

# Nitrate and potassium concentrations in cotton petiole extracts as influenced by nitrogen fertilization, sampling date and cultivar

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## Abstract

The effect of nitrogen fertilization levels on seed cotton yield responses of cultivars from Andalusia was studied during two consecutive years. To obtain preliminary information for improving nutrient management, the analysis of nitrate and potassium (K) was performed in petiole extracts at critical times (flowering, boll-setting, boll growth) during the growth cycle. Significant differences among cotton cultivars were found for petiole nitrate and K concentrations. The highest petiole nitrate and K concentrations were found in cultivar 'Crema 111' with 4,140 and 14,180 mg kg<sup>-1</sup> respectively. These concentrations were affected by N treatment and sampling date. The relationships among petiole nutrient concentrations and seed cotton yield depended on sampling date, cultivar and year. The highest association between petiole nitrate and yield was found early in the season in 2003 ( $r = 0.918$  and  $r = 0.965$  for 'Crema 111' and 'Sor Angela' respectively). The analysis of petiole extracts for controlling plant nutritional status and optimize seed cotton yield requires a systematic assessment as critical values in nutrient concentration for seed cotton yield may differ depending on cultivar, soil type, sampling date and crop management. The technique of petiole analysis used in this paper might be useful for detecting genotypes with improved nutrient uptake or use efficiency.

**Additional key words:** *Gossypium hirsutum*, mineral nutrition, yield.

## Resumen

### Efecto de la fertilización nitrogenada, fecha de muestreo y cultivar en la concentración peciolar de nitrato y potasio en el algodón

Se ha estudiado el efecto de la respuesta de distintos niveles de fertilización nitrogenada sobre el rendimiento de algodón bruto en cultivares andaluces durante dos años consecutivos. Con el objeto de obtener información para mejorar el manejo nutricional del cultivo, se realizaron análisis de nitrato y potasio (K) en peciolo en periodos críticos del cultivo (floración, fructificación y desarrollo de cápsulas). Los cultivares presentaron diferencias en la concentración de nitrato y K en peciolo, alcanzándose las mayores concentraciones en 'Crema 111' (4.140 and 14.180 mg kg<sup>-1</sup> para nitrato y K respectivamente). Los tratamientos fertilizantes y la fecha de muestreo afectaron a las concentraciones de nitrato y K en los peciolo. Las relaciones entre las concentraciones de nutrientes en el peciolo y el rendimiento de algodón bruto dependieron de la fecha de muestreo, del cultivar y del año. La relación más estrecha entre producción y concentración de nitrato se observó en 2003 y el muestreo temprano ( $r = 0.918$  y  $r = 0.965$  para 'Crema 111' y 'Sor Angela' respectivamente). El uso del análisis del peciolo para controlar el estado nutricional de la planta y optimizar los rendimientos requiere una evaluación sistemática para estimar valores críticos de concentración de nutrientes, ya que estos difieren dependiendo del cultivar, tipo de suelo, fecha de toma de muestra y prácticas culturales. La técnica del análisis peciolar parece útil también para detectar genotipos con mayor eficiencia en el uso y absorción de nutrientes.

**Palabras clave adicionales:** *Gossypium hirsutum*, nutrición mineral, rendimiento.

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## Introduction

In South Western Andalusia, the main cotton production region in Spain with 52,000 ha in 2009, high rates of N fertilization (160-440 kg N ha<sup>-1</sup>) are currently used averaging 266 kg N ha<sup>-1</sup> with mean yields of 3,200 kg seed cotton ha<sup>-1</sup> (Bilbao-Arrese *et al.*, 2005). In the U.S. cotton belt, N applications are variable ranging from 80 to 246 kg N ha<sup>-1</sup>, but in cotton regions similar to Andalusia like California the N application reaches 211 kg ha<sup>-1</sup> (Gerik *et al.*, 1998). For the survival of cotton production in Spain under the present Common Agricultural Policy regulations (Bilbao-Arrese *et al.*, 2005), production costs have been reduced in order to provide economic return to farmers. Furthermore, a more strict environmental regulation on the use of nitrogen fertilizers to avoid eutrophication requires better nutrient management to diminish the impact of agriculture on the environment (Bilbao-Arrese *et al.*, 2005).

