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INHERITANCE OF OKRA LEAF TYPE, GOSSYPOL GLANDS AND TRICHOMES IN COTTON

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ABSTRACT

Cotton growing in Pakistan is facing a great threat from the insect pest perspective; which have developed resistance in the versatile insect pest population against the high doses of chemical sprays used in the past years. Such a growing resistance in the cotton insect pests against the insecticide/pesticide application needs to be redressed. Insect non-preference traits exist in nature which can reduce the insect pest population by ultimately reducing the high cost being spent on the insecticide/pesticide application. In cotton, the traits like okra leaf type, trichomes and gossypol glands confer significant resistance against some of the major insect pests including the sucking and chewing insect complex. These traits are oligogenically controlled. Inheritance of these traits is simple. Crosses were made among the contrasting traits. The segregating populations along with the parents, F_1 and back crosses for each trait under study were subjected to the inheritance studies. Okra leaf type, trichomes and gossypol glands behaved like any incompletely dominant allele. The chi-square well fits to 1:2:1 in the F_2.

Key words: Okra leaf; trichomes; gossypol glands; inheritance; cotton

INTRODUCTION

Insect pests constitute a major factor in hampering the cotton production all over the cotton growing areas of the world. In recent times, insect control has been mostly based on the use of chemical insecticides. During the 1970’s and 80’s the use of insecticides increased tremendously in almost all the cotton producing countries of the world including Pakistan. According to an estimate, in Pakistan about 122.3 million US dollars were spent on the import of insecticides during 2010 - 2011 (GOP, 2010 - 2011). The environmental concerns demand cotton production free from insecticides. Nature has provided cotton with traits like okra leaf type, gossypol glands and trichomes which confer non-preference to the insect pest infestation. It has been reported that okra leaf in cotton confers resistance to the white fly which is a serious pest and vector for cotton leaf curl virus. In upland cotton, okra and normal leaves are two major types. Okra leaf is a deeply lobed leaf shape which is a monogenic trait governed by incompletely dominant (L^0) to normal leaf gene (l^0) in the upland cotton (Niles, 1980; Nawab *et al*., 2011 b). Heavy pubescence confers resistance against pink bollworm, thrips and jassids. Niles (1980) reported that the increase in the plant hairiness is governed by two major genes and a complex of modifier genes. The major gene was designated as H_1 for sparse hairiness. A second major gene, H_2 controlled the finely dense pubescence in an upland mutant designated as ‘pilose’. Gossypol glands confer resistance to *Heliothis armigera*, jassid, and aphids (Niles, 1980; Nawab *et al*., 2011 a). Crosses of high glanding (HG) and normal glanding (NG) parents (Calhoun, 1997) resulted in the high glanding plants in the F_1 whereas, a ratio of 3 HG : 1 NG was observed in F_2 and ratios of 1 HG: 2 segregating: 1 NG amongst $F_{2:3}$ progeny suggesting that high glanding was conferred by a special Gl_3 allele. The genes for these traits (okra leaf, trichomes and gossypol) have higher effect in terms of magnitude and are reported to be governed by oligogenes (Niles, 1980; Endrizzi *et al*., 1984).

In view of the importance of these insect resistant traits there is a need for breeding varieties by incorporating the genes for these naturally conferring insect resistances in elite cotton varieties. In order to get information on the pattern of inheritance for these traits, the information reported herein, would be useful in determining the genetics for these traits in different genetic back grounds.

MATERIALS AND METHODS

The present studies were conducted in the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad (Pakistan). The available germplasm was assessed for the traits i.e; okra leaf type, normal leaf, high gossypol glands, normal gossypol glands, glandless types, pilose hairiness and low/sparse hairiness. Based upon the assessment made on the above mentioned traits, five genotypes were selected. The particulars of the selected genotypes are described in Table 1. The selected germplasm was selfed for four generations by growing twice a year, in a glasshouse and field during 2003 to 2004, to ensure homozygosity for the traits under study. Crosses were attempted in four groups to obtain F_0 seed during February through March, 2005. The crossing scheme is given in Table 2. The selfed seed of the parents was obtained by covering their floral buds with butter paper bags. The F_1 and their parents were planted during the normal crop season of 2005-06. The F_1 plants of each cross were divided in three groups for developing BC_1, BC_2 and F_2 for each combination. After developing these crosses, the six generations of the four crosses were sown during the normal crop season of the year 2006-07. The experiment in the field was laid out in a Randomized Complete Block Design layout with three replications of each of the six generations of the four crosses. A single plot per replication was assigned to each of the parents and their respective F_1, while, three plots per replication were assigned to each of the backcrosses and four plots per replication were assigned to raise the F_2 population of each cross. The length of the plot was maintained at 4.5 m, accommodating approximately 16 plants spaced 30 cm apart. The distance between the rows was 75 cm. All other agronomic and cultural practices were kept uniform to minimize the experimental error.

