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CROP BREEDING AND
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Root distribution in common bean populations used in breeding programs

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ABSTRACT - *This study aimed to assess the root distribution of mutant common bean populations along the profile and to select bean genotypes with higher metric values for the trait root distribution, with a view to their inclusion in a bean breeding program. In this field study the genotypes IPR Uirapuru, IPR Chopim, Pérola and IAPAR 81 were evaluated and three doses (0, 100 and 200 Gy) of mutagen gamma rays (Co⁶⁰), nested in the depths of the soil profile. A completely randomized design was used with two replications. The trait root distribution was measured in the presence (1) and absence (0) of roots. The experiment was arranged in a mixed factorial design where the factors genotypes and doses were crossed and depth was nested within the levels of these factors, and the regressions showed significant differences of the genotypes. Analysis of variance revealed an interaction of genotype x dose, nested in the factor depth of the soil profile, and the regressions showed significant differences among the genotypes. The most promising segregating populations were those derived from the cultivars IPR Uirapuru and Pérola, since with increasing mutagen doses their root number increased significantly.*

Key words: *Phaseolus vulgaris* L., generalized linear models, Bayesian analysis, mutant populations, abiotic stresses.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a staple food in Brazil, and an important protein source. Apart from the value as food, the economic importance of this crop is also considerable. According to the survey CONAB (2008), this grain is grown on about 3,894.48 million ha in the 2008/09 growing season, with a yield of 3,500.16 million tons. Although these estimates are impressively high, the values were affected by changes over the growing seasons, both in terms of planted area as well as yield. These changes were caused primarily by abiotic factors, such as cold, drought and low-fertility soils, as observed in the highlands of Santa Catarina.

Therefore, the breeding of common bean genotypes that are more adapted to abiotic adversities can reduce these fluctuations in yields. Different factors may affect the development and hamper common bean cultivation, such as: *i)* low soil phosphorus (P), aggravated by the fact that soil correction with P fertilization is not a viable option for many producers in developing countries (Beebe et al. 2006), *ii)* high soil aluminum, where a significant increase in bean yields can be achieved by breeding Al-tolerant genotypes

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(Rao 2001) and *iii)* the reduction in water availability is worldwide one of the most impacting abiotic stresses on major agricultural crops (Rodriguez-Uribe and O'Connell 2006). Therefore, many breeding programs have focused on root traits to increase yields and reduce the expenses of producers (Henry et al. 2008).

Root distribution can be defined as the presence of roots in a particular gradient (Lynch 1995). Common bean genotypes with a well-developed root distribution can enable plants to survive periods of persistent water stress and compete effectively for essential nutrients for their development.

The use of induced mutations is a useful process in the area of plant breeding. The main advantage is that the mutant genotype can be altered slightly, unlike procedures involving crosses of genetically distinct varieties (Coimbra et al. 2004). The identification of segregating common bean populations with great root distribution is therefore important for studies of genetic variation, function, regulation and gene action, allowing the introduction of new technologies (Sreenivasulu et al. 2007) for the incorporation of genes in superior cultivars by classical methods of plant breeding (eg, by backcrossing), or genetic transformation (Kahl and Lavi 2001).

The response variables in experiments are often ratios, especially in agriculture. More than a dozen published papers describe the different root traits (morphology, topology, etc.) only qualitatively. The reason is, that the variability between measurements is excessively high, so a comparison of the treatments by analysis of variance and consequently in comparison tests of means is hampered. An appropriate analysis of this kind of experiment involves essentially the theory of generalized linear models (McCullagh and Nelder 1989). The root distribution can be handled advantageously by means of Bayesian methods, which allow probabilistic relations of the parameters and the incorporation of hyperparameters into the model by hierarchical *a priori* distributions (Giannotti et al. 2006).

In this context, the purpose of this study was to assess the root distribution along the profile between mutant populations (M_3) based on the Bayesian approach and select common bean genotypes with higher metric values for the trait, with a view to using them in the breeding program of the Instituto de Melhoramento e Genética Molecular (IMEGEM) of the Universidade do Estado de Santa Catarina (UDESC).

