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Hybrids with abnormal development were obtained when particular genotypes of Phaseolus vulgaris L. were crossed. The phenotypic aberrations of the F_1s included retarded growth, chlorosis of the trifoliate leaves and the formation of adventitious roots on the hypocotyls. The expression of the abnormalities was dependent on the temperature. At high temperature regime (30°C/25°C, day/night), the onset of the abnormal phenotypes occurred at an early stage of development (two weeks after planting) and essentially no new growth was observed between the second and the sixth week. However, at lower temperature regimes (25°C/20°C and 20°C/15°C, day/night), the F_1s exhibited normal and vigorous growth. The abnormalities observed on F_1s at high temperature is referred to as the "crippled" phenotypes. Genetic analysis indicated that the abnormal development is conditioned by two loci, DL_1 and DL_2 (for dosage-dependent lethal). The severity and the expression of the mutant phenotypes are dependent on the allelic dosage as well as the temperature. F_1s are heterozygous for DL_1 and DL_2. Plants homozygous dominant for both loci (four dominant alleles) are classified as lethals and perish soon after germination. Plants homozygous for one of the locus but heterozygous for the other locus (three dominant alleles) are classified as sub-lethals which also

perish but at a slower rate than the lethals. The expression of the lethal and the sub-lethal phenotypes is not influenced by the temperature. The developmental controls of DL_1 and DL_2 were examined through grafting experiments and hydroponic studies. Phenotypes of mutant classes were duplicated by unions of scions and stocks derived from different genotypes. These results indicate that DL_1 and DL_2 regulate a root and shoot factor respectively which contribute to the mutant phenotypes. The allelic dosage of DL_1 in the root and DL_2 in the shoot rather than the genotype of the whole plant determine the severity of the abnormal development. Plants heterozygous for both loci with a temperature-dependent expression of the mutant phenotype were used to determine physiological components involved. One of the primary morphogenetic events associated with mutant expression is the restricted root growth at high temperature which can be alleviated by the addition of cytokinin in hydroponic solution. The differential expression of DL_1 and DL_2 in the root and the shoot systems and the effect of cytokinin in restoring normal growth of heterozygotes suggest that the two loci may be related to the regulation of hormonal biosynthesis or metabolism.

DEVELOPMENTAL MUTANTS OF PHASEOLUS VULGARIS L.

by

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Dedicated to my wife,

Celine Wang Shii, and

to my two daughters

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DEVELOPMENTAL MUTANTS OF PHASEOLUS VULGARIS L.

Introduction

In the course of hybridizing Phaseolus vulgaris L., it was noted that morphologically abnormal hybrids were often recovered when genotypes with diverse origins were used as the parents (Coyne 1965, Provvidenti and Schroeder 1969, Rheenew 1979, York and Dickson 1975). As the occurrence of abnormal F_1s was limited to certain genotypic combinations, it was speculated that the abnormal development was primarily of a genetic nature rather than pathological or nutritional. However, the genetic basis of the developmental abnormalities was not known partly due to the inconsistent observations taken under field conditions.

An exploratory study conducted at OSU indicated that the expression of morphological abnormalities of these hybrids was related to temperature. This observation allows a detailed genetic study possible by utilizing controlled environmental chambers. In addition, the physiological components of the mutants were also examined through grafting experiments and hydroponic studies. The major findings are reported in this thesis in manuscript forms. The first chapter has been published (Shii et al. 1980) and the second has been submitted for publication (Developmental Genetics). Co-authors Drs. D. Mok and M. Mok provided guidance and advice during the conduct of the experiments and also critically reviewed the manuscripts. Dr. Steve Temple of CIAT supplied the initial plant materials and also assisted in the preparation of the first publication.

REVIEW OF LITERATURE

As plant hormones play a central role in maintaining normal development in plant systems, developmental mutants associated with hormonal derangements are often used to investigate the genetic regulation of plant growth and development. In addition, the mutants investigated during the course of the thesis research appear to be related to hormonal functions, therefore, the following review is centered on previous studies concerning mutants of this type.

Tumorous hybrids obtained from interspecific matings of certain Nicotiana species, such as N. glauca and N. langsdorffii have been the subject of intensive studies (Kehr and Smith 1954, Kostoff 1930, Naf 1958). Several lines of evidence support the hypothesis that a higher level of hormone production is related to tumorous growth. Tumorous hybrids were found to contain a larger quantity of extractable auxin relative to the parental species (Bayer 1965, 1967, Bayer and Ajuha 1968, Kehr and Smith 1954). Treatment of young seedlings of tumorous hybrids with a solution containing indole acetic acid (IAA) and kinetin resulted in premature formation of tumors (Schaeffer 1962). The most conclusive evidence was obtained by comparing growth regulator requirements of normal versus tumorous plants, for growth in vitro. Cultured tissues derived from the hybrids N. suaveolens-langsdorffii and N. glauca-langsdorffii did not require auxin nor cytokinin for growth, whereas the parental species and a non-tumorous N. glauca-langsdorffii hybrid, a mutant obtained by irradiation (Izard 1957), did not exhibit this auxin and cytokinin independence (Kehr and Smith 1954, Schaeffer and Smith 1963, Schaeffer et al. 1963). However, it has been suggested that a reduction of the endogenous auxin level

triggers the onset of tumor formation (Ames 1970, 1974, Ames and Mistretta 1975, Ames et al. 1969). The age differences of the plant materials may have caused the discrepancy between these and earlier studies.