Rating system for leaf shape: In order to classify leaf shape, a qualitative system of classification including a visual rating of leaf shape was used. Leaf shape was categorized into, normal leaf (grade-I), sub-okra (grade-II) and narrow okra (grade-III) following Nawab *et al*. (2011 b).

Qualitative grading system for trichomes: Trichomes represent the presence of small hairs on the cotton plant. The trichome density on leaves was estimated following method proposed by Bourland *et al*. (2003). Three leaves at random from upper, middle and lower portion of the selected plants were used to assess the trichome density rating. A rating system of trichomes on the abaxial surface of leaf, using a scale of 1 for sparsely (non) hairiness, 2 for moderate number of trichomes and 3 for (pilose) hairiness was carried out.

Quantification of gossypol glands: The gossypol glands present on the surface of the unopened bolls were quantified on a spectrophotometer according to the protocol (A.O.C.S., Official Method, 1989).

Statistical Analysis: The segregating ratios of plants in F_2 and back crosses for the traits, okra leaf type, gossypol glands and trichomes were tested for their fitness to a theoretical ratio through chi-square test (Harris, 1912).

RESULTS AND DISCUSSION

The inheritance studies were conducted for three important insect non-preference traits, namely as okra leaf type, trichomes and gossypol glands in *Gossypium hirsutum* L. The results were depicted in order to study the inheritance pattern associated to these traits, on the basis of the F_2 data and test/back cross populations. Chi-square test was employed to test the differences of the observed versus the expected segregating phenotypic ratios.

Okra leaf type: Inheritance studies for okra leaf type were studied in three crosses. It is easy to explain from the Fig.1 that the leaf type segregated into three major different types of shapes. Almost an equal number of plants studied exhibited okra and normal leaf types, while a large number of plants exhibited intermediate leaf type (sub-okra) in the F_2 generation. The two homozygous extremes for leaf type: okra and normal were easily distinguishable (Fig. 1). Chi-Squared values and probabilities of goodness of fit of segregation ratios of F_2 and backcross generations are shown in Table 3. Non-significant chi-squared values were observed for the segregating ratios in F_2 and backcross generations of the three crosses. Observations of 1 normal : 2 sub-okra :1 okra, leaf types were observed in the F_2 populations of the three crosses. The segregation in the backcrosses with parent-I and parent-II also fit to the theoretical ratio of 1:1 further confirms the incomplete pattern of inheritance. The segregation of the leaf shape in F_2 generation into three classes: okra leaf, normal and the intermediate leaf shape of sub-okra and fitting into the theoretical 1:2:1 monohybrid ratio of incomplete dominance in the present studies lies in close proximity to the findings of Rahman and Khan (1998) and Nawab *et al*. (2011 b).

Trichomes/Hairiness: The inheritance pattern for hairiness/trichomes was studied in the three cross combinations. It is evident in the entire three cross combinations, that leaf trichomes segregated into three distinct classes (Fig. 2). Chi-Squared values and probabilities of goodness of fit of segregation ratios of F_2 and backcross generations in a study of inheritance of leaf trichomes trait in three cross combinations are shown in Table 4. Non-significant chi- squared values were observed for the segregating ratios in F_2 and backcross generations of the three crosses. The F_2 data regarding number of trichomes were categorized into three main classes; pilose hairy, sparse/normal hairy and intermediate hairy (Nawab *et al*., 2011 a & c) as shown in Fig. 2. The major gene is designated as H_1 for sparse hairing. A second major gene, H_2 controls the finely dense pubescence in an upland mutant designated as 'pilose'. In the F_1 populations, both H_1 and H_2 showed incomplete dominance (Niles, 1980; Rahman and Khan, 1998; Nawab *et al*., 2011 a). The segregation in the backcrosses with parent-I and parent-II also fit to the theoretical ratio of 1:1 which further confirmed the incomplete dominance pattern of inheritance (Table 4). The non-significant chi-square values in F_2 for trichomes in all the crosses fit well against the theoretical monohybrid ratio of 1:2:1.This ratio is in proximity to the understanding got from Figure 3a, suggesting that inheritance of this trait can be manipulated easily in a breeding programme. However, a very small proportion of plants fell in another intermediately resembling hairiness category. This phenotypic expression of the intermediate hairy state in heterozygous condition was probably affected by the genetic background of the parents indicating, modifying gene effects (Falconer and Mackay, 1996; Rieseberg *et al*., 1996; Xu *et al*., 1997; Rahman and Khan, 1998; Schwarz-Sommer *et al*., 2003).

