THE DISTRIBUTION AND EFFECTS OF GENES CAUSING F_1 WEAKNESS IN *ORYZA BREVILIGULATA* AND *O. GLABERRIMA*[1]

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ABSTRACT

Weakness of F_1 plants is frequently found in hybrids between strains of *Oryza breviligulata* (wild) and *O. glaberrima* (cultivated rice) endemic to West Africa. A set of two complementary dominant weakness genes, W_1 and W_2, was found to control the observed F_1 weakness. Many *breviligulata* strains had W_1, while most of the *glaberrima* and semi-wild strains had W_2 or were free of both. In the weak F_1 plants, tissue differentiation in adventitious roots seemed to be disturbed. Modifier genes affecting the expression of the weakness genes appear to be present also.

ORYZA glaberrima Steud. and *O. breviligulata* Chev. et Roehr. are annual grasses endemic to inland areas of West Africa. *Oryza glaberrima*, a cultivated rice, may have been derived from the apparently closely related wild species, *O. breviligulata*, independently of the more commonly cultivated *O. sativa* L. which is a derivative of the Asian form of *O. perennis* (MORISHIMA, HINATA and OKA 1963). Fields of *glaberrima* often contain wild and semi-wild plants belonging to *breviligulata*, as well as occasional hybrid swarms between the two (OKA and CHANG 1964). Furthermore, both species are sometimes found growing together with *O. sativa* and *O. perennis* subsp. *barthii*.

Frequently, the F_1 hybrid plants between strains of *breviligulata* and *glaberrima* were observed to be weak. This suggested to us that an amount of genetic differentiation might have occurred in this plant group. We decided therefore to investigate the pattern of differentiation by studying the distribution and effects of the genes causing F_1 weakness.

MATERIALS AND METHODS

Fifty-three *O. glaberrima* (including semi-wild forms) and 19 *O. breviligulata* strains collected by OKA and CHANG (1964) in various West African countries were the essential materials used. Each strain consisted of 2 or 3 plants derived from a single plant of the original population. In addition, four *glaberrima* strains (W025 from Sierra Leone; W416, W440 and W446 from Guinea) and a *breviligulata* strain (W042 from Guinea), which were genetically pure, were used for genetic analysis. In order to observe the distribution of F_1 weakness genes, two *glaberrima* (W025 and GN6-8 from Guinea) and three *breviligulata* (W042, ML22 from Mali, and TC2 from Tchad) strains were selected as test strains.

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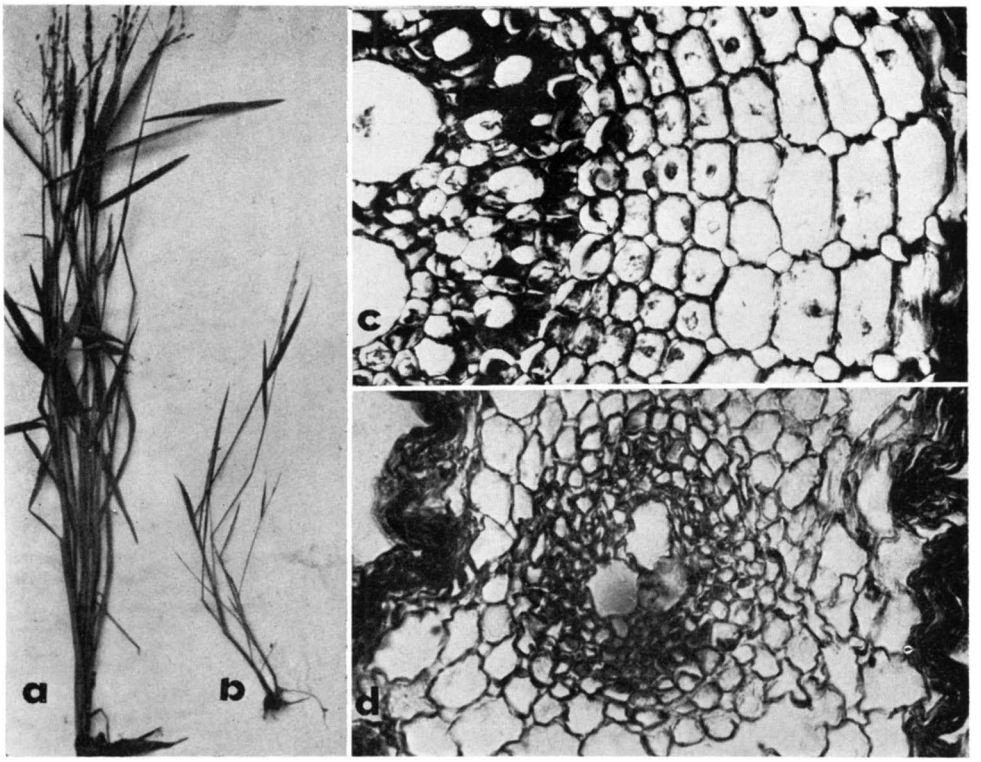

