# Natural selection in four common bean traits

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## ABSTRACT

Three segregating bulk populations derived from the cross between one early (Manteigão Fosco 11) and three normal cycle (Carioca MG, Ouro and Milionário) dry bean cultivars were assessed for natural selection effects. The populations were advanced from $F_2$ to $F_{13}$. A seed sample was kept to represent the generation and the remnant used to obtain the following generation. Populations from each cross and their respective parents were assessed in randomized complete blocks with four replications, in three sowing seasons (February, July and November). The traits growth habit (determinate or indeterminate), weight of 100 grains (g), reaction to *Colletotrichum lindemuthianum* and grain yield were scored. Natural selection effects were observed on grain yield (the increase was 4.8% per generation), growth habit and weight of 100 grains in all crosses. Individuals with indeterminate growth habit and smaller seeds were predominantly kept. The reaction to *C. lindemuthianum* was neutral to natural selection.

**KEY WORDS:** *Phaseolus vulgaris*, natural selection, common bean, segregating population, bulk breeding method.

## INTRODUCTION

The bulk breeding method is widely used in self pollinating plant species. According to this method, the segregating population increase homozygosity to the $F_5$ or $F_6$ generations, with or without small artificial selection pressure. From this point the majority of the loci have reached the homozygous state and families are obtained and submitted to more intense artificial selection.

The natural selection is one of the advantages attributed to this method, i.e., with the advance of generations, only the more adapted individuals remain in the population. The issue that arises is whether natural selection acts in the direction required by the breeders.

Grain yield studies on some species tried to answer this question in the late 1920's (Harlan and Martini, 1938; Soliman and Allard, 1991; Corte, 1999). The longest study has been carried out on rye since 1929 (Allard, 1988; Soliman and Allard, 1991). Results from the assessments made on more than fifty generations show a grain yield gain of approximately 1% per generation. In the common bean crops, studies using mixtures of cultivars to simulate a segregating population (Cardoso and Vieira, 1976) and populations derived from inbred line crosses conducted by the bulk method (Hamblin, 1975, Hamblin 1977) have been carried out as well. The results for grain yield, however, were inconclusive.

The process of natural selection in the advance of common bean segregating populations is questioned under tropical/subtropical conditions with many sowing seasons. In the majority of the bean cropping areas in Brazil, many sowing seasons are used due to the diversity of environmental conditions. Corte (1999) carried six common bean populations with the respective five parents from the $F_2$ generation to the $F_{18}$ generation by the bulk method, in three sowing periods throughout the year. In each generation, the segregating populations and the parents were assessed in replicated experiments. Using the parents as an environmental variation indicator, this author found that the grain yield of the segregating populations increased by 2.4% per generation. This result showed the strong effect of natural selection. It would be important, however, to assess the segregating populations in the different generations, simultaneously, to avoid the effect of environmental variation in the genetic progress estimates and confirm the results obtained so far.

Data on natural selection effects on other traits are available for rye (Allard, 1988, Soliman and Allard,

1991) but no reports have been found on the common bean. Therefore, the present study was carried out to assess the effects of natural selection in common bean grain yield and other traits during the advance of homozygosis in populations by the bulk method.

## MATERIAL AND METHODS

Experiments were carried out in the experimental area of the Department of Biology of the Federal University at Lavras (UFLA) in a dark red distrophic lattosoil. Lavras is located in the southern part of the state of Minas Gerais, Brazil, at an altitude of 910 m. South 21º41´S latitude, and West 45º00´ longitude.

