RECURRENT INTERCROSSING COUPLED WITH NEUTRON IRRADIATION AS A MEANS OF INCREASING GENETIC VARIABILITY IN NAVY BEANS (PHASEOLUS VULGARIS L.)

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ABSTRACT

RECURRENT INTERCROSSING COUPLED WITH NEUTRON IRRADIATION AS A MEANS OF INCREASING GENETIC VARIABILITY IN NAVY BEANS (PHASEOLUS VULGARIS L.)

By Antonio M. Pinchinat

The hypothesis has been proposed that a combination of irradiation and recurrent intercrossing would generate more variability in a complex trait than would either method alone. To test it, 10 homozygous lines of navy beans were crossed in all possible combinations. Each of the resulting 45 first cycle intercrosses was simultaneously selfed for seed increase and out-crossed to 4 unrelated hybrids. These second cycle intercrosses were also selfed afterwards to produce enough seed for the subsequent treatments. Then each group, including the original parents, was split into two lots: one to be neutron-irradiated and the other kept as control. They were grown in bulk for two years and as single plant progenies the third year.

The statistical analysis of the recorded data indicated that the combination of recurrent intercrossing and irradiation increased both variances and coefficients of variability of yield and its components more than did either alone. Intercrossing alone rated better than the radiation treatment per se. But only a few isolated significant changes in treatment means emerged. Apparently

the neutron dosage applied was not high enough to produce drastic effects.

The second cycle intercrossing alone or with radiation excelled the first cycle intercrossing in increasing mean seed weight (Z) means, and without radiation, in increasing seed number per pod (Y) variances. Their effects otherwise were similar.

Bean yield per plant (W) was seen to be strongly correlated with number of pods (X), average seed number per pod (Y) and mean seed weight (Z), as postulated by the geometric concept of yield. Component X showed negative correlations with both Y and Z, which in turn were positively correlated with each other. The nature of these correlations does not preclude the existence of different gene systems governing the components. Correlation patterns did not sensibly vary with treatments.

Hybridization alone or with radiation increased regression values of yield on its components more consistently than did irradiation alone. Combined with radiation, hybridization made yield more dependent on number of seed per pod and hence would facilitate yield improvement through manipulation of the components. This combination induced enough variability to permit isolation of transgressive recombinant lines for further selection.

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TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

Genetic studies on beans (Phaseolus vulgaris L.) have been relatively limited even though the plant has agronomic features advantageous to such investigations. Almost wholly self-pollinated, the species possesses an intermediate haploid chromosome number (n=11) and a well defined set of fitness components (2,5,9). Its life cycle is comparatively short (85-110 days) and each plant produces ample quantities of seed.

The first commercial artificially induced navy bean mutant, Sanilac (12,13), was developed at Michigan State University through X-irradiation and subsequent backcrossing. Irradiation thus offers a means of inducing variability in an elite population without degrading it, as is often the case with wide outcrossings, by the introduction of disparate germ plasm.

Theoretically, recurrent cycles of intercrossing are expected to result in a gradual reduction in size of initially intact gene blocks. The present experiment was aimed primarily at enhancing genic recombination in a self-pollinated species by combining neutron irradiation and recurrent intercrossing. The effectiveness of this technique might be reflected in measurable changes in some fitness

components or yield factors, and permit the isolation of lines improved in one or more performance characteristics.

REVIEW OF LITERATURE

Hybridization

The success of hybridization as a breeding method rests on the release of variability in a population through gene recombination. Hagedoorn (18) remarks that the variability caused by crossing even closely related subspecies may be enormous. Most published reports on genetic recombination have dealt with cross-fertilizing species. Dobzhansky and his group (10) reached the conclusion that genetic variance of viability in natural populations of Drosophila prosaltans scarcely depends on newly arisen mutants. Most of the variance traced back to recombination within the accumulated store of genetic variants. A single generation of intercrossing between sets of second chromosomes produced an unexpectedly large amount of genetic variability as compared to the wild population. Similar results were obtained with several other Drosophila species (41,42,43). Milkman (30) believes that recombination in the chromosomes contributed by randomly chosen pairs of parents in Drosphila melanogaster could essentially generate the full range of phenotypic variation characterizing the population.

Crossing early- and late-heading timothy plants enabled Van Dijk (47) to enlarge the diversity of forms of this grass. In a long-term study of composite-crosses with barley, Harlan (23) demonstrated that steady improvement in

yield and other agronomic traits can be made in later generations, presumably through increased recombination. Donnelly and Clark (11) found inter- as well as intra-specific crosses in Vicia capable of generating potentially useful variants for plant and seed types as early as the F_2 generation.

Hybridization and the Breakage of Linkage Blocks

Mitchell (31) attempted to:

(1) Break down initial gene sequences in X-chromosomes of Drosphila melanogaster

(2) Accumulate new linkages and

(3) Promote continued change of the new linkages through recombination in successive generations.

The mating system he devised allowed genotypic variability to depend mainly on the rate of recombination of sex-linked genes.

He found that:

(a) the only major source of increased variability in the populations was through accumulation of linkages of genese derived from the X-chromosomes of the two parental inbred lines

(b) recombination raised the frequency of non-fecund pairs and lowered the mean survival of the offspring of fecund pairs

(c) accumulation of linkages in the X-chromosomes increased the variance of developmental rate, but did not create any significant change in means.

Multiple inversion heterozygosity, he suggested, resulted in an accelerated rate of release of potential variability in the structurally homozygous portion of the genome.

If increased recombination were to reduce variance where linkages are predominantly in coupling phase, the reverse should take place in the repulsion phase of linkage, according to Adams (1). He also reasons that successive intercrossings should progressively disrupt homeostatic linkage segments in self-pollinated species. To support his arguments, he drew some figures from papers by Hanson (19,20,21). The reported theoretical considerations indicated that four generations of recurrent intercrossing in plants would cut intact segment lengths from 1/2 to 1/8 of the original chromosome lengths. And, the calculated average recombination frequencies per chromosome, under these conditions, would be at least 38%.

Intercrossing, however, does not always increase recombination. Results obtained by Stephens (46) from certain crosses in cotton bear on this point. In an interspecific cross, crossing-over in the non-homologous terminal region differentiating the two species dropped from 20 to 8%. Yet this drop was compensated for by slight, but general, increases in other marked segments of the chromosome.

