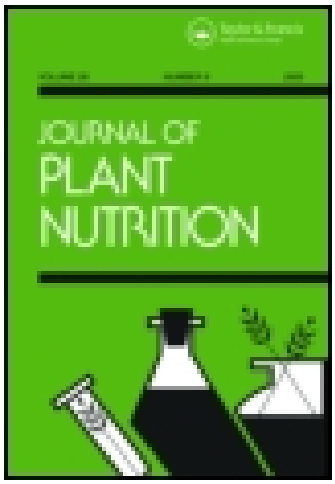

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Diagnosis of Sugar Beet (*Beta vulgaris* L.) Nutrient Imbalance by DRIS and CND-clr Methods at Two Stages during Early Growth

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ABSTRACT

High yield of sugar beet require adequate mineral nutrition. To be diagnosed across interacting nutrients using appropriate interpretation models, the plant must be sampled at a critical physiological stage. This study aimed to develop and validate norms at the 7-leaf and well-developed rosette stages, for diagnostic purposes using the Diagnosis and Recommended Integrated System (DRIS) and Compositional Nutrient Diagnosis based on centered log ratios (CND-clr). Data on nutrient concentrations and plant performance were obtained from 409 plots in West-Central Poland. With respect to the growth stages, for physiological and practical reasons, the 7-leaf stage is preferable for diagnostic purposes. At this growth stage, the high-yield subpopulation characterized by higher concentration of potassium and sodium compared to other nutrients. CND-clr indices were closely related to DRIS indices ($R^2 > 0.93$). The CND-clr indices, however, better explained the differences in the white sugar yield within the validated dataset than the DRIS indices.

Keywords: beet yield, quality, leaf analysis, macronutrients, sodium, critical nutrient indices

INTRODUCTION

Yield potential of sugar beet has been evaluated by Kenter et al. (2006) for European regions extending from northern France through Germany to eastern Poland at the level of 150 Mg ha^{-1}. However, the maximum attainable yields of currently cultivated varieties are 85.6 in France, 82.8 in Germany, and 80.2 Mg ha^{-1} in Poland. In contrast, yields harvested by farmers in these countries are much lower, showing at the same time a large disparity. In France, farmers harvest 86%, in Germany 68%, but in Poland only 50% of the current yield potential of sugar beets (Supit et al., 2010; FAOSTAT, 2012). The key reasons for these differences are attributed to differences in soil fertility levels. Soils in Poland and eastern Germany originate from postglacial material, which are naturally poor in colloids. As a result, they are characterized by low water retention and a natural shortage of nutrients (Grzebisz and Diatta, 2012). In this region, conditions for plant growth undergo a continuous change from humid (France, northern Germany) to be more continental in eastern Germany and Poland (Jongman et al., 2006). Therefore, both soil and climatic factors, negatively affecting sugar beet growth during a particular vegetative season, are responsible for high year-to-year yield variability (Kenter et al., 2006; Supit et al., 2010).

The effect of soil nutrient availability on crop plant growth and/or yield is well described in the scientific literature. Frequently cited production laws, such as Sprengel's "law of the minimum" and Mitscherlich's "law of constant activity," evaluate the level of harvested yield according to the most limiting element (Claupen, 1993). Therefore, concentration of a particular

nutrient in indicative plant parts is used as a diagnostic tool to make a significant evaluation of the nutritional status of the crop plant. The critical nutrient range (CNR) approach allows one to interpret plant nutrient status, using the following rating: deficiency, sufficiency, luxury consumption, and excess (Bates, 1971). It is well recognized that plant stress, resulting in growth disturbance, is related not only to deficiency of a particular nutrient, but also to inadequate relations between nutrients (Fageria, 2001).

In the 1970s, the diagnosis of crop plants' nutritional status was extended from the analysis of one to multiple, interrelated pairs of nutrients. At present, there are two groups of most frequently applied procedures. The first, older one is the Diagnosis and Recommendation Integrated System (DRIS) Beaufils and Sumner, 1976) and modified (M)-DRIS (Hallmark et al., 1987). The second one comprises the Compositional Nutrient Diagnosis using centered log ratios (CND-clr) (Parent and Dafir, 1992) and recently Compositional Nutrient Diagnosis using isometric log ratios (CND-ilr) (Parent, 2011). The preliminary procedure of DRIS and M-DRIS methods relies on bivariate nutrient ratios [nitrogen (N)/phosphorus (P), N/potassium (K), etc.]. The DRIS indices are empirical without a clean and distinct outline of the covariance matrix for conducting multivariate statistical analyses. The attribute of the CND-clr method, in contrast to DRIS, is an accurately stated covariance matrix, allowing the computation of ratios originating from nutrient concentration that are mutually exclusive (Parent, 2011). Up to now, CND norms for diagnosing nutrient imbalance have been developed for such plants as potato (Parent el al., 1994; Khiari et al., 2001a; Bélanger et al., 2005), corn (Khiari et al., 2001b; Magallanes-Quintanar et al., 2006), orange trees (Hernández-Caraballo et al., 2008), *Opuntia ficus-indica* (Blanco-Macías et al., 2010), eucalypt (Silva et al., 2004), *Aloe vera* L. (Garcia-Hernández et al.,

2006), banana (Raghupathi et al., 2002; Wairegi and Van Asten, 2011), and coffee plants (Wairegi and Van Asten, 2012). The scientific literature, however, is lacking of data dealing with optimal ratios between nutrients for the sugar beet, a raw material for sugar production in temperate climates.

The main aims of sugar beet growers are to i) harvest a maximal yield, and ii) increase the quality of beets (Märländer et al., 2003). The achievement of both targets requires a complex evaluation of growth conditions, the practical applicability of which depends on the reliability of a diagnostic test for a well-defined stage of crop plant growth. Classically, the diagnosis of sugar beet nutritional status has been carried out in the period extending from mid-June to the beginning of July (Urlich and Hills, 1990; Bergmann, 1992). It should be pointed out that these norms were established two or three decades ago. The main attribute of currently cultivated sugar beet varieties is much higher vigor of growth. As a result, plants are highly sensitive to external factors, such as the supply of nutrients at very early stages of growth (Malnou et al., 2006; Hergert, 2010). Moreover, most of these norms do not contain data about optimal sodium concentration in sugar beets. As reported by Haneklaus et al. (1998), ca 62% of German soil cropped with sugar beets has an unfavorable K:sodium (Na) ratio. In comparison, in Denmark, only 10% of soils show this disadvantage. This discrepancy is probably related to both soil sodium availability and distance to the sea. Rainfall is considered an important source of this nutrient for growing plants. In Poland, the measured amount of sodium from this source extends from 20 at the Baltic seaside to 1-3 kg Na ha^{-1} in the central parts of the country (Werner et al., 2011).

