UNIVERSIDAD AUTÓNOMA AGRARIA ANTONIO NARRO

SUBDIRECCIÓN DE POSGRADO

ESTEQUIOMETRÍA EN *Opuntia ficus-indica* L. (Miller) VARIEDAD ‘ROJO PELÓN’

Tesis

Que presenta EVELYN HERNÁNDEZ VDAL
como requisito parcial para obtener el Grado de
DOCTOR EN CIENCIAS EN PRODUCCIÓN AGROPECUARIA

Torreón, Coahuila, México Mayo 2021

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Torreón, Coahuila, México

Mayo 2021

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Tesis

Elaborada por EVELYN HERNÁNDEZ VIDAL como requisito parcial para obtener el grado de Doctor en Ciencias en Producción Agropecuaria con la supervisión y aprobación del Comité de Asesoría

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Torreón, Coahuila

Mayo, 2021

A mi madre y mi padre, Aurora Vidal Santos y, Enrique Hernández Rodríguez
a quienes amo profundamente. Este trabajo es un homenaje a sus vidas, porque a pesar de no contar con su presencia física siguen teniendo un efecto fortalecedor en mí. Porque cada día de sus vidas cuente.

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CARTAS DE ACEPTACIÓN Y ENVÍO DE LOS ARTÍCULOS

Aceptación de artículo

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Fecha: El mié, 28 de octubre de 2020 a la(s) 21:09
Asunto: Your Submission JSSP-D-20-00851R2
Para: Ricardo David Valdez-Cepeda <vacrida@gmail.com>

Dear Dr. Valdez-Cepeda,

We are pleased to inform you that your manuscript, "Boundary-line approach macro-nutrient standards for Opuntia ficus-indica (L.) Miller variety 'Rojo Pelón' fruiting", has been accepted for publication in
Journal of Soil Science and Plant Nutrition.

You will receive an e-mail in due course regarding the production process.

Please remember to quote the manuscript number, JSSP-D-20-00851R2, whenever inquiring about your manuscript.

With kind regards,
María de la Luz Mora, Ph.D.
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Dr. Bernardo Murillo Amador <bmurillo04@cibnor.mx>

para mí, Fidel, Francisco, Leticia, Ricardo

14:35 (hace 9 horas)

Hola,

Anselmo González Torres ha enviado el manuscrito "CND standards for Opuntia ficus-indica (L.) Miller variety 'Rojo Pelón' fruiting (April 2021).docx " a Journal of the Professional Association for Cactus Development.

Si tiene cualquier pregunta no dude en contactarme. Le agradecemos que haya elegido esta revista para dar a conocer su obra.

Dr. Bernardo Murillo Amador

Dr. Bernardo Murillo-Amador

Editor-in-Chief

Journal of the Professional Association for Cactus Development http://www.jpacd.org/jpacd

ÍNDICE DE CONTENIDO

ÍNDICE DE FIGURAS

RESUMEN

Las concentraciones óptimas y los rangos de suficiencia de nutrimentos son útiles para el diagnóstico correcto y las mejoras del estado nutricional de las plantas cultivadas. Las concentraciones críticas o los equilibrios de nutrimentos para algunos cultivos se han determinado utilizando los principios del enfoque de curva límite (LCL) y el diagnóstico de nutrimentos compuesto (DNC). Sin embargo, los requerimientos de nutrimentos para la fructificación de *Opuntia ficus-indica* siguen siendo prácticamente desconocidos. De acuerdo con lo anterior, los objetivos de este trabajo de investigación fueron identificar las concentraciones óptimas de macronutrimentos vinculadas al rendimiento máximo y rangos de suficiencia al 90% de rendimiento máximo por cladodio de fructificación de un año para *Opuntia ficus-indica* (L.) Miller variedad “Rojo Pelón”. En ese sentido, una base de datos (n=228) de rendimiento (fruto (g cladodio^{-1}) y concentración de los nutrimentos N, P, K, Ca y Mg se utilizó con el objetivo de desarrollar los estándares nutrimentales mediante la línea o curva límite y DNC, para posteriormente compararlos. Las concentraciones medias de nutrimentos estimadas por medio de DNC difieren levemente de las estimadas con la LCL. Lo que se puede observar es que las concentraciones medias estimadas de N y K vinculadas a los estándares de DNC son levemente inferiores a las concentraciones óptimas estimadas por LCL (0.958% a 1.02% y 3.507% a 3.518%, respectivamente). Por otro lado, las concentraciones medias calculadas de P, Mg y Ca asociadas a los estándares de DNC mayores a las concentraciones óptimas estimadas por LCL, es decir, 0.318% versus 0.304%, 1.448% versus 1.383 y 4.228% versus 3.665%, para P, Mg y Ca, respectivamente. Esas diferencias pueden deberse a que los rendimientos de fruto objetivo varían entre las técnicas DNC y LCL, es decir de 1666.67 g cladodio^{-1} a

1901.13 y 1984.41 g cladodio^{-1}, respectivamente. Los rangos estimados con LCL suelen ser más amplios que los establecidos con DNC para algunos nutrimentos; sin embargo, las diferencia son mínimas. Estos resultados sugieren que las técnicas de línea de curva límite y diagnóstico de nutrimento compuesto permiten establecer normas nutrimentales confiables; por lo tanto, estas técnicas son complementarias y no mutuamente excluyentes.

Palabras claves: Fruto, tuna, cladodios, nitrógeno, nutrimentos, magnesio.

ABSTRACT

Optimal concentrations and ranges of nutrient sufficiency are useful for correct diagnosis and improvements in the nutritional status of cultivated plants. Critical concentrations or nutrient balances for some crops have been determined using the principles of the boundary-line approach (B-LA) and Compositional Nutrient Diagnosis (CND). However, the nutrient requirements for the fruiting of *Opuntia ficus-indica* remain practically unknown. In accordance with the above, the objectives of this research work were to identify the optimal concentrations of macronutrients linked to maximum yield and ranges of sufficiency at 90% of maximum yield per one-year fruiting cladode for *Opuntia ficus-indica* (L.) Miller variety “Rojo Pelón”. In this context, a database (n = 228) of fruit yield (g cladode^{-1}) and concentration of the nutrients N, P, K, Ca and Mg was used to develop the nutrient standards by means of the B-LA and CND, and later to compare them. The nutrient concentrations estimated by means of the CND differ slightly from those estimated with the BLA. It can be appreciated that the estimated mean concentrations of N and K linked to the CND standards are slightly lower than the optimum concentrations estimated by the BLA (0.958% vs 1.02%, and 3.507% vs 3.518%, respectively). On the other hand, the calculated mean concentrations of P, Mg and Ca associated with the CND standards are higher that the optimum concentrations estimated by the BLA, that is, 0.318% versus 0.304%, 1.448% versus 1.383 and 4.228% versus 3.665%, for P, Mg and Ca, respectively. The CND and BLA techniques estimated yields of 1666.67 g cladode^{-1} to 1,901, 13 and 1984.41 g of cladode^{-1}, respectively. The nutrient ranges of sufficiency estimated with BLA are wider than those established with the CND for some nutrients; however, the differences are small. These results suggested that the B-LA and CND techniques allow establishing reliable nutrient norms; therefore, these techniques are complementary and not mutually exclusive.

Keywords: Fruit, prickly pear, cladodes, nitrogen, nutrients, magnesium.

1. INTRODUCCIÓN

El cultivo de *Opuntia ficus-indica* L. Miller para la producción de frutas se ha convertido en una importante actividad agrícola en México (Gallegos Vázquez *et al*., 2013). México es el mayor exportador de tuna, obteniendo el 80% de la producción mundial estimada en 468,100 t año^{-1} con una superficie plantada con nopal tunero de 45,733 ha (SIAP, 2019), siendo este el sexto frutal más importante del país (Inglese, 2019). En las regiones templadas semiáridas de México, el nopal puede ser un cultivo en condiciones de secano confiable y rentable debido a su resistencia a sequías y temperaturas congelantes extremas (Valdez *et al*., 2001). A pesar de la importancia de *O. ficus-indica*, poca información sobre los requerimientos nutrimentales de este cultivo existe en la literatura. La determinación de niveles óptimos de concentración de nutrimentos podría dar lugar a un aumento de los rendimientos y mejoras en la calidad de los frutos (Habib, 2000; Mostashari *et al*., 2018). Por lo tanto, lo necesario es conocer las concentraciones óptimas y los rangos de suficiencia de nutrimentos útiles para el diagnóstico correcto y la mejora del estado nutrimental de las plantas cultivadas (Blanco-Macías *et al*., 2009, 2010). Las técnicas utilizadas para el desarrollo de normas nutrimentales son diversas, no obstante, las de mayor confiabilidad son: línea de curva límite (LCL) y diagnóstico de nutrimento compuesto (DNC). Algunos investigadores han demostrado la precisión en el diagnóstico de los desequilibrios cuando se utilizan normas desarrolladas localmente (Blanco-Macías *et al*., 2010; Bendaly Labaied *et al*., 2018). En el caso de *O. ficus-indica* existe poca investigación acerca de los requerimientos nutrimentales y la mayoría se centra en las respuestas a la fertilización o con el fin de evaluar la materia seca y fresca (Galizzi *et al*., 2004; Blanco-Macías *et al*., 2006; Blanco-Macías *et al*., 2010; Alves. 2017; Teixeira *et al*., 2019). Sin embargo, los estudios sobre los requerimientos nutrimentales de *O. ficus-indica* para la producción de frutas (tuna) se encuentran desactualizados y fragmentados; por estas razones, lo necesario es establecer las normas nutrimentales de la variedad *O. ficus-indica* 'Rojo Pelón' y de esta manera mejorar las prácticas de fertilización.

1.1. Hipótesis

1. La técnica de curva límite delimita la frontera de las concentraciones óptimas de macronutrimentos que maximizan el rendimiento de fruto de *O. ficus-indica* variedad 'Rojo Pelón'.
2. Las concentraciones de macronutrimentos estimadas mediante la técnica de curva límite son comparables a las obtenidas por la técnica de diagnóstico de nutrimento compuesto; por lo tanto, ambas técnicas son complementarias y no mutuamente excluyentes.

1.2. Objetivos

1. Identificar, mediante la técnica de curva límite (CL), las concentraciones óptimas de macronutrimentos (N, P, K, Ca, Mg) y rangos de suficiencia al 90%, vinculados al rendimiento máximo de fruta en cladodio de fructificación de un año de edad de *O. ficus-indica* (L.) Miller variedad 'Rojo Pelón'.

2. Desarrollar normas de diagnóstico de nutrimentos compuesto (DNC) para la producción de frutos de *Opuntia ficus-indica* (L.) Miller variedad 'Rojo Pelón' y compararlas con las establecidas por medio de la técnica de curva límite.

2. REVISIÓN DE LITERATURA

2.1. Estequiometría en los cultivos

La estequiometría es el estudio del balance de masa de múltiples elementos químicos en los ecosistemas o plantas, analiza las restricciones y consecuencias de estos balances de masa durante procesos bioquímicos, con base en leyes universales de física, química y biología. El balance de nutrimentos tiene un papel importante en los procesos de crecimiento y desarrollo de las plantas. Los equilibrios de elementos múltiples indican la capacidad de fijación, absorción y la eficacia en la utilización de los nutrimentos por la planta.

La estequiometría de las plantas tiene una estrecha relación con la ley del mínimo de Liebig (Ågren *et al*., 2018). La estequiometría contempla la importancia de las proporciones relativas entre los elementos para el crecimiento de las plantas, los rendimientos máximos y las proporciones de los nutrimentos en concentraciones ideales o en balance. El enfoque de la estequiometría, así como el de la ley del mínimo, indica que el rendimiento de las plantas está en función de los factores limitantes; por lo tanto, el conjunto de proporciones entre nutrimentos en sus concentraciones ideales no limita el crecimiento de las plantas; pero cualquier disminución en relación con las proporciones haría de algún elemento un factor limitante. Por el contrario, cualquier aumento de un nutrimento no incrementaría el crecimiento o rendimiento, inclusive el exceso de algún nutrimento podría originar toxicidad en las plantas.