Nitrogen fertilization in cotton requires a balanced approach as inadequate N limits yield and profits and excessive N is more likely and equally troublesome (Hearn, 1981). High soil N produces rank growth, delays maturity, increases attractiveness to insects and incidence of boll rot, and complicates harvest management (Guthrie *et al.*, 1994; Gerik *et al.*, 1998). Over-fertilization also may be harmful to the environment if N pollutes ground or surface water or contributes to air quality problems (UNEP and WHRC, 2007). Closely related to N nutrition, K requirement in cotton plants, deserves special attention because of the high uptake rate and the relative inefficiency of cotton as a K absorber and the high demand of new fast-growing cultivars (Kerby and Adams, 1985; Oosterhuis, 1999).

The seasonal changes in plant N utilization and the variation in soil N availability generated by the N cycle require to perform some kind of plant nutrient monitoring (Gerik *et al.*, 1998). The analysis of nutrients like N and K in petioles is an aid in the fertilization of the current crop which may provide a forewarning of imminent nutrient deficiencies or surpluses (Sabbe and Mackenzie, 1973). Petiole analysis is the most common plant assay because it is indicative of plant nutrient status, and speed and simplicity of analysis (Gerik *et al.*, 1998; Oosterhuis, 1999). Models have been developed for estimating desired nutrient levels in petioles for expected yields according to commodity prices and production factor costs (Grimes *et al.*, 1973). The petiole analysis has been used for monitoring N and K

nutrition in different cotton regions to optimize nutrient use (Grimes *et al.*, 1973; Lutrick *et al.*, 1986; Constable *et al.*, 1991; McConnell and Mozaffari, 2004; Mozaffari *et al.*, 2005). Petiole N concentration was even useful to study the variation in soil N availability and plant N uptake under different irrigation regimes (Leidi *et al.*, 2001).

Although nitrogen fertilization is not one of the main costs, N misuse may increase the production costs and environmental hazards. In an attempt to reduce cotton production costs by optimizing N use efficiency and improving the cost-benefit ratio, a study was conducted to determine the yield response of Andalusian cotton cultivars to N fertilization while monitoring plant nutrient status by the analysis of petioles at critical stages.

## Material and methods

In 2003 and 2004, fertilizer N trials were performed using 'Crema 111' and 'Sor Ángela', cotton cultivars of medium-long season and short-season respectively. The experiments were performed at IFAPA Centro Las Torres-Tomejil (Alcalá del Río, Sevilla) on a sandy loam soil (Typic Xerofluvent). The soil had an electrical conductivity of 0.34 dS m<sup>-1</sup>, field water capacity of 22.4% and pH of 7.68. Monthly temperatures, rainfalls, evapotranspiration (ET<sub>0</sub>) and solar radiation recorded during the crop seasons are presented in Figure 1. The soil nutrient contents at planting (for 0-30 and 30-60 cm depth, respectively) were: in 2003, N, 853-772 mg kg<sup>-1</sup>, K, 253-161 mg kg<sup>-1</sup>; in 2004, N, 778-680 mg kg<sup>-1</sup>; K, 210-151 mg kg<sup>-1</sup>. Soil N was determined colorimetrically after Kjeldahl digestion (Houba *et al.*, 1986). Soil K was extracted with 1 N ammonium acetate and determined by flame emission spectrophotometry (Houba *et al.*, 1986). Planting was performed under plastic mulching the 28 April 2003 and the 24 April 2004 to get 150,000 plants per hectare.

## Nitrogen treatments

A ground fertilization before sowing was provided with 30 units of P (NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>) and 150 units of K (K<sub>2</sub>SO<sub>4</sub>). Nitrogen fertilization treatments were applied after sowing and consisted of seven different levels of 34.5% ammonium nitrate to provide 6, 40, 80, 120, 160, 200 and 240 kg N ha<sup>-1</sup>.

