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Multivariate Diagnosis of Nutrient Imbalances in Apple using Compositional Nutrient Diagnosis and Principal Component Analysis

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ABSTRACT: In order to improve yields and quality while maintaining tree sustainability, multivariate nutrient diagnostic norms to be derived for proper nutrient and sustainable soil fertility management in apple orchards. Foliar nutrient content vs. yield performance data bank was established for 280 apple (*Starking delicious*) orchards from seventy locations in Kinnaur region of Himachal Pradesh to diagnose nutrient imbalances in apple using compositional nutrient diagnosis and principal component analysis for efficient nutrient management in orchards. The mean foliar N, P and K concentrations were 2.27, 0.243 and 1.53% respectively. The mean Ca (1.56%) concentration in leaf was quite higher compared to Mg (0.31%) concentration. The diagnostic norms for major nutrients (N, P, K, Ca and Mg) were developed by using compositional nutrient diagnosis (CND) technique. The CND norms for N (V_N), P (V_P), K (V_K), Ca (V_{Ca}), and Mg (V_{Mg}) for apple were 0.219, -1.998, -0.198, -0.137 and -1.763 respectively. The application of Principal component analysis (PCA) indicated that four nutrient elements (N-K-Ca-Mg+) were found integrated with first principal component. The nutrient indices developed indicated that P, Ca and Mg nutrient elements were the most common yield limiting nutrients. The CND norms and the indices developed can be used for identifying the hidden hunger of various nutrients in apple for evolving nutrient management strategies and enhancing yield and quality of apple orchards in the Kinnaur region of Himachal Pradesh.

Keywords: Apple, compositional nutrient diagnosis (CND), nutrient imbalances, principal component analysis (PCA).

INTRODUCTION

Mineral nutrients often limiting plant growth and the assessment of plant mineral requirements are fundamental for crop management. Foliar analysis is a common method for diagnosing the mineral imbalance in apple like in many other crops. Providing adequate nutrition to fruit trees is utmost concern among apple growers for enhancing growth, yield and quality of apple and is mandatory for sustainable production. In India, apple is important commercial fruit crops of Himalayan states viz. Jammu and Kashmir, Himachal Pradesh, Uttaranchal and Arunachal Pradesh but Jammu and Kashmir (J&K) and Himachal Pradesh are the only principal apple growing states in the country. In Himachal Pradesh, apple is one of the most important fruit crop, since it occupies 49 percent of total area under fruit crops with an estimate production of 777 thousands tones over an area of 110.7 thousand hectares (NHB, 2017). The yield levels of apple (7.02 t/ha) in the state are, however, far below the international standards (30 t/ha) and improper nutrient management is likely to be the major factors contributing to poor yield and quality, though no local nutrition guidelines are available. Nutritional imbalances in the soil cause nutritional disorders and consequently affect both quality and quantity of fruit. Hence, its nutrient requirements have to be carefully monitored for higher productivity through leaf analysis. Comprehensive leaf nutrient diagnostic norms and information on interactions among different nutrients are not available for apple.

Several approaches were adopted by different researchers for the diagnosis of nutrient imbalances and developing nutrient diagnostic norms. Nutrient ratios in plants are diagnosed using nutrient concentration or dual ratio (Diagnostic and recommendation integrated system, DRIS) in selected tissue. The use of dual ratio in DRIS instead of nutrient concentration could reduce effect of nutrient concentration, diluting or accumulation in plant tissues. However, when multi-nutrient interaction exists in plant, it cannot be explained by DRIS effectively. The compositional nutrient diagnosis (CND) (Parent and Dafir, 1992), that has been proposed for nutritional diagnosis includes physiological concept, such as nutrient interaction and provides undistorted variates amenable to Principal

Component Analysis (PCA). The CND norms are multivariate norms that give due weightage to all the elements, including unmeasured factors and therefore, have higher diagnostic sensitivity (Anjaneyulu *et al*., 2008; Ganeshamurthy, 2019). Presently, there are no information in the literature on the use of multivariate nutrient diagnosis and PCA in apple. The present investigation was carried out to develop multivariate diagnosis of nutrient imbalances using compositional nutrient diagnosis (CND) and principal component analysis (PCA) approach in apple crop.

