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Multivariate diagnosis of nutrient imbalance in rose grown under open and protected conditions using compositional nutrient diagnosis and principal component analysis

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ABSTRACT

A data bank of nutrient concentration VS yield was developed for rose grown under open and protected conditions through a survey to develop multivariate nutrient diagnostic norms using compositional nutrient diagnosis. Three hundred and twenty leaf samples were collected when flower buds were of pea size and were analyzed for different nutrients. The compositional nutrient diagnostic norms for identification of yield-limiting nutrients were developed. Although, the mean nutrient concentrations in leaves between open and protected conditions showed marginal difference, the compositional nutrient diagnosis norm values differed with higher diagnostic sensitivity. The interaction among different nutrients was explained by principal component analysis on log-transformed data. The principal component analysis extracted 4 significant principal components under protected conditions namely ($N^+P^+Fe^- Zn^-$), ($Ca^- S^+$), ($K^+ Mn^-$) and ($P^- Mg^+$), which explained nearly 70.30% of variance, while only 2 significant principal component, viz ($N^+P^+Ca^-Mg^- S^+Fe^-Mn^-Zn^+$) and ($K^+Ca^+Mg^+Fe^-Zn^-$) were formed under open conditions explaining nearly 74.26% of variance. The principal component analysis indicated that K had an overwhelming interaction with Ca and Mg. The multivariate technique revealed greater imbalance of Fe and Zn, followed by K under protected conditions and Ca, Mg, Fe and Zn under open conditions. Thus, the multi-nutrient interactions need to be understood for proper management of nutrients to sustain yield and quality of cut flowers in rose.

Key words: CND indices, Nutrients, Norms, Protected conditions, Rose

Understanding of nutrient composition and their interactions in ornamental crops is essential to maintain the quality of cut flowers. Rose is one of the popular commercial ornamental crops, which is being grown under open conditions in India. However, with the introduction of liberalization policy in agriculture, rose cultivation in poly-houses became popular for export of quality flowers. The nutrient removal by rose plants is higher under protected cultivation when compared to open cultivation due to removal of long stalks/stems frequently (Gurav *et al*. 2005). Application of N, P and K was found to increase number of flowers/plant, stalk length and number of flowers/shoot under polyhouse conditions. Response for application of Zn, Fe, and Cu was also reported in rose (Joshi *et al.* 2002). Since nutrients play an important role in maintaining yield and quality, the nutrient imbalance if any need to be identified well in advance. For identification of nutrient imbalance, several approaches were adopted, the recent being Compositional Nutrient Diagnosis (Parent and Dafier 1992), which provides undistorted variates amenable to principal component analysis. There are a few reports in the literature on the use of bivariate (DRIS)/multivariate (CND) nutrient diagnosis in ornamental plants (Mourao Filho 2004). With the advancement of interpretation techniques, it was observed that the diagnostic precision increased, when a large number of nutrients are included in the interpretation process. Therefore, the present investigation was carried out to develop multivariate diagnostic norms for rose under open and protected conditions and to understand interaction among different nutrients that govern yield and quality of cut flowers.

MATERIALS AND METHODS

A survey was conducted in Karnataka and Tamil Nadu and 320 leaf samples were collected from various open and protected cultivation units of rose. At each site, composite samples of recently matured 5th leaflets (Jones *et al.* 1991) were collected from 50 plants to develop multi-nutrient norms. It is essential to select a specific part of the same physiological age at a definite location on the plant at definite

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stage of growth (when flower buds were of pea size). The leaf samples were decontaminated by washing samples in sequence with tap water, 0.2% detergent solution, N/10 HCl and finally with double distilled water. They were dried at 60–65°C for 48 hr. After complete drying, the samples were powdered in a Cyclotec Mill. The samples were analyzed for different nutrients by digesting 1 g tissue in di-acid mixture (9:4 ratio of nitric acid and perchloric acid) by using standard analytical methods (Bhargava and Raghupathi 2005). Phosphorus was analyzed by vanado-molybdate method, K by flame-photometer and S turbidity methods. Calcium, magnesium and the micronutrients Fe, Mn, Cu and Zn were analyzed by using Atomic Absorption Spectrophotometer (Perkin-Elmer-A-Analyst–200). Nitrogen was estimated by micro-kjeldhal method separately using 0.5 g plant sample.

