Chapter III

MATERIALS AND METHODS

The investigations to study the effect of radiation on different genotypes of Triticum aestivum L. of diverse origin were conducted at the experimental farm of the Punjab Agricultural University, Ludhiana during 1968, 1969 and 1970. Four pure-line varieties viz. NP 824, C 273, Kalyan 227 and S 354 and F2 seeds of three crosses, viz. NP 824 x C 273, Kalyan 227 x C 273 and S 354 x Kalyan 227, were irradiated with gamma rays. The origin and important characters of the parent varieties are given below:

<table>
<thead>
<tr>
<th>Variety</th>
<th>Place of origin</th>
<th>Pedigree</th>
<th>Important characters</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP 824</td>
<td>New Delhi</td>
<td>Developed from the cross F1 (W 245(44-25; 75) x (C 513 x NP 165)) F1</td>
<td>Tall, medium duration, bearded, bold and long ears, glumes white, medium hard and amber grains, resistant to yellow rust, loose smut and lodging, high yielding under high fertility and normal sown conditions.</td>
</tr>
<tr>
<td>Variety</td>
<td>Origin</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>-----------</td>
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<td>------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>C 273 Punjab</td>
<td>Developed from the cross (C 209 x C 591)</td>
<td>Tall, bearded, long and mid dense ears, grains hard, bold and amber, resistant to lodging and does well on average to rich soils</td>
<td></td>
</tr>
<tr>
<td>Kalyan 227 Punjab (to be referred as K 227)</td>
<td>Selected from S 227 strain developed from the cross (FmnK58-3) N 10 B Y 54</td>
<td>Semi-dwarf (2-gene) bearded long ears, profuse tillering, glumes light red, thick straw, broad erect and dark green leaves, grain deep amber, resistant to rusts, loose smut and lodging, heavy yielder</td>
<td></td>
</tr>
<tr>
<td>S 354 Mexico</td>
<td>Developed from the cross [(Fn-K 53 -N) N 10 B Y 54] HA 1 60 II-1259-3y-2c-2y-1c</td>
<td>Semi-dwarf (2-gene), bearded long ears, profuse tillering, glumes white glabrous, grain small and red, thick straw, resistant to brown and black rust, medium yielder</td>
<td></td>
</tr>
</tbody>
</table>

The three crosses involving four ecologically and genetically diverse varieties, were attempted during 1965-66 and F₁'s of the three crosses were grown during 1966-67. The seed from F₁ plants and the parental varieties were kept for 15 days in a desiccator to stabilize the moisture content before irradiation. Three hundred dry seeds of each of the three crosses and their respective parents were exposed to acute radiation doses of 10, 20 and 50 Kr gamma rays from 200Ci Co⁶⁰ source installed at the Indian Agricultural Research Institute, New Delhi.
The material was studied in R$_1$, R$_2$ and R$_3$ during 1967-68, 1968-69 and 1969-70, respectively. During all the three years, the sowing was done in a split-split plot design with the genotype groups (each comprising a hybrid and its parents) in main plots and radiation doses in the sub plots. Within each sub plot, rows of the parents and the cross were randomized. Row to row and plant to plant spacings of 30 cm and 15 cm respectively were maintained in all years. In R$_1$, R$_2$, and R$_3$, each sub plot (treatment) had eight randomized rows – two of each parent and four of the hybrid. The main spikes of all the plants were marked and were bagged to eliminate any chances of out crossing. All morphological variants showing macro-mutations along with the sterile plants were rejected for the study of polygenic variation. The data were recorded for the eight quantitatively inherited traits on all competitive plants separately.

From the R$_1$, normal appearing plants in each radiation treatment and the control, random samples of 50 main spikes were drawn, of which 30 were from the hybrid and 10 each from the parental lines and these formed spike to row progenies in R$_2$ sub plots thus there were 1800 entries in all (600 spike rows) in this year. The single plant observations were recorded on all competitive normal appearing plants in each line.

On the basis of mean progeny yield in the R$_2$, seven best spike row progenies of the hybrid and three of each parent
variety were selected in the control and 10 and 20 Kr treatments. Three to four best plants were selected from each selected line. In the selection response study during Qach sub plot comprised of 40 randomized lines (9 single plant progenies of each parent and 22 of the hybrid). This way, each selected line of the previous year was represented with a family of 3 to 4 lines. A total of 1080 entries (360 plant progenies) were studied in this year. Again the data were collected on individual competitive plants.

