SUMMARY

The Diagnosis and Recommendation Integrated System (DRIS) can improve interpretations of leaf analysis to determine the nutrient status. Diagnoses by this method require DRIS norms, which are however not known for oil content of soybean seeds. The aims of this study were to establish and test the DRIS method for oil content of soybean seed (maturity group II cultivars). Soybean leaves (207 samples) in the full flowering stage were analyzed for macro and micro-nutrients, and the DRIS was applied to assess the relationship between nutrient ratios and the seed oil content. Samples from experimental and farm field sites of the southernmost Brazilian state Rio Grande do Sul (28° - 29° southern latitude; 52° - 53° western longitude) were assessed in two growing seasons (2007/2008 and 2008/2009). The DRIS norms related to seed oil content differed between the studied years. A unique DRIS norm was established for seed oil content higher than 18.68% based on data of the 2007/2008 growing season. Higher DRIS indices of B, Ca, Mg and S were associated with a higher oil content, while the opposite was found for K, N and P. The DRIS can be used to evaluate the leaf nutrient status of soybean to improve the seed oil content of the crop.

Index terms: nutritional diagnosis, plant nutrition, nutritional balance, Glycine max (L.) Merrill.
RESUMO: SISTEMA INTEGRADO DE DIAGNOSE E RECOMENDAÇÃO (DRIS) PARA DIAGNÓSTICO DO TEOR DE ÓLEO EM GRÃO DE SOJA

O Sistema Integrado de Diagnose e Recomendação (DRIS) pode melhorar a interpretação da análise foliar, contribuindo com a avaliação do estado nutricional. Para diagnósticos obtidos com esse método, é imprescindível que as normas DRIS sejam previamente estabelecidas, o que ainda não foi efetuado para o teor de óleo do grão de soja. Os objetivos deste trabalho foram estabelecer as normas DRIS e avaliar esse método, em relação ao teor de óleo do grão usando o método DRIS. As amostras foram obtidas em parcelas experimentais em lavouras comerciais do Estado do Rio Grande do Sul (28° e 29° de latitude sul; e 52° e 53° de longitude oeste), nas safras 2007/2008 e 2008/2009. As normas DRIS relacionadas com o teor de óleo do grão diferiram entre os anos agrícolas. Foi estabelecida uma única norma DRIS para o teor de óleo do grão maior que 18,68 %, com os resultados do banco de dados do ano 2007/2008. O acréscimo dos valores dos índices DRIS de B, Ca, Mg e S foi acompanhado do maior teor de óleo, ocorrendo o contrário com os índices de K, N e P. O DRIS pode ser usado para avaliar o status de nutrientes de folha de soja, com o objetivo de melhorar o teor de óleo do grão dessa cultura.

Termos de indexação: diagnose nutricional, nutrição vegetal, equilíbrio nutricional, Glycine max (L.) Merrill.

INTRODUCTION

Soybean (Glycine max (L.) Merril) is the major source of oil and protein in the international market. The economic importance of this crop is mostly due to the high oil and protein concentrations in soybean seed (Câmara et al., 2004). Previous studies related to oil and protein content in soybean seed suggested high variations across cultivars (Maestri et al., 1998; Rangel et al., 2004, 2007) and across environmental conditions and factors (Bachlava & Cardinal, 2009; Carrera et al., 2009; Proulx & Naeve, 2009), mainly during reproductive growth (Wilson, 2004).

The plant nutritional status varies according to the factors that influence the soil availability of nutrients (Taiz & Zeiger, 2009). The most widely used method for the diagnosis of plant nutritional status is based on critical leaf nutrient content. However, this method does not allow assessing the relationship among nutrients. On the other hand, the Diagnosis and Recommendation Integrated System (DRIS) has been proposed as an alternative to critical leaf nutrient content. DRIS can be used to assess the interaction of nutrient leaf analysis (Urano et al., 2007; Farnezi et al., 2009), and most studies with this approach focus on nutrient balance, fertilization practices and crop yield (Urano et al., 2007; Bailey et al., 2009; Farnezi et al., 2009). Besides yield, nutrient imbalances or deficiencies may affect the oil content of the seeds. However, few studies have been conducted to assess the effect of nutrient balance on oil content of soybean seeds (Seguin & Zheng, 2006).