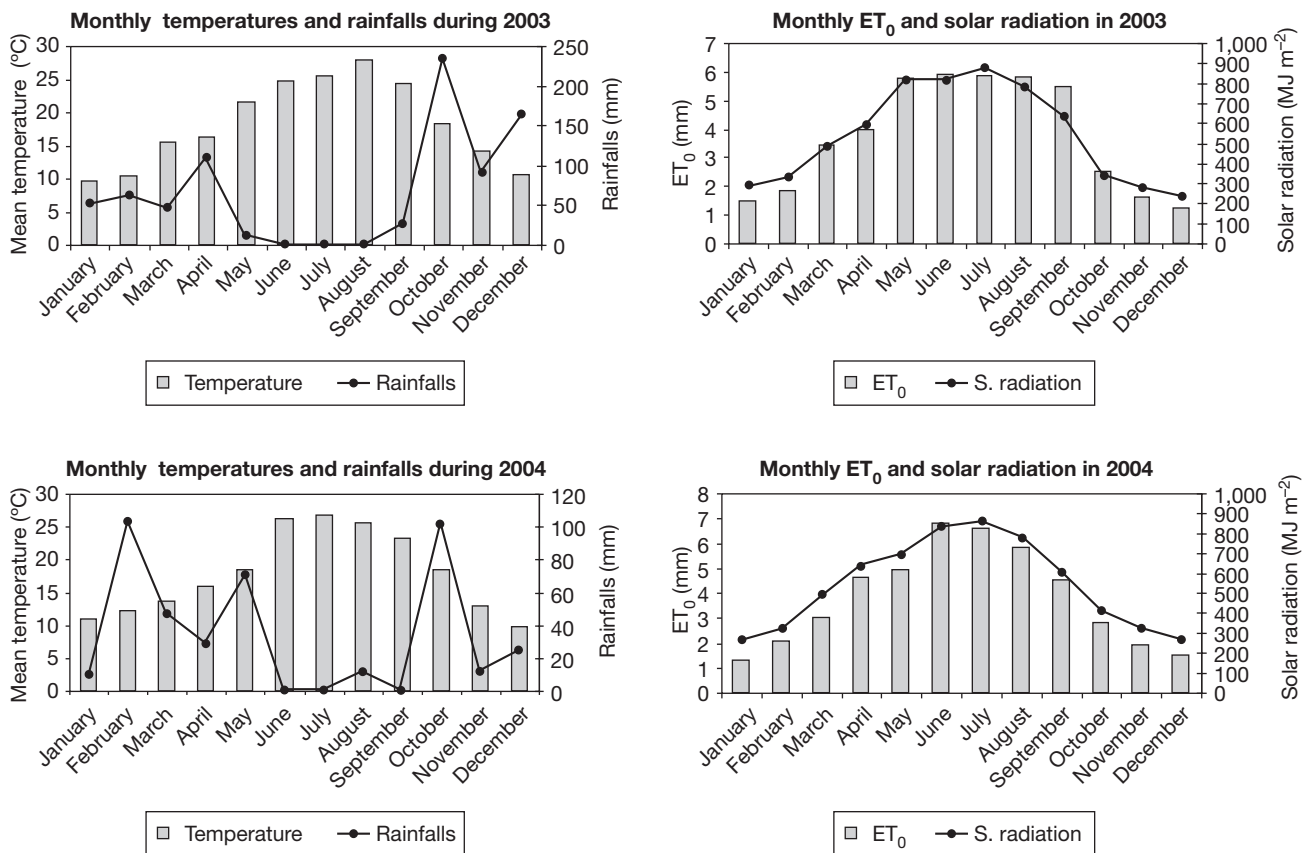


Figure 1. Monthly temperatures, rainfalls, ET<sub>0</sub> and solar radiation at IFAPA Las Torres-Tomejil in 2003 and 2004.

## Irrigation

Furrow irrigation was applied, in 2003 using a total volume of 3,690 m<sup>3</sup> water in five applications (from 4<sup>th</sup> June to 14<sup>th</sup> August). In 2004, a lower total water volume (3,100 m<sup>3</sup>) was used because of soil stored moisture from autumn-winter rains and temperatures lower than average in the hottest months (July and August) (see Fig. 1).

