Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

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Selection for new types of dwarf dry bean (Ph. vulgaris): a statistical analysis of productivity and heritability of the total N content

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **72** (1982), n.4, p. 243–252.

Accademia Nazionale dei Lincei

<http://www.bdim.eu/item?id=RLINA_1982_8_72_4_243_0>

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Accademia Nazionale dei Lincei, 1982.

SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Genetica. — *Selection for new types of dwarf dry bean* (Ph. vulgaris): *a statistical analysis of productivity and heritability of the total* N *content* (*). Nota di LUIGI CONTI, presentata (**) dal Socio C. BARIGOZZI.

RIASSUNTO. — Vengono riportati i risultati della selezione operata sulla progenie ottenuta da un incrocio tra « Borlotto nano » (da sgranare, a tenore proteico medio–alto, con buone caratteristiche agronomiche ma non resistente alle comuni virosi) e « Degli Ortolani » (rampicante, mangiatutto, con basso tenore proteico ma resistente ai virus BCMV e BYMV e tollerante a CMV).

Questa selezione ha consentito di ottenere due linee entrambe resistenti a BCMV, una con elevata produzione e l'altra con alto tenore proteico.

Il contenuto proteico, che è il carattere più importante tra quelli studiati, è risultato altamente ereditabile ($h_n^2 = 0{,}70$).

INTRODUCTION

The cultivation of the dry bean (*Ph. vulgaris*) has progressively decreased in Italy in recent years (Allavena, 1979), in spite of its importance as a source of food (Bozzini, 1979).

This situation is the result of economic factors. These depend not only on the market but also on the conditions of the Italian germoplasm which is unable to meet the requirements of both grower and consumer.

The main requirements are dwarfness, precocity and contemporaneity of maturation. Dwarfness is particularly important for facilitating mechanical harvesting.

Other important characteristics are high productivity and resistance to natural events (such as drought and sudden temperature changes) and diseases. Among these viruses must be considered particularly those produced by the viruses BCMV, BYMV and CMV, the latter also being dangerous for Cucurbitaceae.

Protein content exhibiting a balanced amino acid composition is of paramount importance, whenever not associated with antinutritional and toxic factors. Seed colour is also important because the Italian consumer accepts only rather large seeds such as the typical " Borlotto " (pink background with purple variegation) or the white bean.

The dwarf cultivars presently grown in Italy are the " Borlotto lingua di fuoco", "Borlotto nano", "Taylor's horticultural" and the white "Cannellino".

(*) This investigation was supported by C.N.R. (Progetto finalizzato miglioramento genetico – subprogetto leguminose da granella).

(**) Nella seduta del 24 aprile 1982.

All these cultivars, however, are sensitive to viruses. There is thus a clear need to produce new varieties in which the above characteristics are associated.

A prerequisite for undertaking such a task is to know the importance of genetic control of the different characters. In particular, the most important ones, according to preliminary information, are the heritability of productivity and of protein content of the seed.

These two characters are genetically complex, hence the need to estimate the degree of association between them in order to obtain a correlated response to selection.

The genetic variability of the yield and of its components (both qualitative and quantitative) was studied in the segregant population derived from the cross between two varieties of our collection which may meet the needs of national agriculture. The results obtained are discussed with regard to the requirements of selection in view of Italian needs. The results obtained are also discussed in connection with future breeding work.

From the collection of bean cultivars existing at Minoprio, two were selected for the reason given below: " Degli Ortolani " (the greengrocers bean) and the " Borlotto nano " (dwarf Borlotto).

Materials and methods

The two following cultivars were used:

a) " *Degli Ortolani* " (indicated as P_1). This cultivar from the Lake Como area is a green bean climber resistant to viruses BCMV and BYMV and tolerant to CMV, as demonstrated by tests both in the open field and in the greenhouse.

b) " *Borlotto nano* " (dwarf Borlotto, indicated as P_2). This cultivar, having a pink seed colour with purple variegation, is characterized by good productivity of dry seed and medium-high protein content. It has a very high sensitivity to viruses.

The two cultivars were crossed, and F_1 and F_2 were rapidly obtained. Moreover, F_1 was backcrossed with both parental cultivars to obtain $B_1 (F_1 \times P_1)$ and $B_2 (F_1 \times P_2)$. In May 1979 all these generations were utilized and an open field experiment was set up, based on three randomized blocks, comprising 4 plots for P_1, P_2 and F_1, 20 plots for F_2 and 7 for B_1 and B_2.

Each experimental plot comprised 8 plants, the row distance being 2 m and the plant distance 0.50 m.