The aims of this study were to i) determine the norms of sugar beet nutritional status by applying DRIS and CND-clr methods, ii) evaluate the diagnostic value of DRIS and CND-clr, and iii) compare the yield prediction value of both methods at two early but distinct stages of sugar beet growth.

MATERIALS AND METHODS

Trials with sugar beet (*Beta vulgaris* L.) were carried out in the period 1997-2005 on commercial farms. They were located in areas of intensive sugar beet cropping in Central-Western Poland (51–53°N and 16–18° E). In total, 408 observations were conducted. According to the World Reference Base for Soil Resources (WRB) classification system, the majority of studied soils have been classified as Luvisols (85%). The others belong to Phaeozems, Cambisols, and Podzols. Soils are characterized by sandy loam and light loam textures. Chemical properties of the topsoil were variable. The soil pH, measured in a suspension of 1 M potassium chloride (KCl), varied from 4.3 to 7.6. Contents of plant-available P, K, magnesium (Mg), and Na were in the following ranges: 10.3–194.7, 30.5–294.1, 14.8–146.7, and 1.4–29.1 g kg^{-1}, respectively. Plant-available P and K were extracted using the Egner-DL method (calcium lactate, HCl, pH 3.5) and Mg and Na were extracted using 0.0125 M calcium chloride ($CaCl_2$) solution (Fotyma and Dobers, 2008). During the study, total annual precipitation ranged from 369 to 728 mm and average yearly temperature from 7.5 to 9.6°C. It was assumed that soil fertility level, climate, and applied technological measures allow 60 Mg ha^{-1} of beets to be harvested. All procedures, including cultivation, farmyard manure application, seed bed preparation, sowing time, variety

selection, and protection were performed by farmers according to good agricultural practices. Rates of P and K mineral fertilizer application were adjusted to the current soil fertility level.

Sugar beet tissues were sampled at the 7-leaf stage (Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie; BBCH 17, approximately in mid June; BBCH 43 – coding system of growth stages) (Meier, 2001) and at the well-developed rosette stage (BBCH 43, the beginning of July). At the first date, the whole leaf biomass was subjected to chemical analyses. At the second one, only leaf blades of young but fully developed leaves were collected. The representative sample from each observation site consisted of 25 individuals, randomly chosen plants. Tissues were then dried at 60°C, and next ground in a mill. Analysis for total N was conducted by the standard Kjeldahl digestion. Concentration of K, Na, Ca, and Mg, after dissolving the ashes (550°C; 5 h) in dilute nitric acid (HNO_3), was determined by atomic absorption spectrometry and phosphorus by the vanado-molybdate method (Jones and Case, 1990).

Sugar beets were hand-harvested at the stage of technological maturity (BBCH 49) from an area of 10.8 m^2. The evaluation of qualitative parameters of storage roots, i.e., concentration of sucrose (SC), α-amino-N (AmN), K, and Na was performed by using a Venema auto-analyzer (Venema, Groningen, Holland Typ IIG). Representative root samples (25–30 roots per field, randomly collected) were first washed, ground to get a uniform pulp, and clarified with 0.3% aluminum sulfate solution. Potassium and Na concentrations were determined by flame photometry and AmN was analyzed by the fluorometric ortho-phthaldialdehyde (OPA) method. Sucrose concentration in the fresh root was determined by polarimetry. White sugar yield (WSY)

and standard molasses loss (SML) were calculated according to the Brunswick formula (Buchholz et al., 1995):

$$SML = 0.12 (K + Na) + 0.24 AmN + 0.48 \quad (1)$$

$$WSC = SC – SML – SFL \quad (2)$$

$$WSY = (BY \times WSC)/100 \quad (3)$$

where SML is standard molasses loss (%), K + Na is sum of potassium and sodium concentration in beet (mM 100 g^{-1} fresh matter), AmN is α-amino-N concentration in beet (mM 100 g^{-1} fresh matter), SC is sucrose concentration (% on beet fresh matter), SFL is standard factory loss (0.6%), BY is beet yield (Mg ha^{-1}), and WSY is white sugar yield (Mg ha^{-1}).

The total set of data (n = 409) was divided randomly into two groups: a calibration group (n = 368) and a control group (n = 41). The first one was used to determine Diagnosis and Recommendation Integrated System (DRIS) and Compositional Nutrient Diagnosis into centered log ratios (CND-clr) norms, and the second one was applied to validate the norms.

DRIS Method

The DRIS provides a means of ordering nutrient bivariate ratios (N/P, K/N, …, X/Z) into a meaningful expression called the DRIS index (Beverly, 1991). The procedure for selecting the proper ratio, e.g. N/P instead of P/N, was based on obtaining values > 1. An example of calculation of DRIS indices is illustrated below:

$I_N = 1/5[f(N/P) - f(K/N) + f(N/Na) + f(N/Ca) + f(N/Mg)]$ (4)

where

fN/P = 100 [(N/P)/(n/p) - 1] k/CV when N/P > n/p (5)

fN/P = 100 [1 - (n/p)/(N/P] k/CV when N/P < n/p (6)

where I_N = DRIS index; f(N/P), … f(N/Mg) = DRIS functions; N/P = nutrient ratio in the plant under diagnosis; n/p = value of the norm (mean value of high-yielding subpopulation); CV = coefficient of variation for high-yielding subpopulation; k = sensitivity coefficient = 10.

The DRIS indices can have negative or positive values summing up to zero. The more negative the index, the more lacking would be the nutrient relative to others. The sum of DRIS indices, taking into account their absolute values, is termed the nutrient imbalance index, N*II* (Walworth and Sumner, 1987).

CND-clr Method

The CND scores were computed from nutrient concentration (in g kg^{-1} DM), following Parent and Dafir (1992). The filling value R was calculated as follows:

R = 1000 – (N + P + K + Na + Ca + Mg) (7)

Log-centered ratios (V scores) were then computed using the geometric mean:

$G = (N \times P \times K \times Na \times Ca \times Mg \times R)^{1/7}$ (8)

and

$V_N = \ln(N/G)$; $V_P = \ln(P/G)$; $V_K = \ln(K/G)$; .....; $V_R = \ln(R/G)$ (9)

where V_N, V_P, V_K, …. V_R are the log-centered ratios for N, P, K, …. and R.

Nutrient norms are the means and standard deviations (SDs) of the row-centered log ratios of the high-yield subpopulation (indicated by asterisks: V^*_X and SD^*_X). CND indices were computed as follows:

$$I_N = (V_N - V^*_N)/SD^*_N \qquad (10)$$

where I_N = index for N.

