

Compositional meta-analysis of the nutrient profile of potato cultivars

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While several potato (Solanum tuberosum L.) cultivars of different maturity groups (e.g. early, mid-season, late) are being selected each year as a result of successful breeding for disease resistance and market requirements, their nutrient management is based on past experience and few experiments. Nutrient profiles from leaf analysis can guide fertilization and liming programs of potato cultivars. Since leaf analytical data are strictly positive and compositional, nutrient profiling using raw data is spoiled by non normal distribution, resonance and spurious correlations. Compositional data analysis provides log ratio transformations that avoid such problems. Our objective was to derive nutrient profiles from tissue analysis using isometric log ratio (ilr) coordinates and meta-analysis for classification of cultivars into uniform nutrient management groups. The dataset comprised 678 potato fields producing more than 28.5 Mg marketable tuber ha⁻¹, i.e. above Quebec average, of the early-, mid-, and late-season cultivars. The first mature leaf from top was sampled at the beginning of flowering for N, P, K, Ca, and Mg analysis. Anionic (N, P) and cationic (K, Ca, Mg) nutrients were arranged into binary partitions representing positive and negative nutrient interactions. Groups of cultivars were compared to 'Superior' using *ilr* mean and standard deviation in the mixed model of meta-analysis. We minimized the within-group heterogeneity (I² value) by allocating cultivars iteratively between *ilr* groups. We derived group-specific *ilr* norms to compute the Aitchison distance. The critical value for nutrient imbalance was 0.38. To guide correcting nutrient deficiencies with appropriate nutrient management techniques, nutrient composition can be altered numerically by a perturbation vector on nutrients that lead to the largest and most negative *ilr* differences from *ilr* norms until the Aitchison distance falls below critical value.

1. Introduction

Nutrient requirements of annual crops like potato (*Solanum tuberosum* L.) are derived from fertilizer trials and soil tests using crop response models (Meslted and Peck, 1984) as well as soil test partitioning and crop response probability (Nelson and Anderson, 1984). Leaf analysis is less common to guide fertilization of the potato because nutrient norms have been developed for samples generally collected at beginning of blooming or at full bloom (Bergmann, 1988; Parent et al., 1994) that is too late for timely fertilizer application.

Potato cultivars are classified broadly into maturity groups (e.g. very early to early, mid-season, late, and very late). Since new cultivars inherit from their parents specific traits for nutrient absorption and assimilation, nutrient profiles as described by leaf analyses may vary widely among cultivars of the same maturity group. In addition to genetic effects, sensitivity to water, grazers or disease stress during growth that may hasten or delay wilting and date of top kill may also impact on nutrient needs, especially nitrogen. However, it is impossible to conduct a sufficient number of trials to generate cultivar-specific fertilizer recommendations for different markets and agro-ecosystems, thus increasing the risk of under or over fertilization with economic, agronomic and environmental consequences. Leaf



analyses may be useful to classify new cultivars into groups showing the same nutrient profile and to take advantage of fertilizer trials conducted on a limited number of cultivars.

Raw leaf analytical data are strictly positive data that form a closed space between 0 and 100% (Lagatu and Maume, 1935; Parent and Dafir, 1992; Parent et al., 1994) rather than a real space between $-\infty$ and $+\infty$. Non conformity to Gaussian laws, resonance and spurious correlations occur among components in the constrained compositional space (Aitchison, 1986; Butler, 2005). Any change in the proportion of one component must affect the proportions of other components in a closed space. In the leaf analytical space, resonance and spurious correlations result from nutrient interactions (Bates, 1971), luxury consumption and natural variation in nutrient concentration within tolerance ranges even at high yield level (Bergmann, 1988). The log transformation often used for data proportions allows data to spread into the real space (minus and plus signs) but disregards the relative information contained in the closed compositional space (Aitchison, 1986). Compositional data must be projected in the real space using log ratio transformations (Aitchison, 1986).