El establecimiento de estándares nutrimentales tomando en consideración el balance de los nutrimentos es el resultado de un avance en la comprensión de los mecanismos detrás de las relaciones estequiométricas, así como la apreciación de la existencia de patrones a gran escala (Sterner y Elser. 2002). Las plantas requieren para su crecimiento del orden de 30 elementos, algunos en mayores cantidades (C, H, O, N, P, K, Ca, Mg, S), mientras que otros se requieren en cantidades mínimas (Fe, Zn, Mo, Mn, etc.). La función de los nutrimentos dentro de las plantas se ha estudiado previamente; pero los requerimientos de los elementos para algunos cultivos están pobremente considerados, así como la dependencia entre los elementos. La relación más investigada es la de C: N: P porque N y P comúnmente limitan el crecimiento, y proporciona la base estructural, formando un 50% de la materia seca de la planta (Elser *et al*., 2007); sin embargo, la proporción de estos nutrimentos, así como sus requerimientos son muy variables y dependen de muchos factores, en especial del tipo de planta y el ambiente en el que se desarrolla.

2.2. Análisis de concentraciones de nutrimentos en hojas

El análisis de la composición química de las plantas es complejo debido a que la planta integra múltiples factores (Sumner y Boswell, 1981). Por lo tanto, el efecto de los factores se expresa en la composición química de la planta y como consecuencia en el rendimiento (Robinson 1980; Dow y Roberts, 1982). El estado nutrimental de las plantas es más perceptible a los cambios del ambiente, por lo que su interpretación es importante para establecer diagnósticos y recomendaciones.

2.3. Relación entre concentración de nutrimento y el rendimiento

Las concentraciones de nutrimentos foliares tienen relación con las necesidades de las plantas y del suministro del suelo. Por lo tanto, el estudio del equilibrio dinámico de elementos de las plantas, es importante para establecer concentraciones óptimas de nutrimentos para el crecimiento, desarrollo y producción de frutos.

La relación entre la concentración de nutrimentos y el rendimiento puede representarse mediante una distribución normal de los datos y un gráfico de dispersión. Chapman (1967) determinó que esta relación entre concentración y rendimiento puede clasificarse en cinco regiones importantes. En el primer cuadrante, con concentraciones y rendimientos mínimos, se clasifica la primera zona llamada región de deficiencia; en esta región, la concentración del nutrimento es mínima y consecuentemente limita el rendimiento del fruto. El segundo cuadrante es de especial cuidado; a esta región se le conoce como marginal porque la concentración sigue siendo deficiente; sin embargo, esta deficiencia no es observable y por eso también es llamada hambre oculta (Barker y Pilbeam, 2015). Los síntomas observables de desequilibrio de nutrimentos a menudo aparecen cuando se han generado daños importantes e irreversibles; estos daños pueden provocar un crecimiento escaso y un rendimiento reducido de los cultivos. El tercer cuadrante en el grafico nos indica una región en la que es posible obtener un 90% de rendimiento de los cultivos; a esta región se le considera de suficiencia ya que las concentraciones de los nutrimentos se encuentran en concentraciones ideales u óptimas y en balance; esta región forma la parábola del gráfico y justo en el vértice de la curva se pueden identificar la concentración óptima y el rendimiento máximo para el cultivo involucrado. La cuarta región comprende la segunda zona marginal;

en este caso, la concentración es mayor a la necesaria para la planta, lo que inducirá a toxicidad con daños poco visibles. Finalmente, en la quinta región se encuentran las concentraciones mayores a las requeridas por las plantas; por lo tanto, esta zona es llamada región de toxicidad e indica que una mayor concentración del nutrimento no representa mayor rendimiento, sino por el contrario, este exceso se traduce en pérdidas económicas y de producción (Figura 1).

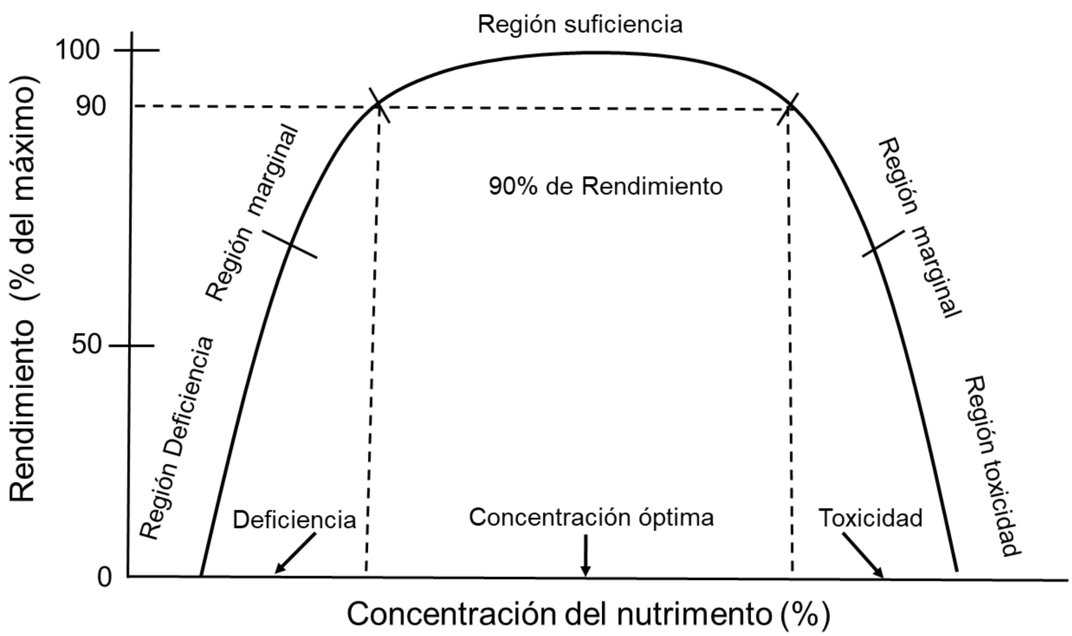

Figura 1. Diagrama de representación de concentraciones críticas y óptimas, rangos de deficiencia, suficiencia marginal y toxicidad (Chapman, 1967).

2.4. Técnicas de diagnóstico nutrimental

Las técnicas que se han desarrollado para estimar concentraciones óptimas de los cultivos son diversas. Algunas carecen de soporte y suelen ser poco rígidas en el análisis. Otras técnicas omiten que el desarrollo y rendimiento de las plantas están vinculados a diversos factores y no exclusivamente a la concentración de nutrimentos. Los rendimientos máximos se obtienen cuando los nutrimentos y los demás factores involucrados en el crecimiento se encuentran en niveles óptimos o balanceados, es decir, que existe menos posibilidad de interacciones, o bien que, si se presentan, la respuesta sea favorable, obteniendo respuestas mayores (sinergismos) o menores (antagonismos) que la suma de esos efectos individuales de los factores (Sumner y Fariña, 1986).

2.4.1. Valor critico

La técnica de valor crítico (VC) representa la composición mineral de los tejidos vegetales, expresada en concentraciones o valores relativos, formando valores numéricos básicos y tomando en consideración a los nutrimentos de forma individual, es decir, en un enfoque univariado (Bates, 1971). La interpretación del análisis foliar utilizando la técnica de VC para determinar deficiencia, suficiencia o exceso de nutrimentos es afectada en gran medida por las interacciones de los nutrimentos y por la edad de las plantas. La técnica de VC, por lo tanto, se basa en solo comparar las concentraciones de nutrimentos observados con los valores de referencia; cuando la concentración de nutrimentos en cuestión es menor que el valor de referencia se asume una deficiencia. Este enfoque ha sido criticado por no tener en cuenta las interacciones entre nutrimentos (Wilkinson, Grunes y Sumner 2000; Marschner 2011). Su interpretación no es confiable y carece de soporte debido a que establece la relación de la concentración de algún elemento mineral con el rendimiento y no considera que los rendimientos máximos se obtienen por el balance de los nutrimentos y por los niveles ideales de los demás factores responsables del crecimiento; esta técnica descarta la posibilidad de que se presenten sinergismos o antagonismo entre los nutrimentos.

2.4.2. Sistema Integrado de Diagnóstico y Recomendación (DRIS)

El DRIS fue elaborado con la finalidad de mejorar el diagnóstico en comparación con VC, teniendo en cuenta interacciones entre pares de elementos expresadas como proporciones de nutrimentos (Walworth y Sumner,1987). El DRIS respalda sus principios mediante el modelo Sumner y Fariña (1986) (Figura 1). La letra A representaría una deficiencia de X o bien un exceso de Y, y que esto puede corregirse incrementando el valor de X, permitiendo tener un rendimiento B determinado también por otros factores. En escala, entre más se corrijan los factores limitantes se reflejará en un aumento del rendimiento. Por lo tanto, así se forma un grupo integrados de normas representativas de diversos factores como son: suelo, clima, planta y prácticas de manejo (Walworth y Sumner, 1987; Sumner, 1986). Por consiguiente, la exactitud de los diagnósticos foliares depende de la validez de las normas en situaciones específicas (Walworth y Sumner, 1987). A pesar de considerar las proporciones de nutrimentos y soportarse en un análisis bivariados se han reportado inconsistencias entre VC y DRIS (Alkoshab et al., 1988), pero en general el DRIS muestra mayor eficiencia diagnóstica (Walworth y Sumner, 1989).

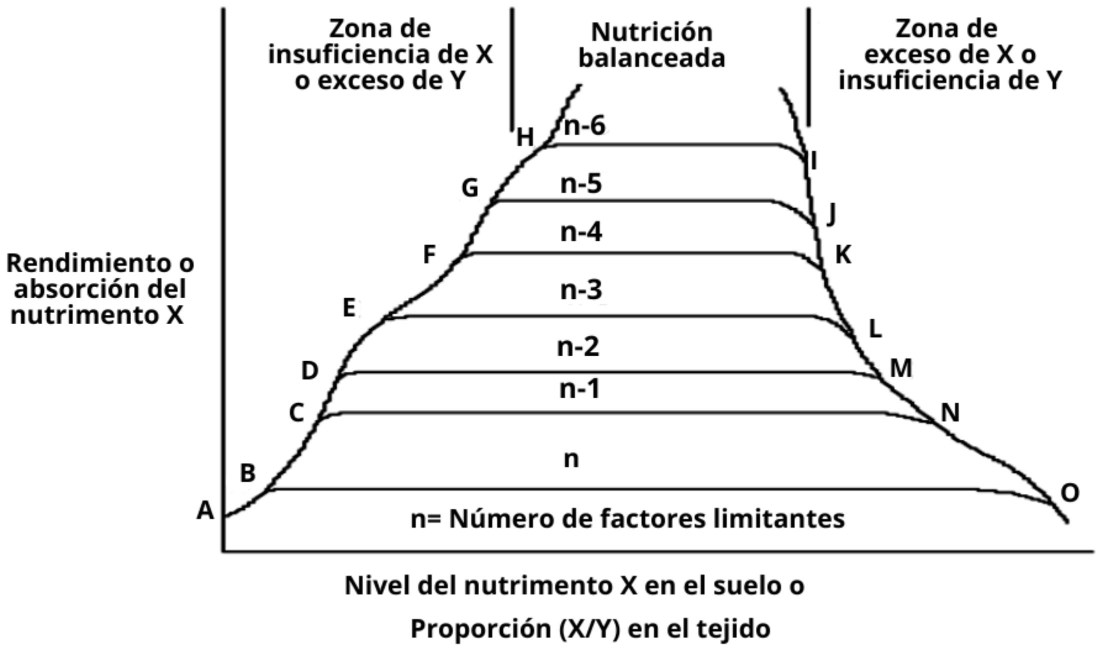

Figura 2. Diagrama de la respuesta de las plantas a los factores limitantes (Sumner y Fariña, 1986)

2.4.3. Técnica Diagnóstico de Nutrimento Compuesto (DNC)

La técnica DNC permite estimar proporciones entre varios nutrimentos (multivariables) (Quesnel *et al*., 2006) también, DNC tiene una matriz de covarianza bien definida y calcula las proporciones a partir de los valores de concentración que son mutuamente excluyentes (García *et al*., 2006). DNC está respaldado por la teoría de diagnóstico con respecto al análisis de datos composicionales. Por lo tanto, DNC es la expansión multivariable de VC y DRIS debido a que toma en cuenta todas las posibles interacciones entre nutrimentos y los índices de nutrimentos del DNC se componen de dos funciones separadas; una que considera las diferencias entre los niveles de nutrimento y otra que examina diferencias entre los balances de nutrimentos (según se define por medios geométricos de nutrimento), de especímenes individuales y específicos. Estas funciones indican que la insuficiencia de nutrimentos se puede corregir agregando un solo nutrimento o tomando ventaja de múltiples interacciones de nutrimentos para mejorar el equilibrio entre nutrimentos en conjunto. Las normas del diagnóstico de nutrimento compuesto (DNC) se estiman por medio de una serie de procedimientos utilizados previamente por, Khiari, Parent y Tremblay (2001a 2001b).

