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Diagnosis of Nutrient Imbalance in Wheat Plant by DRIS and PCA Approaches

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Abstract

The foliar diagnosis can be considered as a useful tool to assess the nutritional status of plants. The diagnosis and recommendation integrated system (DRIS) has been developed for this purpose. In this study DRIS norms were determined for wheat plant (*Triticum aestivum* L.) in the Moghan region, northwest of Iran. A data bank created from nutrients concentrations of flag leaf and yield was used to subdivide data into low and high yielding subgroups based on average yield ± SD. Calculated DRIS indices showed the nutrients requirement order as fallow: Zn>Mn=Fe=Cu>N=P>B>K>Ca>Mg. Based on nutrient application potential response (NAPR) method, N, P, K, Ca, Mg and B were placed in the negative response class and Fe, Zn, Cu and Mn were placed in the positive response class. The result of principal component analysis revealed that nutrient concentration in the low and high yielding subgroups and whole data set explained 54.88%, 68.65% and 63.03% of the total variance, respectively. The involvement of several nutrients in a single PC indicated that the diagnosis of any nutrient imbalance is not possible in isolation. This study showed that in this region, macronutrients and micronutrients are in the adequate status (positive DRIS indices) and deficiency state (negative DRIS indices), respectively. Furthermore, NAPR method indicated the positive response of crops if micronutrients were added to the soil.

Keywords: Diagnosis and Recommendation Integrated System (DRIS); Nutrient Application Potential Response (NAPR); Nutrient imbalance; Principal Component Analysis (PCA)

Introduction

Diagnose of nutritional status in plants by determination of nutrients content is made by the comparison of the nutrient content with reference values. The foliar diagnosis can be considered as a useful approach to assess the nutritional status of plants (Pereira *et al*. 2012). Diagnosis and recommendation integrated system (DRIS) was developed for such diagnosis (Pereira *et al*. 2012). Calculated DRIS indices shows the effect of each nutrient on the plant nutritional balance. These indices are expressed by positive or negative values, which indicate that the referred nutrient is in excess or deficiency, respectively (Walworth and Sumner 1987).

Soil fertilization is a costly process. Therefore, the nutrient diagnostic method should identify the nutrients that limit yield in order to improve the quantity and quality of field crops. Nutrient application potential response (NAPR) is a criterion for interpretation and classification of DRIS indices based on the probability of highest fertilizer response to the nutrients (Wadt 2004).

Principal component analysis (PCA) uses the covariance or correlation matrices of the variables to find new independent variables (principal components-PC) that account for the largest portion of the total variation in a multidimensional data set. These new variables are linear combinations of the original variables (Hammer *et* *al*. 2001). This method helps to identify groups of correlated variables (i.e. nutrients concentrations or other soil and plant characteristics) based on the loadings using soil or plant samples. The objective of this work was to establish appropriate norms and identify the most limiting nutrients by DRIS method and to assess the status of the nutrition by PCA for the wheat plant in the Moghan region, northwest Iran.

Materials and Methods

Location

The study site was located in the Moghan Agro-Industrial Company of Parsabad Moghan, Ardabil Province, Iran (latitudes 39°23' to 39°42' N and longitude 47°25' to 48°23' E). The average total precipitation for the last 25 years was 332 mm. The means of minimum and maximum temperatures were 2.3°C and 35°C, respectively.

Soil and plant analysis

Composite soil samples (top 30 cm) were collected from each field at the beginning of the growing season. Samples were subjected to determine chemical properties and soil essential nutrients status (Follett and Lindsay 1971; Wolf, 1971; Jackson 1973; Soltanpour and Schwab 1977; Lindsay and Norvell 1978; Page *et al*. 1982). The results are presented in Table 1.

Leaf samples were collected randomly from 96 wheat fields at the flag leaf emergence stage (GS-39) (Zadoks *et al*. 1974; Singh *et al*. 2005). Total N was measured by micro-Kjeldahl method (Isaac and Johnson 1976). For measuring other nutrients, leaf samples were digested by dry ash and extracted by mixing diluted hydrochloric acid and nitric acid (Jones 2001). The digests were analyzed for P, K, Ca, Mg, Fe, Zn, Mn, Cu and B content. Mg, Fe, Mn, Zn and Cu were determined by atomic absorption spectroscopy, while, K and Ca were measured by flame emission (Jackson 1973) and P by ascorbic acid-reduced molybdophosphoric blue colorimetry (Page *et al.* 1982). Boron was measured by colorimetric method of azomethine-H (Wolf 1971).