The CND-clr nutrient imbalance index of a diagnosed specimen was CND r^2:

$$CND\_r^2 = I^2_N + I^2_P + I^2_K + I^2_{Na} + I^2_{Ca} + I^2_{Mg} + I^2_R \qquad (11)$$

The CND r^2 values were distributed like chi-square values (χ^2) for d + 1 degree of freedom for d analytical results and the filling value. The critical CND r^2 is of 14.1 for a χ^2 having seven degrees of freedom and probability level $\alpha = 0.05$. The critical CND r^2 can be validated by summing up all critical squared CND indices (I^2_X) that were independently determined by the Cate-Nelson partitioning procedure (Khiari et al., 2001c).

Calibration Method

In the first step of computation, pairs of variables, represented by WSY as the dependent value and the nutrient ratio (X/Z) for DRIS or V_x for the CND-clr procedure as the independent one, were established. Then they were arranged in decreasing order from the highest to the lowest variable "WSY". In the next step, to iterate a partition of the database between two subpopulations (using an Excel spreadsheet; Microsoft Corp., Redmond, WA,USA), the Cate-

Nelson procedure was applied (Khiari et al., 2001c). The variance ratios for each iteration were computed as follows:

$f_i(V_x)$ = (variance of V_x; n_1) / (variance of V_x; n_2) (12)

where $f_i(V_x)$ is the ratio function between two subpopulations for element X at the *i*th iteration, $I = n_i - 1$; V_x is the CND-clr ratio or DRIS expression for the nutrient ratio.

The yield cut-off for determining low- and high-yield subpopulations was determined after examining the cubic cumulative variance ratio functions, for DRIS $F^c_i(X/Z)$ and for CND $F^c_i(V_x)$. The latter describes the relationships between cumulative values of variance quotients (y) and the WSY (x). The yields at inflection points of those functions were computed as follows:

$Ip = -b / 3a$ (13)

where Ip = inflection points; b, a = function parameters.

The highest value of the inflection point allows one to define a cut-off point splitting both yield subpopulations. With respect to the CND method, the cut-off points can also be used to determine the theoretical critical χ^2 for qualifying a sample in the high-yield subpopulation (Khiari et al. 2001c). The obtained nutrient ratios (DRIS) and V_x scores (CND), along with concomitant CV and SD coefficients, representing the high-yielding subpopulation, respectively, are considered as DRIS and CND norms. Statistical differences between nutrient ratios and V_x scores for both subpopulations were evaluated by one-way ANOVA (STATISTICA 9; Statsoft Inc., Tulsa, OK, USA).

Validation of Norms

DRIS and CND norms were validated using a separate set of data (n = 41). A set of nutritional indices (I_X) has been calculated as well as N*II* and CND r^2 for each observation. Next, linear regression was applied to evaluate respective relationships between the computed indices. The coefficient of determination (R^2) depicted the closeness of this relationship.

To determine threshold value of I^2_X as well as N*II* and CND r^2, the white sugar yields (WSYs) were partitioned into two groups using the Cate-Nelson iterative procedure (Nelson and Anderson, 1977). First, DRIS and CND indices (I_X) were recalculated as squares of DRIS and CND indices (I^2_X). Then, the critical values of I^2_X were computed. Through this process, a series of variance ratios (F) were obtained for divisions made at various levels of I^2_X. The critical level of I^2_X was taken as that level of I^2_X-division for which F ratio was maximized. In the next step, the Cate-Nelson graphical procedure was applied to define critical WSYs. This was achieved by maximizing the number of points in the negative quadrants (Figure 1). The critical range of I_X indices was determined by computing the root squares of critical I^2_X values. The optimal values of indices have lower and upper limits symmetrical with respect to zero. Any observation below or above this range indicates a status of nutritional imbalance. It is possible, based on the critical ranges, to establish the probability of achieving a fixed yield of a given crop plant (Parent et al., 1994).

RESULTS

Yields of beets (BY) varied from 20.6 to 84.6 Mg ha $^{-1}$, between experimental fields, with the mean amounting to 57.4 Mg ha $^{-1}$. The average sucrose concentration (SC) in sugar beet roots was at a high level (18.2 g kg^{-1} fresh weight), ranging from 13.2 to 21.4 g kg^{-1}. Concentrations of non-sugar impurities, especially of AmN, were highly variable, compared with the SC (Table 1). The white sugar yields (WSY) were in the range of 3.7–13.3 Mg ha^{-1}. The WSY depended mostly on BY (R^2 = 0.71; $P \leq 0.001$; n=409). However, including SC, significantly increased the determination coefficient value of multiple regression:

WSY = -9.688 + 0.158 BY + 0.577 SC; R^2=0.97; $P \leq 0.001$; n=409 (14)

Therefore, WSY can be treated as a synthetic measure of both the BY and its quality. Consequently, it has been taken as the criterion to calibrate DRIS and CND norms.

The average concentration of nutrients (compositional vectors) in the diagnostic tissue samples showed a marked tendency to decrease during the period from the 7th leaf to the fully developed rosette (Table 2). The highest relative decrease was attributed to Ca (-40%), followed by Na (-33%), and K (-30%). The smallest drop was recorded for N and P. The second characteristic refers to year-to-year variability of nutrient concentration. At the 7th leaf stage (BBCH 17), the highest coefficient of variation (CV) was noted for Na followed by Mg and P. At the fully developed rosette stage (BBCH 43), it increased for Ca, Mg, and Na and simultaneously decreased for P concentration. At both stages, N followed by K showed the lowest values of coefficient of variation. The total set of nutrient concentrations was divided into

two subpopulations based on the WSY potential. Regardless of the analyzed plant tissue, the cut-off point represented upper quartile of WSYs (10.1 Mg ha^{-1}). In general, high-yielding subpopulations were characterized by similar values of means, but lower of CVs compared to the whole dataset. The highest differences between two yield categories were found at BBCH 17 for K and Na concentrations (Table 2).

The white sugar yields at inflection points of cubic cumulative variance ratio functions were methods- and growth stage-dependent (data not shown). For the DRIS method, the WSY cut-off at inflection points amounted to 10.3 Mg ha^{-1} for N/Ca at BBCH 17 and 9.4 Mg ha^{-1} for K/Mg at BBCH 43. The share of the population assigned to the high-yielding subpopulation was 24.7% and 38.0%, for both pairs of nutrients, respectively. For the CND method, the ratio of magnesium (V_{Mg}) showed the WSY cut-off at 9.4 Mg ha^{-1} at BBCH 17 and 9.3 Mg ha^{-1} at BBCH 43. The proportion of top yields was 39.4% at BBCH 17 and 41.6% at BBCH 43. Therefore, critical χ^2 values with seven degrees of freedom were 5.4 and 5.1, respectively, for qualifying a sample in the high-yielding subpopulation.

At BBCH 17, the high-yielding subpopulation differed significantly from the low-yielding one for ratios of K/N, K/Ca, K/Mg, K/P, N/Na, and Ca/Na. Top sugar yields were attributed to the relative higher concentration of K compared to N, P, Ca, and Mg, but at the same time to the lower K in relation to Na (Table 3). At BBCH 43, the compositional vectors for P, taking into account N/P, K/P, Ca/P, and N/Ca pairs, show stronger relationships with high-sugar yields than all others. Significant differences between both subpopulations were also attributed to K/Ca and Ca/Mg ratios. Mean values obtained for the high-yielding subpopulation along with CV values are presented as the DRIS norms (Table 4).