Gossypol glands: The inheritance studies for gossypol glands were studied in two cross combinations (Fig. 4 & 5). In the cross HRVO-1 × Acala 63-74 [normal glanding × glandless (Fig. 4)], three classes of glandless, low intermediate and normal glanding genotypes were obtained on the basis of the F_2 data as shown in Table 5. Similarly, in the cross HRVO-1 × HG-142 [normal glanding × high glanding (Fig. 5)] three classes of high, high intermediate and normal glanding types were observed from the F_2 data (Table 5). The segregation ratios showed non-significant chi-squared values for the segregation ratios in F_2 and backcross generations of the two crosses. The F_2 of both the crosses segregated in the ratio of 1 : 2 : 1. In contrast, Calhoun (1997) showed two main classes of glandless and normal glanding in the F_2 of the cross of normal glanding and glandless for gossypols on the calyx. His studies were however, based on visual classification, and so the differences between the intermediate and glandless classes obtained in F_2 might not be identified. However, the present studies confirmed the statistically significant differences among the parents and their F_1s which justified the distinctness of three classes as these were the same as produced as a result of the segregation in F_2. The differences among the different classes are also evident from the pictorial view as presented in Figures 4 & 5.

Table 1: Distinctive morphological features of the upland cotton accessions assessed for the traits under study

S. No.	Variety/ Cultivar	Parentage	Distinct Features	Origin
1	Acala 63-74	-	Normal leaf, Glabrous & Glandless	Exotic
2	CIM 446	CP 15/2 × S 12	Normal leaf & Light hairy	CCRI, Multan, Pakistan
3	FH 1000	S 12 × CIM 448	Normal leaf & Light hairy	CRI, Faisalabad, Pakistan
4	HRVO-1	B-557/2/Gambo Okra/Rajhans/3/Rajhans	Okra leaf, Pilose hairiness & Normal glanding	CRI, Faisalabad, Pakistan
5	HG- 142	-	Normal leaf & High glanding	Exotic

Table 2: Scheme of crossing

S. No.	Cross	Traits Considered
1	HRVO-1 × FH-1000	Okra leaf, Pilose hairiness × Normal leaf, Light hairy
2	HRVO-1 × CIM-446	Okra leaf, Pilose hairiness × Normal leaf, Light hairy
3	HRVO-1 × Acala 63-74	Okra leaf, Pilose hairiness, Normal glanding × Normal leaf, Glabrous, Glandless
4	HRVO-1 × HG-142	Normal glanding × High glanding

Table 3: Chi-Squared values and probabilities of goodness of fit of segregation ratios of F_2 and backcross generations in a study of inheritance of okra leaf type trait

Cross	Generation	Expected Phenotypic Ratios	Observed value			Expected value			χ^2 value	Probability
			Normal leaf	Sub-okra	Okra leaf type	Normal Leaf	Sub-okra	Okra leaf type		
HRVO-1 × FH 1000	F_2	1: 2:1	43	76	31	37.5	75	37.5	1.95	0.25-0.10
	BC_1	1:1	-	42	48	-	45	45	0.40	0.75-0.50
	BC_2	1:1	38	52	-	45	45	-	1.88	0.25-0.10
HRVO-1 × CIM 446	F_2	1: 2:1	30	80	40	37.5	75	37.5	2.00	0.50-0.25
	BC_1	1:1	-	50	40	-	45	45	1.11	0.50-0.25
	BC_2	1:1	39	51	-	45	45	-	1.60	0.25-0.10
HRVO-1 × Acala 63-74	F_2	1: 2:1	39	69	42	37.5	75	37.5	1.08	0.75-0.50
	BC_1	1:1	-	44	46	-	45	45	0.04	0.90-0.25
	BC_2	1:1	49	41	-	45	45	-	0.71	0.50-0.25

