

EFFECT OF POTASSIUM ON NITROGEN FIXATION, NITROGEN
TRANSPORT, AND NITROGEN HARVEST INDEX OF BEAN

Key words: *Phaseolus*, beans, nitrogen fixation, harvest index,
potassium, ureides

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ABSTRACT

Four cultivars of *Phaseolus vulgaris* L., Carioca, Venezuela-350, Rio Tibagi and Negro Argel, were grown to maturity in a glass-house with a low (1 mol m^{-3}) and a high (4.5 mol m^{-3}) potassium supply. Potassium stimulated nitrogenase activity (acetylene reduction) and the accumulation of ureides in pod walls of Carioca and Negro Argel, cultivars which had the highest rates of acetylene reduction. In three of the four cultivars the higher level of potassium nutrition resulted in a greater partitioning of above ground N to the seeds. The proportion of xylem sap N in the form of the ureides, allantoin and allantoic acid (allantoate), was only slightly increased by the higher rate of potassium supply. Seed yields were increased by high potassium in the cultivars Venezuela-350 and Negro Argel. The results suggest that potassium stimulates the transport of nitrogenous compounds to developing fruits.

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INTRODUCTION

The stimulation of both nodulation and nitrogen fixation by potassium has been well documented for a number of grain and forage legumes (2). Most of the evidence indicates an effect of potassium on either nodulation (nodule number or mass) or on nodule productivity (moles nitrogen fixed per unit time per unit mass of nodule). Proposed reasons for these effects include an increased transport of photosynthates to nodules, increased root growth, and increases in nitrogen assimilating enzymes in nodules such as glutamine synthetase, asparagine synthetase, glutamate-oxaloacetate aminotransferase (GOT) and glutamate synthase (GOGAT) (3,4,5,6). Other effects of potassium on metabolism such as osmotic effects on water transport cannot be ruled out.

Most of the reported work has concentrated on potassium effects at one or a few points of the growth cycle. The amount of data on the effect of potassium on yields of grain legumes is limited and varies from increases in yield to little or no effect (7,8).

This study reports the effect of potassium supply on nitrogen fixation and seed yield parameters in four cultivars of Phaseolus vulgaris L. grown in glasshouses.

MATERIALS AND METHODS

Plants were grown in 3.5 l plastic pots filled with a mixture of washed sterilised sand and vermiculite (1:2 v/v) in a glasshouse at EMBRAPA-UAPNPBS, km 47, Rio de Janeiro, Brazil during February-April 1983. Seeds of four cultivars of Phaseolus vulgaris L. cv Carioca, Venezuela-350, Rio Tibagi and Negro Argel obtained from EMBRAPA-CNPAP, Goiania, Brazil, were surface sterilised with 0.2% HgCl₂ (9) and inoculated with 1 ml inoculant for each 15 seeds (approx. 10⁸ cells ml⁻¹ of Rhizobium leguminosarum biovar phaseoli, strain C05 obtained from CENA-Piracicaba, Brazil). Each pot initially contained 5 seeds which were thinned to 2 plants per pot two days after emergence. The experiment was laid out in a

complete randomised block design with four replicates for each treatment. Each pot was fed 200 ml of nutrient solution with no nitrogen source every 4 days and watered daily with distilled water. Two nutrient solutions were used, a low and high potassium treatment. Nutrients common to either treatment were 3.1 mol m^{-3} $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2.9 mol m^{-3} $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, and trace elements (all mmol m^{-3}) $1 \text{ MnSO}_4 \cdot 4\text{H}_2\text{O}$, $0.1 \text{ CuSO}_4 \cdot 5\text{H}_2\text{O}$, $0.1 \text{ ZnSO}_4 \cdot 7\text{H}_2\text{O}$, $5 \text{ H}_3\text{BO}_3$, 10 NaCl , $0.5 \text{ NaMoO}_4 \cdot 2\text{H}_2\text{O}$ and 5 ferric citrate. The high potassium solution also contained 1.45 mol m^{-3} KH_2PO_4 , and 1.5 mol m^{-3} K_2SO_4 (total $\text{K}^+ = 4.45 \text{ mol m}^{-3}$, total monovalent cations 4.45 mol m^{-3}). The low potassium solution contained 1.45 mol m^{-3} NaH_2PO_4 , 1 mol m^{-3} Na_2SO_4 and 0.5 mol m^{-3} K_2SO_4 (total $\text{K}^+ = 1 \text{ mol m}^{-3}$, total monovalent cations 4.45 mol m^{-3}). Final pH of each solution was 6-6.2. Three harvests were taken 35, 50 and 60 days after planting approximately equivalent to flowering and early pod formation, pod formation and filling, and seed filling and pod senescence (yellowing) respectively.