The soil was prepared according to the usual agronomic techniques. Mulching was used.

For each plant the following characters were considered:

1) flowering date from sowing (in days)
2) harvesting date from sowing (in days)
3) pod number per plant at harvesting

4) seed number per pod
5) weight of 50 seeds (in g)
6) seed length (in mm)
7) seed width (in mm)
8) seed thickness (in mm)
9) yield per plant (in g)
10) total nitrogen content, expressed as percentage transformed into protein content
11) plant size (dwarf, semi-climber, climber).

For each plant grown in the open field a classification into six degrees was made for the following three characters:

1) general aspect of the plant
2) time of flowering
3) resistance to viruses.

On the basis of this classification 600 dwarf F_2 types were selected and sown in the greenhouse. This was appropriately heated, in January 1980, in order to multiply the F_3 seeds to be used for further experiment in the open field in spring-summer 1980.

From this experiment (concluded in autumn 1980) nearly 6000 F_4 plants were obtained (10 from each of the 600 plants of the former generation), and the following evaluated:

a) homogeneity of each plot
b) precocity of flowering
c) precocity of maturation.

Evaluation of both production and protein content was carried out on both control and selected plants.

Following this method the 10 best lines were selected, taking 1 plant per line.

The 10 progenies were reduced to the 5 best by further selection based on characters observed in the open field and on protein content. Having multiplied these in the greenhouse (F_5), sufficient material was harvested for a comparative production test to be performed in 1981.

The design was once again based on 4 complete randomized blocks. In addition to the 5 lines, 4 test cultivars were grown: " Borlotto nano " (dwarf Borlotto), " Taylor's horticultural ", " P_2 " and " P_{71} " (resistant to BCMV) recently obtained by Salamini (Salamini *et al.*, 1978).

The last experiment carried out in 1981 was based on 4 randomized blocks comprising plots of two binary rows of 40 plants each. The distance between binary rows was 70 cm, between plots 1.20 m and between plants in a row 3–5 cm. Data were not collected on all lines because one turned out to be too late, although its virus resistance and tolerance make it quite interesting.

The commercial cultivar " Borlotto nano " showed no uniformity and was thus discarded.

Protein content was measured as total N content, determined by using a Technicon Autoanalyzer. The total N content was transformed into protein content by multiplying the values obtained by 6.25.

STATISTICAL ANALYSIS

1979 Experiment.

For each character the collected data for all generations of the experiment (P_1, P_2, F_1, F_2, B_1, B_2) were submitted to the analysis of variance according to the randomized block scheme. For quantitative genetical analysis the single plant was considered as the experimental unit. Whenever observation on the same plant was repeated, the mean was used.

Since the bean is an autogamous species, both cultivars ("*Borlotto nano*" and "*Degli ortolani*") can be considered as pure lines, with restricted genetic variability. Environmental and genetic variance components were estimated according to the method proposed by Mather (1949).

For determining heritability in the narrow sense the formula

$$h_N^2 = \frac{{}^1/_2\,D}{{}^1/_2\,D + {}^1/_4\,H + E}$$

was utilized, where D is the additive genetic component, H the non-additive genetic component and E the environmental variability.

Some characters are strongly influenced by the vegetative habit of the plant. Therefore for some characters (e.g. the pod number per plant and production of the plant) only the distribution frequency is given.

The association between characters was evaluated on a phenotypical basis, as a simple correlation as indicated by the formula

$$r = \frac{\Sigma\, x_1 \cdot x_2}{\sqrt{\Sigma x_1^2 \cdot \Sigma x_2^2}} \, .$$

1981 Experiment.

Data on yield tests of F_5 lines were submitted to analysis of variance and to the Duncan test in order to evaluate the significance of intervarietal differences.

RESULTS

Variability and heritability of the characters.

The characters considered, observed in F_1, F_2, B_1 and B_2, are given in Table I. One notices, in general, that F_1, which is a climber, shows values closer to the "Degli Ortolani", while F_2 is more or less intermediate. Both backcrosses are closer to the values of the parental cultivar used.

Owing to its particular importance, I wish to emphasize that this behaviour extends also to the N content.