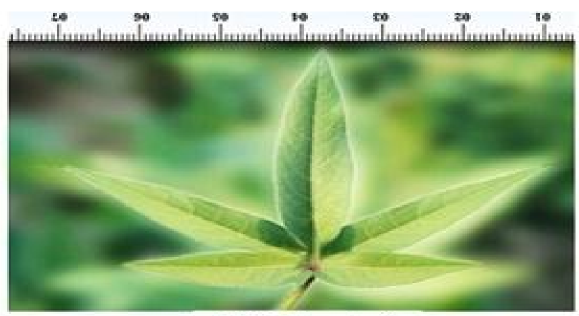
Table 4: Chi-Squared values and probabilities of goodness of fit of segregation ratios of F_2 and backcross generations in a study of inheritance of leaf trichomes trait

Cross	Generation	Expected Ratios	Observed value			Expected value			χ^2 value	Probability
			Sparse Hairy	Medium Hairy	Pilose Velvet	Sparse Hairy	Medium Hairy	Pilose Velvet		
HRVO-1 × FH 1000	F_2	1: 2:1	34	79	37	37.5	75	37.5	0.55	0.90-0.75
	BC_1	1:1	-	53	37	-	45	45	2.84	0.10-0.05
	BC_2	1:1	36	54	-	45	45	-	3.60	0.10-0.05
HRVO-1 × CIM 446	F_2	1: 2:1	41	69	40	37.5	75	37.5	0.97	0.75-0.50
	BC_1	1:1	-	37	53	-	45	45	2.84	0.10-0.05
	BC_2	1:1	48	42	-	45	45	-	0.40	0.75-0.50
HRVO-1 × Acala 63-74	F_2	1: 2:1	35	74	41	37.5	75	37.5	0.50	0.90-0.75
	BC_1	1:1	-	47	43	-	45	45	0.18	0.75-0.50
	BC_2	1:1	52	38	-	45	45	-	2.18	0.25-0.10

Table 5: Chi-Squared values and probabilities of goodness of fit of segregation ratios of F_2 and backcross for gossypol gland quantification of cotton bolls in crosses HRVO-1 × Acala 63-74 and HRVO-1 × HG-142

Cross	Generation	Expected Pheno-typic Ratios	Observed value			Expected value			χ^2 value	Prob.
			Normal glanding	Intermediate glandless	Glandless	Normal glanding	Intermediate glandless	Glandless		
HRVO-1 × Acala 63-74	F_2	1:2:1	48	68	34	37.5	75	37.5	3.91	0.25-0.10
	BC_2	1:1	-	49	41	-	45	45	0.18	0.75-0.50
Cross	**Generation**	**Expected Pheno-typic Ratios**	**Observed value**			**Expected value**			**χ^2 value**	**Prob.**
			Normal Glanding	**Intermediate glanding**	**High glanding**	**Normal glanding**	**Intermediate glanding**	**High Glanding**		
HRVO-1 × HG 142	F_2	1:2:1	40	71	39	37.5	75	37.5	0.44	0.90-0.75
	BC_2	1:1	-	42	48	-	45	45	0.40	0.75-0.50