With respect to CND-clr method, at BBCH 17, high-yielding crops differed from others in the V_N log ratio. In general, top-yielding crops were characterized by a narrower log ratio of N compared to other nutrients, especially to K (Table 5). At the next growth stage (BBCH 43), there were significant differences between both studied subpopulations for V_P and V_{Ca} log ratios. The high-yield subpopulation characterized by significantly higher V_P and lower V_{Ca} than low-yield subpopulation. The CND-clr norms are the means and standard deviations (SDs) of the row-centered log ratios of the high-yield subpopulation (Table 5).

Nutritional indices were calculated by using the DRIS and CND norm for each object belonging to the validated subpopulation (n = 41). For most nutrients, DRIS and CND indices were strongly interrelated, as underlined by respective coefficients of determination, R^2, ranging from 0.93 to 0.97. The only exception was N at BBCH 43 (R^2 = 0.81). The DRIS N*IIs* showed a linear relationship with CND r^2 indices. However, values of the R^2 coefficients for the computed indices were, in general, much lower compared to nutrient indices (I_X). At BBCH 17, the R^2 coefficient was at the level of 0.65, increasing at BBCH 43 up to 0.85 (Figure 2).

Data used for the validation are an independent part of the whole population. They have been gathered from different environmental and agrotechnical conditions. According to the principle of Sprengel, plant yield is limited by the factor being at minimum, for instance water, soil pH, diseases, nitrogen, etc.. As shown in the study, the closer to zero IN indices were, the higher WSYs were observed, irrespective of the method used and growth stage of sugar beet (Figure 3). Similar relationships were also obtained for the other indices (data not shown). Therefore, for describing the relationships between WSY and DRIS, CND indices, the critical ranges of IX indices as well as DRIS NII and CND r2 were determined by using Cate-Nelson procedure.

In Figure 1, the data in the upper left corner represent the sugar beet plants being diagnosed as nutritive balanced and having a high yield. The data in the lower-right corner represent plants diagnosed as nutrient unbalanced and having a low yield. The lower corner on the left part of figure comprises data on plants well supplied with nutrients, but limited in WSY potential due to the presence of other, non-nutritional factors or random effects. The sufficiency ranges of DRIS and CND indices (I_X) can be computed as the square root of critical values (I^2_X) and are as follows for DRIS I_N at BBCH 17 growth stage (Table 6):

N deficient: $I_N < -3.2$

N sufficient: $-3.2 < I_N < +3.2$

N excess: $I_N > 3.2$.

The other critical ranges of DRIS and CND indices are demonstrated in Table 6 and 7. As shown in the study, the DRIS and CND index sufficiency ranges differed among nutrients and were stage specific. Theoretically, narrower the range shows a higher WSY being harvested. Based on the DRIS the broadest range was obtained for Ca and the narrowest for N, Mg, P, and Na (Table 6). For CND, the broadest range was an attribute of Ca again and to the component R. The nitrogen index (I_N) characterized by a narrow range, showed at the same time high critical WSYs (11.0-11.5 Mg ha^{-1}). This index showed also the highest F ratio of the conducted analysis of variance (Table 7).

The critical DRIS N*II* obtained by the Cate-Nelson partitioning procedure was 35.0 for cut-off yield at 10.8 Mg ha^{-1} at BBCH 17 and 24.0 for cut-off yield at 11.0 Mg ha^{-1} at BBCH 43. The critical CND r^2 amounted to 5.0 and 1.8, respectively, to cut-off yields at 10.9 and 11.0 Mg ha^{-1}. It is worth noting that the partition of the validation group was statistically significant using

only DRIS N*II* at BBCH 17 (F = 5.18*) and CND r^2 at BBCH 43 (F = 15.1***). In comparison, critical CND r^2 obtained by summing of the squared CND indices (I^2_X) amounted to 5.9 and 4.7, respectively to both studied stages of sugar beet growth (Table 6).

DISCUSSION

The yield of beets, averaged across the period 1997-2005, amounted to 57.4 Mg ha^{-1}. This was slightly lower than the assumed target. By comparison, in Germany, the average yield of this crop in this period was 56.8 Mg ha^{-1}. However, compared to average growing conditions in Poland, the obtained average yield was 35% higher (FAOSTAT, 2012). It can be concluded that the BY was high, indirectly corroborating the thesis of a high level of applied cropping technology. White sugar yield (WSY) has been taken as the criterion to calibrate DRIS and CND norms in the study. Several researches have revealed that selection of the reference subpopulation is an important factor for the nutrient imbalance diagnosis effectiveness and success. In scientific literature, there are many methods for selection of the reference subpopulation. For DRIS, the whole population is divided into two-subpopulations or categories taking into account the arbitrarily chosen yield, descriptive statistics, or economic effects (Beaufils and Sumner. 1976; Sharma et al., 2005; Sema et al., 2010). There is also proposed to consider variance ratios of nutrient expressions to discriminate between sub-populations (Walworth and Sumner 1987). For CND methods, the selection of the high-yielding sub-population was conducted across multiple ratios using a cumulative variance function fitted to cubic equation (Khiari et al., 2001c), Boltzman equation (Hernández-Caraballo et al., 2008),

Boundary-line method (Quesnel et al., 2006; Blanco-Macías et al., 2010), chi-square distribution function (Parent et al., 1994), and Aitchison or Mahalanobis distances (Parent, 2011). In the current study, the first method was applied. An application of this procedure allowed the discrimination of high-yield subpopulations of WSY that were higher than the experimental mean (9.0 Mg ha^{-1}). It is worth noting that yield cut-off values for dual ratios (DRIS) were on average higher (10.3 and 9.4 Mg ha^{-1}) compared to multiple ratios calculated by CND-clr method (9.4 and 9.3 Mg ha^{-1}).

Classically, the diagnosis of sugar beet nutritional status has been carried out in the period extending from mid-June to the beginning of July (according to the BBCH coding system 43). Using the nutrient concentration at BBCH 43, it can be documented that the crop was generally well supplied with P, K, Ca, and Mg. Only the average concentration of N appeared moderately below the critical value as proposed earlier (Bergmann, 1992). This discrepancy can be explained by a higher growth rate of the current varieties, in turn diluting N content slightly faster (Märländer et al., 2003). It is well recognized that the rate of leaf dry matter growth, and also of fibrous roots, at the earliest stages of growth best fits an exponential model. From the 7^{th} leaf stage to the fully developed rosette, the increase in the dry matter of the canopy follows a linear model (Milford et al., 1985). This change is probably the main reason for the much higher variation of nutrient concentration at BBCH 17 than at BBCH 43. At the first critical stage, as documented in Table 2, all nutrients achieved maximum concentration followed by a subsequent dilution. However, the rate of decrease was nutrient specific, showing limited dilution of N and P but much higher of cations.