Effects of Ionizing Radiations on the Genetic Material

That ionizing radiations can cause hereditary changes in living matter has been known since 1927. Their mutagenic action was demonstrated by Muller (33), Stadler (44,45), and Goodspeed and Olson (16) among others. Sparrow and his associates (40) have compiled a bibliography on the effects of ionizing radiations on plants, for the period 1896-1955. At the latter date, Bacq and Alexander (6) gathered and published the fundamentals of radiobiology. Sparrow (39) in 1961 surveyed the current types of ionizing radiation and their cytogenetic effects. Apart from so-called point mutations, high energy radiations induce a wide array of chromosome aberrations. Breakage and translocation of fragments of chromatids and chromosomes may lead to new gene associations and rare or totally

unexpected variants. Deletions, or losses of chromatin, may induce lethal, aberrant, or fully viable novel types, depending on the metabolic importance of the lost portion. Cytologically the aberrations may appear as bridges, rings, fragments or other configurations, although minute deletions may escape direct detection.

Abnormal configurations followed X-irradiation of Lilium anthers by Mitra (32). After X-ray treatment of Drosophila melanogaster, Schacht (37) recovered a total of 20 translocations: 17 between chromosomes II and III, and 3 between Y and II. More important, he also demonstrated 18 induced cross-overs: 11 singles, 6 doubles and 1 triple. Similar irradiation - enchanced crossing over was noticed by Wittinghill and Davis (48) and Scossiroli (38). In X-rayed roots of onion seedlings, Cohn (8) identified two classes of chromosome breaks based on average time of restitution. Some breaks underwent rapid restitution (15 min. or less), some remained open for a relatively longer period (4 hrs. or more). The longer the breaks remained open, the greater the opportunity for chromosomal interchange. Heinz (24) reported a wide range of translocations in tetraploid Dactylis seed bombarded with neutrons for one-half hour.

Comparing Effects of Some Current Ionizing Radiations

Reviewing some studies with X-rays and neutrons (fast and thermal), Elliott (14) concluded that the two types of radiation differ sharply in some of their effects, at least in barley. Neutrons, he notes, allow:

(1) more uniform seedling height and survival of N_1 plants,

(2) higher survival of low fertility N_1 progeny, and

(3) higher frequencies of chromosomal aberrations and mutations.

Bacq and Alexander (6) reported the damage caused by densely ionizing radiations in the various stages of spermatogenesis of Drosophila virilis. In all stages, fission neutrons showed a higher relative biological efficiency (RBE) than X-rays of comparable energies. Kulik (26) reported that treatments with gamma rays excelled thermal neutron irradiation in inducing potentially useful mutants in tomatoes. But, for the production of chromosome aberrations, he deemed neutrons as superior to gamma rays. For a given level of chromosome damage under conditions of aeration, Evans and his co-workers (15) found fast neutrons above 10 times more effective than gamma rays. In the absence of air, the estimated effect ratio ran as high as 18:1, in favor of neutrons. Biological effectiveness

of fast neutrons for radiation damage in field peas reached 40 times that of gamma rays, according to Hvostova and Nevzgodina (25). Studies of the genetic effects of beta rays, have been very limited compared with those of X-rays and neutrons (34).

Expressing his reasons for suggesting the use of fast neutrons, Dr. Osborne (35) remarked:

(1) Current knowledge (up to 1961) puts neutrons ahead of gamma rays in producing gross chromosomal changes

(2) With thermal neutrons - in which most ionization results from capture by atomic nuclei and subsequent disintegration - the chemical composition of the target is extremely important.

(3) With fast neutrons - where ionizations result mostly from collision, chemical composition is of little importance, and moisture and storage effects are trivial.

The foregoing survey of the literature exposes the great potentiality of ionizing radiations, especially neutrons, as a means of inducing genetic variability. Coupling irradiation with intercrossing should enhance genic recombination, assuming independent action of the two breeding procedures.

As Lerner (28) points out for polygenic characters, neither the number of loci nor the magnitudes of the effects produced by allelic substitutions can be ordinarily known. The usual available information consists of individual phenotypic measurements for one or more generations and of pedigree relationships between the members of the population. From these, means and statistics of higher order, such as variances and covariances, can be computed as needed.

Yield (W) in navy beans, has been regarded as the product of 3 components: pod number per plant (X), average seed number per pod (Y), and mean seed weight (Z). Camacho and his collaborators (7) reported negative correlations between the yield components in kidney beans and, as expected, a positive correlation between yield and each component. Considering the genotypic correlations, an increase in X seemed to cause a decrease in Y, and an increase in Y corresponded to a reduction in Z. If linkage were to be invoked from the results, he commented, genetic association would be close between X and Y and between Y and Z, but weak between X and Z.

If increased recombination shows up as variability, it might reduce variance in the traits under investigation where:

(1) linkages, if present, are predominantly in the coupling phase, and

(2) increased genetic variance leads to decreased phenotypic variance due to a gain in homeostasis. On the other hand, the breaking of repulsion phase linkages would tend to enhance variances. Treatment with 15 Kr of X-rays reduced means and increased variances in yield of F_2 peanut progenies and their parent population, according to Gregory (17). The larger variances, often were associated with the smaller means.

MATERIALS AND METHODS

Plant Materials

Ten parental lines of navy beans (Phaseolus vulgaris L.) were used that had been previously field-grown for at least six generations. They were tested for genotypic homogeneity and only two off-type plants for maturity showed up, one in each of two lines. They were all white-seeded, and two, Sanilac and Michelite, are standard commercial varieties. They differed in growth habit, maturity, and yield components but all were fully inter-fertile.

The whole study comprised two stages. The first stage, on which the present paper is based, involved the ten parental lines (P), all their possible first cycle intercrosses (I_1), and second cycle intercrosses (I_2). The second stage, currently underway, will comprise a third (I_3) and a fourth (I_4) cycle of intercrossing, in addition to the parental lines.

As each generation of intercrossing became available, equal amounts of beans harvested from four plants at random in each line were planted to produce selfed seed. Sufficient material was thus obtained to allot sizable seed samples for each of a control (C) and a radiation (R) series.