(a) Normal leaf



(b) Okra leaf

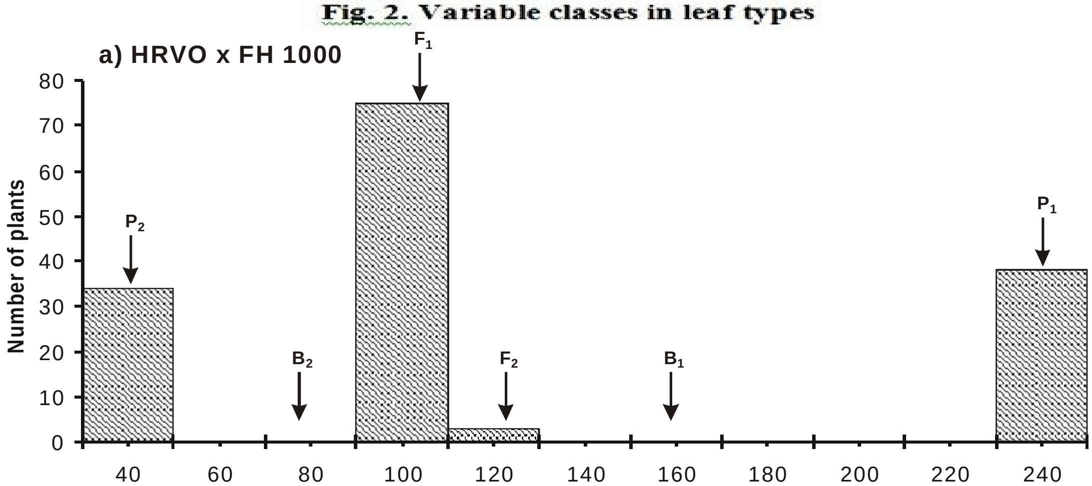
(c) Sub-okra leaf

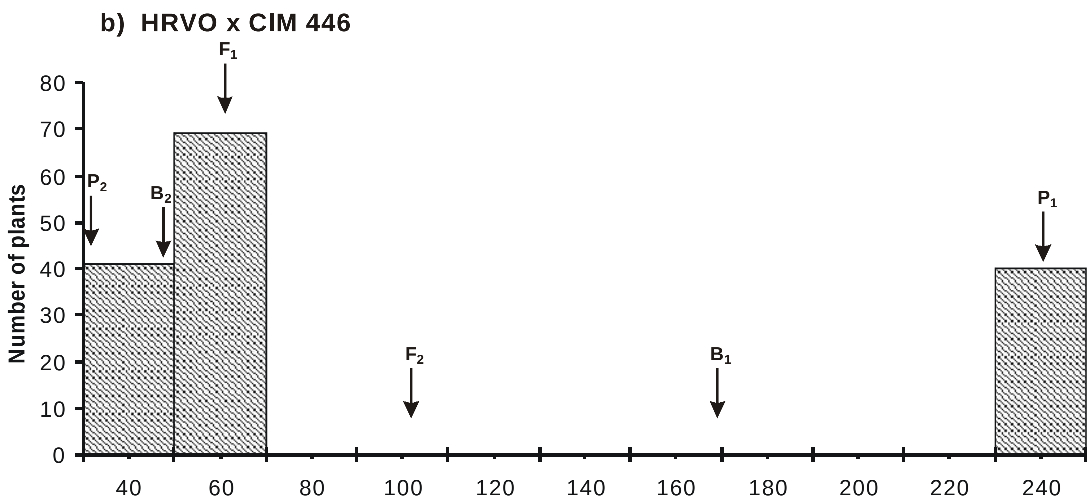
Fig. 1. Inheritance of leaf morphology

(a) Pilose hairiness (P_1)

(b) Normal hairiness (P_2)