FIGURE 1.—A normal (a) and a weak (b) plant, and cross sections of their root tips (c and d, respectively).

The plants were grown in concrete beds under short day (12–13 hr/day) photoperiods, at Misima, Japan. Crosses were made by the warm-water emasculation method (43°C, 7 min).

The physiological and anatomical observations were carried out on the F_1 plants of W025 × W042. For the organ culture experiments, the basic medium of LIMSMAIOR and SKOOG (1965) was used. Sterilized seeds were germinated on moistened filter paper in Petri dishes. When the seminal root and shoot had both attained a length of about 1 cm, they were separated by a cut at the center of the mesocotyl. The root was cultured in a flask containing liquid medium, in darkness at 25°C. The shoot was cultured on agar medium in a test tube, in an illuminated incubator at 25°C.

For the study of the growth rate of callus tissue derived from roots, sterilized seeds were germinated on a medium containing 1 ppm kinetin and 5 ppm 2,4-D in addition to the basic medium of LIMSMAIER and SKOOG. When the induced callus attained a fresh weight of about 50 mg, it was transplanted to another dish which contained the basic medium fortified with 0.5% yeast extract, 1 ppm kinetin, 5 ppm 2,4-D and 10 ppm IAA For the anatomical observations, the roots were fixed with Carnoy's fluid and paraffin-sectioned. They were stained according to Heidenhein's hematoxylin method.

RESULTS

Genetic analysis of F_1 weakness: In contrast to the growth rate of normal rice plants and their hybrids, the growth rate of the weak F_1 plants begins to decline about 30 days after germination. Typically such hybrids flower, but they produce

TABLE 1

Segregation for weakness in crosses of (A × B) × C design, where the F_1 plants of A × B and B × C are normal and those of A × C are weak

Cross combination (A) (B) (C)	Normal plants	Weak plants	Ratio
(W025 × W416) × W042	18	14	
(W025 × W440) × W042	15	13	
(W025 × W446) × W042	14	19	
Total	47	46	1 : 1

few or no tillers and short roots. Their dry weight is also low (Figure 1a and b). For the genetic analysis, crosses of (A × B) × C design were made, where A × B and B × C produce normal F_1 plants but the F_1 of A × C is weak. Three such crosses were observed, and all gave a ratio of 1 normal to 1 weak as shown in Table 1. This indicates that weakness results from interaction between two complementary dominant genes, which segregate independently. The F_2 of W025 × W042, an A × C cross, segregated into 9 weak and 5 normal plants. Another F_2 population observed (ML22 × ML18, both parents being *breviligulata* from Mali) segregated into 23 weak and 16 normal plants. Though the number of F_2 plants observed was small because of the weakness of the F_1 hybrids, the ratios may be taken as 9 : 7. The weakness gene of W042 is denoted by W_1 and that of W025 by W_2. Thus, the genotype of an A plant is $++W_2W_2$, of a B plant is $++++$, and that of a C plant is W_1W_1++.

