AN ABSTRACT OF THE THESIS OF

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Title: The Expression and Inheritance of a Leaf Distortion in the Common Bean Phaseolus vulgaris L.

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The expression and inheritance of a leaf distortion of beans was studied in a group of the Oregon State University breeding lines and a commercial cultivar. Of seven parents involved, two were without distortion and classified normal, and the remainder were affected by various degrees of distortion. The distortion affected only the trifoliolate leaves and never appeared on the primary leaves. It consisted in a clearing of the leaf area between the veins. The clear region appeared to be a zone of slower growth compared to the normal region. The veins became distorted and the resulting leaf had an abnormal shape. Variations of the distortion were observed within and among lines.

Distorted lines were intercrossed and crossed with normal lines. Genetic data were obtained in the field and greenhouse as scores, using a 0 - 5 system where 0 indicated no distortion and 5 severe distortion. The scores of parental lines, the F_1, F_2 and backcross generations provided the base for interpretation of genetic ratios and calculation of heritability estimates. Reciprocal differences were observed in a cross between a severe and a mild parent. This cross could not be used for

inheritance study and estimation of heritability as computations necessitated pooling of reciprocals. The character appeared to involve one major locus with three alleles: one, A of 'G50', completely dominant over a of the distorted lines, and a' of 'Oregon 1604', recessive to a. Modifying genes acting in an additive manner were assumed to be responsible for the differences in degree of distortion found among the distorted lines. Heritability estimates of the distortion, based on regression of F_2 on F_1, and using three methods were 81, 72 and 85 percent.

Factors affecting the expression of the distortion were studied in a field experiment involving the effects of temperature, soil fertility and age of plants. The increase of any of these three factors decreased the degree of severity of the distortion. However, these effects were not evident on low-scoring plants.

In the greenhouse, groups of plants were grown in a warm lighted room, and in a colder room with no additional lighting. The distortion was most severe in the cold room. Plants grown in the warm room had low average distortion scores which were not significantly different from the scores of plants grown in the field.

No differences in endogenous auxin level could be detected between the normal and the distorted types when callus tissues from each type were cultured on solid media containing various concentrations of 2,4-D.

The Expression and Inheritance of a Leaf Distortion in the Common Bean *Phaseolus vulgaris* L.

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INTRODUCTION

The bean is a crop of world-wide importance. Most studies have been related to development of varieties which are higher yielding under various combinations of environmental conditions. The role of the leaf in yield production is especially important. Work has been or is being done on breeding bean cultivars for various leaf characteristics, such as leaf angle or leaf width. Well-known in many genera of plants is the occurrence of leaf abnormalities which involve unusual leaf coloration or leaf shape or both. An abnormality resembling injury caused by the herbicide 2,4-dichlorophenoxyacetic acid was found by Frazier *et al.* (1958) in progeny of crosses between 'Logan' bush cultivar and 'Blue Lake' pole cultivar where the bush type was the female parent. The distortion was thought to be cytoplasmic because it was not transmitted through backcrossing if 'Blue Lake' was used as female parent. However, the distortion, or a condition identical in appearance to that described by Frazier *et al.*, has continued to appear in lines involved in the Oregon State University bean breeding program. Its physiological cause is unknown. Certainly the character is undesirable in a commercial crop; therefore its elimination in the selection program is necessary. The genotype x environment interaction may disguise the character under some conditions, permitting it to be retained through part of the breeding process. The design of a proper experiment to determine factors affecting its expression and its mode of inheritance was considered worthwhile to facilitate the elimination of this character.

The objectives of the present study included:

1. Investigation of the effect of environment on the expression of the leaf distortion in several bean lines and cultivars.

2. Determination of the inheritance of the distortion.

3. Study of possible physiological mechanisms involved in the occurrence of such a distortion.

REVIEW OF LITERATURE

Types of Leaf Abnormalities in Beans

Normally, in beans, two types of leaves are borne on the mature plant: the primary leaves, simple and opposite, with a cordate base, and the trifoliolate leaves or secondary leaves, composed of three leaflets, and arranged in an alternate manner on the stem. The leaves are uniformly green in color, lighter or darker depending on the cultivar. The leaf surface is uniform, marked by three main veins issued from the base of the blade, and several veinlets forming a netted appearance. The general shape of a leaflet is deltoid ovate with acute tip and entire margin. Several authors reported cases that deviate in many respects from the normal type. These cases can be classified in two groups according to their origin: spontaneous abnormalities and induced abnormalities. Spontaneous leaf abnormalities in beans are of various types. One such type involves the shape and the size of the leaflet. Hofman (1928) observed in lines of the Navy bean (*Phaseolus vulgaris*) selected for uniformity, a fasciation of the primary and secondary leaves, and the occurrence of more than two cotyledons. Burkholder and Muller (1926) first reported a leaf abnormality which they termed "pseudo-mosaic" as it resembled the mosaic disease in appearance, but differed from it in that the leaflets were longer and narrower and tended to twist. Similar twisting of the developed leaflets have been mentioned by Parker (1934).

More common are those abnormalities which affect the color of the leaf. Such abnormalities involving a deficit in the production of chlorophyll in certain portions of the leaf are termed variegations. A typical picture of variegation is given by Parker (1934) who described it as "characterized by irregular patches of chlorophyll-deficient leaf tissue. All gradations from normal green through light green and yellowish green to pure white may be observed on a single leaf." Later (1942) the same author found in commercial planting of 'Wisconsin Refugee' and 'Idaho Refugee' of the same parentage, two types of variegation. One type affected the primary leaves and later the trifoliolate leaves on plants that had survived, and another type occurred only on the trifoliolate leaves. On the primary leaves, he observed the characteristic symptoms of variegation but with considerable variation, from absence of chlorophyll with only small islands of green and yellow tissue, to light yellowish green or deep yellow color with patches of dark green. On the trifoliolate leaves, he observed a sectored pattern where only a portion of the leaf was variegated and the remaining portion normal green.

Other authors alluded to the common bean mosaic when describing the variegation. Burkholder and Muller (1926) called "pseudo-mosaic" a situation where the leaf was dark green with light green or yellowish areas which appeared to be caused by an anastomosing of the veins and a production of less chlorophyll compared to the darker areas. Harrison and Burkholder (1936) observed on Wisconsin Refugee, which is immune to the common mosaic, symptoms different from that produced by the true disease. This new disease was characterized by a malformation of the true leaves accompanied by a mosaic pattern of yellow, green and dark green. Horsfall _et al_. (1937) called "one-sided mosaic" a new disease which they noticed in plantings of 'Idaho Refugee' and 'Wisconsin Refugee'. This disease affected only one side of a leaf, the other side being normal. Zaumeyer (1938) first reported in crosses involving 'Corbett Refugee' source of mosaic resistance, a case of variegation which he described as resembling mosaic, yet differing from it as attempts to infect healthy susceptible plants failed. The variegation ranged from absence of chlorophyll in the leaves (in which case the plants often died) to the formation of islands of variegation in plants that could reach maturity. Variegation also affected greatly the shape and the size of the leaflet. As in the case of one-sided mosaic the variegation may occur on one side of the leaflet. Zaumeyer (1942) noted that in this case, the leaflet became distorted and curled toward the affected portion as a result of a differing growth rate between the affected and the unaffected areas, the affected tissue growing slower than normal. This sectoring was also common in a variegated leaf situation called "mottle leaf" reported by Conroy and Wilson (1952). The symptoms appeared as a mottling of the primary and the secondary leaves. Although it resembled a virus disease, it was not transferrable to healthy plants. Coyne (1969) reported a similar case of variegation in which there was a sectored pattern or a mottled pattern on the trifoliolate leaves. Moreover, the severity of the variegation was determined by the time when it affected the leaflet; variegation occurring at an early stage in the leaflet development caused the leaf to become crinkled and distorted, while a late variegation resulted in more or near normal plants. Negative results were obtained when transmission of this virus infection-like abnormality was attempted through grafting.

Parker (1933) observed another type of chlorophyll-deficiency which he termed "yellow-spot". This abnormality was characterized by circular yellow spots on the primary and the secondary leaves. This character was variable, since in some cases spots coalesced and formed irregular patches and in other cases the spots were fewer. A similar deficiency was reported by Johansen (cited by Parker, 1933) as a bud mutation which resulted in a yellowish-green color termed "aurea". A variation of this abnormality was found by Smith (1934) in a selection of a cross between two bean cultivars, 'Robust' and 'Pink'; the mutants were pale, with leaves lighter colored than normal.

Abnormality is not always confined to the leaves but also may affect the growth of the plant. As Zaumeyer (1938) pointed out, extreme variegation stunted the affected plants. Among the "mottle leaf" types mentioned by Conroy and Wilson (1952) appeared rosette-form plants with short internodes and yellow leaves which they called "dwarfed mottle". Davis and Frazier (1964) also reported stunted plants in progenies of crosses between Phaseolus vulgaris cultivars. Similarly, Coyne (1965) observed aberrant plants termed "crippled" which were very stunted and had crinkled leaves, in the F_2 and F_3 populations derived from the P. vulgaris crosses Yellow Eye PI 209806 X 'GN #1', and 'Dark Red Kidney' X 'GN #1'.

The need for wider genetic variability, especially in a self-pollinating crop as bean has led to the application of external agents known to increase the mutation rate. Effects of mutagens on plants cover a wide range of modifications which may be external or internal, but only the modifications in leaf characteristics will be considered in this review. Mutagens have been grouped into physical and chemical types. Among the physical agents, ionizing radiations are most common. Genter and Brown (1941) used a Coolidge cathode X-ray tube to irradiate seed of field beans (P. vulgaris). The second generation of such irradiated material gave mutants with a large variety of leaf shapes and sizes including long and narrow "willow leaf" type, narrow primary leaves, thick leathery leaves, uni- to pentafoliolate, and rounded leaves. Chlorophyll abnormalities appeared as yellow, light green or white seedlings, mottled primary leaves, and mosaic appearance. Mutants showed various growth habits from dwarf, rosette-form to bushy plants, to slender vines. Chlorophyll mutations were most observed in materials treated with ionizing radiation. Swarup and Gill (1968) obtained yellowish and yellowish-green plants; Biserka (1971) reported yellowish, variegated, albino and dark green leaves, all different from the normal plants. Associated with chlorophyll-deficient types were puckered leaves, very small sized or large sized leaves (Swarup and Gill, 1968) or short plants with curled leaves (Biserka, 1971). In a series of experiments designed to study the mutation frequency and mutation spectrum induced by radiation, Moh and Alan (1965, 1970), Moh (1968), Moh and Nanne (1969) used various doses of gamma radiation and obtained bean leaf mutants which they called 'Yellow Mosaic', 'Wrinkled Leaf', 'Pepper Mutant' and 'Curly Leaf'. The yellow mosaic mutant had the appearance of a bean plant affected by bean yellow mosaic virus, with yellow chlorotic spots on the primary and the secondary leaves. In addition, the secondary leaves exhibited a narrower and more elongated shape than the normal leaf, and had a later development, a characteristic shared with the three other mutants.

Chemical mutagens form a large group of very diverse compounds producing diverse alterations on the genetic structure of the plant. The effect of ethylene oxide and oxygen and disodium salt of ethylenediamine tetraacetic acid (EDTA) was studied in leguminous plants other than Phaseolus vulgaris but similar reactions can be expected. Zacharias and Ehrenberg (1962) showed that ethylene oxide and high pressure oxygen produced leaf spots in Vicia faba and other legumes. Rancelis and Zemaitite (1971) reported that EDTA and other mutagenic factors induced spotted-leaves mutations in fodder beans.

Other chemicals were tested on bean plants but their genetical effects were not reported. Hofman (1927) in the course of his study on the effect of chloral hydrate on the production of aberrant types of beans, obtained a fasciation of the stem, petioles and leaves, a modification of the trifoliolate leaf symmetry and the production of various shapes, from uni- to pentafoliolate types, and of various sizes. Zimmerman (1943) called attention to the existence of many hormone-like compounds such as the substituted phenoxys, which induce modifications in the pattern of leaves and other organs when applied to the plant. Burton (1947) studied the effect of 2- and 4-chlorophenoxyacetic acid, and 2,4-dichlorophenoxyacetic acid (2,4-D) on bean leaves. The common reaction of these three compounds was the production of darker and smaller leaves than normal, with prominent light-colored veins. Leaves produced later were even smaller and narrower in plants treated with 2-chlorophenoxyacetic acid and 2,4-D; the veins and the midribs were fasciated and occupied a large portion of the leaf. In addition, 2-chlorophenoxyacetic acid produced narrow and straplike leaves with dark green and wrinkled margins. Watson (1948) in treating leaves of *P. vulgaris*, 'Red Kidney' with 2,4-D distinguished between three types of injury according to the degree of severity of the injury and the location of the leaves on the plant. Near the base of the plant occurred pale green or yellow leaves with a mottled appearance. Darker green tissue was observed in vein eyelets close to the larger veins and along the margin. A common feature of the injuries at the base was the constriction of the base of the leaflet where the veins come close and a deficit in chlorophyll. Injuries located on the upper part of the plant were characterized by narrow, linear to lanceolate leaves with prominent veins. A rugose condition of the blade was observed along with a narrowing of the base which turned pale green. However, no mottling occurred in these types of injury. The most severe of all of the injury types were found in the middle part of the plant. Greatly reduced leaf size and altered leaf shape, severe constriction of the base of the leaf and lack of chlorophyll were the common features of these injuries.

Natural chemical substances also induce leaf modifications when applied externally. Moh (1970) showed that cycasin[1] (methylazoxymethanol-β-D-glucoside) induced in the bean plant chlorophyll mutations including mottling and variegation, and morphological mutations such as wrinkled leaf and dwarf growth habit.

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[1]Cycasin is a chemical compound occurring naturally in cycads of the Cycadaceae family (Gymnosperms).

Genetics of Leaf Abnormalities

Inheritance of spontaneous leaf distortions is of several types but the characteristics are usually controlled by one or a few recessive genes. The pale characteristic was inherited as simple recessive (Smith, 1934). The mosaic-like variegation described by Coyne (1969) was also controlled by a single recessive gene; the F_2 generation from crosses between normal and variegated plants gave a 3:1 ratio of normal to variegated. Two complementary recessive genes were found to control the characteristic pseudo-mosaic described by Burkholder (1926) and the crippled morphology resembling virus symptoms reported by Coyne (1965). In each case the F_2 generation segregated 15 normal to one variegated. The variegation in progenies from crosses involving Corbett Refugee (Zaumeyer, 1938, 1942) was also due to two recessive genes. Variegated plants were believed to be homozygous recessive for both variegated genes, but the failure of those plants to breed true led the author to suggest the existence of inhibiting factors carried by these plants. Wade (1941) found that three recessive genes were involved in the inheritance of variegation, the normal character being controlled by three complementary dominant genes. Coyne (1966, 1967) suggested that variegation was essentially due to the action of two genes: an unstable gene which mutates to dominant in presence of a mutator gene. A third gene was expressed only in some environments.