Hybridization

The breeding work took place in the Plant Science greenhouse of Michigan State University, East Lansing. Crossing the 10 parents in all possible combinations gave 45 F_1's which after selfing became 45 F_2's. Simultaneously each F_1 was crossed to four unrelated ones to make up an expected total of 180 second cycle intercross lines. Actually 178 such lines were recovered and subsequently selfed. Thus the original material subjected to neutron irradiation in 1961 consisted of selfed seed of:

(1) 10 parents (P)

(2) 45 first-cycle intercrosses (I_1)

(3) 178 second-cycle intercrosses (I_2)

About half of the seed of each line in each class served as the control group. Both treatment series were field-planted that same year, and their progenies grown in 1962 and again in 1963. Both the first-cycle and second-cycle intercrosses have been grown for three generations after their initial production. No differential viability in emergence was noted in any year. In 1962, stands were thinned after emergence to prevent undue competition and possible elimination of weak plants. The 1963 nursery consisted of single plant progenies taken at random from the field in 1962. It was assumed that no appreciable changes had occurred in the parental material.

Neutron Irradiation

In May 1961, Dr. S.T. Osborne, of the University of Tennessee, irradiated the seeds in the graphite reactor of the Oak Ridge National Laboratory, using an enriched uranium plate beneath the sample cart. He calculated the neutron flux and set the other environmental conditions inherent to treatment. The material received a dose of fast neutrons (1-2 Mev) equivalent to about 739 rep, for a total exposure time of half an hour. Rather crude observations had previously indicated that for an expected 70-80% seedling emergence such a dose and rate would work satisfactorily. To get reliable data on possible breakage of presumed linkage groups large irradiated as well as control populations were required.

Osborne and Lunden (36) have provided detailed information on plant and seed irradiation at Oak Ridge. Table 1, drawn from their article, describes the composition of the neutron source.

Although thermal neutrons constituted over half the total flux, they contributed less than 3% of the effective dose. This is, therefore, essentially a fast neutron source.

Table 1. Neutron Spectrum of Graphite Reactor with U^{235} Plate (Univ. of Tenn.)

Monitor foil	Min. energy perceived (eV)	Neutrons per cm^2/ sec.	Percentage of total flux	Rep/hr.	Percentage of total rep.
Au	2.5×10^{-2}	4.2×10^{8}	54.5	43.2	2.9
Pu	2.5×10^{4}	1.9×10^{8}	24.7	280.8	19.0
Np	7.5×10^{5}	1.1×10^{8}	14.3	705.6	47.8
U	1.5×10^{6}	3.5×10^{7}	4.5	252.0	17.1
S	2.5×10^{6}	1.3×10^{7}	1.7	147.6	10.0
Al	8.6×10^{6}	2.4×10^{6}	0.3	46.8	3.2
Totals		7.7×10^{8}	(100.0)	1476.9	(100.0)

Field Layouts and Statistical Approach

All the lines were grown in the field in 1961, in single row plots 10 feet long and 32 inches apart. Seedlings averaged 4 inches apart in the row. This spacing, was adequate to allow survival of individuals of differential vigor. To minimize accidental outcrossings, the control set was planted 10 days before the irradiated series. The two treatment groups were separated in the nursery by a 25 yard wide strip of soybeans. Each row was harvested in bulk. Two I_2 plots were missing in the control group and one in the radiation group. The other lines did not suffer any loss.

In 1962, each plot included 2 replications per treatment. Isolation in space only was maintained. Each replication consisted of single rows, 20 feet long and 32 inches apart. To reduce plant competition, the rows were thinned to 30 plants each after seedling emergence. At harvest, 5 plants were randomly chosen per replication to compose family sets of 10 sublines per treatment for each original line. Each plant subline had to have enough well filled pods to guarantee a minimum of 40 seeds for each sample. For each parent, however, 15 one-plant samples were retained per line to provide for more check rows in the following year's nursery. So, assuming no missing plots, the material harvested would consist of:

(1) 150 parental samples (P) in both treatment groups

(2) 450 I_1 sublines in both treatment groups

(3) 1760 I_2 sublines in the control group

(4) 1770 I_2 sublines in the radiation group.

Relatively few plots were missing or did not yield enough seed to meet the requirements for sample selection. Each randomly-selected plant sample was separately threshed, and its yield (W) and yield components (X,Y,Z) measured. Mean seed weight (Z) was calculated on the basis of 100 seeds per sample. Number of pods per plant (X) was obtained by counting pods with at least one viable seed. Average number of seeds per pod (Y) was derived from the equation $W = X \cdot Y \cdot Z/100$. The samples were stored in a warehouse at 40°F to await the next planting season.

In 1963, the field layout consisted of single-row plots, 10 feet long and 32 inches apart. The seed was sown about 3 inches apart in the row as in regular planting. Control and radiation blocks alternated throughout the nursery in a systematic fashion. In spite of slight eptam damage in some spots where herbicide concentration was perhaps critical, stands were satisfactorily uniform. Growth habit, maturity dates (starting from seeding date),

and plant appearance were recorded. A few isolated off-type plants were set aside for further study.

A uniform 4-foot section containing about 15 plants was harvested from each plot. This method was thought to adjust for differential competition resulting from occasional missing plants. Due to the large number of samples that had to be handled, some modifications in measuring yield and its components appeared necessary. Total yield (W) was measured on a plot basis. Average number of seeds per pod (Y) was arrived at by taking 20 random pods in each plot and counting the seed. The procedure to get mean seed weight (Z) was not changed. Number of pods per plant (X) was obtained on a plot basis, by using the same equation $W = X.Y.Z/100$. Distribution, means, ranges, correlations, variances and coefficients of variation of the measured variables were tabulated so as to compare the effectiveness of the:

(1) hybridization phase alone

(2) neutron irradiation treatment alone

(3) combination neutron-hybridization.

The simple correlations and analysis of variance routines used were programmed by the University's Computer Laboratory staff (3,4).