Distribution of the weakness genes: Each of the 19 *breviligulata* and 53 *glaberrima* strains from West Africa was crossed with each of the five test strains, and the growth of the resulting F_1 hybrids was closely observed. Weakness was noted in over 20 per cent of the 398 crosses, as shown in Table 2. Comparing the incidence of weakness among the F_1 plants revealed no other weakness genes than W_1 and W_2 in the study strains. Among the test strains, W042 and ML22 had W_1 and W025 and GN6–8 had W_2, while TC2 was free of both.

The strains used for crosses were observed for 29 metric characters, and correlation coefficients between characters were computed. The resulting correlation matrix (29 × 29) was then subjected to principal component analysis by KEN-

TABLE 2

Incidence of F_1 weakness between strains of O. breviligulata *and* O. glaberrima

Cross combination	Number of crosses observed*	Crosses showing F_1 weakness	Percent
breviligulata × *breviligulata*	51	14	27.5
breviligulata × *glaberrima* *glaberrima* × *breviligulata*	198	63	31.8
glaberrima × *glaberrima*	149	6	4.0
Total	398	83	20.9

* With test strains carrying W_1 or W_2 (excluding crosses with TC2).

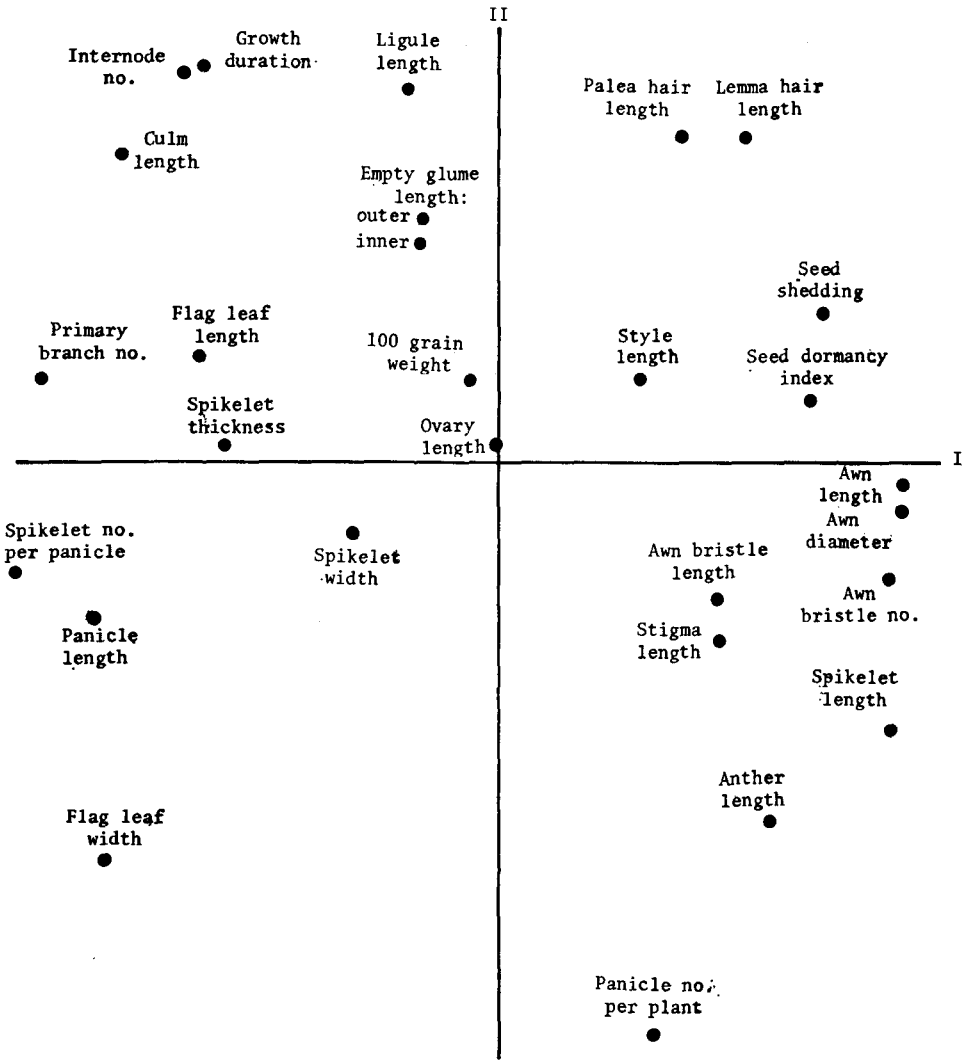

FIGURE 2.—Twenty-nine characters of *breviligulata* and *glaberrima* strains scattered on the plane defined by the first and second principal components extracted from their correlations.