Yellow spot character (Parker, 1933) was shown to be incompletely dominant, the F_1 plants being moderately spotted and the F_2 giving a 1:2:1 ratio. Intensely spotted plants were homozygous dominant for the factor yellow spot. This was one of the rare cases where dominance is involved in the inheritance of spontaneous leaf distortion. In other instances inheritance was due to cytoplasmic factors. Parker (1934) reported a variegation which was believed to be cytoplasmically determined as the F_1 progenies were similar to the female parent in a cross between a variegated leaf type and a normal plant. Normal F_1 plants when selfed produced only one plant with a slight variegation, which was thought to be the result of some cytoplasm coming into the egg with the male nucleus.

Artifically induced mutations, like those naturally occurring, are in general simply inherited. Radiation-induced leaf abnormalities (Moh, 1965, 1968; Moh and Allan, 1970) were controlled by a simple recessive gene. The mutants segregated in simple Mendelian ratios except in the case of the "pepper mutant" (Moh and Nanne, 1969) where a 3:1 ratio was not obtained due to a deficit of recessive mutants. It was assumed that the mutant gene could not be transmitted during fertilization due to the competition with the normal gene carried in the normal pollen.

The character unifoliolate leaves is due to a simple recessive uni. Plants manifesting this character may also show a few bifoliate and trifoliate leaves (Yarnell, 1965). Fasciation and opposite leaf insertion observed in the Navy bean by Hofman (1927) were not heritable variations as they appeared at a lesser frequency in the progeny of deviates than in the progeny of normal types. When these aberrant types were induced by treatment with chloral hydrate they persisted through six generations. However, they appeared to be non-heritable as the proportion of aberrant types decreased in each succeeding generation. The progeny of crosses between normal plants and induced aberrant types showed no modifications.

Influence of Environment on the Expression of Leaf Abnormalities

The character pseudo-mosaic observed by Burkholder and Muller (1926) varied considerably in intensity depending on the location and the year. In the greenhouse it almost disappeared whereas in the garden the abnormality was more severe when planting was late in the season. Light intensity affected the degree of yellow color of the mosaic pattern of the yellow mosaic mutant obtained by Moh and Alan (1965). The spots were bright yellow when the plants were grown outdoors and were pale yellow or even pale green when grown in the greenhouse under shade. The expression of the variegated rogue in *Phaseolus vulgaris* L. found by Coyne (1969) was related to temperature. At 27°C most plants were normal and only a few were slightly variegated. At temperatures of 16 and 21°C severe symptoms appeared on some plants while others had slight or no symptoms. This negative relationship between temperature and the mutant expression has been observed in various chlorophyll-deficient types reported in the literature (e.g. Sheridan and Palmer, 1977).

MATERIALS AND METHODS

Plant Materials

Origin and Description

Seven bean cultivars or breeding lines were used in the study. They are as follows:

'Oregon 1604' is a commercial cultivar from Oregon State University derived from the cross Oregon 190 x Oregon 58. It is a bush 'Blue Lake' type because its background involved crosses between bush cultivars and the pole bean 'Blue Lake'. It is classified normal with respect to the leaf abnormality.

'Gallatin 50' (G50) is a commercial bush cultivar, provided by the Gallatin Valley Seed Company, which originated as a white-seeded selection from 'Tendercrop'. This cultivar is also normal.

The remaining five lines are OSU breeding selections of bush 'Blue Lake' type.

The degree of abnormality among these lines range from mild to severe. The degree of distortion for all parents, based on preliminary observations in the greenhouse, is given in Table 1.

Characteristics of the Distortion

The leaf distortion under study is observed on bean breeding lines in the field and in the greenhouse. It is confined to the trifoliolate leaves and never appears on the primary leaves. Affected leaves show interveinal clearing (Figure 1) which appears light yellow, green yellow

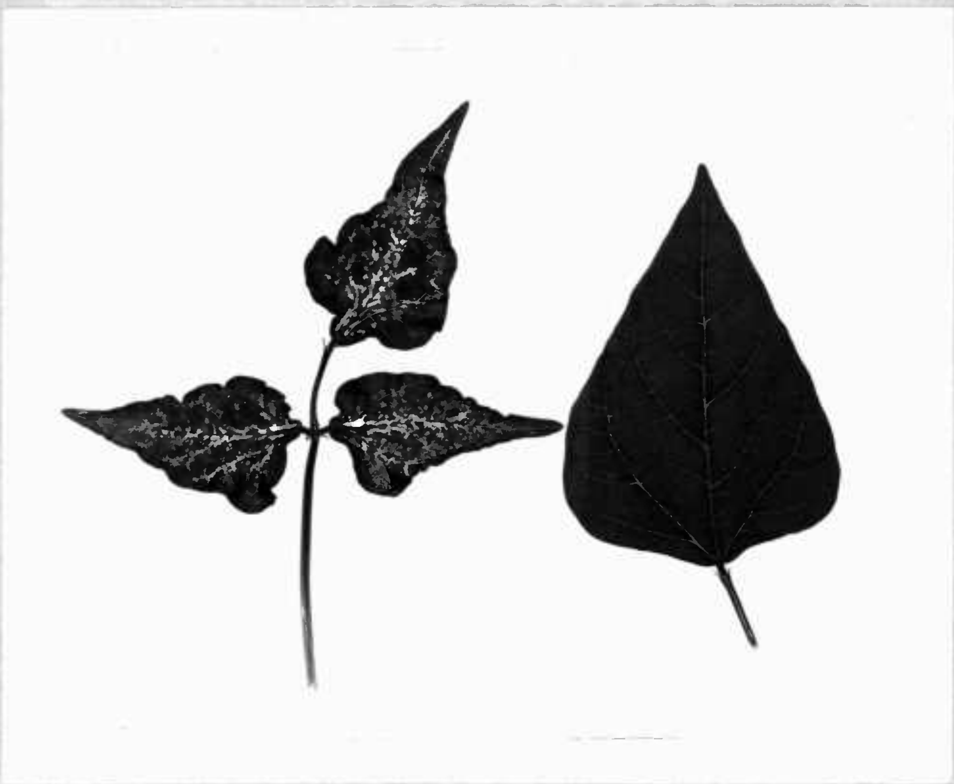

Figure 1. Severe distortion showing interveinal clearing. Left: severe; right: normal.

Table 1. Origin and distortion type of seven parental bean lines and cultivars.

Name	Parentage	Distortion class	Normal leaf color
'Oregon 1604'	Oregon 190 x Oregon 58	none	dark green
'Gallatin 50'	Tendercrop	none	light green
4035	OSU 2217-29 x 2718	slight	dark green
4607	1604-6 x B4840-14-1	slight	dark green
4622-1	1604-6 x 4840-14-1	moderate	dark green
4622-2	1604-6 x 4840-14-1	moderate	dark green
4594	1604-4 x 2313-1	severe	dark green

or even light green in the less affected types. The distortion resembles the effect of 2,4-D or similar herbicides or may resemble a mosaic disease as it assumes a mottled appearance. Later-formed leaves may be narrower and shorter than normal when fully expanded. The margins may be excessively curled but no puckering of the leaf surface occurs as in the mosaic disease. The transition between light and dark areas is not well-defined as in mosaic. The dark areas appear normal; the light green or yellowish areas appear thinner and even tend to be transparent in the severe cases.

Narrowing of the leaf base is characteristic of the distortion. The shape and the symmetry of the leaflet may be severely distorted due to the difference in growth rate between the green and the light areas, the latter growing more slowly. The growth habit of the plant is not affected as it is in the case of the true mosaic disease where stunting of the plant is frequent. The lighter areas take a much darker color upon aging. Gross variation in leaflet number and form was occasionally associated with the distortion (Figure 2).

Figure 2. Various leaflet shapes and numbers associated with distortion. Upper left: normal.

Expression of the distortion is variable, ranging from very mild to very severe. The very mild types show a slight discoloration at the base of the leaflets of compound leaves accompanied by a narrowing of the leaf base (Figure 3). The moderate types are characterized by a discoloration along the veins in addition to that at the base. In the severe types, the discoloration tends to cover the entire leaf surface. Considerable deformity may occur, giving rise to twisted leaflets smaller than normal while the leaf size of the mild and moderate types may not change (Figure 3).

Classification and Scoring System

Preliminary observations in the greenhouse showed that symptoms are best expressed when the plant is three to four weeks old, i.e. just before blooming time, when the plant has four to five true leaves. The degree of distortion varied slightly within lines. To eliminate subjectivity in scoring as much as possible, each plant was rated according to the greatest degree of distortion observed. Based on the extent of the surface discoloration and the distortion, scores from 0 to 5 were assigned to individual plants, 0 indicating the normal condition, 1 through 5 indicating the presence of distortion from very mild to very severe. The ratings are as follows:

Score	Description of the leaf	Classification
0	uniformly green, no defective shape	normal
1	discoloration at the base; leaf base narrower than normal	slight

continued

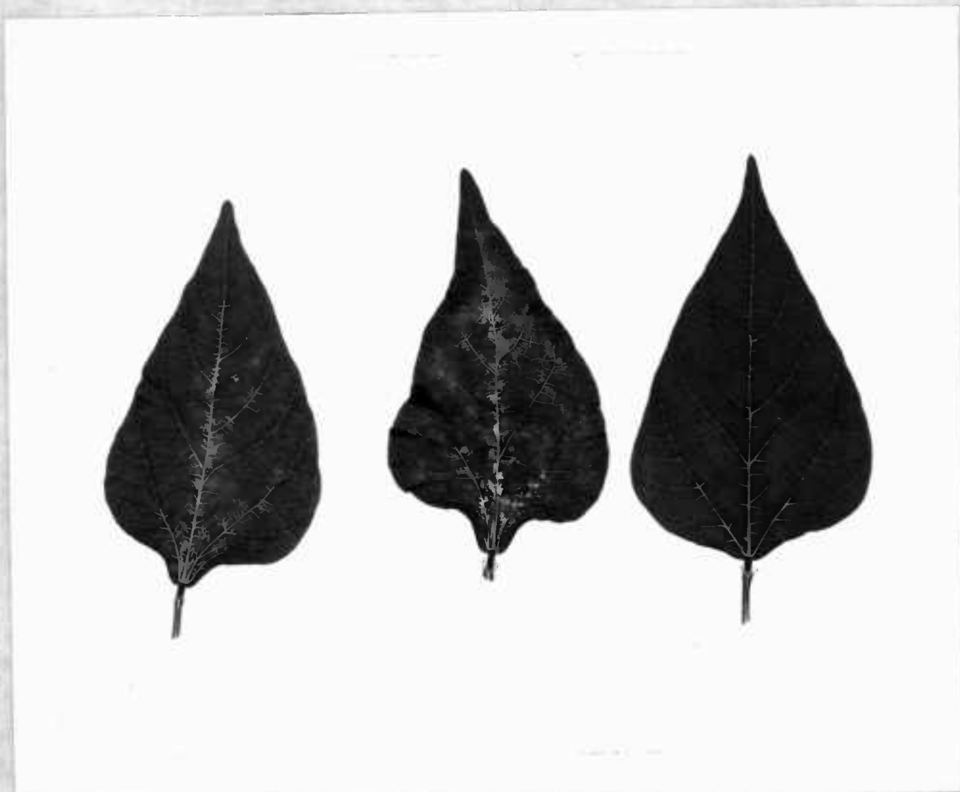

Figure 3. Mild (left) and moderate (middle) distortion as compared with normal (right).

2	discoloration at the base and continuing as thin lines or scattered spots along main vein; no severe distortion	moderate
3	same as 2 but more extensive around the main vein; slight distortion of veins and leaf shape	moderate
4	intense discoloration along primary and secondary veins; margin curled	severe
5	discoloration affecting the entire leaf except the margins; severely curled	very severe

Genetic Studies

To study the inheritance of the distortion, crosses were made between normal and distorted lines. Lines showing different degrees of distortion were also intercrossed.

Crossing Technique

The flower buds to be pollinated were emasculated one day before opening. The buds were opened with pointed forceps, the outer petals being kept on the flowers; the keel was opened and partially removed and the stamens inside were all removed with caution not to break or injure the pistil. Stigma surfaces were examined with magnifying glasses to ascertain absence of self-pollen. Prepared flowers were pollinated by rubbing on the stigma a pollen-covered stigma from newly opened flowers. Then, the outer petals were reclosed over the stigma and secured by tape to protect the stigma from desiccation and possible contamination.

In the field, during summer, moist tissue paper was wrapped around the pollinated bud and fixed with an adhesive band to preserve moisture.

The tissue paper was removed after five days to prevent pod-distortion. The forceps and the fingers of the manipulator were washed in ethanol after each operation to avoid mixing of pollen. In the greenhouse, during spring, crossing was made any time of the day as long as flowers were available. In the field, during the hot summer months, crossing was usually done in the morning from 8 a.m. to 10 a.m. Success of pollination ranged from 40 to 50 percent in the greenhouse and was much higher in the field. Crosses made for this study are listed in Table 9. For all the combinations listed, reciprocal crosses were made initially and in the backcrosses.

Chronology of Work

March - April 1976:	F_1 crosses made in the greenhouse
June - August 1976:	F_1 and parents planted in the field
	F_1 backcrossed to the parents in the field
September 1976:	F_2 and backcross seeds obtained from field plantings
December 1976:	F_2 populations planted in the greenhouse
January 1977:	F_2 plants scored in the greenhouse
March 1977:	F_1, F_2 and backcross progenies planted in the greenhouse
April 1977:	Scoring of F_1, F_2 and backcrosses in the greenhouse
May 1977:	F_1, F_2 and backcross progenies planted in the field
June 1977:	Scoring of F_1, F_2 and backcrosses in the field
	Second set of F_1, F_2 and backcrosses with checks planted
July 1977:	Scoring of second planting in the field

Studies on the Expression of the Distortion in Several Environments

Greenhouse Tests

A preliminary experiment was conducted in the greenhouse during the winter of 1975 and spring of 1976 to determine the best conditions for the greenhouse tests. Plant materials were grown in 3.78 liter plastic pots in a mixture of greenhouse soil and 5 g per can of a commercial fertilizer of formula 9.6 N - 12.7 P - 7.9 K. Plants were distributed in three different locations in the greenhouse. Each set of plants included all seven parents. The first set was placed in a warm room at a temperature of approximately 27°C, equipped with additional lighting; the second set was kept in the greenhouse hallway where day temperature fluctuated between 15° and 27°C during winter and early spring; the third set was grown in a 15°C room with no supplemental lighting.