Xylem Sap Collection

For each harvest 8 plants per cultivar and treatment were decapitated below the cotyledonary node with a razor blade, the cut ends were rinsed with distilled water and dried with tissue paper. Thereafter bleeding (xylem) sap was collected at intervals of 1-3 min with calibrated micropipettes. Usually a collection time of 3-7 min was sufficient to collect the 150-200 μl sap necessary for analysis of the nitrogenous compounds from 8 plants.

Analysis of Sap Constituents

Amino-, amide-, ammonium-, ureide- and total-N in sap samples were analysed as described previously (10) and modified for use in tropical conditions after Boddey *et al* (11). Potassium was measured by flame photometry.

Nitrogen Fixation

Nitrogen fixing activity was estimated by acetylene reduction after Mague and Burris (12). Plants were decapitated at the cotyledonary node and roots sealed into 30 ml glass bottles and

incubated for 30 min in an atmosphere of 12% (v/v) acetylene. One ml gas samples were removed at the beginning and end of the incubation period. Acetylene and ethylene gases were analysed using a Perkin-Elmer F-11 gas chromatograph fitted with a hydrogen flame ionization detector and a 50 cm stainless steel column (0.32 cm external diam.) filled with Poropak N (80-100 mesh and operated at 40°C with a carrier gas (N₂) flow rate of 40 ml min⁻¹. Errors in the use of acetylene reduction in closed systems have been reported (13). However when an additional experiment was performed using excised roots in a continuous flow system run at a rate of 100 ml min⁻¹ with 12% acetylene and removing gas samples after 1,3,4,5,7,10,15 and 30 min, there was no significant decrease in the rate of acetylene reduction due to the presence of acetylene (14). The results obtained from the closed system can therefore be considered to be free from substantial errors reported by Minchin *et al* (13).

Plant Dry Weight, Total N and Ureides

After each harvest roots were washed free of substrate with distilled water and plants were separated into roots plus nodules, stems, leaves plus petioles, pods and seeds and oven dried to constant weight at 70°C. Total N in plant tissues was determined using a semi-micro Kjeldahl method (11). Ureides were extracted from dry plant material with 0.1 M phosphate buffer pH 7.0 containing 50% ethanol at 80°C for 5 min. After centrifugation extracts were analysed for ureides as in Boddey *et al* (11).

RESULTS

Nitrogenase Activity, Plant Dry Weight and Total N

In general the increased supply of potassium had little effect on nitrogenase activity (acetylene reduction) during development with the exceptions of Negro Argel at 50 and 60 DAP and Carioca at 60 DAP, when rates of acetylene reduction were greater in plants supplied with 4.45 mol m⁻³ K⁺ (Fig 1). Nitrogenase activity was significantly lower in Rio Tibagi compared with the other cultivars at all three harvests.

