Exercise 10

Aim: To verify the Mendel's Law of Independent Assortment

Principle: In a dihybrid cross, the segregation of one gene pair is independent of the segregation of the other pair. It means that genes of two different traits assort independently to give a probability ratio equal to segregation probability ratio of one allele pair X segregation probability ratio of other allele pair, which comes to, $(3:1) \times (3:1) = 9:3:3:1$

Requirement: Plastic beakers; 64 plastic beads each of yellow, green, red and white to represent, yellow and green colour of seed coat and red and white flowers respectively and napkin/hand towel

Procedure

Students are to work in pair.

The following steps are to be followed sequentially:

- (i) Place 64 beads of each colour in four separate beakers.
- (ii) Put the beakers containing the yellow and red beads on your left side, and those containing the green and white beads on your right side. The beakers on your left side represent plants bearing yellow seed and red flower (dominant character YY, RR). Beakers on the right side represent plants bearing green seeds and white flowers (recessive character yy, rr). These are the two parental types having contrasting forms of two different characters.
- (iii) Stir the beads in each beaker with a pencil/pen. Each bead now represents alleles in the male and female gametes.
- (iv) Pick up one yellow, one green, one red and one white bead, and put them together on the napkin spread on the table.
- (v) Continue picking up and putting together of the beads of all colours as mentioned in the previous step, till all the beads are utilised.
- (vi) Note that in all, 64 such 4-bead clusters are obtained representing the $\rm F_1$ individuals. Ascertain their genotype and phenotype.
- (vii) Next step is to cross these F_1 individuals to raise the F_2 generation. Let us suppose half of the 4-bead clusters (32 clusters) represent the male parents and the remaining half (32 clusters) the female parents. Now put the 32 red and 32 white beads together in one beaker (numbered-I), and similarly put 32 yellow and 32 green beads together in other

LABORATORY MANUAL: BIOLOGY

beaker (numbered-II). These two beakers represent F_1 female. Similarly put remaining 32 red + 32 white beads in beaker numbered-III, and 32 yellow and 32 green one in beaker numbered-IV to represent the F_1 male. The arrangement can be presented as below

Female F ₁	Male F ₁
32 red + 32 white (Beaker I)	32 red+32 white (Beaker III)
32 yellow + 32 green (Beaker II)	32 yellow + 32 green (Beaker IV)

- (viii) Stir the beads in each beaker with a pencil. In order to raise the $\rm F_2$ generation, pick up (with eyes closed) one bead from the beaker-I of female and one bead from the beaker-III of the male, and put into the palm of the partner student. Similarly, pick up one bead each from the beaker-II of female and beaker IV of male to put in the palm of the partner. This partner would now keep all the four beads together (to represent the $\rm F_2$ individual). Continue this process till all beads are utilised. At the end, 64 $\rm F_2$ individuals (each represented by a 4-bead cluster) are obtained.
- (ix) Determine the genotype and phenotype of each of the $64~F_2$ individuals and write down the number of individuals of different genotypes and phenotypes in the tabular form (given below), remembering that Y (yellow seed colour) is dominant over y (green seed) and R (red flower) is dominant over r (white flower).
- (x) Repeat the whole procedure (steps i to ix) six times, and tabulate your results.

Observation

Tabulate the results as follow:

Symbol (-) indicates the presence of corresponding dominant or recessive allele e.g. Y or y and R or r.

Summarise your results (adding together the data of all the six repeats)

F1 Generation

(a)	Total number of individuals:	
(b)	Phenotype (s)	
(c)	Genotype (s)	

EXERCISE 10

	Genotype		Phenotype		
& repeat of offsprings Y-R- Y-rr yyR-	yyrr	Yellow	Yellow	Green	Green
No.		Red	White	Red	white
F1 1. 2. 3. 4. 5. 6. Total F2 1. 2. 3. 4. 5. 6.		Red	White	Red	white

F2 Generation

1. 2	Generation				
(a)	Total number of indi	viduals			
(b)	Phenotypes				
(c)	Number of individuals in each phenotypic class:				
	Number		Phenotype		
(d)	Phenotypic ratio				
(e)	Genotypic ratio				

LABORATORY MANUAL: BIOLOGY

(f)	Number of individuals of each genotypic class:			
	Number	Genotype		
(g)	Genotypic Ratio			

Discussion

The four phenotypic classes in the F2 generation are in ratio of 9:3:3:1 as expected from the **Law of Independent Assortment**. The genotypic ratio would be (1:2:4:2): (2:1):(2:1):1.

Note

- 1. In case six repeats of the experimental procedure are not feasible due to time limitations, either the number of repeats be slashed down to three or the data from single repeat of six different pair of students may be pooled together to make the final calculations.
- 2. This Law of Independent Assortment was later found to be true only for traits present on two different homologous pair of chromosomes, that is, the two are not linked together. The linked traits do not assort independently, rather they are inherited together (linked) except when crossingover separates them.
- 3. It is quiet likely that you may not find your data exactly in the expected ratio, instead almost approximate to it. The statistical significance of this deviation from the exact expected ratio due to probality can be checked using chi-square (χ^2) test, about which you will study in higher classes.

Questions

- 1. Linked traits fail to assort independently. Explain.
- 2. How is independent assortment of alleles important from the point of view of variation?