A combination of complete N. glauca and N. langsdorffii genomes was not required for tumor formation, since in some aneuploids of the interspecific hybrid tumorous growth was observed (Ahuja 1963) and not in others (Kehr and Smith 1954). In tumorous hybrids of N. longiflora and N. debnyei-tabacum, a specific longiflora chromosome is likely to be involved in tumor production, as one longiflora chromosome added to a debnyei-tabacum complement was sufficient to induce tumor (Ahuja 1962). The inability to characterize individual chromosomes within a species or to distinguish chromosomes of different species prevented the identification of the chromosomes responsible for tumor formation.

The inheritance of tumor formation was studied by analyzing tumor forming ability of progeny derived from intermatings between tumorous and non-tumorous N. glauca-langsdorffii amphidiploids. However, results were not conclusive, since the genetic ratio of backcross progeny was not substantiated by the pattern of F_2 segregation (Smith and Stevenson 1961). Further genetic analysis is impaired due to the sterility of diploid F_1s of distantly related species and mostly autosyndetic pairing of the amphidiploids (Smith 1962).

Tumor formation was observed in tomato plants upon the introduction of a dominant gene, originated from Lycopersicon chilense, into the genome of L. esculentum (Martin 1966). A higher level of endogenous auxin was suggested to be the cause of tumor formation, since a lower

concentration of auxin was required for optimal growth of hybrid tissue as compared to parental tissue in culture (Ahuja and Doering 1967, Doering and Ahuja 1967). However, the difference in auxin requirement between cultures derived from tumorous and normal plants was small. In addition, tumor formation was greatly influenced by the environment; tumorous growth was not observed under field grown conditions (Doering and Ahuja 1967). Further investigation of the relationship between gene action and hormone production is difficult due to the minor physiological differences and the inconsistent expression of the tumor.

Dwarf plants have been used widely to study the genetic regulation of gibberellin (GA) production and utilization. The occurrence of single-gene dwarfs is frequent in plants (Pelton 1964), but the physiological anomalies are not identical.

The dwarfing growth habit in maize (Phinney 1956) and in beans (Brian and Hemming 1955) could be overcome by the application of GA. The hypothesis that dwarfism is the result of lower amount of endogenous GA was confirmed by comparing extractible GA in dwarf and tall plants of maize (Phinney 1961) and beans (Gotoh 1970, Gotoh and Esashi 1973). The mutant loci in maize leading to the five dwarf types (d-1, d-2, d-3, d-5 and an-1) each control a different step in the biochemical pathway leading to the production of a gibberellin (Katsumi et al. 1964, Phinney 1961).

The correlation between dwarfing and lower GA content was not found in dwarfs of peas (Hota and Israelstam 1977, Jones 1968, Kende and Lang 1964, Radley 1958) and a dwarf of bean (Proane and Greene 1968) obtained by irradiation. However, normal plant height

could be restored by application of GA (Brian and Hemming 1955, Moh and Alan 1967). Available evidence suggests the presence of higher levels of inhibitors in these dwarfs (Higgins and Bonner 1974, Kohler and Lang 1963, Morales and Greene 1972).

There is no clear relationship between endogenous GA content and plant height in rice. Some dwarfs contained either no or lower amounts of extractable GA, others had the same GA content as normal plants (Suge and Murakami 1968, Suge 1975, 1978). Also the responses to GA application differed widely among dwarf varieties (Harada and Vergara 1971, 1972). Thus it seems that different kinds of dwarf mutants occur in rice. The inability to synthesize a sufficient amount of GA appears to be the cause of some of these dwarf mutants.

Dwarfs and semi-dwarfs of wheat did not respond to the application of GA (Allan et al. 1959, Gale and Marshall 1973, McVittie et al. 1978, Radley 1970). In addition, dwarf and semi-dwarf derivatives of the variety Norin-10 were found to have higher endogenous GA as compared to variety with normal plant height (Gale and Marshall 1973, Radley 1970). These findings led to the conclusion that an insensitivity to GA was the basis of short stature in Norin-10 dwarfs (Fick and Qualset 1975). However, the production of α-amylase did not exhibit insensitivity when endogenous GA was supplied (Radley 1970). Dwarfs of a different genetic origin, Tom Thumb derivatives, were found to be GA insensitive in both growth and α-amylase production (Gale and Marshall 1973).

Monosomic analysis has been employed to localize the dominant gene for GA insensitivity in the varieties Tom Thumb (Morris et al. 1972) and Minister Dwarf (Gale et al. 1975, McVittie et al. 1978). In

both varieties the GA insensitivity gene was located on chromosome 4A. In Norin-10, two major genes are responsible for GA insensitivity, one of which is located on chromosome 4D (Gale et al. 1975).

Although GA insensitivity was reported to be a major cause of dwarfing in these genetic sources, several other chromosomes have been demonstrated to have an effect on plant height in wheat. In the variety Bersee, monosomics for chromosomes 2A, 2B and 2D were shorter than the euploid. These monosomics responded to the application of GA. It appears that these chromosomes contain genes for GA synthesis (Gale and Law 1973).

Dosage effects of different chromosomes were examined in order to locate genes involved in GA action in barley (Carlson 1972). The activities of α-amylase and protease were increased in barley aleurone when extra copies of chromosome 2 were present, while only α-amylase activity was elevated with extra copies of chromosome 6. It appears that chromosome 2 is involved in the expression of GA action.

Other types of mutants which are related to growth regulator metabolism are the knotted mutant in maize and the flacca and diageotropica mutants in tomato. The knotted phenotype is characterized by hollow, fungus-like outgrowth (knots) mainly appearing in the leaf blade (Bryan and Sass 1941) and is inherited as a simple dominant character. The location of the mutation is on chromosome 1. A dosage effect of the kn allele was suggested as knkn plants had a faster rate of knot formation than plants heterozygous for this mutation (Rhodes and Meyers Jr. 1966). The physiological basis is associated with an increase in IAA-oxidase inhibitors (Gelinas and Postlethwait 1969).

