



Full Length Article

Cooking Quality of Common Beans as Influenced by Different Nitrogen Levels and Time of Application

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ABSTRACT

This study was conducted to evaluate the influence of N fertilization on the yield, cooking quality and nutritional characteristics of the common bean. Treatment combinations were two cultivars (cvs. IAPAR-81 & Uirapuru) and four levels of topdressing N (0, 40, 80 & 120 kg N ha⁻¹). Observations were taken at two stages of plant development, at three trifoliolate leaf (V4) and flowering phases (R6). The seed traits analyzed were; hydration time, hydration capacity, cooking time, phytate content, phosphorus content, soluble and total protein content and 100 seed weight. The results revealed that N topdressing led to changes in cooking time, but these changes were dependent on the genotype, the plant growth stage and N level. The dose and stage recommended for N topdressing of common beans (V4, 40 kg N ha⁻¹), showed a shorter cooking time for the cv. IAPAR (32 min) than cv. Uirapuru (43 min). This short cooking time was associated with higher hydration time, total proteins, phytate and 100 seeds weight and lower hydration capacity. The cv. Uirapuru showed a decrease in cooking time at both stages with increased N topdressing, but was more pronounced at the R6 stage, with higher yield. These data led us to recommend delayed N topdressing for this variety.

Key Words: *Phaseolus vulgaris*; Cooking quality; Hydration time; Phosphorus

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) seeds contain about 20-25% protein and are a major source of vegetable proteins, especially in Brazil (Sgarbieri & Whitaker, 1982), China, India and other developing countries. A major factor responsible for underuse of common bean is prolonged cooking requirements before consumption (CONAFE, 2005).

A number of efforts have been devoted to optimize soaking and cooking characteristics of legumes (Silva *et al.*, 1981). Although bean breeding programs have been successfully applied to a wide range of bean varieties to reduce cooking time, this research has taken a long time to obtain required results and is dependent on interrelations between field conditions and storage conditions (Carbonell *et al.*, 2003; Dalla Corte *et al.*, 2003; Ramos *et al.*, 2005; Rodrigues *et al.*, 2005; Coelho *et al.*, 2007a). Therefore, research that considers the use of manuring procedures to reduce the cooking time of bean controls the nutritional level (Silva *et al.*, 2006) and bean condition during storage associated with genotypic character (in order to prevent hard to cook phenomenon) have been observed in previous reports (Coelho *et al.*, 2007a).

Nitrogen (N) is the element taken up from the soil by the plant and into the grain in major amounts. N and phosphorus (P) directly affect the yield and protein content in the grains and we need to consider the available N and P level and the plant development stage (Silveira & Damasceno, 1993; Ahmad *et al.*, 2003; Gutiérrez-Rodríguez *et al.*, 2006). Although this available N relationship is essential, the genotype effect is more important (Furtini *et al.*, 2006). Albeit the N topdressing and molybdenum application did not affect the common bean yield, they influenced the technological characteristic of the grains, with increase in protein contents, cooking time and time for maximum hydration (Silva *et al.*, 2006).

Most of the N-related studies have not addressed cooking characteristics and nutritional quality of the bean grain. We believe that protein contents are associated with cooking time. This is because those proteins are stored in globoids, which have greater contents of minerals and phytate in the grain (Lott *et al.*, 1985), since phytate can be an indicator of the cookability of fresh common beans (Coelho *et al.*, 2007a). Another important factor is the protein contents of the grains, since a negative correlation between phytate and protein contents has been noted (Raboy, 1990; Raboy *et al.*, 1991). This is not entirely

surprising given the association between protein and phytate in protein storage bodies, but the regulatory mechanisms are not entirely known.

Limited information is available on the factors influencing cooking time. Since the bean grains that have been produced under the same environmental conditions of soil, fertilizer and climate, the most probable causes of the observed differences appear to be genotype and/or the management conditions during the crop cycle. The objective of this study was to analyze and compare two different cultivars of common beans under four levels of N, topdressed at two stages of development of bean plants and their effect on the cooking time of the fresh harvested grains.

