AGRICULTURAL SCIENCE IN FINLAND

Vol. 4: 495–501.

Determination of barley nitrogen status with chlorophyll meter for high β-amylase in grains

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The production of β-amylase is of great importance in two-rowed spring barley cv. *Kymppi* (*Hordeum vulgare* L.) in the Finland, where long-day conditions favour high enzyme activities. Nitrogen (N) fertilization of a crop is the main means of manipulating barley β-amylase activity for industrial purposes. In this study, leaf chlorophyll content determined with a portable chlorophyll meter (Minolta SPAD-502) in the field, was used to predict N availability of cv. *Kymppi* for β-amylase production. Critical chlorophyll meter readings (SPAD values) were calculated from data deriving from experiments with various N fertilizer levels using the Cate-Nelson procedure. According to the results of this study it can be stated that the critical SPAD values at pollination (i.e. pollen grains on well-developed stigmatic hairs, GS 52–58) are 37 SPAD units for grain yield and 41 SPAD units for β-amylase activity. The optimum grain yield occurred at 41 SPAD units and optimum β-amylase activity was reached at 45 SPAD units. Determination of leaf chlorophyll content using the chlorophyll meter led to more appropriate fertilizer application recommendations and subsequently increased β-amylase activity in grains. Grain protein concentration could be an effective diagnostic tool for post-harvest evaluation of grain β-amylase activity in cv. *Kymppi*.

Key words: β-amylase activity, grain protein concentration, grain yield, chlorophyll content, plant tissue N status

Introduction

β-amylase is an important barley (*Hordeum vulgare* L.) protein, determining the technical quality of starch used for various industrial processes in Finland (Helenius 1992). Cultivars with high β-amylase activity are often reported to be high in grain protein concentration (Harris and Banasik 1952). However, Helenius (1992) indicated that although increases in grain protein concentration led to increased β-amylase activity in grain, β-amylase activity differed among barley varieties: two-rowed *Kymppi* had – and

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Manuscript received December 1995

still has – the highest β-amylase activity among barley varieties grown in Finland.

Nitrogen (N) fertilization of a crop is the main means of manipulating barley β-amylase activity for industrial purposes (Hayter and Riggs 1973). Previously, Peltonen et al. (1995) showed that leaf chlorophyll content, determined in the field with a portable Minolta SPAD-502 chlorophyll meter (developed by the Soil-Plant Analyses Development Section of Minolta Camera Company, Ramsey, NJ)[1], accurately indicated plant N status of small-grain cereals, allowing N fertilizer requirements to be determined accurately. Applying N fertilizer on this basis improved production economics and improved the physical input-output ratio during grain yield formation (Peltonen et al. 1995).

The objectives of this research were to quantify relationships between yield, grain protein concentration, β-amylase activity and SPAD values measured with a Minolta chlorophyll meter. The specific goals were to evaluate the potential for calibrationing the meter to optimize cv. *Kymppi* N status and predict the need for supplemental N fertilization for increasing β-amylase activity. Finally, the β-amylase stability of an optimized production system was evaluated using SPAD values.

Material and methods

Field trials

Field trials (Series 1) included five separate experiments at Experimental Farms of the University of Helsinki (Viikki and Suitia in 1993 and 1994) and at the Experimental Farm of Kemira Ltd. (Kotkaniemi in 1993) with two-rowed spring barley cv. *Kymppi*. A row spacing of 12.5 cm and 500 viable seeds m^{-2} were used. Nitrogen (NH_4NO_3-N) was applied to three 10 m^2 replicated blocks at 0, 50, 100, 150 and 200 kg N ha^{-1} at Viikki and Suitia, and 0, 40, 80, 120 and 160 kg N ha^{-1} at Kotkaniemi. Nitrogen fertilizer was applied 7 cm deep at sowing. P, K, Ca, S, Mg, Zn and Mn were also applied at sowing according to Finnish soil test recommendations: phosphorus availability in particular may influence calibration of the SPAD 502 meter (Follet et al. 1992). This program yielded a series of samples with increasing grain yield, grain protein concentration and β-amylase activity. Standard crop protection procedures (herbicide and fungicide) were implemented.

The plots were harvested when grains reached the caryopsis hard stage (GS 92, Zadoks et al. 1974). Yield was determined at 15% grain moisture content. The grain samples from each of the three replicates were combined and mixed thoroughly. Grain protein concentration was determined from whole grain meal using the standard method 46–11 of the AACC (1983). β-amylase activity was determined according to a method described by Helenius (1992).