The flacca mutant wilts as a result of the failure of stomata

closure which was reported to be caused by extremely low abscisic acid and a relatively high concentrations of endogenous auxin and cytokinins in the leaves (Tal and Imber 1970, Tal et al. 1970, Tal and Nevo 1973, Tal 1979).

The diageotropy (dgt) in tomato is expressed in both roots and shoots and is conditioned by a single recessive gene located on the long arm of chromosome 1 (Zobel 1972). Grafting experiments demonstrated that a polarly transported substance derived from the shoots is responsible for the mutant phenotype (Zobel 1973). The cause of the diageotropic response was suggested to be a blockage of the auxin-stimulated ethylene production in the shoots (Zobel 1973, 1974) and the genetic lesion is probably related to steps prior to the formation of ACC (1-aminocyclopropane-1-carboxylic acid) in the ethylene biosynthetic pathway (Bradford and Yang 1980).

CHAPTER 1

Expression of Developmental Abnormalities in Hybrids of *Phaseolus vulgaris* L.: Interaction Between Temperature and Allelic Dosage

INTRODUCTION

The hybridization program at CIAT (Centro Internacional de Agricultura Tropical, Cali, Colombia) includes a large number of parent genotypes of *Phaseolus vulgaris* with diverse geographic origin. A number of parental combinations produced abnormal F_1 hybrids. The primary phenotypic abnormalities, when grown at Cali, were stunted growth and yellowing and chlorosis of the young trifoliate leaves. Development typically ceased after the formation of two or three trifoliate leaves and F_2 seeds could seldom be obtained. The occurrence of abnormal F_1s dictated the elimination of otherwise desirable combinations of genotypes from further breeding efforts. As the occurrence of abnormal F_1s was limited to specific parental combinations, it was suspected that the abnormal development was of a genetic nature rather than pathological or nutritional. In an initial effort to investigate the cause of the abnormal hybrids, identical F_1 populations were grown in the greenhouse at Corvallis. Similar morphological deformity was not observed, however, among greenhouse-grown F_1s where the temperature was maintained at approximately 21°C/16°C (day/night). This initial observation suggested the possible influence of environment on the expression of abnormalities. The present paper describes the effects of temperature (under controlled environment) on growth and development of F_1s and the inheritance of the developmental abnormalities.

MATERIALS AND METHODS

Phaseolus vulgaris genotypes P691 and P725 (CIAT promising numbers) were used as parents. (P691 is also identified as G4489 or Cuilopa 72 obtained from Guatemala. It has purple flowers and black seeds. The growth habit is indeterminate. P725 is also identified as G3804 or Bolivia 6 I 1095, obtained from Venezuela. It has pink flowers and beige colored seeds. The growth habit is determinate.) Reciprocal F_1 (P691 x P725 and P725 x P691), F_2 and backcross populations were generated.

Seeds were planted in Jiffy Mix Plus planting medium (obtained from George Ball Pacific Company, California) in one gallon pots. Plants were grown in growth chambers at three temperature regimes, 30°C/25°C, 25°C/20°C, and 20°C/15°C (day/night). The photoperiod was 12 hours and the light intensity was approximately 275 μ einsteins/m^2sec.

To determine the effect of temperature on the expression of developmental abnormalities, 12 plants each of P691, P725, P691 x P725 and P725 x P691 were grown at each of the three temperature regimes. The experiment was repeated three times. The growth rate of each genotype was measured weekly as the average number of trifoliate leaves per plant. The proportion of chlorotic leaves on F_1 plants was calculated as percent of the total number of trifoliate leaves.

For genetic analysis, segregating F_2 and backcross populations were grown at 30°C/25°C and 25°C/20°C and classified into phenotypic categories.

RESULTS

The influence of the temperature regime on the developmental sequence of the parents (P691 and P725) and the reciprocal F_1s is illustrated in Figures 1 and 2. At the high temperature (30°C/25°C) regime, the abnormal development of the F_1 plants was apparent two weeks after planting (Figure 1A). The phenotypic abnormality at that time was the chlorosis of the trifoliate leaves. After three weeks, severe chlorosis of the leaves developed (Figure 1B). Essentially no new leaves were formed between the second and the fourth week (Figures 1C and 2A). At the fifth week, however, new growth was initiated and the newly formed leaves were green (Figures 2A and 2B). Some of the older leaves which were chlorotic began to develop chlorophyll. The time of initiating new growth and the greening of previously chlorotic leaves varied among F_1 plants. At the seventh week, some F_1 plants had four sets of green trifoliates, while others had just begun to initiate new growth (Figure 1D). The severely restricted growth of the F_1 plants contrasted sharply with the rapid and normal development of the parents (Figures 1A and 2A).

In addition to the chlorosis and necrosis of the first few sets of trifoliate leaves and the retarded growth in the first four weeks, all F_1s formed aerial root primordia on the hypocotyls. These primordia were visible at the second week and became quite pronounced at the third week (Figure 1E). There was no reciprocal cross difference in the expression of these abnormal phenotypes.