MATERIALS AND METHODS

The research was carried out in Kinnaur region which extends from 30°22′40″ to 33°12′40″N latitude and 75°47′55″ to 79°04′20″E longitude and the altitude from 1600 to 3500 m above mean sea level during 2016-17. It is a north eastern frontier district in the Indian western Himalayas. By virtue of its location Kinnaur experiences the cool temperate climate and is ideally suited for the production of quality temperate fruits. The mean maximum and minimum temperatures ranges from 18 to 32° and -4 to -20°C, respectively. The study area typically representing a dry temperate region with less precipitation in the leeward side of the mountains whereas moderate precipitation is received by the well exposed slopes between the months of December to February and sometimes even up to mid march.

A survey was conducted in apple growing orchards of Kinnaur region to establish nutrient concentration vs. yield data bank for developing leaf diagnostic norms. Seventy locations were selected randomly to collect two hundred eighty leaf samples from middle of terminal shoots of current year growth index tissue in the periphery of tree from 15th July to 15th August to represent a composite sample (Bhargava and Chadha, 1993). At each location four orchards of 15-18 years old were selected and 50 leaves per tree from twenty uniform and healthy trees were collected randomly at shoulder height from all sides to form a composite and representative sample. The information on yield was obtained based on the visual performance and observation of the orchards and interaction with the orchardists.

The foliar samples were purged by washing in a sequentially with tap water, 0.2% detergent solution, N/10 HCl and, finally, with double distilled water. Leaf samples were dried at 65-70°C. After complete drying, the samples were powdered in a cyclotec mill and wet digested with standard procedure and analyzed for N by Kjeldahl method (Jones *et al*., 1991). Another part of the sample was digested with di- acid (HNO_3: $HClO_4$). Phosphorus was analyzed by vanado-molybdate yellow method. Potassium (K), Calcium (Ca) and magnesium (Mg) were measured by using Atomic Absorption Spectrophotometer.

Compositional Nutrient Diagnosis (CND)

Compositional Nutrient Diagnosis norms were deduced by using the procedure suggested by Parent and Dafir (1992) for tree crops and the selection of the high-yielding reference subpopulation may be carried across multiple ratios using a cumulative variance function fit to cubic or Boltzmann (Hernandez *et al.*, 2008) equations as given in Fig. 1. V^*_N to V^*_{Mg} and SD^*_N to SD^*_{Mg} are CND norms i.e., mean and standard deviation of each row-centered log ratios in the high-yielding population. The normalized variables $(V_N - V_N^*)/SD^*_N$ to $(V_{Mg} - V^*_{Mg})/SD^*_{Mg}$ are CND nutrient indices.

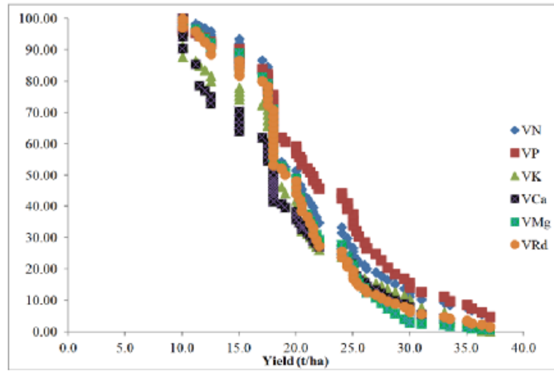

Fig. 1. Relationship between apple yield and cumulative variance ratio functions in S^5 for yield cutoff between high and low yielding subpopulations at inflection point in the cubic pattern.

In order to compute the principal components analysis (PCA) of the row-centered log ratio in the high-yielding subpopulation, the database of leaf nutrient content and crop yield was used. Principal component analysis (PCA) was performed on log-transformed data of original nutrient concentration and could lead to greater understanding of nutrient interactions in the plant and effect of fertilization treatments on leaf composition. PCA lessens the number of interdependent variables to a smaller number of independent PCs that are linear combinations of original variates (Schleppi *et al.*, 2000). The PC loadings in Eigen vectors having values greater than the selection criterion (SC) only are considered significant. The selection criterion was computed as:

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

RESULTS AND DISCUSSION

A. Nutrient concentration range

The mean foliar N concentration was 2.27% and ranged from 1.80 to 2.60% (Table 1). Maximum yield in apple was reported when N concentration in the leaf ranged between 2.20 and 2.60% (Awasthi, 2005); Sharma *et al.* (2018). The mean P concentration was 0.243% and varied from 0.18 to 0.33% which was comparable to the values (0.11 to 0.32%) published earlier by Upadhayay and Awasthi (1993); Singh *et al.* (2000). The mean K concentration was 1.53% and showed less variation (1.20 and 1.80%) in the study area owing to a conglomeration of muscovite, smectite, vermiculite and kaolinite in these soils (Bhandari and Sharma, 1981).