Compositional nutrient diagnosis norms were developed by adopting the procedure as outlined by Parent and Dafir (1992). The full composition array for the nutrient proportions (D) in plant tissues was described by the following simplex (S^D) contained to 100%:

S^D=[(N, P, K, … R): N>O, P>O, K>O, …, R>O; N+P+K+…+R =100%]

Where 100% is the dry matter content (ie the invariable sum of all the components or the full relative composition of the diagnostic tissues). N, P, K are the nutrient concentrations and R is the filling value between 100% and sum of the nutrient concentrations. The value of R is thus composed of undetermined components as well as experimental error and was required to be linearized compositional data. The bounded sum constraint to 100% of compositional data was alleviated by correcting nutrient concentrations by geometric mean (G) of all the D components including R.

$$G = [N \times P \times K \times \ldots \times R]^{1/D}$$

Row centered log-ratios were generated for V_N to V_{Zn} as follows:

$$V_N = \ln (N/G), \ldots, V_{Zn} = \ln (Zn/G)$$

Expressions such as N/G, … Zn/G are multi-nutrient ratios, since each nutrient is divided by geometric means of all the components (the determined nutrients and the filling value). The row-centered log-ratios are linearized (undistorted) estimates of the original components that are fully compatible with PCA. V^*_N to V^*_{Zn} and SD^*_N to SD^*_{Zn} are the compositional nutrient diagnosis norms (indicated by asterisks), i e mean and standard deviation of each row centered log-ratios of the population. The standardized variables $(V_N - V_N^*)/SD^*_N$ to $(V_{Zn} - V^*_{Zn})/SD^*_{Zn}$ are the CND nutrient indices. The indices are calculated by using the formula

$$I_N = (V_N - V^*_N)/SD^*_N, \ldots\ldots I_{Zn} = (V_{Zn} - V_{Zn}^*)/SD^*_{Zn}$$

Independent values for V_N to V_{Zn} can be introduced in the equation for diagnostic purpose.

A reasoned application of principal component analysis could lead to the greater understanding of the effect of fertilization treatments on leaf composition. The principal component analysis reduces the number of interdependent variables into smaller number of independent principal components that are linear combinations of original variates. Principal component analysis *w*as performed on log transformed nutrient concentration data prior to statistical computation that followed normal distribution.

To be declared as significant, principal components must have Eigen values >100/P, where P is the total number of varieties under diagnosis. Alternatively, principal components showing Eigen values <1 were considered non-significant. Only principal component loading in Eigen vectors having values greater than the selection criterion (SC) are given significance. The selection criterion was computed as follows:

$$SC = 0.50/(\text{PC Eigen values})^{0.5}$$

RESULTS AND DISCUSSION

Nutrient concentrations

The average N concentration in leaf samples grown under open conditions showed only a marginal difference when compared to N level under protected conditions and the values were below the optimum values published earlier (Jones *et al.* 1991). The mean P concentration was at the same range under open and protected conditions (Table 1). The mean K

Table 1 Mean and range of nutrients in rose grown under protected and open conditions

Nutrient	Mean	Maximum	Minimum	Mean	Maximum	Minimum
	Protected condition			*Open condition*		
N (%)	2.26	3.26	1.52	2.14	2.9	1.56
P (%)	0.16	0.25	0.10	0.16	0.23	0.10
K (%)	2.23	2.96	1.30	1.48	3.0	0.80
Ca (%)	1.07	2.54	0.37	0.84	1.90	0.38
Mg (%)	0.40	0.75	0.24	0.28	0.61	0.15
S (%)	0.202	0.33	0.12	0.14	0.19	0.09
Fe (ppm)	168.50	322	99	124.14	233	1.20
Mn (ppm)	104.13	264	47	61	130	27
Zn (ppm)	25.30	72	9	24.33	42	7

concentration was much higher under protected conditions when compared to the mean K concentration under open conditions. The mean Ca and Mg concentration was also much higher under protected conditions. The differences in K, Ca and Mg concentrations may be mainly due to high humidity and temperature under protected conditions. Similar trend of higher concentrations of S, Fe and Mn under protected conditions were observed, while Zn concentration was same. The differences in nutrient concentrations between these two populations can also be attributed to the removal of long stalks/stems frequently under protected conditions (Gurav *et al.* 2005).