Although DRIS has been proposed as a universal method (Beaufils, 1973), recent studies have pointed out that the establishment of regional DRIS norms or standards enables more accurate diagnoses of the plant nutritional status (Silva et al., 2005; Farnezi et al., 2009). DRIS has proved to be a promising method for the calibration of optimal nutrient concentrations in soybean (Urano et al., 2007).

DRIS norms for soybean have not been established for the state of RS, thus restricting the application of this method. Also, the scientific literature does not discuss DRIS norms based on oil concentration as a response variable. Moreover, other methods for the interpretation of leaf analysis have not been investigated for the improvement of the nutritional status of soybean given the increase in seed oil content. The aims of this study were to establish and test the DRIS method for oil content of soybean seeds.

MATERIAL AND METHODS

Location

Soybean plants assessed for the development of DRIS norms were grown in the experimental area of the University of Passo Fundo (UPF), whereas the plants assessed for the validation of these norms were from farm fields of the Planalto Médio Region of the State of Rio Grande do Sul (28° - 29° southern latitude; 52° - 53° western longitude), in the 2007/2008 and 2008/2009 growing seasons. The regional climate was classified according to Köppen as Cfa characterized as subtropical wet, without a clearly defined dry season, average temperature higher than 22 °C in the hottest month (Cunha, 1997), average annual...
temperature of 17.5 °C and average annual rainfall of 1,787.8 mm (Embrapa Trigo, 2008). The assessed plants were not irrigated. The soils of the assessed areas were a Dystroferric Red Nitosol and a Humic Dystrophic Red Latosol. These soils with no physical or nutritional constraints had been under no-tillage for over 10 years. The variability in soil fertility across experimental plots and farm fields was a prerequisite for this research, for investigating differences in the nutritional status of leaf samples.

**Database, sampling protocol and analysis**

Two data sets (2007/2008 and 2008/2009 growing seasons) were constructed, both to establish the DRIS norms and to test them. To establish the norms, 84 (2007/2008) or 94 (2008/2009) samples were analyzed, while to test the DRIS method, 15 (2007/2008) or 14 (2008/2009) samples were assessed. The cultivars BRS 243 RR (2007/2008) and Fundacep 55 RR (2008/2009), both belonging to maturity group II, were used to establish DRIS norms. The soybean cultivars 6445 RR, 6001 RR, Apolo RR, Fundacep 53 RR, and Fundacep 55 RR were evaluated in the farm fields, all of maturity group II.

Rainfed crops were managed in compliance with the recommended cultural practices. In the experimental area, plots (3.5 x 6 m), and black oats (Avena strigosa Schreb) was used as cover crop prior to soybean in both growing seasons. The sampled farm fields covered approximately 1 ha. Leaf samples consisted of 30 recently matured trifoliate leaves, with petioles (Farias et al., 2007), randomly collected during full flowering (R2 stage) (Neumaier et al., 2000). The total concentrations of N, P, K, Ca, Mg, S, B, Zn, Cu, Mn, and Fe in the leaf tissue were determined by the method described by Tedesco et al. (1995).

Seed samples were obtained from the whole area of experimental plots and farm fields with a combine harvester, to determine the oil content by near-infrared reflectance spectroscopy (NIRS) (van Kempen & J. Jackson, 1996). This method had been calibrated previously by determining the oil content of 120 soybean seed samples using the Soxlet method (AOAC, 1995) and hexane as solvent. The results were expressed as percentage of dry mass (65 °C).