MATERIAL AND METHODS

Mutant populations

Four common bean genotypes, two of the commercial group carioca (Pérola and IAPAR 81) and two of the black group (IPR Chopim and IPR Uirapuru) were irradiated in 2006 at the Oncology Center at the Universidade Federal de Pelotas (UFPel). Approximately 2000 seeds of each bean genotype were treated with physical mutagen ^{60}Co gamma rays, resulting in the fixed M_1 generation. The total irradiated and absorbed doses 0, 100, 200 and 400 G_y (*Gray*) individually, for each treatment of the physical mutagen used in this study.

The seeds of the M_1 generation were sown in the field in the 2006/07 growing season, where each treatment consisted of an experimental unit of 20 rows (10m x 0.5 m). The highest dose (400 Gy) was lethal to the embryo. The rows were bulk harvested simply to advance to M_2. The seeds of the progenies (M_2) were sown in the field in the 2007/08 growing season and bulk harvested again to proceed to the M_3 generation.

Field experiment

During the 2008/09 growing season, 12 mutant common bean populations (M_3) were evaluated in a completely randomized, mixed factorial design with two replications, where genotypes and doses (mutagen employed - gamma rays - Co^{60}) are crossed and the depth factor is nested in genotypes and doses. Each experimental unit consisted of four 4-m rows spaced 0.5 m apart, representing a measurable area of 3.6 m². The final plant density was 200,000 plants per hectare (15 plants per meter).

Leaf Area Index

The leaf area index was determined indirectly, by the device Accupar LP – 80 (Decagon Devices, Inc.). The basic principle of operation of the equipment is to include variables such as light transmission and penetration and the structural traits of the canopy (leaf angle and distribution). The field measurements consisted of four reference readings over the canopy and nine readings below the canopy (ground level) for each experimental unit.

Root distribution

The root system of common bean was assessed by the profile method described by Bohm (1979). At

flowering of the genotype, profiles were opened perpendicular to the sowing direction, 5 cm away from the plants and the roots were exposed with a long sharp tool. Then a rectangle (width 0.5 m, height 0.3 m) was laid on the profile and subdivided into small squares (sides 0.05 m), to photograph the plant root distribution. The 7.2 Mp digital camera was placed at a standard distance of 60 cm from the grid. Based on the pictures, root distribution was determined according to presence (1) and absence (0) of roots at depths of 10, 20 and 30 cm.

Statistical analysis

The theory of classical linear models generally assumes that the errors are normally distributed. In the area of agronomy, the response variable often has normal distribution, e.g., root distribution measured by presence and absence. An alternative is therefore to model the response variable directly, as described by the authors Nelder and Wedderburn (1972). The basic idea is to estimate the parameters of a linear model using the maximum likelihood method based on data distribution.

The following statistical model was used in the experiment:

$y_{ij} = \mu + g_i + D_j + gD_{ij} + g(d)_k + D(d)_l + gD(d)_m + e_{ijklm}$

where, μ: effect of the overall mean; g_i: effect of the ith level of factor G (genotype) ; D_j: effect of the jth level of factor D (doses) gD_{ij}: effect of interaction between the factors studied GxD; $g(d)_k$: effect of the kth level of factor G nested in d (depth); $D(d)_l$: effect of the lth level of factor D nested in d; $gD(d)_m$: effect of the mth level of GxD interaction nested with factor d; e_{ijklm}: effect of error.

The computational part of this article, i.e., the true means (MV) and the transformed means (MT), variances and credibility intervals were calculated using software package SAS 9.1.3 (SAS Institute Inc. 2007). The main feature is that it contains a broad class of models with several distributions available, so the sample of the joint posterior distribution can be constructed with the most appropriate specification. Bayesian analysis by generalized linear models was performed based on the *BAYES* statement in the PROC GENMOD procedure of SAS. In the Bayesian analysis, the model parameters are treated as random variables, and conclusions about the parameters are based on the *a posteriori* distributions of these parameters. The *a posteriori* distribution is obtained by Bayes´ theorem. In the

analysis, a chain of 55,000 values was generated for each estimate, to be specifically matched to the trait root distribution. The first 5,000 values were discarded (*burn-in* of 5,000), and from the other 50,000 values every 10th was selected (*thin* = 10), to ensure the independence of the sample. The convergence rate of the Gibbs sampler was tested graphically by both the trace and the *a posteriori* density, and the convergence diagnostics of Geweke, Raftery and Lewis, available in SAS software 9.1.3.