Chlorophyll measurements

Chlorophyll meter readings (SPAD values) were taken at the stage of maximum number of florets per ear primordium (FM), coinciding with Zadoks' GS 37–41, and at pollination (PO), i.e. pollen grains on well-developed stigmatic hairs (GS 52–58). The developmental stage of the inflorescence was determined according to the scale of Waddington et al. (1983), and was considered to be reached when at least 60 % of plants were at that stage. It has been indicated (Peltonen 1992) that these developmental stages are important for timing of supplemental N applications. Thirty randomly selected plants per plot were used for determining the chlorophyll content. Determination of the SPAD value was made from mid-length on the uppermost fully expanded leaf.

Statistical analysis of critical readings

The critical levels of leaf chlorophyll were established using Cate-Nelson analysis (Cate and Nelson 1971, Nelson and Andersen 1977). The

[1] Trade and company names are included for the benefit of the readers and do not imply any endorsement or preferential treatment of the product by the authors.

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Cate-Nelson II model partitioned the data for critical SPAD values into two group in terms of grain yields; N fertilizer responsive and non-responsive. The Cate-Nelson III model partitioned the data into three groups; N fertilizer responsive, transitional and non-responsive. Grain yield was expressed as a percentage yield $Y_i/Y_{max} \times 100$ where Y_i is the grain yield when N_i kg ha^{-1} was applied, and Y_{max} is the maximum grain yield obtained in an experiment. The increase in grain protein concentration (P) and in β-amylase activity (β) were calculated as $\triangle P_i = P_i - P_0$ and $\triangle \beta = \beta_i - \beta_0$, where P_0 and β_0 is control of grain protein and β-amylase activity, respectively. Actual grain yield, grain protein concentration and β-amylase activity, as functions of the SPAD values, were consistently the best in terms of the coefficient of determination (R^2).

On-farm trials

The accuracy of the critical and optimal SPAD values for optimizing N fertilizer application for maximum β-amylase production was studied using independent barley (cv. *Kymppi)* data from 24 on-farm trials (Series 2) in southern Finland in 1995. Nitrogen fertilizer application at sowing ranged from 41 to 115 kg N ha^{-1}. The SPAD values were measured at the pollination (PO) developmental stages as in Series 1. The barley fields were grouped into categories of near optimum plant N status and excess N, according to the critical SPAD values for Series 1.

Results and discussion

The temperatures during crop growth in 1993 and 1994 were close to the mean for the period 1961–1990, but approximately 60 – 80 mm more rain fell prior to heading in 1993 and 1994 than during 1961–1990. This weather promoted very high grain yields and a very good response to N fertilizer in Finland. The grain yield, grain protein concentration and β-amylase activity for all experiments are presented in Table 1. The maximum grain yields obtained in Series 1 were 7292 kg ha^{-1} in 1993 and 6252 kg ha^{-1} in 1994. These maximum yield levels were clearly higher than

Table 1. Grain yield, grain protein concentration and β-amylase activity as functions of N application rate for spring barley cv. *Kymppi* grown 1993 and 1994.

Location and year	Trait	N1[a]	N2	N3	N4	N5
Viikki 1993	Grain yield kg ha-^{1}	3933	6140	6786	7292	7220
	Grain protein %	8.8	9.4	10.2	11.5	12.4
	β-amylase activity W.K.	80	87	111	149	235
Suitia 1993	Grain yield kg ha^{-1}	4023	5680	6028	7058	6570
	Grain protein %	9.4	9.6	11.9	12.1	13.3
	β-amylase activity W.K.	95	115	165	212	267
Kotkaniemi 1993	Grain yield kg ha^{-1}	2040	4290	4930	5310	5600
	Grain protein %	6.9	8.3	11.0	12.9	14.1
	β-amylase activity W.K.	77	87	129	200	253
Viikki 1994	Grain yield kg ha^{-1}	4355	5449	5951	5799	5598
	Grain protein %	9.8	12.1	12.8	13.5	15.8
	β-amylase activity W.K.	131	176	229	256	332
Suitia 1994	Grain yield kg ha^{-1}	3530	5660	6092	6164	6252
	Grain protein %	9.4	11.1	13.3	14.3	15.4
	β-amylase activity W.K.	102	134	212	231	253

[a] N1 = 0, N2 = 50, N3 = 100, N4 = 150 and N5 = 200 kg N ha-^{1} at Viikki and Suitia
N1 = 0, N2 = 40, N3 = 80, N4 = 120 and N5 = 160 kg N ha-^{1} at Kotkaniemi

annual mean yields in Finland, which, according to Mukula and Rantanen (1989), are about 3000 kg ha^{-1} for spring barley. The higher the N fertilizer rate the higher was β-amylase activity and grain protein concentration. The maximum β-amylase activity and grain protein concentration values were obtained at Suitia in 1993 and at Viikki in 1994.