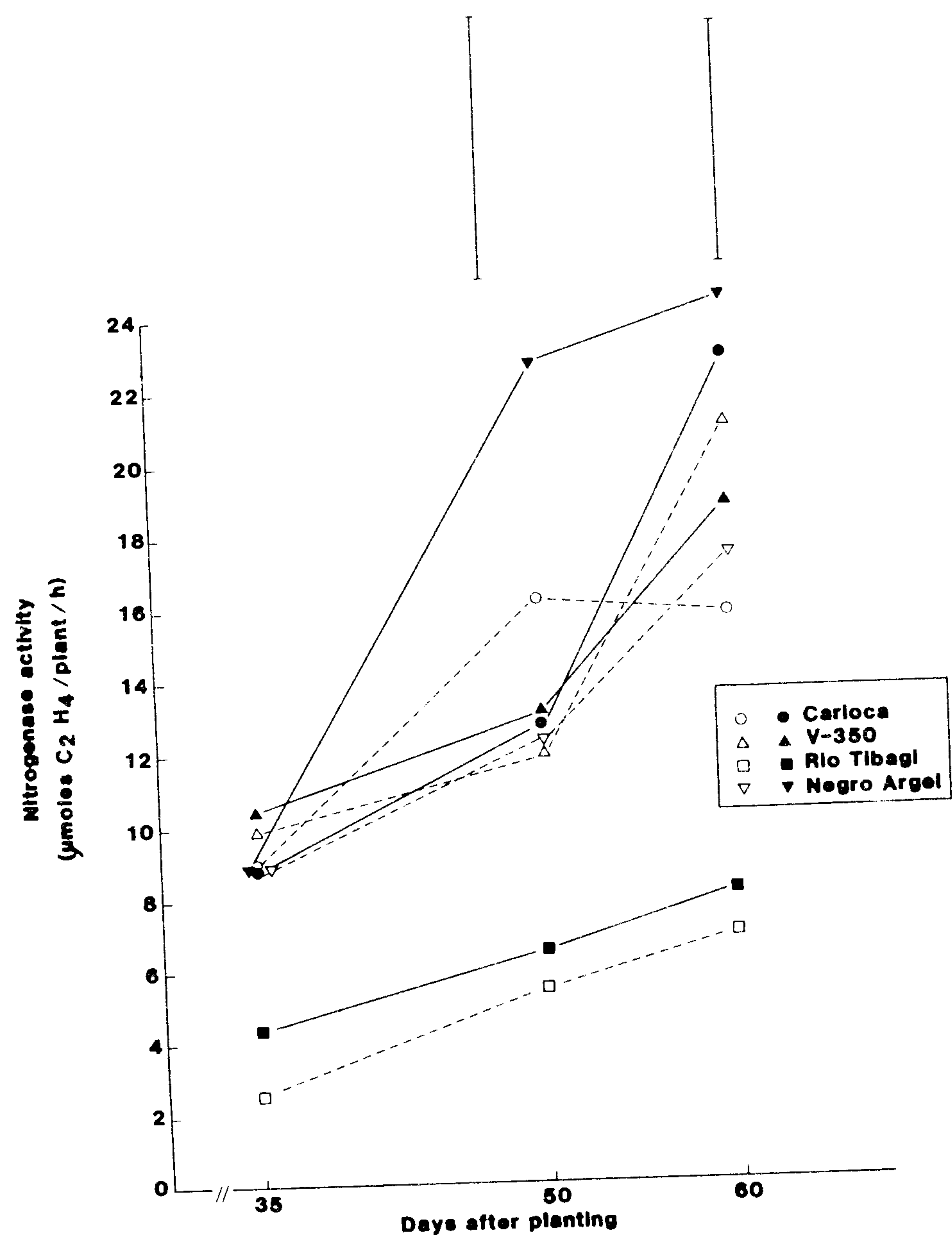


FIGURE 1. The effect of Potassium Supply on Nitrogenase activity. Plants grown with either 1 mM K⁺ (open symbols) or 4.5 mM K⁺ (closed symbols). Error bars are LSD (P= 0.05).