DALL's (1961) method using an electronic computer. The first and second components extracted from the correlation matrix contributed 32% and 16% to total multi-dimensional variance, respectively. They gave a distribution of the 29 characters as shown in Figure 2. The first component, having its principal effects on awn characters, seed shedding, seed dormancy, spikelet number and primary branch number per panicle, etc., seems to represent the variation between the wild and cultivated types. The second component, carrying principal effects on duration of growth, panicle number per plant and various size characters, appears

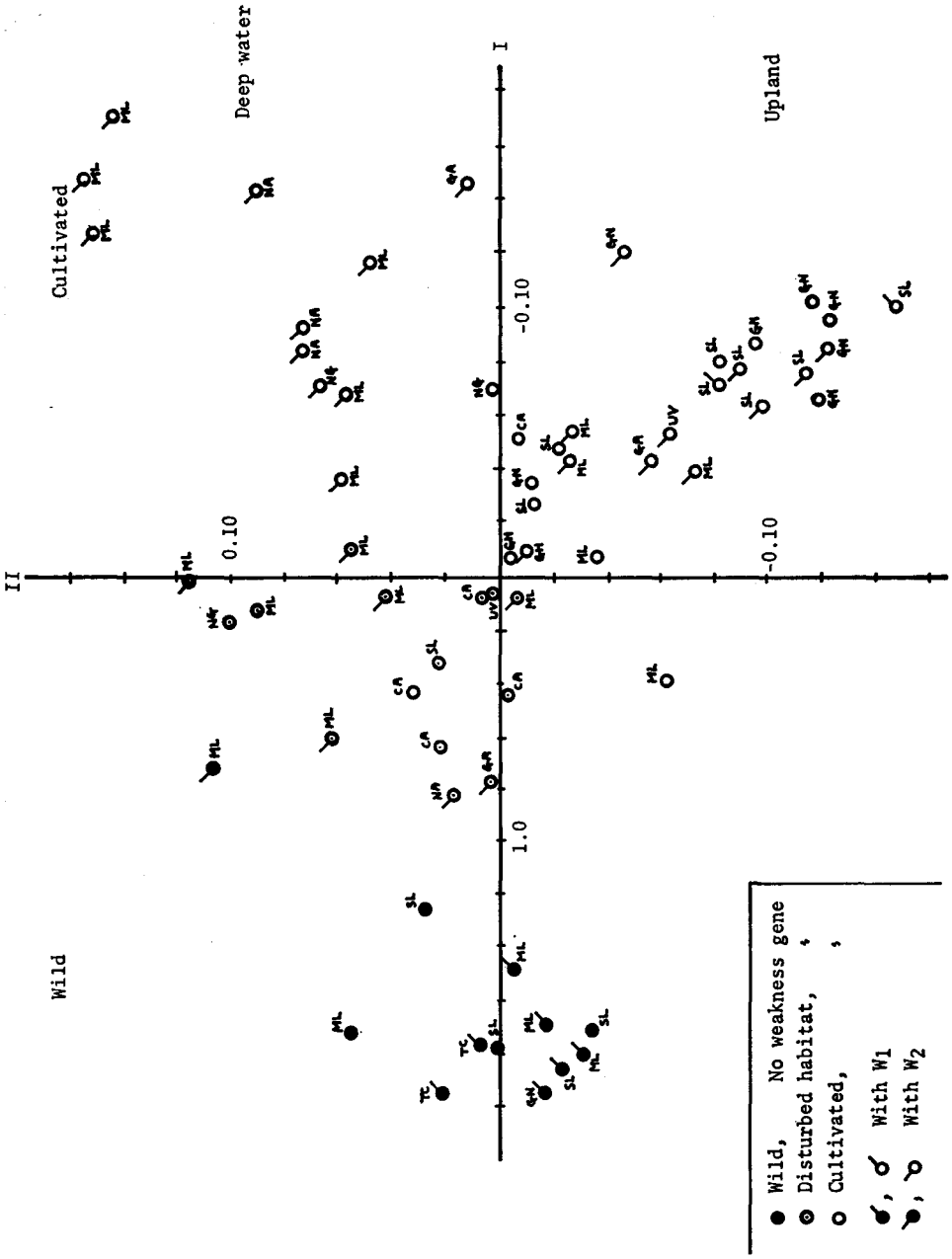

FIGURE 3.—Sixty-five strains of *breviligulata* (wild) and *glaberrima* (cultivated) scattered according to the scores given by the first and second component vectors. Their weakness genes, W_1 and W_2, are denoted by ♂ and ♀, respectively. CA–Cameroon, GA–Gambia, GH–Ghana, GN–Guinea, ML–Mali, NA–Nigeria, NG–Niger, SL–Sierra Leone, TC–Tchad, UV–Upper Volta.

to reflect the variation between deep water and upland types. In West Africa, the strains are differentiated into these deep water and upland ecological types. The plants growing in deep swamps are generally large in size and late maturing as compared with those growing in upland conditions.

The strains were plotted according to the values of their first and second component vectors in Figure 3. The distribution of the strains suggests that the wild populations (*breviligulata*) might have differentiated into deep water and upland types in the course of being domesticated. The weakness genes they carry are shown by "♂" (W_1) and "♁" (W_2), respectively. The truly wild strains growing in swamps of varying depth either had W_1 or had no weakness genes. A few slightly domesticated wild strains, which were adapted to deep water conditions, had W_2. The W_2 gene was also distributed among the cultivated strains. This pattern of distribution is similar to that found for the Ne_1 and Ne_2 genes controlling F_1 necrosis in wheat (TSUNEWAKI 1969). No particular trend was found in the geographical distribution of these genes, although their frequency is high in Mali, the center of distribution of these species, and is low in Sierra Leone and Cameroon, the margin of the distribution area.