Determination of nutrient's norms and DRIS index

The wheat grain yield for the selected fields was obtained from the Moghan Agro-Industrial Company. The entire population was divided into low and high yielding subgroups. The mean yield for entire population was 4042.8 kg/ha with a standard deviation (SD) of 1194.6. The criteria for dividing population into low and high yield subgroups were as follows (Sharma *et al*. 2005):

Low yielding (kg/ha) ≤ (Mean yield-SD)

High yielding (kg/ha) ≥(Mean yield+SD)

Means, variances and coefficients of variation were determined for each possible nutrient ratio for both high and low yielding subgroups according to Beaufils (1973). F test was used to select the appropriate nutrient ratio (Walworth and Sumner 1987).

$$\left[\frac{S^2\left(\frac{A}{B}\right)\text{non}-\text{reference group}}{S^2\left(\frac{A}{B}\right)\text{reference group}}\right] > \left[\frac{S^2\left(\frac{B}{A}\right)\text{non}-\text{reference group}}{S^2\left(\frac{B}{A}\right)\text{reference group}}\right]$$

DRIS index (I_A) and related functions ($f\left(\frac{A}{B}\right)$) were calculated as follows:

$$I_A = \frac{\left[f\left(\frac{A}{B}\right) - \left(\frac{B}{A}\right)\right]}{n} \quad ; \quad \begin{array}{l} \text{if } \frac{A}{B} > \frac{a}{b} \;\rightarrow\; f\left(\frac{A}{B}\right) = \left(\frac{\frac{A}{B}}{\frac{a}{b}} - 1\right) \cdot \frac{1000}{CV} \\ \text{if } \frac{A}{B} < \frac{a}{b} \;\rightarrow\; f\left(\frac{A}{B}\right) = \left(1 - \frac{\frac{a}{b}}{\frac{A}{B}}\right) \cdot \frac{1000}{CV} \\ \text{if } \frac{A}{B} = \frac{a}{b} \;\rightarrow\; f\left(\frac{A}{B}\right) = 0 \end{array}$$

Table 1. Soil properties at the experimental sites

	pH	EC	O.C	$CaCO_3$	Total N	Ca†	Mg†	Na†
		dS/m		……………………………(%)……				
Min.	7.7	1.67	0.06	3	0.03	0.89	0.0093	0.043
Max.	8.2	7.71	2.32	21	0.18	2.88	0.42	0.16
Mean±SD	7.3±0.17	1.17±0.46	0.98±0.38	10.33±4.0	0.09±0.03	1.17±0.21	0.19±0.07	0.07±0.02
%CV	2.32	39	38	38	33	18	0.36	28
	K‡	P‡	Cu‡	Mn‡	Fe‡	Zn‡	B‡	
		………………………………	(mg/kg)………………………					
Min.	391	4.4	0.42	0.96	0.19	0.04	0.02	
Max.	1726	40	6.96	8.87	10.86	1.62	2.63	
Mean±SD	760±226	17.±5.2	2.4±1	5.25±1.45	4.81±2.15	0.52±0.24	1.19±0.6	
%CV	30	30	41	27	44	46	50	

†: Exchangeable ‡: Available

where A/B and a/b are the dual ratios of the measured nutrients in the flag leaf at non-reference and reference populations, respectively, S^2 is the variance, CV is the coefficient of variation and n is the number of DRIS functions for that the A nutrient was involved.

Another method for the interpretation of the DRIS indices was proposed by Wadt (2004) that called NAPR. In this method, according to relationships in the Table 2, five categories of NAPR were established that indicate the status of nutrient balance (Pereira *et al.* 2013):

$$NBI = |I_N| + |I_P| + |I_K| + \cdots + |I_{Zn}|$$

$$NBI_a = \frac{NBI}{n}$$

Where, NBI and NBIa are nutrient balance index and its average, respectively and n is the number of DRIS indices involved in the analysis.

PCA

A PCA was performed on the nutrient concentration data for the low and high yielding populations and for DRIS indices separately (Sharma *et al.* 2005). Only PC loadings in the eigen vectors having values greater than the selection criterion (SC) were considered as the significant loads. The selection criterion was as follows (Raghupathi *et al*. 2004):

$$SC = \frac{0.5}{(\text{PC eigen values})^{0.5}}$$

Excel software and/or Statistical Package for the Social Sciences (SPSS 23.0 software), were used for the purpose of computations and statistical analysis of the data.

Results and Discussion

Significant differences ($p \leq 0.01$) between high and low yield subgroups were observed for micronutrient concentrations and grain yield (Table 3).