MATERIALS AND METHODS

Growth conditions. A field experiment was conducted during the 2006/2007 planting season, with two commercial genotypes of common bean, cv. IAPAR-81 (carioca colored-group) and cv. Uirapuru (black bean group). The experiment was carried out in Lages, Santa Catarina State, Brazil, at 27° 52' South Latitude, 50° 18' East Longitude, 930 m above sea level (EPAGRI, 2006).

Prior to planting, N, phosphorus (P₂O₅) and potassium (K₂O) were applied at 20, 73, 63 kg ha⁻¹ of N, P₂O₅ and K₂O, respectively based on the recommendation of the Chemical and Fertility Committee for Santa Catarina and Rio Grande do Sul State, Brazil (CQFS-RS/SC, 2004). The experimental setup was design with three replicates and arranged in split plots. The sowing density was 12 seeds m⁻¹ in each plot and consisted of four rows of plants spaced at 0.5 m. The N topdressing treatments were 0, 40, 80 and 120 kg N ha⁻¹ applied in two stage of development of the plant, at three trifoliolate leaf (V4) and flowering phases (R6).

At harvest, the seeds from the two central rows of each plot were collected and dried to a standard moisture content of 12%. The samples were stored at 10°C and 40% relative air humidity. The seeds were analyzed in relation to weight of 100 seeds, productivity, total and soluble protein, total P and phytic acid, water soaking and cooking time.

Total proteins quantification. The total proteins were determined by measuring the total N content of the samples, where % protein=N content (%) × 6.25 according to Kjeldahl (AOAC, 1995). Total N was determined by micro Kjeldhal methods as described by Tedesco *et al.* (1985).

Soluble proteins extraction and quantification. The dry seeds, without husk, were ground by cold mortar and pestle. To fine powder (100 mg) 1 mL of 0.5 M NaCl buffer (pH 2.4) was added then the solution was stirred for 30 min at room temperature on a rotator shaker. The samples were centrifuged at 10.000 × g for 20 min to clarify the supernatant and stored at -20°C. Proteins were measured using colorimetric Bradford assay (1976). To the protein solution, 5 mL of the Bradford reagent was added and the absorbance was measured at 595 nm. A bovine serum

albumin (BSA) dilution curve was used as standard.

Quantification of total P and phytic acid. Total P content was determined calorimetrically following nitric/perchloric acid digestion, using the metavanadate method, which is based on the formation of a yellow vanadomolibdofosforic complex (Bernhart & Wreath, 1955). Phytate was quantified according to Latta and Eskin (1980) method. A 500 mg sample was extracted, with 20 mL of 2.4% HCl (0.65 N) for 2 h at room temperature on a rotatory shaker (2000 × g). The extract was centrifuged for 15 min and the supernatant decanted and filtered through Whatman No. 1 filter paper. A 3 mL aliquot of the filtrate was diluted to 18 mL with distilled water and the diluted sample was passed through a 200-400 mesh AG1-X8 chloride anion exchange resin, taking care that no more than 3 mg phytate per 0.5 g of resin was applied. Inorganic P was eluted with 0.07 M NaCl followed by elution of phytate with 0.7 M NaCl. Phytate was determined colorimetrically, with sodium phytate standard based on the pink color of the Wade reagent, which is formed upon the reaction of ferric iron and sulfosalicylic and has an absorbance maximum at 500 nm. In the presence of phytate, the iron is sequestered and unavailable to react with sulfosalicylic acid, resulting in a decrease in pink color intensity.