2.4.4. Enfoque de línea de curva limite (LCL)

El enfoque de línea de curva límite (LCL) fue desarrollado por Webb (1972) y se ha utilizado para estimar concentraciones óptimas de nutrimentos. Esta técnica describe que los desempeños de los mejores especímenes en la muestra analizada deben ser tomados como modelos, en el supuesto de que existen otras razones que explican un desempeño no ideal y que está relacionado con diversos factores bióticos y abióticos (Blanco-Macías *et al*., 2010). Por tanto, la exactitud de los diagnósticos foliares depende de la validez de las normas y situaciones específicas (Walworth y Sumner, 1987).

La LCL es un método frecuentemente utilizado para evaluar la nutrición y estado de las plantas cuando la relación entre las variables siendo analizadas han sido influenciadas por otros factores que interactuaron. Las concentraciones críticas de nutrimentos en las hojas que limitan el crecimiento son determinadas comúnmente por regresión en respuesta a la variación de un solo factor. Sin embargo, una serie de factores que afectan el crecimiento y desarrollo de las plantas existen; por ejemplo, en diferentes años, las ecuaciones ajustadas pueden diferir de las ajustadas en años anteriores, especialmente debido a interacciones entre otros factores que determinaron al rendimiento (Walworth *et al*., 1986). Varios métodos de interpretación del análisis nutrimental de las hojas existen; los basados en el nivel crítico o los rangos de suficiencia son los más utilizados. Sin embargo, Camacho *et al*. (2012) y Blanco-Macías et al. (2010) afirman que los diagnósticos nutrimentales y los métodos que determinan los valores de referencia para regiones específicas pueden proporcionar resultados más precisos que los que diagnostican estados nutrimentales de plantas en general.

La técnica de curva límite sugerida por Webb (1972) consiste en graficar el rendimiento en función de las características evaluadas, eliminando algunos puntos y dejando sólo los puntos de la línea de borde. Esta línea se ajusta a un modelo polinomial para obtener el valor de concentración óptima o los rangos de suficiencia de nutrimentos en hojas. Webb (1972) declaró que las relaciones entre las variables generalmente se confunden con otros factores que interactúan, es decir, la dispersión de datos no sólo es el resultado de errores de medición y variabilidad biótica, sino también de interacciones con otros factores ambientales.

El método de la línea de curva límite se ha desarrollado para varios cultivos. Por ejemplo, *miscanthus, Arundo donax y Triticosecale* (Lewandowski y Schmidt, 2006), *Acer saccharum* (Vizcayno-Soto y Côté, 2004), *Abies alba* (Quesnel *et al*., 2006), Zea mayz (Walworth *et al*., 1986), *arecanut* (Bhat y Sujatha, 2013), arándano silvestre (Lafond, 2013), *Mangifera indica* (Ali, 2018) y *Opuntia ficus-indica* (Blanco-Macías *et al*., 2009; 2010). Este método se ha utilizado ampliamente para evaluar el estado nutrimental de las plantas, principalmente para determinar el nivel crítico o rango de suficiencia en hojas. Quesnel *et al*. (2006) concluyeron que el método se considera apropiado para este propósito, de manera similar al método de DNC. Por lo tanto, la línea de curva límite es una alternativa para calcular los niveles críticos y el rango de suficiencia en la hoja. Los datos del estado nutricional a través de la técnica de curva límite permiten una estimación de la concentración óptima y del rango de suficiencia de los nutrimentos en la hoja que maximizan el rendimiento de los cultivos.

2.5. Estado actual de conocimiento sobre nutrición en *Opuntia ficus-indica* (L.) Miller

El cultivo de *Opuntia ficus-indica* (L.) Miller para la producción de fruta es una actividad agrícola importante en México (Gallegos Vázquez *et al*., 2013). México es el mayor exportador mundial de tuna (Inglese, 2019), siendo Estados Unidos de América el principal país de destino de las exportaciones mexicanas, seguido de países como Canadá, Alemania y El Salvador. Las exportaciones de estos frutos generan divisas al país de 8.9 millones de dólares (SIAP, 2019). A pesar de la importancia de este cultivo, el conocimiento del manejo de los huertos aún es escaso (Inglese *et al*., 2002). El cultivo de nopal tunero requiere de insumos para aumentar los rendimientos; el manejo adecuado de los huertos se asocia con mayores rendimientos y mejor calidad de los frutos. Para mejorar la productividad y calidad de fruta se debe generar mayor conocimiento de la influencia del ambiente, el manejo de las huertas, el crecimiento de la fruta, la maduración y los requerimientos nutrimentales (Ochoa, 2003). La determinación de niveles óptimos de concentración de nutrimentos podría dar lugar a un aumento de los rendimientos y a una mejora de la calidad del fruto, lo que a su vez aumentaría los ingresos de los agricultores. Por lo tanto, lo importante es establecer las concentraciones óptimas de nutrimentos, desarrollar estándares de calidad de la fruta e implementar un manejo de huertas y prácticas de fertilización apropiadas. Las normas nutrimentales generadas podrían aportar conocimiento en el aumento de la producción anual de frutos de *O. ficus-indica* en regiones que cumplan con las condiciones climáticas de referencias de esta investigación.

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4. CAPITULO 1. Boundary-Line Approach Macro-Nutrient Standards for *Opuntia ficus-indica (*L.) Miller Variety “Rojo Pelón” Fruiting

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ORIGINAL PAPER

Boundary-Line Approach Macro-Nutrient Standards for *Opuntia ficus-indica* (L.) Miller Variety "Rojo Pelón" Fruiting

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Abstract
The aim of this research work was to identify the Boundary-Line Approach (B-LA) macro-nutrient optimum concentrations linked to maximum yield and sufficiency ranges at 90% maximum yield per 1-year-old fructification cladode for *Opuntia ficus-indica* (L.) Miller variety "Rojo Pelón". Four years' (2012–2015) data of yield per fructification cladode and macro-nutrient concentration [nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), or magnesium (Mg)] were used to elaborate bivariate scatter diagrams (n = 228). Selection from 9 to 11 points was performed to estimate quadratic functions as boundary-lines in each bivariate scatter diagram. The vertices allowed estimation of the optimum macro-nutrient concentrations: ln N = 2.32 (10.20 g kg^{-1}), P = 3.04 g kg^{-1}, K = 35.18 g kg^{-1}, Ca = 36.65 g kg^{-1}, Mg = 13.83 g kg^{-1}. The target maximum yield per fructification cladode varies between 1901.13 and 1984.41 g. The estimated sufficiency ranges at 90% of the maximum yield are ln N = 2.07–2.59 (7.86–13.40 g kg^{-1}), P = 2.54–3.54 g kg^{-1}, K = 25.36–45.01 g kg^{-1}, Ca = 27.96–45.35 g kg^{-1}, and Mg = 10.3–17.38 g kg^{-1}. These nutrient standards may be used as a reference for maximizing yield per fructification cladode in *O. ficus-indica*, specifically for the variety "Rojo Pelón".

Keywords: Nitrogen · Phosphorus · Potassium · Calcium · Magnesium · Cactus pear

1 Introduction

Tissue analysis can be a useful tool for estimating plant nutrient status, maximizing crop yield, and evaluating fertilizer requirements. Then, the use of tissue analysis as a diagnostic criterion requires knowledge about the relationships between yield and plant nutrient concentrations (Reis Junior and Monnerat 2003). Good relationships between crop performance and plant nutrient status are expected when an involved nutrient is a limiting factor (Dow and Roberts 1982; Blanco-Macías et al. 2010). Improvements in nutrient status should be led by a balanced and adequate supply of macro- and micro-nutrients for growth and yield focused on nutrient requirements. Thus, it is necessary to know optimum nutrient concentrations (i.e., nutrient requirements) and/or sufficiency ranges of nutrients useful for correct diagnosis and improvements of nutrient status of cultivated plants (Blanco-Macías et al. 2009, 2010).

This contribution is part of Evelyn Hernández-Vidal's Ph. D. thesis

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Commonly, optimum, critical concentrations, and sufficiency ranges are key in plant nutrient diagnosis. Moreover, the determination of nutrient critical values and nutrient balances in plant-diagnostic models (Walworth et al. 1986) has been carried out by using the principle of the Boundary-Line Approach (B-LA). Also, the B-LA has been used to describe the relationship between soil nutrient concentrations and crop yields (e.g., Evanylo 1990). The B-LA considers the upper limits of the scatter of points in a bivariate scatter diagram and would delineate the response of the dependent variable to a particular independent variable when other variables were not limiting. The scatter of points below the boundary line may be due to errors in measurement, variability in biological data, and overall variation caused by other interacting or controlling factors (Webb 1972).

B-LA standards have been developed for several crops, including *Miscanthus sinensis* Andersson (Eulalia grass), *Phalaris arundinacea* L. (Reed canary grass), and *x Triticosecale* Wittm ex *A. Camus* (Triticale) (Lewandowski and Schmidt 2006), *Acer saccharum* Marshall (Sugar maple) (Vizcayno-Soto and Côté 2004), *Picea glauca* (Moench) Voss (White spruce) (Quesnel et al. 2006), *Zea mays* L. (Maize) (Walworth et al. 1986), *Areca catechu* L. (Arecanut) (Bhat and Sujatha 2013), *Vaccinium angustifolium* Aiton (Wild lowbush blueberry) (Lafond 2013), *Mangifera indica* L.(Mango) (Ali 2018), and *Opuntia ficus-indica* (L.) Miller (Nopal) (Blanco-Macías et al. 2009, 2010). However, in the *O. ficus-indica* case, the B-LA nutrient standards were developed under the basis of relationships between 1-year-old cladode macro-nutrient concentrations and produced yearly cladode fresh matter (Blanco-Macías et al. 2009, 2010). Recently, sufficiency ranges have been proposed for *O. ficus-indica* dry matter production by Alves (2017) and Teixeira et al. (2019).

Studies on *Opuntia* nutrient requirements are outdated and fragmented (Mayer and Cushman 2019) although *O. ficus-indica* species is becoming an important crop in various countries around the world. It is cultivated due its tender shoots are widely used for human consumption as vegetables, its mature cladodes are frequently used for animal feed, and its fruits are produced and considered of high-value in at least 18 countries (Russell and Felker 1987; Blanco-Macías et al. 2010). Its whole fruit, pulp, flowers, seeds, and peel contain various groups of bioactive compounds including phenolic acids, flavonoids, anthocyanins, carotenoids, betalains, sterols, lignans, saponins, vitamin E, and vitamin C (Tahir et al. 2019). The identified bioactive compounds have demonstrated to be endowed with biologically relative activity such as antioxidant, antimicrobial, anticancer, anti-diabetes mellitus, hypertension, hypercholesterolemic, rheumatic pain, antiulcerogenic activity, gastric mucosa diseases, and asthma (Tahir et al. 2019). For instance, its fruit extract has pH-sensitive, antioxidant, and antimicrobial abilities (Yao et al. 2020).