The alternate arrangement of the control and radiation plots plus other precautions to insure high environmental uniformity in the field aimed at minimizing experimental error. The control parent (CP) lines being sufficiently homozygous and homogeneous, no significant genotypic differences were expected within them. Thus the error variance value found for the control-parent treatment could be taken as a measure of the effect of environment on all the genotypes. A significant phenotypic difference between this value and that of another treatment could be ascribed primarily to the combined effect of genotype, treatment, environment, and their interactions. Similarly, any two treatments might be compared and appropriate inferences drawn. Then, for sake of convenience the error variances can be expressed as:

1. Control-parent (CP): σe^2
2. Radiation-parent (RP): $\sigma e^2 + \sigma^2_{RP}$
3. Control-1st cycle intercrossing (CI_1): $\sigma e^2 + \sigma^2_{RI_2}$
4. Radiation-1st cycle intercrossing (RI_1): $\sigma e^2 + \sigma^2_{RI_2}$
5. Control-2nd cycle intercrossing (CI_2): $\sigma e^2 + \sigma^2_{CI_2}$
6. Radiation-2nd cycle intercrossing (RI_2): $\sigma e^2 + \sigma^2_{RI_2}$

RESULTS

Treatment Effects on Correlations and Regressions

Correlated variation of two characters may be due to similar actions on both characters by genes or chromosomes on the one hand or by environmental influences on the other (22). To evaluate the effects of treatments on the correlations between yield (W) and its components (X,Y,Z) and among the components themselves, the homogeneity chi-square test, described by LeClerg *et al* (27), was used. Table 2 summarizes the overall results.

Hybridization and radiation, singly or combined, apparently did not provoke any appreciable changes in correlation patterns. Three of the homogeneity chi-square values look somewhat high and could be an indication of real differences between treatment effects on the correlations involved. To test this possibility, critical comparisons of r-value for XY (χ^2 = 12.50), YW (χ^2 = 14.05) and YZ (χ^2 = 15.10) are sorted out in Table I in the appendix section. None of the t-values obtained exceeds the 1% level of probability for significance. The individual r-values in each correlation class can be considered drawn from the same population and averaged.

Table 3, which sums up differences between mean correlation coefficients, permits these generalizations:

Table 2. Correlation Coefficients Between Bean Yield (W) and its Components (X,Y,Z) and Among the Components

Treatment		D.f.	Correlation					
Group	Breed[Δ]		XY	XZ	YZ	XW	YW	ZW
Control (c)	P	134	-.454**	-.284**	.003	.767**	-.013	.187
	I_1	425	-.442**	-.281	.181**	.807**	-.008	.196**
	I_2	1674	-.339**	-.296**	.122**	.800**	.118**	.154**
Radiation (R)	P	138	-.371**	-.390**	-.019	.753**	.144	.024
	I_1	416	-.382**	-.322**	.153**	.748**	.160**	.170**
	I_2	1684	-.303**	-.266**	.034	.792**	.167**	.153**
Homogeneity χ^2		5	12.50	2.77	15.10	8.24	14.05	3.41
Average r-value		4465	-.344**	-.291**	.090**	.791**	.126**	.157**
Ave. coeff. determin. (r^2), %			11.83	8.47	0.81	62.57	1.59	2.46

Δ : P = parent; I_1=1st cycle intercross, I_2=2nd cycle intercross

** : Significant at 1%

Table 3. Test of Significance of Differences Between Mean Correlation Coefficients

Comparison[1]	Difference	t-value[2]
XY - XZ	.059	2.81**
XY - YZ	.269	12.81**
XZ - YZ	.210	10.00**
XW - ZW	.917	43.67**
XW - YW	.948	45.14**
ZW - YW	.031	1.48
XW - XY	.715	34.05**

1. The corresponding mean r-values appear in Table 2

2. Def = ∞, so t.01 = 2.576

** Significant at 1%

(1) The three yield components (X,Y,Z) form strong positive associations with seed yield (W).

(2) The phenotypic correlation XW is the strongest of all. Statistically the average correlation coefficients ZW and YW do not differ appreciably.

(3) A positive correlation YZ opposes the negative ones: XY and XZ, all three being significant.

In Table 4, however, the calculated values of the regression of yield on each component, even without elaborate statistical analysis, showed that the regression values increase with intercrossing in both the control and the radiation series.

These values are practically of the same magnitude for both the first and second cycles of intercrossing within each series, except for the control series in YW. The non-significant values at CP and CI_1 for YW contrast sharply with the high value at CI_2.

Where it is not negligible, the regression value for YW is overwhelmingly greater than the values for XW and ZW at the same treatment level.

Radiation <u>per se</u> increased the regression value YW over that of control, left XW unchanged and decreased ZW.

The combination hybridization-radiation increased the regression value for YW, decreased that of ZW, but left that of XW practically unchanged.

Table 4. Regression of Bean Yield on its Components

Treatment		D.f.	Linear regression					
			XW		YW		ZW	
Group	Breed[Δ]		b value	F-value	b value	F-value	b value	F-value
Control (c)	P	134	0.63	191.85**	-1.59	0.02	5.58	4.87
	I_1	425	0.70	792.61**	-1.19	0.03	7.33	16.94**
	I_2	1674	0.75	2976.29**	16.83	23.60**	6.01	40.24**
Radiation (R)	P	138	0.62	181.20**	14.33	2.94	0.75	0.08
	I_1	416	0.66	529.74**	19.42	10.87**	5.82	12.44**
	I_2	1684	0.74	2839.21**	21.78	48.09**	5.79	40.39**

Δ: P = parent, I_1=1st cycle intercross, I_2=2nd cycle intercross

**: Significant at 1%

Effects of Treatments on Variance, Means and Coefficients of Variability

Variances

Comparisons of population variances appear in Table 5 for X, Table 6 for Y, Table 7 for Z, and Table 8 for W. The inference of significance in these tables, as in the subsequent ones, is again drawn at the 1% level of probability.

Hybridization as such (I_1, I_2), generated excess variance over the parents, both within the control and radiation series, except for Y. For Y, no significant difference was established within the radiation set; in the control group only the second cycle intercrossing variance exceeded parental variance.

In general, the second and the first cycle intercrossing variances did not differ significantly for Y, where I_2 exceeded I_1 in the control group.

Radiation plus the first cycle of intercrossing (RI_1) produced higher variances over the control plus the first cycle of intercrossing (CI_1) in both Y and Z; their differences were negligible in X and W.