Water absorption. The water uptake was monitored by soaking 16 g of beans in 100 mL of distilled water at 25°C until a complete soaking was achieved (Berrios *et al.*, 1999). The water content of the bean grains was evaluated at each hour by measuring the amount of water absorbed until reaching a constant absorption. At hourly intervals, the water was drained from the samples and beans were blot dried prior to weighing. The value of absorption of water in the grains was expressed in percentage of absorbed water and the calculations based on grams of absorbed water per 100 g of grains, through use of the following equation:

$$\frac{\text{Weight of the grain-weight of the grain dry}}{\text{weight of the dry grain}} \times 100.$$

Cooking time. Prior to cooking, grain samples were soaked in deionized water. A modified Mattson-type cooker (Proctor & Watts, 1987) was used to determine the cooking time of the individual beans. This cooker utilized 25 stainless steel, cylindrical, piercing tip rods (82 g each) in contact with the surface of the bean. The cooker was then placed into a 2-L beaker containing 1.4 L of boiling water. Bean grains were judged as “cooked” when the 2 mm diameter tip of the brass rods passed through the beans. The cooking time was reported as the time required for 60% of the grains to be cooked, as indicated by plungers dropping and penetrating individual beans.

Data analysis. The data were analyzed for variance analyses, unfolding the degrees of freedom in the situations, where there was interaction among the tested factors (SAS Institute, 2004). The effect of doses was tested through the use of regression polynomial analysis (Rosse & Vencovsky, 2000).

RESULTS AND DISCUSSION

Firstly, the time and capacity of hydration, the total proteins, phytic acid content and weight of the 100 seeds did not fit the regression analyses model. Secondly, the regression model was adjusted for total P and soluble proteins to the effect of dose. Finally, the cooking time was the only characteristics significantly affected by genotype, dose and developmental stage. In no fit regression case, the cv. IAPAR-81 was significantly higher than cv. Uirapuru in most cases, except for hydration capacity (Table I). The total proteins and phytic acid content was around 28% and 0.88%, respectively. These values are similar to those documented by other studies that related genetic diversity to protein and phytic acid content, which was 20–27% (Antunes *et al.*, 1995; Santalla *et al.*, 1999; Dalla Corte *et al.*, 2003) and 0.7–1.48% (Coelho *et al.*, 2002), respectively.

The weight of 100 seeds had little variation, around 24 to 27 g in both genotypes, as with commercial cultivars (Coelho *et al.*, 2007b). The hydration capacity varied from 94 to 99% for both the genotypes and these were very similar to values reported for Carioca Precoce and Pérola cultivars, respectively (Ramos *et al.*, 2005). The highest variation (15 to 115%) was found in commercial cultivars when the grains were soaked for four hours in distilled water (Costa *et al.*, 2001). This contrasting data between genotypes might be due to the rigid tegument junction of cotyledons, elasticity, porosity and colloid traits (Esteves *et al.*, 2002).

In the second case, when the adjustment of the regression model was observed, the concentration of soluble protein began to increase from 83 to a maximal value of about 115 g kg⁻¹ when 75.31 kg N ha⁻¹ was applied as topdressing (Fig. 1). But total protein did not show a significant increase ($P < 0.05$), because the N in dry bean grains is stored temporarily as free amino acids and other compounds, not proteinic ones. In agreement with other authors (Andrade *et al.*, 2004), protein content increased slightly (~1%). However, the P content decreased in the grains with the increase in N topdressing and the variation values were small (~3.9 to 4.7 g kg⁻¹) (Fig. 1).

Lastly, only the cooking time showed significant response in function of cultivar dose and stage of N topdressing (Fig. 2). This effect was noted at both crop developmental stages (V4 & R6), where the decrease of cooking time was observed in cv. Uirapuru. However, with cv. IAPAR-81 the cooking time increased linearly only at R6 stage. This behavior can be explained by genotypic traits. Particularly, cv. Uirapuru had a shorter cooking time in both stages, but more pronounced at R6 stage (5 min) as compared to V4 stage (1 min). Similarly, the P content also decreased significantly. However, the soluble proteins increased by 75.31 kg N ha⁻¹ (Fig. 1) than in the absence of N topdressing. This indicated that the R6 stage and 75 kg N topdressing were the treatments that resulted in better crop performance in relation to agronomic traits and quality

Fig. 1. Soluble protein content and phosphorus as a function of the level of N topdressing kg ha⁻¹ in two common bean cultivars (IAPAR-81 and Uirapuru), UDESC, LAGES, SC, season 2006/2007