Information on *O. ficus-indica* nutrition allows for discarding the prevailing common opinion about cactus crop needs low inputs to give high yields. A low number of studies have docu- mented the macro- and micro-element concentrations in clad- odes. Several of them have been focused on the mineral contents of cladodes to demonstrate their forage potential. Other studies have been focused on element contents in tender pads and fruits to determine their nutritional contribution to the human diet. A few research works have involved 1-year-old cladode mineral contents and biomass or fruit production relationships.

For instance, macro-nutrient concentrations in 1-year-old fruiting cladodes of *Opuntia dillenii* are as follows (Kalegowda et al. 2015): phosphorus, P = 0.152 g kg^{-1}; potassium, K = 22.43 g kg^{-1}; calcium, Ca = 29.38 g kg^{-1}; and magnesium, Mg = 9.86g kg^{-1}. In addition, estimated macro-element contents in 1-year-old cladodes of *O. ficus-indica* were as follows: nitro- gen (N) = 11.36 g kg^{-1}, P = 0.8 g kg^{-1}, K = 47.8 g kg^{-1}, Ca = 50g kg^{-1}, and Mg = 14.2 g kg^{-1} when trees were growing under field conditions (Mayer and Cushman 2019), whereas the concentrations were N = 42.21 g kg^{-1}, P = 7.1 g kg^{-1}, K = 73.6 g kg^{-1}, Ca = 41.6 g kg^{-1}, and Mg = 21.2g kg^{-1} when plants were growing under greenhouse conditions (Mayer and Cushman 2019).

Other research works have been focused on the effect of soil fertilization on fruit production. For example, nitrogen fertilization (0, 60, 120 kg ha^{-1}) did not affect flower bud formation in *O. ficus-indica* (Nerd and Mizrahi 1994). No *O. ficus-indica* fruit response was obtained in the case of fertilizer application even comparing application rates of 100 kg ha^{-1} N, 50 kg ha^{-1} P, 100 kg ha^{-1} K, and 50 kg ha^{-1} Mg to the control that had never been fertilized (Karim et al. 1997; Galizzi et al. 2004). Nitrogen and phosphorus fertilization (0–0, 0–80, 40–40, 60–0, and 60–80 kg ha^{-1} N-P_2O_5) had no effect on *O. ficus-indica* fruit yielding in the first year; however, the doses 60 kg ha^{-1} N or 80 kg ha^{-1} N-P_2O_5 alone increased the fruit yield by +3 and + 6.1 kg $plant^{-1}$, respectively, compared with the control (Arba et al. 2017). Those results suggest that fertilizer/response is difficult due to the high moisture content of cladodes and the large mass of *Opuntia*'s buffers nutrient changes (Felker and Bunch 2009).

All these prior research works did not relate cladode nutrient concentrations with yield or quality of yield in terms of fruit (prickly pear or cactus pear) per 1-year-old cladode through statistical trends or functions. This means that nutrient storage at the plant and cladode levels and best fit plant requirements for fruiting remain practically unknown (Inglese et al. 1995). So, we hypothesize that *O. ficus-indica* plants pose macro-nutrient requirements in terms of optimum concentrations and sufficiency ranges for producing fruits. Then, the aim of this research work was to identify the B-LA macro-nutrient optimum concentrations related to maximum yield and sufficiency ranges at 90% maximum yield per 1-year-old fructification cladode for *O. ficus-indica* variety "Rojo Pelón".

2 Material and Methods

2.1 Study Site

An orchard was established in 2006 at the experimental field of the "Centro Regional Norte Universitario Centro Norte" of the "Universidad Autónoma Chapingo" at 22° 44′ 49.6″ north latitude, 102° 46′ 28.2″ west longitude and 2296 masl, near the city of Zacatecas, Mexico. The regional climate is classified as BS1kw (w), i.e., temperate semiarid climate, with an average annual temperature that varies between 12 and 18 °C, an average annual rainfall of 472 mm, and most of the precipitation (> 65%) occurs from June to August.

The soil at the site had a clay loam texture, a very slightly alkaline pH (7.5), and high content matter (3.23%). Extractable nutrient levels were as follows: availability for inorganic N was low (15 mg kg^{-1}), very high for P (40.5 mg kg^{-1}), medium for K (230 mg kg^{-1}), high for Ca (4371 mg kg^{-1}), moderately high for Mg (569 mg kg^{-1}), mod- erately low for iron (Fe, 7.85 mg kg^{-1}), very high for copper (Cu, 7.47 mg kg^{-1}), excessive for zinc (Zn, 14.6 mg kg^{-1}), moderately for manganese (Mn, 6.13 mg kg^{-1}), and medium for boron (B, 1.59 mg kg^{-1}). The high content of organic matter can be due to the plot has been used as a fruit orchard during the previous 50 years, involving regular organic soil amendment with cow manure and incorporation of tree's fo- liage on the ground. The high content of Ca may be associated with the calcareous origin of the soil.

The orchard was established using 20 mother cladodes. Plant density was 625 plants ha^{-1}. Then, 20 trees with a natural vessel-shaped structure were growing. The management of the orchard consisted of removing weeds each year at the end of spring and summer through minimum tillage. Fertilization, irrigation, and other agronomic practices were not performed.

2.2 Sample Collection and Analysis

Two hundred twenty-eight fruiting cladodes and 1744 fruits of *O. ficus-indica* variety "Rojo Pelón" were considered in this research. Fruiting cladodes and their fruits were taken during four consecutive years (2012–2015). The collection was carried out as follows: 2012, 60 cladodes and 474 fruits; 2013, 52 cladodes and 364 fruits; 2014, 56 cladodes and 420 fruits; and 2015, 60 cladodes and 516 fruits. All cladodes were selected from the uppermost part of the trees to ensure they were 1-year-old. We selected cladodes having from 1 to 15 fruits; four cladodes having each of these numbers of fruits were selected from different plant orientations (north, south, east, and west). None of these cladodes had young shoots. All fruits were harvested when most of the fruits showed peel coloration change indicating the beginning of fruit ripeness.

All 1-year-old cladodes and their fruits were identified. Each fruit weight was registered. Besides, all 228 detached fruiting cladodes were cleaned with distilled water and immediately weighed. Afterward, the cladodes were cut into slices and dehydrated to constant weight in an oven at 75 °C for 36 h, and then their dry weights were registered.

Dry tissue of cladode samples was milled and then digested with a mixture of hydrochloric and nitric acids ($HCl:HNO_3$, 3:1). Afterward, these samples were used to determine macro-nutrient concentrations. The N concentration was determined by the Kjeldahl method, whereas the P content was estimated by reduction with the molybdo-vanadate technique using an optical photo spectrometer (Thermo Spectronic, Helios Epsilon model, USA®). The K, Ca, and Mg concentrations were determined with an atomic absorption spectrophotometer (UNICAM Solar model 9626).

2.3 Boundary-Line Approach Standards

A database of N, P, K, Ca, and Mg concentrations (g kg^{-1}) in all 228 fruiting cladodes and their fruit weights (g) was used to develop boundary-line approach standards for *O. ficus-indica* variety "Rojo Pelón" as described by Blanco-Macías et al. (2010) and Ali (2018). The boundary-line is formed when all values for two variables are plotted and a line enclosing most of these points is established (Michael et al. 1985). The boundary-line represents the limiting effect of the independent variable on the dependent one (Webb 1972; Lark 1997); then, it is assumed that all values below such a line result from the effect of another independent variable or a combination of variables that are limiting the dependent variable (Webb 1972). Detailed information regarding B-LA can be obtained from Blanco-Macías et al. (2010) and Ali (2018).

Boundary-lines can be described by quadratic functions as maximization curves; therefore, we split each macro-nutrient concentration range into 9 to 11 classes and choose the observation linked to each class's highest yield per fruiting cladode. These datasets were used to fit quadratic functions ($Y = aX^2 + bX + c$). Estimation of the maximum value of the yield per fruiting cladode (dependent variable) and the optimum value of the macro-nutrient concentration (independent variable) was performed by calculating the vertex [vertex $X = -b/2(a)$] in each bivariate case. Besides, macro-nutrient sufficien- cy ranges and critical concentrations at 90% of maximum yield per fruiting cladode were calculated by solving the esti- mated quadratic functions ($([-b \pm \sqrt{b2-4(a)(c)}]/2(a))$).

3 Results

In this research work, yield per fructification cladode dependence on macro-nutrient concentrations in 1-year-old cladodes of *O. ficus-indica* variety "Rojo Pelón" was studied to define its macro-nutrient standards. Thus, relationships were evidenced through the B-LA.

Table 1 Basic statistics of *Opuntia ficus-indica* (L.) Miller variety "Rojo Pelón" yield per fructification cladode and N, P, K, Ca, and Mg concentrations in 1-year-old fruiting cladodes (n = 228)

Statistic	Yield (g cladode^{-1})	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})
Mean	795.16	11.67	2.86	32.06	38.45	13.65
Standard deviation	469.79	3.58	0.55	11.69	9.47	2.85
Coefficient of variation	59.08	30.69	19.38	36.48	24.62	20.88
Minimum	60.00	6.00	1.56	13.12	13.00	5.94
Maximum	2186.00	21.70	4.20	65.36	63.50	21.50

Basic statistic estimators of yield per fructification cladode and nutrient concentrations in 1-year-old fruiting cladodes can be appreciated in Table 1. The estimated fruiting cladode mean nutrient concentrations are N = 11.67 g kg^{-1}, P = 2.86 g kg^{-1}, K = 32.06 g kg^{-1}, Ca = 38.45 g kg^{-1}, and Mg = 13.65 g kg^{-1}, whereas the mean yield per fructification cladode is 795.16 g. Also, results suggest that yield shows high variability (CV = 59.08%); P, Mg, and Ca concentrations have moderate variability (CV = 19.38%, CV = 20.88%, and CV = 24.62%, respectively); and K and N show high variability (CV = 36.48% and CV = 30.69%, respectively). Variability is an important issue to get the planned aim. Therefore, our database can be used to identify the dependence of yield per fruiting cladode on cladode nutrient concentrations for the *O. ficus-indica* variety "Rojo Pelón".

It deserves to be noted that concentrations of P, K, Ca, and Mg were normally distributed but those of N did not. Thus, N concentration values were transformed to the natural logarithm (ln); so, ln N values were involved in the analysis.

3.1 Boundary-Line Approach Standards

Bivariate scatter diagrams involving fruit yield and each nutrient expression show that most of the data is grouped at the graph bottom, that is, at lower fruit yields. As a result, it was easy to choose several boundary points, which were used to estimate the boundary-line in each scatter diagram. In fact, from 9 to 11 boundary points (Table 2) were selected to be involved in the estimation of reliable boundary-lines as pointed out by Schmidt et al. (2000). The estimated quadratic boundary-lines were good-adjusted (R^2 values > 0.8) as can be appreciated in Table 2.

The estimated vertexes using parameter values of the quadratic functions indicate optimum concentrations could be as follows: ln N = 2.32 (N = 10.20 g kg^{-1}), P = 3.04 g kg^{-1}, K = 35.18 g kg^{-1}, Ca = 36.65 g kg^{-1}, and Mg = 13.83 g kg^{-1}. The corresponding estimated maximum yields per fructification cladode varies between 1901.13 and 1984.41 g (Fig. 1).

The estimated boundary-line sufficiency ranges at 90% maximum yield are the following: ln N = 2.07–2.59 (N = 7.86–13.40 g kg^{-1}), P = 2.54–3.54 g kg^{-1}, K = 25.36–45.01 g kg^{-1}, Ca = 27.96–45.35 g kg^{-1}, and Mg = 10.3–17.38 g kg^{-1}. The linked 90% maximum yields per fruiting cladode changes between 1711 and 1785.96 g (Fig. 1).