Crop yield in-season performance is highly sensitive to deficiency or imbalance of nutrient supply. Therefore, the rational choice of the crucial stage of sugar beet growth with respect to yield of sugar (WSY) should take into account two contradictory aspects. The first one refers to the expected WSY decrease due to a shortage of a particular nutrient at a critical stage of growth. The second one concerns technological measures aimed at correcting the rate of crop growth via fertilizer application, if necessary. In the conducted study, the nutritional status of sugar beet was evaluated in two stages: 7-leaf (BBCH 17) and fully developed rosette (BBCH 43). The results obtained using DRIS and CND-clr validation underline a very close predictive value of both growth stages. However, the evaluation of sugar beet nutritional status at BBCH 43 is an *ex-post* procedure, because in general, it is too late to avoid yield losses. It is necessary to take into account the fact that the appearance of consecutive leaves in a sugar beet plant up to the stage of BBCH 17 is governed by accumulated temperature, circa 900°C d, from sowing (accumulated daily mean air temperature above 3°C as a physiological base). This amount of heat is required to reach 85% of canopy cover, which occurs at BBCH 43 (Werker and Jaggard, 1997; Kenter et al., 2006). This physiological target is a prerequisite for high-sugar yield, provided that a well-developed plantation has taken up 120 kg N ha^{-1} (Malnou et al., 2006). It is well documented that N is the nutrient most limiting beet productivity (Hergert, 2010). The application of low N rates results in decreased root tonnage. However, too high rates of N leads to reduced sugar content and increased concentration of impurities, such as AmN, K, and Na (Hoffmann and Märländer 2005). Management and N uptake by sugar beet plants depends on the many factors, especially on the availability of other nutrients. Moreover, some nutrients play an important role in water uptake during the growing season. In crop plants, this function is

attributed mainly to K but in sugar beets also to Na (Subbarao et al., 2003; Cakmak, 2005). Therefore, content of soil available K and Na and their supplies to sugar beet are extremely important in regions experiencing frequent drought during the growing season. An adequate K/Na ratio in beet leaves controls beet yield (Haneklaus et al., 1998; Wakeel et al., 2009). As reported by Haneklaus and et al. (1998), the sugar beet requirement for Na increases at early stages of growth. However, there is a lack of standardized data on sugar beet nutritional ranges for Na at 7-leaf growth stage.

The conducted study implicitly showed that at the 7-leaf stage, high-yielding plants differed from others in K/N, K/Ca, K/Mg, K/P, N/Na, and Ca/Na ratios. Top WSY resulted from a higher share of K with respect to N, P, Ca and Mg, but lower regarding Na. For ratios calculated by the CND method, a significant difference was obtained for V_N only. High-yielding plants were characterized by a narrower ratio of N with respect to other investigated nutrients, mainly K. The obtained results, on the one hand, implicitly indicate the necessity to regulate content of available K in Polish soils (Grzebisz and Diatta, 2012). However, they stress the specific positive response of sugar beet to Na (Subbarao et al., 2003).

In contrast to the first growth stage, at BBCH 43, the main compositional vectors determined WSY were concentration of P and Ca. In general, the high-yield subpopulation characterized by significantly higher V_P but lower V_{Ca} than low-yield subpopulation. The P-Ca antagonism in plant tissues has been previously reported by some authors (Parent et al., 1994; García-Hernández et al., 2006; Magallanes-Quintanar et al., 2006). This negative relationship may result from reduced activity of P in the soil solution due to forming low solubility of Ca-P

minerals, especially on soils rich in exchangeable Ca^{2+}, and thus reducing P uptake by plants (Hinsinger, 2001).

The validation of the DRIS and CND norms is traditionally carried out by factorial schemes, based on the design of the fertilization experiments (Sumner, 1979; Khiari et al., 2001c, Bélanger et al., 2005). An alternative method of validation is to determine whether the frequencies of the most limiting nutrients are randomized or not, e.g. by chi-square test (Silva et al., 2004). In this study, the validation CND norms as well as DRIS norm was carried out by using the Cate-Nelson iterative procedure. Conventional statistical analyses (analysis of regression) were not applied since observations forming the validation subpopulation have been collected at different growth conditions, such as years, soils, and agro-techniques. According to Khiari et al. (2001c), Cate-Nelson method gives a possibility to obtain nutrient sufficiency ranges. Moreover, F ratios (or R^2) of iterative variance analysis reflect the strength of relationships between DRIS or CND indices and yield in the validation data set. According to some authors both methods showed the same diagnostic value (Serra et al., 2010; Wairegi and van Asten, 2011; Wairegi and van Asten, 2012). Silva et al. (2004) showed that diagnosis concordance level among the methods was procedure-dependent, and varied according to the nutrient concentration in tissues samples. As shown in this study, DRIS and CND-clr indices were significantly, positively correlated. However, CND-clr indices exhibited higher statistical significance compared to DRIS, for discriminating differences in WSY in the validation group of data. At both stages, significant F values of iterative ANOVA procedure were obtained more frequently with CND than DRIS indices, being in line with other studies (Khiari et al., 2001c, Kumar et al., 2003). Application of the CND-clr method corrects inherent biases generated by

DRIS (Parent and Dafir, 1992; Parent et al., 2012). It is worth noting that CND I_N was the most important discriminator between the balanced (high-yield) and unbalanced (low-yield) subpopulations within the control database. Thus, the study confirms the importance of yield-forming impact of nitrogen taken up by sugar beet crop at early stages of growth (Malnou et al., 2006). According to Parent et al. (1995), CND method could improve the shape of the yield-N response curve since the CND expression for N status adjusts N concentration to other nutrient concentrations in the tissues. In contrast to I_X indices, ambiguous results were obtained while comparing N*II* and CND r^2. At BBCH 17, the population partition was statistically more effective for N*II* compared to CNDr^2. An inverse relationship was obtained at BBCH 43. This outcome seems to be only seemingly contradictory to the results of correlation analysis. As documented, both indices showed a significant relationship. The main causes of the observed inconsistency are rooted in differences of cut-off points. They are the key discriminator of the chemical composition of high-yield subpopulations of sugar beet, and thus DRIS and CND norms, especially at BBCH 17. Another possible explanation was that N*II* and CND r^2 are, in fact, sum of individual indices. As a consequence, there is an increasing probability of some uncontrolled observations, which bias the Cate-Nelson analysis of variance (Nelson and Anderson, 1977).