Critical chlorophyll meter readings

Leaf chlorophyll content, determined with the chlorophyll meter, correlated strongly with grain yield at the stage of maximum number of florets per ear primordium (FM), but poorly with grain protein concentration and β-amylase activity (not shown in Table 2). In turn, leaf chlorophyll content at the stage of pollen grains on well-developed stigmatic hairs (PO) correlated more strongly with grain yield, grain protein concentration and β-amylase activity. Therefore, the Cate-Nelson analyses were performed only on data taken at this developmental stage. Cate-Nelson III model produced significantly higher R^2 values than Cate-Nelson II models. Both models for critical and optimum SPAD units are shown in Table 2.

Table 2. Cate-Nelson equations describing grain yield, grain protein concentration and β-amylase activity in relation to leaf chlorophyll (SPAD) levels, and suggested critical chlorophyll meter readings for spring barley cv. *Kymppi* at PO[a].

Equation	R^2
Grain yield = $3576 + 1868 \times X_1 + 733 \times X_2$	0.84***
where $x_1 = 0$ if <37 SPAD and 1 if >37 SPAD	
where $x_2 = 0$ if <41 SPAD and 1 if >41 SPAD	
Grain protein concentration = $9.5 + 4.7 \times X_1 - 1.8 \times X_2$	0.74***
where $x_1 = 0$ if <41 SPAD and 1 if >41 SPAD	
where $x_2 = 0$ if <45 SPAD and 1 if >45 SPAD	
β-amylase activity = $109 + 143 \times X_1 - 51 \times X_2$	0.72***
where $x_1 = 0$ if <41 SPAD and 1 if >41 SPAD	
where $x_2 = 0$ if <45 SPAD and 1 if >45 SPAD	

*** Significant at the 0.001 probability level.

[a] PO = stage of pollen grains on well-developed stigmatic hairs, coinciding with Zadoks' GS 52–58.

These results show that the application of sufficient N to spring barley cv. *Kymppi,* according to the recommendations based on leaf chlorophyll content (SPAD units) at PO, ranking "low" in N would result in an average response of 3576 kg ha^{-1} in grain yield as compared with an average response of 1868 kg more grain ha^{-1} for the "medium N" class. In class "high", the grain yield would change only by 733 kg ha^{-1} compared with class "medium". For grain protein concentration and β-amylase activity the critical SPAD values were 41 units and the optimum SPAD values 45 units. This indicates that the use of N fertilization in excess of the amount needed for an optimum grain yield (SPAD values exceeding 41 units) will generally result in increased grain protein concentration and β-amylase activity, but not necessarily in grain yield. The likely reason for this is that the photosynthetic rate, which is important in determining yield potential, will not increase to the same extent as the nitrate reductase activity of the plant (Lawlor et al. 1987, Joy and Peltonen 1993). In this study, chlorophyll content within the zone of adequacy resulted in 14.2 % of grain protein and 252 W.K. of β-amylase activity. However, if the SPAD values ranked "high", indicating over optimum N status, there would be a 51 W.K. decrease in the β-amylase activity of grains (Table 2).

Effect of optimized N management on β-amylase production stability using SPAD values

Yields were high in 1995 in on-farm trials (Series 2) and generally there were strong N responses. There were also large differences between applied N fertilizer and β-amylase activities in on-farm trials when the 24 fields were grouped into zones of N deficiency, optimum N status and excess N in plants using SPAD values (Table 3). Surprisingly, the better N fertilized fields suffered N deficiency for β-amylase production. Data indicated that 11 of the 24 growers succeeded in optimizing the N management

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without information on plant N status. Plant N status was supraoptimal only in two of 24 spring barley fields surveyed, although the lower dose of N fertilizer was fell within the optimum SPAD zone. The plant N deficiency among 11 fields examined resulted in 19 W.K. lower β-amylase activity than for optimal N status. Based on these data, growers are advised to pursue more vigorously the N fertilization program in their barley fields to promote β-amylase production.