Figure 1. Study area and plant sampling locations

Table 3. Comparison of leaf nutrient concentration between low and high yielding wheat subgroups using t-test

	High yielding wheat (n=20)		Low yielding wheat (n=76)		t- test	
	Mean±SD	CV%	Mean±SD	CV%	F	p-value
Yield (kg/ha)	5631±366	6.5	3624±961	26.5	14.71	0.00**
N (%)	2.42±0.76	31.4	2.44±0.47	19.2	10.64	0.913ns
P (%)	0.39±0.09	23.2	0.37±0.08	21.6	2.21	0.574ns
K (%)	2.32±0.62	26.7	2.33±0.65	27.9	0.40	0.961ns
Ca (%)	0.61±0.16	26.2	0.61±0.14	22.9	1.74	0.984ns
Mg (%)	0.57±0.12	21.0	0.58±0.16	27.5	3.76	0.790ns
Cu (mg/kg)	9.86±4.54	46.0	5.99±1.94	32.3	23.95	0.00**
Mn (mg/kg)	24.61±7.32	29.7	16.31±3.09	18.9	14.17	0.00**
Fe (mg/kg)	113.54±54.71	48.1	70.38±24.89	35.3	53.43	0.00**
Zn (mg/kg)	12.01±5.81	48.3	6.66±2.42	36.3	12.35	0.00**
B (mg/kg)	6.12±2.34	38.2	6.42±2.44	38.0	0.001	0.617ns

CV: Coefficient of variation, ns and **: Non-significant and significant at 1% levels of probability, respectively.

DRIS indices

The DRIS indices for nutrients were presented in Figure 2. Mean DRIS indices for micronutrients in the flag leaf tissue were negative. According to Figure 2, the requirement order of nutrients in the study region was as: Zn > Mn = Fe = Cu > N =P > B > K > Ca > Mg.

NAPR is based on the highest probability of the fertilizer response to the nutrients (Wadt 1996). The NBIa was chosen to be a value that reflects the average deviations of each dual ratio relative to the reference value (Pereira *et al*. 2013). High percentage of studied fields in terms of macronutrients were in the class of negative response. On the other hand, micronutrients (Zn, Mn, Fe, and Cu) were in the positive response class with high probability (Figure 3).

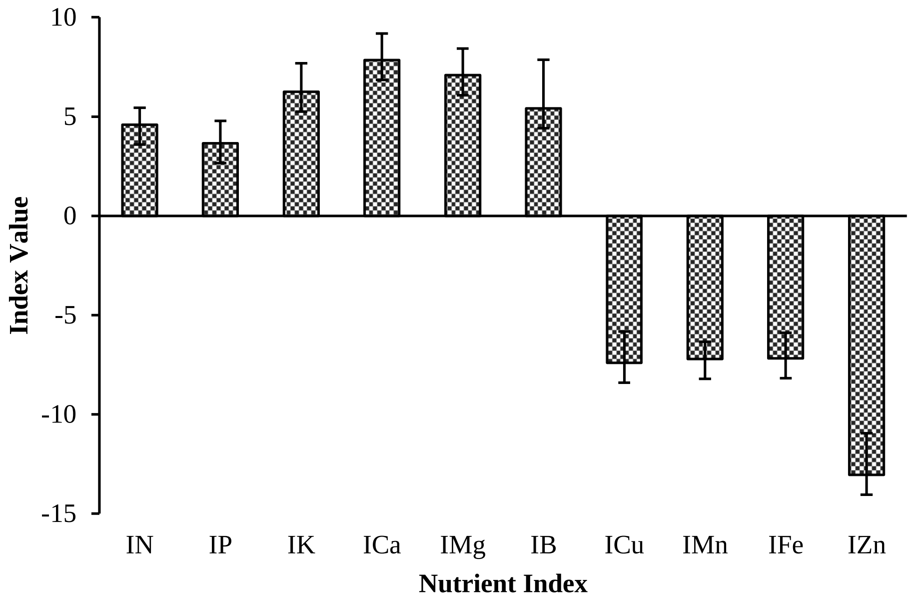

Figure 2. Nutrient index values for the flag leaf tissue

It is well known that optimum plant growth depends not only on the total amount of nutrients but also on their availability. Nutrient availability is controlled by physico-chemical properties of the soil, such as texture class, organic carbon, calcium carbonate, pH and electrical conductivity (Bell and Dell 2008). The soils of the studied region were characterized by low organic carbon content, high pH and salinity/alkalinity problems (Table 1). These soil conditions are not favorable for adequate availability of micronutrients. Gartrell (1981) stated that availability of copper is influenced by soil pH and balance of macro- and micronutrients. High rates of N fertilizer greatly accentuate Cu deficiency. Factors causing the Fe deficiency of plants include low Fe supply from soil, high calcium carbonate content and high P application (Fageria *et al.* 1990). Factors such as pH, precipitation and redox reactions control Mn availability. When the soil pH drops below 5.5, Mn toxicity may be evident, whereas above pH 6.5, Mn deficiency may occur (Rosas *et al.* 2007).