Compositional nutrient diagnosis norms

The compositional nutrient diagnosis norm for N (V_N) under protected conditions was 2.112 and 2.308 under open conditions. The compositional nutrient diagnosis norm for P (V_P) was –0.536 under protected conditions that was much higher compared to open conditions (–0.304) although the mean P concentration showed no apparent difference. A similar difference in compositional nutrient diagnosis norm values was noticed for other elements, although the nutrient concentration values indicated a different picture. This is mainly because compositional nutrient diagnosis norms are multivariate norms with due weight-age for all the other elements including the unmeasured factors. The sum of the tissue components is 100% and therefore the sum of the row centered log ratios including the filling value is zero (Table 2). The compositional nutrient diagnosis norm value developed for rose was difficult to decipher compared to the nutrient concentration expressed as% or ppm. However, the compositional nutrient diagnosis norms are having higher diagnostic precession compared to the univariate values as in case of critical value approach or bivariate values as in case of diagnosis and recommendation integrated system. Considerable differences are observed in compositional nutrient diagnosis norms developed for open and protected conditions indicating the need for separate nutrient management strategies for both. In general, N and Zn norms were lower under open conditions and K, Mg, S, Fe and Mn norms were higher under protected conditions.

Table 2 Multivariate nutrient diagnosis (CND) norms for rose under protected and open cultivation

CND variate	CND norm	SD	CND norm	SD
	Protected condition		*Open condition*	
V_N	2.112	0.1554	2.308	0.1690
V_P	–0.536	0.2025	–0.304	0.2167
V_K	2.103	0.1677	1.912	0.2137
V_{Ca}	1.289	0.4383	1.298	0.3535
V_{Mg}	0.362	0.2322	0.215	0.2601
V_S	–0.314	0.2190	–0.447	0.2012
V_{Fe}	–2.801	0.2189	–2.921	0.5872
V_{Mn}	–3.343	0.3359	–3.635	0.3559
V_{Zn}	–4.726	0.2919	–4.535	0.4081
V_R	5.853	0.1285	6.108	0.1225

Compositional nutrient diagnosis indices

The compositional nutrient diagnosis indices were developed for selected protected and open cultivation units for diagnosis of nutrient imbalance (Table 3). Under protected cultivation units, Fe and Zn were the most common yield-limiting nutrient followed in importance by K. Under open cultivation, Fe and Zn, followed by Ca and Mg were the most common yield-limiting factors. However, it was observed that no single nutrient was solely responsible for low yield in different units and therefore, it is essential to make an individual unit assessment for nutrient recommendation.

Principal component analysis

The principal component analysis (PCA) conducted on log-transformed data produced 4 significant principal components explaining about 70.30% of variance (Table 4) under protected conditions. Since principal components are linear contrasts among the nutrients, interpretation of principal components should consider the sign of the variate. The first principal component was positively correlated with

Table 3 CND indices for selected protected and open cultivation units of roses

N	P	K	Ca	Mg	S	Fe	Mn	Zn
				Protected condition				
0.357	0.079	–0.018	0.380	0.406	0.263	–0.435	0.714	–0.580
1.456	0.217	0.191	0.239	0.158	0.112	–0.296	0.258	–0.200
0.040	0.759	0.072	0.152	0.158	0.276	–0.345	0.379	–0.685
0.655	0.326	0.066	1.140	0.895	0.171	–0.753	0.647	–0.213
1.628	0.119	–0.626	0.234	0.017	0.308	–0.342	1.935	–0.356
				Open condition				
0.334	1.068	0.238	–0.669	–0.514	0.614	–0.308	0.052	–0.302
0.109	0.496	0.322	–0.524	–0.770	0.946	–0.345	0.171	–0.537
1.738	0.585	0.393	–0.722	–0.737	1.612	–0.198	–0.980	–0.378
0.519	0.221	0.338	–0.518	0.213	1.198	–0.168	–1.206	–0.540
0.873	0.079	0.161	–0.120	–0.123	–0.311	–0.142	–0.508	–0.050