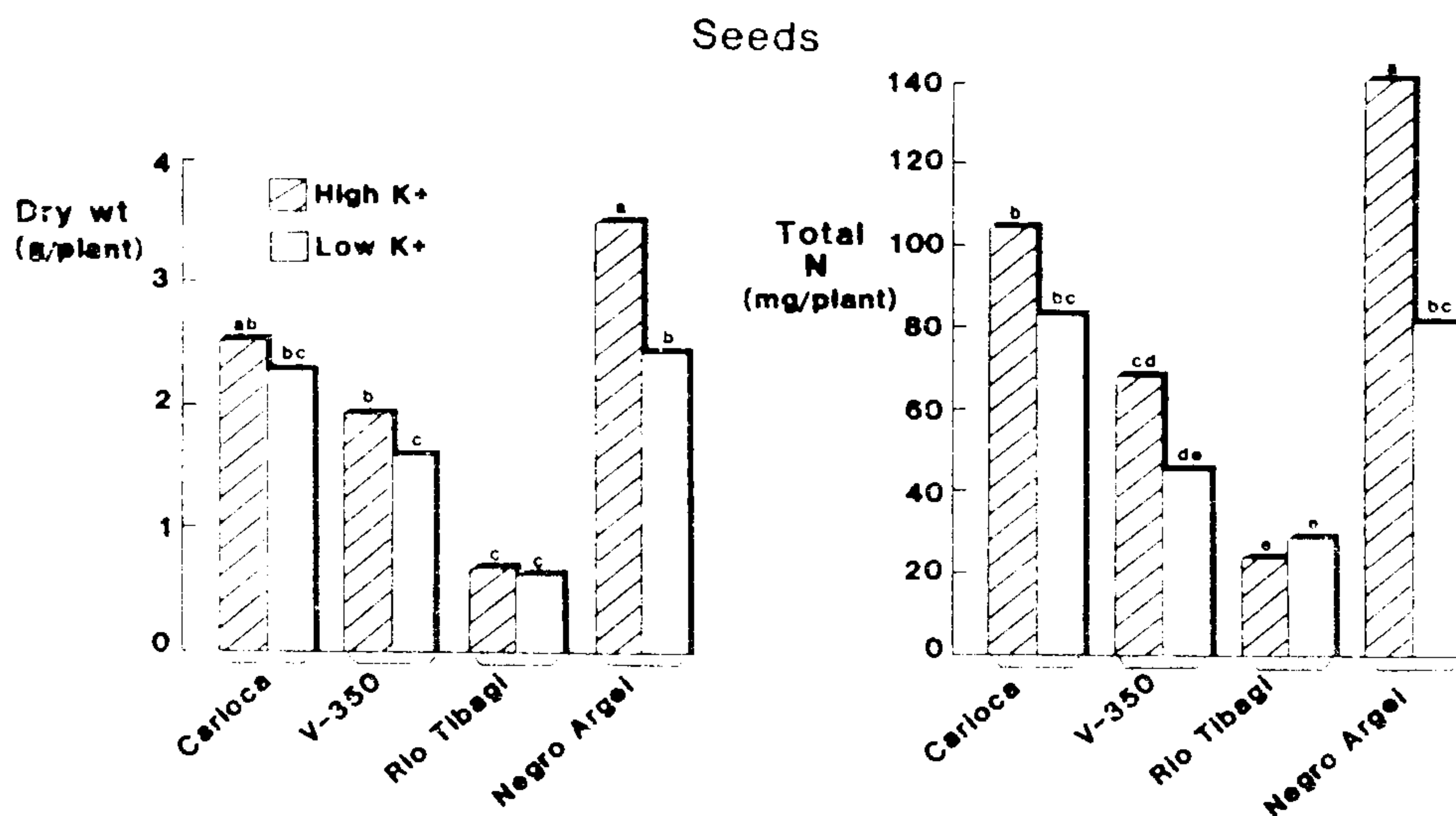


FIGURE 2. Dry weight and total N of seeds per plant. Columns with the same lettering do not differ significantly ($P = 0.05$, Tukey).

Potassium supply had no significant effect on N or dry weight of roots, stems or leaves or on total plant N (results not shown). After 60 days growth the amounts of total plant N were 211, 202, 116 and 250 mg N plant⁻¹ for Carioca, V-350, Rio Tibagi and Negro Argel respectively (treatment means). However the increased supply of potassium resulted in greater seed dry weights in V-350 and Negro Argel, and greater seed N in Negro Argel (Fig 2).

Transport of N in Xylem Sap

Although there was a slight increase in the percentage of the sap N as ureide-N in cultivars receiving the greater supply of potassium, in general potassium had no effect on either the composition or translocation rate (rate of exudation x total N ml⁻¹ sap) of xylem sap N (Table 1). Similarly potassium had no effect on the relative proportions of the ureide-N as allantoin or allantoic acid (allantoate) with the latter being the predominant

TABLE 1. Effect of Potassium supply on the Composition of Xylem Sap N

	K ⁺ Supply		
	low	high	
N concentration $\mu\text{mol N ml}^{-1}$ sap	51.8	60.9	n.s.
% composition			
Ureide-N	84.0	89.7	*
α -Amino-N	6.6	5.2	n.s.
Amide-N	2.6	1.8	n.s.
Ammonium-N	3.4	2.8	n.s.
Other-N	3.4	0.5	*
rate of exudation			
$\mu\text{mol N plant}^{-1} \text{ min}^{-1}$	130.8	158.9	n.s.

