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Preliminary studies on the influence of boron on forage quality of the pasture legume *Desmodium ovalifolium*

Estudios preliminares sobre el efecto del boro en la calidad nutritiva de la leguminosa forrajera *Desmodium ovalifolium*

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Abstract

The knowledge about genotype x environment interactions on quality components of tropical legumes is rather limited. In relation to plant nutrition it is assumed that micronutrients could have a major influence on forage quality since they are often essential constituents of enzymes and other proteins. Among the micronutrients boron (B) is required in plants in larger quantities than any other, although its function is not entirely clear yet. In this paper the role of B in plants is reviewed and results of an exploratory glasshouse experiment with two *Desmodium ovalifolium* genotypes are presented. They suggest a correlation between B concentration in the soil and forage quality traits of the species. Boron seems to reduce soluble tannin content and astringency in leaves, resulting in a higher *in vitro* digestibility of leaves. An indepth study under highly controlled climate chamber conditions is proposed.
Key words: micronutrients, genotype x environment interaction, condensed tannins, Near Infrared Reflectance Spectroscopy (NIRS)

Resumen

Los conocimientos sobre las interacciones genotipo x medio ambiente respecto a los componentes de valor forrajero de leguminosas tropicales son bastante escasos. Para efectos de la nutrición de plantas se asume que los microelementos pueden influenciar el valor forrajero significativamente ya que algunos de ellos son componentes esenciales de enzimas y otras proteínas. Entre ellos, el boro (B) se requiere en la planta en cantidades mayores que cualquier otro microelemento aunque su función aún no es completamente clara. En el presente trabajo se revisa el papel del B en la planta y se presentan los resultados de un experimento exploratorio de invernadero realizado con dos genotipos de *Desmodium ovalifolium*. Los resultados sugieren la existencia de una correlación entre la concentración de B en el suelo y factores de valor nutritivo: El boro parece reducir tanto el contenido de los taninos solubles en las hojas como la astringencia de los

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taninos con lo cual la digestibilidad *in vitro* de las hojas aumenta. Como conclusión se propone realizar un estudio a fondo bajo condiciones de cámara de crecimiento altamente controladas.

Palabras clave: micronutrimentos, interacciones genotipo x medio ambiente, taninos condensados, Near Infrared Reflectance Spectroscopy (NIRS)

Introduction

Micronutrients have a major influence on plant growth, in general, since they are often essential constituents of enzymes and proteins (4). Their influence on forage quality, however, remains to be determined. It is known that deficiency of micronutrients often leads to increased contents of phenols (tannins) and decreased lignification. Brown *et al.* (3) report for manganese (Mn) deficiency in wheat decreasing lignin content while phenols and flavonoids accumulated. Shkol'nik *et al.* (22) related an increased content of phenols to boron (B) deficiency.

Plants require boron in larger quantities than any other micronutrient although its function is not entirely clear yet. Boron is not a component of enzymes, but an essential mineral element for all vascular plants (15). It is easily bound to cell walls and thus less mobile with low retranslocation (2). Boron is needed in meristematical tissues as a cell wall stabilising complex (8). Lewis (13) considers boron to be an extracellular nutrient. Marschner (15) describes B as responsible for the reduction of cellulose and hemicellulose fiber mobility and indicates a relationship between demand for B and lignification and xylem differentiation.

Plant boron deficiency leads to K-efflux increases, changing thereby the potential of the cell wall and resulting in the loss of membrane integrity; furthermore, the chlorophyll content is reduced as a consequence of the loss of amyloplast and chloroplast membrane integrity (10). Insufficient root growth, death of plant apices, and abnormal fructification, sometimes sterility, are reported by Srivastava *et al.* (26). Shkol'nik *et al.* (22) suggest a shift to increased secondary metabolism under B deficient conditions, leading to an accumulation of phenols and chinons whereas lignification is restricted; on their turn, high contents of phenols cause a higher polyphenoloxidase activity and result in toxic oxygen radicals leading to the loss of membrane integrity.