Notes were taken as soon as the symptoms appeared on the trifoliolate leaves and continued at regular intervals until blooming time. Three plantings were made in the greenhouse, one in January, the second in February and the third in March, 1976. The symptoms appeared first in plants in the hallway followed by those in the warm lighted room whereas they appeared one month later on the most severe lines in the cold room, the other lines showing no symptoms. Since the distortion was poorly expressed in the cold room it was decided that subsequent studies would be done only in the warm room and in the greenhouse hallway. However, the best symptoms were obtained from the plants grown in the hallway and genetic studies were based on those plants.

Field Tests

In the field the experiment involved the combined effect of temperature and fertility level on the expression of the distortion. The experiment was conducted at the Vegetable Research Farm of Oregon State University, Corvallis, Oregon. Included in this study were normal cultivars, 'G50' and 'Oregon 1604' and line 4594 which was severely distorted in the greenhouse.

A band of fertilizer was applied under alternate rows prior to planting at the rate of 56 kg N, 74 kg P and 46 kg K per hectare. The other rows were left without fertilizer. Rows were one meter apart. Overhead sprinklers provided adequate irrigation during the growing season. Weeds were controlled by hand and by mechanical weeding. Control of environment was difficult to achieve in the field, but a differential in temperature was obtained by using white plastic tents and screen plastic shades over the plants (Table 4). These were placed just before the symptoms were expected to appear. The plants were observed at regular intervals from the four- or five-leaf stage up to blooming time.

Laboratory Tests

Since the distortion was similar to 2,4-D injury, a test was conducted in the laboratory to compare the reaction to 2,4-D of the distorted lines and the normal lines. Methods of tissue culture were used for the study. Tissues for culture were obtained from two normal lines 'G50' and 'Oregon 1604' and two distorted lines, 4622-1 and 4622-2.

The materials were prepared as follows. Two seeds of each line were surface-sterilized by immersion in ethanol for 3 minutes followed by treatment with sodium hypochlorite for five minutes and rinsing in sterilized distilled water. The seeds were then germinated in 125 ml Erlenmeyer flasks containing filter paper soaked with sterile water. When the hypocotyl attained a length of 2 to 3 cm, its middle portion was sliced into small disks 1.5 to 2.0 mm thick, before transfer to the culture medium.

The stock culture medium was composed of inorganic salts, sucrose, vitamins and growth factors. Its composition (Table 2) was that of the revised medium (RM-1964) for tobacco tissue cultures (Linsmaier and Skoog, 1965). Sucrose and myoinositol were added at concentrations of 30 g per liter and 100 mg per liter respectively. The concentration of kinetin was 5 μM; 2,4-D was tested at 1 μM and 5 μM. The pH of the medium was adjusted to 5.7 with 1N NaOH or 1N HCl. Agar to solidify the culture medium was added last at 30 g per liter. The medium was autoclaved at a pressure of 0.75-1 atm at 120°C for 15 minutes and distributed to 125 ml Erlenmeyer flasks.

Each flask containing 50 ml of the culture medium received three small cylinders of hypocotyl. The cultures were kept in the dark at 28°C. Callus growth was maintained by transferring healthy pieces of tissue from the previous culture on fresh medium after a 21-day period. At the end of the second passage the effects of increasing concentrations of 2,4-D were tested. The concentration of kinetin was 2.5 μM. Other constituents of the medium were at the same concentration as in the stock medium. The tissues were grown for 28 days at the end of which the fresh weight was measured.

Table 2. Composition of the stock culture medium for bean callus culture.

Compound	Concentration (mg/ℓ)
Inorganic salts[1]	
NH_4NO_3	1,650
KNO_3	1,900
H_3BO_3	6.2
KH_2PO_4	170
KI	0.83
$Na_2MoO_4 \cdot 2H_2O$	0.25
$CoCl_2 \cdot 6H_2O$	0.025
$CaCL_2 \cdot 2H_2O$	440
$MgSO_4 \cdot 7H_2O$	370
$MnSO_4 \cdot H_2O$	22.3
$ZnSO_4 \cdot 7H_2O$	8.6
$CuSO_4 \cdot 5H_2O$	0.025
Na_2 . EDTA	37.3
$FeSO_4 \cdot 7H_2O$	27.8
Organic constituents	
Vitamins	
Thiamine, HCl	1
Nicotinic acid	5
Pyridoxine	0.5
Myo-inositol	100
Growth factors	
2,4-dichlorophenoxyacetic acid	0.2 and 1
Kinetin	0.1
Carbohydrates	
Sucrose	30,000
Agar	10,000

[1]Constituent of the revised medium RM-1964.

RESULTS AND DISCUSSION

Effect of Environment

Comparison of Field and Greenhouse

The mean distortion scores of the parental bean lines grown in several environments are presented in Table 3. The variability among lines as shown by the value of the standard deviation was high in the low temperature (LT-GH) compared with that in the high temperature greenhouse (HT-GH) and in the field.

The mean score of plants grown in LT-GH was significantly higher than the means of plants from HT-GH and from the field. No significant difference existed between the means of plants from the latter two locations. Also, significant differences were only observed for the severely distorted lines 4622-1, 4622-2 and 4594. The two mild distortion lines 4035 and 4607 did not seem to be affected by the change of environment. The progenies from crosses between various distorted and normal lines followed the same pattern of variation. The mean and median of parents and progenies (F_1, F_2 and backcrosses) from four representative crosses are shown in Figure 4.

Field Experiment

In cloudy weather the temperature was at the same level under the screen shades and in the open field (Table 4). Under the plastic tents it was significantly higher than in the open in either sunny or cloudy weather. When it was sunny there was a significant difference between the three environments; the temperature was highest under the plastic

Table 3. Average distortion scores of parental lines in three environments.

Line	LT-GH[1] $\bar{x}_1$	$t_{(\bar{x}_1-\bar{x}_2)}$	HT-GH[2] $\bar{x}_2$	$t_{(\bar{x}_2-\bar{x}_3)}$	Field[3] $\bar{x}_3$	$t_{(\bar{x}_3-\bar{x}_1)}$
'OSU 1604'	0	-	0	-	0	-
'G50'	0	-	0	-	0	-
4035	0.26	1.29ns	0	1.02ns	0.3	0.32ns
4607	0.70	0.34ns	0.60	0.29ns	0.54	1.90ns
4622-1	1.81	2.11*	1.25	0.76ns	1.10	6.21**
4622-2	2.20	3.27**	1.39	-0.55ns	1.49	7.72**
4594	3.24	8.16**	1.27	0.59ns	1.17	18.04**
s	1.2611		0.6534		0.5997	

[1]LT-GH: low temperature greenhouse (hallway)

[2]HT-GH: high temperature greenhouse (warm room)

*significant difference at the 5% level of probability

**significant difference at the 1% level of probability

ns non-significant difference

Table 4. Mean temperatures (°C) obtained from three environments under two weather conditions.

	Plastic	Shade	Open	$LSD_{.05}$	$LSD_{.01}$
Cloudy	22.3	20.2	20.4	1.02	1.37
Sunny	33.2	29.8	30.9	1.13	1.52

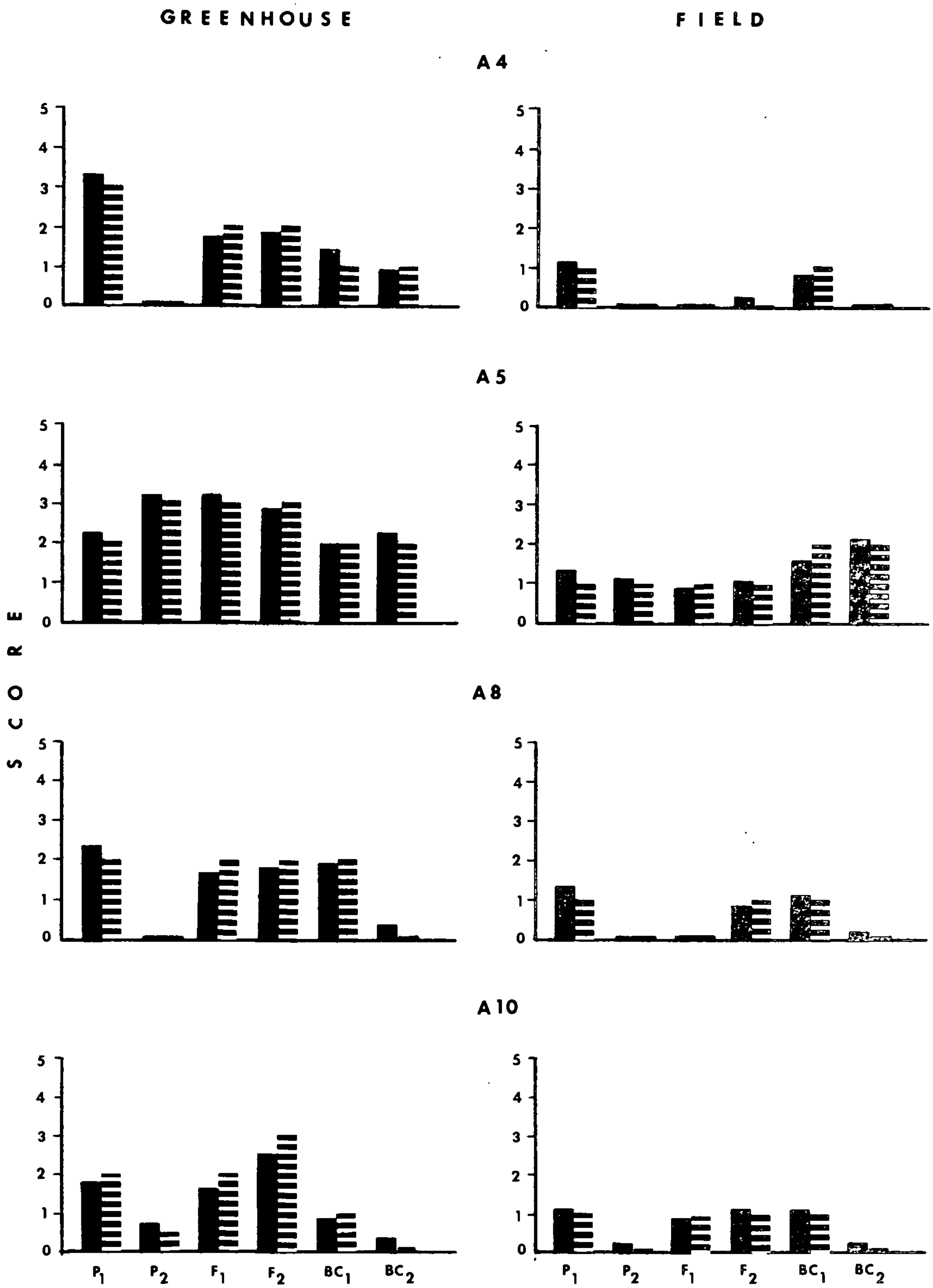

Figure 4. Mean ■ and median ☰ distortion scores of four crosses in the low-temperature greenhouse and in the field.

tents and lowest under the screen shades. However, temperature under the latter was not very different from that in the open field, as indicated by the five percent LSD (Table 4).

The effects of fertility level, temperature and date of observations (age of the plants at the time of observation) were studied. The analysis of variance (Table 5) shows that all three factors influenced the expression of the distortion, and that significant interaction existed between fertility level and temperature, and between temperature and age of the plants.

Tables of mean scores were used to examine the main effects and the interactions (Tables 6 and 7). The use of fertilizer decreased the severity of the distortion. Temperatures higher than those in the open field had the same effect. Aged plants were less distorted than young plants.

The average score for distortion increased significantly from fertilized to non-fertilized soil under the shade and in the open field whereas no significant difference was observed between fertilized and non-fertilized under the plastic tent where the temperature was the highest. Conversely the differences in average score for distortion were significant between the three temperature treatments for fertilized and non-fertilized soil, the lowest score being observed among the plants in the plastic tents and the highest in the open field.

Table 7 shows that the average distortion did not change significantly during the first three weeks of observation for the plants under the plastic tents, whereas it decreased significantly in the shade and in the open field as the plants grew older.

Table 5. Analysis of variance for influence of fertilizer, temperature, and date of observations on the expression of distortion.

Source	df	MS	F
Replication	3	0.1326	1.90
Treatment	23	3.2622	
Fertilizer	1	6.4067	91.92**
Temperature	2	17.5663	252.03**
Date	3	8.6663	124.34**
Fertilizer x Temperature	2	0.9282	13.32**
Fertilizer x Date	3	0.0636	<1
Temperature x Date	6	0.8826	12.66**
Fertilizer x Temperature x Date	6	0.0248	<1

**significant at 0.01 probability

Table 6. Effects of fertilizer and temperature treatment on mean distortion scores.

Treatment	Plastic tent	Screen shade	Open field	Mean for fertilizer
Fertilized	0.39	0.99	1.55	0.98
Non-fertilized	0.51	1.68	2.29	1.49
Mean for temperature	0.45	1.33	1.92	

Difference between interaction means: $LSD_{.05} = 0.19$ $LSD_{.01} = 0.25$

Difference between mean effects: Fertilizer: $LSD_{.05} = 0.11$

$LSD_{.01} = 0.15$

Temperature: $LSD_{.05} = 0.13$

$LSD_{.01} = 0.18$

Table 7. Effect of temperature treatment and date of observations on mean distortion scores.

Treatment	Week 1	Week 2	Week 3	Week 4	Mean for temperature
Tent	0.69	0.57	0.41	0.12	0.45
Shade	2.19	1.54	1.05	0.56	1.33
Open field	3.00	2.19	1.52	0.97	1.92
Mean for date	1.96	1.43	1.00	0.55	

Difference between interaction means: $LSD_{.05} = 0.26$ $LSD_{.01} = 0.36$

Difference between main effects: Temperature: $LSD_{.05} = 0.13$

$LSD_{.01} = 0.18$

Date: $LSD_{.05} = 0.15$

$LSD_{.01} = 0.21$

Genetic Studies

Behavior of Parents

Among the seven lines used for this study, two, 'Oregon 1604' and 'G50' never showed the symptoms of distortion. The other five lines expressed a range of distortion in the greenhouse as well as in the field. The 95 percent confidence interval of their mean was calculated. They were grouped into different classes with respect to the distortion based on whether their confidence intervals overlapped. Two lines were judged to belong to the same class on this basis (Table 8).

In the greenhouse, at low temperature, three distortion classes were identified:

mild: 4035, 4607

moderate: 4622-1, 4622-2

severe: 4594

Table 8. Confidence intervals (95% probability) of mean distortion scores of five distorted lines in three environments.