## Pesticides and growth regulators

Presowing (benfuresate) and preemergent (fluometurone) herbicide treatments were applied both years. Insecticides for controlling white fly (*Bemisia* sp.), spiny bollworm (*Earias* sp.), *Heliothis* sp. and red bollworm (*Pectinophora* sp.) were sprayed 6 times in 2003 and 4 times in 2004 rotating compounds to prevent resistance (cypermethrin, endosulfan, azynphos-methyl, chlorpiriphos). In 2003, a total of 1.8 L ha<sup>-1</sup> of mepiquat chloride was applied in four treatments, while in 2004

a total of 1.6 L ha<sup>-1</sup> of this product was distributed in three applications. Before harvest, 400 and 300 g ha<sup>-1</sup> of Drop (thidiazuron) were applied in 2003 and 2004 respectively.

## Petiole analyses

Petiole sampling was performed at beginning of flowering (77-83 days after sowing, DAS), full flowering (91-92 DAS), first open boll (101-106 DAS), mid-open bolls (119 DAS only in 2003) and bolls fully opened (134-136 DAS). Twenty recently expanded leaves (3<sup>rd</sup>-4<sup>th</sup> leaf from top) with four replicates were collected for analysis of nitrate and potassium in the petioles. The leaves were set into plastic bags and maintained in an ice-box. In the laboratory, the petioles were separated, chopped and frozen until analysis. The sap was then extracted by centrifugation (9200 g, 5 min) in Eppendorf tubes. In 2003, nitrate analysis was performed with a Cardy nitrate meter (Horiba Ltd., Kyoto, Japan) while in 2004 the Reflectoquant method (Merck

KGaA, Darmstadt, Germany) was used. Potassium concentration in petiole sap extracts was measured by atomic absorption spectrophotometry using Cs-AI in the dilutions to avoid element ionization (AAS Perkin-Elmer 1100B, Operator's Manual).

## Harvest

Seed cotton was manually picked two times each year (in 2003, 17<sup>th</sup> September and 15<sup>th</sup> October; in 2004, 5<sup>th</sup> and 25<sup>th</sup> October) from central rows of the plot.

## Experimental design

The experimental unit was 4 rows 10 m long at 0.95 m between rows in a split-plot design with four replications. ANOVA, mean comparison and regression analyses were performed using the Statistix vers. 7.0 package (Analytical Software, Tallahassee, USA). Calculation of non-linear regressions were performed with Sigmaplot vers. 8.02 (Systat Software, Chicago, USA).

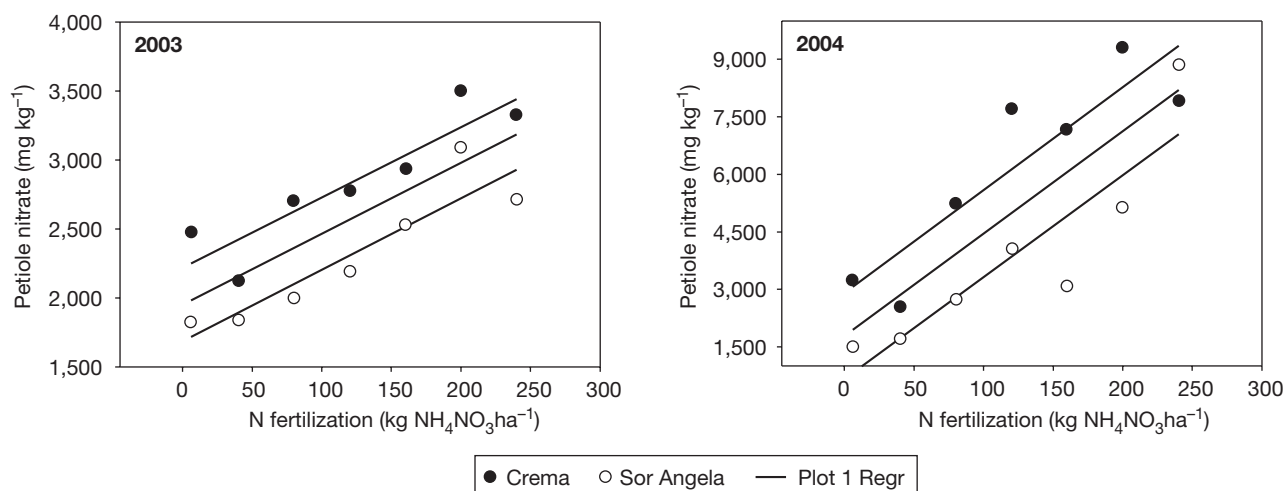
## Results and discussion

The concentration of nitrate in petioles was affected by the amount of applied N and significant differences in concentration between cultivars were found (Table 1, Fig. 2). Petiole nitrate concentration decreased along the crop season (Table 1) following changes in nitrogen uptake rates due to the variation in crop nutrient