Boron deficiency occurs mainly in areas with high precipitation, humidity and light intensity (1, 14), heavily leached soils with high contents of Fe- and Aloxides (10), high pH (23), and pronounced dry season stress inducing complexation of polyborates (11). Most of these conditions are typical for the humid tropics and are considered as limiting factors for forage plant production and thus for livestock nutrition.

Desmodium heterocarpon (L.) DC. subsp. *ovalifolium* (Prain) Ohashi, widely known under its earlier name *Desmodium ovalifolium*, is used as a forage legume in the humid tropics because of its aggressive, stoloniferous growth habit and adaptation to lowfertility soils (9). However, livestock performance with this legume in association with grasses can be poor (7), mainly because of low acceptability to grazing cattle (21). Low forage quality in *D. ovalifolium* is associated with high tannin contents (12), which are variable among accessions (20), and influenced by edaphic and climatic factors (17, 25).

Since results of a collaborative Centro Internacional de Agricultura Tropical (CIAT) – University of Hohenheim project with focus on the identification of environmental factors that affect forage quality of *D. ovalifolium* genotypes (19) indicated a possible positive correlation between boron application and forage quality, an exploratory glasshouse experiment was designed in order to verify the observed trends. It was considered that increasing forage quality traits of *D. ovalifolium* through boron application could result in an important contribution to enhance the utilisation of this legume which because of its persistence and adaptation to acid, lowfertility soils is of particular promise for the humid tropics.

Materials and methods

In the glasshouse facilities of CIAT near Cali, Colombia, a total of 16 pots were filled with soil (4 kg) from La Pista, Carimagua: an acid (pH 4.6), sandy soil with low contents of micronutrients (0.13, 0.72, 0.22, 0.40 mg/kg of Cu, Mn, Zn, B, respectively) and P (1.9 mg/kg, Bray II). The equivalents of 50, 50, 500, 10 kg ha^{-1} of P, K, Ca, and S, respectively, were applied in each pot to avoid any nutrients getting into minimum. The treatments consisted of a boron application of 0.003 g boric acid (H_3BO_3)/pot and no boron application. Eight pregerminated seedlings/pot of two *Desmodium ovalifolium* genotypes, CIAT 3793 and CIAT 13110, were transplanted in four replicates (Completely Randomised Design) and grew for a total of 125 days before they were harvested and the leaves analysed for forage quality. Pots were watered daily to the field capacity of the soil. Mean glasshouse temperature ranged from 24-26°C. Forage quality analysis included *in vitro* dry matter digestibility (IVDMD) and tannin astringency, and contents of crude protein (CP), soluble condensed tannins (SCT), bound condensed tannins (BCT), acid detergent fiber (ADF), neutral detergent fiber in sulphite acid (NDF-S), and nitrogen in acid detergent fiber (N-ADF). These parameters were determined using Near Infrared Reflectance Spectroscopy (NIRS) (NIRSystems 6500, Silver Spring, USA). Data were analysed using the General Linear Models (GLM) procedure of SAS (18).

Results

The results of this exploratory experiment indicate a significant effect of boron application on IVDMD, SCT and astringency (table 1). IVDMD increased 3.8 % while SCT and tannin astringency decreased 1.2 and 1.1 %, respectively. No significant differences between treatments were observed with any other parameter included in the study. In table 2 the forage quality traits of the two genotypes are presented. Whereas SCT contents did not differ significantly, tannin astringency was significantly higher in CIAT 3793. Both IVDMD and CP contents were significantly higher for CIAT 13110 (45.36 vs. 41.15 % and 12.96 vs. 10.76, respectively).