Neutron irradiation alone (RP) did not differ from the control-parent (CP), except for Y in which the neutron treatment was associated with a higher variance. But,

Table 5. Comparisons of treatment error variance for number of pods per plot (X)°

Treatment Group	Breed Δ	Source of variation	D.f.	M.sq.	treatments comparison	F-value of treatments comparison
Control (c)	P	Between lines	9	37861.20	C I_1/P	2.13**
		Error	126	2882.75	I_2/P	1.88**
	I_1	Between lines	44	15459.78	I_1/I_2	1.13
		Error	382	6130.23	R I_1/P	1.93**
	I_2	Between lines	175	14485.66	I_2/P	1.78**
		Error	1500	5428.25	I_1/I_2	1.09
Radiation (R)	P	Between lines	9	26507.91	RP/CP	1.12
		Error	130	3235.99	RI_1/CI_1	1.02
	I_1	Between lines	44	10924.07	RI_2/CI_2	1.06**
		Error	373	6252.67		
	I_2	Between lines	176	12144.59		
		Error	1509	5753.52		

° : A harvested plot contained approximately 15 plants

Δ : P=Parents, I_1=1st cycle intercross, I_2=2nd cycle intercross

**: significant at 1%

Table 6. Comparisons of treatment error variances for mean seed number per pod (Y)

Treatment Group	Breed[Δ]	Source of variation	D.F.	M.sq.	Treatments comparison		F-value of treatments comparison
Control (C)	P	Between lines	9	1.37	C	I_1/P	1.18
		Error	126	0.17		I_2/P	1.41**
	I_1	Between lines	44	0.73		I_2/I_1	1.20**
		Error	382	0.20	R	I_1/P	1.15
	I_2	Between lines	175	0.53		I_2/P	1.15
		Error	1500	0.24		I_2/I_1	1.00
Radiation (R)	P	Between lines	9	1.17		RP/CP	1.59
		Error	130	0.27		RI_1/CI_1	1.55**
	I_1	Between lines	44	0.69		RI_2/CI_2	1.29**
		Error	373	0.31			
	I_2	Between lines	176	0.52			
		Error	1509	0.31			

Δ : P=Parent, I_1=1st cycle intercross, I_2=2nd cycle intercross

**: Significant at 1%

Table 7. Comparisons of treatment error variances for mean seed weight (Z)

Treatment Group	Breed$^{\Delta}$	Source of variation	D.f.	M.sq.	Treatments comparison		F-value of treatments comparison
Control (c)	P	Between lines	9	35.35	C	I_1/P	1.41**
		Error	126	1.72		I_2/P	1.53**
	I_1	Between lines	44	15.52		I_2/I_1	1.09
		Error	382	2.42	R	I_1/P	2.04**
	I_2	Between lines	175	12.44		I_2/P	1.94**
		Error	1500	2.64		I_2/I_1	1.05
Radiation (R)	P	Between lines	9	28.60		CP/RP	1.13
		Error	130	1.52		RI_1/CI_1	1.28**
	I_1	Between lines	44	15.90		RI_2/CI_2	1.11**
		Error	373	3.10			
	I_2	Between lines	176	12.24			
		Error	1509	2.95			

Δ :P=Parent, I_1=1st cycle intercross, I_2=2nd cycle intercross

**: Significant at 1%

Table 8. Comparisons of treatment error variances for seed yield per plot (W)°

Treatment Group	Breed△	Source of variation	D.f.	M.sq.	Treatments comparison		F-value of treatments comparison
Control (C)	P	Between lines	9	25225.12	C	I_1/P	2.55**
		Error	126	1960.28		I_2/P	2.60**
	I_1	Between lines	44	7868.76		I_2/I_1	1.02
		Error	382	4995.96	R	I_1/P	1.79**
	I_2	Between lines	175	9504.29		I_2/P	1.88**
		Error	1500	5099.49		I_2/I_1	1.05
Radiation (R)	P	Between lines	9	8940.27		RP/CP	1.45
		Error	130	2837.67		RI_1/CI_1	1.02
	I_1	Between lines	44	5928.60		RI_2/CI_2	1.05**
		Error	373	5093.25			
	I_2	Between lines	176	7963.73			
		Error	1509	5339.52			

° : A harvested plot contained approximately 15 plants

△ : P=Parent, I_1=1st cycle intercross, I_2=2nd cycle intercross

** : Significant at 1%

radiation plus the second cycle of intercrossing (RI_2) led in all cases to higher variances over the second cycle of intercrossing alone (CI_2).

Means

Comparisons of treatment means are arranged in Table 9 for X, Table 10 for Y, Table 11 for Z, and Table 12 for W. Differences in magnitude are very limited and generally not impressive.

Hybridization (I_1, I_2) means did not exceed the parent means (P), except for two cases. For Y, both I_1 and I_2 were greater than P in the control lot; for Z, only I_2 exceeded P in the radiation set.

The second cycle intercrossing (I_2) and first cycle intercrossing (I_1) means did not differ notably, except for Z, where I_2 exceeded both the control and the radiation sets.

Radiation alone (RP) or combined with hybridization (RI_1, RI_2) produced means not statistically different from the control counterparts (CP, CI_1, CI_2) except for Y. There the CI_2 mean was slightly greater than RI_2.

Coefficients of Variation

Comparisons of coefficients of variation appear in Table 13 for X, Table 14 for Y, Table 15 for Z, and Table 16 for W.

Table 9. Comparisons of differences between treatment means for number of pods per plot (X)[1]

Treatment Group	Breed[Δ]	Number observations	Mean	Standard deviation	Comparison		Difference	t-value
Control (C)	P	136	314.79	72.21	C	I_1-P	6.01	0.81
	I_1	427	320.80	84.22		I_2-P	3.05	0.47
	I_2	1676	317.84	79.84		I_1-I_2	2.96	0.65
Radiation (R)	P	140	319.83	68.87	R	P-I_1	1.31	0.19
	I_1	418	318.52	82.13		P-I_2	1.38	0.22
	I_2	1686	318.48	80.13		I_1-I_2	0.07	0.02
						RP-CP	5.04	0.59
						CI_1-RI_1	2.28	0.40
						RI_2-CI_2	0.61	0.22

1 : A harvested plot contained approximately 15 plants

Δ : P=Parent, I_1=1st cycle intercross, I_2=2nd cycle intercross

Table 10. Comparisons of differences between treatment means for mean seed number per pod (Y)