Many nutrient dual ratios have diagnostic value in plants (Bergmann, 1988; Malavolta, 2006). Using *n* nutrients, n(n-1) ratios and their inverse can be computed by the Diagnosis and Recommendation Integrated System (DRIS) (Beaufils, 1973). Interactions may be positive (nutrient product) or negative (nutrient ratio). The zero-sum constraint of DRIS nutrient indices for balance was no more attained where nutrient products rather than ratios were used (Walworth and Sumner, 1987). Nutrient ratios and DRIS are often viewed as complementary to nutrient concentration standards (Mourão Filho, 2005) although there is no model integrating both diagnoses. Parent and Dafir (1992) proposed an interactive concept expanding dual to multi-ratios as centered log ratios (Compositional Nutrient Diagnosis – CND-*clr*). On the other hand, the concept of dual ratios and products can be expressed as isometric log ratios (*ilr*) with orthonormal basis (Egozcue & Pawlowsky-Glanh, 2006) using sequential binary partitions of nutrients representative of positive and negative nutrient interactions in plants (CND-*ilr*).

Meta-analysis is a procedure to analyze datasets pursuing the same objective (Borenstein et al., 2009) such as nutrient profiling of potato cultivars. Cultivar groups can be arranged a priori and tested against a control. Their effects sizes over control are compared using fixed, random, and mixed models. The fixed model considers that *ilr* means of each cultivar are true means rather than random means. The random model considers that differences between cultivars must account for both the within and between sources of variation for cultivars of the same group. The within-group heterogeneity measures the internal coherence of the group in the mixed model.

Our objective was to derive groups of nutrient profiles for potato cultivars using *ilr* coordinates and meta-analysis. This novel procedure is designated as compositional meta-analysis and could be instrumental to build fertilization programs for new potato cultivars.

2. Material and methods

The potato dataset comprised 678 field observations in the St-Lawrence Lowlands of southwestern Quebec, Canada. There were early (17 'Norland'), mid- (56 'Atlantic', 79 'Chieftain', 154 'Kennebec', 38 'Shepody', and 286 'Superior'), and late (35 'NorDonna', 13 'Russet Burbank') season cultivars. We rejected areas producing less than 28.5 Mg marketable tuber ha⁻¹, i.e. below Quebec average. The first mature leaf from top was sampled at the beginning of flowering and analyzed for N by micro-Kjeldahl following the Devarda treatment. After ashing in a muffle furnace and acid dissolution of the ashes (Jones and Case, 1990), tissue P, K, Ca, and Mg concentrations were determined by colorimetry for P and by atomic absorption spectroscopy for metals. We excluded B and cationic micronutrients since

analyses were performed on a limited number of samples and fungicide sprays contaminated the samples with cationic micronutrients.

The compositional space of leaf tissue was described as follows (Aitchison, 1986): $x = C(N, S, P, K, Ca, Mg, F_v) = 1000g kg^{-1}$ (1)

Where \mathcal{C} is closure operator and F_{ν} is the filling value computed by difference between 1000 g kg⁻¹ and analytical results. The *ilr* coordinates were computed as follows (Egozcue and Pawlowsky-Glahn, 2006):

$$x_{i}^{*} = \sqrt{\frac{rs}{r+s}} \ln \frac{g(x_{+})}{g(x_{-})},$$
(2)

Where r and s are numbers of components in plus (+) and minus (-) groups, respectively, $g(x_+)$ is geometric mean of components in the plus group x_+ and $g(x_-)$ is geometric mean of components in the minus group x_- . There were 5 *ilr* coordinates from six components (five analytical results plus the filling value). The first partition was the one between nutrient and the filling value, whereby nutrients interact to promote plant growth. We contrasted anionic (N, P) against cationic (K, Ca, Mg) species and arranged other non overlapping binary partitions based on common physiological knowledge such as the N:P ratio (Güsewell, 2004) and antagonisms among cationic species (Bergmann, 1988; Malavolta, 2006) (Table 1).

Sequential non-overlapping partitions

1	1	1	1	1	1	-1	5 1	0.913 Nutrients vs. filling value
2	1	1	-1	-1	-1	0	2 3	1.095 Anionic vs. cationic species
3	1	-1	0	0	0	0	1 1	0.707 [N] vs. [P]
4	0	0	1	-1	-1	0	2 1	0.816 [K] vs. [Ca, Mg]
5	0	0	0	1	-1	0	1 1	0.707 [Ca] vs. [Mg]

Table 1. Ilr coordinates of the 5-elements and filling value (F_v) for potato in central Quebec.

