# Plant tissue tests for predicting nitrogen fertilizer requirements in spring wheat

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PELTONEN, J. 1992. Plant tissue tests for predicting nitrogen fertilizer requirements in spring wheat. Agric. Sci. Finl. 1: 509-517 (Univ. Helsinki, Dept. Plant Production, SF-00710 Helsinki, Finland.)

Application of nitrogen (N) at sowing (basal N) alone is not always adequate for maximum yield and quality formation in wheat (*Triticum aestivum* L.). Because uptake and utilization of N by the plant is influenced by many environmental and varietal factors, supplementary N may be needed during the growing season, too. Additional N can be applied at particular stages of the plant's development (phenology) to produce the best result from its use.

The applicability of plant tissue N concentration as a diagnostic tool for measuring the N status of a wheat stand to guide economical use of additional N application was reviewed here. On the basis of grain protein concentration data, growers producing spring wheat with consistently low protein concentration are advised to pursue a more vigorous and better planned N fertilization programme in their crops. Plant tissue N testing provides a useful method for the producer to annually optimize wheat grain yield and grain protein concentration. Knowledge of both these 'critical components' as determined by pre-harvest N levels of plant tissue and post-harvest grain protein concentration can be utilized for making both basal and supplemental N fertilizer recommendations.

Key words: spring wheat (Triticum aestivum L.), critical tissue N, grain yield, protein content

### Introduction

The present recommendation by the leading fertilizer supplier in Finland for basal N fertilizer application for spring wheat in southern Finland varies from 110 to 140 kg N ha<sup>-1</sup> depending on soil type and previous cropping. These recommendations provide the framework on which the grower must base the nutritional requirement of his crop. However, precise determination of the optimum amount of N to apply is not easy, because N fertilizer requirements are influenced by many factors: the total demand of a crop for N, mineralized N present in the soil at the time of N application, and N mineralization during the growing season (WEHRMANN et al. 1982). Therefore, fertilizer recommendations are generalizations, which are only applicable in growing seasons and on farms where growing conditions coincide with the mean values of the field trials used as the basis for the recommendation. In actual fact, yield responses of spring wheat to N fertilization vary widely within one region, between regions, and from one year to another (HEIKKILÄ 1980). According to the author, the economical (90-180 kg N ha<sup>-1</sup>) as well as biological (120-200 kg N ha<sup>-1</sup>) optima for basal N application rate vary greatly. More precise recommendation may be possible by developing a proper scientific foundation as to timing of N for a crop.

Nitrogen uptake efficiency by plants is strongly regulated by environmental factors such as soil and air temperature (SPIERTZ 1977, LAWLOR et al. 1988), drought (which reduces fertilizer solubility and stresses the plant), or excessive rains (which may cause N leaching and water logging) (PELTO-NEN et al. 1990), and management practices such as irrigation (ELONEN et al. 1975), as well as time, form and rate of N fertilizer applied (FAJERSSON 1961, RAININKO 1966, JAAKKOLA 1978, SVENSSON and LINDAHL 1989). Therefore, soil testing for establishing the N fertilizer recommendations made on the basis of short term N mineralization measurements have been unreliable (SCHARPF and WEHRMANN 1976, STANFORD 1982). Adverse sowing conditions such as heavy rain can lead to denitrification losses of N fertilizer (ESALA 1991) and lead to the need for adjustment of N later. There are also varietal differences in N uptake efficiency (SETH et al. 1960, MIKESELL and PAULSEN 1971, NOAMAN and TAYLOR 1990). The situation may be affected by leaf shading, which becomes more significant at high plant population densities leading to translocation of N from shaded leaves (CAMPBELL and READ 1968, WILLEY and HOLLIDAY 1971, BREMNER 1972). It has also been shown that prolonged root activity and leaf duration positively affect N accumulation into the grain (SPIERTZ and ELLEN 1978, HERZOG and STAMP 1983). Because the utilization of N by a plant is controlled by so many factors, supplemental N may be needed if basal N application at sowing is insufficient or not optimal for wheat growth.