Culture experiments with weak F_1 plants: In order to investigate the physiological basis of the weakness of the F_1 hybrids, the growth of W025, W042 and their F_1 plants was observed in different conditions, i.e., in soil, water culture, and sterile culture of excised organs. In water culture (Kasugai's solution containing inorganic nutrients only), the F_1 plants showed hybrid vigor in the early stages of growth. Their growth rate began to decline about 60 days after germination (Figure 4). The F_1 hybrids had a larger number of adventitious roots than the parents, but the roots did not elongate and were slender. The weak plants had a low rate of tillering each producing less than four tillers in the field.

When the shoot and root of the seedlings were excised and cultured separately, no significant difference in the growth of the shoot was found between normal and weak plants. However, the growth of the roots from weak F_1 plants was significantly poorer than the growth of the roots from normal plants (Table 3).

About 50 days after germination, adventitious roots of water-cultured plants were sectioned to observe their internal structure. As also shown in Table 3, the roots of the weak F_1 hybrids had smaller diameters and smaller numbers of cell layers than those of the parents in both the meristematic and elongating regions. A normal root had five to six xylem points, but a weak root had only two or three. Furthermore, the size of root cells and their periclinal arrangement were irregular in weak plants, as shown in Figure 1a. The weak F_1 plants showed a significantly larger standard deviation of the size of cortical parenchyma cells than did the parental plants (Figure 5). The standard deviation of the distance from the center of the root to the respective cell layers was also much larger than in normal plants. The cells in the endodermis, central cylinder parenchyma, exodermis and epidermis also showed irregularities in size and arrangement, and were not clearly distinguishable.