**DRIS establishment**

The total leaf-nutrient content data were processed by software developed at UPE, generating the DRIS norms and nutrient indices. One of the binary relations of nutrients of direct or inverse order was used to compute the DRIS indices. The largest variance ratio obtained between the high- and low-yielding subpopulations adopted for the selection of direct (A/B) or inverse (B/A) nutrient relations was used as criterion. From the binary relations, DRIS indices (IA) may then be calculated for nutrients, using the generalized equations proposed by Beaufils (1973), where n is the number of relations of direct order, k the sensitivity constant, CV the coefficient of variation and m is the number of relations of inverse order:

\[
I_A = \sum_{i=1}^{n} f\left(\frac{A}{B} \right) \frac{K}{CV} x 100 + f\left(\frac{A}{C} \right) \frac{K}{CV} x 100 + \ldots + f\left(\frac{A}{N} \right) \frac{K}{CV} x 100 + \ldots + \sum_{m=1}^{n} f\left(\frac{B}{A} \right) \frac{K}{CV} x 100 + \ldots + f\left(\frac{N}{A} \right) \frac{K}{CV} x 100 \right] / (n + m)
\]

After obtaining the DRIS indices for the nutrients, the nutritional balance index (NBI) of the sample was calculated. This index consists of the sum of all DRIS indices in absolute values, as described by Wadt (1996):

\[
NBI = |I_A| + |I_B| + |I_C| + \ldots + |I_N|
\]

The mean NBI (mNBI) was calculated by dividing the NBI by the number of preliminary indices (per nutrient) used in the calculation (Wadt, 1996):

\[
NBI_m = \frac{|I_A| + |I_B| + |I_C| + \ldots + |I_N|}{n}
\]

The DRIS norms were obtained from the mean values of the existing binary relations between the analyzed nutrients and the standard deviation(s) (Malavolta, 2006). The DRIS indices were interpreted by classifying the nutrients according to their potential response to fertilization, as proposed by Wadt (1996). The classes of positive, negative and zero responses were designated as limiting factors due to deficiency, excess, and null (without limitation), respectively (Silva et al., 2005).

The reference subpopulations consisted of samples with higher oil contents than the mean-plus-one standard deviation (18.68; 21.38 and 20.62 %, in the 2007/2008 and 2008/2009 growing seasons and for the combined data set, respectively). These mean values can be considered adequate since, in general, soybean has a seed oil content of 11.29 - 32.45 % (Albrecht et al., 2008). The number of reference population samples was 13 in the harvest of 2008; 24 in the harvest of 2009; and 35 in the combined data set. These numbers of samples are higher than the minimum of 10 % of samples from the database, which is considered adequate by Letzsch & Sumner (1984). Different samples were evaluated in each year and for each experimental plot.

**Statistical analysis**

Normal distribution and other descriptive statistical parameters were calculated by the Sisvar software (Ferreira, 2000). The t test was used to compare the contents of leaf nutrients and seed oil, obtained from the 2008 and 2009 harvests and from their combined data set. The norms were analyzed according to the DRIS indices, as proposed by Wadt.
(1996), using the chi-square test, in addition to the correlation between oil content (dependent variable) and NBI, using Excel (Microsoft Office® 2007).

To assess the nutritional status diagnosed with the proposed norms, plants from farm fields (14 and 15 samples, in 2008 and 2009, respectively) were evaluated by using the DRIS norms.

**RESULTS AND DISCUSSION**

Ranges for nutrient concentration in leaves and seed oil content of the reference subpopulations (oil content >18.71, 21.36 and 21.05 % in 2007/2008 and 2008/2009 and in the combined data set, respectively) across growing seasons are shown in table 1. The observed ranges in our study (N 32.8-59.4 g kg⁻¹; P 1.2-5.6 g kg⁻¹; K 10.2-46.7 g kg⁻¹; Ca 5.0-12.7 g kg⁻¹; Mg 1.8-5.0 g kg⁻¹; S 0.8-3.5 g kg⁻¹; Zn 19.34-75.61 mg kg⁻¹; Cu 3.81-13.72 mg kg⁻¹; Mn 26.74-35.15 mg kg⁻¹; Fe 30.19-179.38 mg kg⁻¹; B 14.48-52.47 mg kg⁻¹ and oil 16.27-22.47 %) were similar to the range reported elsewhere in studies on soybean nutrient status (Urano et al., 2007) or on seed oil (Maestri et al., 1998; Bonato et al., 2000; Chung et al., 2003; Fasoula et al., 2004; Albrecht et al., 2008; Bellaloui et al., 2009). On the other hand, the results were lower than the oil content range of 17.2 to 28.5 % reported by Dardanelli et al. (2006), since in this last study some of the analyzed cultivars were from soybean maturity groups with higher oil content yield.