**Table 1.** Nitrate and potassium concentration ( $\text{mg kg}^{-1}$ ) in petioles from cultivars Crema 111 and Sor Angela as affected by different sampling dates (DAS, days after sowing) in 2003. Means and results of the analysis of variance performed jointly and each cultivar independently. Means followed by the same letter are not significantly different (LSD test,  $p < 0.05$ )

	Nitrate		K	
	Crema 111	Sor Angela	Crema 111	Sor Angela
<i>DAS</i>				
77	3,409 <sup>ab</sup>	2,770 <sup>a</sup>	11,484 <sup>a</sup>	10,165 <sup>ab</sup>
92	4,076 <sup>a</sup>	2,960 <sup>a</sup>	14,180 <sup>b</sup>	12,737 <sup>c</sup>
106	4,142 <sup>a</sup>	2,733 <sup>a</sup>	11,779 <sup>a</sup>	11,153 <sup>a</sup>
119	2,905 <sup>b</sup>	1,639 <sup>b</sup>	12,038 <sup>a</sup>	9,142 <sup>bd</sup>
134	1,781 <sup>c</sup>	1,457 <sup>b</sup>	11,146 <sup>a</sup>	8,603 <sup>d</sup>
<i>ANOVA</i>				
Date (D)	$p < 0.01$		$p < 0.001$	
N level (F)	$p < 0.01$		$p < 0.001$	
Cultivar (C)	$p < 0.001$		$p < 0.001$	
C*D	$p < 0.05$		$p < 0.05$	
C*F	ns		ns	
D*F	$p < 0.001$		ns	
C*D*F	ns		$p < 0.05$	

requirements (Gerik *et al.*, 1998). Similar results have been obtained under several conditions of soil, cultivars and management practices (Baker *et al.*, 1972; Sunderman *et al.*, 1979; Constable *et al.*, 1991). Significant interactions were observed between sampling time and fertilizer N level, as well as between sampling time and cultivar for the petiole nitrate concentration (Table 1, Fig. 2). Petiole nitrate concentration was



**Figure 2.** Effect of nitrogen fertilizer level on petiole nitrate concentration in cultivars Crema 111 and Sor Ángela in 2003 and 2004. Mean values represent five and four different sampling dates for 2003 and 2004 respectively.

**Table 2.** Nitrate and potassium concentration ( $\text{mg kg}^{-1}$ ) in petioles from cotton cultivars Crema 111 and Sor Ángela as affected by different sampling times (DAS, days after sowing) in 2004. Means and results of the analysis of variance performed jointly and each cultivar independently. Means followed by the same letter are not significantly different (LSD test,  $p < 0.05$ ).

	Nitrate		K	
	Crema 111	Sor Ángela	Crema 111	Sor Ángela
<i>DAS</i>				
83	5,806.3 <sup>ab</sup>	4,695.9 <sup>a</sup>	13,195 <sup>a</sup>	12,988 <sup>a</sup>
91	7,322.2 <sup>a</sup>	5,118.4 <sup>a</sup>	12,861 <sup>a</sup>	13,786 <sup>a</sup>
101	4,246.8 <sup>b</sup>	3,421.4 <sup>ab</sup>	13,588 <sup>a</sup>	15,203 <sup>b</sup>
136	7,360.1 <sup>a</sup>	2,238.9 <sup>b</sup>	8,199 <sup>b</sup>	9,962 <sup>c</sup>
<i>ANOVA</i>				
Date (D)	$p < 0.001$		$p < 0.001$	
Fertilizer N (F)	$p < 0.001$		$p < 0.001$	
Cultivar (C)	$p < 0.001$		$p < 0.001$	
C*D	$p < 0.001$		$p < 0.05$	
C*F	$p < 0.001$		ns	
D*F	$p < 0.001$		$p < 0.01$	
C*D*F	ns		ns	