Table 4 Principal component analysis loadings performed on log-transformed data

Variate	Protected condition				Open condition	
	PC1	PC2	PC3	PC4	PC1	PC2
N	0.805*	0.265	0.065	0.138	0.907*	–0.110
P	0.489*	0.346	0.000	–0.606*	0.882*	–0.209
K	–0.093	0.171	0.784*	–0.191	–0.075	0.854*
Ca	0.131	–0.943*	0.216	–0.060	–0.530*	0.662*
Mg	0.059	–0.009	–0.018	0.932*	–0.450*	0.847*
S	0.172	0.662*	0.252	–0.277	0.835*	–0.223
Fe	–0.537*	0.138	–0.508*	–0.018	–0.633*	–0.633*
Mn	–0.069	0.105	–0.750*	–0.131	–0.573*	0.077
Zn	–0.628*	0.233	0.098	0.244	0.749*	–0.457*
Eigen values	2.166	1.689	1.27	1.145	4.795	1.889
Variance (%)	24.70	18.76	14.12	12.72	53.27	20.98
Selection Criteria	0.339	0.384	0.443	0.467	0.228	0.363

* Significant loadings

N and P and negatively correlated with Fe and Zn and designated as ($N^+P^+Fe^- Zn^-$) indicating that N and P behaved in one direction, Fe and Zn in the opposite direction (Fig 1)

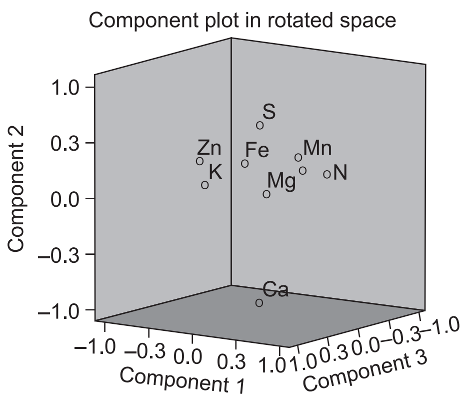

Fig 1 Component in rotated space for log transformed data under protected conditions

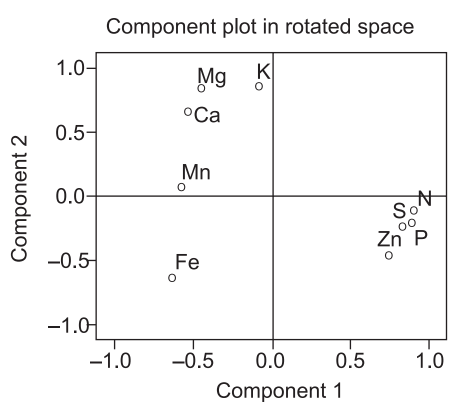

Fig 2 Component in rotated space for log transformed data under open conditions

under protected conditions. In the second principal component, the antagonistic effect between Ca and S was evident and was designated as (Ca^-S^+). In the third principal component, K was positively correlated, while Fe and Mn behaved in the opposite direction. In the forth principal component, P and Mg behaved in opposite direction indicating that build up of Mg resulted in substantial decrease in P concentration under protected conditions. The loading for N was significant only in the first principal component indicating that N was associated with P, Fe and Zn, and had least interaction with other nutrients.

Under open conditions, the PCA conducted on log transformed nutrient concentration data showed only two significant principal components explaining nearly 74.26% of the variance. The first principal component ($N^+P^+Ca^-Mg^-S^+Fe^-Mn^-Zn^+$) explained nearly 53.27% of the variance although the full nutrient interaction was not explained by it alone but as many as 5 nutrients ($K^+Ca^+Mg^+Fe^- Zn^-$) showed significant interaction in principal component. Excluding K, all other nutrients showed a significant loading in principal component. Nitrogen, P, S and Zn formed one group while Ca, Mg, Fe and Mn formed the other group. Although it is difficult to interpret such nutrient interactions on physiological basis, the influence of K appears to be much less critical in open conditions, whereas K had overwhelming influence under protected conditions with other nutrients. The compositional nutrient diagnosis multivariate technique revealed that application of major nutrients could result in greater imbalance of Fe and Zn under protected conditions, whereas it resulted in greater imbalance of Ca, Mg, Fe, Mn and Zn under open conditions. Thus, the multi-nutrient interactions need to be understood for proper management of nutrients in rose.

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