Observations

The data were recorded on individual plants in all the three years on the following characters.

1- Days to earing. It was taken as days from seeding to full emergence of the first (main) spike of the plant.

2- Plant height. Plant height was measured at maturity in centimeters from the ground level to the tip of the spike (excluding the awns) of the longest tiller.

3- Spikes per plant. Spike bearing tillers per plant were counted at maturity.

4- Spike length. The length of main spike was measured in centimeters from the lowest point of the rachis to the tip of the spike excluding the awns.

5- Spikelets per spike. The number of fertile spikelets were counted on the main spike.

6- Grains per spike. The grains set in the main spike were taken as number of grains per spike.
7- **100-grain weight.** The grains of main ear threshed separately were weighed on a Torsion balance in grams. This was converted to weight per 100-grains.

8- **Grain yield per plant.** The total grain yield of a single plant was recorded in grams.

**Statistical Methods**

The single plant observations in the R$_1$ generation along with untreated control were statistically analysed as:

(a) Mean

$$\bar{X} = \frac{\sum x_i}{N}$$

(b) Variance of a treatment

$$S^2 = \frac{n_1 S_1^2 + n_2 S_2^2 + n_3 S_3^2}{n_1 + n_2 + n_3}$$

(c) Standard error of mean

$$s_{\bar{X}} = \sqrt{\frac{S^2}{N}}$$

(d) Comparison of means by t-test

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{S_1^2}{N_1} + \frac{S_2^2}{N_2}}}$$

This t-value was compared against table value at $n_1 + n_2$ degree of freedom.
(e) Coefficient of variation

\[ C.V. = \frac{\sqrt{S^2}}{\bar{x}} \times 100 \]

where

- \( x \) = variable
- \( \sum x \) = sum of all observations
- \( n \) = degree of freedom (N-1)
- \( N \) = number of observations
- \( S^2 \) = variance in a treatment over replications
- \( \bar{x}_1, \bar{x}_2 \) = means of sample one and sample two
- \( N_1, N_2 \) = number of observations of sample one and sample two
- \( S^1, S^2 \) = variance of sample one and sample two.

The data collected in the R₂ and R₃ generations on spike and plant row progeny, replicated thrice were subject to the analysis of variance. The analysis of variance within R₂-progenies based on single plants was performed as in the R₁ generation. The means of progenies in a treatment were calculated and used for analysis of variance according to randomized block design. The analysis of variance in both these generations had the following forms.
Two types of F-ratios were calculated; (a) against error variance, (b) against control variances in the case of irradiated populations. These were tested against the table value of 'F' for the corresponding degrees of freedom.

A similar type of analysis of variances was also performed on treatment means to test the differences between treatments in both the generations, the partitioning of the d.f. was as under:

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>s.s.</th>
<th>(expected) m.s.</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replications</td>
<td>(R-1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entries</td>
<td>(E-1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entries in P₁</td>
<td>(P₁-1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entries in P₂</td>
<td>(P₂-1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entries in hybrid</td>
<td>(H-1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P₁ vs. P₂</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hybrid vs. Parents</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>(R-1)(E-1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>(RE - 1)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[
\text{Analysis of variance within a treatment}
\]

\[
\text{Source} \quad \text{d.f.} \quad \text{s.s.} \quad \text{(expected) m.s.} \quad \text{F}
\]

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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>(H-1)</td>
<td></td>
<td></td>
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<td>Error</td>
<td>(R-1)(E-1)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>(RE - 1)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
The treatment means were compared with that of control mean by critical differences:

\[ \text{C.D.} = t \times \text{S.E.d}' \]

where

\[ t = \text{table value at error degrees of freedom} \]
\[ \text{S.E.d}' = \text{standard error of difference} \]

\[ = \sqrt{\frac{2 \sigma^2 e}{r}} \]