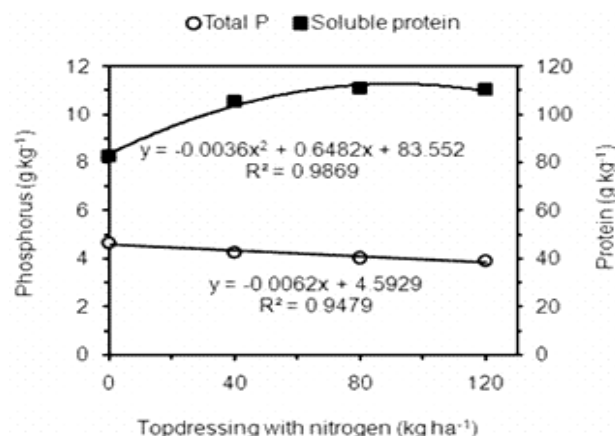
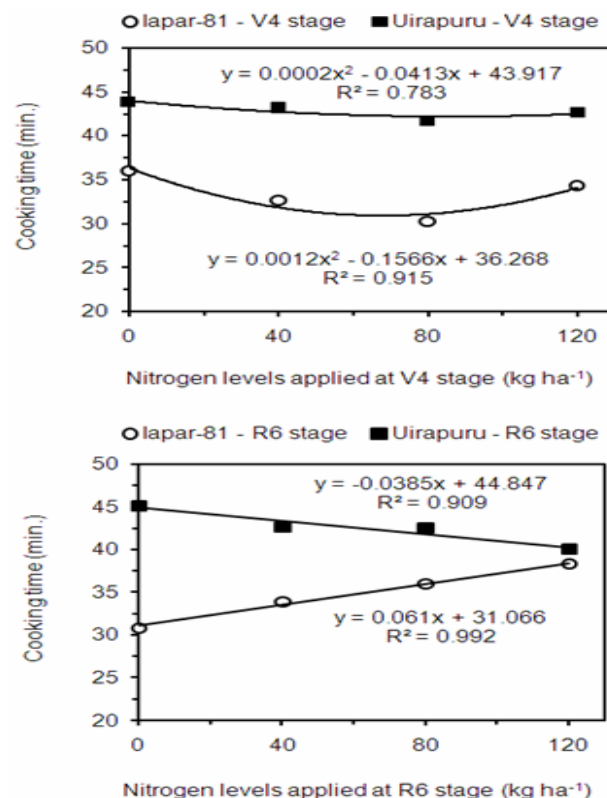


Fig. 2. Cooking time for two cultivars of common bean (Uirapuru and IAPAR-81) as a function of the level of N topdressing and stage of plant growth (a) V4 stage or (b) R6 stage. UDESC, LAGES, SC, season 2006/2007



characteristics. This was noted by the higher soluble content and shorter cooking time. In addition, it was also observed that the yield was higher at R6 stage (2162 kg ha⁻¹) when compared to V4 stage (1483 kg ha⁻¹). These responses are indicative of N topdressing in the R6 stage for cv. Uirapuru (Table II).

The increase in N topdressing had no influence on dry grain yield, but there was significant interaction between genotypes \times stages of N topdressing (Table II). The cv. IAPAR-81 showed a higher yield than cv. Uirapuru, about 693 kg ha⁻¹ at V4 stage. The N topdressing at R6 stage led to an increase in yield only for cv. Uirapuru around 679 kg ha⁻¹. The yield of cv. IAPAR-81 was 2100 kg ha⁻¹ and for cv. Uirapuru it was from 1483 to 2162 kg ha⁻¹. These results were higher than the average reported in Santa Catarina state for first season crops, around 910 kg ha⁻¹ (IBGE, 2007). However, we did not find a close relationship between N topdressing doses and yield. The application of N in topdressing did not represent an increase in yield only a genotype effect has been reported in common bean (Furtini *et al.*, 2006), which can be explained by a high concentration of nitrate in plant tissue associated with decreased nitrate reductase activity (Andrade *et al.*, 2004). In addition, other authors noted an increase in the yield of common beans as a result of an increase in the N topdressing dose when the crop is succeeded by black oat/pearl millet/bean in a no-tillage system (Farinelli *et al.*, 2006).