Discussion and conclusions

Results indicate improved forage quality through B application which seems to reduce tannin astringency and SCT content resulting in higher IVDMD. There was, however, no correlation with protein or fiber contents. Reduced tannin content and astringency are likely due to the character of B to complex phenolics

Table 1. The effect of boron treatments on forage quality of *Desmodium ovalifolium* under glasshouse conditions. Means (of two plant genotypes) within a row followed by the same letter are not significantly different (Duncan's Multiple Range Test; p< 0.01).

Parameter	With Boron	Without Boron
IVDMD (%)	45.46a	41.59b
CP (%)	12.27a	11.64a
SCT (%)	6.69b	7.93a
BCT (%)	3.00a	2.96a
Astring	5.90b	7.02a
ADF (%)	33.89a	33.56a
NDF-S (%)	45.48a	45.32a
N-ADF (%)	0.59a	0.55a

IVDMD = *In vitro* dry matter digestibility; CP = crude protein; SCT = soluble condensed tannins; BCT = bound condensed tannins; Astring = tannin astringency (g protein precipitated/ g condensed tannin); ADF = acid detergent fiber; NDF-S = neutral detergent fiber in sulphite acid; N-ADF = nitrogen content in acid detergent fiber (N-ADF).

Table 2. Forage quality parameters of two *Desmodium ovalifolium* genotypes under glasshouse conditions. Means (of two B treatments) within a row followed by the same letter are not significantly different (Duncan's Multiple Range Test; p< 0.01).

Parameter	CIAT 3793	CIAT 13110
IVDMD (%)	41.15b	45.36a
CP (%)	10.76b	12.96a
SCT (%)	7.21a	7.51a
BCT (%)	2.96a	2.99a
Astring	7.17a	5.90b
ADF (%)	34.53a	33.00a
NDF-S (%)	44.64a	46.06a
N-ADF (%)	0.56a	0.58a

IVDMD = *In vitro* dry matter digestibility; CP = crude protein; SCT = soluble condensed tannins; BCT = bound condensed tannins; Astring = tannin astringency (g protein precipitated/ g condensed tannin); ADF = acid detergent fiber; NDF-S = neutral detergent fiber in sulphite acid; N-ADF = nitrogen content in acid detergent fiber (N-ADF).

as reported by Cakmak *et al.* (5). Nevertheless, Ruiz *et al.* (16) state also the opposite effect of phenol accumulation in leaves of tobacco plants when applying very low or very high levels of B. An intermediate B application, however, resulted in decreased phenol levels. Therefore it appears that the two predominant forms in which B is found in leaves (forming complexes with pectins and phenols, or in the free form) determine and explain the effect of this element on the phenol metabolism (16). Sinha & Chatterjee (24) reported similar observations in pearl millet by applying deficient and excess levels of B.

Since the observed results in *D. ovalifolium* have to be considered as preliminary because of limited data sets, an indepth study is required in order to elucidate the effect of B – or other micronutrients – on forage quality of *D. ovalifolium*, which is also considered as highly valuable for the work with other hightannin forage legumes in the tropics. Such an indepth experiment should include a larger number of genotypes, since the presented data showed significant differences between genotypes. This stresses the importance of the assessment and evaluation of the genetic variability within a germplasm collection. The experiment should be conducted under highly controlled climate chamber conditions where *D. ovalifolium* plants are grown in nutrient solution. The objectives of an experiment should be the identification of the extent to which B influences the nutritional quality in general, the identification of soil and plant B concentration levels which have a negative effect on fora-ge quality, and the establishment of guidelines for fertilization recommendations. Such recommendations require careful consideration, since the range between deficiency and excess of available B is considered to be very narrow (6). However, they can be very important for the improved management of *D. ovalifolium* and might enable farmers to benefit to the full extent from new selected lowtannin genotypes currently multiplied by CIAT. Genotypes of *D. ovalifolium* with high nutritive value and low condensed tannin contents will be of major interest for developing persistent grass/legume production systems in the humid tropics. There they might represent a significant technological breakthrough as there is presently only one persistent, however, expensive herbaceous legume technology option available (*Arachis pintoi*).

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