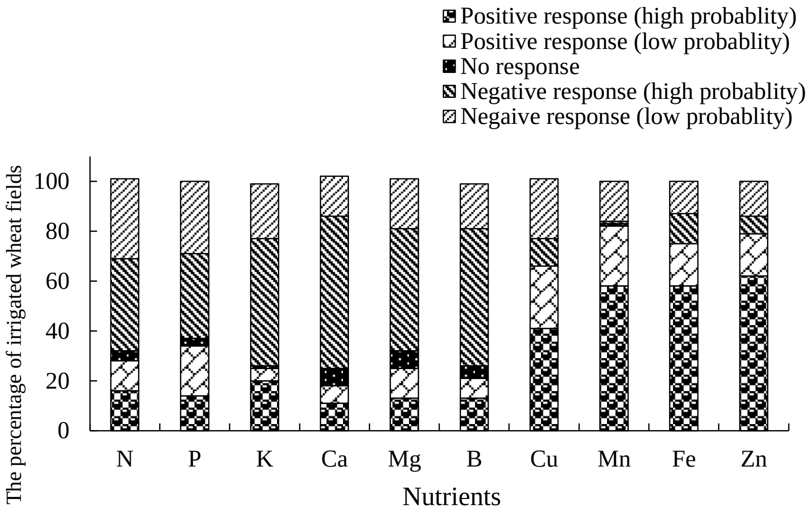

Figure 3. Percentage of nutrient application potential response (NAPR) of wheat plant diagnosed by DRIS indices

The deficiency of zinc is associated with several unfavorable environmental conditions for its uptake and utilization. The apparent recovery of Zn in calcareous soils of Iran is often less than 5%, because of the high capacity of these soils for retention of Zn (Reyhanitabar *et al.* 2007). High levels of phosphorus may induce zinc deficiency in crop plants due to antagonistic interaction of these elements in terms of plant absorption. Zinc deficiency is also reported in soils having low organic matter content (Fageria *et al.* 2002). Zinc deficiency is most likely to occur in plants growing on calcareous soils. Adsorption and occlusion of zinc by carbonates are the major causes of poor zinc availability and the appearance of zinc deficiency in calcareous soils (Mengel *et al.* 2001).

PCA

The four PCs in the low and high yielding subgroups and whole data set explained 54.8%, 68.6% and 63% of total variance, respectively (Table 4). The first principal component of the low yielding subgroup had positive loadings for Mg (0.69) and B (0.67) and negative loading for Zn (-0.60) that were expressed as (B+ Zn- Mg+). The P, Ca and Fe variables were placed in the second PC that showed inverse relationship of P and Ca with Fe (P+ Ca+ Fe-). The PC3 and PC4 were designated as (K+ Cu+ Mn+) and (N+), respectively.

The PCA conducted on the high yielding subgroup also placed several nutrients in four PCs. PC1 and PC2 were designated as (N+ Mg+ Fe-) and (P+ Ca+ B+ Cu-), respectively. N and Mg which were in the PC1, changed in the same direction and they had the maximum variance which represented their importance. On the other hand, in the second PC, a contrasting relationship of P, Ca and B with Cu was obtained. The results showed that high scores for B (0.85) and Cu (-0.72) were strongly correlated with PC2 in the high yielding group. The two eigen values of PC3 (P+ K+ Ca+ Mg+) and PC4 (Zn+ Mn+) explained about 27% of the total variance.