Line	LT-GH[1]	HT-GH[2]	Field
4035	0.103 - 0.417	0	0.187 - 0.413
4607	0.412 - 0.988	0 - 1.203	0.458 - 0.622
4622-1	1.526 - 2.094	0.998 - 1.502	1.021 - 1.179
4622-2	1.980 - 2.420	1.140 - 1.639	1.432 - 1.548
4594	2.968 - 3.512	1.016 - 1.523	1.092 - 1.247

[1]LT-GH: low temperature greenhouse

[2]HT-GH: high temperature greenhouse

The two lines 4035 and 4607 were grouped in the same class since their intervals overlapped slightly but were distinctly separated from the next higher class interval.

In the field, 4594, which was the most severe line in the greenhouse, was only moderately severe, and 4622-2, classified as moderately severe in the greenhouse, became severe. The two mild lines, 4035 and 4607, were very stable in all tests.

Behavior of the Progenies

Crosses were made in several ways, intercrossing normal and distorted lines or two distorted lines of the same class or of different classes. Originally, 11 combinations were made with their reciprocals. In one cross, A2, between a severe (4594) and a mild distortion line (4035) a reciprocal difference was apparent in that, when the severe parent was female, severity was generally increased. A chi-square of 101.67 (df = 11) was obtained in the comparison of reciprocals by contingency tables. This cross was not included in the calculation of genetic ratios and heritability estimates. In another cross, A7, between 4622-2 and 4035, there was some reciprocal difference which may have been due to error in classification. The chi-square was 12.02 (df = 9). This cross and the remaining nine crosses were used for the study of inheritance with their reciprocals combined.

Considering the wide range of degree of distortion existing among the different lines used in this study, it was necessary to consider the concept of penetrance which, in this case, is the observed proportion of the expected distorted types under a certain environment.

(1) Greenhouse Tests

Greenhouse data are listed in Table 9. Crosses tested were grouped into four categories based on the mean of the parents.

(a) Crosses between normal lines and severe or moderately severe lines: A3, A4, A6, and A8. Crosses A3 and A6 had a common parent, 'G50' which was always free from symptoms.

In cross A3, the second parent, 4594, was the most distorted line in the greenhouse with an average distortion score of 3.24 (Table 9). All plants tested showed the abnormality and were scored in classes 2 through 5. Due to problems of germination, only one F_1 plant reached maturity, the others being at seedling stage at the time of observation. This F_1 plant was free from abnormality (Table 10). The F_2 generation showed a high proportion of normal or zero-scoring plants (91 percent) and only a few plants (9 percent) were distorted with scores ranging from 1 to 3 (Table 10). This fit a theoretical ratio of 15 normal to 1

Table 9. Average distortion scores of parents and progenies in the greenhouse, April 1977.

Cross	P_1			P_2			MP	F_1	F_2	BC_1	BC_2
	Line	Class	Score	Line	Class	Score					
A1	4594	severe	3.24	4622-1	moderate	1.81	2.52	2.83	2.62		
A3	4594	severe	3.24	'G50'	normal	0	1.62	0	0.16		
A4	4594	severe	3.24	'Oregon 1604'	normal	0	1.62	1.75	1.79	1.42	0.83
A5	4622-2	moderate	2.20	4594	severe	3.24	2.72	3.25	2.57	2.22	3.03
A6	4622-2	moderate	2.20	'G50'	normal	0	1.10	0	0.20		
A7	4622-2	moderate	2.20	4035	mild	0.26	1.23	1.33	1.09		
A8	4622-2	moderate	2.20	'Oregon 1604'	normal	0	1.10	1.57	1.72	1.98	0.38
A10	4622-1	moderate	1.81	4607	mild	0.70	1.26	1.57	2.47	0.22	0.36
A11	4622-2	moderate	2.20	4607	mild	0.70	1.45	1.67	2.91		
A12	4607	mild	0.70	'Oregon 1604'	normal	0	0.35	0.75	1.13		

Table 10. Frequency distribution of distortion scores for greenhouse populations, 1977

Cross	Family	Low temperature greenhouse								High temperature greenhouse							
		Number of plants scored						Total	% distorted	Number of plants scored						Total	% distorted
		0	1	2	3	4	5			0	1	2	3	4	5		
A1	P_1	0	0	10	13	16	2	41	100	0	11	4	0	0	0	15	
	P_2	1	10	16	4	1	0	32	97 ± 6.0	0	6	2	0	0	0	8	
	F_1	0	0	4	6	2	0	12									
	F_2	1	16	56	38	22	8	141	99 ± 1.4	1	41	28	4	0	0	74	99 ± 2.6
A3	P_1	0	0	10	13	16	2	41	100	0	11	4	0	0	0	15	
	P_2	25	0	0	0	0	0	25	0	9	0	0	0	0	0	9	
	F_1	1	0	0	0	0	0	1									
	F_2	150	7	5	3	0	0	165	9 ± 4.4	38	45	4	0	0	0	87	56 ± 10.4
A4	P_1	0	0	10	13	16	2	41	100	0	11	4	0	0	0	15	
	P_2	41	0	0	0	0	0	41	0	0	10	0	0	0	0	10	
	F_1	0	3	9	0	0	0	12									
	F_2	54	45	72	42	16	10	239	77 ± 5.3	24	34	23	5	1	0	87	72 ± 9.4
	BC_1	2	11	10	1	0	0	24	92 ± 11.0								
	BC_2	24	16	11	2	0	0	53	55 ± 13.4								
A5	P_1	1	18	42	14	7	3	85	99 ± 2.3	0	11	4	0	0	0	15	
	P_2	0	0	10	13	16	2	41	100	0	11	7	0	0	0	18	
	F_1	0	0	1	1	2	0	4		0	11	24	13	0	0	48	100
	F_2	1	12	43	26	14	6	102	99 ± 1.9								
	BC_1	1	3	21	7	1	2	45	98 ± 4.4								
	BC_2	1	1	4	19	6	2	33	97 ± 5.8								
A6	P_1	1	18	42	14	7	3	85	99 ± 2.3	0	11	7	0	0	0	18	
	P_2	25	0	0	0	0	0	25	0	9	0	0	0	0	0	9	
	F_1	3	0	0	0	0	0	3									
	F_2	69	11	3	0	0	0	83	17 ± 8.1	68	12	0	0	0	0	80	15 ± 7.8

Table 10. Continued

Cross	Family	Low temperature greenhouse: Number of plants scored 0	1	2	3	4	5	Total	% distorted	High temperature greenhouse: Number of plants scored 0	1	2	3	4	5	Total	% distorted
A7	P_1	1	18	42	14	7	3	85	99 ± 2.3	0	11	7	0	0	0	18	
	P_2	23	8	0	0	0	0	31	26 ± 15.4	5	0	0	0	0	0	5	
	F_1	1	5	2	1	0	0	9									
	F_2	49	23	18	10	8	0	108	55 ± 9.4	5	62	16	0	0	0	83	94 ± 5.1
A8	P_1	1	18	42	14	7	3	85	100	0	11	7	0	0	0	18	
	P_2	41	0	0	0	0	0	41	0	10	0	0	0	0	0	10	
	F_1	0	3	4	0	0	0	7									
	F_2	48	59	75	29	21	6	238	80 ± 5.1	38	41	9	1	0	0	89	57 ± 10.3
	BC_1	1	8	23	2	1	1	36	97 ± 5.4								
	BC_2	26	11	2	0	0	0	42	31 ± 14.0								
A10	P_1	1	10	16	4	1	0	32	97 ± 6.0	0	6	2	0	0	0	8	
	P_2	15	10	4	1	0	0	30	50 ± 18.0	6	2	2	0	0	0	10	
	F_1	0	3	4	0	0	0	7									
	F_2	2	8	19	23	7	1	60	98 ± 4.5	2	54	30	3	0	0	89	98 ± 3.1
	BC_1	5	14	2	0	0	0	22									
	BC_2	17	2	3	0	0	0	22									
A11	P_1	1	18	42	14	7	3	85	99 ± 2.3	0	11	7	0	0	0	18	
	P_2	15	10	4	1	0	0	30	50 ± 18.0	6	2	2	0	0	0	10	
	F_1	1	1	3	1	0	0	6									
	F_2	3	3	10	5	4	9	34	91 ± 9.5	1	16	23	7	0	0	48	98 ± 4.0
A12	P_1	15	10	4	1	0	0	30	50 ± 18.0	6	2	2	0	0	0	10	
	P_2	41	0	0	0	0	0	41	0	10	0	0	0	0	0	10	
	F_1	1	3	0	0	0	0	4									
	F_2	68	64	49	15	4	1	201	66 ± 6.5	30	39	8	0	0	0	77	61 ± 11.0

distorted with P = 0.10 - 0.25 (X^2 = 2.27). The average score of 0.16 was approaching that of the normal parent. Based on this ratio, it appeared that the normal character was completely dominant over the distorted and that the difference was due to two major genes. However, the observed ratio was intermediate between 15:1 and 3:1 and it is possible that there was a single major gene difference with incomplete expression of distortion in the F_2 (X^2 for 3:1 was 21.30).

In cross A6 the distorted parent was 4622-2. This line was classified as moderately severe with a mean of 2.20 (Table 10). About 99 percent of the plants observed during the experiment were distorted with scores ranging from 1 to 5, the majority scoring 1, 2 and 3 (Table 10). The problem of F_1 survival arose also in this cross. Three F_1 plants were observed, none showing distortion. In the F_2 generation 69 plants were normal and 14 plants distorted (Table 10). This is a poor fit to the theoretical ratio 15 normal to 1 distorted (X^2 = 15.97) but gives a 3 normal to 1 distorted ratio with X^2 = 2.93 (P = 0.05 - 0.10). The F_2 population gave an average score of 0.20. The data from this cross resemble those from A3 except for F_2 ratio. It can be concluded that the two distorted lines 4594 and 4622-2 were of the same major genotype but differed in modifying factors which acted to suppress the distortion in the less affected parent.

In crosses A4 and A8 the normal parent was 'Oregon 1604' which remained symptomless throughout the study.

In cross A4, with 4594 as the distorted parent, all F_1 plants tested showed symptoms, with an average score of 1.75 which was close to that of the mid-parent (Table 10). The F_2 population was composed of 54 normal plants and 185 distorted, with scores in classes 1 through 5 (Table 10). The ratio of normal to distorted plants fit a theoretical ratio of 1:3 with a probability of 0.25 - 0.50. The F_2 population had an average distortion score of 1.79 which was not significantly different from that of the F_1. Mean scores suggest one gene difference between the normal and the distorted parent with additive gene action involved in the inheritance of the distortion. However, additivity was not apparent in the frequency distribution of the F_2 population due possibly to the arbitrary nature of the classification system. The normal plants which accounted for 25 percent of the population were grouped in a single class, whereas the severely distorted ones which should constitute another 25 percent fell in classes 3, 4 and 5 (Table 10). This resulted in an asymmetrical distribution which was skewed in the direction of the normal class (Figure 5). Backcrossing the F_1 to the severely distorted parent (BC_1) resulted in 92 percent distorted plants (Table 10), but the average distortion score of 1.42 was lower than that of the F_1. This apparent dominance of the normal character in BC_1 is not consistent with F_1 and F_2 behavior. Backcrossing to the normal parent (BC_2) gave 55 percent distorted plants, the remaining being normal (Table 10). The proportion of distorted to normal plants fit a 1:1 ratio with a probability of 0.25 - 0.50. The average distortion score of 0.83 was intermediate between those of the F_1 and the normal parent. These backcross results, for the most part, confirm the one-gene hypothesis with additive gene action. The low score obtained in BC_1 may be due to the presence of some 0-scoring plants, which was unexpected.

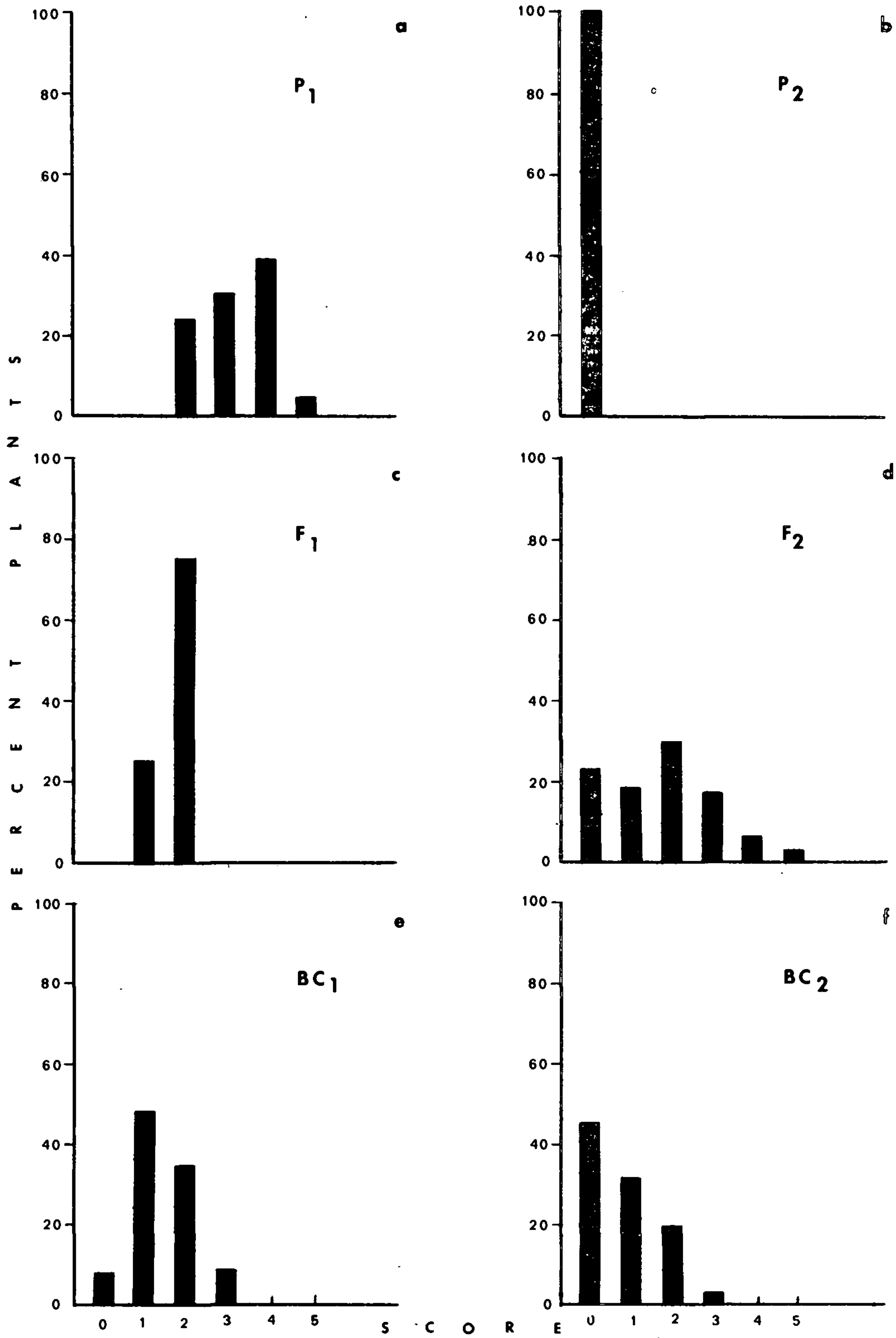

Figure 5. Frequency distribution of greenhouse distortion scores for parents and progenies from cross A4 (4594 x 'Oregon 1604').