RESULTS AND DISCUSSION

The results of analysis of variance by the generalized linear model showed significant effects at 5% probability for all sources of variation evaluated in relation to the variable root distribution (Table 1). The results show differential responses of the genotypes regarding the effect of increasing concentrations of the mutagen gamma rays (Co^{60}) at different depths, due to the significant interaction between these factors, which implies in the need for decomposition. Therefore, the genotypes described as possibly tolerant to abiotic stresses were those with the comparatively highest values at each depth for the variable root distribution, even if their means were not high, mainly in the layer 21 - 30 cm. These populations were chosen due to the greater chance of containing genes controlling the cell capacity to maintain water and nutrient absorption under water stress and for their greater nutrient uptake capacity.

For the variable root distribution, with the application of the equations of logistic regression, the parameters were obtained to the second polynomial degree, represented by the values of the chi-square test

Table 1. Analysis of deviance (ANODEV) considering the nested or sequential model and its respective corresponding numbers of degree of freedom (DF), deviance, residual Chi-square (ChiSq) for the trait root distribution at different depths (d) in M_3 mutant common bean populations

Source	DF	Deviance	ChiSq	Pr >ChiSq
Intercept	-	13.808	-	-
Dose (D)	2	13.673	134.57	0.0001
Genotype (G)	3	13.656	17.47	0.0006
D*G	6	13.570	86.15	0.0001
D(d)	6	6.982	6.588.04	0.0001
G(d)	6	6.965	16.31	0.0122
d(D*G)	12	6.794	171.41	0.0001

and the respective significance of the polynomial (Table 2). Analyzing genotype IAPAR 81 at a depth of 10 cm, and IPR Chopim at 30 cm, it can be seen that the maximum point of the doses of mutagen gamma rays (Co[60]) ranged from 52 to 157 Gy, demonstrating that the significant increase in the trait root distribution often occurred at lower rates in the first 10 inches of soil (Figure 1). This fact is relevant in terms of breeding, since if a program is designed to create genotypes recommended for high-input agriculture, the lowest doses of mutagen gamma rays can result in a higher probability of selection of genotypes with a greater number of roots at the soil surface. On the other hand, if the objective of the breeding program is the selection of genotypes adapted to the conditions of low-input agriculture, as given on the small bean-producing farms in Santa Catarina, the highest doses of the mutagenic agent would probably increase the frequency of mutants with greater root distribution throughout the soil profile.

Table 3 presents the summary *a posteriori* for each parameter obtained by both the Bayesian and the frequentist analysis. Since a non-informative *posteriori* distribution was used for the interaction (genotype x dose) effect nested in depth, and because the interaction between the factors indicates a significant effect, both the mean and standard deviation of the *a posteriori* distribution were very similar for all estimates obtained by maximum likelihood, comparatively. The fitting of *a posteriori* distribution is much more difficult and interesting in situations where there is no objective theoretical basis for the fitting. Also according to this author in these situations the *a posteriori* distribution represents simply the expectation of the researcher. The *a posteriori* distribution (Table 3) estimated by the Bayesian method can be interpreted considering that this distribution represents an approximation of the true probability distribution of the parameters, as observed in the collected data. The data of Table 3 also show that

Table 2. Results of the analysis of variance for the trait root distribution in mutant M_3 common bean genotypes with the decomposition of the variation attributable to the mutagen dose (Co[60] gamma rays), for each common bean genotype at the studied depths, in the linear and quadratic polynomial component