Table 4. Principal component analysis of nutrient concentration for high yielding and low yielding subgroups and the whole data set using DRIS index

	High yielding group				Low yielding group				Whole data set			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
N	0.667*	0.060	-0.176	0.249	0.054	-0.091	-0.133	0.831*	0.638*	-0.117	-0.267	0.347
P	0.048	0.385*	0.677*	-0.271	-0.281	0.501*	-0.151	-0.222	0.579*	-0.214	0.255	-0.031
K	-0.155	-0.096	0.617*	-0.010	-0.109	0.131	0.672*	-0.143	0.190	-0.151	0.716*	-0.191
Ca	0.239	0.467*	0.701*	0.175	0.134	0.563*	0.139	0.391	0.702*	-0.093	0.049	0.111
Mg	0.650*	-0.238	0.473*	-0.337	0.690*	0.029	0.088	-0.292	-0.142	-0.284	0.056	-0.714*
Cu	-0.193	-0.722*	0.221	0.040	-0.245	0.151	0.558*	0.287	-0.122	-0.393*	0.099	0.77*
Mn	-0.029	-0.024	0.301	0.828*	0.324	-0.253	0.709*	-0.139	-0.285	0.173	0.658*	0.404
Fe	-0.853*	-0.138	0.039	-0.051	-0.021	-0.772*	-0.099	0.088	0.064	0.788*	0.135	0.077
Zn	0.204	0.204	-0.061	0.801*	-0.602*	-0.240	-0.017	0.213	-0.217	0.651*	-0.401	0.094
B	-0.089	0.857*	0.022	-0.154	0.674*	-0.014	-0.17	0.069	-0.504*	-0.439*	0.524*	-0.138
Eigen value	2.252	1.917	1.395	1.311	1.67	1.40	1.292	1.11	1.979	1.67	1.413	1.246
% Variance	22.5	41.68	55.63	68.74	16.74	30.83	43.75	54.89	19.78	36.48	50.62	63.08
Selection criteria	0.332	0.361	0.423	0.437	0.386	0.421	0.440	0.474	0.355	0.387	0.421	0.448
	PC1=N+Mg+Fe-				PC1=B+Zn-Mg+				PC1=N+P+Ca+B-			
	PC2=P+Ca+Cu-B+				PC2=P+Ca+Fe-				PC2=Cu-Fe+Zn+B-			
	PC3=P+K+Ca+Mg+				PC3=K+Cu+Mn+				PC3=K+Mn+B-			
	PC4=Zn+Mn+				PC4=N+				PC4= Mg-Cu+			

PCA on whole data set indicated involvement of several nutrients in PC1 and PC2 that was designated as (N+ P+ Ca+ B-) and (Cu- Fe+ Zn+ B-), respectively. The PC3 (K+ Mn+ B-) and PC4 (Mg- Cu+) explained 26.58% of the total variance. The involvement of several nutrients in a single PC indicated that the diagnosis of any nutrient imbalance is not possible in isolation.

The communality for a given variable can be interpreted as the proportion of variation in that variable explained by the extracted factors (Malhotra 2004). The communalities, considering four factors for the high and low yielding groups and the whole data set were high for almost all variables (Table 5).

Productivity, stability and sustainability of soils are influenced by micronutrients (Bell and Dell 2008) and macronutrients (Fageria 2001). High phosphate content of soils or high fertilization with phosphate may reduce the uptake of zinc and other nutrients (Kizilgoz and Sakin 2010). Thus, indiscriminate use of macronutrients may affect uptake of micronutrients.

Table 5. Communality of PCA for nutrients concentration in relation to high and low yielding subgroups and the whole dataset using DRIS index

Variable	N	P	K	Ca	Mg	Cu	Mn	Fe	Zn	B
	High yielding group									
Communality	0.541	0.683	0.414	0.798	0.815	0.609	0.777	0.751	0.72	0.766
	Low yielding group									
Communality	0.719	0.403	0.502	0.507	0.653	0.476	0.69	0.615	0.466	0.459
	Whole data set									
Communality	0.612	0.448	0.608	0.516	0.614	0.773	0.707	0.65	0.64	0.741

Conclusion

From the foregoing discussion, it becomes evident that continuous fertilizer application over years on wheat fields at different levels produced specific effect on the leaf micronutrient concentration. It appears that soils may have been deficient in the micronutrients especially zinc that allow for significant differences in yield and consequently differences in the DRIS indices between high yielding and low yielding subgroups. The order of plant requirement to nutrients was as: Zn>Mn=Fe=Cu>N=P>B>K>Ca>Mg. Conducted PCA on DRIS indices from three cases including low and high yielding subgroups and the whole data set resulted in four PCs that explained 54.8%, 68.6% and 63% of the total variance, respectively. The inclusion of several nutrients in a single PC suggested that the diagnosis of any nutrient imbalance is not possible in isolation. This study showed that in this region, macronutrients and micronutrients are in the adequate (positive DRIS indices) and deficiency (negative DRIS indices) status, respectively. Furthermore, NAPR method indicated the positive response of crops when micronutrients are added of to the soil.

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