4 Discussion

Mean macro-nutrient concentrations (Table 1) and the estimated optimum concentrations suggest that the requirement descending order is Ca > K > Mg > N > P. On the other hand, the extreme concentrations (minimum and maximum, Table 1) indicate that the requirement decreasing order is K ≥ Ca > N ≥ Mg > P. Both orders suggest *O. ficus-indica* variety "Rojo Pelón" plants tend to concentrate much more Ca and K than N in their 1-year-old fruiting cladodes, proving that they are calcitrophic organisms (Lüttge 2004). These requirement orders may be explained due to *Opuntia* spp. fruits growing on 1-year-old cladodes concentrate much more K and Ca than Mg, N, and P (e.g., Lamghari El Kossori et al. 1998; Kalegowda et al. 2015); also, these macro-nutrient cations (K, Ca and Mg) are competing nutrients (Marschner 2012); indeed, Ca concentration may decrease as K content increases as driven by K antagonism or luxury consumption (Marschner 2012).

Table 2 Statistics of boundary-lines fitted by second-degree functions: $Y = ax^2 + bx + c$, where Y is *Opuntia ficus-indica* (L.) Miller yield (g) per fructification cladode; x is the nutrient concentration (g kg^{-1}) in 1-year-old fruiting cladode; a, b and c are regression coefficients; R^2 is the coefficient of determination; and n^y is the number of points used to estimate regression equation coefficients

		Regression coefficients			
Nutrient (x)	n^y	a	b	c	R^2
ln N (g kg^{-1})	11	− 2765.600	12,883.00	− 13,092.00	0.83
P (g kg^{-1})	10	− 764.750	4652.00	− 5171.10	0.88
K (g kg^{-1})	9	− 2.052	144.39	− 555.98	0.97
Ca (g kg^{-1})	10	− 2.517	184.51	− 1480.00	0.87
Mg (g kg^{-1})	10	− 15.294	423.32	− 1014.60	0.83

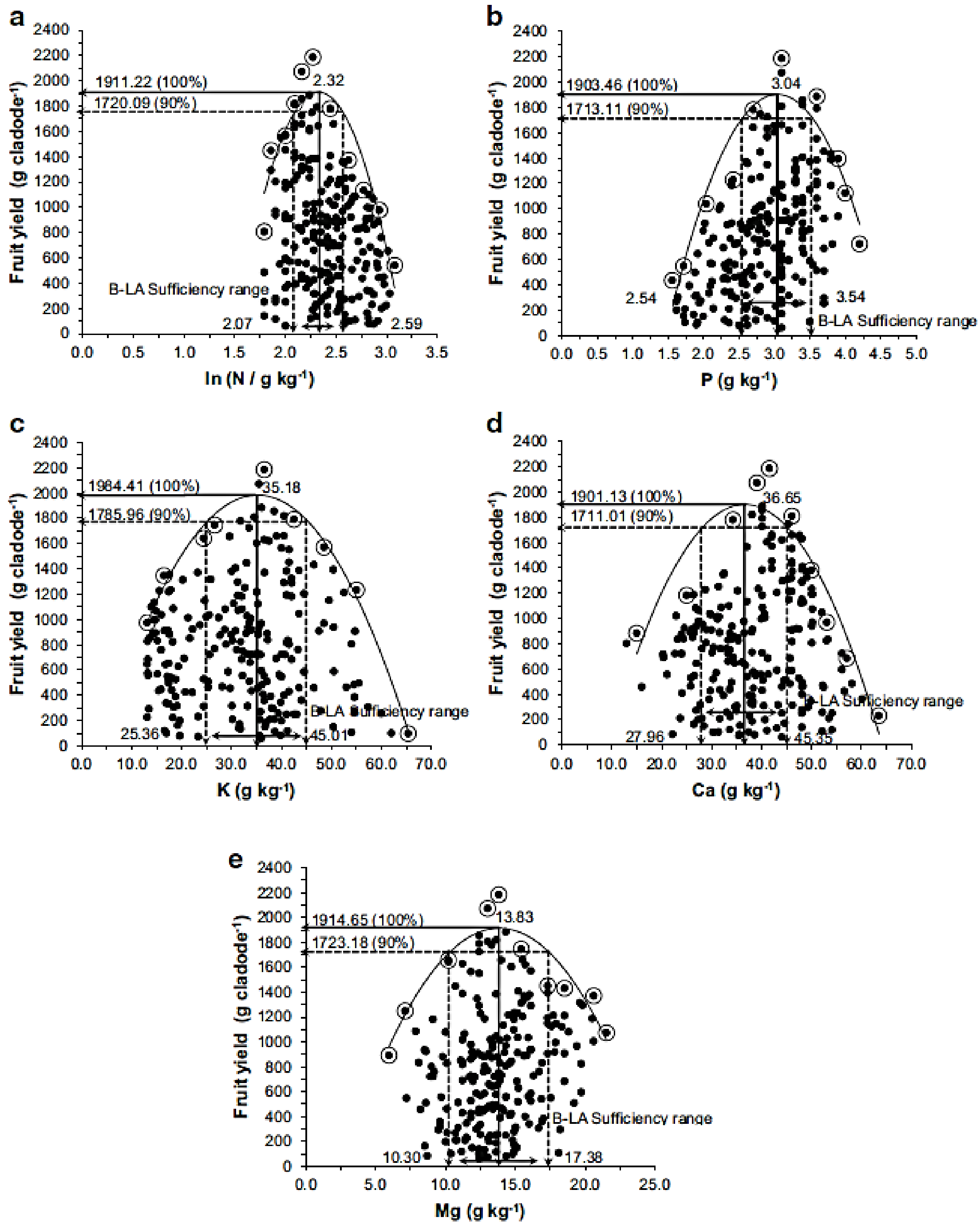

Fig. 1 Relationships between nutrient concentrations (g kg^{-1}) in 1-year-old fruiting cladodes and *Opuntia ficus-indica* (L.) Miller variety "Rojo Pelón" fruit yield (g $cladode^{-1}$) (n = 228) showing boundary-lines described by second-degree functions using the independent variables ln nitrogen (**a**), phosphorus (**b**), potassium (**c**), calcium (**d**), and magne- sium (**e**). See Table 2 for fitted quadratic functions

There is noticeable that fruiting occurred on cladodes with concentrations surpassing the following minimum values: N = 6 g kg^{-1}, P = 1.56g kg^{-1}, K = 13.12 g kg^{-1}, Ca = 13, and Mg = 5.94 g kg^{-1}. The mentioned fruiting cladode N minimum concentration (i.e., 6 g kg^{-1}) is strongly higher than the N concentration (1.8g kg^{-1}) reported by Blanco-Macías et al. (2010) as the minimum required for the production of vegetative buds in *O. ficus-indica*. On the other hand, the Mg minimum concentration (5.94g kg^{-1}) is lower than the Mg concentration (7.8 g kg^{-1}) reported by Blanco-Macías et al. (2010) as the minimum required for *O. ficus-indica* vegetative growth. However, minimum concentrations of P, K, and Mg appear to be similar for both purposes, that is, for the production of fruits and new clad- odes. The noted discrepancies could be due to differences in plant age, type of sampled cladode (with new cladodes or fruits), plant density, and the genotype, among other factors. It is widely known flowers and cladodes appear simultaneously in spring; the flowers occur mostly at the crown edge of 1-year-old clad- odes whereas new cladodes usually develop on 2-year-old or even older cladodes (Inglese et al. 1994).

The minimum macro-nutrient concentrations required for producing fruits might be an important issue to avoid variation between productivity levels in successive years. It can provide a convenient index for predicting which 1-year-old cladodes will produce fruits. They should be used together with the proposed boundary-line standards to perform reliable nutrient diagnosis, and then, adequate fertilization practice can be recommended having in mind the management of nutrient balance for fruit production.

Notably, this is the first study to our knowledge proposing boundary-line approach macro-nutrient standards for *O. ficus-indica* fruiting. It was possible because normal distributions were found for macro-nutrient concentrations in fructification cladodes (N content was ln distributed) and bivariate observations (yield per 1-year-old fructification cladode against macro-nutrient concentrations, Fig. 1).

Prior works were mainly focused on the estimation of *O. ficus-indica* nutrient requirements for biomass production (cladode's dry matter or fresh matter) as can be appreciated in Table 3. There are interesting differences between the Brazilian (Alves 2017; Teixeira et al. 2019) and the Mexican cases (Blanco-Macías et al. 2010; Valdez-Cepeda et al. 2013b; and the current work). The Brazilian P sufficiency ranges are notably lower than the Mexican ones. Brazilian K sufficiency ranges lie within that of the present case but their extreme limits are lower than those pointed out by Blanco-Macías et al. (2010) and Valdez-Cepeda et al. (2013b), and the lower value of the K sufficiency range of the present case is markedly lower than the others. The extreme values of the Brazilian Ca and Mg sufficiency ranges are lower than those of the Mexican ranges. In general, the upper limits of the Brazilian N sufficiency ranges tend to be higher than the Mexican ranges, and the N sufficiency range of the current case is strongly lower than the others.

The noted differences between the Brazilian and Mexican sufficiency ranges could be mainly linked with the involved genotype, environmental conditions, and management practices. For instance, Brazilian experimental sites are much more humid and warmer than the Mexican sites. Besides, the appreciated discrepancies between the results of the current case and the others could also be associated with the type of sampled cladodes (vegetative cladodes or fruiting cladodes). The lower N sufficiency range and the lower limit of the K sufficiency range in mature fruiting cladodes may be explained because these organs serve as a source of N and K for flowering and fruit development. This means that 1-year-old fruiting cladodes may support reductions in dry weight (García de Cortázar and Nobel 1992) between flowering and fruit growth. Fruiting occurs when the cladode dry weight surpasses 14.4 g referred to as "cladode excess dry weight" in *O. ficus-indica* variety Rojo Pelón (Valdez-Cepeda et al. 2013a), and of course, all involved 1-year-old fruiting cladodes in our work had dry weights higher than that cladode excess dry weight.

We propose standards that involve optimum concentrations (N = 10.20 g kg^{-1}, P = 3.04 g kg^{-1}, K = 35.18 g kg^{-1}, Ca = 36.65 g kg^{-1}, Mg = 13.83 g kg^{-1}); the target maximum yield per fructification cladode varies between 1901.13 and 1984.41 g. In addition, the norms involve macro-nutrient suffi- ciency ranges at 90% maximum yield (N = 7.86–13.40 g kg^{-1}, P = 2.54–3.54 g kg^{-1}, K = 25.36–45.01 g kg^{-1}, Ca = 27.96–45.35 g kg^{-1}, and Mg = 10.3–17.38 g kg^{-1}. All these optimum contents and sufficiency ranges indicate that *O. ficus-indica* plants require macro-nutrients in the following descending order Ca > K > Mg > N > P. Both nutrient standard types may be used as a reference for maximizing yield per fructification cladode in *O. ficus-indica*, specifically for the variety "Rojo Pelón". Uses of nutrient standards can be found in the scientific literature. For instance, in a surprising experience, Arba et al. (2017) pointed out that they successfully used the Compositional Nutrient Diagnosis approach nutrient standards for *O. ficus-indica* fresh matter production as developed by Valdez-Cepeda et al. (2013b) to identify the best soil fertilization dosage for *O. ficus-indica* cv. "Moussa" fruit yield and fruit size improvements. Nonetheless, future works should dedicate efforts to increase the database by involving more *O. ficus-indica* fruit production sites and to a refinement of target values for soil available nutrient concentrations by using other varieties and species; in fact, plants take up

Table 3 Macro-nutrient sufficiency ranges in 1-year-old cladodes for *Opuntia ficus-indica* (L.) Miller with different purpose as proposed by several authors

Source	Response	Target yield	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})
Blanco-Macías et al. (2010)	Fresh matter	> 27.01 kg Plant^{-1}	8.40–20.30	2.40–4.20	38.20–50.80	31.80–45.20	14.30–20.90
Valdez-Cepeda et al. (2013b)	Fresh matter	> 27.01 kg Plant^{-1}	6.20–16.60	2.70–4.10	33.70–50.90	28.50–56.50	11.80–20.60
Alves (2017)	Dry matter	> 21.80 t ha^{-1} cycle^{-1}	12.70–18.50	1.00–1.80	31.60–44.10	23.20–32.80	9.50–14.30
Teixeira et al. (2019)	Dry matter	> 18.12 t ha^{-1} cycle^{-1}	13.03–18.21	0.80–2.12	30.74–45.04	23.3–32.76	9.07–14.69
This work	Fruit yield	> 1901 g cladode^{-1}	7.86–13.40	2.54–3.54	25.35–45.02	27.96–45.34	10.30–17.38

most of the mineral nutrients that they require from the soil; then, information on soil fertility should also be collected.