In conclusion, optimal timing of N to reduce annual variation in grain yield and quality in wheat requires a more detailed knowledge of N requirements of the wheat crop during its growth and development, and hence a way of accurately assessing the total N demand of the crop must be available. This review article examined whether the technique of tissue N testing can provide a useful method for optimizing wheat grain yields and protein concentration.

# Relationship between tissue nitrogen concentration and crop yield

Crop yield and quality of yield are determined by internal and external growth factors. The former are governed by genetic factors, and the latter by environmental factors (SIMAN 1974). Nutrient supply is an external factor that can be regulated quite easily. The increased N concentration increases total plant dry-mass, leaf area of main shoots slightly but of tillers greatly (LAWLOR et al. 1988). The photosynthesis per unit area increases with increasing N supply (LAWLOR et al. 1989). Cool conditions increase the root dry-mass, the root to shoot ratio and the N content in plant dry-mass (LAWLOR et al. 1988). All this relies, however, upon the interactive effects of weather and soil factors, and may be modified by the occurrence of pest, weeds and disease control (WEHRMANN et al. 1982, HAGROVE et al. 1983, NEEDHAM 1983, PELTONEN and KARJALAINEN 1992).

Plant analysis can be used to determine a crop's nutritional status at various developmental stages to guide the fertilizer programme or as a means of obtaining valuable economic information on the nutrient availability in soils. BENZIAN and LANE (1981) have indicated that there are four general types of curves of yield responses to N fertilizer application: positive linear, convex without a maximum, convex with a maximum, and negative linear. The authors concluded that as a rule the grain protein concentration of wheat increased linearly, whereas many of the grain yield curves reached a maximum and then declined with further additions of N. Therefore the use of N fertilization in excess of that needed for a maximum yield only seemed to result in increased grain protein concentration (BENZIAN and LANE 1981). The nitrogen concentration of the wheat plant decreases during growth although N uptake continues until maturity (SIMAN 1974, ANGUS and MONCUR 1985, HARPER et al. 1987, PELTONEN 1992). These authors have shown that the N concentration decreased from 50 - 60 g kg<sup>-1</sup> at floral initiation to 20 - 30 g kg<sup>-1</sup> at anthesis.

The factors contributing to variation in plant tis-

sue N concentration can be classified into variation (a) in plant developmental stages, and variation (b) in N supply (SIMAN 1974). The variation (a) is genetically controlled and its presence is revealed by the downward trend in N concentration as the plant matures irrespective of the N supply. The variation (b) caused by N supply is related to changes in growth which depend on the N available in soil. It is necessary, therefore, to separate the variation as a result of the developmental stage from that dependent on the N supply. Variation (a) can be partly eliminated by sampling at identical developmental stages, whereby the variation in the tissue N concentration is only dependent on the N supply (SIMAN 1974). In contrast, sampling based only on a fixed dry matter weight level (DMwlevel), as suggested by Møller Nielsen and Friis-NIELSEN (1976), is not sufficiently accurate to explain the variation in N concentration described above (PELTONEN 1992).

For the interpretation of internal nutrient concentration, ULRICH and HILLS (1967) presented a concept of the growth (as yield response) and nutrient concentration curve. According to the authors, the nutrient concentration values can be divided into zones related to their impact on yield. These zones in the calibration curve are as follows: (i) the zone of deficiency, i.e. the growth increases sharply but there is little change in the concentration of the nutrient, (ii) the zone of transition, i.e. both the nutrient concentration and growth increase, and (iii) the zone of adequacy, when the nutrient concentration increases, but there are no more changes in growth. STEENBJERG (1951) found that plants which were extremely deficient in one nutrient had a higher concentration of this nutrient at maturity than plants that were less deficient in the same nutrient. When small amounts of this nutrient were supplied, it stimulated the vegetative growth to such an extent that the increased yield production caused decreases in the nutrient concentration. It is noteworthy that this "Steenbjerg effect" was also observed in the work of MØLLER NIELSEN and FRIIS-NIELSEN (1976) in the mature crop, but not in the young plants with increasing N applications.