Values are means of treatments, three harvest dates and four cultivars.

ureide comprising, on average, 73% of the sap ureide-N. There was a positive linear relationship between the concentration of allantoate, the anionic form of allantoic acid, and potassium in sap samples (Fig 3).

Ureide Content of Tissues

In general the increased supply of potassium had little or no effect on the accumulation of ureides in leaf and stem tissue although there were some significant differences between cultivars (Table 2). With the cultivars Carioca and Negro Argel, increased

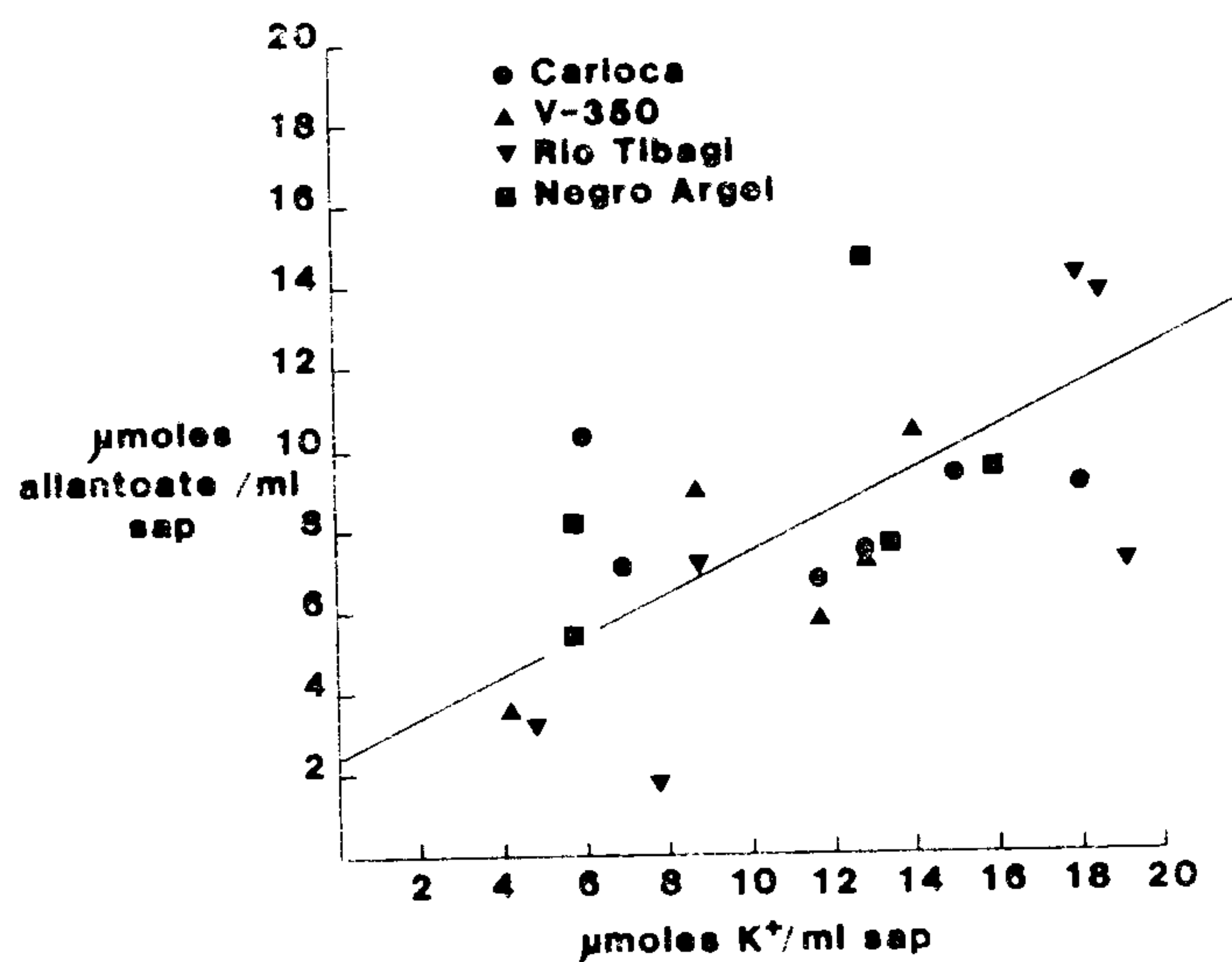


FIGURE 3. The relationship between Potassium and Allantoate in Xylem Sap. Data from each of three harvests. $\text{Allantoate} = 2.5 + 0.503K^+$. $r^2 = 0.73$

potassium supply resulted in greater amounts of ureides in pod walls but not seeds (Fig 4).

Partitioning of N Between Seed and Vegetative Parts

In three of the four cultivars the proportion of the plant N in the seed at maturity compared with that remaining in the vegetative parts was increased by the higher rate of potassium supply (Fig 5).

DISCUSSION

The results demonstrate that, depending on cultivar, increasing supplies of potassium can increase nitrogenase activity and seed yield of Phaseolus as reported previously for other grain legumes (2,6). The effects of potassium on the ureides in the xylem sap were not as great as those reported previously for soybeans (6). The finding that potassium can increase the ureide concentration of pod walls and also the partitioning of N between