The seed oil content variability was lower in samples from the experimental area than from the field sites, mainly in 2007/2008 (s = 0.66; CV = 3.67 %) (Table 1), as indicated by the DRIS norms. In this year, the mean oil content (18.01 %) was lower than that of 2008/2009 (20.17 %) and the mean values of these two years (19.39 %). This year effect was associated to rainfall (862 mm in 2007/2008 and 400 mm in 2008/2009), as well-described in studies on the effect of water availability on soybean (Kravchenko & Bullock, 2002; Albrecht et al., 2008; Descriptive statistic

<table>
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<tr>
<th>Nutrient</th>
<th>Oil</th>
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<td>P</td>
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<td>Fe</td>
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<tr>
<td>B</td>
<td>mg kg⁻¹</td>
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Table 1. Descriptive statistics of leaf nutrient contents in soybean (R2 stage) with high(1) oil content, based on data of the 2007/08 and 2008/09 growing seasons and the means of these years

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(1) Oil content > 18.71, 21.36 and 21.05 % in 2007/08, 2008/09 growing seasons and for the combined set of these years, respectively. (2) Standard deviation. (3) Coefficient of variation. (4) Samples of 2007/08 and 2008/09 growing seasons.
The correlation between seed oil content and NBI varied with the analyzed database. In 2007/2008, this correlation was negative ($r=-0.647$; $p=0.002$) and positive in 2008/2009 ($r=0.628$; $p=0.002$) as well as for the two-year data set ($r=0.630$; $p=0.002$). Therefore, the DRIS norms of the second year and for the combined set of both years did not result in an appropriate nutritional diagnosis, unlike the norm for 2007/2008. These results suggest that a unique norm enabled an appropriate diagnosis of oil content of soybean seeds. In this way, the norms for 2008/2009 and for the combined set of both years were not shown in the paper, whereas the DRIS norm of the 2007/2008 growing season is displayed in Table 2.

The difference between the norms may be attributed to the cultivar or environmental effects. Since the cultivars used in the study region vary from year to year, because new cultivars are introduced and others are withdrawn, it is not simple to evaluate the consistency of results between years. However, all analyzed cultivars were from the same maturity group and the interest here was to evaluate the effect of weather variations, rather than of cultivars. In this way, these results permitted the analysis of a different range of environmental conditions each year and the repeatability of the two studied years. Aside from the annual differences, the appropriate diagnosis found with DRIS norms for 2007/2008 may indicate that the nutritional status of cultivar BRS 243 RR, used in this year, is closer to farm field cultivars than the cultivar Fundacep 55 RR, used to establish the norms of the 2008/2009 growing season.

Sulfur was often present in the binary ratios found for suitable nutritional balance and high oil content (Table 2). This is due to the role S plays as a constituent of cystine, cysteine, and methionine, which are essential amino acids for the formation of plant proteins and biotin and thiamine, as well as of enzymes that synthesize essential substances for the plant (Dechem & Nachtigall, 2007). Cysteine and methionine are precursors to coenzyme A synthesis (Epstein & Bloom, 2006; Marschner, 2012), which participate in Acetyl-CoA synthesis and in the first step of the biosynthetic pathway of fatty acids (Taiz & Zeiger, 2009). Then, it explains the importance of S in the nutrition of oil plants and likely explains the positive correlation between the higher DRIS indices for this nutrient and seed oil content (Table 5).