In A8 where the distorted parent was 4622-2, all F_1 plants were distorted and gave an average score of 1.57 which was closer to that of the distorted parent than that of the normal one (Table 9). The F_2 generation produced 48 normal plants and 190 distorted plants scoring 1 through 5 with a large proportion in the mild classes (Table 10). The average score in the F_2 was 1.72 which was not significantly different from that of the F_1 ($t_{.05}$ = 0.30 with df = 243). Observed ratio in the F_2 fit a theoretical ratio of 1 normal to 3 distorted with a probability of 0.05 - 0.10. The backcrosses of the F_1 to the distorted parent (BC_1) gave 35 distorted out of 36 observed plants (Table 10). It had an average score of 1.98, intermediate between the F_1 and the distorted parent, which suggests additivity. The backcross progeny of the F_1 to the normal parent (BC_2) did not satisfactorily fit the 1:1 ratio because of the proportion of normal plants being higher than expected (Table 10). This gave a mean distortion score of 0.38 which approached that of the normal parent. The graphic representation of the frequency distribution of parents and progenies is shown in Figure 6. The results from this cross are very similar to those obtained from A4. Mean scores of progenies seemed to indicate additive gene action. However, it was not possible to obtain a 1:2:1 ratio in the F_2 due to the continuous nature of severity differences. By grouping the distorted plants into one group, a ratio of 1 normal to 3 distorted was obtained, which suggested dominance of the distortion over the normal condition.

Thus, when a classification of normal versus distorted was used, the distortion was dominant over the normal condition, but when mean scores were used, the difference between normal and distorted was

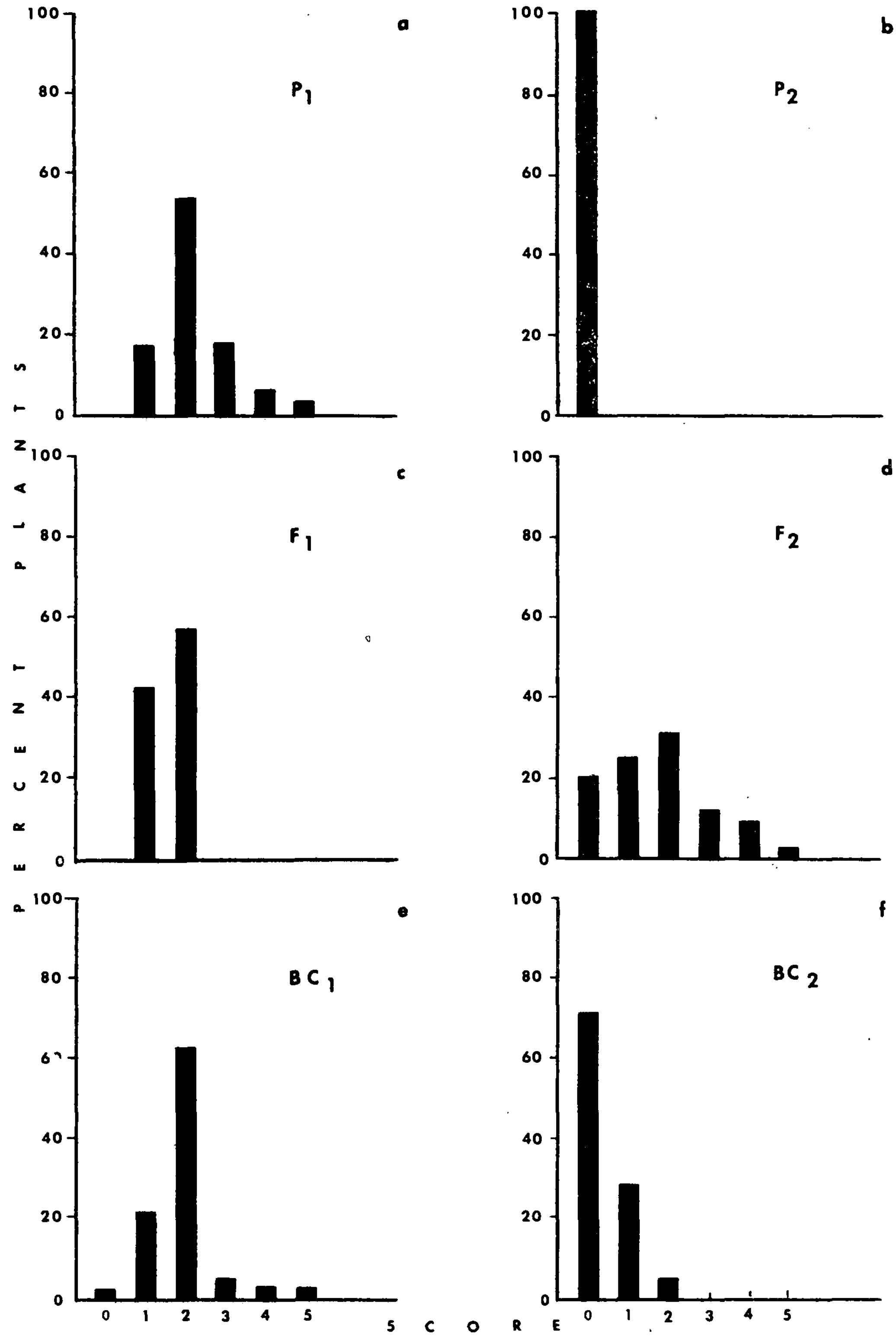

Figure 6. Frequency distribution of greenhouse distortion scores for parents and progenies from cross A8 (4622-2 x 'Oregon 1604).

additive in nature.

(b) Cross between a normal line and a mild line: A12. The normal line was 'Oregon 1604'. The mild distortion parent was 4607 with an average score of 0.70 (Table 9). Only one out of the four observed F_1 plants was symptomless (Table 10). The F_2 generation gave an average score of 1.13 which exceeded that of the distorted parent (Table 9). In the F_2 population 68 plants were normal and 133 distorted scoring 1 through 5, being more concentrated in classes 1 and 2 (Table 10). The ratio of normal to distorted did not fit a theoretical 1:3 ratio due to the high number of normal plants which indicates a low penetrance of the distorted genotype.

(c) Crosses between mild and severe lines: A7, A10, A11. In cross A7 the severe line was 4622-2 and the mild line 4035. Most plants of 4035 did not show distortion. Although this line could be heterogygous it is more likely that expression is inconsistent in such very mild types. Most F_1 plants were distorted and had an average score of 1.33, close to that of the mid-parent. The ratio of 1 normal to 1 distorted (X^2 = 0.93) observed in the F_2 was not considered to support any particular genetic ratio. This is to be expected in view of the incomplete expression of distortion in the mild parent.

Crosses A10 and A11 had a common parent, the mild distortion line 4607. No distortion was expressed in 50 percent of the plants. In cross A10 the severe parent was 4622-1 which had a mean score of 1.81. About 97 percent of the plants showed distortion and were in classes 1 to 4 (Table 10). In the F_2 population, no distinct segregation of the character was observed. The pattern of distribution in the F_2 was similar to that of the severe parent 4622-1 (Figure 7). About 97 percent of the observed F_2 plants were distorted, scoring from 1 to 5 but most of the plants scored 2 and 3 (Table 10). This gave an average score of 2.48 which was higher than that of the severely distorted parent (Table 9). The backcross progenies apparently followed the distribution patterns of their respective parents (Figure 7), however, due to the low number of individuals observed (Table 10) no firm conclusion can be drawn from the results.

In cross A11 the severe parent was 4622-2 with a mean score of 2.20. All observed F_1 plants but one were distorted with a mean score of 1.62 which was higher than that of the mid-parent (Table 9). In the F_2 population 94 percent of the plants showed symptoms.

(d) Crosses between two severe lines: A1 and A5. In A1 (4594 X 4622-1), all F_1 plants were distorted and gave an average score of 2.83 (Table 9). In the F_2 population the mean score was 2.48 (Table 9) with 98 percent of the plants showing the symptoms (Table 10).

In A5 (4622-2 X 4594), all F_1 plants were distorted, with an average score of 3.25 which was similar to that of the most severe parent. About 99 percent of the F_2 plants were distorted with an average score of 2.57 (Table 9). This behavior was similar to that of the F_2 population from A1. The backcross progeny to the first and second parent yielded respectively 98 percent and 100 percent of distorted plants. Patterns of distribution of the parent populations and the progenies are shown in Figure 8. Results of intercrossing distortion lines of different severity thus indicate that the lines are of the same genotype with respect to the major gene locus hypothesized,

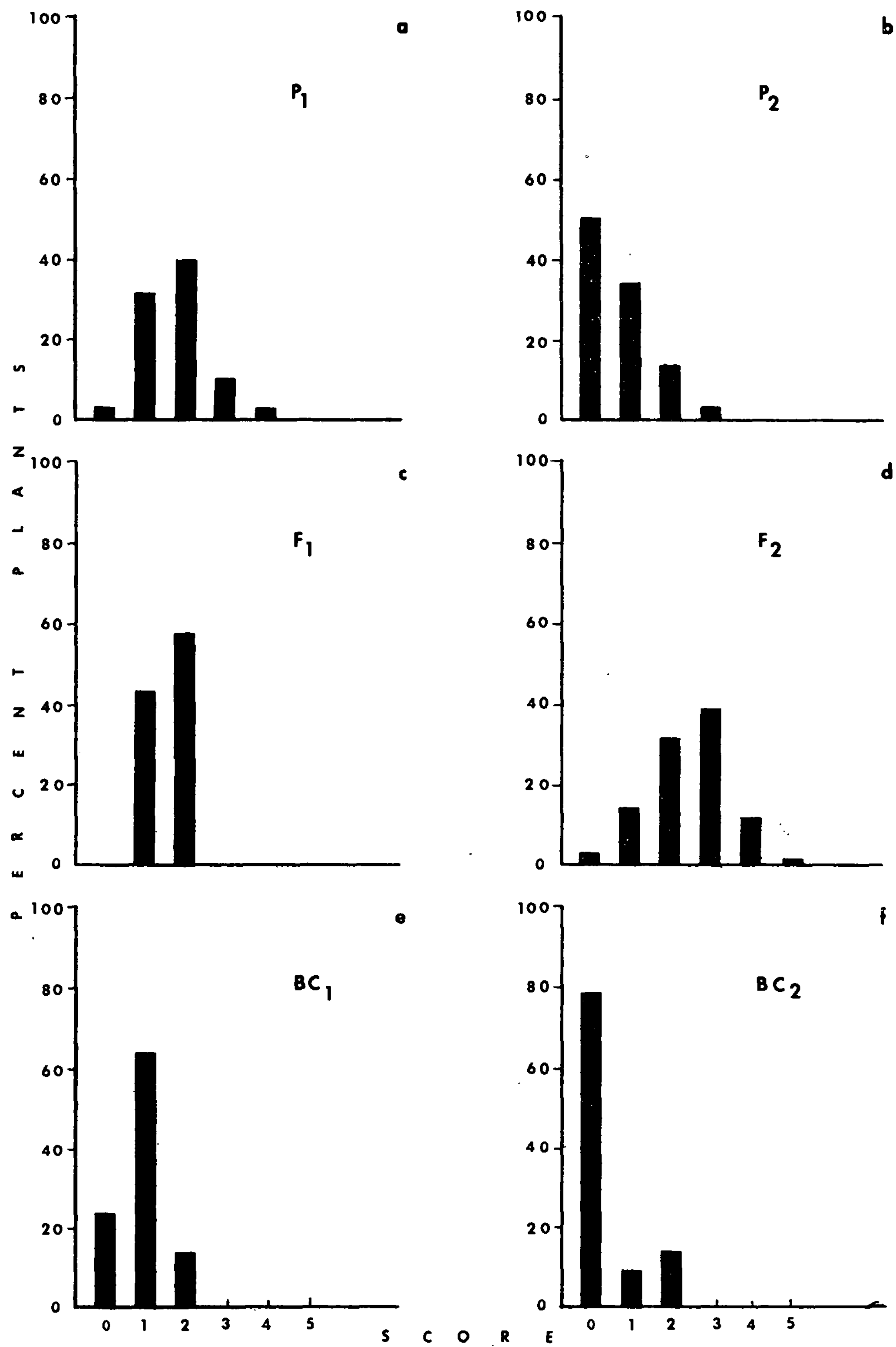

Figure 7. Frequency distribution of greenhouse distortion scores for parents and progenies from cross A10 (4622-1 x 4607).