At the 25°C/20°C regime, the growth rates of hybrids were approximately equal to those of the parents (Figure 2C). The development of both parents and reciprocal F_1s was apparently normal up to the fourth

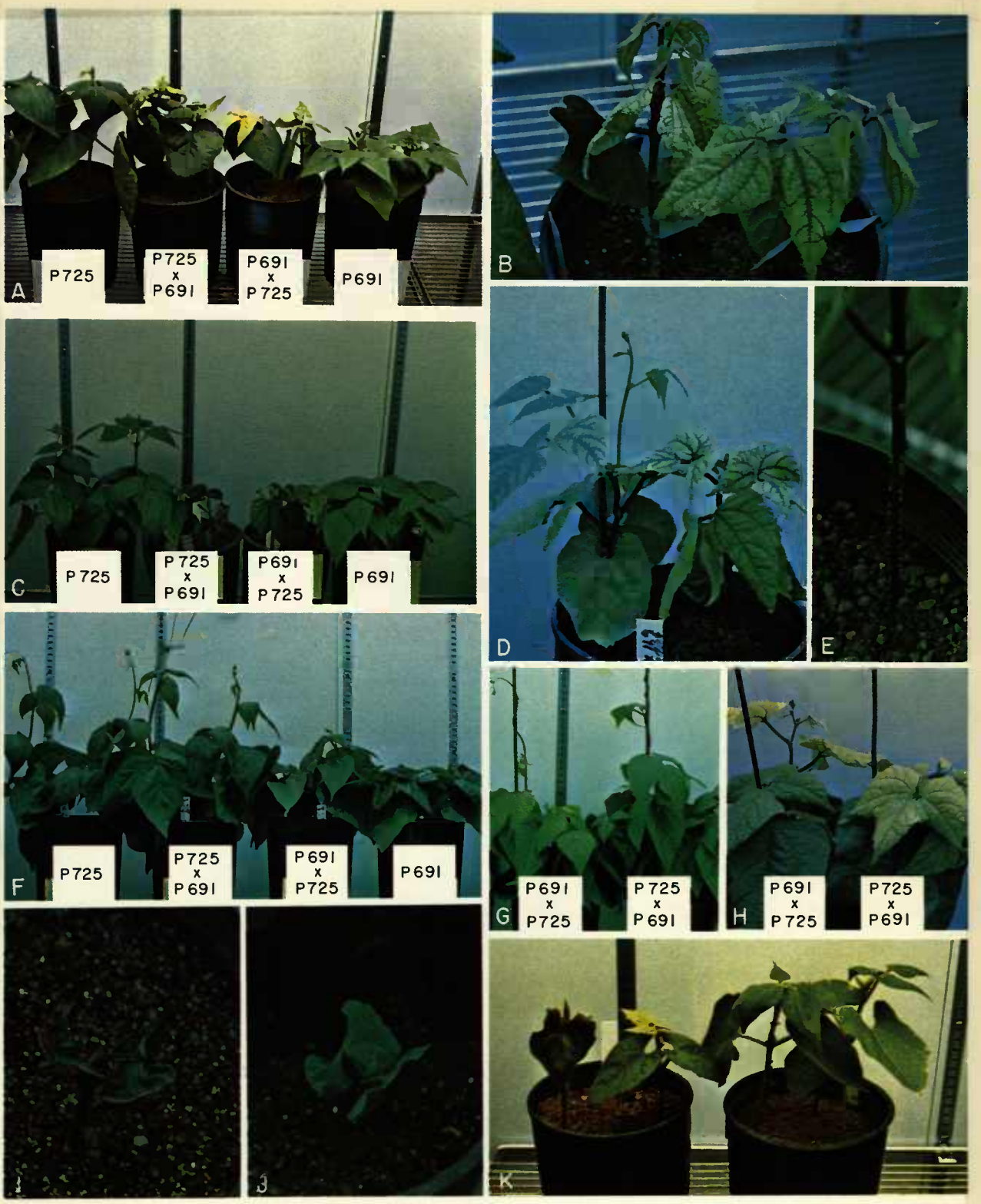


FIGURE 1—Morphological abnormalities in progeny of P691–P725. *A*—parents and F_1's two weeks after planting at 30°C/25°C. *B*—P691 × P725 three weeks after planting at 30°C/25°C. *C*—parents and F_1's four weeks after planting at 30°C/25°C. *D*—formation of normal leaves of P691 × P725 seven weeks after planting at 30°C/25°C. *E*—aerial root primordia on P691 × P725 three weeks after planting at 30°C/25°C. *F*—parents and F_1's four weeks after planting at 25°C/20°C. *G*—F_1's five weeks after planting at 25°C/20°C. *H*—F_1's seven weeks after planting at 25°C/20°C. *I*—lethal F_2, two weeks after planting at 30°C/25°C. *J*—sub-lethal F_2, two weeks after planting at 30°C/25°C. *K*—backcross progeny (P691 × F_1), two weeks after planting at 30°C/25°C.