Fig. 2. Variable classes in leaf types





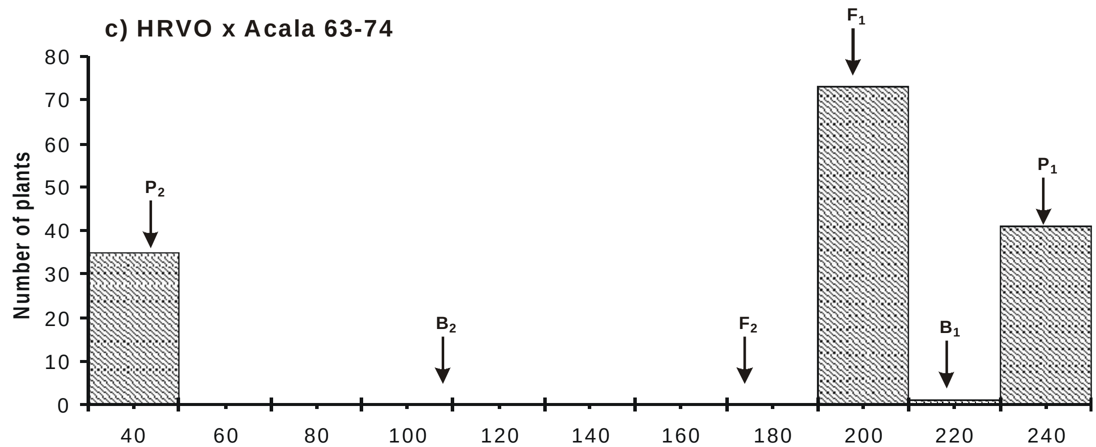
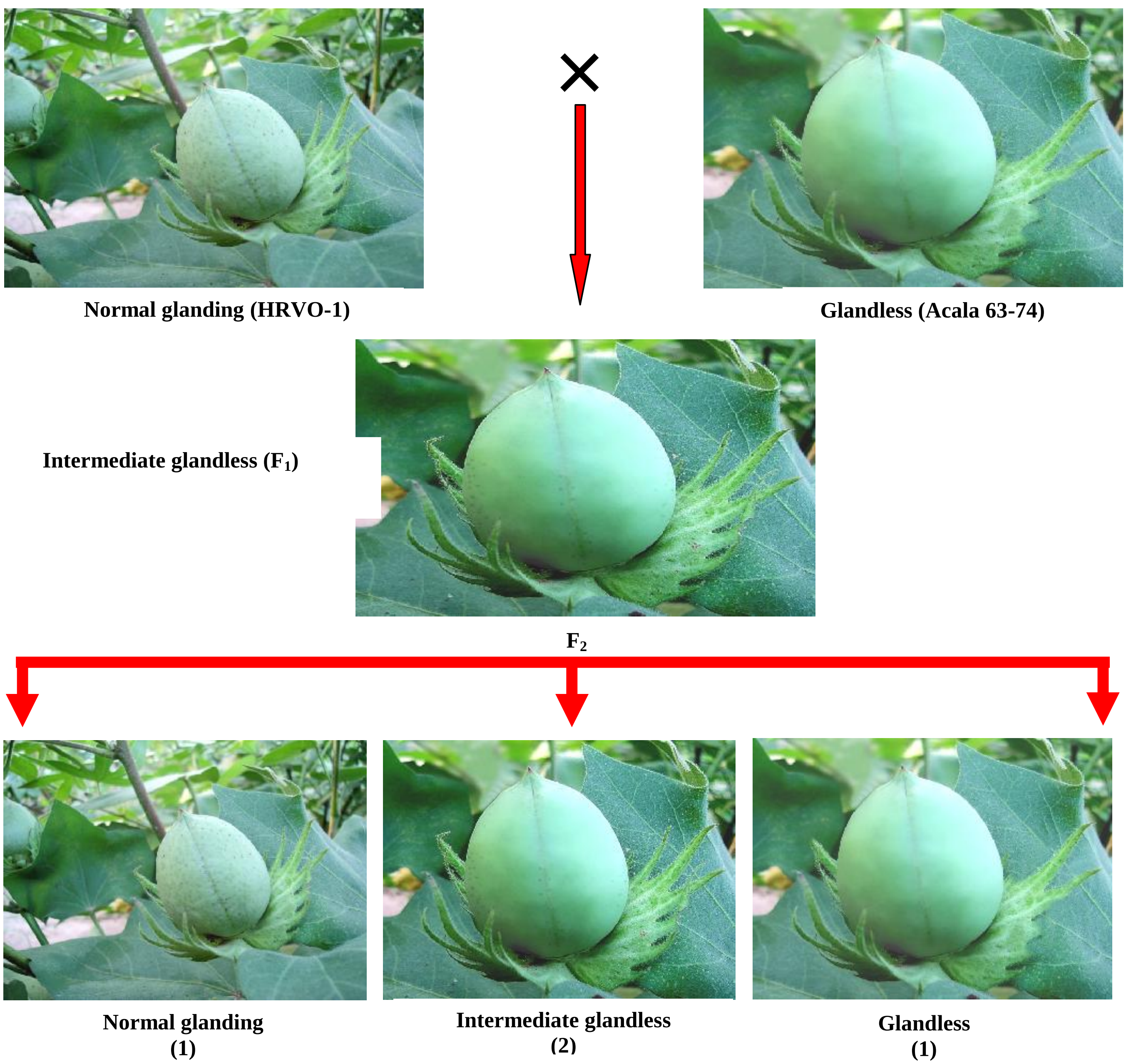


Fig. 3. Frequency distributions for number of leaf trichomes in F_2 generations of three crosses.