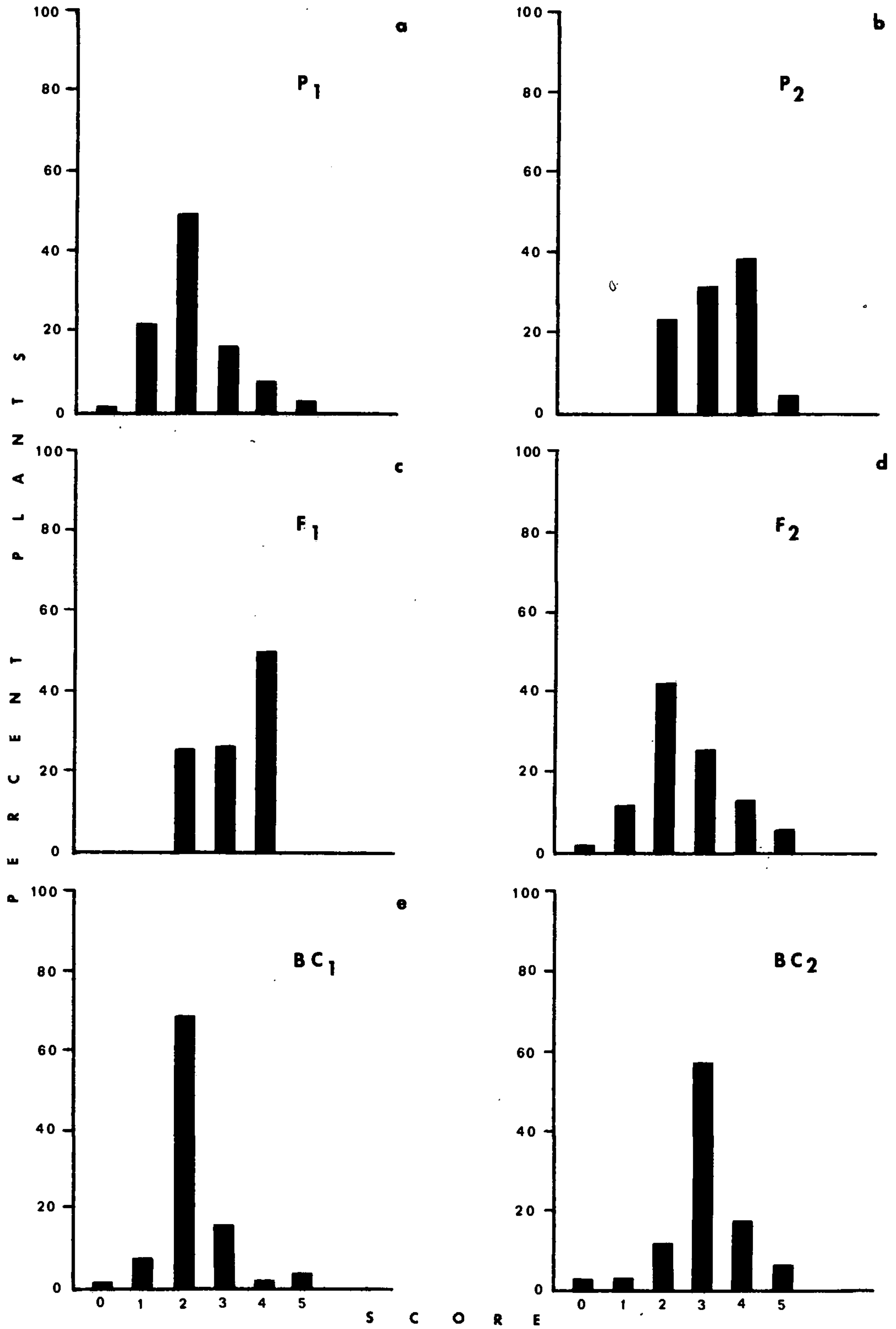

Figure 8. Frequency distribution of greenhouse distortion scores for parents and progenies from cross A5 (4622-2 x 4594).

but differ in modifying or minor genes.

(2) Field Test

In the field, there was generally less expression and lower severity of distortion. Few plants were observed in the distortion classes 4 and 5, the majority scoring in the lower classes. None of the parental lines had 100 percent incidence of distortion (Table 12). Three major distortion classes could be identified: a mild class with 4035 and 4607, a moderate class with 4622-1 and 4594, and a severe class with 4622-2. The four types of crosses observed in the greenhouse were also tested in the field (Table 11).

In crosses between normal and severe or moderately severe lines (A3, A4, A6, A8) all observed F_1 plants were normal. In the F_2 populations there were more normal plants than distorted, except in cross A8 where the distorted plants slightly predominated. However, in all four crosses, the frequency distribution by distortion scores is strongly skewed in the direction of the normal parent. This skewness is further expressed in the distribution of the backcross progeny in A8 and A4 (Figures 9 and 10). These distributions suggest dominance of the normal condition over the distorted. However, no genetic ratios could be derived from the data except for the 'G50' crosses (A3 and A6). In these cases a ratio of 15 normal to 1 distorted was approached in the F_2 with a chi-square of 14.15 for A3 and 16.32 for A6. The apparent dominance in the remaining crosses was considered as resulting from the incomplete penetrance of the distorted genotype under field conditions. Allowing for the lower expression of distortion in the field, data

Table 11. Average distortion scores of parents and progenies in the field, May and June 1977 combined.

Cross	P_1			P_2			MP	F_1	F_2	BC_1	BC_2
	Line	Class	Score	Line	Class	Score					
A1	4594	moderate	1.17	4622-1	moderate	1.10	1.13	0.66	0.89		
A3	4594	moderate	1.17	'G50'	normal	0	0.58	0	0.11		
A4	4594	moderate	1.17	'Oregon 1604'	normal	0	0.58	0	0.54	0.83	0.01
A5	4622-2	severe	1.49	4594	severe	1.17	1.33	0.9	1.09	2.12	1.57
A6	4622-2	severe	1.49	'G50'	normal	0	0.74	0	0.12		
A7	4622-2	severe	1.49	4035	mild	0	0.30	0.23	0.51		
A8	4622-2	severe	1.49	'Oregon 1604'	normal	0	0.74	0	0.79	1.16	0.19
A10	4622-1	moderate	1.10	4607	mild	0.54	0.82	0.92	1.13	1.05	0.45
A11	4622-2	severe	1.49	4607	mild	0.54	1.02	0.9	1.34		
A12	4607	mild	0.54	'Oregon 1604'	normal	0	0.27	0	0.52		

Table 12. Frequency distribution of distortion scores in populations tested in the field, May and June 1977 combined.

Cross	Family	Number of plants scored 0	1	2	3	4	5	Total	% distorted
A1	P_1	22	189	51	3	4	0	269	92 ± 3.3
	P_2	13	149	21	4	1	0	188	93 ± 3.6
	F_1	6	4	2	0	0	0	12	
	F_2	274	519	97	33	2	0	925	70 ± 2.9
A3	P_1	22	189	51	3	4	0	269	92 ± 3.3
	P_2	261	0	0	0	0	0	261	0
	F_1	11	0	0	0	0	0	11	0
	F_2	786	68	9	4	0	0	867	9 ± 5.3
A4	P_1	22	189	51	3	4	0	269	92 ± 3.3
	P_2	314	0	0	0	0	0	314	0
	F_1	21	0	0	0	0	0	21	
	F_2	574	219	106	20	2	0	921	38 ± 3.1
	BC_1	52	40	27	2	0	0	121	57 ± 7.2
	BC_2	150	2	0	0	0	0	152	1 ± 1.8
A5	P_1	45	382	196	46	9	0	678	93 ± 1.9
	P_2	22	189	51	3	4	0	269	92 ± 3.3
	F_1	3	8	2	0	0	0	13	
	F_2	62	349	84	11	0	0	506	88 ± 2.8
	BC_1	0	24	67	40	0	0	131	100
	BC_2	2	29	30	3	1	0	65	97 ± 4.2
A6	P_1	45	382	196	46	9	0	678	93 ± 1.9
	P_2	261	0	0	0	0	0	261	0
	F_1	14	0	0	0	0	0	14	
	F_2	831	72	10	3	2	0	918	10 ± 1.9
A7	P_1	45	382	196	46	9	0	678	93 ± 1.9
	P_2	102	20	5	3	0	0	130	21 ± 7.1
	F_1	21	4	1	0	0	0	26	
	F_2	623	242	78	30	3	0	976	36 ± 3.0
A8	P_1	45	382	196	46	9	0	678	93 ± 1.9
	P_2	314	0	0	0	0	0	314	0
	F_1	16	0	0	0	0	0	16	
	F_2	337	371	86	27	6	1	829	59 ± 3.3
	BC_1	34	24	29	9	1	0	97	65 ± 9.5
	BC_2	91	22	0	0	0		113	20 ± 7.3

continued

Table 12. Continued

Cross	Family	Number of plants scored						Total	% distorted
		0	1	2	3	4	5		
A10	P_1	13	149	21	4	1	0	188	93 ± 3.6
	P_2	118	91	13	1	0	0	223	47 ± 6.6
	F_1	2	22	0	0	0	0	24	
	F_2	139	483	127	41	12	0	802	83 ± 2.6
	BC_1	16	56	19	1	0	0	92	83 ± 7.7
	BC_2	54	17	8	1	0	0	80	32 ± 10.3
A11	P_1	45	382	196	46	9	0	678	93 ± 1.9
	P_2	118	91	13	1	0	0	223	47 ± 6.6
	F_1	2	6	1	0	0	0	9	
	F_2	62	221	103	36	9	2	433	86 ± 3.3
A12	P_1	118	91	13	1	0	0	223	47 ± 6.6
	P_2	314	0	0	0	0	0	314	0
	F_1	12	0	0	0	0	0	12	
	F_2	490	272	44	19	3	1	829	67 ± 3.2

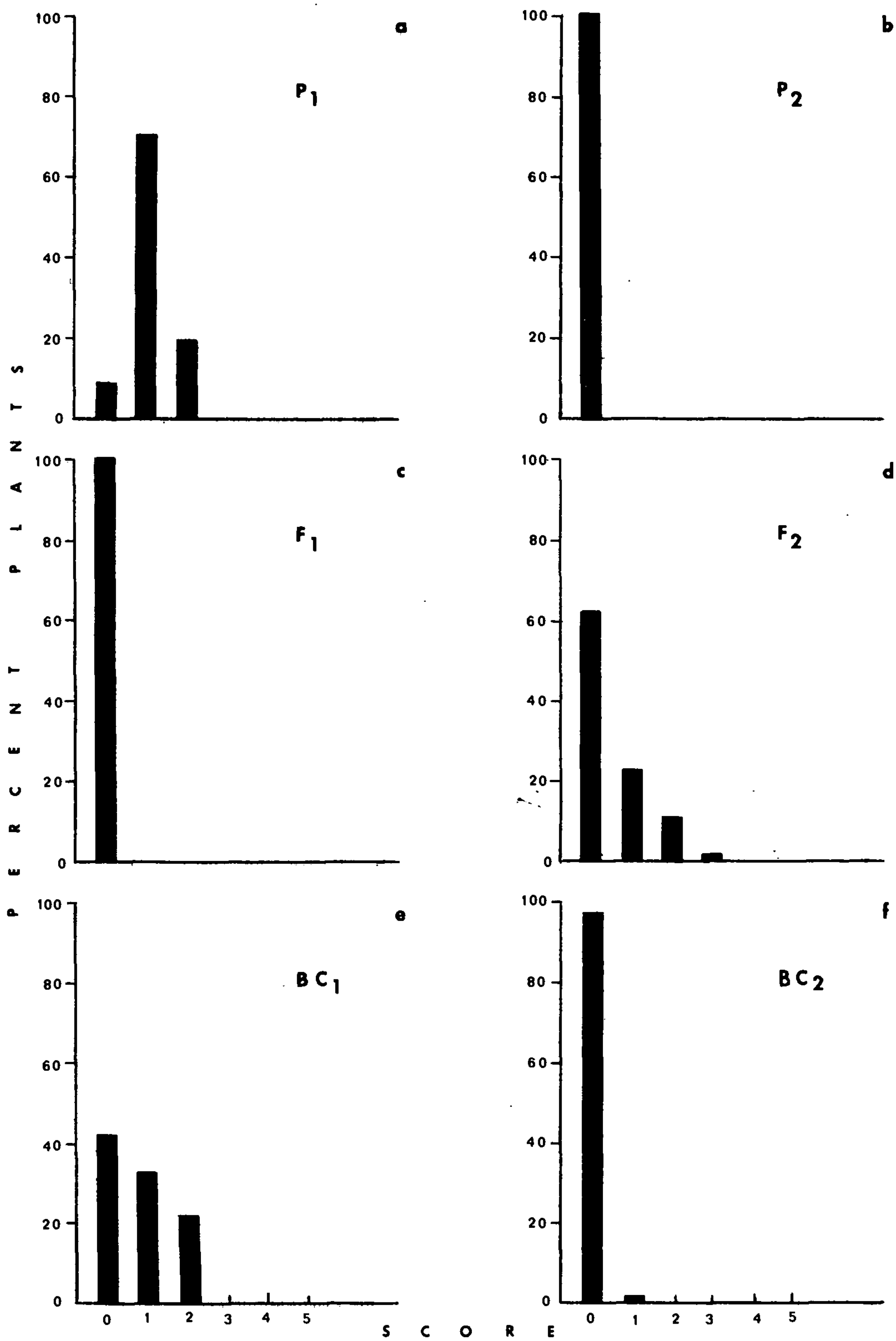

Figure 9. Frequency distribution of field distortion scores for parents and progenies from cross A4 (4594 x 'Oregon 1604').

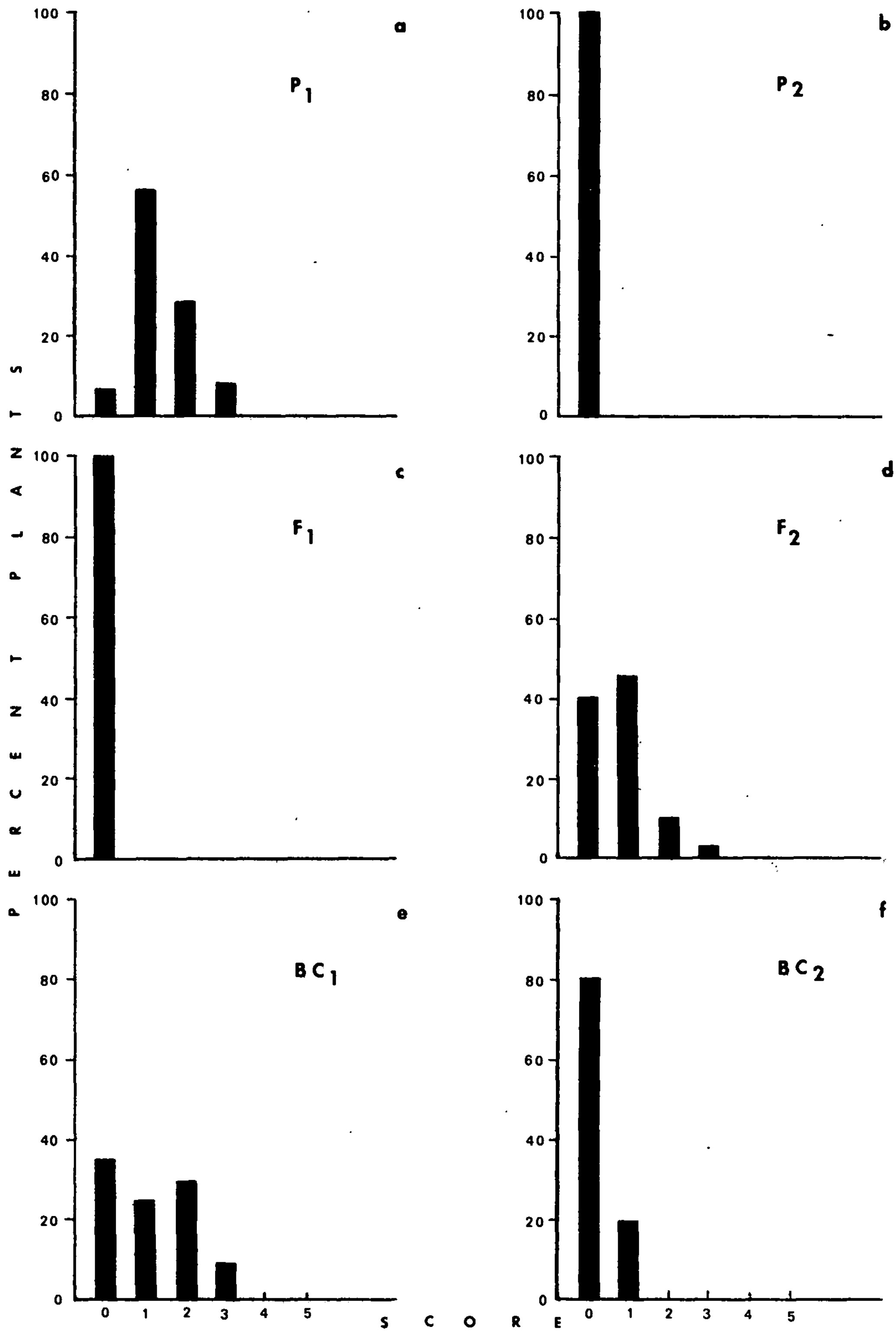

Figure 10. Frequency distributions of field distortion scores for parents and progenies from cross A8 (4622-2 x 'Oregon 1604').

obtained from the field support the greenhouse results with respect to the relative behavior of 'G50' and 'Oregon 1604' crosses.