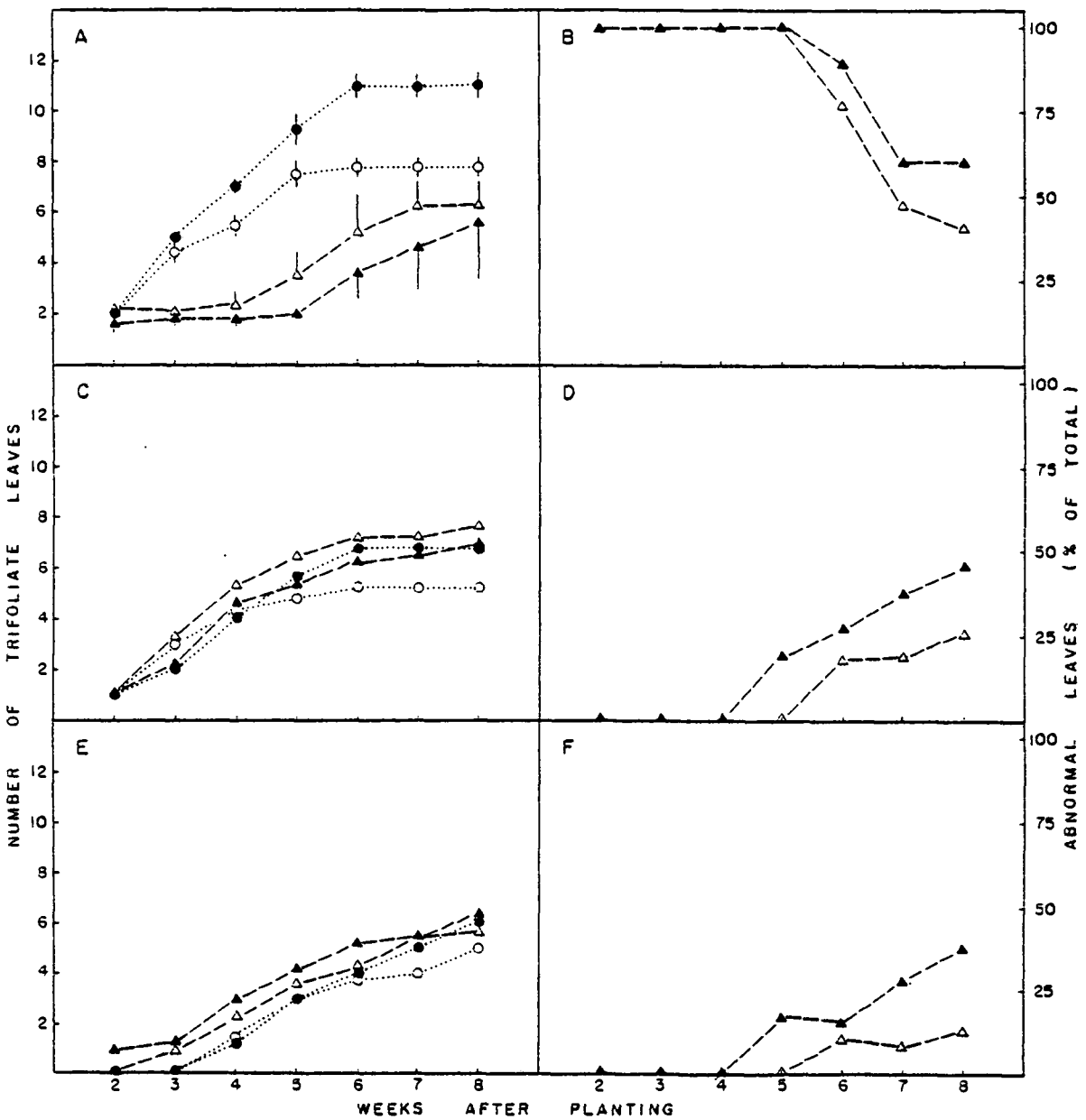
Figure 2. The effect of temperature regimes on the growth (A,C,E) of parents and F_1; and the proportion of chlorotic leaves (B,D,F) on F_1s.

A and B, 30°C/25°C (day/night); C and D, 25°C/20°C; E and F, 20°C/15°C.

Parents: P691 (●·····●); P725 (○·····○)

Hybrids: P691 x P725 (▲----------▲)

P725 x P691 (△----------△)


A
B
C
D
E
F
NUMBER OF TRIFOLIATE LEAVES
ABNORMAL LEAVES (% OF TOTAL)
WEEKS AFTER PLANTING
2
3
4
5
6
7
8
12
10
8
6
4
2
100
75
50
25

week (Figure 1F). At the fifth week, yellowing of new leaves occurred on some F_1 plants of P691 x P725 (Figure 1G). By the sixth week, most of the F_1 plants had newly formed leaves which were chlorotic (Figures 1H and 2D). The development of green and yellow leaves on F_1's was not clearly sequential since some green leaves turned yellow and some yellow leaves developed chlorophyll. The formation of aerial root primordia on the hypocotyls coincided with the appearance of chlorotic leaves. The occurrence of these abnormalities was not statistically different between the reciprocal hybrids.

The development of F_1 plants grown at 20°C/15°C was similar to that at 25°C/20°C (Figures 2C and 2E). However, the expression of the abnormal phenotypes was observed only in 11 of 12 F_1s of P691 x P725 and 8 of 12 F_1s of P725 x P691. (The apparently normal F_1s were later proven to be true hybrids. As P691 had purple hypocotyls and flowers and P725 had green hypocotyls and pinkish white flowers, the purple hypocotyls of apparently normal F_1s of P725 x P691 indicated they were hybrids. In addition, all apparently normal F_1s of reciprocal crosses gave rise to F_2 progeny which segregated for morphological abnormalities as well as for hypocotyl color. The purple hypocotyl and flower color was found to be a simple dominant trait in the present study.) Thus, there was incomplete penetrance of the abnormal phenotypes at the lower temperature regime.

The inheritance of the morphological abnormalities was studied using F_2 and backcross populations grown at 30°C/25°C. Phenotypic classes and the frequencies of individuals within each class were determined. Among F_2 populations, normal and F_1-like plants were recovered. In addition, two classes of abnormal seedlings not pre-