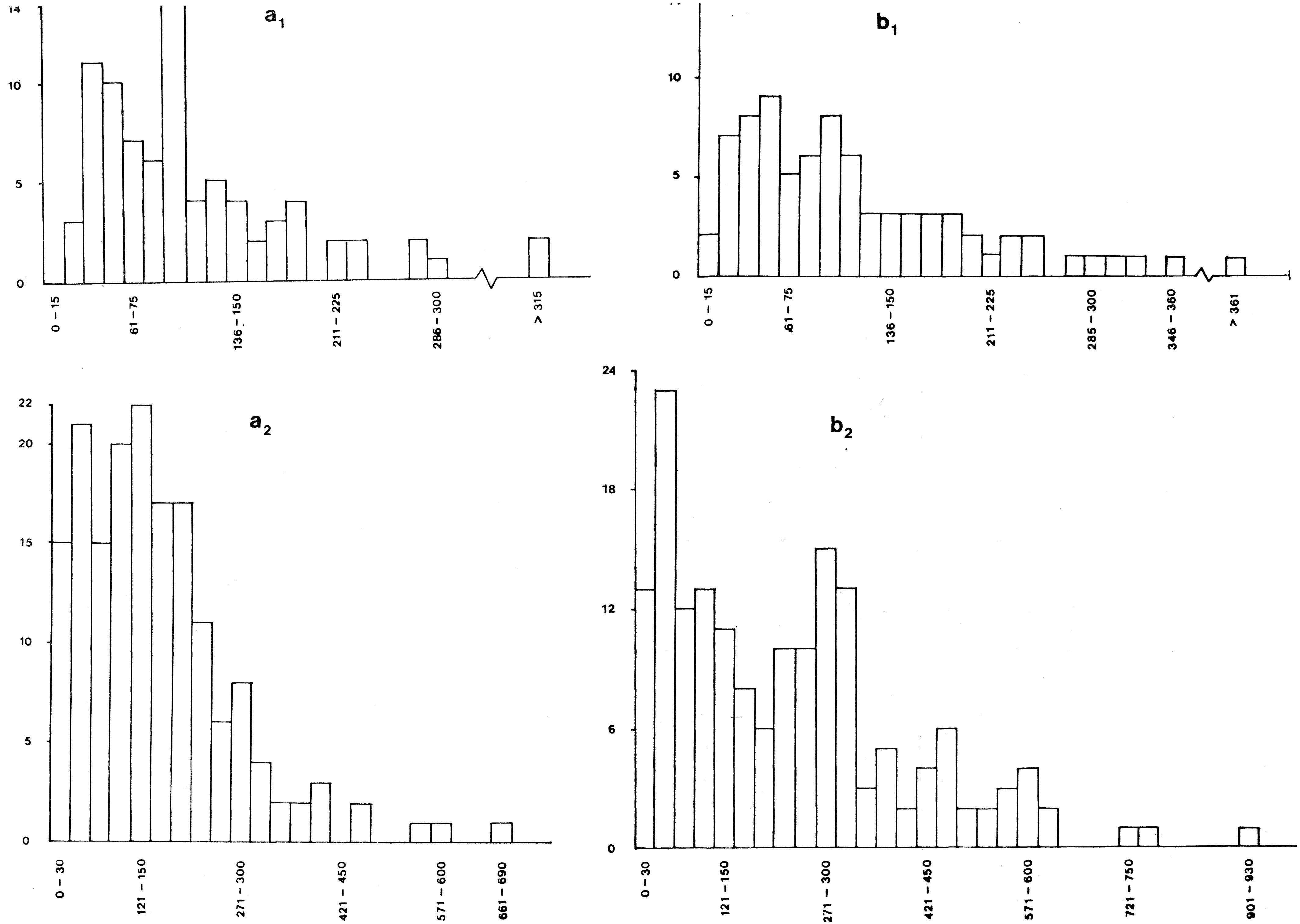

Fig. 1. – *a*) Pod number per plant in F_2: a_1) dwarfs and semidwarfs; a_2) climbers; *b*) Yield per plant in F_2: b_1) dwarfs and semidwarfs; b_2) climbers.

TABLE I.

Characters of Parents (expressed in means) and of F_1, F_2, B_1 *and* B_2.

	Flow. date 1	Harv. date 2	Pod n° per plant 3	Seed n° per pod 4	Weight 50 seeds 5	Seed length 6	Seed width 7	Seed thickness 8	Yield per plant 9	Total nitrogen % 10
DO (1)	51.86	138.22	157.21	5.79	16.22	12.53	6.78	5.76	302.22	20.91
BL (2)	39.93	113.00	38.50	3.58	23.20	13.36	8.57	6.68	62.52	27.09
F_1	46.77	137.96	162.20	4.36	18.52	13.72	7.26	5.97	256.99	24.04
F_2	47.74	131.60	143.23	3.93	18.02	13.11	7.28	5.96	196.24	24.86
B_1 (3)	50.08	236.67	172.85	4.57	17.57	13.16	6.96	5.85	272.66	23.56
B_2 (4)	44.51	124.94	102.03	3.59	20.38	12.82	7.85	6.42	149.56	26.01

(1) Degli Ortolani.
(2) Borlotto nano.
(3) Backcross to Degli Ortolani.
(4) Backcross to Borlotto nano.