In the cross between a normal and mild line (A12) all the F_1 plants were normal. The F_2 population had a higher number of normal than distorted plants and gave an average distortion score as high as the mild parent. The distorted plants were spread over all the five classes of distortion but the majority scored in class 1 (Table 12). These results also suggest dominance of the normal character due to the incomplete expression of distortion in the field.

The third type of cross, between a mild line and a severe line included A7, A10 and A11. In the F_1, normal and distorted plants were both observed but with a higher porportion of distorted plants in A10 and A11. This distribution of normal and distorted plants was further emphasized in the F_2 population which had a distribution similar to that of the severe parent (4622-1 for A10 and 4622-2 for A11). In A7 the proportion of normal plants exceeded that of distorted in the F_1 and F_2 generations. The same remarks made for the first and second types of crosses apply for this cross.

The crosses between severe or moderate lines (A1 and A5) involved 4594, common parent to both crosses, 4622-1, the second parent for A1 and 4622-2 for A5. They showed respectively 92, 93 and 92 percent distortion and had about the same distribution (Table 12). The F_1 in both crosses had an average score lower than that of both parents. In the F_2 a high proportion of distorted plants was observed but not as high as in the parents (Figure 12).

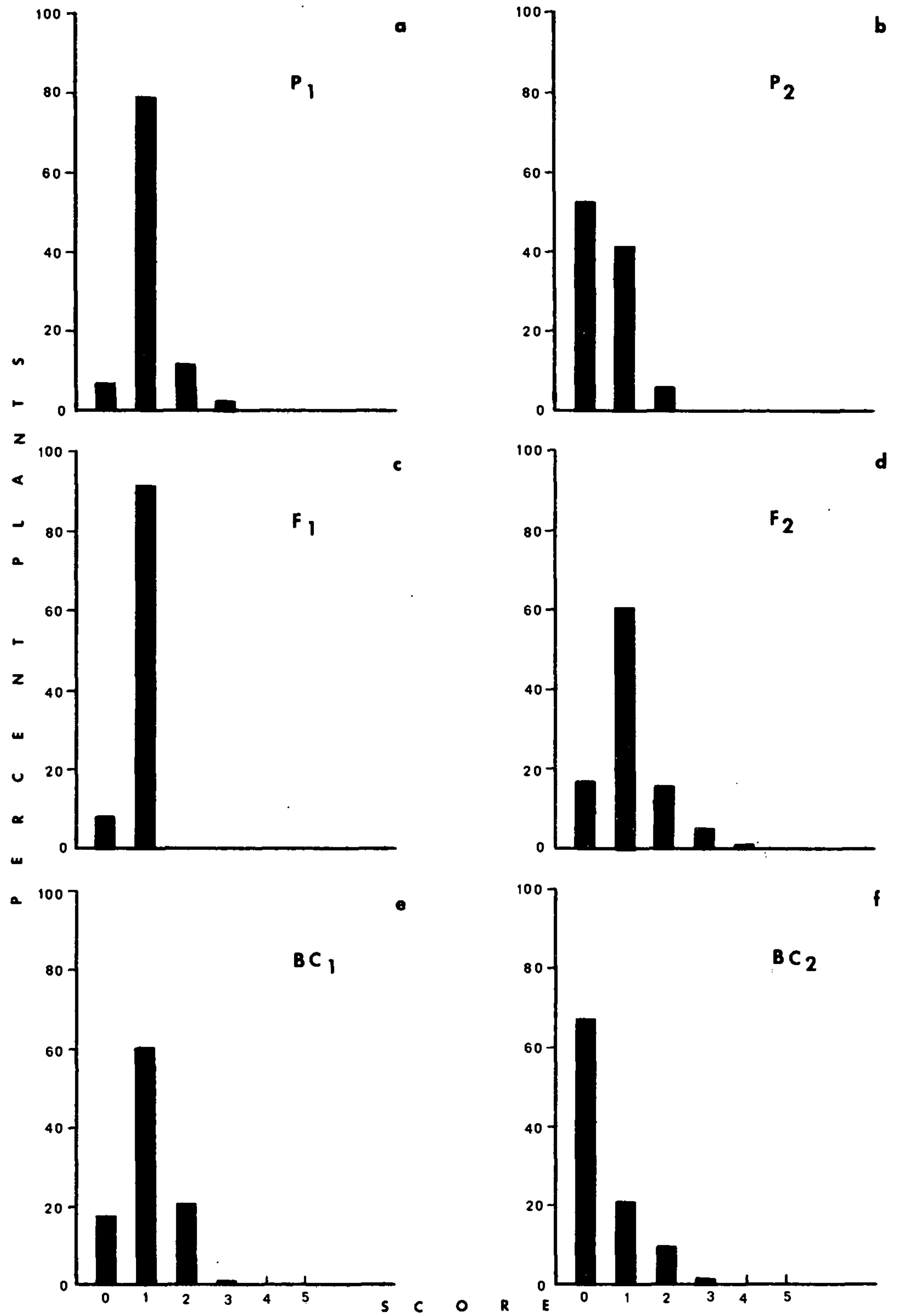

Figure 11. Frequency distribution of field distortion scores for parents and progenies from cross A10 (4622-1 x 4607).

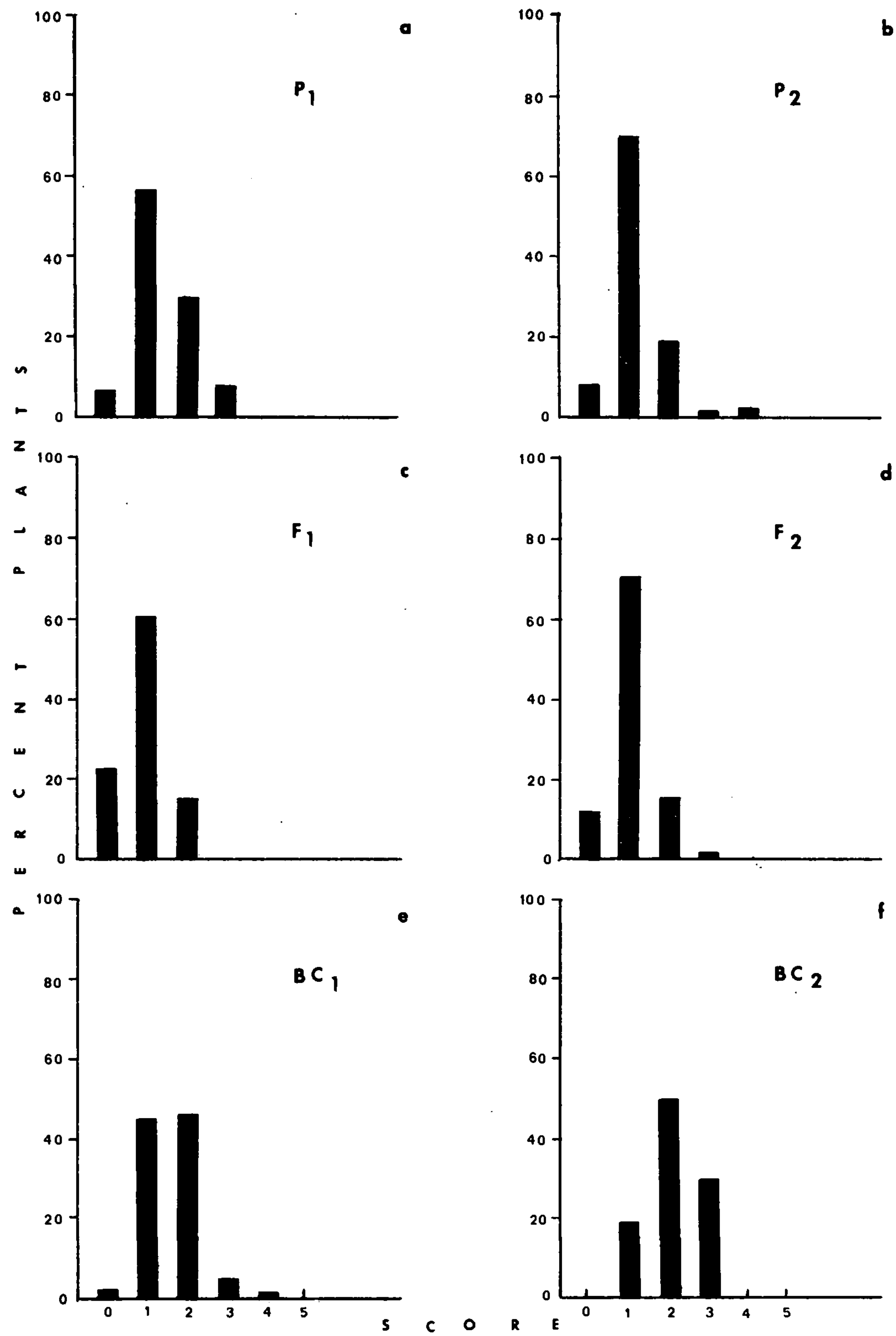

Figure 12. Frequency distribution of field distortion scores for parents and progenies for cross A5 (4622-2 x 4594).

Heritability Estimates

Because of the low expression of the distortion in the field, the heritability estimate was based only on data obtained from the greenhouse. Heritability represents the proportion of the total variance due to the additive effect of genes. It is the heritable portion of the total variance, which determines the degree of resemblance between parents and offspring and is expressed by the ratio V_A/V_P where V_A is the breeding value and V_P the phenotypic value (Falconer, 1960). The phenotypic value can be measured directly as the individual whereas the breeding value is only determined by the influence of this individual on the next generation. The regression of offspring on the mid-parent is a direct estimate of the ratio V_A/V_P (Falconer, loc. cit.). In a self-pollinated population the regression of offspring on parent is an appropriate estimate of heritability if the parent is non-inbred (Smith and Kinman, 1965). This applies for the regression of F_2 on its F_1 parent. Mahmud and Kramer (1951) suggested the use of regression in self-pollinated plants in terms of percent deviation from the mean and proposed the formula

$$h^2 = \frac{\bar{x}}{\bar{y}} b_{yx} \times 100$$

where $\bar{x}$ = mean of the earlier generation

$\bar{y}$ = mean of the later generation

b_{yx} = the regression of y on x.

Where heritability estimates greater than one are obtained, Frey and Horner (1957) suggested the formula of heritability in "standard units":

$$h^2 = b_{yx} \frac{sx}{sy}$$

which is identical to the correlation coefficient.

Each of these formulae was applied to estimate the heritability of the distortion character (Table 13). Heritability estimates based on the regression of the F_1 on the mid-parent were above 100 percent except for the estimate in standard units which was 72.8 percent. Such estimates greater than 100 percent may result from unpredictable environmental variations such as temperature changes and daylight intensity which cause parents and progeny (F_1) to express the distortion differently. The estimation of heritability in standard units reduces such environmental effects. Based on the regression of the F_2 on the F_1 heritability values were 80, 69 and 85 percent. These estimates are relatively high, perhaps because of the influence in the regression calculations of the 'Oregon 1604' and 'G50' crosses.

The precision of the estimate of heritability is indicated by its standard error (Table 13). The standard error is that of the regression coefficient and is the square root of its variance. The variance of the regression coefficient is given by the formula:

$$s^2_b = \frac{1}{n-2} \left(\frac{s^2y}{s^2x} - b^2\right)$$

where n = number of paired observations of x and y

x = the value of the parent

y = the value of offspring

s^2x and s^2y = the variances of x and y respectively

b = the regression coefficient of y on x.

Table 13. Estimates of heritability of the distortion by regression of F_1 on mid-parent and regression of F_2 on F_1 using three methods.

	Methods		
	Falconer[1]	Mahmud & Kramer[2]	Frey & Horner[3]
Regression of F_1 on MP	110.7 ± 85.03[4]	112.5	72.8
Regression of F_2 on F_1	81.0 ± 40.0	71.6	85.5

[1] h^2 estimated by regression coefficient: $h^2 = b_{yx} \times 100$

[2] h^2 estimated by percent deviation of regression coefficient from the mean:

$$h^2 = \frac{\bar{x}}{\bar{y}} b_{yx} \times 100$$

where $\bar{x}$ = mean of parent
$\bar{y}$ = mean of offspring

[3] h^2 estimated in units of standard deviations:

$$h^2 = \frac{sx}{sy} b_{yx} \times 100$$

where sx = standard deviation of parent
sy = standard deviation of offspring

[4] Confidence intervals with 95 percent probability

The regression of the F_2 on the F_1 yielded more precise estimates of heritability than the regression of F_1 on mid-parent.

Tissue Culture

Good callus growth was obtained in cultures grown on a stock medium containing 5 µM of 2,4-D. Cultures grown on 1 µM 2,4-D medium could not be maintained because rapid browning of tissue occured.

'G50' developed voluminous white soft callus. The remaining three lines formed comparatively small and firm callus.

Subcultures were tested on five concentrations of 2,4-D: 0.1, 0.3, 1, 3 and 10 µM. Callus tissues derived from 'G50' continued to have a higher growth rate compared with those from the remaining three lines. However, optimal growth was attained at the same level of 2,4-D for all four genotypes (Figure 13). This behavior indicates that there was no significant difference in their level of endogenous auxins.