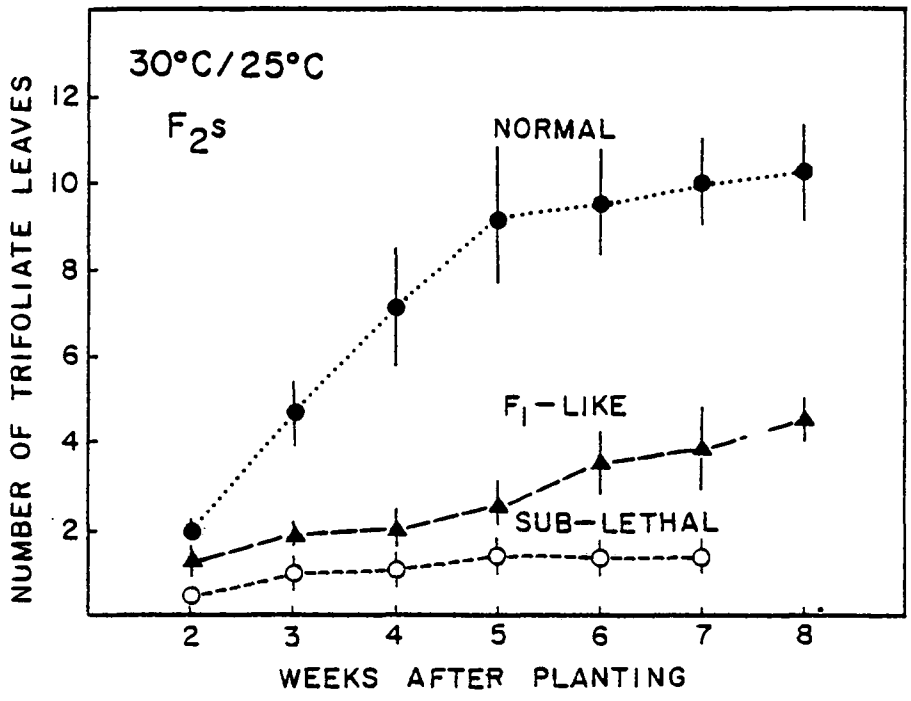


Figure 3. Growth of three phenotypic classes, normal (●·····●), F_1-like (▲----------▲), and sub-lethal (○·····○), of reciprocal F_2 populations grown at the temperature regime of 30°C/25°C (day/night).

viously observed were encountered. The first one was a lethal which had only very small primary leaves and perished soon after germination (Figure 1I). The second class of seedlings had normal primary leaves which enlarged very slowly. The development of these seedlings, designated as sub-lethals, was limited to two or less sets of small and malformed trifoliate leaves (Figure 1J) and followed by death. The growth rates of three classes of F_2 plants, the normal, F_1-like, and sub-lethal at the 30°C/25°C regime differed significantly (Figure 3). Root primordia were formed on hypocotyls of F_1-like as well as sub-lethal plants. The development and the fate of lethal and sub-lethal seedlings grown under the 25°C/20°C regime were the same as those grown under the 30°C/25°C regime. This independence from temperature in phenotypic expression further distinguished them from the F_1 phenotypes. F_2 plants classified as F_1-like developed in similar ways as the F_1s when grown at the same temperature regime.

The frequency distributions of F_2 progeny, classified into the normal, F_1-like, sub-lethal, and lethal categories grown under 30°C/25°C is presented in Table 1. The number of plants within each class did not differ from the 7:4:4:1. Backcross populations grown under the 30°C/25°C regime gave three phenotypic classes of plants, normal, F_1-like and sub-lethal (Figure 1K). The frequency distribution of individuals of each class in backcross populations was not significantly different than the ration of 2:1:1 (normal:F_1-like:sub-lethal).

DISCUSSION

The contrasting development of parents and F_1 plants and the distinct segregation of phenotypic classes in progeny populations

Table 1. Frequency distributions of F_2 and backcross populations and the goodness of fit to expected segregation ratios.

Population	No. of plants within each phenotypic class				Expected ratio	Goodness of fit (p)
	Normal	Temperature-dependent (F_1-type)	Sub-lethal	Lethal		
F_2 of P691 x P725	65	35	37	13	7:4:4:1	0.75
F_2 of P725 x P691	90	49	55	19	7:4:4:1	0.40
F_2 backcross to P691	24	14	14	0	2:1:1:0	0.85
F_2 backcross to P725	26	13	12	0	2:1:1:0	0.97

suggest that the abnormal phenotypes are of a genetic origin. Furthermore, the concomitant occurrence of a host of morphological abnormalities affecting growth, chlorophyll development and aerial root formation, indicate that a developmental disorder with diverse phenotypic expressions may be involved. The genetic differences between phenotypic classes are likely related to nuclear genes, as no reciprocal cross difference was observed. A genetic hypothesis is formulated to interpret the phenomenon based on progeny distributions (Figure 4).

As Phaseolus vulgaris is cleistogamous, and both parents are homozygous lines with normal phenotypes, the abnormal phenotypes of the F_1s may be conditioned by two heterozygous loci. The genes involved are tentatively designated as DL_1 (for dosage dependent lethal) and DL_2. The former is arbitrarily assigned as being derived from P691 and the latter from P725, such that parental genotypes would be DL_1 $DL_1dl_2dl_2$ and $dl_1dl_1DL_2DL_2$ respectively. The F_1s ($DL_1dl_1DL_2dl_2$) are phenotypically abnormal under high temperature. At lower temperature regimes, the phenotypic expression is either delayed or incomplete. F_2 individuals that are homozygous dominant at both loci are lethal and the expected frequency is 1/16. Plants homozygous dominant at one locus and heterozygous at the other locus (or having three doses of the dominant alleles) will display the phenotype of sub-lethal with the expected frequency of 1/4. The remaining F_2 individuals are homozygous recessive for at least one of the two loci, and have normal phenotypes. The expected phenotypic ratio of lethal:sub-lethal:temperature-dependent:normal is 1:4:4:7. The observed F_2 distribution (Table 1) did not differ significantly from this ratio. The phenotypic classes recovered in the backcross populations, and their frequencies, serve as a test of

the assigned genotypes of parents and F_1 based on the proposed genetic hypothesis. The expected phenotypes are the sub-lethal, the temperature-dependent, and the normal with a ratio of 1:1:2. The observed progeny distribution did not differ significantly from the expected (Table 1). Thus the proposed hypothesis explains adequately the progeny distributions observed in the present study.