Table 1: Mean and range of different nutrient concentrations for apple.

Nutrient (%)	Mean	Minimum	Maximum
N	2.27	1.80	2.60
P	0.243	0.18	0.33
K	1.53	1.20	1.80
Ca	1.56	1.10	2.10
Mg	0.306	0.24	0.45

B. Compositional Nutrient Diagnosis norms

The CND norms as means and standard deviations of V_N, V_P, V_{Ca}, V_{Mg}, V_K and V_{Rd}, for high yielding subpopulation (>23.4 t ha^{-1}), as well as the corresponding optimum ranges, means and standard deviations of nutrients are presented in Table 2. CND norms for N (V_N), P (V_P) and K (V_K) for apple were 0.219, -1.998 and -0.198 respectively. The norms derived indicated higher requirement of N and K compared to P that might be due to their continuous requirement. Similarly, high CND norm for N and K was reported in some fruit crops (Raghupathi *et al.*, 2002) indicating higher N and K requirement. CND norms are multivariate standards with due credit to all the other elements and unmeasured factors. The CND norm values developed were difficult to interpret compared to nutrient concentrations, expressed as % or ppm, although, CND norms have better diagnostic precision compared to the bivariate values (Walworth and Sumner 1987).

The Ca norm (-0.137) was many times high as that of Mg (-1.763) and, therefore, its requirement was much higher, compared to Mg. The higher norm value noticed for Ca was mainly due to fact that he apple orchards of the area are well supplied with available Ca because the soils are young and least leached due to less rainfall in the region, which might have overwhelming influence on calcium uptake (Sharma, 2018). This finding corroborates with the results observed by Anjaneyulu and Raghupathi (2010).

C. Principal component analysis

In order to compute the principal components analysis (PCA) of high-yielding subpopulation, the data of per cent leaf nutrient content and crop yield was used. Based on the PCA, four PCs with Eigen values >1.0 has been selected with accumulated variance equal 90.89% (Table 3). The first principal component was positively correlated with V_P, and V_{Mg} and negatively correlated to V_N, V_K, and V_{Ca} (Table 3). The second principal component was positively correlated with V_R, and negatively correlated with V_{Ca} only. The third principal component correlated positively with V_P and negatively correlated with V_{Mg}. Finally, the fourth principal component showed positive correlation with V_N and negative with V_K.

Table 2: CND norms based on the high-yield subpopulation (>23.4 t ha^{-1}) of apple crop in the NW Himalayan region.

CND variate	CND Norm	SD
V_N	0.216	0.0812
V_P	-2.018	0.0619
V_K	0.180	0.0945
V_{Ca}	-0.160	0.1484
V_{Mg}	-1.79	0.0927
V_R	3.92	0.1912

Table 3: Principal component analysis (PCA) loadings performed on log-transformed data.

Nutrient	PC1	PC2	PC3	PC4
N	**-0.383***	0.350	0.299	**0.730**
P	0.259	-0.221	**0.884***	-0.322
K	**-0.646***	-0.077	-0.281	**-0.561**
Ca	**-0.508***	**-0.721***	-0.199	0.306
Mg	**0.868***	0.139	**-0.464***	0.073
Residue	**-0.448***	**0.772***	-0.013	-0.206
Eigen values	1.843	1.312	1.204	1.094
Selection criterion (SC)	0.368	0.4365	0.456	0.478
Explained variance (%)	30.718	21.873	20.073	18.231
Accumulated variance (%)	30.718	52.591	72.664	90.895

*Significant loading

Higher range of Ca concentration (1.10 to 2.10%) was observed, whereas, a majority of the samples were in the optimum range with regard to Mg (0.24 to 0.45%) as these soils are young and least leached (Sharma and Sood, 2019). Since PCs are the linear contrasts among nutrients, interpretation of PCs considers the sign of the variate. The structures devised by PC-1 (N-K-Ca-Mg+R-), PC-2 (Ca-R+), PC-3 (P+Mg-) and PC-4 (N+K-) and explained about 90.89% of the variance indicating that N, K, Ca and R (residue, which is a reflection of dry matter accumulation in the plant) showed the tendency to build-up in the same direction while Mg behaved in an opposite direction and are endorsed by some rules of plant and soil nutrition relative to interaction between nutrients, but other structures should be studied carefully for a better understanding.