TABLE 2. Effect of Potassium supply on Ureide concentration in vegetative tissues.

Days after planting	$\mu\text{moles ureide-N g}^{-1}$ dry wt					
	Leaves			Stems		
	35	50	60	35	50	60
Cultivar						
Carioca	7.9a*	66.8b	8.3a	24.9a	27.7bc	15.2a
Venezuela-350	8.3a	65.8b	5.3b	21.5a	24.8c	11.6ab
Rio Tibagi	8.4a	56.0c	4.5b	14.7b	50.6a	8.9b
Negro Argel	7.2a	72.5a	5.5b	24.0a	40.6ab	10.4b
High K ⁺	8.5a	74.7a	6.2a	25.1a	39.8a	12.4a
Low K ⁺	7.4a	55.9b	5.7a	17.5a	31.1a	10.6a
CV%	23.0	13.2	18.2	22.8	29.9	24.1

* Results are means of 8 replicates. Values followed by the same letter are not significantly different at the 5% level (Tukey).

seeds and the rest of the above ground tissues has not been previously recorded for grain legumes. This effect is analogous to that reported for cereal grains where potassium increased the translocation of assimilates from the vegetative parts to the ears of wheat (15). In cereals this effect was thought to occur via promotion of phloem loading and transport.

Although N translocation rates in xylem sap did not differ greatly with potassium supply there was a positive relationship between allantoate, the predominant form of N in sap samples, and potassium, suggesting that potassium may have a stimulating