The nutritional diagnoses of K, Mg, S and B differed between the norms of the two growing seasons (Table 3). This is due to the role S plays as a constituent of cystine, cysteine, and methionine, which are essential amino acids for the formation of plant proteins and enzymes. Then, it explains the importance of S in the nutrition of oil plants and likely explains the positive correlation between the higher DRIS indices for this nutrient and seed oil content (Table 5).
3). On the other hand, the nutritional status of N, P, Ca, Zn, Mn, Cu, and Fe calculated using the norms from the two analyzed growing season was quite similar. The frequency values of potential response fertilization (Table 4) corroborate the results obtained by the chi-square test (Table 3).

The correlation coefficient values for seed oil content and DRIS indices were similar when calculated for the three different norms (Table 5). A correlation was found between these variables using the proposed norms, except for Fe and Mn concentrations. Seed oil content was positively correlated with the leaf contents of B, Ca, Mg and S. The most negative correlation coefficients were observed for the DRIS indices of Cu, K, N, P, and Zn (Table 5).

The positive correlation found between seed oil content and B indices can be explained by the role this nutrient plays in the plants. These functions have not been sufficiently clarified yet (Taiz & Zeiger, 2009), although this nutrient plays a role in water uptake and carbohydrate metabolism and, when its levels are low, sugars accumulate in plant tissues (Dechen & Nachtigall, 2007). This latter effect reduces the formation of fatty acids by the plant (Taiz & Zeiger, 2009) and, consequently, reduces seed oil content. Therefore, at appropriate B levels, the formation of fatty acids by the plant is adequate, enhancing the seed oil content.

The oil bodies consist of a mono phospholipid membrane, which could explain the positive correlation between Ca indices and seed oil content. Amongst the functions of Ca in plants, Ca binds proteins, known as annexins, and phospholipids. Annexins, among other functions, many of which are not fully known, participate in processes such as phospholipid metabolism (Epstein & Bloom, 2006). Magnesium activates many enzymes, mainly phosphorylation enzymes, which are essential for photosynthesis, respiration, and synthesis reactions of organic compounds, e.g., of lipids (Epstein & Bloom, 2006; Dechen & Nachtigall, 2007). Possibly, the higher lipid synthesis explains the correlation observed between the DRIS index of Mg and oil content.

The negative correlation of Cu concentration with seed oil content in soybean (r=-0.805; Table 5) is not closely related to the role of this nutrient, as it does not participate in lipid synthesis in plants. Copper directly influences N2 fixation and, therefore, protein synthesis in leguminous plants (Dechen & Nachtigall, 2006; Malavolta, 2006). Potassium, P and N are important for protein synthesis, and their concentrations are negatively correlated with oil content (Bonato et al., 2000; Bellaloui et al., 2009). This can be inferred from the negative correlation between DRIS indices of N and seed oil content, as shown by the data for the assessed years (-0.627; -0.589 and -0.596; in 2007/2008, 2008/2009 and the combined data of these growing seasons, respectively) (Table 5). Also, higher concentrations of K, P and N favored protein formation to the detriment of fatty

![Table 4. Frequency of potential response to fertilization (PRF) for soybean leaf samples (R2 stage) assessed with the DRIS norms obtained from seed oil content in different growing seasons](image)

Table 4. Frequency of potential response to fertilization (PRF) for soybean leaf samples (R2 stage) assessed with the DRIS norms obtained from seed oil content in different growing seasons

<table>
<thead>
<tr>
<th>PRF</th>
<th>B</th>
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acid synthesis, whose effect possibly explains the negative correlation between the DRIS indices of these nutrients and seed oil content. This also applies to the results obtained for Zn, as this nutrient participates in protein synthesis by activating proteins in DNA transcription (Epstein & Bloom, 2006).

**CONCLUSIONS**

1. The Diagnosis and Recommendation Integrated System can be used to evaluate the leaf nutrient status of soybean for breeding for the seed oil content of this crop.

2. A unique DRIS norm of soybean was established, of a seed oil content higher than 18.68 %, based on the database of the 2007/2008 growing season.

**LITERATURE CITED**


Diagnosis and recommendation integrated system (DRIS) of soybean seed...