Variation of macro-nutrient concentrations in 1-year-old fructification cladodes may be explained because old cladodes function as stems and branches. So, these organs play a key role in the reallocation of nutrients toward vegetative and floral buds (new cladodes and fruits). It is known differences in nutrient conservation from one growing season to the next can cause changes in growth responses of perennial plants to dual nutrient (e.g., N/P) supply ratios (Güsewell et al. 2003). Some species show the same patterns in nutrient concentrations and dual nutrient ratios, whereas others have high nutrient contents and low dual ratios even at nutrient-poor soils (Güsewell et al. 2003). Then, an evidenced variation of macro-nutrient contents may be linked to atmospheric conditions; so, future research work should be considering environmental (or weather) variables and fruit yield relationships.

In general, our results provide compelling pieces of evidence about yield increasing and decaying trends departing from macro-nutrient optimum concentrations or extreme values of their sufficiency ranges at 90% maximum fruit yield in *O. ficus-indica* variety "Rojo Pelón" (Fig. 1). Strongly, our results appear to confirm the validity of several scientific laws and principles; for instance, Sprengel's "Law of the Minimum" (van der Ploeg et al. 1999), Mitscherlisch's "Law of Diminishing Returns", and Wallace's "Law of the Maximum" (Velayutham 2017), and "Critical Levels of Deficiency and Toxicity". Our results may also be supported on the "Response Model" of the "Diagnosis-Recommendation Integrated System" as pointed out by Sumner and Farina (1986) and Walworth and Sumner (1987). All the reliable five boundary functions were estimated because each bivariate dataset contained several instances in which the involved nutrient (N, P, K, Ca, or Mg) was limiting the fruit yield as crop response. In other words, each estimated boundary-line might be representing the response of fruit yield to the corresponding macro-nutrient in a model expressing the "Law of the Minimum" (Lark et al. 2020). Besides, the decaying trend after the upper concentration (Critical Level of Toxicity) of each macro-nutrient sufficiency range might be expressing the "Law of the Diminishing Returns".

The proposed optimum macro-nutrient concentrations and sufficiency ranges for *O. ficus-indica* variety "Rojo Pelón" fruiting have limitations due to nutrients are treated separately. For instance, these standards do not account for nutrient interactions and nutrient balances. Nutrient concentrations (as compositional data) are parts of a whole (Parent et al. 2013)—really a complex system—bounded between zero and the unit of measurement, i.e., 1, 100%, 1000 g kg^{-1}, or 1,000,000 mg kg^{-1}. Thus, several properties of compositional data were not considered because these nutrient norms do not involve the corresponding compositional space. As a consequence, future works should develop nutrient norms useful to perform diagnosis and recommendations focused on nutrient concentrations in plant tissue or organ of reference as compositional space (e.g., Parent et al. 2013). From this point of view, the compositional or ionomic profile of plants can reflect effects or adaptations to the local environment, especially to levels of soil factors (e.g., salinity and pH) and atmospheric conditions (i.e., weather). In some cases, local adaptations could be driven by ionomic loci (Huang and Salt 2016).

5 Conclusions

There can be no doubt knowing macro-nutrient requirements for *Opuntia ficus-indica* (L.) Miller fruit production and quality improvements is an important horticultural issue. These requirements can be used as references to improve yields. In this context, the yield per fructification cladode dependence on each macro-nutrient concentration in 1-year-old fruiting cladodes of *O. ficus-indica* variety "Rojo Pelón" was evidenced through the boundary-line approach. The estimated optimum contents are N = 10.20 g kg^{-1}, P = 3.04 g kg^{-1}, K = 35.18 g kg^{-1}, Ca = 36.65 g kg^{-1}, and Mg = 13.83 g kg^{-1}; the corresponding estimated maximum yields per fructification cladode vary between 1901.13 and 1984.41 g. In addition, the calculated macro-nutrient sufficiency ranges at 90% maximum yield (between 1711 and 1785.96 g) are N = 7.86–13.40 g kg^{-1}, P = 2.54–3.54 g kg^{-1}, K = 25.36–45.01 g kg^{-1}, Ca = 27.96–45.35 g kg^{-1}, and Mg = 10.3–17.38 g kg^{-1}. These nutrient standards may be used as a reference for maximizing yield per fructification cladode in *O. ficus-indica*, specifically for the variety "Rojo Pelón" through the application of chemical and/or organic fertilizers.

Notable differences were evidenced when these results were compared with *O. ficus-indica* requirements for fresh and dry matter production under different environments. Nonetheless, these standards do not account for nutrient interactions and nutrient balances. Then, future works should develop nutrient norms and diagnosis focused on macro- and micro-nutrient concentrations in 1-year-old fruiting cladodes as compositional spaces. So, researchers should consider the ionomic profile of specific genotype plants that can reflect effects or adaptations to a local environment, especially to levels of soil factors and atmospheric conditions, mainly weather.

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Data Availability Data will be available through request.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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5. CAPITULO 2.

Compositional Nutrient Diagnosis (CND) standards for *Opuntia ficus-indica* (L.) Miller variety ‘Rojo Pelón’ fruiting

Compositional Nutrient Diagnosis (CND) Standards for *Opuntia ficus-indica* (L.) Miller Variety 'Rojo Pelón' Fruiting

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Short running title: CND Standards for *Opuntia ficus-indica* Fruiting

Abstract

In this study, the aim was to develop the compositional nutrient diagnosis norms (CND) norms for *Opuntia ficus indica* variety 'Rojo Pelón' fruiting. Statistical analyses involved a dataset of fruit yield and macro-nutrient concentrations of 228 1-year old cladodes from healthy plants. A cutoff yield (1,166.67 g cladode^{-1}) between the low- and high-yield subpopulations was determined after examining six cumulative variance ratio functions related to yield per 1-year old cladode. Means and standard deviations of row-centered log-ratios V_X^* of five nutrients (N, P, K, Ca, and Mg) and a filling value R_d, which includes all nutrients not chemically analyzed. Estimated preliminary CND norms (mean ± standard deviation of the row-centered log-ratios) are: $V_N^* = -1.114 \pm 0.219$, $V_P^* = -2.194 \pm 0.076$, $V_K^* = 0.163 \pm 0.259$, $V_{Mg}^* = -0.708 \pm 0.157$, $V_{Ca}^* = 0.401 \pm 0.100$, and $V_{Rd}^* = 3.452 \pm 0.095$. These CND standards are associated with the following 1-year old fruiting cladode mean concentrations: N=0.958%, P=0.318%, K=3.507%, Ca=4.228%, and Mg=1.448%. Then, the order of macro-nutrient requirements is as follows: Ca>K>Mg>N>P.

Keywords: Macro-nutrients; Fruit yield per cladode; Nitrogen; Phosphorus; Potassium; Calcium; Magnesium.

Introduction

Nutrient status in plants may be identified through tissue analysis. Chemical analysis of tissue of reference allows identifying optimum nutrient concentrations or nutrient norms (or standards) linked to maximize crop yields. So, nutrient diagnosis taking into account identified nutrient optimum concentrations as a reference may allow estimating nutrient imbalances or disorders. Commonly, nutrient disorders are not observable, e.g. hidden hunger or toxicity (Barker and Pilbeam, 2015). The observable symptoms of nutrient imbalance often appear when significant and irreversible damage has been generated; these damages may cause scarce growth and low crop yield. Therefore, the opportune determination of nutrient status through correct diagnosis under the basis of nutrient norms could result in practical recommendations that increase yields and improve fruit quality (Habib, 2000; Mostashari et al., 2018).

There are widely known nutrient norms developed locally that may allow for correct field diagnoses useful to improve plant nutrient status through agricultural practices such a foliar or soil fertilization (Blanco-Macías et al., 2010). Some researchers have shown the precision in diagnosing imbalances when using locally developed norms (Bendaly Labaied et al., 2018). Nutrient norms can be developed according to various techniques. The most known techniques are the Critical Value Approach (CVA) (Bates, 1971), the Diagnosis and Recommendation Integrated System (DRIS) (Walworth and Sumner, 1987), and the Compositional Nutrient Diagnosis (CND) (Parent and Dafir 1992; Blanco-Macías et al., 2006; García-Hernández et al., 2006).

The CVA method does not consider interactions between or among mineral nutrients; these interactions may be important issues for plant nutrient balance. On the other hand, the DRIS and CND approaches involve nutrient interactions (Bhaduri and Pal, 2013). Various authors indicate that there are little differences between DRIS and CND for establishing nutrient norms (Khiari et al., 2001b; Barłóg, 2016; Mostashari et al., 2018; Barros de Morais et al., 2019; Bendaly Labaied et al., 2020). Nonetheless, other researchers endorse the CND arguing that it is more efficient to determine the nutritional status of crops because of its sound mathematical and statistical bases (Kumar et al., 2003; Blanco-Macías, 2009; René et al., 2013; Valdez-Cepeda et al., 2013; Barros de Morais et al., 2019).

Up to now, CND norms for nutrient status diagnosis have been developed for various crops such as *Carya illinoinensis* (Wangenh.) K. Koch (García-Hernández et al., 2009), *Aloe vera* L. (García-Hernández et al., 2006), *Coffea arabica* L. and *Coffea canephora* Pierre ex A. Froehner (Wairegi and Van Asten, 2012), *Phoenix dactylifera* L. (Bendaly Labaied et al., 2020), *Vitis vinifera* L. (Mostashari et al., 2018), *Eucalyptus grandis* W. Hill ex Maid. (Costa da Silva et al., 2004; Barros de Morais et al., 2019), *Zea mays* L. (Khiari et al., 2001a; Magallanes-Quintanar et al., 2006), *Musa* spp. AAA (Wairegi and Van Asten, 2011), and *Opuntia ficus-indica* L. Miller (Magallanes-Quintanar et al., 2004; Blanco-Macías et al. 2006; Valdez-Cepeda et al., 2013), among others. Recently, the Boundary-Line Approach (B-LA) was used by Hernández-Vidal et al. (2021) to estimate macro-nutrient standards for *O. ficus-indica* (L.) Miller variety 'Rojo Pelón' fruiting; the estimated optimum concentrations were N=1.02%, P=0.304%, K=3.518%, Ca=3.665%, and Mg=1.383% as linked to maximum fruit yield that varies between1901.13 and 1984.41 g cladode^{-1}. However, the B-LA does not consider interactions between mineral nutrients like the CVA does.

In the case of *O. ficus-indica*, the CND norms have been determined for dry and fresh matter production (Magallanes-Quintanar et al., 2004; Blanco-Macías et al., 2006; Valdez-Cepeda et al., 2013). In fact, the species *O. ficus-indica* is an important crop around the world (López-García et al., 2016). It is widely used for vegetable production in Mexico and fruit production in at least 25 countries around the world (Valdez-Cepeda et al., 2013). Hitherto, *Opuntia ficus-indica* L.variety 'Rojo Pelón' is being used for fruit production in Mexico's Northern-Central Region. Despite its social and economic importance, there is little information about the nutrient requirements of this variety; this means that its CND norms remain unknown yet. Therefore, this work aimed to develop CND norms for *O. ficus-indica* variety 'Rojo Pelón' for fruit production.

Materials and methods

Study site

This research was carried out in the experimental field of the 'Centro Regional Universitario Centro Norte' of the 'Universidad Autónoma Chapingo'. It is located at 22° 44' 49.6'' North latitude, 102° 46' 28.2'' West longitude, and 2 296 masl, near the Zacatecas City, Mexico. The regional climate is classified as BS1kw (w), with an average annual temperature that varies between 12°C and 18°C, and an average annual rainfall of 472 mm. Most of the precipitation (65%) occurs from June to August.