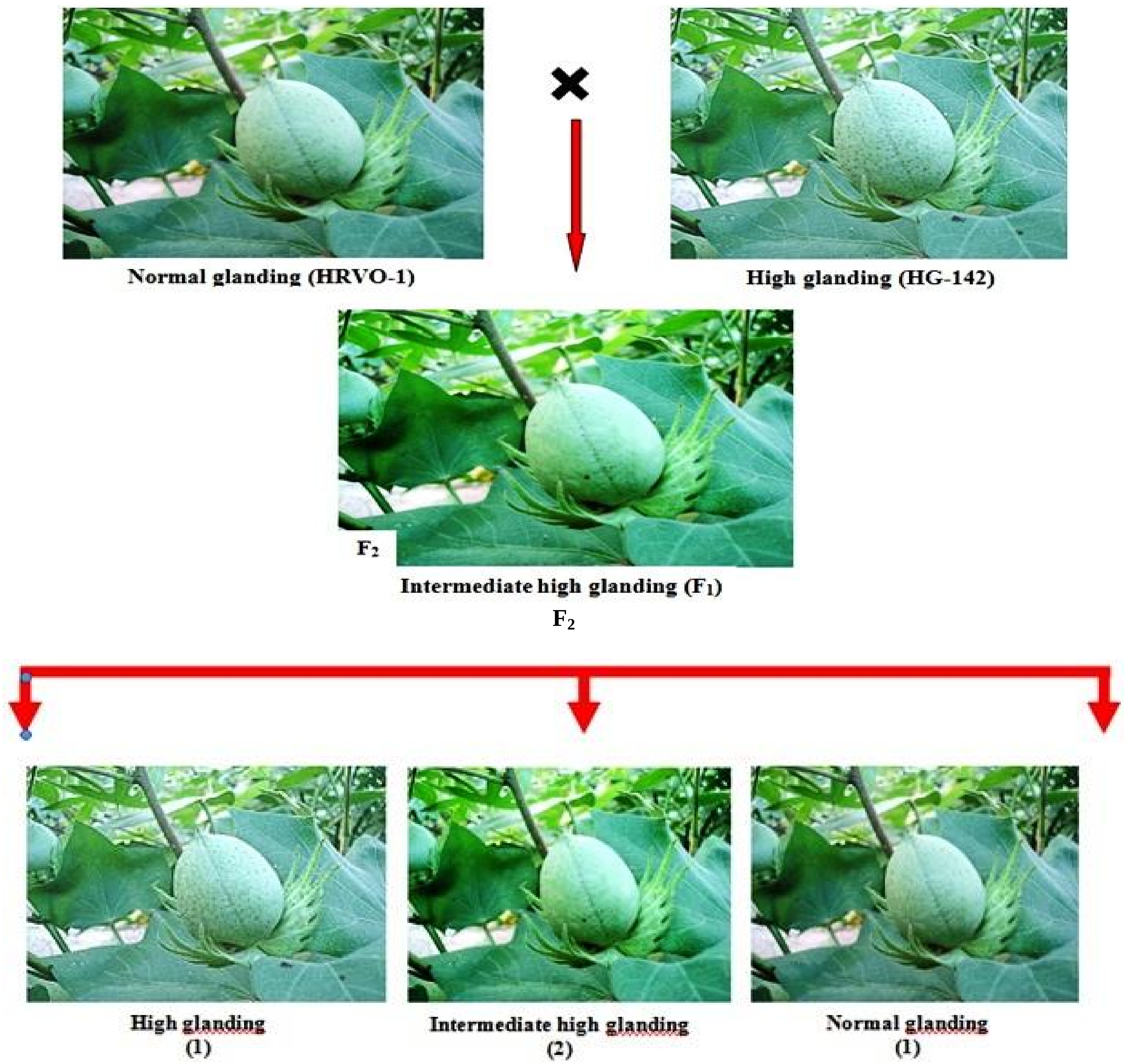


Fig. 5. Inheritance pattern in F_2 for the cross HRVO-1 × HG-142

Conclusions: From the present research it was easy to learn and confirm the inheritance pattern involved for okra leaf, number of trichomes and gossypol glands. The rating system devised for okra leaf morphology and trichomes is very easy, understandable and effective in classifying the genotypes. The previous studies conducted on the genetics of gossypols were based on visual observations which were misleading in depicting its mode of inheritance but the gossypol quantification based results are considered to be more reliable to understand the inheritance pattern involved for this trait.

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