The difference between two compositions was computed as the Aitchison distance across *ilr* coordinates computed as follows (Egozcue & Pawlowsky-Glanh, 2006):

$$d_a^2(x,y) = \sum_{i=1}^{D-1} (x_i^* - y_i^*)^2 \text{ and } \mathcal{A} = \sqrt{d_a^2(x,y)},$$
(3)

Where y_i^* is reference composition and \mathcal{A} is Aitchison distance.

Compositional analyses were conducted in R (van den Boogaart et al., 2000). We determined the critical value of Aitchison distance using the Cate-Nelson procedure (Nelson and Anderson, 1984). Using the Excel package and the chi-square homogeneity test (Hollander and Wolfe, 1999), we compared the number of successful and unsuccessful classification to an equal distribution between successful and unsuccessful cases. We conducted Manova and Anova (completely random design) and meta-analysis using the R package. Normality was verified using the Anderson-Darling test (Aitchison, 1986). The *ilr* data that were not normally distributed were transformed according to Yeo and Johnson (2000) and results of statistical analyses were compared to the non transformed *ilr* data. We made groups of cultivars for conducting meta-analysis in R (Schwarzer, 2010). Since the reference cultivar compared to each other cultivar was repeated, we divided the number of observations in the reference cultivar by the Box factor, k-1, where k is total number of cultivars (Huynh and Feldt, 1976). We selected 'Superior' as reference since it was the one with the largest number of observations. The effect size of each cultivar against 'Superior'

was computed as standardized mean difference corrected by Hedge's g. Difference between groups of cultivars was determined by the meta-analysis using the mixed model and the χ^2 distribution for testing the value of the Q statistics (Borenstein et al., 2009).

3. Results and discussion

The median tissue nutrient concentrations of potato cultivars are presented in Table 2. There were large discrepancies among cultivars, especially in N and K concentrations. All *ilr* coordinates but *ilr3* were normally distributed (P > 0.01) (Fig. 1).

	Cultivar									
Nutrient	Norland	Atlantic	Chieftain	Kennebec	Shepody	Superior	NorDonna	Russet Burbank		
	$g kg^{-1}$									
Ν	56.5	51.0	57.0	56.0	60.0	56.8	50.0	51.0		
Р	4.5	4.1	4.3	4.2	4.4	4.0	4.0	3.6		
K	50.7	69.3	54.8	53.8	51.0	57.5	66.3	61.9		
Ca	12.7	13.1	11.1	10.0	11.5	11.8	14.2	10.4		
Mg	5.5	7.7	5.6	6.1	5.3	5.8	8.1	7.4		

Table 2. Median values of nutrient concentration and the filling value for potato cultivars in Quebec.



Figure 1. Anderson-Darling normality test for ilr coordinates of potato cultivars (output of the R package).



Manova and Anova across *ilr* values were highly significant (P < 0.01). The Anova on Yeo-Johnson transformed *ilr3* (A-D test with P = 0.023) showed a higher and more significant F value (data not shown) compared to untransformed *ilr3*. Median *ilr* coordinates of potato cultivars are presented in Table 3.

Ilr	Norland	Atlantic	Chieftain	Kennebec	Shepody	Superior	NorDonna	Russet Burbank
1	-3.643	-3.577	-3.667	-3.711	-3.703	-3.675	-3.563	-3.642
2	0.141	-0.280	0.047	0.050	0.125	-0.012	-0.395	-0.203
3	1.813	1.800	1.835	1.845	1.870	1.873	1.830	1.915
4	1.149	1.562	1.583	1.623	1.525	1.606	1.494	1.482
5	0.635	0.391	0.509	0.375	0.532	0.496	0.360	0.260

Table 3. Median values of *ilr* coordinates for eight potato cultivars in Quebec producing > 28.5 Mg ha⁻¹.

There were marked differences among mid-season cultivars, especially *ilr2*. The early cultivar 'Norland' appeared to be different than others since *ilr2* and *ilr5* were higher and *ilr4* lower. The *ilr2*, *ilr4* and *ilr5* of late-season cultivars were close to each other but appeared different compared to others. Multiple comparisons are difficult to interpret due to overlap among means. Meta-analysis provided a useful grouping procedure that minimizes the within-group heterogeneity. A first comparison between cultivars using 'Superior' as control showed that the distribution of the *ilr3* means did not vary whether *ilr3* values were Yeo-Johnson transformed or not to conduct meta-analysis (Fig. 2).