According to Khiari et al. (2001c), the proportion of low-yield specimens in calibration population is a chi-square value of the cumulative function which can we use to define a critical CND r^2. In this study, they amounted to 5.4 and 5.1, in respective growth stage. For validation, the independent data set should to be characterized by a similar value (Bélanger et al., 2005, Garcia-Hernández et al., 2006). As shown, critical values of CND r^2 obtained summing CND I^2_X

indices mostly represent the nutritionally balanced sugar beet plants at BBCH 43 than at BBCH 17 (5.9 and 4.7, respectively). Despite this, it should be noted that at both growth stages crucial values of CND r^2 oscillated around the crucial values for the calibration population.

The presented data implicitly underline the advantage of the CND-clr method for WSY prediction compared to the DRIS one. However, this method does not have a conceptual basis to make a simple interpretation of nutrient interactions. According to Parent (2011) the CND procedure, in the form of log ratio (ilr) coordinates, has overcome this difficulty. Some authors, however, propose to use PCA analysis to determine nutrient interaction for indices obtained by CND-clr method (Raghupathi et al., 2002; García-Hernández et al., 2006; Wairegi and van Asten, 2012). In addition, the CND-clr method allows a simple comparison of nutritional indices (e.g. I_N, I_P, I_K, etc.) for particular nutrients, calculated by DRIS method. The latter one is the most frequently applied to evaluate a nutrient imbalance. Therefore, the clr transformation is useful to conduct exploratory analyses on compositional data (Parent et al., 2012).

CONCLUSION

Data obtained at both growth stages can be used to diagnose of nutrient imbalances and to predict white sugar yield. Taking into account both physiological and agronomic applicability, the 7th leaf stage is preferable for making in-season correction of sugar beet nutritional status. At this growth stage, plant tissues of the high-yield subpopulation were characterized by a higher concentration of K compared to N, P, Ca, and Mg, but lower with regards to Na. The obtained results implicitly indicate the necessity to regulate content of available K and Na in Polish soils. Both statistical methods showed almost the same diagnostic value. However, white sugar yield prediction was statistically more significant applying the CND-clr procedure, especially by using of I_N.

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TABLE 1 Statistical overview of sugar beet yields and quality (n = 409)

Yield and quality characteristics	Descriptive statistics						
	Mean	SD	CV, %	Min.	Max.	Median	Skew-ness
Beet yield, Mg ha^{-1}	57.4	10.4	18.1	20.6	84.6	57.3	-0.12
Sucrose conc., %	18.2	1.4	7.9	13.2	21.4	18.3	-0.19
AmN, mmol kg^{-1}	22.2	8.9	39.9	2.4	46.2	20.9	0.30
K, mmol kg^{-1}	44.8	10.8	24.2	4.8	79.8	44.1	2.98
Na, mmol kg^{-1}	6.0	3.0	49.6	1.8	29.3	5.4	-0.05
WSY[A], Mg ha^{-1}	9.0	1.6	18.0	3.7	13.3	9.0	-0.04

[A] White sugar yield calculated by using the Brunswick formula (Buchholz et al., 1995)

TABLE 2 Statistical overview of nutrient concentration in plant tissue samples (g kg^{-1} DM) in relation to growth stages and white sugar yield (WSY) categories

Growth stage	Nutrient	Whole population (n = 409)			High-yield subpopulation [A] (n = 102)		
		Mean	SD	CV, %	Mean	SD	CV, %
BBCH 17	N	40.6	4.1	10.0	40.2	3.5	8.7
	P	3.9	1.8	45.6	3.7	1.4	38.6
	K	58.9	11.4	19.3	62.0	11.8	19.1
	Na	10.4	5.1	48.9	11.0	4.7	42.8
	Ca	11.2	3.2	29.1	10.8	2.8	25.8
	Mg	4.8	1.9	40.0	4.6	1.5	33.3
BBCH 43	N	37.2	4.2	11.3	37.4	3.3	8.8
	P	3.6	1.1	30.7	3.8	1.1	29.0
	K	41.5	8.0	19.2	40.7	7.3	17.8
	Na	7.0	3.5	54.9	7.7	4.2	50.2
	Ca	6.8	2.8	40.6	6.2	2.5	40.4
	Mg	4.1	1.8	43.5	3.9	1.4	35.3

[A] The WSY cut-off at 10.1 Mg ha^{-1} (related to the upper quartile of dataset)

TABLE 3 The mean and coefficient of variation CV (%) of nutrient ratios, for high- and low-yield subpopulations of sugar beet at BBCH 17 growth stage

Nutrient ratios	High-yield [A)]		Low-yield	
	Mean	CV	Mean	CV
N/P	11.97	29.2	11.84	31.6
K/N	1.57***	20.2	1.43	20.2
N/Na	4.41*	45.0	5.09	51.7
N/Ca	4.12	40.6	3.96	39.0
N/Mg	9.71	34.0	9.62	38.2
K/P	19.41*	40.3	17.33	41.8
K/Na	6.96	52.9	7.37	58.6
K/Ca	6.31**	37.9	5.53	34.0
K/Mg	15.20*	38.6	13.53	39.1
Ca/P	3.26	42.4	3.36	45.7
Ca/Na	1.21*	62.7	1.44	64.5
Ca/Mg	2.58	41.9	2.71	50.4
P/Mg	0.91	48.7	0.91	51.5
Na/Mg	2.72	64.9	2.58	75.1
Na/P	3.30	56.9	3.06	64.0

*, ** and*** The variance ratio between high- and low-yield subpopulations significant at $P \leq 0.05$; 0.01 and 0.001 respectively.

[A)] The means and CV (%) of ratios for high-yield subpopulation are the DRIS norm.

TABLE 4 The mean and coefficient of variation CV (%) of nutrient ratios, for high- and low-yield subpopulations of sugar beet at BBCH 43 growth stage

Nutrient ratios	High-yield [A)]		Low-yield	
	Mean	CV	Mean	CV
N/P	10.34***	23.5	11.71	31.6
K/N	1.09	19.7	1.13	19.0
N/Na	6.91	55.3	6.77	58.4
N/Ca	7.19***	50.1	5.91	42.9
N/Mg	10.79	32.2	11.23	63.8
K/P	11.28***	30.2	13.16	34.5
K/Na	7.52	61.0	7.62	59.9
K/Ca	7.47**	38.3	6.59	41.2
K/Mg	11.58	32.2	12.41	57.6
Ca/P	1.81***	64.7	2.35	60.2
Ca/Na	1.16	69.9	1.28	61.5
Ca/Mg	1.77*	51.0	2.11	66.0
P/Mg	1.11	42.8	1.01	59.7
Na/Mg	2.00	54.4	1.94	51.8
Na/P	2.01	59.8	2.26	59.4

*, ** and*** The variance ratio between high- and low-yield subpopulations significant at $P \leq 0.05$; 0.01 and 0.001 respectively.

[A)] The means and CV (%) of ratios for high-yield subpopulation are the DRIS norm.