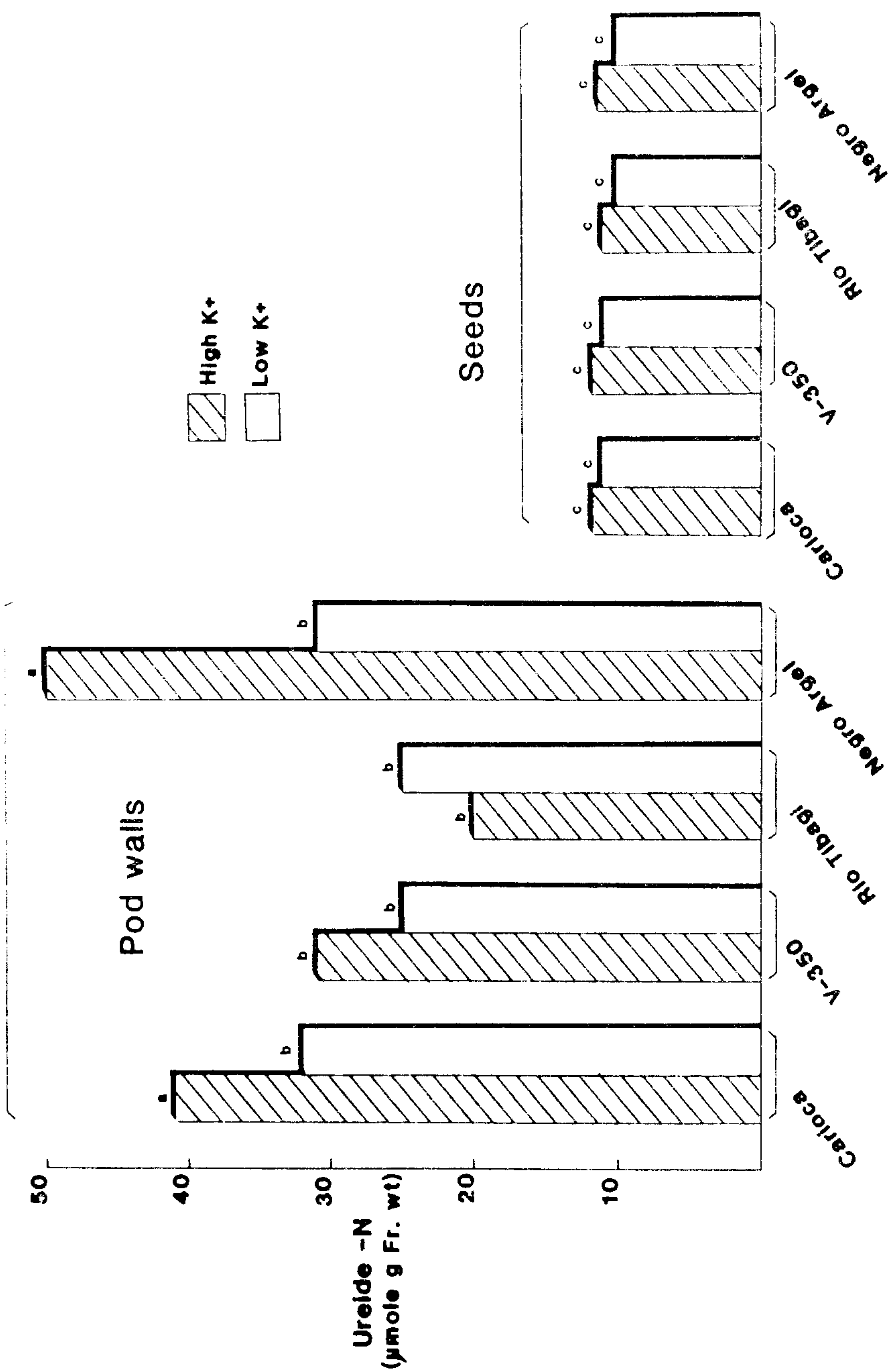


FIGURE 4. Ureide-N concentrations in pod walls and seeds. Data from third harvest. Columns with same lettering do not differ significantly ($P = 0.05$, Tukey).