The occurrence of morphologically abnormal hybrids in Phaseolus vulgaris was reported by Provvidenti and Schroeder (1969). These authors considered the abnormalities, seedling wilt, leaf-rolling, and apical chlorosis as three separate traits. However, the phenotypes were similar to those observed in the present study. Coyne (1969) observed "crippled" progeny among F_2 populations. The phenotypic abnormalities included stunted growth and shrunken leaves. It was suggested that the abnormalities were caused by two pairs of recessive alleles. Two brief reports, by York and Dickson (1975) and Rheenew (1979), described abnormal F_1s following intraspecific crossing of P. vulgaris. The frequency of normal to abnormal individuals in F_2 populations was not significantly different from a 7:9 ratio. As no distinction among abnormal progeny was mentioned, and all studies were conducted in the field, it is not clear if the two genes suggested to be responsible are similar to DL_1 and DL_2.

The present study centered on two genotypes as a model system for the genetic analysis of seedling lethals. It is likely that the interaction between DL_1 and DL_2 is sufficient to account for other lethals encountered in crossing programs at CIAT. Future testing of other parents would verify this possibility. The disruption of development of F_1s at temperature regimes higher than 30°C/25°C may be more severe

Figure 4. Proposed genetic hypothesis for the occurrence of abnormal plant types resulting from the crosses between P691 and P725.

P691 x P725

$DL_1DL_1dl_2dl_2$ Normal × $dl_1dl_1DL_2DL_2$ Normal

F_1

$DL_1dl_1DL_2dl_2$

Temperature-dependent abnormal

F_2

$1DL_1DL_1DL_2DL_2$	$2DL_1DL_1DL_2dl_2$ $2DL_1dl_1DL_2DL_2$	$4DL_1dl_1DL_2dl_2$	$1DL_1DL_1dl_2dl_2$ $1dl_1dl_1DL_2DL_2$ $2DL_1dl_1dl_2dl_2$ $2dl_1dl_1DL_2dl_2$ $1dl_1dl_1dl_2dl_2$
1 Lethal	4 Sub-lethal abnormal	4 Temp. Dep.	7 Normal

F_1 BC to P725

$1DL_1dl_1DL_2DL_2$	$1DL_1dl_1DL_2dl_2$	$1dl_1dl_1DL_2DL_2$ $1dl_1dl_1DL_2dl_2$
1 Sub-lethal	1 Temp. Dep. abnormal	2 Normal

than observed in the present study. Specifically, the reinitiation of growth and formation of chlorophyll at later growth stages may not occur. This assumption may explain the cessation of growth of F_1s grown at Cali where the maximum temperature often reaches 33°C. Furthermore, environmental factors such as relative humidity and light intensity may also influence the phenotypes of the heterozygotes.

As the presence of dominant alleles of DL_1 and DL_2 affects many aspects of growth, it is likely that the two loci may be related to the regulation of certain key processes of normal development. Although the physiological changes associated with DL_1 and DL_2 are unknown, it is reasonable to speculate that derangement in the hormonal metabolism may be involved.

SUMMARY

The occurrence of abnormal F_1s in crosses of Phaseolus vulgaris was found to be conditioned by two independent heterozygous loci, tentatively designated as DL_1 (for Dosage Dependent Lethal) and DL_2. The expression of morphological abnormalities of F_1s was influenced by temperature; the abnormal phenotype being accentuated by high temperature. The severity of the developmental derangement was dependent on the allelic dosage. Plants homozygous dominant at both loci were lethal and plants which are homozygous dominant at one locus and heterozygous for the other also perished but at a slower rate. As the presence of dominant alleles of DL_1 and DL_2 affects many aspects of growth, it appears that the two loci may be related to the regulation of key process of normal development. However, the specific physiological changes associated with the morphological abnormalities are unknown.

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CHAPTER 2

Developmental Controls of Morphological Mutants of *Phaseolus vulgaris* L.: Differential Expression of Mutant Loci in Plant Organs.

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ABSTRACT

Developmental controls of morphological mutants of Phaseolus vulgaris L. conditioned by two independent loci, DL_1 and DL_2, were examined through grafting experiments and hydroponic studies. Phenotypes of mutant classes were duplicated by unions of scions and stocks derived from different genotypes. Results indicate that DL_1 and DL_2 regulate a root and shoot factor respectively, contributing to the mutant types. The allelic dosages of DL_1 in the root and DL_2 in the shoot rather than the genotype of the whole plant per se determine the severity of the mutant expression. Plants heterozygous for both loci with a temperature-sensitive expression of the mutant phenotype were used to determine physiological components involved. The primary abnormal developmental event associated with the appearance of mutant phenotypes, the restricted root growth at high temperature, could be overcome by the addition of cytokinin in hydroponic solution. These observations suggest that DL_1 and DL_2 may be related to the regulation of hormonal function or metabolism.

Key Words: temperature-sensitive mutant, cytokinin, hormonal metabolism.

INTRODUCTION

Hybrids with abnormal development were obtained when particular genotypes of Phaseolus vulgaris were crossed (1). The phenotypic aberrations included retarded growth, chlorosis of the trifoliate leaves and the formation of adventitious roots on the hypocotyls. The expression of the abnormalities was temperature-dependent. At a high temperature regime (30°C/25°C, day/night), the onset of the abnormal phenotypes occurred at an early stage of development (two weeks after planting) and essentially no new growth was observed between the second and the sixth week. However, at lower temperature regimes (25°C/20°C and 20°C/15°C, day/night), the F_1s exhibited normal and vigorous growth. Only a slight yellowing of the leaves and the appearance of adventitious root primordia on the hypocotyls at the sixth week distinguished them from the parental genotypes. The abnormalities observed on F_1s at high temperature are referred to as the "crippled" phenotypes.