Segregating populations of the following crosses were obtained: Manteigão Fosco x Carioca MG; Manteigão Fosco x Ouro and Manteigão Fosco x Milionário. The Carioca MG cultivar has type II indeterminate growth habit with a 90-day (normal) life cycle, small grains, and a brown-striped cream-colored tegument and carries the Co-2 allele which gives resistance to some *Colletotrichum lindemuthianum* races. EMGOPA 201-Ouro has a type II indeterminate growth habit, normal cycle, small grains, and a yellow tegument and carry the Co-5 allele which gives resistance to some *C. lindemuthianum* races. It will be called Ouro in this paper. The Milionário cultivar has type II indeterminate growth habit, normal cycle, small grains, black tegument and is resistant to some *C. lindemuthianum* races. Manteigão Fosco is a cultivar with Determinate type I growth habit, short cycle (80 days), large grains, and a dull dark brown tegument and is susceptible to *C. lindemuthianum*.

The three populations were advanced by the bulk method in three sowing seasons during the year. A seed sample was stored at the end of each generation in a cold chamber and the remnant used to obtain the following generation. The process was repeated up to the $F_{13}$ generation when the populations were assessed.

The populations from each cross were assessed in a randomized complete block design with four replications, in three sowing seasons (February, July and November). Twelve segregating generations ($F_2$ to $F_{13}$) and the parents were analyzed. The plot consisted of four 5-meter long rows with a sowing density of 16 seeds per meter. The plants were fertilized with 400 kg/ha of the 4-14-8 N, $P_20_5$, $K_20$ formula at sowing and 150 kg/ha of ammonia sulfate in side-dressing, 20 days after emergence. Supplementary sprinkler irrigation was used to prevent water shortage.

The following traits were assessed from a sample of a hundred plants in the two central rows: a)growth habit; b) grain yield; c) weight of 100 grains, obtained by weighing a sample of 100 grains; and, d) the reaction of the $F_2$ to $F_{13}$ populations of the Manteigão x Ouro cross for resistance to *C. lindemuthianum*, race 89. For this analysis a 50-seed sample was removed from each generation and sown in plastic trays containing sterilized soil. The parents were sown on all trays as controls. Seven days after emergence, the plants were spray inoculated with a fungus suspension containing 1.2 x $10^6$ conidia/ml, following the methodology proposed by Mendonça (1996). The reaction of each individual plant was scored seven days after the inoculation, and those without and with symptoms were considered resistant and susceptible, respectively.

The grain yield data from each experiment were submitted to analysis of variance per sowing period, considering all the effects except the experimental error as fixed effects. A joint analysis of variance of the three crosses was then performed. The linear regression coefficient (b) was estimated for the regression of the mean grain yield (dependent variable, y) on the generation number (independent variable, x). The genetic gain was estimated in percent (G%) per generation using the expression $G\% = \frac{b_i}{F_{2i}} x 100$, where: $b_i$ is the linear regression coefficient obtained for the $i^{th}$ population and $F_{2i}$ is the mean of the referred $i^{th}$ population in the $F_2$ generation.

The linear regression coefficient for the weight of 100 grains was also estimated, similarly to that for grain yield. The b estimates of the populations were compared by the t test following the expression presented by Gomez and Gomez (1984).

The segregation for growth habit and reaction to *C. lindemuthianum* was studied using the chi-square test ($\chi^2$) to compare the observed with the expected frequencies in the different inbreeding generations.

## RESULTS AND DISCUSSION

Common beans can be cultivated three times a year in the region, with enormous variation in the environmental conditions. The wet season crop is sown in October-November and is characterized by high temperatures and rainfall. Excessive rainfall is usually a limiting factor at harvesting time, causing in many cases, considerable losses in yield and product quality. The dry season crop is sown in February-March when the temperatures are normally high at sowing and at initial plant development stages but decrease from flowering onwards. In this period, water is the limiting factor because the crop cycle coincides with the end of the rainy period in the region. The winter crop is sown in July-August and, contrary to the dry season crop, is characterized by low temperatures at the beginning of the crop which can delay plant development and lengthen the cycle. In this period, there is almost no rain, and the crop has to be irrigated. These different environmental conditions contributed to the mean grain yield variation from 1,506 kg/ha in the wet season to 2,050 kg/ha in the dry season (Table 1).