# Selection of plant part and methods for plant analyses

Four parameters for testing plant tissue have been suggested as indicators of N fertilizer requirements for wheat: (i) stem nitrate nitrogen (NO<sub>2</sub>-N) concentration (GARDNER and JACKSON 1976, PAPASTYLIANOU and PUCKRIDGE 1981), (ii) whole plant N concentration (ENGEL and ZUBRISKI 1982, ROTH et al. 1989, VAUGHAN et al. 1990a), (iii) leaf N concentration (VAUGHAN et al. 1990a), and (iv) crop N uptake (ROTH et al. 1989). The stem NO<sub>3</sub>-N test has been proposed as a satisfactory method of predicting N deficiency in wheat (PAPASTYLIANOU and PUCKRIDGE 1981, WEHRMANN et al. 1982), but some researchers have reported that critical NO<sub>3</sub>-N levels can vary greatly from site to site (BERINGER and HESS 1979, ROTH et al. 1989, VAUGHAN et al. 1990a) and can change rapidly over time (ROTH et al. 1989). Therefore, more samples were needed to achieve a confidence level of accuracy for the NO<sub>3</sub>-N test than for testing the whole plant N concentration (ROTH et al. 1989). Total N concentration can be misleading in some cases because dry matter yield can either be low or high at the same level of N applied to crop if the amount of N taken up and the amount of dry-mass produced vary in such a way that the concentration of N in tissue remains similar (SIMAN 1974). Critical N uptake levels are too variable to be used alone for monitoring a crop's N status (BERINGER and HESS 1979, ROTH et al. 1989, PELTONEN 1992). The critical level of N uptake can be, however, used as an indicator of whether the high N concentration is due to the low dry-mass production or high N uptake level of the wheat plant (SIMAN 1974, ROTH et al. 1989).

According to ALDRICH (1973), there are two general analytical methods for plant analyses: (i) total or quantitative (total chemical analysis or spectrographic analysis) and (ii) relative quantity or semiquantitative (rapid tissue tests). The total or quantitative analysis measures both the elements that have already been incorporated into plant tissue and those that are still present as soluble constituents of the plant sap. The determination may be made by the Kjeldahl method or by chemical separation followed by weighing the constituents being determined. Other techniques measure the quantity of an element by the amount of light emitted following excitation in an appropriate manner by heat and high voltage (emission spectrograph) or by Xray bombardment (X-ray spectrograph) or by Xray bombardment (X-ray spectrograph). The semiquantitative analysis, e.g. the rapid NO<sub>3</sub>-N tissue tests (WEHRMANN et al. 1982, SCAIFE and STEVENS 1983, NITSCH and VARIS 1991), measures the unassimilated, soluble contents of the plant sap (ALDRICH 1973). An advantage of "rapid tests" is that they can be used in the field, or when laboratory facilities for total chemical analyses are not readily available.

# Estimation of nitrogen utilization by wheat plant

Analyzing soil for inorganic N (NO<sub>3</sub>-N + NH<sub>4</sub>-N) (WEHRMANN and SCHARPF 1979, WEHRMANN et al. 1982) provides a valuable means of revealing the amount of N available for plant growth. However, the amount of available N present in the soil depends on soil type, the cultivation system, preceding crop, straw disposal, crop species or even the cultivar cultivated, pests, weeds and diseases, and soil water supply (HARGROVE et al. 1983, NEEDHAM 1983, PELTONEN and KARJALAINEN 1992).

If available N is a limiting factor, protein concentration often becomes more a function of total grain yield than of the genetic potential of a particular cultivar to produce protein. In this situation, the highest yielding cultivars will suffer the greatest reductions in protein even if they have genetic potential for protein production (FOWLER and de la ROCHE 1984). The analysis of grain protein has been successfully used in Colorado and North Dakota to evaluate post-harvest previous N management practices and to guide future N fertilizer recommendations (GOOS 1984). Previously GOOS et al. (1982), GOOS (1984) and VAUGHAN et al. (1990b) indicated that simple Cate-Nelson models (CATE and NELSON 1971, NELSON and ANDERSEN 1977) are sufficient for the calibration of critical levels of N in the soil and N in the plant. In Cate-Nelson model II the data for critical N concentration are interpreted as N responsive or N nonresponsive categories in terms of grain yield and/or grain protein concentration. In contrast, Cate-Nelson model III separates the data into three groups: N fertilizer responsive, transitional, and non-responsive data.