COLUMNS

1–2 in days
5–9 in grams
6–7–8 in mm
10 in %

Regarding the plant habit, the dominance of climbing is confirmed, as well as the dominance of variegation and of the bronze colour (Barigozzi and Conti, 1981).

The variances of P_1, P_2 and F_2, which are estimates of the environmental component, are strongly heterogeneous for all characters associated with the vegetative habit (harvesting date, pod number per plant and yield per plant). For this reason the data obtained for climbers and for dwarfs must be kept separate. Date of flowering, seed number per pod, length, width and thickness of the seed and percent of protein content have been submitted to the usual statistical treatment.

The histograms in Fig. 1 represent the following characters:

a) the pod number in F_2 in climbers and in dwarfs, and *b*) the yield per plant in F_2 in climbers and in dwarfs. The considerable heterogeneity depends on the different vegetative habits, which also produce intermediate phenotypes (Evans, 1973).

Table II shows the heritability in the narrow sense (h^2_N) of the characters, the variability of which is not affected by vegetative habit. Negative values of the variance components have been considered as the result of sampling error. Being related to the estimation procedure adopted, this may give this result whenever the value of the components is very small.

TABLE II

Components of variance and of h^2_N.

	Flow. date	Seed n° per pod	Seed length	Seed width	Sed thickness	Total nitrogen %
$^1/_2$ D	56.470	51.390	10.070	24.32	14.860	4.011
$^1/_4$ H	—4.240	24.590	56.370	1.15	—2.880	—1.177
E	14.710	20.250	42.630	12.14	16.400	1.750
h^2_N	0.793	0.534	0.092	0.65	0.475	0.697
% H	—	0.256	0.517	0.03	—	—
% E	0.207	0.210	0.391	0.32	0.525	0.303

Therefore negative values have been considered zero. The six characters considered in Table II allow some conclusions to be drawn: *flowering date* (appearance of the first flower) reveals a high degree of heritability: $h^2_N = 0.79$).

Slightly lower is the heritability of the *seed number per pod* ($h^2_N = 0.53$). The heritability of the protein content (0.697) is higher.

TABLE III

Correlation coefficients.

	Flow. date	Harv. date	Seed n° per pod	Seed length	Seed width	Seed thickness	Total nitrogen %
Flow. date	—	.4526 **	.1835 **	.0511	—.2227 **	—.0833	—.0557
Harv. date	—	—	.3547 **	.1159 *	.0490	—.0073	—.1383 *
Seed n° per pod	—	—	—	.0416	—.1636 **	—.1624 **	—.2500 **
Seed length	—	—	—	—	.2783 **	.2470 **	.1293 *
Seed width	—	—	—	—	—	.4593 **	.0470
Seed thickness	—	—	—	—	—	—	—.0333

For each item: n° of observations 250;
probability level of the hypothesis H_0 (* $P < 0.0.5$; ** $P < 0.01$).

Table III shows the correlation coefficients of all characters, the heritability of which was calculated. In this table harvesting date is also included, although its heritability could not be calculated because of the heterogeneity. Dimensional components (thickness, width and length) are positively correlated. Only thickness and width are negatively correlated with the seed number per pod. This character is positively associated with both the harvesting date and the flowering date.

The correlation value between protein content and seed number per pod is negative ($r = -0.25$ **), while between protein content and seed length it is positive ($r = 0.129$ *).

Selection

Selection in the F_2 generation was essentially made on the basis of vegetative habit, pod colour and seed colour. The other characters were evaluated for selection in F_3 (spring 1980).

This procedure allowed 10 families (F_4) to be selected from a total of 600.

Within the 10 families, the best plants were selected as candidates for the preliminary comparative trials.

Fig. 2 shows the yield of dry seed expressed in grams of the best 10 plants. For comparison, yields of a commercial variety and of both parental lines are also indicated. These data provide preliminary indications concerning the agronomic qualities.