Treatment Group	Breed△	Number observations	Mean	Standard deviation	Comparison	Difference	t-value
Control (c)	P	136	5.27	0.50	C I_1-P	0.13	2.65**
	I_1	427	5.40	0.51	I_2-P	0.14	3.13**
	I_2	1676	5.41	0.52	I_2-I_1	0.01	0.35
Radiation (R)	P	140	5.24	0.57	R I_1-P	0.07	1.26
	I_1	418	5.31	0.59	I_2-P	0.05	1.00
	I_2	1686	5.29	0.57	I_1-I_2	0.02	0.63
					CP-RP	0.03	0.47
					CI_1-RI_1	0.09	2.41
					CI_2-RI_2	0.12	6.00**

△ :P=Parent, I_1=1st cycle intercross, I_2=2nd cycle intercross

**:Significant at 1%

Table 11. Comparisons of differences between treatment means for mean seed weight (Z)

Treatment Group	Breed[Δ]	Number observations	Mean	Standard deviation	Comparison		Difference	t-value
Control (c)	P	136	18.87	1.99	C	$P-I_1$	0.08	0.41
	I_1	427	18.79	1.94		I_2-P	0.45	2.54
	I_2	1676	19.32	1.91		I_2-I_1	0.53	5.05**
Radiation (R)	P	140	18.83	1.81	R	I_1-P	0.21	1.14
	I_1	418	19.04	2.11		I_2-P	0.59	3.68**
	I_2	1686	19.42	1.98		I_2-I_1	0.38	3.33**
						CP-RP	0.04	0.17
						RI_1-CI_1	0.25	1.79
						RI_2-CI_2	0.10	1.49

Δ : P=Parent, I_1=1st cycle intercross, I_2=2nd cycle intercross

**: Significant at 1%

Table 12. Comparisons of differences between treatment means for seed yield per plot (W)[1]

Treatment Group	Breed[4]	Number observations	Mean	Standard deviation	Comparison		Difference	t-value
Control (c)	P	136	307.85	59.26	C	I_1-P	12.05	1.95
	I_1	427	319.90	72.75		I_2-P	19.78	3.65**
	I_2	1676	327.63	74.56		I_2-I_1	7.73	1.95
Radiation (R)	P	140	310.11	56.86	R	I_1-P	6.02	1.01
	I_1	418	316.13	71.98		I_2-P	11.88	2.32
	I_2	1686	321.99	74.92		I_2-I_1	5.86	1.48
						RP-CP	2.26	0.32
						CI_1-RI_1	3.77	0.76
						CI_2-RI_2	5.64	2.19

1 : A harvested plot contained approximately 15 plants

4 : P=Parent, I_1=1st cycle intercross, I_2=2nd cycle intercross

**: Significant at 1%

Table 13. Coefficients of variation for number of pods per plot (X)[1]

Treatment Group	Breed[Δ]	No. observations	C.V.°	Comparison		Difference	t-value
Control (c)	P	136	22.9	C	I_1-P	3.4	1.94
	I_1	427	26.3		I_2-P	2.2	1.44
	I_2	1676	25.1		I_1-I_2	1.2	1.13
Radiation (R)	P	140	21.5	R	I_1-P	4.3	2.62**
	I_1	418	25.8		I_2-P	3.7	2.61**
	I_2	1686	25.2		I_1-I_2	0.6	0.57
					CP-RP	1.4	0.71
					CI_1-RI_1	0.5	0.37
					RI_2-CI_2	0.1	0.15

1 : A harvested plot contained approximately 15 plants

° : Transformation of C.V. drawn from Ag. Jour. Vol. 28, No. 1, Jan. 1934

Δ : P=Parent, I_1=1st cycle intercross, I_2=2nd cycle intercross

** : Significant at 1%

Table 14. Coefficients of variation for mean seed number per pod (Y)

Treatment		No. observations	C.V.°	Comparison		Difference	t-value
Group	Breed[Δ]						
Control (c)	P	136	9.5	C	$P-I_1$	0.1	0.15
	I_1	427	9.4		I_2-P	0.1	0.17
	I_2	1676	9.6		I_2-I_1	0.2	0.56
Radiation (R)	P	140	10.9	R	I_1-P	0.2	0.26
	I_1	418	11.1		$P-I_2$	0.1	0.14
	I_2	1686	10.8		I_1-I_2	0.3	0.70
					RP-CP	1.4	1.59
					RI_1-CI_1	1.7	3.40**
					RI_2-CI_2	1.2	4.80**

° : Transformation of C.V. drawn from Ag. Jour. Vol. 28, No. 1, Jan. 1934

Δ : P=Parent, I_1=1st cycle intercross, I_2=2nd cycle intercross

**: Significant at 1%

Table 15. Coefficients of variation for mean seed weight (Z)

Treatment Group	Breed[Δ]	No. observations	C.V.°	Comparison		Difference	t-value
Control (c)	P	136	10.5	C	$P-I_1$	0.2	0.27
	I_1	427	10.3		$P-I_2$	0.6	0.90
	I_2	1676	9.9		I_1-I_2	0.4	1.00
Radiation (R)	P	140	9.6	R	I_1-P	1.5	2.14
	I_1	418	11.1		I_2-P	0.6	0.98
	I_2	1686	10.2		I_1-I_2	0.9	2.09
					CP-RP	0.9	1.05
					RI_1-CI_1	0.8	1.54
					RI_2-CI_2	0.3	1.20

° : Transformation of C.V. drawn from Ag. Jour. Vol. 28, No. 1, Jan. 1934

Δ : P=Parent, I_1=1st cycle intercross, I_2=2nd cycle intercross

Table 16. Coefficients of variation for seed yield per plot (W)[1]

Treatment Group	Breed[Δ]	No. observations	C.V.°	Comparison		Difference	t-value
Control (c)	P	136	19.3	C	I_1-P	3.4	2.33
	I_1	427	22.7		I_2-P	3.5	2.73**
	I_2	1676	22.6		I_2-I_1	0.1	0.11
Radiation (R)	P	140	18.3	R	I_1-P	4.5	3.21**
	I_1	418	22.8		I_2-P	5.0	4.13**
	I_2	1686	23.3		I_2-I_1	0.5	0.54
					CP-RP	1.0	0.60
					RI_1-CI_1	0.1	0.09
					RI_2-CI_2	0.5	0.85

1 : A harvested plot contained approximately 15 plants

° : Transformation of C.V. drawn from Ag. Jour. Vol. 28, No. 1, Jan. 1934

Δ : P=Parent, I_1=1st cycle intercross, I_2=2nd cycle intercross

** : Significant at 1%

Hybridization as such (I_1, I_2) did not change the coefficients much. In the radiation group, both second cycle intercrossing (I_2) and first cycle intercrossing (I_1) were superior to parent (P), for X and W. In the control set, only I_2 markedly surpassed P for W.