On the basis of grain yield and grain protein data collected during 1968 - 1988, Cate-Nelson model II predicted that if the grain protein concentration is less than 11.2 percent in southern Finland, yield losses would be associated with N deficiency (Figure 1). On the basis of these data, growers producing spring wheat with consistently low protein concentrations are advised to pursue a more vigorous and better planned N fertilization programme in their crops. In years of drought, yields are usually low and the protein content will generally be high (KONTTURI 1979). However, in such years, the N requirement for maximum yield will also be lower. Analysis of the critical levels of grain protein has been found to be applicable in both good and poor years (Goos 1984). However, the author pointed out that there have been situations where the model has not been validated. For example, where there is early season N deficiency but N is available through root uptake later, the often observed high protein concentration in the grain is a result of yield loss due to N deficiency. Such situations have been rare in the data of PELTONEN et al. (1990) concerning the yields in 1968-1988 in southern Finland.

# Tissue nitrogen levels related to grain yield and grain protein concentration

Plant tissue analysis can provide an effective means of monitoring the nutritional status of a crop at an advanced developmental stage. If critical tissue N concentrations are known, potential deficiencies can be identified before visual symptoms appear. Also the sufficiency of N in tissue can be monitored. If N deficiencies are identified early enough, additional N can be applied to the crop before yield



Fig. 1. Relative yields of spring wheat as related to grain protein concentration in southern Finland (after PELTONEN 1992).

Growth stage	Plant part	N analyzed	Critical value (g kg <sup>-1</sup> )	Country	Reference
Feekes 10.5	Whole plant	Total N	18-26	USA	ENGEL and ZUBRISKI (1982)
Feekes 10.5	Top two leaves	Total N	36-45	USA	ENGEL and ZUBRISKI (1982)
Feekes 10.5	Flag leaf	Total N	<35	USA	HAGROVE et al. (1983)
Feekes 9-10	Whole plant	Total N	24-35	USA	ENGEL and ZUBRISKI (1982)
Feekes 9-10	Top two leaves	Total N	41-50	USA	ENGEL and ZUBRISKI (1982)
Feekes 9-10 <sup>a</sup>	Whole plant	Total N	<28	Finland	PELTONEN (1992)
Feekes 9-10 <sup>a</sup>	Leaves	Total N	30-38	Finland	PELTONEN (1992)
Feekes 7	Whole plant	Total N	19-27	USA	VAUGHAN et al. (1990a)
Feekes 7	Leaves	Total N	34-41	USA	VAUGHAN et al. (1990a)
Feekes 5-6	Whole plant	Total N	42-51	USA	ENGEL and ZUBRISKI (1982)
Feekes 5	Whole plant	Total N	25-33	USA	VAUGHAN et al. (1990a)
Feekes 5	Leaves	Total N	34-38	USA	VAUGHAN et al. (1990a)
Feekes 1-2 <sup>a</sup>	Whole plant	Total N	<43	Finland	PELTONEN (1992)
Feekes 10	Stems	NO3-N	3-9	USA	GARNER and JACKSON (1976)
Feekes 7-8	Stems	NO3-N	5-10	USA	GARNER and JACKSON (1976)
Feekes 3-4	Stems	NO3-N	6-10	Australia	PAPASTYLIANOU (1984)
Feekes 3-4	Stems	NO3-N	7-12	USA	GARNER and JACKSON (1976)

Table 1. Critical N values related to grain yield in spring wheat reported in the literature.

a = the apex developmental stages: early double-ridge stage and stage when stigmatic branches of carpel have formed have been estimated to correspond to the Feekes scale.

and quality are reduced. A summary of critical N levels in wheat tissue at different growth stages has been presented in Table 1. Previously VAUGHAN et al. (1990a) concluded that the observed differences between critical N levels could be due to the differences in the growing conditions in varying geographical regions and/or wheat classes.