The highest and lowest mean yields were obtained from crosses Manteigão Fosco x Carioca MG (1,875 kg/ha) and Manteigão Fosco x Ouro (1,661kg/ha), respectively. A significant season x cross interaction was also observed (Table 1). Genotype x season interaction is common for the common bean in the region (Ramalho et al., 1998; Takeda et al., 1991; Abreu et al., 1990).

The mean yield of the early parent Manteigão Fosco in all experiments was 1,261 kg/ha, while the average performance of the Carioca MG, Ouro and Milionário parents was 2,087 kg/ha. The early cultivar yielded 39.6% lower than the mean of the other parents (Table 1).

**Table 1 -** Grain yield (kg/ha) of the parents and the different crosses in the three growing seasons. Means from the 12 generations ($F_2$ to $F_{13}$).

| Crosses | Sowing period | | | Means |
|---|---|---|---|---|
| | November | February | July | |
| Manteigão Fosco x Carioca MG | 1584 | 1890 | 2218 | 1897 |
| Manteigão Fosco x Ouro | 1365 | 2121 | 1519 | 1668 |
| Manteigão Fosco x Milionário | 1636 | 2121 | 1540 | 1766 |
| Mean | 1528 | 2044 | 1759 | 1777 |
| Parents | | | | |
| Manteigão Fosco | 926 | 1539 | 1319 | 1261 |
| Carioca MG | 1940 | 2066 | 2368 | 2125 |
| Ouro | 1560 | 2489 | 1658 | 1902 |
| Milionário | 1953 | 2545 | 2210 | 2236 |
| Mean | 1595 | 2160 | 1889 | 1882 |
| General Mean | 1561 | 2102 | 1824 | |

The mean yield of the segregating populations in the different generation was different. In spite of the non-significance of the cross x generation interaction, indicating that the generation effect was similar in the different crosses, the linear regression equations were obtained for the individual crosses (Figure 1). In all cases, the estimates of the determination coefficient($R^2$) estimates were high (higher than 82%), indicating that there was a good fit of the data to the regression line. These values are much larger than those reported by Corte (1999) who used a different methodology to assess the effect of natural selection. The linear regression coefficients (b) were positive and different from zero, varying from 58.1 kg/ha to 82.2 kg/ha for the crosses Manteigão Fosco x Carioca MG and Manteigão Fosco x Milionário, respectively.

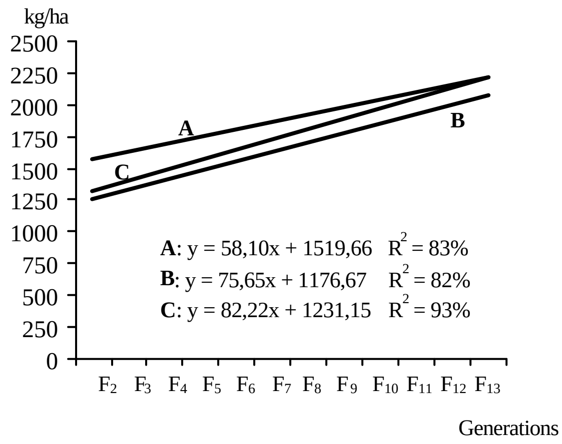


**Figure 1 -** Linear regression equations between the number of self-pollinating generations ($F_2$ to $F_{13}$) and grain yield (kg/ha) for the three crosses. A: Manteigão Fosco x Carioca MG; B: Manteigão Fosco x Ouro and C: Manteigão Fosco x Milionário.

The joint estimate for the three crosses (b = 72.0 kg/ha) indicated an average yield increase of 4.8% per generation compared to the $F_2$ mean. It should be pointed out that Corte (1999) assessed these same populations for each generation and estimated a 2.4% gain per generation. These results confirm many of those reported in the literature, which show the process of natural selection increasing grain yield in populations conducted by the bulk method, especially when the generation advance occurs over a longer period (Allard, 1988; Solimand and Allard, 1991). Some results reported in the literature were not conclusive about the natural selection action, probably due to the smaller number of advanced generations in the populations (Hamblin, 1977; Hamblin and Morton, 1977; Cardoso and Vieira, 1976).