| Genotype	Depth (cm)	Component	DF	ChiSq	Pr>ChiSq
IAPAR 81	10	Linear	1	46.69	0.0001
IAPAR 81	10	Quadratic	1	37.15	0.0001
IAPAR 81	20	Linear	1	106.43	0.0001
IAPAR 81	20	Quadratic	1	7.36	0.0001
IAPAR 81	30	Linear	1	17.90	0.0001
IAPAR 81	30	Quadratic	1	21.71	0.0001
IPR Chopim	10	Linear	1	8.95	0.0001
IPR Chopim	10	Quadratic	1	33.22	0.0001
IPR Chopim	20	Linear	1	70.32	0.0001
IPR Chopim	20	Quadratic	1	49.01	0.0001
IPR Chopim	30	Linear	1	30.89	0.0001
IPR Chopim	30	Quadratic	1	16.03	0.0001
IPR Uirapuru	10	Linear	1	21.81	0.0001
IPR Uirapuru	10	Quadratic	1	32.62	0.0001
IPR Uirapuru	20	Linear	1	43.16	0.0001
IPR Uirapuru	20	Quadratic	1	36.42	0.0001
IPR Uirapuru	30	Linear	1	30.89	0.0001
IPR Uirapuru	30	Quadratic	1	16.03	0.0001
Perola	10	Linear	1	21.81	0.0001
Perola	10	Quadratic	1	32.62	0.0001
Perola	20	Linear	1	43.16	0.0001
Perola	20	Quadratic	1	36.42	0.0001
Perola	30	Linear	1	8.84	0.0001
Perola	30	Quadratic	1	14.14	0.0001
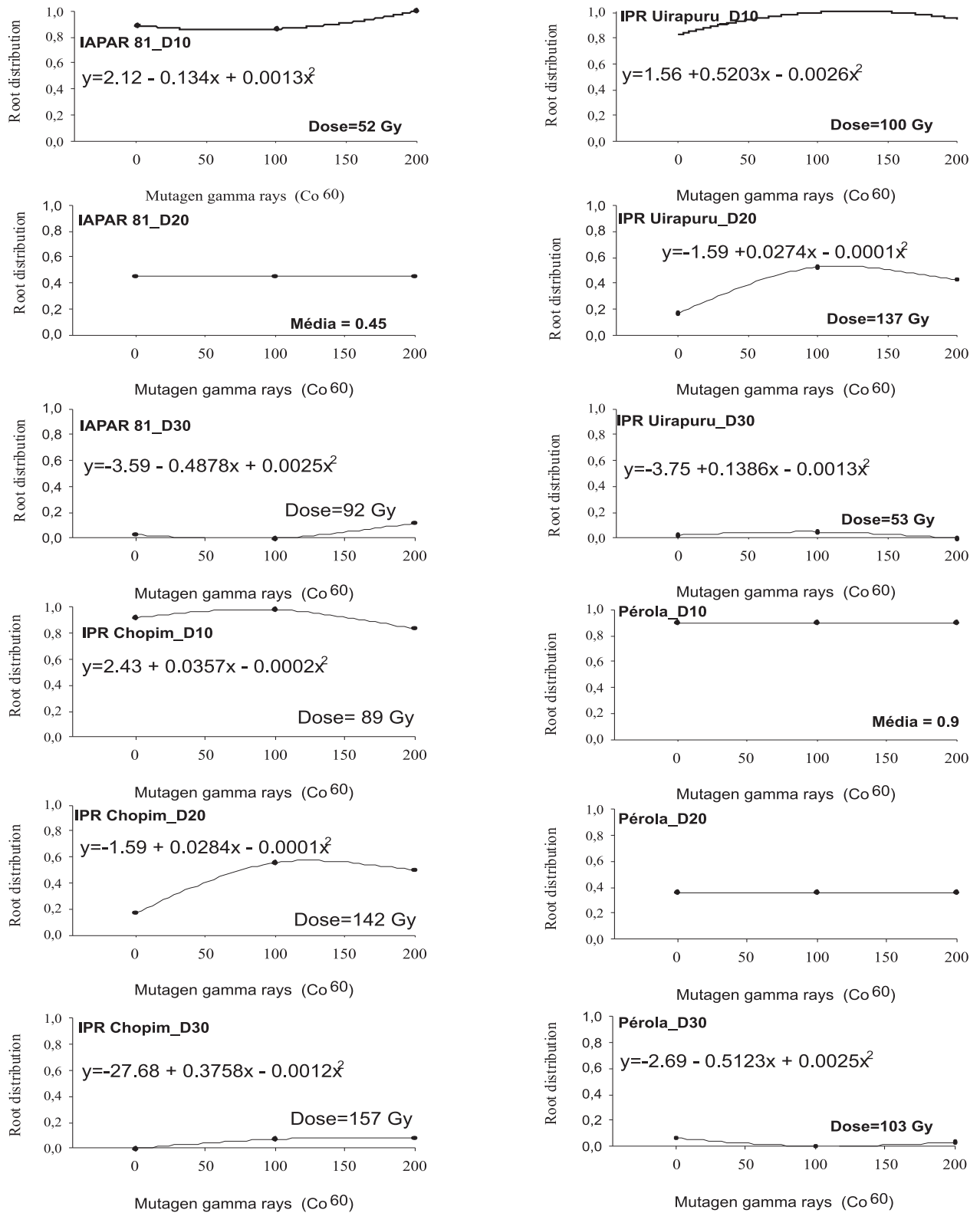
Figure 1. Fitting of the logistic linear model for the trait root distribution