affected by applied N but the variation depended on cultivar and sampling dates. Similar results were recorded in 2004, when petiole nitrate concentration varied according to the fertilizer N level, sampling date and cultivar (Table 2, Fig. 2). Other authors have reported the relationship of petiole nitrate vs. N fertilization limited only to first bloom stage (Bronson *et al.*, 2001). Differences in petiole nitrate concentration between 2003 and 2004 might be attributed to the different

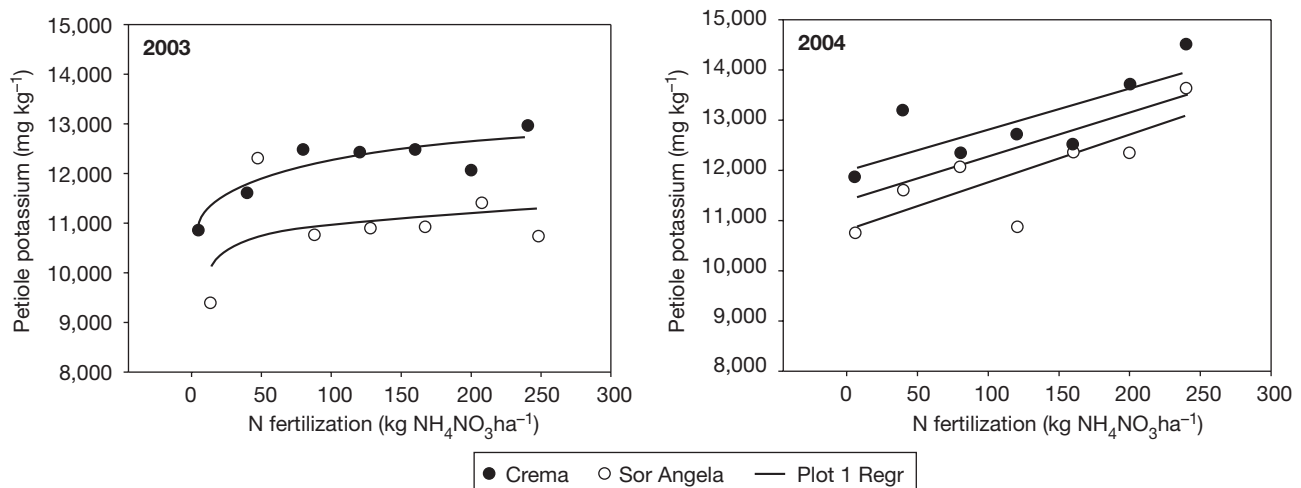
**Table 3.** Correlation coefficients (Pearson) for the relationships among concentration of K and nitrate in petioles, N fertilization treatment and sampling date

	2003	2004
K vs. Nitrate	$r = 0.66^{***}$ , $n = 70$	$r = 0.17^*$ , $n = 223$
K vs. N treatment	$r = 0.25^*$ , $n = 70$	$r = 0.25^{***}$ , $n = 223$
K vs. Date	$r = -0.35^{**}$ , $n = 70$	$r = -0.49^{***}$ , $n = 56$

\*\*\*\*\*: statistical significance for the correlation coefficient at  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$  respectively.

methodologies used to determine nitrate.

In both experimental years, the concentration of K in petioles was significantly associated to petiole nitrate (Table 3) and N applied (Table 3, Fig. 3). In 2003, the relationship K vs N fertilization fitted better a two-parameter power equation. Such K-N relations might be explained by a promotion in K uptake in cotton by N fertilization as reported by Halevy *et al.* (1987). A synergism in the uptake of N and K occurs as K is the main counterion for root nitrate uptake and xylem transport to shoots (Lips *et al.*, 1987; Marschner, 1995), *i.e.* increasing N uptake rates require increasing uptake of K for balancing charges (Peuke *et al.*, 2002). The concentration of K in petioles was significantly affected by sampling time and cultivar (Tables 1 and 2). Root nutrient uptake is related to nutrient availability but strongly regulated by growth demand (Marschner, 1995). When root uptake was reduced at the final stages of growth, petiole nutrient concentration reflects the balance on what roots are presently absorbing and how much is being translocated to sinks (*e.g.* deve-