The data above indicate that the capacity of SPAD values for predicting crop N status and β-amylase activity at given developmental stages is especially promising. Calculations using Series 1 for the category of adequacy, where the SPAD readings increased from 41 to 45, increased β-amylase activity by 143 W.K. and required about 130 kg more N ha^{-1}, when compared with the category where the grain yield reached a plateau (data not shown). Thus, to increase β-amylase activity by 10 W.K. with N fertilization, 11 kg more N ha^{-1} was required, which corresponded to 40 FIM ha^{-1} input [calculated using a N (foliar urea) cost of 1.68 FIM kg^{-1}]. The model developed provides useful information for calculating the price per unit β-amylase activity as a routine part of marketing cv. *Kymppi*. In addition, based on the knowledge of this physical input-output ratio, growers could express the calculations in economical terms, knowing the current fertilizer cost and price of barley grain and the unit increase in β-amylase activity.

Table 3. Comparison of the effect of the intensiveness of N fertilization, based on chlorophyll meter readings, on β-amylase production in spring barley cv. *Kymppi* in 24 on-farm trials.

Intensiveness of N fertilization according to SPAD readings	Mean applied N fertilizer rate[a]	β-amylase activity
	kg ha^{-1}	W.K.
< Critical SPAD	104 (11)	128
Adequate SPAD	94 (11)	147
> Optimum SPAD	70 (2)	160

[a] Number of growers in parentheses

Post-harvest evaluation of β-amylase activity with grain protein concentration

Grain protein concentration of barley cv. *Kymppi* correlated strongly with β-amylase activity (Fig. 1), thus confirming findings of Harris and

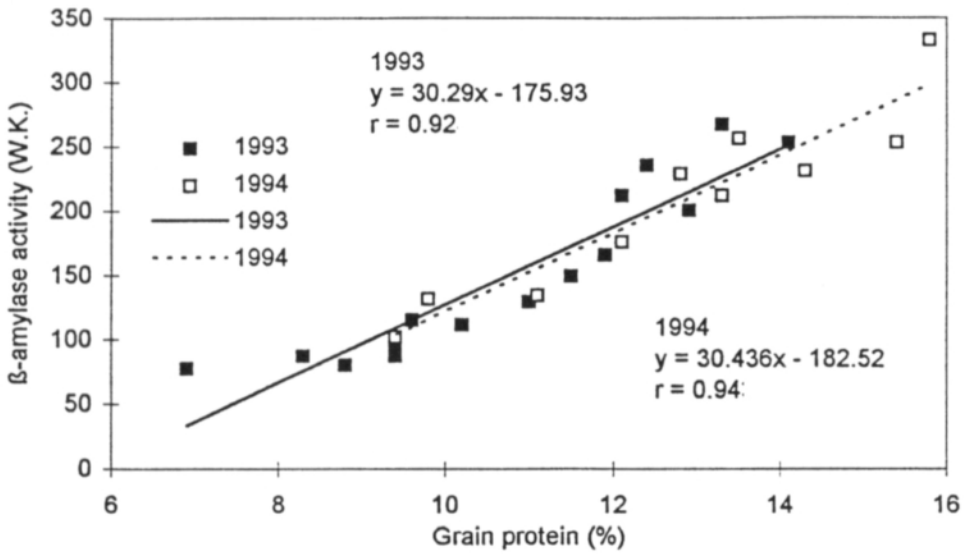

Figure 1. Relationship between grain protein concentration (%) and β-amylase activity (W.K.) in 1993 and 1994.

Table 4. Suggested critical tissue chlorophyll (SPAD) levels for spring barley cv. *Kymppi* at PO[a] in Finland.

Traits	< Critical[b]	Adequate[c]	> Optimum[d]
Grain yield kg ha^{-1}	37	37–41	46
Grain protein concentration %	41	41–45	48
β-amylase activity W.K.	41	41–45	48

[a] PO = stage of pollen grains on well-developed stigmatic hairs, coinciding with Zadoks' GS 52–58.
[b] Critical levels were calculated from Cate-Nelson II models.
[c] Lower and upper limits for adequate SPAD values were developed from Cate-Nelson III models. Adequacy level can be given if Cate-Nelson III models are an improvement in terms of R^2 in respect to Cate-Nelson II models.
[d] Mean values for class of > optimum SPAD, where there were no more increases in grain yield, grain protein concentration and β-amylase activity.