On the other hand, the calluses induced from the root of normal and weak plants grew equally well in the test tube cultures (Figure 4d), indicating that the

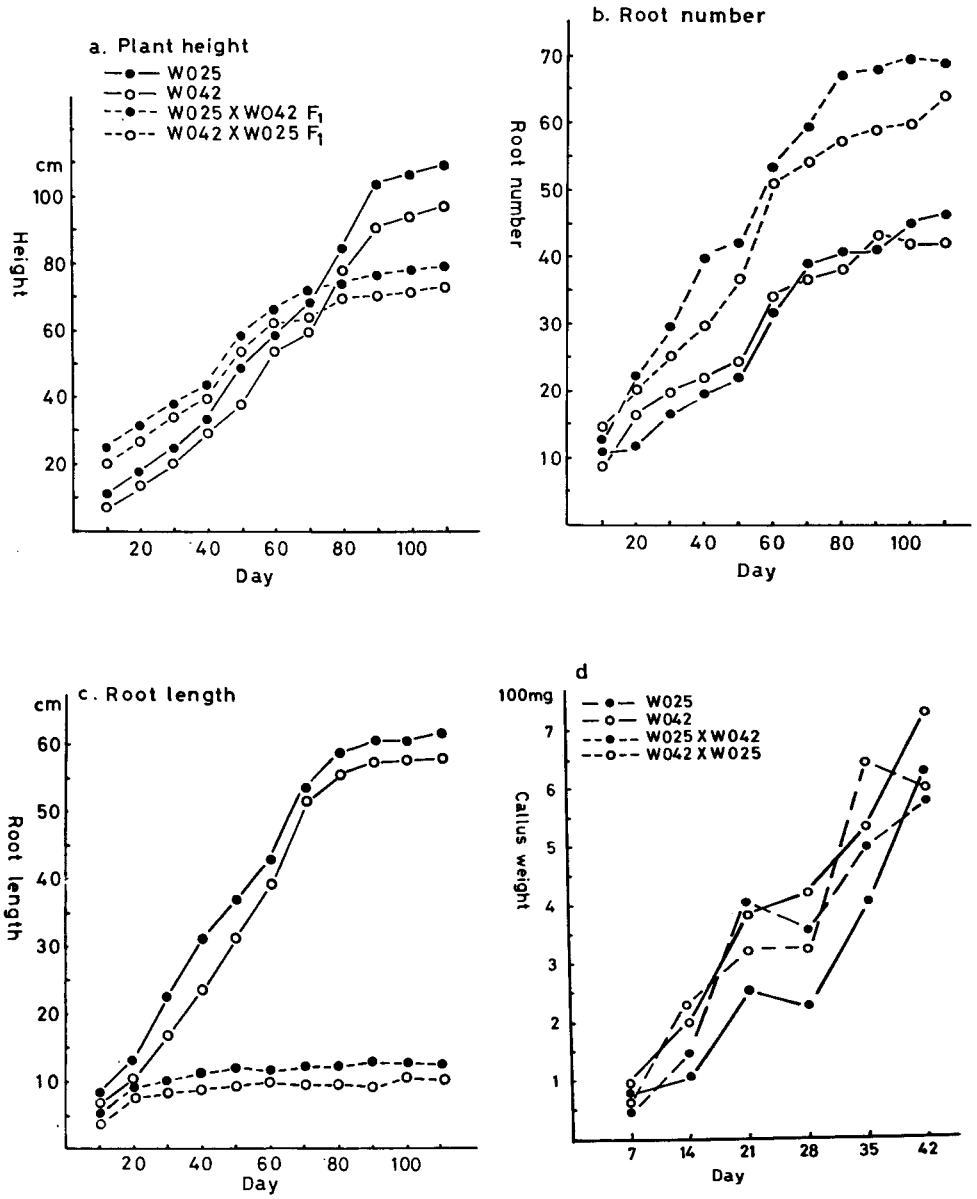

FIGURE 4.—Growth curves of weak F_1 plants and their parents in water culture (a, b, c) and of calluses induced from their roots (d).

growth of undifferentiated tissues is not affected by the weakness genes. We conclude from these observations that the weakness genes disturb the differentiation of cells in the adventitious roots. The disturbance seems to take place in the region proximal to the growing point, about 1,500 micra from the tip.