TABLE 5 The means and standard deviations (SD) of row-centered log ratios (V_X), for high- and low-yield subpopulations of sugar beet at BBCH 17 and BBCH 43 growth stages

Log ratios (V_X)	High-yield [A)]		Low-yield	
	Mean	CV	Mean	CV
BBCH 17				
V_N	0.598*	0.093	0.624	0.103
V_P	-2.101	0.348	-2.100	0.338
V_K	0.985	0.215	0.958	0.210
V_{Na}	-0.833	0.437	-0.875	0.476
V_{Ca}	-0.720	0.285	-0.713	0.297
V_{Mg}	-1.599	0.305	-1.582	0.350
V_R	3.670	0.109	3.689	0.135
BBCH43				
V_N	0.716	0.146	0.700	0.151
V_P	-1.601***	0.272	-1.720	0.290
V_K	0.788	0.169	0.807	0.183
V_{Na}	-1.051	0.470	-1.088	0.417
V_{Ca}	-1.145***	0.377	-1.001	0.324
V_{Mg}	-1.598	0.264	-1.603	0.370
V_R	3.672	0.109	3.688	0.136

*, ** and*** The variance ratio between high- and low-yield subpopulations significant at $P \leq 0.05$; 0.01 and 0.001 respectively.
[A)] The means and CV (%) of ratios for high-yield subpopulation are the DRIS norm.

TABLE 6 Critical squared DRIS indices (I^2_X) for the validation subpopulation using the Cate-Nelson partitioning procedure, together with their respective white sugar yields cut-off (WSY_{crit}), critical ranges of DRIS indices, and variance ratios (F)

Growth stage	Nutrient	WSY_{crit} (Mg ha^{-1})	Critical I^2_X	F ratio	Lower limit of I_X	Upper limit of I_X
BBCH 17	N	10.0	10.1	0.95	-3.2	+3.2
	P	11.0	4.1	3.09	-2.0	+2.0
	K	11.0	20.6	2.82	-4.5	+4.5
	Na	11.0	1.6	4.27*	-1.3	+1.3
	Ca	8.8	311.0	5.31*	-17.7	+17.7
	Mg	9.5	6.0	0.20	-2.5	+2.5
BBCH 43	N	10.5	17.9	2.93	-4.2	+4.2
	P	11.7	10.2	2.76	-3.2	+3.2
	K	10.0	48.0	1.13	-6.9	+6.9
	Na	11.5	1.6	8.43**	-1.3	+1.3
	Ca	10.4	52.6	1.88	-7.3	+7.3
	Mg	11.5	0.1	1.28	-0.3	+0.3

* and** The variance ratio between WSYs of balanced and unbalanced subpopulations significant at $P \leq 0.05$ and 0.01 respectively

TABLE 7 Critical squared CND indices (I^2_X) for the validation subpopulation using the Cate-Nelson partitioning procedure, together with their respective white sugar yields cut-off (WSY_{crit}), critical ranges of CND indices (I_X), and variance ratios (F)

Growth stage	Nutrient	WSY_{crit} (Mg ha^{-1})	Critical I^2_X	F ratio	Lower limit of I_X	Upper limit of I_X
BBCH 17	N	11.5	0.04	9.96**	-0.20	+0.20
	P	11.0	0.02	4.77*	-0.15	+0.15
	K	9.3	1.02	1.35	-1.01	+1.01
	Na	8.5	1.84	4.32*	-1.36	+1.36
	Ca	8.5	2.69	5.31*	-1.64	+1.64
	Mg	10.4	0.01	2.24	-0.12	+0.12
	R	9.5	0.26	3.84	-0.51	+0.51
	$CNDr^2 = \sum I^2_X$	---	5.89	---	0	5.89
BBCH 43	N	11.0	0.01	4.50*	-0.08	+0.08
	P	9.0	1.45	4.05*	-1.20	+1.20
	K	10.1	0.65	1.30	-0.81	+0.81
	Na	11.2	0.03	4.29*	-0.17	+0.17

Ca	10.9	0.17	1.75	-0.41	+0.41
Mg	10.2	0.51	2.83	-0.71	+0.71
R	8.2	1.91	2.76	-1.38	+1.38
$CNDr^2 = \sum I^2_X$	---	4.73	---	0	4.73

* and ** The variance ratio between WSYs of balanced and unbalanced subpopulations significant at $P \leq 0.05$ and 0.01 respectively

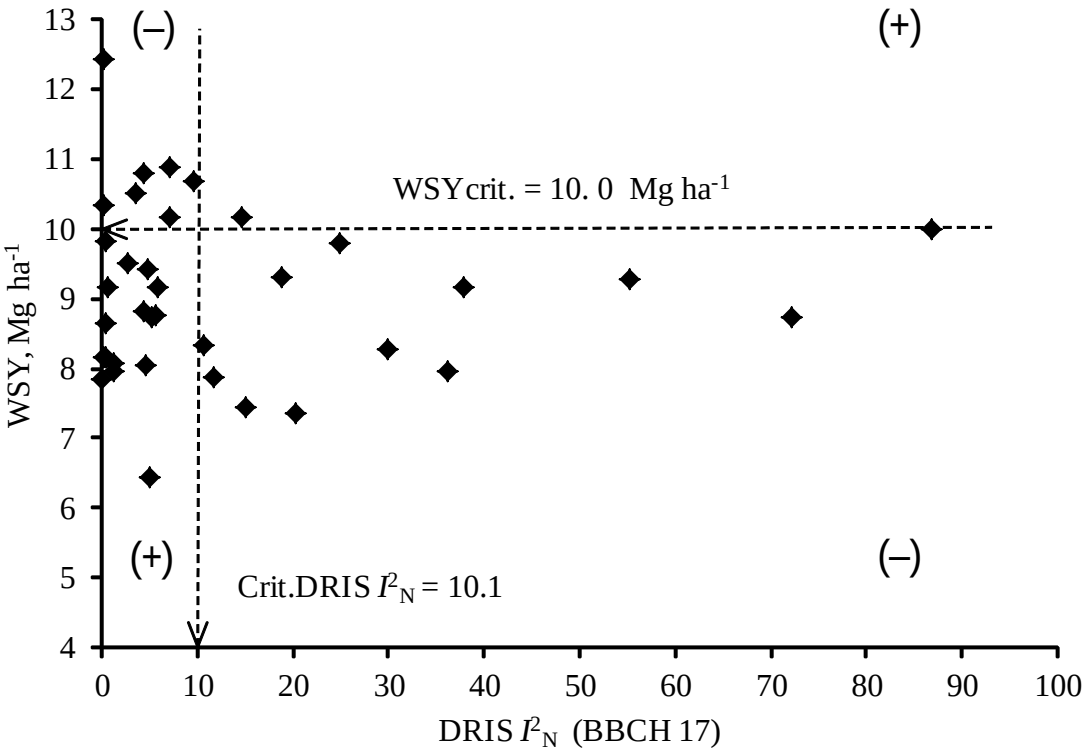

FIGURE 1 Cate-Nelson partitioning procedure – relationship between nutrient imbalance index DRIS I^2_N and white sugar yield (WSY) of sugar beet for the validation database at BBCH 17 growth stage