An orchard was established in 2006, using 20 mother cladodes. The plant density was 625 plants ha^{-1}. Then, 20 trees with a natural vessel-shaped structure were growing. The management of the orchard consisted of removing weeds each year at the end of spring and summer through minimum tillage. Fertilization, irrigation, and other agronomic practices were not performed.

Experimental data

To develop CND norms, a data set of nutrient concentrations and fruit yield per 1-year old cladodes was used. A sample of two hundred twenty-eight fruiting cladodes of *O. ficus-indica* variety 'Rojo Pelón' and a total of 1744 fruits were considered in this research. All cladodes were selected from the uppermost part of the trees to ensure they were 1-year old. Fruiting cladodes and their fruits were taken for four consecutive years (2012-2015). Sampling involved the selection of cladodes having from 1 to 15 fruits, and four cladodes having each of these numbers of fruits were selected when possible. Sampled cladodes with each number of fruits were taken from sections of the plant oriented to North, South, East, and West when most of the fruits showed peel color breakage.

Weights of each cladode and their fruits were registered. Besides, all 228 detached fruiting cladodes were cleaned with distilled water and immediately weighted. Afterward, the detached cladodes were cut into slices and dehydrated to constant weight in an oven at 75°C for 36 hours, and then their dry weights were registered.

Dry tissue of cladode samples was milled and then digested with a mix of acids. Afterward, these samples were used to determination of nutrient concentrations. The N concentration was determined by the Kjeldahl method, whereas that the P content was estimated by reduction with the molybdo-vanadate technique using an optical photo spectrometer (Thermo Spectronic, Helios Epsilon model, USA®). The K, Ca, and Mg concentrations were determined with an atomic absorption spectrophotometer (UNICAM Solar model 9626).

Theory of the CND approach

The following description of the theory of the CND approach was modified from Khiari et al. (2001). Plant tissue composition forms a *d*-dimensional nutrient arrangement, *i.e.*, simplex (S^d) made of d+1 nutrient proportions including d nutrients and a filling value defined as (Parent and Dafir, 1992):

$$S^d = \left[(N, P, K, ..., R_d): N > 0, P > 0, K > 0, ..., R_d > 0, N + P + K + ... + R_d = 100\right], \qquad (1)$$

where 100 is the dry matter concentration (%); N, P, K, … are nutrient proportions computed as:

$$R_d = 100 - (N + P + K + ...) . \qquad (2)$$

The nutrient proportions become scale invariant after they are divided by the geometric mean (G) of the d + 1 components, including R_d (Aitchinson, 1986), as follows:

$$G = \left[N \cdot P \cdot K \cdot ... \cdot R_d\right]^{\frac{1}{d+1}} . \qquad (3)$$

Row-centered log ratios are computed as:

$$V_N = ln\left(\frac{N}{G}\right), V_P = ln\left(\frac{P}{G}\right), V_K = ln\left(\frac{K}{G}\right), \dots, V_{R_d} = ln\left(\frac{R_d}{G}\right), \text{ and} \tag{4}$$

$$V_N + V_P + V_K + \dots + V_{R_d} = 0, \tag{5}$$

where V_X is the CND row-centered log-ratio expression for nutrient X. The sum of tissue components is 100%, as in Equation 1, and the sum of their row-centered log ratios, including the filling value must be zero, as in Equation 5.

Thereafter, the database is partitioned between two subpopulations using the Cate-Nelson procedure, once the observations have been ranked in a decreasing yield order (Khiari et al., 2001ab). In the first partition, the two highest yield values form one group (group A) and the remainder of yield values forms another group (group B); thereafter, the three highest yield values form the group A. This process is repeated until the two lowest yield values form group B, and the remainder of yield values forms the group A. At each iteration, group A comprises n_1 observations, and group B comprises n_2 observations for a total of n observations ($n = n_1 + n_2$) in the whole database. For the two subpopulations obtained during each iteration, one must compute the variance of the CND V_X values. Then the variance ratio for component X can be estimated as:

$$f_i(V_X) = \frac{Variance\ of\ V_X\ n_1\ observations}{Variance\ of\ V_X\ n_2\ observations}, \tag{6}$$

where $f_i(V_X)$ is the variance ratio function between two subpopulations for nutrient X at the ith iteration $(i = n_i - 1)$ and the V_X is the CND row-centered log-ratio expression for nutrient X. The first variance ratio function computed for the two highest yields is put on the same line as the highest yield, and so on, thus leaving three empty bottom lines.

The cumulative variance ratio function is the sum of variance ratios at the ith iteration from the top. The cumulated variance ratios for a given iteration are computed as a proportion of the total sum of variance ratios across all iterations to compare the discrimination power of the V_X between low- and high-yield subpopulations on a common scale. The cumulative variance ratio function $F_i^C(V_X)$ can then be computed as:

$$F_i^C(V_X) = \left[\frac{\sum_{i=1}^{n-1} f_i(V_X)}{\sum_{i=1}^{n-3} f_i(V_X)} \right] \cdot [100], \tag{7}$$

where n–1 is the partition number and n is the total number of observations ($n_1 + n_2$). The denominator is the sum of variance ratios across all iterations and is a constant for nutrient X. The cumulative function $F_i^C(V_X)$ related to yield (Y) shows a cubic pattern:

$$F_i^C(V_X) = aY^3 + bY^2 + cY + d\,. \tag{8}$$

The inflection point is the point where the model shows a change in concavity. It is obtained by the second derivation of Equation 8:

$$\frac{\partial F_i^C(V_X)}{\partial Y} = 3aY^2 - 2bY + C \tag{9}$$

$$\frac{\partial^2 F_i^C(V_X)}{\partial Y^2} = 6aY - 2b\,. \tag{10}$$

The inflection point is then obtained by equating the second derivative Equation 10 to zero. Therefore, the solution for the yield cutoff value is *–b/3a*. The highest yield cutoff value across nutrient expressions can be selected to determine what minimum yield target for a high-yield subpopulation will be classified as high yield, whatever the nutrition expression. CND norms are computed using means and standard deviations corresponding to the row-centered log-ratios V_X of d nutrients for high-yield specimens, that is, V_N^*, V_P^*, V_K^*, …, V_R^*, and SD_N^*, SD_P^*, SD_K^*, …, SD_R^*, respectively.

Once CND norms have been developed, an independent database can validate them. Validation of CND norms has been reported by Parent and Dafir (1992), Parent et al. (1994), and Khiari et al. (2001ab). CND norms can also be used for diagnostic purposes:

$$I_N = \frac{\left(V_N - V_N^*\right)}{SD_N^*}, I_P = \frac{\left(V_P - V_P^*\right)}{SD_P^*}, I_K = \frac{\left(V_K - V_K^*\right)}{SD_K^*}, \ldots, I_{R_d} = \frac{\left(V_{R_d} - V_{R_d}^*\right)}{SD_{R_d}^*}, \tag{11}$$

where I_N, …, I_{R_d} are the CND indices.

Independence among compositional data is ascertained by row-centered log-ratio transformation (Aitchison, 1986). CND indices, as defined by Equation 11, are standardized and linearized variables as dimensions of a circle (d + 1 = 2), a sphere (d + 1 = 3), or a hypersphere (d + 1 > 3) in a d +1 dimensional space. The CND nutrient imbalance index of a diagnosed specimen is its CND r^2 and is computed by:

$$r^2 = I_N^2 + I_P^2 + I_K^2 + \ldots + I_{R_d}^2 . \tag{12}$$

Its radius, r, computed from the CND nutrient indices, thus characterizes each specimen. The sum of d + 1 squared independent, unit-normal variables produces a new variable having a chi-square distribution with d + 1 degrees of freedom (Ross, 1987). Because CND indices are independent, unit-normal variables, the CND r^2 values must have a chi-square distribution function. This is why it is recommended that the highest yield cutoff value (highest discrimination power) among d + 1 nutrient computations be retained to calculate the proportion of the low-yield subpopulation below yield cutoff used as a critical value for the chi-square cumulative distribution function. As defined by Eqs. [11] and [12], the closer to zero that CND indices and thus the CND r^2 or chi-square values are, the higher the probability to obtain a high yield. Theoretically, at the critical chi-square value of zero where the ideal nutrient balance is reached, 100% of the population would be expected to produce low target yields when a high critical chi-square value is set.

Results and Discussion

Descriptive statistics

In this research work, fruit yield per cladode and macro-nutrient concentrations in 1-year old cladodes of *O. ficus-indica* variety 'Rojo Pelón' was studied to define its CND standards. Basic statistic estimators of fruit yield per cladode and macro-nutrient concentrations in 1-year old fruiting cladodes can be appreciated in Table 1.

Table 1. Basic statistics of *Opuntia ficus-indica* L. Miller variety 'Rojo Pelón' fruit yield per cladode and N, P, K, Ca, and Mg concentrations in 1-year old fruiting cladodes (*n* = 228).

Statistic	Yield (g cladode^{-1})	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
Mean	795.16	1.167	0.286	3.206	3.845	1.365
Standard Deviation	469.79	0.358	0.055	1.169	0.947	0.285
Coefficient of Variation (%)	59.08	30.690	19.380	36.480	24.620	20.880
Minimum	60.00	0.600	0.156	1.312	1.300	0.594
Maximum	2186.00	2.170	0.420	6.536	6.350	2.150

The estimated fruiting cladode mean nutrient concentrations are N = 1.167%, P = 0.286%, K = 3.206%, Ca = 3.845%, and Mg = 1.365%, whereas the mean fruit yield per cladode is 795.16 g. Besides, results suggest that fruit yield per cladode show high variability (CV=59.08%), P, Mg, and Ca concentrations have moderate variability (CV=19.38%, CV=20.88%, and CV=24.62%, respectively), and K and N show high variability (CV=36.48% and CV=30.69%, respectively). Variability is an important issue to get the planned objective, that is, our database (*n*=228) can be used to identify the CND standards for *O. ficus-indica* variety 'Rojo Pelón' fruiting.

The compositional nutrient diagnosis norms for simplex S^5

The S^5, i.e., six-dimensional (d + 1) *O. ficus-indica* variety 'Rojo Pelón) simplex comprised the five nutrients N, P, K, Ca, and Mg and the filling value R. The R values were estimated using Eq. [1]. Nutrient concentrations were transformed into CND row-centered log ratios V_N, V_P, V_K, V_{Ca}, and V_{R_d} through Eqs. [2 to 4]. Eq. [6] was used to estimate the $F_i^C(V_X)$ values.

The descriptive statistics of the yield were as follows: mean = 795.16 g cladode^{-1}, minimum = 60 6 cladode^{-1}, maximum = 2186 g cladode^{-1}, and a standard deviation = 469.79 g cladode^{-1} (Table 1). The cutoff yield between the low- and high-yield subpopulations was determined after examining the six cumulative variance ratio functions [$F_i^C(V_N)$, $F_i^C(V_P)$, $F_i^C(V_K)$, $F_i^C(V_{Ca})$, $F_i^C(V_{Mg})$ and $F_i^C(V_R)$] related to yield per 1-year old cladode (Table 2). All six relationships showed a cubic pattern with inflection points at *–b/3a*. Yield cutoff values were 200 g cladode^{-1} for $F_i^C(V_N)$, 1,166.67 g cladode^{-1} for $F_i^C(V_P)$, 333.33 g cladode^{-1} for $F_i^C(V_K)$, 1,666.67 g cladode^{-1} for $F_i^C(V_{Ca})$, 23,333.33 g cladode^{-1} for $F_i^C(V_{Mg})$, and 33.33 g cladode^{-1} for $F_i^C(V_R)$. The theory of the CND approach recommends that the highest yield cutoff value (highest discrimination power) among *d* + 1 nutrient computations be retained to calculate the proportion of the low-yield subpopulation below yield cutoff used as the critical value for the chi-square cumulative distribution function. Notably, the highest value (23,333.33 g cladode^{-1}) and the lowest value (33.33 g cladode^{-1}) are out of the explored yield range, so each was not considered a target yield. This flaw has been found previously by several researchers (e.g. Khiari et al., 2001ab; Magallanes-Quintanar et al., 2004; García-Hernández et al., 2005, 2007; Magallanes Quintanar et al. 2006ab; Hernández-Caraballo et al., 2008).