**Figure 3.** Effect of nitrogen fertilizer level on petiole potassium concentration in cultivars Crema 111 and Sor Ángela in 2003 and 2004. In 2003, the relation is better represented by a power equation ( $y = a \cdot x^b$ ). Mean values represent five and four different sampling dates for 2003 and 2004 respectively.

loping bolls). In previous works, variation in K uptake among cotton cultivars has been found (Halevy, 1976; Brouder and Cassman, 1990; López *et al.*, 2008b) which probably reflects variation in root K uptake as well as differential demand for growth (Keino *et al.*, 1999; López *et al.*, 2008a).

The concentration of nitrate in petioles was negatively associated with sampling dates in 2003 ( $r = -0.46$ ,  $p < 0.001$ ,  $n = 70$ ) reflecting changes in N demand by shoots. In both years of study, petiole K concentration showed a negative association with sampling date (Table 3). The reduction in petiole K concentration might be reflecting both decay in root uptake activity in maturing plants (Oosterhuis, 1994, 1999) and the great demand of K imposed by growing bolls (Leffler and Tubertini, 1976).

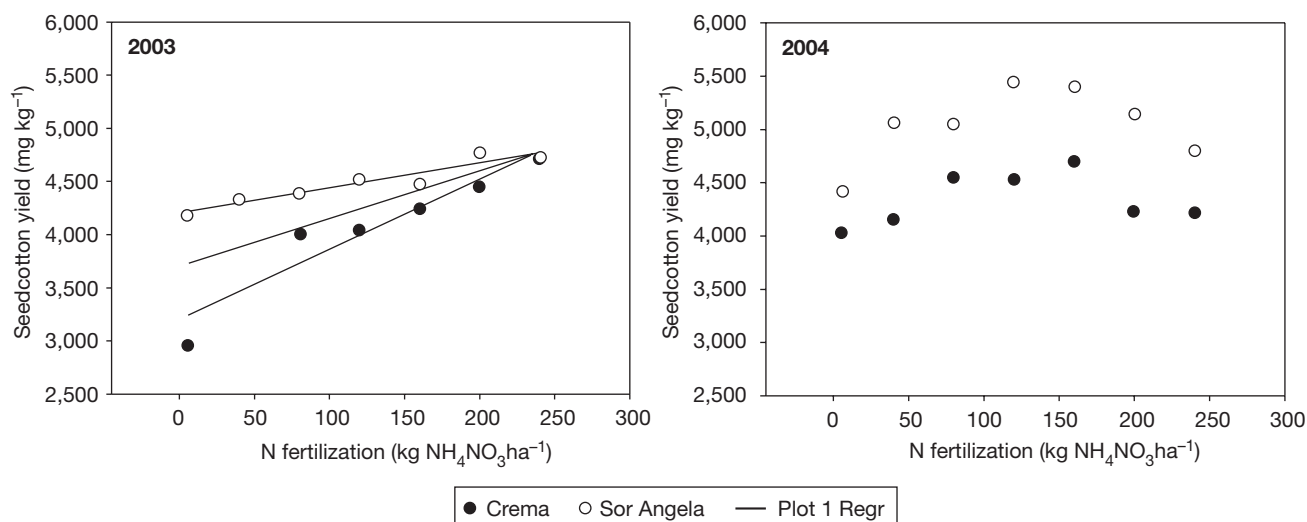
The highest seed cotton production was obtained with the highest level of fertilizer N in both cultivars in 2003 although the situation changed in 2004 (Fig. 4). In 2004, the greatest yield was obtained when fertilizer N application was between 120-160 kg ha<sup>-1</sup>. More favourable climatic conditions in 2004 at the end of the cycle might have promoted regrowth and delayed maturity under high N resulting in greater number of unopened bolls (Guthrie *et al.*, 1994; Oosterhuis and Stewart, 2004). Unfortunately, plant mapping was not carried out in these experiments to attribute lower yields at high N to an increase in unharvestable bolls. As in the year 2003, in 2004 there was a significant effect of the cotton cultivar on seed cotton yield but no significant interaction cultivar by N treatment was observed.