Phenotypic and genotypic variances

From the within treatment analysis of variance the genotypic and phenotypic variances were calculated.
\[ \sigma^2_g = \frac{M_v - M_e}{r} \]

where

- \( M_v \) = progeny mean square
- \( M_e \) = error mean square
- \( r \) = number of replications

\[ \sigma^2_p = \sigma^2_g + \sigma^2_e \]

where

- \( \sigma^2_p \) = phenotypic variance
- \( \sigma^2_e \) = error variance

Coefficient of variation

\[ C.V. (p) = \frac{\sqrt{\frac{\sigma^2_p}{x}}}{x} \times 100 \]

\[ C.V. (g) = \frac{\sqrt{\frac{\sigma^2_g}{x}}}{x} \times 100 \]

where

- \( C.V. (p) \) = phenotypic coefficient
- \( C.V. (g) \) = genetic coefficient
- \( x \) = mean
- \( \sigma^2_p \) and \( \sigma^2_g \) = phenotypic and genotypic variances
Heritability in broad sense

\[ h^2 = \frac{\sigma^2_g}{\sigma^2_p} \times 100 \]

Genetic advance

\[ G.A. = K \times h^2 \times \sigma_p \]

where

- \( K \) = a constant for different selection intensities
- \( h^2 \) = heritability estimate
- \( \sigma_p \) = phenotypic standard deviation

Genetic advance expressed as per cent of mean

\[ \frac{G.A.}{\bar{x}} \times 100 \]

The expected genetic advance was expressed as per cent of means over control parents in case of irradiated parental populations and over mid-parent value in the hybrids. But in the \( R^2 \) generation the selected control and selected mid-parent values were used for treated parents and the irradiated and non-irradiated hybrids, respectively.

Testing additivity of variability

The proposal of Gregory (1956) that irradiation-induced variance should be cumulative with that of hybridization was tested by calculating the predicted genetic variances with the formula of Khadr and Frey (1965) where:

Predicted \( \sigma^2_{RH} = \frac{1}{2} \left( \sigma^2_{RP_1} - \sigma^2_{P_1} \right) + \left( \sigma^2_{RP_2} - \sigma^2_{P_2} \right) + \sigma^2_H \)

The subscript, \( R \) refers to radiation and \( P_1, P_2 \) and \( H \) refers to the origin of the treated material that is parent one, parent two and their hybrid, respectively.

To compare the actual and predicted genetic variances of irradiated hybrid, the ratios between actual and expected variances were calculated.
Components of Variation

The components of variation were determined in irradiated parents and treated and untreated hybrids.

Components of variance in parents

By using formulae derived by Kao et al. (1960) for genetic model in the irradiated pure lines, it was possible to obtain coefficients of additive (D) and dominance (H) gene action. These were estimated by solving the following equations:

\[
\begin{align*}
V_{x_2} & = \frac{3}{8}D + \frac{1}{4}H \\
V_{x_2} & = \frac{1}{8}D + \frac{1}{4}H
\end{align*}
\]

The estimates of environmental variance calculated from the respective control parents were subtracted from the phenotypic variance for each of the equations.

Components of variance in hybrids

The components of variance for each character and treatment in the hybrid were worked out according to the formulae of Mather (1949).

The variance components were estimated from the following equations:
The variation within the non-segregating populations of the parents of each cross was used as an estimate of $E_1$, $E_2$ and $E_3$.

\[
E_1 = \frac{s_1^F + s_2^F}{n_1 + n_2 - 1} \quad \text{(environmental variation from plant to plant in } F_2)\]

\[
E_2 = \frac{s_1^F + s_2^F}{n_1 + n_2 - 1} \quad \text{(environmental variance from plant to plant in } F_2)\]

The environmental variation ($E_3$) among the $F_3$ progeny means was deduced from the variation of plot means of non-segregating parents in the same way.

The estimates of additive (D), dominance (H) and environmental variances ($E_1$, $E_2$, $E_3$) were obtained by applying the method of least squares.