To avoid such a flaw, Valdez-Cepeda et al. (2013) recommended using the unrestricted Boltzmann equation instead of the traditional cubic function.

Table 2. Fruit yields of *Opuntia ficus indica* variety 'Rojo Pelón' at inflection points (–b/3a) of cumulative variance functions [$F_iC(V_X)$] for row-centered log-ratios (n=225) in the survey population (n=228).

Nutrient	$F_i^c(V_x)= aY^3+bY^2+cY+d$	R^2	Yield at –b/3a (g cladode^{-1})
N	$F_i^c(V_N) = –5E–09x^3 + 3E\text{-}06x^2 - 0.0236x + 100$	0.99	200.00
P	$F_i^c(V_P) = 2E–08x^3 - 7E–05x^2 – 0.0019x + 100$	0.99	1,166.67
K	$F_i^c(V_K)= –3E–09x^3 + 3E–06x^2 – 0.0078x + 100$	0.98	333.33
Ca	$F_i^c(V_{Ca}) = 6E–09x^3 – 3E\text{-}05x^2 – 0.0026x + 100$	0.99	1,666.67
Mg	$F_i^c(V_{Mg})= 1E–10x^3 – 7E\text{-}06x^2 – 0.0118x + 100$	0.98	23,333.33
R	$F_i^c(V_R)= –6E–09x^3 + 6E\text{-}07x^2 – 0.0206x + 100$	0.99	33.33

As a consequence, 1,666.67 g cladode^{-1} was used to define the high-yield subpopulation. This implies that 22.37% of the population (51 observations) is considered as the high-yielding subpopulation, while the low-yield sub-population includes 77.63% of the population (177 observations). This result agrees with those from other researchers, who found that a high-yielding subpopulation represents a lower percentage of the whole population (e.g. Parent et al., 1994; Magallanes-Quintanar et al., 2004; Blanco-Macías et al., 2006; García-Hernández et al., 2004, 2006; Blanco-Macías et al., 2009).

As a remarkable result, the preliminary CND norms as means and standard deviations (V_X^* and SD_X^*) of the CND row-centered log-ratios for the high-yield (>1,666.67 g cladode^{-1}) subpopulation, as well as their corresponding nutrient optimum ranges (means and standard deviations) in 1-year old fruiting cladodes, are shown in Table 3. The estimated mean concentrations are N=0.958%, P=0.318%, K=3.507%, Mg=1.448%, and Ca=4.228%. As a remarkable result, the order of nutrient requirements is as follows Ca> K> Mg> N> P. This means that *O. ficus-indica* variety 'Rojo Pelón' plants tend to concentrate more Ca and K than N in their 1-year old fruiting cladodes, confirming they are calcitrophic organisms (Lüttge, 2004). This result also indicates that they may have a high nitrogen use efficiency (Raven and Spicer, 1996).

Table 3. The preliminary compositional nutrient diagnosis (CND) norms (V_X^* means and their standard deviations) for d = 5 nutrients in a high-yield (>1,666.67 g cladode^{-1}) subpopulation of *Opuntia ficus-indica* (L.) Miller variety 'Rojo Pelón', and their corresponding nutrient optimum ranges (mean nutrient concentrations and their standard deviations) in 1-year old fruiting cladodes.

Row-Centered Log-Ratio	Mean	Standard Deviation	Nutrient	Mean (%)	Standard Deviation (%)
V^*_N	-1.11463	0.21916	N	0.95823529	0.20446717
V^*_P	-2.19444	0.07689	P	0.31807843	0.03920808
V^*_K	0.16356	0.25908	K	3.50713725	0.93183052
V^*_{Mg}	-0.70845	0.15711	Mg	1.44847059	0.28891351
V^*_{Ca}	0.40159	0.10077	Ca	4.2284902	0.62320226
V^*_{R5}	3.45238	0.09502			
$\sum^* V_X$	0.00000				

The estimated mean nutrient concentrations (Table 3) differ slightly from those proposed by Hernández-Vidal et al. (2021) as optimum concentrations for *O. ficus-indica* (L.) Miller variety 'Rojo Pelón' fruiting through the B-LA. There can be noted that estimated N and K mean concentrations linked to the CND standards are slightly lower than the optimum concentrations estimated by Hernández-Vidal et al. (2021) (0.958% versus 1.02%, and 3.507% versus 3.518%, respectively). On the other hand, the calculated P, Mg, and Ca mean concentrations associated with the CND standards are higher than the optimum concentrations proposed by Hernández-Vidal et al. (2021), that is, 0.318% versus 0.304%, 1.448% versus 1.383, and 4.228% versus 3.665%, for P, Mg, and Ca, respectively. Those differences may be due to we used a target fruit yield =1,666.67 g cladode^{-1} whereas that Hernández-Vidal et al. (2021) linked their estimations to maximum fruit yield that varies between1,901.13 and 1,984.41 g cladode^{-1}. In other words, we used a target fruit yield lower than those considered by Hernández-Vidal et al. (2021). Nonetheless, our results have the advantage of involving multivariate nutrient ratios through the CND approach whereas those from Hernández-Vidal et al. (2021) do not.

The preliminary CND norms were used to estimate nutrient indices I_N, I_P, I_K, I_{Ca}, I_{Mg}, and I_{Rd} through Eq. [11]. Also, CND r^2 values were computed using Eq. [12]. The CND r^2 values were distributed like chi-square values ($R^2 > 0.988$; $p < 0.001$; Figure 2). Eighty-five percent of the observations were below the yield cutoff of 1,666.67 g cladode^{-1}, and the corresponding chi-square value was 2.5. Then, this value must be considered when validating the preliminary CND norms because the independent dataset ought to be characterized by a similar value. By taking into account that more observations from high-yielding cladodes or specimens must be added to the database, the chi-square value of 5.1 could change due to the high-yielding subpopulation may provide more weight for defining yield cutoff than the low-yielding subpopulation according to the theory of the CND approach.

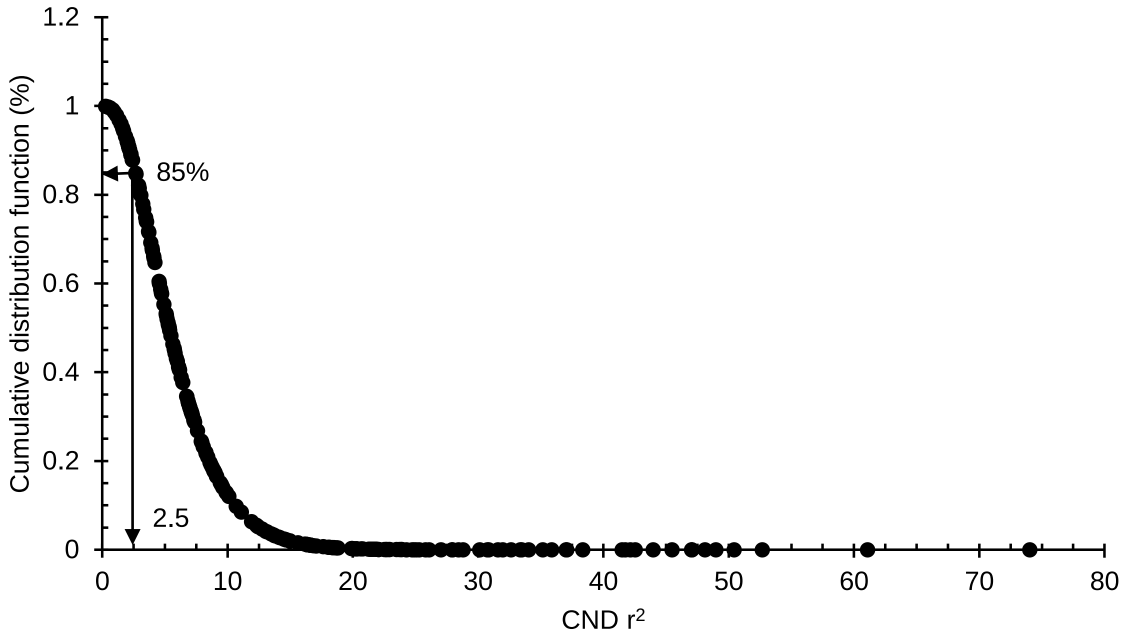

Figure 1. The chi-square cumulative distribution function with 6 degrees of freedom for obtaining theoretical threshold compositional nutrient diagnosis (CND) r^2 value (2.5) in S^5 for a yield cutoff at 85% of the low-yield subpopulation.

Conclusions

This is the first study to our knowledge carried out to estimate Compositional Nutrient Diagnosis (CND) standards for *Opuntia ficus-indica* (L.) Miller variety 'Rojo Pelón' fruiting taking into account a target yield of 1,666.67 g $cladode^{-1}$ or higher. The proposed preliminary CND norms (mean ± standard deviation of the row-centered log-ratios) are: $V_N^* = -1.114 \pm 0.219$, $V_P^* = -2.194 \pm 0.076$, $V_K^* = 0.163 \pm 0.259$, $V_{Mg}^* = -0.708 \pm 0.157$, $V_{Ca}^* = 0.401 \pm 0.100$, and $V_{Rd}^* = 3.452 \pm 0.095$. These CND standards are associated with the following 1-year old fruiting cladode mean concentrations: N=0.958%, P=0.318%, K=3.507%, Ca=4.228%, and Mg=1.448%. Then, the order of macro-nutrient requirements is as follows: Ca>K>Mg>N>P. Future works should be focused on validation of these macro-nutrient norms taking into account a database involving more high-yielding cladodes or specimens to change the estimated chi-square value of 5.1 and to provide more weight for defining a yield cutoff to divide the population into high- and low- yield subpopulations. Also, this process could be improved by increasing the population and estimating the target yield through the unrestricted Boltzmann equation to describe the relationship between each cumulative variance ratio function and the yield per fructification cladode.

ETHICS STATEMENT

Not apply.

CONSENT FOR PUBLICATION

Not apply.

AVAILABILITY ON SUPPORTING DATA

Data might be available upon request addressed to RD V-C.

COMPETING INTERESTS

The research has no financial or commercial purpose that must be interpreted as a potential conflict of interest in the future.

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AUTHOR CONTRIBUTIONS

Conceptualization, EH-V, RDV-C, and FB-M; Project administration, fieldwork and data registration, RDV-C, and FBM; data organization and statistical analyses, EH-V, RDV-C, FB-M, and AG-T; writing and reviewing of the original draft, EH-V, RDV-C, and AG-T; review, editing of the last manuscript, EH-V, FB-M, FGV-D, LG-A, AG-T, and RDV-C. All authors have read and agree to approve the final version of the manuscript.

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6. CONCLUSIONES

Los requerimientos nutrimentales para la producción de fruta de *O. ficus-indica* y las mejoras de calidad del fruto son un tema de importancia. Estos requerimientos pueden usarse como referencias para mejorar los rendimientos. En este contexto, el rendimiento de fruta depende de la concentración de nutrimentos en cladodios de fructificación de un año de la variedad *O. ficus-indica* 'Rojo Pelón'. la validez de los requerimientos nutrimentales estimados con la técnica de curva límite se evidenció y se confirmó por medio del método de Diagnóstico de Nutrimento Compuesto debido a la similitud de los resultados. Así, los resultados corroboran que dichas técnicas son complementarias y no mutuamente excluyentes. Ambas mostraron diferencias notables cuando sus resultados se compararon con los requerimientos *de O. ficus-indica* para la producción de materia fresca y seca en diferentes ambientes. Los trabajos futuros deben desarrollar normas usando las concentraciones de nutrimentos en cladodios de fructificación de un año como espacios de composición (interacciones entre nutrimentos). Por lo tanto, lo que se debe tener en cuenta son los requerimientos de las plantas de genotipos específicos que pueden reflejar efectos o adaptaciones al entorno local, especialmente lo concerniente a los niveles de factores del suelo y las condiciones atmosféricas, principalmente del tiempo atmosférico.