Figure 2. Forest plots of *ilr3* whether using (upper plot) or not (lower plot) the Yeo-Johnson transformation (Output of the R package). NRL = Norland; ATL = 'Atlantic'; CHI = 'Chieftain'; KEN = 'Kennebec', SHE = 'Shepody'; NOR = 'NorDonna'; RUS = 'Russett Burbank'.

Meta-analyses on *ilr1*, *ilr2*, *ilr4*, and *ilr5* are presented in Fig. 3.







Figure 3. Forest plots of *ilr* values except *ilr3* (Output of the R package). NRL = Norland; ATL = 'Atlantic'; CHI = 'Chieftain'; KEN = 'Kennebec', SHE = 'Shepody'; NOR = 'NorDonna'; RUS = 'Russett Burbank'.



The efficiency of grouping was evaluated using the mixed model (Borenstein et al., 2009). The degree of heterogeneity among cultivars of the same group was considered high if $I^2 > 0.75$, medium if $I^2 \approx 0.50$, and small if $I^2 < 0.25$ (Borenstein et al., 2009). The I^2 was given a value of zero for $I^2 < 0$. Where $I^2 > 0.25$ within the same group, we re-allocated cultivars between groups until $I^2 < 0.25$. The mixed model is a tool to decrease within-group heterogeneity. Results of meta-analysis in Figs. 2 and 3 show I^2 values of 0.89 for *ilr1*, 0.88 for *ilr2*, 0.19 for *ilr3*, zero for *ilr4*, and 0.75 for *ilr5*. For all *ilr* values, at least one confidence interval did not overlap 'Superior' (e.g. ATL and NOR for *ilr1* in Fig. 4), indicating that at least two groups must be formed. Groups are considered homogeneous if I^2 is small within groups and there is both high heterogeneity and significant difference between groups.

The classification of cultivars in Table 4 produced relatively homogeneous groups (I² < 0.25 for *ilr2* to *ilr5* and I² = 0.35 for *ilr1*). Meta-analysis showed that these groups differed significantly from each other (P < 0.05).

Ilr	Group	I ² within		I ² between	χ^2
		G1	G2		
1	[ATL, NOR]; [CHI, NRL, KEN, SHE, SUP, RUS]	0	0.35	0.97	35**
2	[ATL, NOR, RUS]; [CHI, NRL, KEN, SHE, SUP]	0	0	0.98	42**
3	[ATL, NRL, KEN]; [CHI, NOR, RUS, SHE, SUP]	0	0	0.93	14**
4	[NRL, NOR, RUS]; [CHI, KEN, ATL, SHE]; [SUP]	0	0	0.84	6.3**
5	[NRL, ATL, KEN, RUS]; [CHI, SUP, SHE]	0	0	0.95	22**

** Significant at the 0.01 level

Table 4. Median value norms of *ilr* coordinates for eight potato cultivars in Quebec (Norland, Atlantic, Chieftain, Kennebec, Shepody, Superior, Russet Burbank, NorDonna).

The *ilr* norms for the groups selected in Table 5 were computed as median values among those presented in Table 3.

Ilr	Grouping	G1	G2	G3	Nutrient interactions
1	[ATL, NOR];				Nutrients vs. filling value
	[CHI, NRL, KEN, SHE, SUP, RUS]	-3.570	-3.667		
2	[ATL, NOR, RUS];				[N, P] vs. [K, Ca, Mg]
	[CHI, NRL, KEN, SHE, SUP]	-0.280	0.050		
3	[ATL, NRL, KEN];				[N] vs. [P]
	[CHI, NOR, RUS, SHE, SUP]	1.813	1.870		
4	[NRL, NOR, RUS];				[K] vs. [Ca, Mg]
	[CHI, KEN, ATL, SHE];				
	[SUP]	1.482	1.573	1.606	
5	[ATL, KEN, NOR, RUS];				[Ca] vs. [Mg]
	[NRL, CHI, SUP, SHE]	0.383	0.503		

Table 5. Median value norms of *ilr* coordinates for eight potato cultivars in Quebec (Norland, Atlantic, Chieftain, Kennebec, Shepody, Superior, Russet Burbank, NorDonna).