The equations for $D_y$, $H_y$, $E_{1y}$, $E_{2y}$ and $E_{3y}$ were constructed utilizing seven equations as given by Mather (1949). Variance components were calculated from these equations and the
C-matrices as given below:

\[
D = C_{DD}D_y + C_{HH}H_y + C_{DE1}E_{1y} + C_{DE2}E_{2y} + C_{DE3}E_{3y}
\]

\[
H = C_{DH}D_y + C_{HE1}H_y + C_{HE2}E_{1y} + C_{HE3}E_{2y} + C_{HE4}E_{3y}
\]

\[
E_1 = C_{DE1}D_y + C_{HE1}H_y + C_{E1E1}E_{1y} + C_{E1E2}E_{2y} + C_{E1E3}E_{3y}
\]

\[
E_2 = C_{DE2}D_y + C_{HE2}H_y + C_{E2E1}E_{1y} + C_{E2E2}E_{2y} + C_{E2E3}E_{3y}
\]

\[
E_3 = C_{DE3}D_y + C_{HE3}H_y + C_{E3E1}E_{1y} + C_{E3E2}E_{2y} + C_{E3E3}E_{3y}
\]

For estimating the standard errors of these components from the expected and observed variances of these seven equations, error variance was calculated \((S^2)\).

The standard errors were calculated as under:

\[
S.E.D. = \sqrt{C_{DD} \times S^2}
\]

\[
S.E.H. = \sqrt{C_{HH} \times S^2}
\]

\[
S.E.E_1 = \sqrt{C_{E1E1} \times S^2}
\]

\[
S.E.E_2 = \sqrt{C_{E2E1} \times S^2}
\]

\[
S.E.E_3 = \sqrt{C_{E3E1} \times S^2}
\]

For testing the significance, \(t\)-values were calculated as \(t = \frac{D}{S.E.D.}\), for all the parameters. These were compared with the table value.
Average degree of dominance and heritability

The average degree of dominance in parental and hybrid populations was estimated from D and H components as \( \sqrt{\frac{H}{D}} \).

Heritability in broad sense

- In parents: \( \frac{V_{F2} - E}{V_{F2}} \times 100 \)
- In hybrids: \( \frac{V_{F2} - E}{V_{F2}} \times 100 \)

Heritability in narrow sense

- In parents: \( \frac{1/2D}{V_{F2}} \times 100 \)
- In hybrids: \( \frac{1/2D}{V_{F2}} \times 100 \)

Intergeneration heritabilities

The parent-offspring covariance was utilized to give intergeneration heritabilities as:

\( (a) \) degree of determination \( (h^2) = r^2 \times 100 \)

where \( r = \frac{\text{cov. } x \bar{y}}{\sqrt{(\text{var. } x)(\text{var. } \bar{y})}} \) and \( r = \text{correlation coefficient} \).
cov. \( xy \) = covariance between parent (x) and its progeny mean (\( \bar{y} \))

\[ \text{var. } x = \text{variance of parent} \]

\[ \text{var. } \bar{y} = \text{variance of progeny means} \]

(b) Parent-offspring regression

\[ h^2 = \frac{\text{cov. } OP}{\text{var. } P} \times 100 \]

where \( b \) is the regression coefficient of offspring (O) on its parent (P) and was calculated as \( \frac{\text{cov. } OP}{\text{var. } P} \).

(c) Heritability in standard units

The method of Horner and Frey (1957) using standard units \( \frac{x - \bar{x}}{\sigma_x} \) as the basis for the estimation of regression coefficient (b) of offspring on the parents was employed.

\[ h^2 = \frac{\text{cov. } OP}{\text{var. } P} \times 100 \]

This method corrects for certain genotype-environment interactions.

Selection Responses

The selection responses were determined by comparing the predicted and actual genetic gains.

In the \( R_3 \) generation, the control population of parents selected in a similar way as those of irradiated was grown. So
this population was taken as a reference point.

Expected gain (G) = \( h^2 \times (\text{mean of selected population} - \text{mean of unselected population}) \)

where \( h^2 \) is heritability in standard units.

The predicted means of selected and unselected populations were determined by using the formulae of Nickell and Grafius (1969).

Thus prediction coefficients:

\[
\frac{\Delta G + \text{mean of unselected population in } R_2^2}{\text{mean of selected control or mid-parent in } R_2^2} \times 100
\]

or

\[
\frac{\text{mean of unselected population in } R_2^2}{\text{mean of selected control or mid-parent in } R_2^2} \times 100
\]

The predicted means for selected and unselected populations in \( R_3 \) were calculated by multiplying prediction coefficient with mean of selected control or mid-parent value in \( R_2 \) generation.

The difference of actual mean from the unselected and selected predicted means in \( R_3 \) gave the estimates of achieved genetic advances which were converted into percentages. The achieved genetic advance over unselected predicted mean was compared with the expected genetic advance over unselected population.