The generation advance was carried out during three growing seasons per year, under distinct environmental conditions. The fluctuations of the environmental conditions made the natural selection effects unforeseeable. The results obtained for grain yield, however, confirm the positive action of natural selection, and this is an additional advantage for the use of the bulk method in common bean breeding. However, it was clear, as already pointed out, that this advantage would be more evident if the populations were advanced in bulk for some extra generations.

The results of the bulk advancement of generations for mean weight of 100 grains were very similar but in the opposite direction of those obtained for grain yield. There was a clear tendency of weight reduction with the advance of the generation in the three crosses. The lowest response estimate for weight of 100 grains was observed in the Manteigão Fosco x Ouro cross (b = -0.60) (Table 2). On average, the linear regression coefficient was –0.79 that is, compared to the mean weight of 100 grains of the $F_2$ generation (29.8g), the decrease in grain weight was 2.8% per generation of self pollinating homozygosis advance. The individual who leaves the greatest number of descendents, that is, greater number of seeds is more adaptable (Darwin, 1996). As there is normally negative correlation between the grain size and the number of grains per plant (Nienhuis and Singh, 1988; Fernandes et al., 1989), a smaller the seed size will condition greater selective advantage of the individual, that will contribute a greater number of descendents. This explains the rapid reduction in seed size with the advance of generations. Allard (1988) obtained a reduction in the weight of 100 grains in rye up to the twentieth generation, when it stabilized.

Table 3 shows the number of plants with determinate or indeterminate growth habit in the three crosses in the $F_2$ to $F_{13}$ segregating generations. Indeterminate growth habit is controlled by a single dominant gene, named Fin (Singh, 1991; Al-Mukhtar, 1981; Ram and Prasad, 1985). The expected frequency in the $F_2$ generation would be ¾ indeterminate (Fin_) and ¼ determinate (finfin). To test the hypothesis of a single gene segregation, a chi-square test of the data to fit this proportion was run for all the crosses. The chi-square tests $(\chi^2)$ were not significant and the formulated single locus hypothesis was not rejected, which is in line with literature.

Considering the single gene genetic control and assuming absence of natural selection effect, the expected segregation ratio in the $F_3$ generation is 5/8 of the plants with indeterminate growth habit (Fin_) and 3/8 determinate (finfin). The chi-square test for this hypothesis was significant for the three crosses. The chi-square tests were also significant for the other selfing generations when the corresponding expected determinate/indeterminate ratios were tested. Therefore the data did not fit the expected single gene segregation model. According to the Mendelian laws, it was expected that the proportion of the two types of plants would tend to equality with generation advance. For example, of the 100 plants assessed in the $F_{13}$ generation, 50.01 and 49.99 are expected to have indeterminate and determinate growth habit, respectively. This did not happen, however,

**Table 2 -** Weight of 100 grains (g) of the three crosses in the 12 assessed generations.

| Generations | Manteigão Fosco x Carioca MG | Manteigão Fosco x Ouro | Manteigão Fosco x Milionário |
|---|---|---|---|
| $F_2$ | 32.67 | 28.97 | 27.83 |
| $F_3$ | 29.01 | 26.53 | 25.06 |
| $F_4$ | 28.74 | 25.49 | 25.81 |
| $F_5$ | 26.17 | 23.69 | 24.46 |
| $F_6$ | 25.32 | 23.86 | 21.90 |
| $F_7$ | 24.40 | 23.47 | 20.16 |
| $F_8$ | 23.36 | 23.39 | 20.10 |
| $F_9$ | 22.95 | 23.31 | 20.06 |
| $F_{10}$ | 22.57 | 21.77 | 19.92 |
| $F_{11}$ | 22.43 | 21.54 | 18.93 |
| $F_{12}$ | 21.68 | 21.27 | 18.79 |
| $F_{13}$ | 21.01 | 21.17 | 18.35 |
| Média | 25.03 | 23.71 | 21.78 |
| b | -0.93a* | -0.60b | -0.83a |
| $R^2$ (%) | 89 | 86 | 88 |