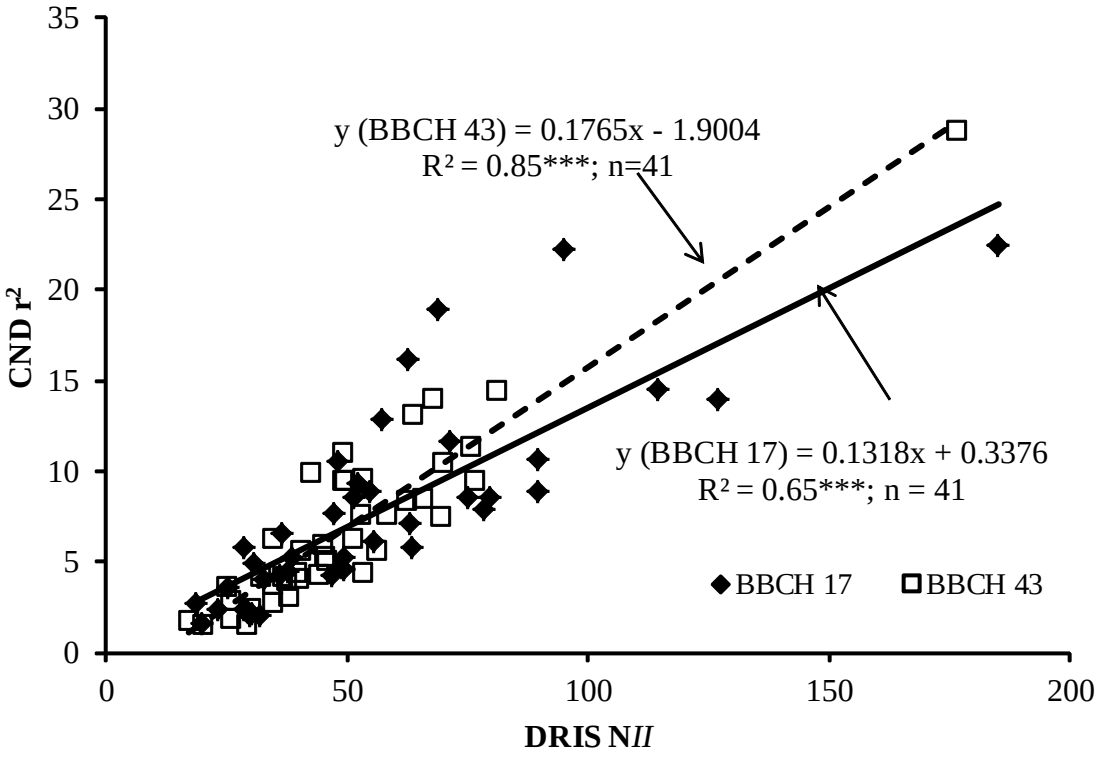

FIGURE 2 Relationships between nutrient imbalance indices N*II* (DRIS) and CND r^2 indices at two growth stages of sugar beet, BBCH 17 and BBCH 43 (n = 41)

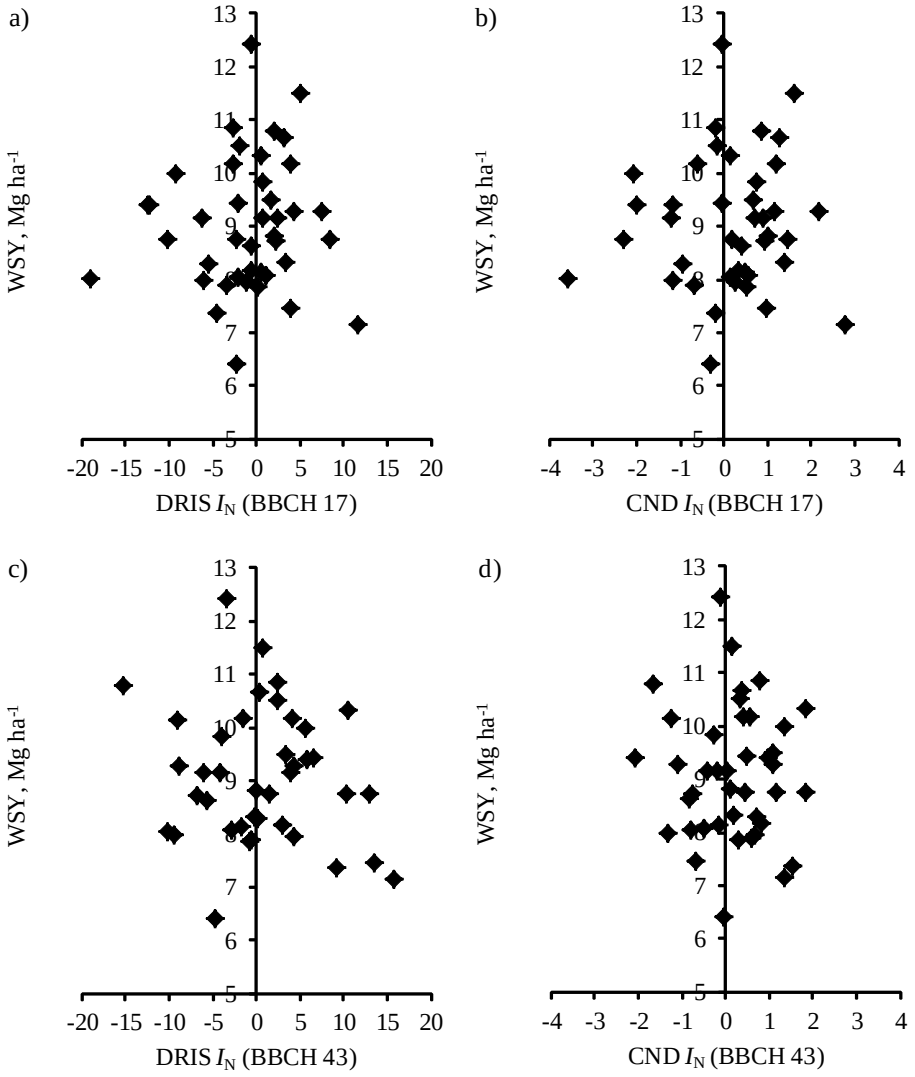

FIGURE 3 Scatter diagrams of DRIS I_N and CND I_N scores plotted against the white sugar yield (WSY) for the validation database at two growth stages of sugar beet: BBCH 17 (a, b) and BBCH 43 (c, d)