Modifiers for the expression of weakness: The weak F_1 plants varied in height

TABLE 3

Growth of excised organs in sterile culture and measurements of root cells, compared between normal and weak plants (Mean for 5 plants)

Character	Normal W025	Normal W042	Weak W025 × W042	Weak W042 × W025
Excised shoot, 30 days culture,				
Length (cm)	20.5	18.2	23.4	22.2
Leaf number	3.5	3.0	3.0	4.0
Dry weight (mg)	6.1	6.3	7.4	8.0
Excised root, 30 days culture,				
Length (cm)	18.1	23.5	8.2	5.3
Adventitious root number	38	56	18	8
Dry weight (mg)	7.1	12.1	3.2	2.1
Water-cultured plants				
Root diameter (mm),				
Meristematic region	0.63	0.71	0.31	0.28
Elongating region	0.84	0.86	0.58	0.51
Number of cell layers,				
Elongating region	54.2	48.1	40.5	36.8
Root-hair region	56.2	58.7	38.8	42.3
Parenchyma cell length (mm)*	0.13	0.14	0.07	0.08
Number of mitotic cells†	16.2	14.4	8.4	4.2

* Cells of the third layer in the upper elongating region.
† Counted in a longitudinal section.

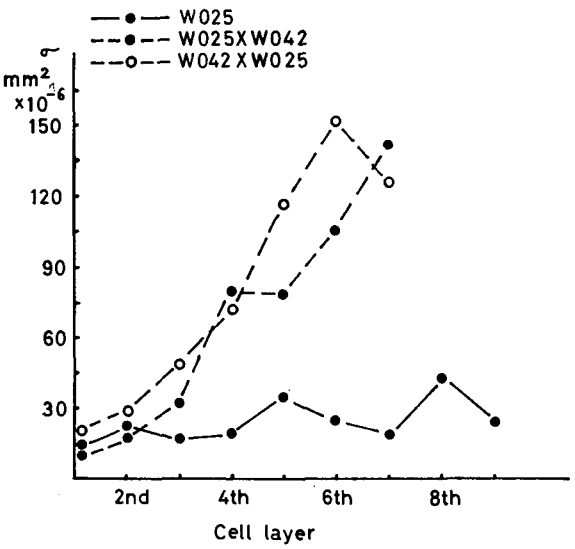

FIGURE 5.—Standard deviations (in $mm^2 \times 10^{-6}$) of the size of cortical parenchyma cells in adventitious roots, in normal and weak plants.

TABLE 4

Mean dry weight (in grams) at maturity of seven strains and their F_1 hybrid plants in a diallel experiment and differences between observed and expected values for weak plants

Number	Genotype		W416	W492	W607	W025	W039	W009	W042
W416	+	+	19.0	...	...	...	...	...	...
W492	+	+	13.8	15.1	...	...	...	...	...
W607	+	+	30.9	27.7	21.7	...	...	...	...
W025	+	W_2	35.2	21.8	41.9	19.3	...	...	...
W039	+	W_2	31.7	23.2	32.7	42.5	33.8	...	...
W009	W_1	+	14.6	23.1	25.7	2.4[1]	12.0[3]	15.3	...
W042	W_1	+	17.4	10.3	25.1	2.9[2]	8.8[4]	15.5	15.5

Weak plant	Combination	Observed	Expected*	Difference
1	W025 × W009	2.4	30.0	27.6
2	W025 × W042	2.9	26.4	23.6
3	W039 × W009	12.0	26.2	14.2
4	W039 × W042	8.8	22.7	13.9

Standard error for difference = 4.14

$$^{*}\gamma_{exp} = \frac{1}{(n-2)(n-3)}\left[(n-1)(\gamma_{s.} + \gamma_{.t} - \gamma_{p_1} - \gamma_{p_2}) + \gamma_{p.} - \gamma_{..}\right]$$