In PC-1, it is reasonable to perceive positive interaction among N, K and Ca; this result may be described as detailed by Marschner, (2012), that N and protein contents are very highly correlated. In PC-3, the antagonistic relation between P and Mg may be deciphered because of dilution and accumulation effects with plant age. The PC-4 showed the inverse direction for N and K inferring the antagonism effects between these nutrients.

N and Mg noticed to be highly and negatively correlated with the first PC. It is important to have N availability in soil in balance with Mg, since this nutrient is a component of proteins. Thus, N deficiency limited the formation of amino acids and proteins. Furthermore, the N and Mg have essential role in the activity of chlorophyll molecule and interactions among nutrients in the rhizosphere. The N deficiency may decrease the action of chlorophyll and consequently reduction of photosynthesis (Marschner, 2012). P (positive) and Mg (negative) affected the third PC, which showed opposite trends between these characters inferring the exorbitant use of P source in soil, discourage the absorption of Mg by plant roots (Marschner, 2012).

Therefore, on the basis of PCA analysis K and Ca formed one group while N and Mg formed the other group in all principal components. Although it is difficult to interpret such elucidated nutrient interactions on physiological basis and no information regarding them has been reported for apple so far. Raghupati *et al*. (2002) used PCA approach in banana for explaining the nutrient interactions. Thus, the relationship extracted by PCA is helpful in understanding the mutual association among different nutrients and these interactions need to be considered for correction of nutrient deficiencies and to evolve nutrient management strategies for apple for higher yield and quality in the region under investigation.

D. CND Indices

In order to identify the nutrient(s) limiting yield, the deduced CND norms were used to determine the nutrient status of the low-yielding sub population in this investigation. With the yield cut off of 23.4 t/ha (Fig. 1), a total of 46 location samples were in this low-yielding sub population. The average of the CND indices is presented for each nutrient in Table 4 and Fig. 2. The concentration of P, Mg and Ca when below the threshold level indicated visual symptoms of nutritional imbalance, which exhibited negative indices (Table 4). Among the forty six location low-yielding orchards studied, P was found to limit yield in as many as thirty six locations, whereas Mg was low in thirty four and Ca in twenty four locations. The major nutrients like nitrogen and potassium had positive indices showing that they were sufficient (Fig. 2). The order in which nutrients were limiting the yield are (P> Mg> Ca) also indicated that most often more than one nutrient were limiting the yield. Thus, the yield limiting nutrients were differing from orchard to orchard though some of the nutrients were more prominent. Similarly, DRIS also identified P and Ca as common yield limiting nutrients in apple (Singh *et al.*, 2000; Ganeshamurthy *et al*., 2019). The results of the present study indicates that the low yields could be due to the low levels of these nutrients. There was an perceptible excessive consumption of N (I_N = 8.58). The detrimental effects of having high values of nutrients like N is that it may accumulate in the leaf at excessive levels at the cost of other nutrients creating nutrient imbalance in the plant system, thus affecting the yield capabilities of the tree and quality of the produce.

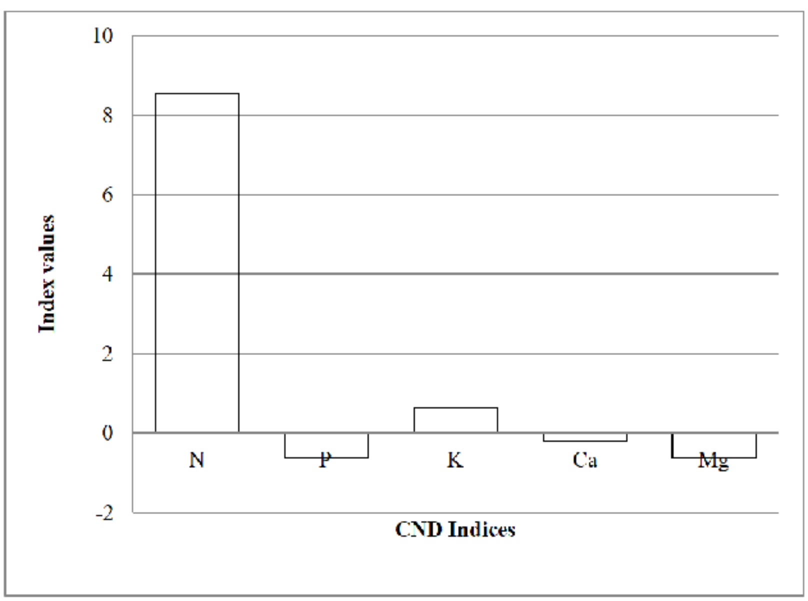

Fig. 2. Mean CND indices for a population of apple plantations in Kinnaur.