* The b values followed by the same letter in a row did not differ at the 5% level of probability by the t test.

because only one plant from the crosses between Manteigão Fosco x Ouro and Manteigão Fosco x Carioca MG and three plants from the cross between Manteigão Fosco x Milionário had determinate habit. The results showed that the generation advance caused a departure of the observed from the expected proportions of the single gene no selection segregation hypothesis. This indicates the natural selection effect, in the sense of eliminating plants with determinate growth habit. Thus, it can be inferred that the plants with determinate growth habit are less competitive in mixtures.

The genetic resistance to *C. lindemuthianum* race 89 is also controlled by a single dominant gene (Mendonça et al., 1998). Therefore, the same observations made about the growth habit are valid for the expected phenotypic resistant/ susceptible proportions. Contrary to the growth habit trait, the chi-square tests for the observed x expected proportions of resistance/susceptible were not significant in the generations, except in the $F_5$ (Table 4). This indicates that in nearly all the cases, the frequencies observed fitted the expected frequencies based on the hypothesis of a single gene segregation in the successive self pollinating generations. It can be inferred that this trait was neutral for natural selection action, or that the pathogen levels during the generation advancement were not expressive preventing its effect as a selective agent.

The results obtained were very favorable to the bulk method, as it is easy to use and brings great versatility to breeding programs, allowing progeny extraction at any moment This study showed that the bulk method which allows natural selection action is a good alternative for breeders. However, if the objective of the program is to select plants with determinate growth habit or large seeds in populations from crosses of contrasting parents for these traits, plants with determinate habit and/ or large seeds should be selected from the $F_2$ onwards. The obtained population could then be advanced by the bulk method to allow for natural selection on the other traits.

## CONCLUSION

Natural selection effect for grain yield was detected. The increase in grain yield was 4.8% per generation. The effect of natural selection was observed in all cases for growth habit and weight of 100 grains. Individuals with indeterminate growth habit and smaller seeds were selected in the populations.

**Table 3 -** Observed and expected frequencies of plants with determinate (D) and indeterminate (I) growth habit of the crosses: A: (Manteigão Fosco x Carioca MG); B: (Manteigão Fosco x Ouro) and C: (Manteigão Fosco x Milionário) in the 12 assessed generations.

| | | | A | | | B | | | C | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | F.E. | | F.O. | | | F.O. | | | F.O. | | |
| Generation | D | I | D | I | $\chi^2_c$ | D | I | $\chi^2_c$ | D | I | $\chi^2_c$ |
| $F_2$ | 25.00 | 75.00 | 29 | 71 | 0.2933 | 25 | 75 | 0.0000 | 23 | 77 | 0.2133 |
| $F_3$ | 37.50 | 62.50 | 20 | 80 | 13.0667** | 19 | 81 | 14.6027** | 22 | 78 | 10.2507** |
| $F_4$ | 43.75 | 56.25 | 27 | 73 | 11.4006* | 9 | 91 | 49.0692* | 19 | 81 | 24.8914* |
| $F_5$ | 46.88 | 53.12 | 13 | 87 | 46.0937* | 22 | 78 | 39.7731* | 10 | 90 | 54.6180* |
| $F_6$ | 48.44 | 51.56 | 19 | 81 | 34.7023* | 10 | 90 | 59.1629* | 5 | 95 | 75.5549* |
| $F_7$ | 49.22 | 50.78 | 9 | 91 | 64.7207* | 6 | 94 | 74.7369* | 1 | 99 | 93.0284* |
| $F_8$ | 49.61 | 50.39 | 7 | 93 | 72.6279* | 5 | 95 | 79.6069* | 3 | 97 | 86.9040* |
| $F_9$ | 49.80 | 50.19 | 6 | 94 | 76.7563* | 6 | 94 | 76.7639* | 2 | 98 | 91.4141* |
| $F_{10}$ | 49.90 | 50.10 | 5 | 95 | 80.6459* | 1 | 99 | 95.6488* | 1 | 99 | 95.6546* |
| $F_{11}$ | 49.95 | 50.05 | 4 | 96 | 84.4589* | 5 | 95 | 80.8202* | 0 | 100 | 99.8032* |
| $F_{12}$ | 49.98 | 50.02 | 3 | 97 | 88.2848* | 2 | 98 | 92.0832* | 2 | 98 | 92.0832* |
| $F_{13}$ | 49.99 | 50.01 | 1 | 99 | 96.0008* | 1 | 99 | 96.0000* | 3 | 97 | 88.3224* |