Banasik (1952). There were no significant differences for levels and slopes of linear regression functions between years (data not shown), indicating that grain protein concentration can be used for post-harvest evaluation of β-amylase activity in *Kymppi* within and between seasons. A grain protein concentration of 11% indicated β-amylase activity of 152–157 W.K. and at least 14% of grain protein should be reached for β-amylase activity to exceed 250 W.K.

Conclusions

In conclusion, the results from this study indicated that making accurate N fertilizer recommendations based on chlorophyll meter (Minolta SPAD-502) readings was economically advantageous. Determination of leaf chlorophyll content (SPAD values) using the chlorophyll meter allowed more appropriate fertilizer application recommendations to be made and promote a subsequent increase in β-amylase activity of a crop. Applying N fertilizer "as needed" resulted in a better physical input-output ratio than with lower or higher N inputs. Based on the knowledge of the physical input-output ratio, one could express the calculations in economical terms, knowing the current fertilizer cost and price of barley grain and unit increase in β-amylase activity.

Chlorophyll content can be assessed rapidly. The meter's cost may be prohibitive for many farmers, but not for large growers, consultants and groups including crop management associations. SPAD values for delimiting responsive and non-responsive ranges at pollination (i.e. pollen grains on well-developed stigmatic hairs, GS 52–58) are summarized in Table 4. The capacity of SPAD values to predict barley N status at this stage is especially promising, because supplemental N, applied as foliar sprayed with urea-ammonium-nitrate (Turley and Ching 1986) could easily be applied at this time of development to increase grain protein concentration and hordein synthesis. The suggested critical SPAD values are suitable under growing conditions where good response of grain yield to N fertilization occurs. The SPAD values for optimum grain yield are 4 SPAD units lower than the SPAD values for optimum β-amylase activity for *Kymppi*. Grain protein concentration could be an effective diagnostic tool for post-harvest evaluation of grain β-amylase activity in *Kymppi*.

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SELOSTUS

Kymppi-ohran β-amylaasin tuotannon optimointi lehtivihreämittarin avulla

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Tutkimuksessa määritettiin Kymppi-ohran lehtivihreäpitoisuuksien raja-arvoja parhaan mahdollisen sadonmuodostuksen ja β-amylaasi-aktiivisuuden kannalta. Lehtivihreämittaukset tehtiin kasvukaudella 1993 ja 1994 pelto-oloihin tarkoitetulla Minoltan SPAD-502 lehtivihreämittarilla. Mittauksissa käytettiin ylimpiä, täysin kehittyneitä lehtiä. Kenttäkokeina olivat typpitasokokeet (0 – 200 kg N ha^{-1}). Lehtivihreämittaukset selittivät β-amylaasi-aktiivisuutta parhaiten tähkälletulovaiheessa (Zadoksin kasvuasteessa 52–58). Taulukossa 4 on yhteenveto Kymppi-ohran lehtivihreän raja-arvoista tässä kasvuasteessa. Lehtivihreän raja-arvo 37 SPAD rajoitti selvästi Kympin sadonmuodostusta. Sadon menetys oli tällöin 1868 kg ha^{-1}. Jos lehtivihreämittaus oli alempi kuin 41 SPAD, laski sadon valkuaispitoisuus 4,7 prosenttiyksikköä ja β-amylaasi-aktiivisuus 143 W.K. Lehtivihreän raja-arvot optimisadolle olivat 37–41 SPAD-yksikköä, ja parhaalle mahdolliselle valkuaispitoisuudelle ja β-amylaasi-aktiivisuudelle 41–45 SPAD-yksikköä. Tutkimus osoitti ohran jyvien β-amylaasin määrän nousevan voimakkaasti vasta sato-optimin jälkeen. Sato-optimin jälkeen jokaista kymmentä β-amylaasi-aktiivisuuden yksikköä kohden tarvitaan lisä-N noin 11 kg ha^{-1}. Paras mahdollinen β-amylaasi-aktiivisuus oli keskimäärin 252 W.K. ja vastaava valkuaispitoisuus 14,2 %. Sadonkorjuun jälkeen voidaan ohran jyvien valkuaispitoisuuden avulla arvioida epäsuorasti jyvien β-amylaasi-aktiivisuutta.