The second cycle intercrossing *per se* (CI_2) and the first cycle intercrossing alone (CI_1) exerted no significant differential effects on the coefficient of variation.

Similarly, radiation alone (RP) or combined with hybridization (RI_1, RI_2) showed no significant differences from the control counterparts (CP, CI_1, CI_2), except for Y. In the case of Y, RI_1 and RI_2 surpassed CI_1 and CI_2, respectively.

Frequency distributions of the original data are shown in Table II for X, Table III for Y, Table IV for Z, and Table V for W, in the appendix section.

DISCUSSION

To analyze the effects of the treatments on yield and its components, a brief review of pertinent reports on the relationships among these fitness factors might be appropriate. Luedders (29) concluded that the yield components in oats were governed by separate gene systems. Archibong (5) noted that number of pods per plant (X) invariably showed strong positive correlations with plant yield (W) in navy beans. Camacho and his associates (7) suggested that yield improvement in kidney beans could be achieved by selecting progenies with a high number of beans per pod (Y) because of the greater genotypic YW correlation value obtained from their data.

Biologically, if not statistically, the correlation patterns emerging from the present data do not preclude similar generalizations. Whereas yield is strongly correlated with number of pods in every case, it regresses more heavily on number of seeds per pod in most instances. In spite of statistically significant correlations among them, the components still can be genetically unrelated one to another or related to a common yet unknown factor. From a developmental standpoint with limited metabolic input, a high number of pods is likely to be associated with a low number of seeds per pod, a low mean seed weight (Z), or both.

The failure of the treatments to bring about any substantial changes in the correlation patterns could indicate either an inefficacy of the intercrossing cycles in these early stages or an inadequacy of the radiation dosage, or both. On the contrary, the rigidity of a developmental scheme for yield might be such as to upset any variation in the components brought about by the treatments and maintain a *status quo* in the correlation patterns.

From the comparison of treatment effects on the regression coefficients of yield on its components, hybridization *per se* (CI_1, CI_2) in general seems to have been more potent than the radiation treatment alone (RP). Yet, when intercrossing is combined with radiation some change developed: while the regression values XW stays roughly the same, YW increases and ZW decreases. Such a trend would imply that with a combination of the two procedures, yield (W) would depend more on average seed number per pod (Y) than on either number of pods per plant (X) or mean seed weight (Z). If so, yield improvement by manipulation of the components would be greatly facilitated as X is most subject to ecological influences and Z must conform to commercial standards.

Granted a few departures, which are normal for metric characters, the relative efficacy of the treatments in

modifying the regression coefficients seems to hold for changes in variances. Hybridization alone very effectively increased variances, but the combination hybridization and radiation led in this respect. Irradiation alone, however, did not differ from the control treatment, likely because of the presumed inadequacy of the dose applied. Moreover, the similarity of effects of the first and the second cycles of intercrossing in all but one case, supports the argument that these early stages may not produce spectacular differential effects, especially on quantitative traits. The significant differences in variances for Y that correspond to non-significant differences in X and Z indicate that Y was the most uniform component.

Generally, irradiation is expected to reduce mean fitness, due to lowered fertility. The failure of the neutron treatment to produce yield and yield component mean statistically distinct from those in the control groups, except in one case, supports the speculation that the neutron dosage might not have been high enough. Within the hybrid groups, certain families with high and low yield and yield component means may have cancelled out so as to become not different from the control populations. The similarity between the first and the second cycles of intercrossing in their effects on fitness means in most of the cases, again conforms with speculations.

The effects of treatments on variances and means show good agreement with earlier reports. Mitchell (31), from crosses in Drosophila, found that hybridization increased variance but brought no significant changes in means. Irradiation with or without hybridization increased variances in peanuts but reduced means, according to Gregory (17), who further pointed out that the large variances were often associated with the smaller means.

The coefficient of variability (C.V.) would tend to increase as means decrease or as variances increase. The higher this index, the greater may be opportunities for selection, if the bulk of variation is not solely due to environmental fluctuations. Where differences in C.V. are noticeable, hybridization alone or coupled with radiation - because of associated increases in variances and little variation in means - raised the percentage values. The neutron treatment alone having produced no major changes in means and variances naturally did not differ from the control.

CONCLUSIONS

In general, the results obtained in the present study have provided support for the idea that a combination of radiation breeding and hybridization would be more effective than either method alone.

Hybridization combined with neutron irradiation did increase both variances and percent of variability more than the other treatments. In this respect, hybridization alone rated better than the radiation treatment *per se*. When some significant changes occurred, hybridization was found to increase and irradiation to decrease means, as normally expected. Only a few such changes were actually recorded because presumably the low and high family means within a line balanced out or the neutron dosage was too low to produce drastic effects.

Hybridization alone or with radiation increased regression values of yield on its components more consistently than did neutron irradiation alone. Combined with irradiation, hybridization made yield variation more dependent on number of seeds per pod (Y) than did the other treatments. Because of its greater stability over both X and Z and its freedom from market specifications, Y offers greater convenience for yield improvement through manipulation by selection.

The second cycle of intercrossing, alone or with radiation, excelled the first cycle in increasing mean seed

weight (Z) means, and, without radiation, in increasing variances of number of seed per pod (Y). Their effects otherwise were similar.

Statistically significant negative correlations seem to link number of pods (X) to either number of seeds per pod (Y) or mean seed weight (Z). Even if developmentally correlated, the three components may still be governed by independent gene systems, or simply related to a common yet unknown factor. The treatments applied did not bring any appreciable changes in correlation patterns.

Claims for disruption of linkage groups may be hard to prove due to unavailability of specified marker genes. But genetic recombination at least has been achieved, as expressed by increased variances and higher coefficients of variability. Lines that transgress the original parents in yield and component means have been isolated and set aside for selection purposes.