Seed cotton yield was highly correlated with N fertilization level in 2003 ( $r = 0.74$ ,  $p < 0.001$ ) but not in 2004 when the relationship yield-N application was

**Table 4.** Correlation coefficients (Pearson) for nitrate and K concentration in petioles at different sampling times (in DAS, days after sowing) vs seed cotton yield for cultivars Crema 111 and Sor Angela during 2003 and 2004 ( $n = 7$ )

Cultivars	2003		2004	
	Nitrate	K	Nitrate	K
	77 DAS		83 DAS	
Crema 111	0.918**	0.911**	0.112	0.289
Sor Angela	0.965***	0.597	0.318	0.220
	92 DAS		91 DAS	
Crema 111	0.848*	0.411	-0.020	-0.465
Sor Angela	0.841*	0.928**	0.261	-0.408
	106 DAS		101 DAS	
Crema 111	0.888*	0.082	-0.524	-0.174
Sor Angela	0.605	0.658	0.588	-0.444
	119 DAS			
Crema 111	-0.652	0.933**	nd	nd
Sor Angela	-0.694	-0.804*	nd	nd
	134 DAS			
Crema 111	-0.441	0.279	0.450	0.629
Sor Angela	-0.326	0.048	0.323	0.359

nd: not determined. \*, \*\*, \*\*\*: statistical significance for the correlation coefficient at  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$  respectively.



**Figure 4.** Effect of nitrogen fertilizer level on seed cotton yield in cultivars Crema 111 and Sor Ángela in 2003 and 2004.

not linear but curvilinear (Fig. 4). Interestingly, the regression analysis of petiole nitrate and seed cotton yield in 2003 showed a positive association mostly in Crema 111 (Table 4). No significant relationships between petiole nutrient concentration and yield were observed in 2004 (Table 4).

Petiole K concentration was also positively related with yield at given dates in 2003 (Table 4). The significant association found at 119 DAS but of opposite sign in Crema and Sor Angela might be due either to genotypic differences in growth cycle or in nutrient use efficiency. The earlier cutout recorded in Sor Angela may have induced significative changes in K demand by bolls and nutrient translocation from vegetative tissues.

The significant differences observed between cotton cultivars in petiole nutrient concentration across N treatments, dates and experimental years certainly reflected differences in nutrient uptake ability by the tested genotypes. In previous studies, differences in K uptake between cotton cultivars were found and related to root growth and morphology (Brouder and Cassman, 1990; Keino *et al.*, 1999; López *et al.*, 2008a). The present study validates former results on cultivar variation in nutrient uptake efficiency by using petiole analysis when applied in different conditions (soil, cultivars, management). In the search for genotypes better adapted to low inputs, differences in the efficiency of uptake or utilization of nutrients might be a rational aim, and petiole nutrient concentration might be a useful tool for the search.

Most studies on cotton nutrition emphasize on the variability and complex yield responses to nutrient availability (Guthrie *et al.*, 1994; Gerik *et al.*, 1998; Oosterhuis and Stewart, 2004). Environmental factors (mostly temperature and available water) (López García and Gutiérrez Más, 2006), genetic variation in nutrient use efficiency and potential yield (Oosterhuis and Stewart, 2004) modify the cotton response to available nutrients and make difficult to design proper fertilization guides. However, there is a need for improving fertilizer use efficiency to reduce the impact of releasing high levels of soluble N in the environment (UNEP and WHRC, 2007). Therefore, more technical research as the presented in this report should be carried out to get reliable indicators of crop nutrient status to maximize cotton yield.

In conclusion, petiole analysis may provide an adequate guide for monitoring fertilization in cotton but an ample database should be generated (gathering

data on environment, crop development and management) to overcome the year-to-year variation in yield response. Furthermore, the variation in nutrient content in petioles may supply additional information on genotypic differences in nutrient uptake and use efficiency.

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