Table 3 . Observed means (μO), transformed means (μT) (ρ= ln (μ/1-μ)) and *a posteriori* means (μP) for the trait root distribution in M$_3$ common bean populations

Genotype	Dose	Depth								
		0-10			11-20			21-30		
		μO	μT	μP	μO	μT	μP	μO	μT	μP
IAPAR 81	0	0.892	2.115	2.134	0.273	-0.979	-0.986	0.027	-3.588	-3.656
	100	0.863	1.836	1.850	0.375	-0.511	-0.509	0.000	-	NC
	200	1.000	-	NC*	0.697	0.833	0.836	0.116	-2.035	-2.042
IPR Chopim	0	0.919	2.432	2.451	0.169	-1.591	-1.603	0.000	-	NC
	100	0.982	4.019	4.095	0.556	0.225	0.224	0.071	-2.578	-2.595
	200	0.837	1.634	1.643	0.500	0.000	0.002	0.080	-2.442	-2.461
IPR Uirapuru	0	0.827	1.564	1.576	0.169	-1.591	-1.600	0.023	-3.746	-3.822
	100	1.000	-	NC	0.525	0.100	0.101	0.050	-2.944	-3.006
	200	0.950	2.944	2.975	0.425	-0.302	-0.306	0.000	-	NC
Pérola	0	0.818	1.504	1.518	0.273	-0.981	-0.987	0.064	-2.689	-2.719
	100	0.911	2.327	2.343	0.333	-0.693	-0.696	0.000	-	NC
	200	0.980	3.892	3.974	0.480	-0.080	-0.081	0.030	-3.476	-3.533

* NC= did not converge

the two most promising mutant populations for the trait root distribution were IPR Uirapuru (black beans) and Pérola (carioca grain), both exposed to a dose of 100 Gy. The average root distribution of these two populations was about 60% of the grid squares, i.e., in more than 36 small squares the roots were distributed along the profile. This result corroborates the logistic regression equation which indicated that lower mutagen doses were more efficient to increase the trait significantly.

Tropical soils are characterized by a high weathering degree and low levels of available P in the a plant-available form, located mainly in subsurface layers, decreasing linearly with increasing soil depth (Bonser et al. 1996). Besides, due to the low mobility in the soil, the absorption of genotypes with a greater root profile is more efficient. However, although common bean responds well to phosphate fertilization, this practice is rare because of the low income of farmers (Horst et al. 2001). Moreover, when applied to the soil it can be fixed in forms not available to plants, e.g., as iron and aluminum oxides (Nielsem et al. 1999). The mutant populations of genotypes IPR Uirapuru and Pérola at 100 Gy may therefore represent an important source of variability in relation to root distribution. Besides, according to Lynch (2007) one of the key points for another revolution in agriculture is related to the study of root traits. Genotypes with greater root distribution will be better adapted to infertile soils and

will significantly improve yields and the sustainability of the agronomic ecosystem, besides reducing the environmental impacts.

Root traits are extremely important because they determine the volume of exploited soil and, consequently, nutrient acquisition, and can thereby contribute to a better adaptation of the genotype to the growth environment. The fact that the roots of most mutant common bean populations evaluated were found in the layer 21-30 cm indicates that plant breeding can be successful in selecting plants with root systems able to acquire immobile nutrients such as phosphorus, for example. However, these traits are usually controlled by many genes of small effect on the phenotype (Falconer and Mackay 1996, Lynch 2007), which can complicate the selection due to the strong effect of environment on the trait.