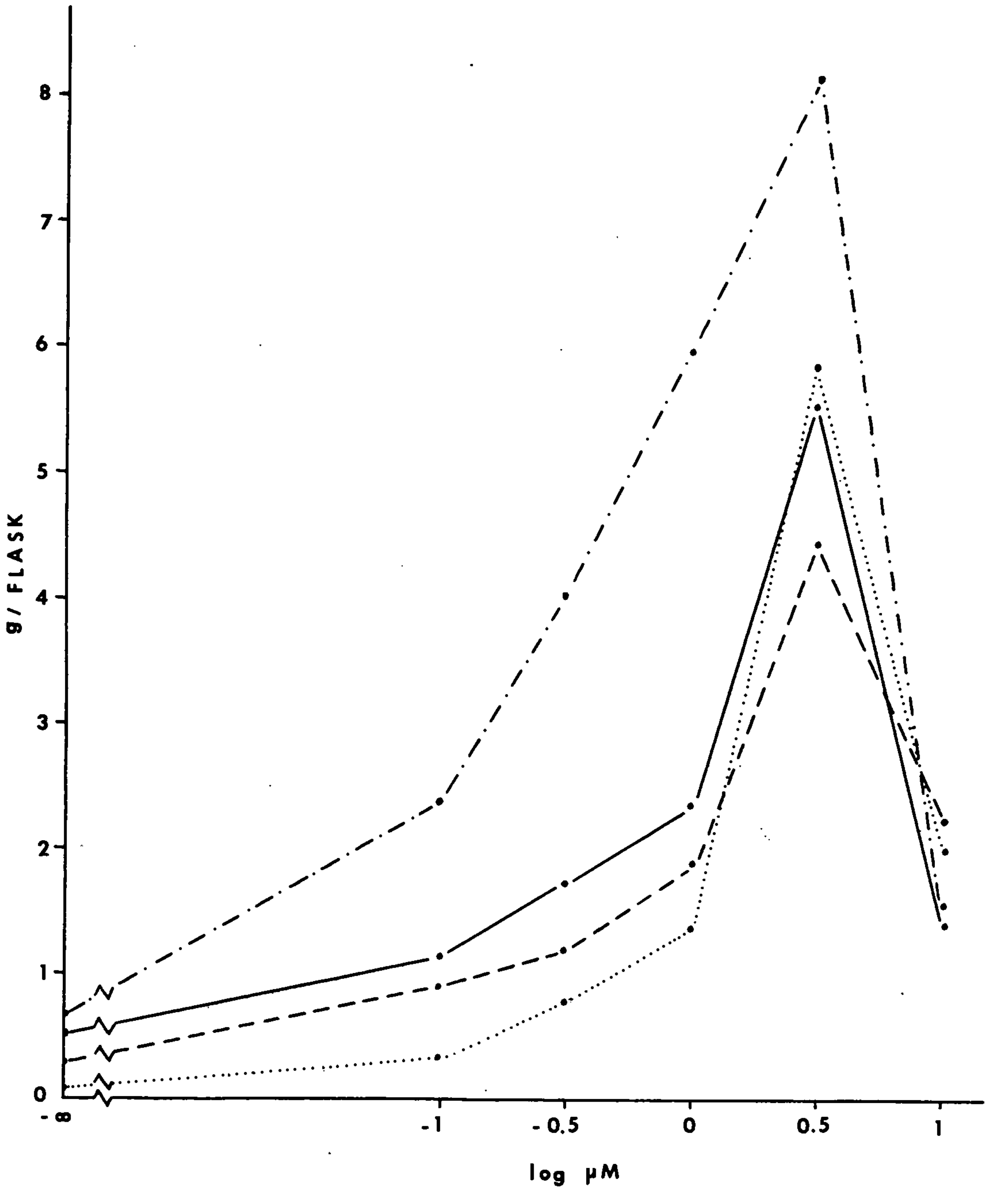

Figure 13. Growth responses of 'Oregon 1604' (·····), 4622-1 (———), 4622-2 (— — —) and 'G50' (—·—·—) to increasing concentrations of 2,4-D. Weight scale reduced by half for 'G50'.

GENERAL DISCUSSION

Inheritance of the Distortion

The idea of quantitative and qualitative characters becomes ambiguous when an abnormality with the general characteristics of a qualitative character is inherited in a quantitative manner. By definition, quantitative characters are those measurable and which usually exhibit a continuous variability. These characters are under the control of polygenes, with slight phenotypic effects, and are particularly susceptible to environmental influences. Qualitative characters are typically controlled by few genes and differences between individuals are discontinuous. However, it is well recognized that no sharp delineation exists between quantitative and qualitative characters. In the leaf distortion under study, a continuous variation was observed, so that distinction between normal and distorted leaves became difficult in the very mild types, and classification of the degrees of distortion into various categories was arbitrary. The normal types did not show variation under any condition, which suggests an absolute or qualitative distinction between normal and distorted plants. For these reasons methods in quantitative inheritance using statistics and in qualitative inheritance using genetic ratios were both adopted for the inheritance study.

The behavior of the F_2 and the backcross progenies showed that the distortion was heritable. Reciprocal differences observed in one of the crosses may have resulted from maternal effect as the phenotype of the distorted parent used as female predominated in the F_1 and the F_2

generations. Usually, such effect does not persist in subsequent generations. Therefore it was not considered important in the inheritance of the distortion. On this assumption it can be stated that nuclear rather than cytoplasmic factors are involved in the inheritance. In certain crosses a ratio of one normal to three distorted was obtained in the F_2 generation. In others the proportion of normal exceeded that of distorted. The results of crosses involving the severely distorted lines and the two normal lines showed that these normal lines 'G50' and 'Oregon 1604', though phenotypically similar with respect to the distortion, are genotypically different. There was a one or two-gene qualitative difference between 'G50' and the severely distorted types, as indicated by the ratio of normal to distorted plants in the F_2. The normal character was completely dominant over the distorted condition. Between 'Oregon 1604" and the distorted types there was one gene difference and no dominance. The genotypes of 'G50' and the distorted types might be represented by <u>AA</u> and <u>aa</u>, respectively where <u>A</u> is dominant over <u>a</u>. The genotype of 'Oregon 1604' would be <u>a'a'</u> where <u>a'</u> is interacting with the allele <u>a</u> of the distorted type to produce intermediate phenotypes. Thus, two major alleles are involved in the inheritance of the distortion. The ratios obtained in F_2 from 'G50' crosses were intermediate between 15:1 and 3:1 theoretical ratios. It is possible that this is a poor expression of the ratio 3:1 in which case the normal character would be controlled by one dominant allele. It should be emphasized that ratios obtained were based on an arbitrary classification where distinction between different classes was not absolute and expression of the distortion was not always complete. Therefore, the inheritance found in this study may only be an approximation to the actual situation.

The mild line 4607 was a common parent in crosses A10, A11, and A12. When crossed with severe lines (cross A10 and A11) distorted plants occurred in the F_1 and F_2. Both parents were probably of the same major genotype. The severe lines were believed to be homozygous for the distortion factor or factors. On this assumption it is expected that in A12 the F_1 would all be distorted. The data showed that some F_1 plants were normal and in the F_2 generation a proportion of normal plants was observed. Similar behavior of the F_2 was observed in cross A7 between a moderate and a mild parent. It is possible that some heterozygosity was still present in the mild lines so that the F_1 segregated into normal and distorted classes and as a result the F_2 contain a proportion of normal plants. Another more probable cause of this reduced expression would be the action of modifier genes which suppress the effect of the allele for distortion. The presence of modifiers may also explain the incomplete penetrance exhibited in 4607, which resulted in a low average score for distortion (0.50 - 0.70). The action of these modifiers is such that the distorted lines may appear phenotypically similar to the normal ones. This was observed in the line 4035 which had a low average score of 0.26 and a reduced penetrance as expressed by the low proportion of distorted individuals (26 percent). Aberrant ratios obtained in other F_2's and backcrosses may also be accounted for by the action of modifiers.

Crosses between two severely distorted lines or moderately severe lines (A1, A5) produced, as expected, only distorted individuals in their F_1 and F_2 generations. Both moderate and severe reactions could be attributed to the hypothetical genotype aa.

Moderate and severe distortion lines along with the mild lines and normal 'Oregon 1604' are essentially of the 'Blue Lake' type in general genetic background. 'G50', conversely, is entirely different in genetic background, lacking the 'Blue Lake' genes. Thus, it is not surprising that 'G50' differs basically from 'Oregon 1604' in factors conditioning the normal or non-distorted condition.

Skewness of the frequency distribution of phenotypic variations in the F_2 indicates the presence of dominance (Grant, 1975). Apparent skewness in the direction of the normal parent was observed in the F_2 populations from certain crosses in the greenhouse. However, this skewness cannot be considered as a general evidence of the dominance of the normal condition. It may be due to the arbitrary nature of the classification system which caused the normal type to fall into one major class and the distorted types into several and probably overlapping classes, the highest ones containing only a few individuals. Skewness was well marked in the F_2 and backcrosses of field population and could not be attributed entirely to the classification system. It resulted from the predominance of low scoring individuals due to very poor expression of the distortion. For this reason no attempt was made to analyze data obtained from the field.

Heritability Estimates

The heritability of a character indicates the extent to which it is transmitted from generation to generation. Characters with high heritability would be expressed in a predicatable manner in the progeny.

Relatively high heritability estimates were obtained from the three methods based on parent-offspring regression. High heritability estimates are typical of qualitative characters, controlled by a few major genes. Some modifier genes may be responsible for the continuous type of variation observed within populations. Heritability greater than 100 percent was obtained when it was estimated by the regression of the F_1 on the mid-parent. This may be due to the fact that certain conditions for the appropriate estimation of heritability were not satisfied. Falconer (1960) pointed out that for the regression of offspring on mid-parent to be usable, the variance should be equal in the two parents. It is evident that in crosses between normal and distorted lines used in this study, such a condition did not exist since the normal lines did not show any variation. In addition, the estimation of heritability by parent-offspring regression assumes random sampling and random mating of the parents. This assumption was not met in our study as the parents were chosen from a base population of breeding lines. Besides non-fulfillment of the conditions stated above, another source of unusual values of heritability may be the effect of environment. Some uncontrollable environmental factors in the greenhouse may have caused differential responses in the parent and the offspring. Such effects were reduced when the heritability was estimated in standard units. The regression of F_2 on F_1 is probably a more satisfactory method for our case. The values of heritability obtained from the three formulas were in close agreement. However, as indicated by Le Cohec (1972) this method is subject to a bias due to the interaction of genotype X environment. Our heritability is certainly overestimated as it was based on one definite set of environmental conditions.

Factors Influencing Expression of Distortion

The character expressed by an individual is the result of interactions between internal and external influences. The nucleus and the cytoplasm constitute the internal factors and the environment in which the individual develops represents the external factors. Nuclear factors have been discussed in the previous section. It has appeared that modifier genes induced changes in the expression of the character. This effect was manifested in the wide range of distortion observed among the various lines used in this study, their average score varying from 0 up to 3. Coyne (1969) explained the range of variegation observed between variegated populations of beans by the difference in time of expression of the gene for variegation during the development of the plant.

External factors are of various types. Responses to changing environment varied with the genotypes. A change in the environment did not induce the distortion in the normal lines, as evidenced by the experiments conducted in the greenhouse and in the field. Similarly, the two mild lines 4607 and 4035 were not much affected by the environment whereas the other distorted lines exhibited wider variability under different conditions in the field and the greenhouse. Conditions in these locations differed in that in the field, temperatures were susceptible to great variations and the light was stronger, while in the greenhouse, temperatures were more stable and the light less intense. The distortion was most severe under the light and temperature conditions of the April test in the greenhouse hallway. High light in the field and high temperature in the warm greenhouse considerably reduced the degree of severity of the distortion. Such a reaction is common among chlorophyll mutants. Coyne (1969) and Sheridan and Palmer (1977) working on chlorophyll-deficient materials reported similar results. Coyne (loc. cit.) suggested that a gene may be present in the chlorophyll-deficient plants (variegated) which, under high temperature causes production of normal plastids. This assumption found support in the work of Ballantine and Forde (1970) who showed that the amount of chlorophyll in the leaves was directly proportional to temperature. The present study does not permit any conclusion on the plastid condition and it can only be said that favorable conditions for plant growth reduced the degree of distortion. This was further emphasized by the fact that reduced soil fertility increased the degree of distortion. Plants grown on fertile soil developed larger and greener leaves than those on soil without fertilizer, and showed little distortion. Those plants under high temperature, with reduced distortion, did not respond to variation in soil fertility levels.

The distortion was more apparent at an earlier than at a later stage of the development of the plant. Closer observations revealed that mild distortion tended to disappear as the leaf became older and the affected areas appeared normal, whereas severe distortion never reverted to the normal condition. In the severely distorted types, the distortion may have affected the leaf during cell division, whereas in the mildly distorted, distortion may have occurred when the cells were fully expanded. These mildly distorted leaves were usually near the base of the plant and the severely distorted at the top.

Nature of the Distortion

The genotype of an organism responsible for a certain phenotypic expression forms a well-balanced system. A change in any of the genes may disturb the balance of that system and results in the appearance of an abnormal character, usually inferior to the normal type. Such characters which reflect a change in a certain gene are transmitted to the next generation without further change in suitable environment and have been called mutations. If this term is used to designate in general any heritable change then it is permissible to call this leaf distortion a mutation. Mutants are often characterized by physiological characters different from normal due to hormonal imbalance. Hormonal differences between the mutant and the normal phenotypes can be detected by appropriate experiments. The method of tissue culture is particularly useful as it permits one to evaluate the growth requirements of different tissues and thus determine any differences in growth regulator levels. In our experiment mutants and normal types had an optimal growth at the same auxin concentration which suggests no difference between the endogenous auxin level of the mutant and that of the normal.

SUMMARY AND CONCLUSIONS

1. One major locus is probably involved in the inheritance of the distortion affecting the trifoliolate leaves of the common bean. Minor or modifying genes are also indicated.

2. Possibly three alleles interact: an allele _a_ of the distorted type which is clearly recessive to an allele _A_ of normal 'G50', and a third allele _a'_ present in normal 'Oregon 1604'. When a classification of normal versus distorted is used, _a_ behaves as dominant over the _a'_ of 'Oregon 1604', but when distortion scores are used, the difference between 'Oregon 1604' and the distorted lines is additive in nature. Distortion lines of different severity are considered to be of the same major genotype, and differ in modifying factors acting in an additive manner. Observed ratios were much affected by these modifying factors.

3. Dominance of normal in crosses of 'G50' X distorted lines was evidenced by the behavior of the F_1 and the skewness of the F_2 variation. The distribution of F_2 phenotypes from crosses of distorted with normal 'Oregon 1604' showed apparent skewness due to the arbitrary nature of classification.

4. The environment strongly influenced the expression of distortion. High light and temperature conditions in the warm greenhouse and in the field reduced the symptom expression. Under low light and low temperature the distortion appeared most severe.

5. Heritability, estimated under a single set of conditions (greenhouse), was high. Selection against the distortion could be done effectively under environmental conditions for maximum expression.

6. Difference between normal and distorted lines was not due to difference in endogenous auxin level.

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