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APPENDIX

Table I. Test of significance of differences between correlation coefficients for the correlations YZ, YW and XY[1]

Correlation	Treatment Group	Breed[Δ]	Approx.Z value	Comparison	Difference	Ł-value[2]
YZ	Control (c)	P	.003	C I_1 - P	.180	1.82
		I_1	.183	I_2 - P	.120	1.33
		I_2	.123	I_1 - I_2	.060	1.11
	Radiation (R)	P	-.019	R I_1 - P	.173	1.77
		I_1	.154	I_2 - P	.053	0.54
		I_2	.034	I_1 - I_2	.120	2.18
				CI_1 - RP	.202	2.06
YW	Control (c)	P	-.013	C I_1 - P	.005	0.05
		I_1	-.008	I_2 - P	.132	1.47
		I_2	.119	I_2 - I_1	.127	2.35
	Radiation (R)	P	.145	R I_1 - P	.016	0.16
		I_1	.161	I_2 - P	.024	0.24
		I_2	.169	I_2 - I_1	.008	0.15
				RI_2 - CP	.182	2.02
XY	Control (c)	P	-.490	C I_1 - P	.015	0.15
		I_1	-.475	I_2 - P	.137	1.52
		I_2	-.353	I_2 - I_1	.122	2.26
	Radiation (R)	P	-.388	R P - I_1	.014	0.14
		I_1	-.402	I_2 - P	.075	0.76
		I_2	-.313	I_2 - I_1	.089	1.62
				RI_2 - CP	.177	1.97

1. The correlation coefficients are drawn from Table 2
Δ P = parent, I_1=1st cycle intercross, I_2=2nd cycle intercross

2. D.f = ∞ , Ł.01 = 2.576

Table II. Frequency distribution of number of pods per plot (X)°

Class interval	Treatment[1]					
	P		I_1		I_2	
	Control	Radiation	Control	Radiation	Control	Radiation
80-129			1	1	2	3
130-179	4	3	9	10	33	27
180-229	8	10	32	40	153	174
230-279	31	25	102	98	374	353
280-329	39	40	109	104	438	457
330-379	33	37	85	71	348	335
380-429	13	17	43	51	194	194
430-479	4	5	25	26	80	89
480-529	4	3	11	12	30	29
530-579			7	4	15	15
580-629			2	1	4	7
630-679			1		2	1
680-729					2	1
730-779					0	0
780-829					0	0
830-879					1	1
Totals	136	140	427	418	1676	1686
Range[2]						
Low	144.00	157.50	136.20	138.60	149.82	143.59
High	561.50	482.50	605.60	568.20	615.06	604.23

° : A harvested plot contained approximately 15 plants

1 : P=Parent I_1=1st cycle intercross, I_2=2nd cycle intercross

2 : 2% of the observations in each treatment were used to evaluate the ranges: 1% for the lower limit
1% for the upper limit

Table III. Frequency distribution of mean number of seeds per pod (Y)

Class interval	Treatment[1]					
	P		I_1		I_2	
	Control	Radiation	Control	Radiation	Control	Radiation
2.35-2.59					1	1
2.60-2.84					0	0
2.85-3.09				1	0	1
3.10-3.34				3	0	2
3.35-3.59				3	2	4
3.60-3.84	1		1	1	4	4
3.85-4.09	1	4	1	3	7	19
4.10-4.34	4	6	4	12	30	55
4.35-4.59	4	9	14	20	54	98
4.60-4.84	16	14	36	35	115	177
4.85-5.09	21	22	54	54	190	216
5.10-5.34	25	19	78	68	289	283
5.35-5.59	20	28	90	80	344	300
5.60-5.84	29	15	68	59	275	240
5.85-6.09	9	14	41	48	218	148
6.10-6.34	5	5	23	20	90	84
6.35-6.59	1	4	11	6	42	43
6.60-6.84			3	5	12	10
6.85-7.09			3		3	1
Totals	136	140	427	418	1676	1686
Range[2]:						
Low	3.90	3.97	4.07	3.21	3.78	3.52
High	6.32	6.45	6.78	6.60	6.73	6.64

1 : P=Parent I_1=1st cycle intercross, I_2=2nd cycle intercross

2 : 2% of the observations in each treatment were used to evaluate the ranges: 1% for the lower limit
1% for the upper limit

Table IV. Frequency distribution of mean seed weight (Z)

Class Interval	Treatment[1]					
	P		I_1		I_2	
	Control	Radiation	Control	Radiation	Control	Radiation
11.0-11.8					1	
12.0-12.8					1	
13.0-13.9				1	0	
14.0-14.9		1	3	3	13	11
15.0-15.9	6	3	21	16	34	42
16.0-16.9	20	13	55	50	108	112
17.0-17.9	24	27	71	66	246	225
18.0-18.9	26	33	95	75	320	322
19.0-19.9	24	25	65	81	374	338
20.0-20.9	12	16	51	46	270	281
21.0-21.9	11	13	40	42	163	193
22.0-22.9	9	4	17	24	84	81
23.0-23.9	4	2	7	4	34	50
24.0-24.9		1	2	5	21	18
25.0-25.9				5	5	9
26.0-26.9					1	3
27.0-27.9					1	1
Totals	136	140	427	418	1676	1686
Range[2]:						
Low	15.40	15.05	14.78	14.50	14.25	14.76
High	23.65	23.80	23.80	25.68	25.09	25.55

1 : P=Parent I_1=1st cycle intercross, I_2=2nd cycle intercross

2 : 2% of the observations in each treatment were used to evaluate the ranges: 1% for the lower limit
1% for the upper limit

Table V. Frequency distribution of seed weight per plot (W)°

Class interval	Treatment[1]					
	P		I_1		I_2	
	Control	Radiation	Control	Radiation	Control	Radiation
80-129			3	1	1	4
130-179	3	2	1	9	18	23
180-229	11	10	33	41	115	132
230-279	27	29	95	74	308	338
280-329	46	46	111	112	454	463
330-379	37	38	106	103	415	374
380-429	9	13	50	52	227	217
430-479	2	2	17	20	90	86
480-529	1		7	4	30	34
430-479			4	1	12	13
580-629					2	1
630-679					3	0
680-729					1	1
Totals	136	140	427	418	1676	1686
Range[2]:						
Low	145.50	156.00	141.40	134.80	154.70	140.88
High	471.00	459.00	552.20	514.40	582.06	560.94

° : A harvested plot contained approximately 15 plants

1 : P=Parent I_1=1st cycle intercross, I_2=2nd cycle intercross

2 : 2% of the observations in each treatment were used to evaluate the ranges: 1% for the lower limit
1% for the upper limit