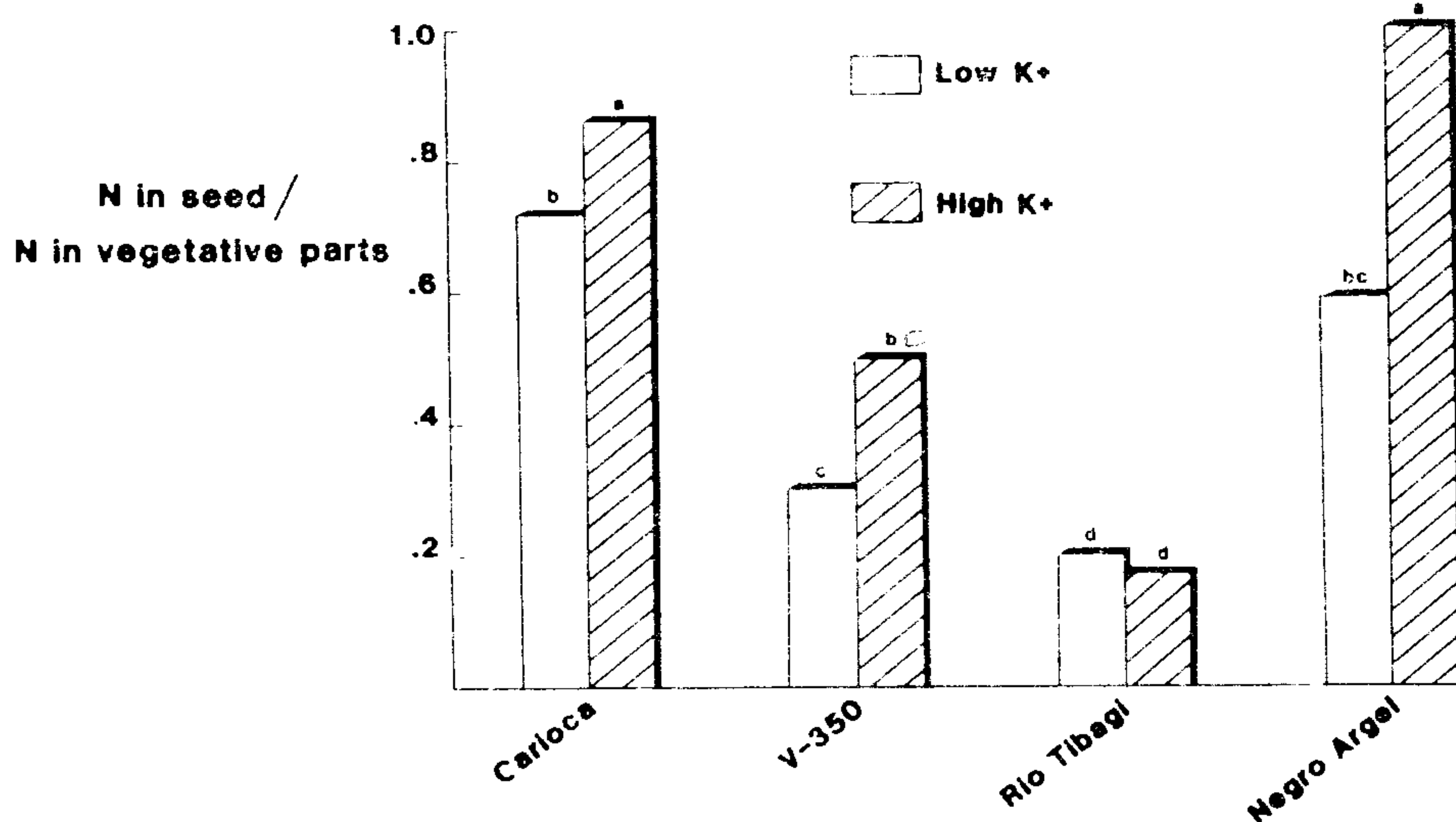


FIGURE 5. Effect of Potassium Supply on N Harvest Index. Columns with same lettering do not differ significantly ($P = 0.05$, Tukey).

effect on the long distance transport of quantitatively important nitrogenous compounds. Potassium has been previously shown to be preferentially distributed to growing or recently matured organs (16) and if potassium is used as the counter ion for allantoate transport in the xylem and phloem this could explain the increased partitioning of ureide-N to developing fruits. In Phaseolus the role of the xylem and phloem in supplying the developing fruit with nutrients has not been fully characterised. In Vigna (cowpea), another legume where ureides are the predominant form of nitrogen transport in nitrogen fixing plants, most of the ureides are delivered to the developing fruit in the xylem rather than the phloem (17). Fruit development and especially pod abortion is particularly sensitive to nutrient/hormone supply at this stage (18) and a potassium-stimulated increase in assimilate transport may result in an increased ability of pod walls and

seeds to act as sinks. Whatever the exact mechanism the results suggest a potassium stimulated transport of nitrogenous assimilates to developing fruits.

The effects of potassium on the partitioning of N reported here are partly similar to those reported for Rhizobium and Bradyrhizobium strain effects with Phaseolus (19) and soybeans (20,21) respectively. Attempts to improve the Rhizobium-legume symbiosis via strain or host plant selection should also take into account potential interactions with increased requirements for minerals such as potassium, especially where legumes are grown on large areas of the tropics where soils are subject to intensive leaching and where legumes respond greatly to applied nutrients (4).

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