The genotypic effect in this study was larger in all evaluated traits. However, analyses of data on cooking time showed significant effects of genotype, dose and stage of N topdressing. The cooking time has been associated with genotype \times environment significant interaction (Carbonell *et al.*, 2003; Dalla Corte *et al.*, 2003; Ramos *et al.*, 2005) and storage condition (Coelho *et al.*, 2007a). If we consider the dose and recommended stage of application (according to the adequate Chemical and Fertility Committee for Santa Catarina and Rio Grande do Sul State, Brazil (CQFS-RS/SC, 2004) of N topdressing on common beans (V4, 40 kg N ha⁻¹), the cv. IAPAR showed a shorter cooking time than cv. Uirapuru (Fig. 2). This was associated with higher hydration time, total protein contents, phytate and 100 seeds weight as well as lower hydration capacity (Table I). The positive relation between phytate and protein content was similar to that described in the literature, only the increases phytate content had a positive correlation with protein content (Raboy *et al.*, 1984; Coelho *et al.*, 2002). In addition, the higher phytate content in the grains just harvested provides an indication of reduced cooking time (Coelho *et al.*, 2007a).

This genotype differential could be explained, because the phytate has the ability to be associated with proteins and minerals and is not degraded by the cooking or the digestion process (Cheryan, 1980). The ions from minerals can cause insolubility of pectinic acid and during the cooking process the middle lamella of the cotyledon cells does not separate (Reyes-Moreno *et al.*, 1994). However, as stored grains age, under temperature of 29°C and relative air humidity of 75%, cv. IAPAR-81 indicated a tendency to develop the hard-to-cook phenomenon of beans (Coelho *et al.*, 2007a), but this effect must be better studied in subsequent work.

It would be very interesting to further investigate the

Table I. Time and capacity of hydration, protein and phytate content and weight of 100 seeds of two cultivars of common bean (IAPAR-81 and Uirapuru), UDESC, LAGES, SC, season 2006/2007

Cultivars	Hydration time (min.)	Hydration capacity (%)	Total protein (%)	Phytate content (%)	weight of 100 seeds (g)
IAPAR 81	9*	94.56*	28.08*	0.88*	26.70*
Uirapuru	7.75	99.18	23.04	0.46	24.11
C.V (%)	2.99	1.44	10.30	11.75	4.80

*significantly different (F-test, $P < 0.01$)

Table II. Yield of IAPAR-81 and Uirapuru cultivars as a result of two stage of N topdressing at plant growth (V4 and R6), UDESC, LAGES, SC, season 2006/2007

Cultivars	Stage 1 (V4)	Stage 2 (R6)
IAPAR - 81	2176,20 a A	2051,49 a A
UIRAPURU	1483,19 b B	2162,42 a A

Means followed by distinct letter (uppercase) within each column and letter (lowercase) within each rows are significantly different by F test ($P < 0.05$)

genetic diversity of protein and micronutrient concentrations, associated with phytate concentration in dry beans and in other legumes. Moreover, regarding the optimal level of these nutrients in the grain, there is little research at the molecular level. Recently, a significant correlation was identified in rice grain between phytate and inorganic P, Fe, Zn, Cu and Mn and the gene (s) responsible for these compounds was not located on the same chromosomal regions, suggesting that they were genetically different (Stangoulis *et al.*, 2007). Additionally, exploring the genetic and control mechanisms of protein accumulation in seeds will be interesting for breeding programs and to improve protein nutrition and amino acids concentrations in populations that consume the dry common bean.

CONCLUSION

The N topdressing application changed the cooking time; but it was dependent on the genotype, the plant growth stage and N applied. Short cooking time was associated with higher hydration time, total proteins, phytate and 100 seeds weight and lower hydration capacity. The cv. Uirapuru showed shorter cooking time in both stages with increased N topdressing, but this was more pronounced at R6 stage, with higher yield. It was recommended to topdress cv. Uirapuru at a later stage.

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