergency. The top of the plant were picked off above the cotyledonary node and placed in a moist chamber. The plants were picked about 6:00 p.m. and the experiment was usually begun at 3:00 p.m. the following day so that the leaves were in the dark (dark cupboard) for about 20 hr. and serve for studies of the synthesis of carotenoids pigments.

Jones et al. 1971 (10) recommended for seedling stage studies to sampling all the above ground portion of the plant when these have less

than 12 inches.

TISSUE SELECTED

One of the most important features of the sampling is that they will come from healthy, vigorous growing plants (16), never from extreme phenotype that do not represent the average plant.

Jones et al. 1971 (10) and Mackay and Leefe, 1962 (14), recommended two or three fully developed leaves at the top of the plant.

Kattan and Fleming, 1956 (11), studying the effect of irrigation at specific stage of development of the bean plants recommended to take trifoliates, fully expanded leaves and free of any damage.

Thomas 1937 (18) recommended that the leaf chosen from a plant would conveniently be the one immediately above the caducous leaves at the base of a stalk.

Jones 1967 (9) found that for snap bean, the degree of zinc deficiency was more closely associated with the zinc content found in mature leaves than other plant part.

Bernstein and Thomson, 1947 (3) and Bandorski, 1949 (2) studying the synthesis of carotenoid pigments, recommended to use 10-15 cordate leaves, removing the midrib from each leaf and using one half as control, wrapped in cheesecloth, both samples were blanched for 2 minutes in boiling water and stores at 20°C until analyzed.

Wade, 1943 (22) (see above) studying ascrobic acid content of snap beans used 4 harvest times of pods at market or canning stage, eliminating variability due to inmaturity or over maturity of pods. The samples were placed in bags and stored at 36°F. Within 30 minutes after picking

and analyzed within 48 hours. Twenty five grams of unselected whole pods were used for analysis, broken and damaged pods were excluded.

An innovation was introduced by Hivon et al. 1951 (8) when they harvest the whole plant and place in a refrigerator until leaves and pods were removed within three hours after harvesting. Duplicated groups of plants from each treatment were processed when they were studying ascorbic acid and ascorbic acid oxidizing in plants deficient in manganese.

Harrington, 1944 (7) studying some factos that influenced the reliability of plant tissues testing, found evidence that support strongly that the conducting tissue such as the stem of beans (and the petiole of spinach) is a more reliable guide than other tissues since the magnitude of differences is greater and the response to a change in available supply is quicker in the same morphological region or tissue when making comparisons, as the differences between tissues is usually greater than the differences between fertilizer treatments.

Fleming, 1956 (5) studying the factors that influence the mineral content of snap beans, in greenhouse experiments used the sample when the early set of pods reached marketable size taken samples of pods and tops (leaves and stem), for chemical analysis.

Sistrunk 1969 (17) differentiating varieties of bush beans by chemical and physical mehtod used pods that were harvested when there were two 6 sieve pods per foot of 40 feet tows in order to obtain sufficent 6 sieve pods. A sub-sample was taken for the analysis of fresh beans. Sampling for fresh analysis were divided further by removing the seed from one half of the sub-sample. Then each lot was cut in 1/4

in. lengths, weighed in duplicated into 2 oz. paper cups, frozen and freeze-dried for 36 hr. at a pressure of 5μ. The dry sample was weighed to obtain total solids before grinding in a Wiley mill to a powder. Dry samples were sealed in plastic vials and sotred at 0°F until analysis were made.

LOCALIZATION OF THE PLANTS

Gallo and Miyasaka, 1961 (6) studying the chemical composition of the beans plant took the sample (one plant including roots) from 1.5m. from the border of the plot.

Fleming, 1956 (5) took the plant samples for chemical analysis from the middle of the row; and from the best plant in the row, consistent in the edible part of the crop. In 1953 a statistical analysis of the K, Ca, O, and Mg content of leaves and stems from a spring crop of bean gave coefficient of variability below 6% for these kind of sampling.

Ulrich and Hills, 1967 (21) recommended a simple system to follow in sampling a field, dividing the field into imaginary quarters at right angles to the plant rows and collected from 25 to 50 leaves per quarter of the field.

SIZE OF THE SAMPLES

There is a goodagreement between the authors for the number of leaves that would represent a good sample. It will vary from 20 to 30 (10,14,16,21) some author recommend to take at least 2 or 3 samples for big plots and for each sample one can make subsamples according with the type of study or the necessities.

Kattan and Fleming, 1956 (11) selected three leaf samples. Each leaf samples consisted of five trifoliates, fully expanded leaves (See above).