(cf. Hayman 1954)

as well as in tiller number according to the hybrid combination, though all of them could be easily distinguished from normal plants. Assuming that there are only two weakness genes, W_1 and W_2, the variation may be due either to modifier genes effecting the expression of the weakness genes, or to additive and non-additive effects of other parental genes. To distinguish between these two possibilities, diallel crosses were made among seven strains and the parental and F_1 lines (4 or more plants per line) were scored for dry weight at maturity. Four of the 21 crosses showed F_1 weakness. The expected values for the four, had they been healthy, were computed as missing values using HAYMAN's (1954) formula, and were compared with the observed values as shown in Table 4. The differences between observed and expected values were found to differ significantly according to which parental strains were used, W025 or W039. This suggests that there may be modifying genes controlling the expression of the weakness genes.

Also, it was found among the F_2 plants of ML22 × ML18 that the mean height at maturity (± standard deviation) was 48.6 ± 11.34 cm for 23 weak and 76.6 ± 8.21 cm for 16 normal plants. The larger standard deviation for weak than for normal plants is a further suggestion of the presence of genes modifying the expression of F_1 weakness.

DISCUSSION

Weakness of F_1 plants was first described by SAX (1921) in wheat, and many cases in crop plants were later reported, e.g., in wheat (*Triticum aestivum*, HEYNE, WIEBE and PAINTER 1943; MORRISON 1957; TSUNEWAKI 1960, 1969; *T. durum*, CALDWELL and COMPTON 1943), and in Gossypium species (GERSTEL

1954). In wild plants, it was found in *Aegilops squarrosa* (NISHIKAWA 1953; ROY 1955) and in Mimulus species (VICKERY 1956a,b).

In wheat, three different sets of complementary dominant lethals are known to express different symptoms, i.e. necrosis (Ne_1 and Ne_2, TSUNEWAKI 1960; HERMSEN 1963), necrosis type-II (Net_1 and Net_2, NISHIKAWA 1962), and chlorosis (Ch_1 and Ch_2, SACHS 1953; TSUNEWAKI and KIHARA 1962). The weakness of F_1 plants found between Indica varieties of *O. sativa* which showed chlorosis, was also due to a set of two complementary dominant lethals, L_1 and L_2 (OKA 1957). In all these cases, the complementary genes were dominant and segregated independently. The weakness of F_1 plants may express itself when the complementary genes are dominant. When they are recessive, the weakness is expressed in the F_2 and later generations resulting in partial breakdown of hybrids, as found in *O. sativa* by OKA (1957). The F_1 weakness dealt with in the present study was also due to two complementary dominant weakness genes that were inherited independently of each other. Our crossing experiment showed that the two weakness genes, W_1 and W_2, were not allelic with those of *O. sativa* reported by AMEMIYA and AKEMINE (1963). They must also differ from L_1 and L_2 of *O. sativa* in view of their different symptoms.

The physiological basis of the weakness has been examined in a few cases. GERSTEL (1954) suggested that the weakness of cotton hybrids could be due to excessive metabolic activity which is mitigated under low temperatures. MOORE (1966) found in wheat that an application of gibberellin restored the growth of dwarf F_1 plants (conditioned by complementary dwarfness genes), suggesting that they might suffer from a deficiency of this substance. AMEMIYA and AKEMINE (1963) suggested that the F_1 weakness they found in rice could be attributed to a reduced respiration rate and to the interruption of mitosis in the roots. In the present material, the weakness genes seemed to interrupt the differentiation of cells in the adventitious roots. Presumably, there may be two alternative systems of genetic control of tissue differentiation for root development. These alternative systems appear to be incompatible when both are present.

We found that the W_1 weakness gene occurred mainly in wild plants, whereas the W_2 weakness gene occurred in those wild plants showing some cultivated characters (adapted to deep-water conditions) and in cultivated varieties. It may be suggested that, as postulated by TSUNEWAKI (1969) for the Ne_2 gene of wheat, W_2 could have arisen in the course of domestication of the wild plants.

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