** indicates a $\chi^2$ test significant at the 1% level of probability.

**Table 4 -** Observed and expected frequencies of resistant (R) and susceptible (S) plants to *Colletotrichum lindemuthianum* for the cross between Manteigão Fosco x Ouro in the 12 assessed generations.

| | Frequencies | | | | |
|---|---|---|---|---|---|
| | Expected | | Observed | | |
| Generations | R | S | R | S | $\chi^2_c$ |
| $F_2$ | 70.50 | 23.50 | 70 | 24 | 0.0005 |
| $F_3$ | 52.50 | 31.50 | 60 | 24 | 2.8571 |
| $F_4$ | 39.38 | 30.62 | 49 | 21 | 5.3778 |
| $F_5$ | 51.53 | 45.47 | 69 | 28 | 12.6330** |
| $F_6$ | 37.64 | 35.36 | 43 | 30 | 1.5750 |
| $F_7$ | 50.78 | 49.22 | 52 | 48 | 0.0594 |
| $F_8$ | 49.89 | 49.11 | 47 | 52 | 0.3370 |
| $F_9$ | 48.19 | 47.81 | 54 | 42 | 1.4080 |
| $F_{10}$ | 47.09 | 46.91 | 47 | 47 | 0.0004 |
| $F_{11}$ | 45.04 | 44.91 | 41 | 49 | 0.7355 |
| $F_{12}$ | 47.52 | 47.49 | 44 | 51 | 0.5206 |
| $F_{13}$ | 49.02 | 49.14 | 40 | 58 | 3.3110 |

** indicates a $\chi^2$ test significant at the 1% level of probability.

Resistance or susceptible reaction to *C. lindemuthianum* was neutral to natural selection.

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## RESUMO

### Seleção natural em quatro caracteres do feijoeiro

Com o objetivo de verificar a ação da seleção natural em populações segregantes do feijoeiro conduzidas pelo método da população (bulk) foram avaliadas três populações segregantes, provenientes do cruzamento da linhagem precoce Manteigão Fosco 11, com três de ciclo normal, Carioca MG, Ouro e Milionário. As populações foram avançadas de $F_2$ a $F_{13}$. Sempre era guardada uma amostra de sementes para representar a geração e o restante utilizado na obtenção da geração seguinte. As populações de cada cruzamento, juntamente com os respectivos pais, foram avaliadas no delineamento de blocos casualizados com quatro repetições, em três épocas de semeadura: fevereiro, julho e novembro. Foram anotados os seguintes caracteres: hábito de crescimento (determinado ou indeterminado), peso de 100 grãos (g), reação ao *C. lindemuthianum* e produção de grãos. Constatou-se efeito da seleção natural para a produtividade de grãos (ganho de 4,8% por geração), hábito de crescimento e peso de 100 grãos. Foram mantidos predominantemente indivíduos com hábito de crescimento indeterminado e com sementes menores. A reação ao *C. lindemuthianum* mostrou-se neutra à ação da seleção natural.

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