WASHING THE SAMPLES

The samples could be washed with any acceptable detergent (mild detergent) and rinsed with destilled water (5, 10, 12). However, such washing should be done rapidly and while the tissue is fresh; washing after the tissue has dried is nearly impossible and is more likely to remove soluble nutrients (12). It is possible to clean the material brushing or wiping with a damp cloth (10).

Unless soil particles and chemical sprays and dust are removed, the results may be unusable or misleading. Such residues must be removed before the plants lose much moisture. Otherwise certain soluble nutrients may be washed out of the tissues in the cleaning process. For all analysis it is desirable to minimize all respiration, molding, and bacterial decomposition simply because they reduce the dry weight of the tissue and thus change the percentages of the elements that are to be measured (1).

The analitical results sometime indicate the probability of contamination of the sample with soil. The amount of aluminium present in the plant sample is valuable guide to the degree of contamination of the sample. Aluminium is not generally recognized as an essencial element for plant growth and only small amounts are present in normal plant material. High values are indicative of contamined sample (15).

DRYING THE SAMPLES

All samples should be dried as rapidly as possible after collection so as to reduce chemical and biological changes to a minimum. Considerable loss in dry weight may occur due to respiration, while proteins are also broken down to simpler nitrogenous compounds, if drying is unduly delayed (15).

In the laboratory, the sample may be reduce by cutting it into small pieces, thoroughly mixing the pieces and a sub-sample (about 100 g. fresh material) must be taken (21). Drying over night in an oven preferable with a forced draft at 60-80°C (12,15,21).

There may be some slight changes in composition of the air dried samples because of respiration, but it is not great consequence. If all samples from a set of plots are treated alike, comparisons are still valid (12).

GRINDING THE SAMPLE

After drying, the samples are gound to pass a 20-40 mesh serum for most purposes and stored in small glass or plastic vials (12,21).

It is not permissible to use a still mill for grinding samples in which iron is to be determined. Still mills are permissible when manganese is to be determined. The methods available preclude the use of any single grinding mill when both copper and iron are to be determined (15).

CHEMICAL ANALYSIS

Several method is possible to use for the chemical analysis. In the present paper it will be presented only a summary; for:

Nitrogen	Macro and Micro Kjeldahl (11)
Phosphorus	The AOAC AC method (11), the molibdenum blue color method (13,23)
K, Ca and Na	Beckman flame photometer (11,13)
Mg	Titan yellow method (13)
Ascorbic acid	Bessey and King (16)

Chapman, 1967 (4) give a suggestive values of nutritional states of selected crops, from this tables some date are summarized in the Table 1 from the upper most mature leaf blade without petiole when plants were 10% in bloom.

Table 1. Some tissue analysis values useful in determining nutrient status.

Nutrient	Unit	Low	Intermedium	High
Cl	%	0.2	<0.6	2.7 - 6.1
Co	p.p.m.	--	1.0	--
Mn	p.p.m.	--	6.8	--
N	%	--	5.1	--
PO_4-P	%	<0.05	0.48-0.55	--
K	%	--	2.0	--
Na	%	--	.02	0.04 - 0.33
Zn	p.p.m.	--	>5	--

Fleming, 1956 (5) from his experiment present a range of K, Ca and P elements in two different places, and different years.

K	1.86 - 3.10
Ca	0.101- 0.525
P	0.295- 0.739

Thomas, Mack and Cotton, 1942 (20) present the results of the periodic analysis during the growth cycle of morphologically homologous leaves of snap beans growth on four differentially fertilized plots from each of two tiers, one tier being left unirrigated and the other receiving moderately heavy irrigation, amounting, during the growth period, to 4.14 inches.

A summary of their results are presented in Table 2.

Table 2. Percent of N, P_2O_5, K_2O, CaO and MgO on the first leave of the base. Yield (Mean for 5 period samples)

Treatment 4-16-4 lbs/acre	Yield Green pods per plot lbs.	N %	P_2O_2 %	K_2O %	CaO %	MgO %
			Irrigated plots			
0	25.7	2.40	0.60	2.86	7.45	0.89
167	27.0	2.40	0.60	3.05	6.82	1.08
333	33.7	2.46	0.59	3.37	7.07	1.24
667	39.9	2.46	0.67	3.73	7.28	1.05
			Non irrigated plots			
0	22.8	2.77	0.48	2.54	6.49	1.27
167	15.4	2.75	0.53	2.14	7.04	1.09
333	17.4	2.94	0.54	2.11	7.97	1.53
667	23.7	3.15	0.61	2.52	7.38	1.35

From this result it is easy to see the changes induced in the composition of the plant in each treatment of fertilized and the differences when irrigated is used.

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FITO: 797-77
Dic. 22, 1977
GE/mdem