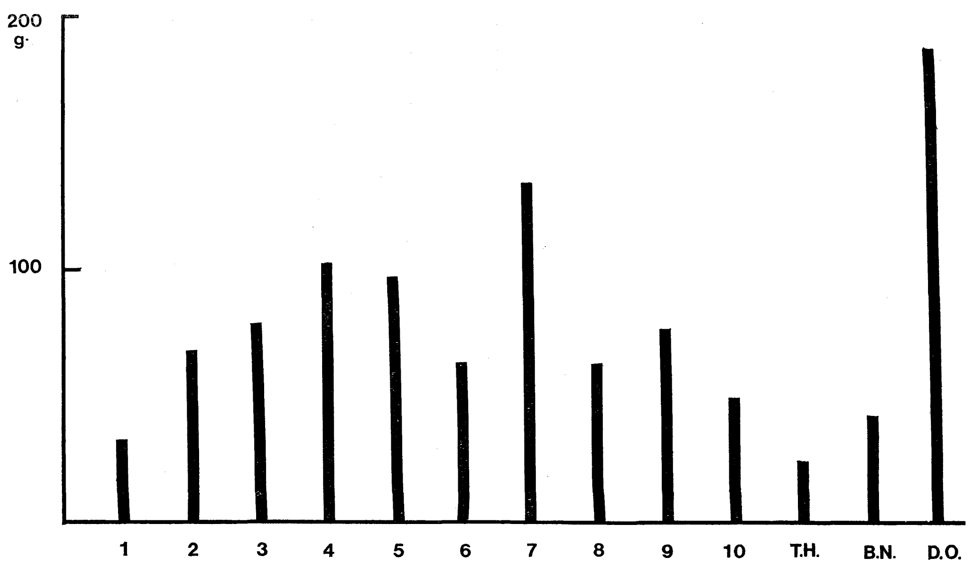

Fig. 2. - Yield per plant of 10 F_4 lines.

The first comparative trial was done in 1981. This comprised the 4 best progenies from the selected material and 4 commercial cultivars. A fifth line will be considered in the future because, in spite of its late maturation, both virus resistance and tolerance were quite high.

The experimental design adopted comprised 4 randomized blocks; the coupled plots consisted of 80 plants. The analysis of variance relative to the average yield revealed the existence of significant differences between lines ($F = 9.11$; $P < 0.01$).

Multiple comparisons were carried out by means of a single Duncan test (Table IV).

At the bottom of the table a continuous line joins the means between which there is no statistical difference.

Cultivars " Taylor's horticultural " (TH) and " P_{71} " failed to show any difference as far as yield is concerned, while there was a significant difference between P_2 and the 4 selected lines. Line 213, moreover, proved significantly different from P_2 and, obviously, from all the rest with a lower yield.

TABLE IV

Duncan test applied to a) the mean yield of the selected lines and of the best cultivars; b) *the mean protein content of the selected lines and of the best cultivars.*

	TH	P_{71}	207	P_2	338	332	213	Standard error of the means
Means *a*	7.26	8.34	11.93	12.64	14.43	15.16	17.48	$s_x = 1.215$

	P_2	213	TH	332	P_{71}	207	338	Standard error of the means
Means *b*	24.57	25.09	25.23	25.55	26.42	26.66	27.26	$s_{\bar{x}} = 0.51$

Probability level $P = 0.05$.

With regard to protein content (derived from the total nitrogen content), analysis of variance proved the existence of variability between the lines ($F = 3.55$; $P < 0.05$).

Line 338, as well as line 207 and cultivar " P_{71} ", did not show any difference in nitrogen content. But, as regards total Nitrogen, line 338 proved significantly different from line 213 and line 332, as well as from the other two cultivars (" P_2 " and " Taylor's horticultural ") (Table IV*b*).

Genetical restistance to viruses will be considered in a subsequent paper.

Discussion

The data described above can be considered under four headings.

1) Fixation of dwarfness. This character proved very easy to fix, since the character is monofactorial. Dwarfness is associated with other favourable traits.

2) Seed colour. Also for this character it was easy to replace the coloration of " Degli Ortolani " with the pink variegated " Borlotto " type, since background colour and variegation are both monofactorial.

3) Total nitrogen heritability (which can be trasformed into protein content) was very high, hence the character is transmissible to subsequent generations. Other authors (Leleji *et al.*, 1972 and Kelly and Bliss, 1975) have obtained lower values. This can be explained by the genetic differences of the material used.

4) At the end of the 4th selection year (1980–81) we obtained two lines, one characterized for high total N content and the other for high yield.

Utilizing the two best lines, one the best for yield and the other the best for protein content, it will be possible to select for high sulphurated aminoacid content.

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