The estimated Pearson correlation coefficients between the traits root distribution and leaf area index (LAI) showed differences in the behavior of the mutant genotypes (Table 4). According to the results, there was a negative correlation between the above traits for genotypes IPR Uirapuru (layers 0-10 and 11-20 cm) and Pérola (layers 11-20 and 21-30 cm). Conversely, a significant positive correlation was observed for genotype IPR Chopim in the 11-20 cm layer. No significant correlation was observed for genotype IAPAR 81, i.e., independent of depth, leaf area is not related to the root distribution.

Table 4. Pearson´s correlation coefficient between the traits root distribution and leaf area index (LAI) separately for each genotype factor, nested in depth

Leaf area per genotype (LAI)	Depth		
	0-10	**11-20**	**21-30**
IAPAR 81	0.16[ns]	0.12[ns]	-0.06[ns]
IPR Chopim	0.08[ns]	0.24[*]	-0.07[ns]
IPR Uirapuru	-0.18[*]	-0.37[*]	0.16[ns]
Pérola	0.05[ns]	-0.30[*]	-0.18[*]

[ns] = non- significant and [*] significant at 5% Pr>|r| H_0=0

In general, common bean has two main mechanisms for adaptation to drought: *i)* stomatal control (O'Toole et al. 1997) and *ii)* development of root system (Kuruvadi and Aguilera 1990). According to Subbarao et al. (1995), this feature of adaptation to water stress favors the development of the root system, rather than an increase of the shoot, under water stress.

In the last decade, there has been significant progress in the understanding of the basic mechanisms for tolerance to acid soils, as well as advance in the development of cultivars better adapted to these environments (Kochian et al. 2004). This breakthrough was possible due to breeding programs focused on the root traits, associating them with a better adaptation of the genotype to the cultivation environment, and consequently, higher grain yield. However, although plant breeding has contributed to higher yields under low fertility and despite being known for at least 120 years (Bates and Lynch 2001), the potential of this technology is not widely recognized. In classical plant breeding, some obstacles must be overcome to increase the efficiency of nutrient acquisition, which is a promising target, for example: *1)* the heritability of traits that influence nutrient use efficiency is low, *2)* the efficiency in nutrient use is indirectly associated with

the creation and selection of highly productive genotypes, *3)* root traits related to nutrient use efficiency may be subject to neutral or negative selection under high fertility experimental conditions and, *4)* root traits related to nutrient use efficiency have very rarely been used as selection criteria, and may represent an untapped reservoir of genes.

It was therefore concluded that: *i)* the dose of 100 Gy increases the genetic variability of common bean to the trait root distribution significantly to a depth of 20 cm, *ii)* there is genetic variability for the trait distribution in common bean root when subjected to the mutagen effect of gamma rays (Co[60]), *iii)* in the mutant populations (M₃) with the most promising segregating common bean plants, derived from the cultivars IPR Uirapuru and Pérola, a significant increase in the number of roots was observed with increasing mutagen doses and *iv)* the genetic variability of the genotypes with black grain (IPR Uirapuru and IPR Chopim) at the depth of 11-20 cm was greater than of the genotypes with carioca grain (Pérola and IAPAR 81), comparatively.

Distribuição de raiz em populações de feijão utilizadas em programa de melhoramento

RESUMO - *Este trabalho teve como objetivo avaliar a distribuição radicular ao longo do perfil entre populações mutantes e selecionar genótipos de feijão com valores métricos superiores para o caráter, para que possam ser utilizados no programa de melhoramento de feijão. O trabalho foi desenvolvido a campo com os genótipos: IPR Uirapuru, IPR Chopim, Pérola e IAPAR 81 e três doses (0, 100 e 200 Gy) do agente mutagênico raios gama (Co⁶⁰), aninhados as profundidade do perfil do solo. O delineamento experimental foi inteiramente casualizado com duas repetições. A variável mensurada foi distribuição radicular quantificada em presença (1) e ausência (0). A análise de variância revelou efeito de interação genótipo x dose, dentro de profundidade, e as regressões estabelecidas mostraram diferenças para os genótipos avaliados. As populações*

segregantes mais promissoras são oriundas das cultivares IPR Uirapuru e Pérola, pois apresentam um aumento significativo no número de raiz com o aumento das doses do agente mutagênico.

Palavras chave: *Phaseolus vulgaris* L., modelos lineares generalizados, análise bayesiana, populações mutantes, estresses abióticos.

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