'Atlantic', 'NorDonna' and 'Russett Brubank' showed significantly higher N:P ratios than others as shown by *ilr2* (Table 5). 'Superior' was apparently the most demanding in potassium while 'Norland', NorDonna' and 'Russett Burbank' were the least demanding. 'Atlantic', 'Kennebec', 'NorDonna' and 'Russett Brubank' were less demanding in Ca or



more demanding in Mg compared to other cultivars. Our results show that cultivar grouping for nutrient management depended on *ilr* coordinate, i.e. the compositional dimension.

The Aitchison distance was computed using Eq. 3 after introducing the *ilr* norms presented in Table 5. The scatter diagram relating yield to the Aitchison distance was partitioned into four quadrants (NW, NE, SW, SE) and the number of points was maximized in the opposite quadrants NW and SE. The NW quadrants represented true high yielders (high yield and small \mathcal{A}), the NE, false high yielders (high yield and high \mathcal{A}), the SW, false high yielders (low yield and low \mathcal{A}), and the SE, true low yielders (low yield and high \mathcal{A}).

The relationship between the Aitchison distance and tuber yield is presented in Fig. 4. The critical Aitchison distance was found to be 0.38. The critical yield separating the high from the low sub-population was found to be 42 Mg tuber ha⁻¹ after maximizing the number of points in opposite quadrants of successful cases Successful classification comprised crops producing more than 42 Mg tuber ha⁻¹ and showing an Aitchison distance below 0.38 (true high yielders) and those producing less than 42 Mg tuber ha⁻¹ and showing an Aitchison distance above 0.38 (true low yielders).

True high yielders and true low yielders made up 64% of this classification (431 successful and 247 unsuccessful cases for classification). Compared to an equal distribution of 50% successful and 50% unsuccessful cases and using a chi-square homogeneity test (Hollander and Wolfe, 1999), the null hypothesis was rejected ($\chi_1^2 = 3.75$, P = 0.05). Hence, the critical Aitchison distance of 0.38 showed diagnostic potential to detect nutrient imbalance in potato cultivars using the *ilr* norms by group presented in Table 5. However, independent fertilizer trials must be conducted to ascertain the critical Aitchison distance of 0.38.



Figure 4. Cate-Nelson partitioning of the relationship between the Aitchison distance across *ilr1* to *il57* and tuber yield in southwestern Quebec, Canada (number of observations per quadrant in parentheses).

The Aitchison distance of a given tissue can be varied using a perturbation vector for diagnostic purposes. A perturbation is a multiplicative operation whereby the original composition x is converted into a perturbed composition X through perturbation vector u as follows (Aitchison, 1986):

$$X = u \oplus x = \mathcal{C}(x_1 u_1, \dots, x_D u_D)$$

(4)



Where C is the closure operator. Since the Aitchison distance can be decomposed into *ilr* differences, imbalance is shown by the greatest *ilr* difference and its direction by the sign of that difference. Nutrient composition can be varied among the largest and most negative *ilr* differences from *ilr* norms using a perturbation vector u until proper Aitchison distance is recovered, i.e. below critical value. Nutrient management can be adjusted accordingly by applying a source of deficient nutrients at the rate and timing sought appropriate to reestablish nutrient balance.

4. Conclusion

The raw nutrient concentrations are not appropriate data to describe the nutrient profile of potato cultivars due their constrained compositional space. Raw data must be log ratio transformed. In this paper, we used isometric log ratios based on sound sequential binary partitions of nutrients that reflected positive and negative interactions in plants.

Running Anova, we found that nutrient profiles overlapped or differed significantly among cultivar groups depending on *ilr* coordinates. Allocating cultivars to minimize the within-group and maximize the between-group heterogeneity in meta-analysis, we found that cultivars showed similar nutrient profiles along some *ilr* coordinates and very different ones along others. The nutrient profiles of new potato cultivars were classified into the appropriate cultivar group to take advantage of fertilizer trials conducted on cultivars of the same group. The Aitchison distance and a perturbation vector are instrumental for diagnostic purposes.

In this paper, we presented an approach to define cultivar nutrient profiles and diagnostic *ilr* norms. Further research is needed to ascertain the critical Aitchison distance using independent fertilizer experiments and to verify the stability of nutrient profiles and *ilr* norms by the meta-analysis of interactions between plant nutrition, climatic conditions, and soils.

5. References

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