The study of SPRATT (1974) indicated that critical  $NO_3$ -N concentration of 300 ppm in dry-matter (DM) of above-ground parts of plant at anthesis was required for optimum grain protein concentration in grains. PELTONEN (1992), in turn, showed that differences in R<sup>2</sup> in tissue N content between different plant parts at anthesis were insufficient to justify any particular choice of plant parts for sampling in the field. Critical N concentrations recommended at anthesis are: 12 g of N kg<sup>-1</sup> in DM of the whole plant and 23 g of N kg<sup>-1</sup> in DM of the leaves (PELTONEN 1992).

The amount of N to be applied when plants are deficient could not be measured in experiments by SIMAN (1974), HAGROVE et al. (1983), ROTH et al. (1989), VAUGHAN et al. (1990a) and PELTONEN (1992). According to BHATIA and RABSON (1976), the relative increase in N required for protein production could be calculated by the system of SIN-CLAIR and de WIT (1975) on the basis of biochemical pathways derived by PENNING de VRIES et al. (1974). This system may provide an effective tool for calculating the supplemental N requirement of the crop if there is lack of N at certain developmental stages. VAUGHAN et al. (1990b) developed the multiple regression models to estimate N fertilizer requirement at various stages of growth. Nitrogen requirement can also be calculated with the concept of a balanced other nutrient composition of the plant by the diagnosis and recommendation integrated system (DRIS) methods (BEAUFILS 1973).

### Conclusions

On the basis of grain protein concentration data, growers producing spring wheat with consistently low protein concentration are advised to pursue a more vigorous and better planned N fertilization programme in their crops. Analyzing previous research data to identify the 'critical level' of grain protein concentration is not difficult, and will provide growers, extension personnel, and fertilizer dealers with a cost-effective means of evaluating the efficiency of N use by the crop and for developing N fertilization recommendations based on those data. Information on the post-harvest grain protein concentration can easily be obtained by wheat growers because grain protein determinations are a routine part of its marketing.

The critical tissue N content could be an effective diagnostic tool for justifying the need of supplementary N fertilizer of a crop during the preharvest phase. The NO<sub>2</sub>-N tests have been reported to vary greatly from site to site, and can change rapidly over time. Therefore, more samples were needed to achieve a confidence level of accuracy for the NO<sub>3</sub>-N test than for testing the whole plant total N concentration. It is concluded that the observed differences between critical N levels could be due to the differences in the growing conditions in varying geographical regions and/or wheat classes. Nitrogen uptake (mg per plant) should be measured, because this information indentifies whether a high N concentration is due to low dry-mass production or high N uptake level of the wheat plant. There is requirement of rapid plant N analysis methods in the field or in the laboratory. When tissue N is determined in the laboratory, the analytical data could be supplemented by a recommendation of the amount of supplemental N fertilizer required to increase tissue N above the critical level.

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Manuscript received September 1992

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## SELOSTUS

### Kasvianalyysit kevätvehnän typpilannoituksen tarkentamisessa

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Tämä kirjallisuustutkimus osoitti, että mikäli typpi on ollut rajoittava tekijä sadon muodostukselle, aiheuttaa se sekä alhaisen sadon että valkuaispitoisuuden (Kuva 1). Toisin sanoen, jos viljelijä tuottaa vehnäsadon jonka valkuaispitoisuus jää alle kriittisen raja-arvon, tulisi hänen lisätä typpilannoituksen määrää viljelyä jatkaessaan. Menetelmän soveltaminen käytäntöön on perusteltua, koska sadon valkuaispitoisuus kuuluu laatuhinnoitteluun.

Mikäli kasvien typen puute havaitaan riittävän aikaisin, ennen näkyviä puutosoireita lehdistössä, täydennystyppeä voidaan lisätä ennen kuin satopotentiaali ja valkuaispitoisuus alentuvat. Nitraattityppeen perustuvat pikatestit ovat kuitenkin epäluotettavia. Sen sijaan nopea, kasvin kokonaistyppeä mittaava analyysi on typpilannoituksen optimoimiseen tarpeellinen. Kriittisiä typpiarvoja kasvin eri kehitysvaiheissa on esitetty taulukossa 1. Kasvianalyysin tulisi sisältää typpipitoisuuden lisäksi suositus mahdollisesta täydennystypen määrästä (kg/ha), joka tarvitaan kohottamaan kasvin typpipitoisuus kriittisen arvon yläpuolelle.