Table 4: CND indices for selected, low yielding orchards of apple.

N	P	K	Ca	Mg	R
9.473411	0.229967	0.563125	0.769178	-2.14343	1.71588
10.2736	0.220839	0.163355	-0.41464	-1.81277	2.548537
9.114397	-0.9086	1.818843	-0.58564	-1.2629	2.26838
8.569979	-0.73625	1.666456	0.197385	-0.95276	0.79809
9.562167	-1.6347	1.763695	-0.53032	-0.34333	0.933663
7.745482	-1.17819	1.710966	0.238233	-0.37493	0.878575
9.60913	-0.15246	1.54691	0.038133	-2.08735	1.848422
9.144492	-0.41349	0.336284	1.328401	-1.61784	1.37712
9.012033	-0.48791	1.13512	1.968091	-2.33408	1.212896
9.586416	-2.21378	0.641059	0.8407	-0.53275	1.824538
10.07039	-0.87098	0.023207	0.330068	-1.23523	2.33276
9.903908	-0.00638	-2.80485	-2.12543	1.904429	1.380716
7.122839	0.27361	1.281557	0.611555	-0.90321	0.253255
9.668128	-0.59751	-0.25421	-2.74913	1.055167	1.986707
7.475255	-0.39313	-0.20179	0.067203	0.516391	0.647374
9.077027	-3.18432	0.902884	0.264039	0.437684	2.228369
9.551155	-1.16269	1.506928	0.818383	-1.77393	1.774279
7.555761	-0.28388	0.495391	-0.10993	-0.33985	1.672461
9.642988	-1.68254	1.293202	-0.25109	-1.04448	2.801091
10.90153	-0.38279	0.961008	-0.55595	-1.90503	2.295126
11.09459	-1.84469	0.142524	-1.37184	0.018909	2.515419
8.425351	0.140627	0.453452	-1.02174	-0.36498	1.611951
8.788946	-1.59089	0.981266	1.826897	-1.15229	0.994289
7.837056	1.858682	0.937919	-1.39408	-1.17827	1.019768
9.741398	0.821203	-0.20368	-0.75148	-1.37118	2.028653
8.6254	-0.20566	-0.36021	-3.94781	2.095222	1.867388
6.586929	-0.82817	-1.1244	0.861233	1.213431	0.704172
8.563898	-0.24021	0.549002	-0.06073	-0.87792	1.730897
6.633204	1.705057	-0.14086	0.890521	-1.29126	0.736412
7.788211	-1.65365	1.545888	0.037194	-0.2438	1.881699
7.382078	-0.85966	0.375609	-2.03126	1.542004	1.531934
8.30081	-0.84297	1.009217	-0.5117	-0.60217	2.417365
7.968169	-2.10075	1.669996	0.968034	-0.73962	2.040453
8.523902	0.260016	1.163074	-0.37051	-1.78396	2.635946
6.934887	-0.08449	0.740169	1.699056	-1.69998	2.003546
8.318309	-1.35584	1.911472	1.189642	-1.86891	2.383249
7.831065	-0.19324	2.55838	-0.52454	-1.18074	0.962913
7.724805	-0.61837	0.333315	-1.19674	0.133225	2.858682
8.092227	0.087508	1.538335	-0.9642	-1.55911	3.201825
7.902515	-1.0131	0.141188	-0.43499	-0.55208	4.068649
7.473007	-1.83073	1.111287	-2.36931	1.535966	2.608451
10.33649	-0.72149	1.158352	-0.37484	-1.78678	2.592705
7.742552	1.553085	-1.34959	0.008296	-0.83285	1.888717
8.392301	-2.4981	-0.22852	0.163799	0.40905	3.50862
4.855174	-1.04669	0.759133	1.71646	-0.18182	2.06462
8.344268	-0.27035	-0.26165	-1.81788	0.109024	3.496882

CONCLUSION

Thus, the results of the present study suggested that multi-nutrient diagnosis developed through CND and nutrient interactions elucidated through PCA identified P, Mg and Ca as the most common yield limiting nutrients and indicate the necessity to regulate content of available N and K in apple orchard soils and, are thus instrumental in evolving nutrient management strategies based on soil and plant analysis.

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