Genetic analyses have indicated that the abnormal development is conditioned by two loci (1), but that the severity of the abnormalities is dependent on the allelic dosage. The F_1s are heterozygous at both loci and the expression of the "crippled" phenotypes is temperature-dependent. Plants homozygous dominant for both loci were lethal and perished soon after germination. (In order to simplify the description of the genetic system, the alleles which confer abnormal development are referred to as "dominant", although heterozygotes can be distinguished from the homozygous plants). Plants homozygous dominant for one of the two loci but heterozygous for the other locus (three dominant alleles) were classified as sub-lethals. These plants formed no more than two sets of small trifoliate leaves and also perished but at a later time

than the lethals (about four to five weeks after planting). The expression of the lethal and sub-lethal phenotypes was not influenced by the temperature. The two loci involved were designated as DL_1 and DL_2 for dosage-dependent lethals, and they were arbitrarily assumed to have been derived from the two parental genotypes used in the genetic study, P691 and P725 respectively.

As the severity of the developmental abnormalities and the expression of the mutant phenotypes are conditioned by allelic dosage and temperature, the DL system is useful in examining the genetic regulation of plant development. The present paper describes experimental results which indicate differential expressions of DL_1 and DL_2 in plant parts and suggest that the developmental processes affected by the genetic factors identified may involve the alteration of hormonal function or metabolism.

MATERIALS AND METHODS

Plant materials: The plant materials included were Phaseolus vulgaris L. genotypes P691 (P_1) and P725 (P_2) and their reciprocal hybrids. The parental genotypes P691 (also identified as G4489 or Guilopa 72) and P725 (G3804 or Bolivia I1095) were kindly provided by Dr. S. R. Temple of CIAT, Colombia.

Environmental conditions: All experiments were performed in growth chambers at two temperature regimes, 30°C/25°C and 25°C/20°C (day/night). The photoperiod was 12 hours and the light intensity was approximately 275 μ einsteins/m^2sec.

Grafting experiments: Seeds were germinated in sterile Jiffy Mix Plus (obtained from Pacific George Ball Co., Ca.). Uniform germination of seeds was achieved by scarifying the seed coats. Five-day old seedlings

were severed at the mid-point of the hypocotyl. Scions and stocks were united by a slant-cut union at this point and the grafts were secured with tape and flexible clamps.

The growth of the graft combination was measured at weekly intervals by the number of trifoliate leaves. The number of adventitious roots on the hypocotyls and the dry weights of shoots and roots were determined six weeks after planting of the seeds. The shoot dry weight represents the dry weight of all parts of the scion (including the hypocotyl) and the root dry weight includes roots only. The data presented are the averages of 15 plants. The experiment was repeated once.

Hydroponic culture: The hydroponic solution contained the inorganic nutrients described by Murashige and Skoog (2) at one-quarter strength. The pH of the culture solution was adjusted to 5.7. Seeds were germinated in sterile Jiffy Mix Plus. Five days after planting, seedlings were washed and transferred to hydroponic culture. Each plant was grown individually in a half-gallon ceramic container. Aeration was provided by bubbling air through the culture solution.

The dry weights of shoots and roots were measured at weekly intervals. The shoot portion included the hypocotyl. Each harvest consisted of four plants per genotype. The experiment was repeated once.

Tests of the effect of N^6-benzyladenine on growth: To test the effect of cytokinin on growth of parents and hybrids, appropriate amounts of N^6-benzyladenine (obtained from Sigma) were added to the hydroponic culture solution to yield concentrations of 0.04, 0.2, 1 and 5 μM. Plants were grown hydroponically as described above. The dry weights of shoots and roots were determined after six weeks. Each harvest consisted of four plants per genotype. The experiment was repeated once.

RESULTS

The grafting experiments were designed to detect possible differential gene expression of the two loci, DL_1 and DL_2, in the root and shoot systems. Initial experiments included graftings between parental genotypes and control graftings (stock and scion derived from the same genotype). The results of these experiments are presented in Figs. 1A and B and in Table 1. The parental genotypes, P_1/P_1 and P_2/P_2 (scion/stock) developed normally at both temperature regimes as measured by the number of trifoliate leaves (Figs. 1A, B), the dry weights of roots and shoots and the low number of adventitious roots on the hypocotyls (Table 1). The development of grafts of the hybrids (F_1/F_1) was restricted at elevated temperature (Fig. 1A, Table 1) whereas at the lower temperature regime, growth was normal (Fig. 1B, Table 1). Thus the development of the controls and their responses to the two temperature regimes were similar to those previously observed on intact plants of the corresponding genotypes. (In this experiment and in the following experiments, the results for reciprocal F_1s have been pooled since no apparent differences between the two types of F_1s were detected.)

Dramatic differences in developmental patterns were observed as a result of intergrafting the two parental genotypes. P_1/P_2 (scion/stock) combinations exhibited normal growth at both temperatures but the growth of the reciprocal combination (P_2/P_1) was severely limited. At high temperature (Fig. 1A), the P_2/P_1 combination died after four weeks; at lower temperature (Fig. 1B) this combination persisted slightly longer (Fig. 2) and died after five weeks. The expression of lethality of P_2/P_1 grafts was not dependent on the temperature. The severity of the abnormal growth could also be measured by the high number of adventitious

Figure 1: Number of trifoliate leaves of graft combinations (scion/stock) at high and low temperature regimes over a period of five weeks.

(A) Reciprocal grafts between P_1 and P_2, and control grafts at 30°C/25°C.

(B) Reciprocal grafts between P_1 and P_2 and control grafts at 25°C/20°C.

(C) Reciprocal graft combinations between parents and F_1s at 30°C/25°C.

(D) Reciprocal graft combinations between parents and F_1s at 25°C/20°C.

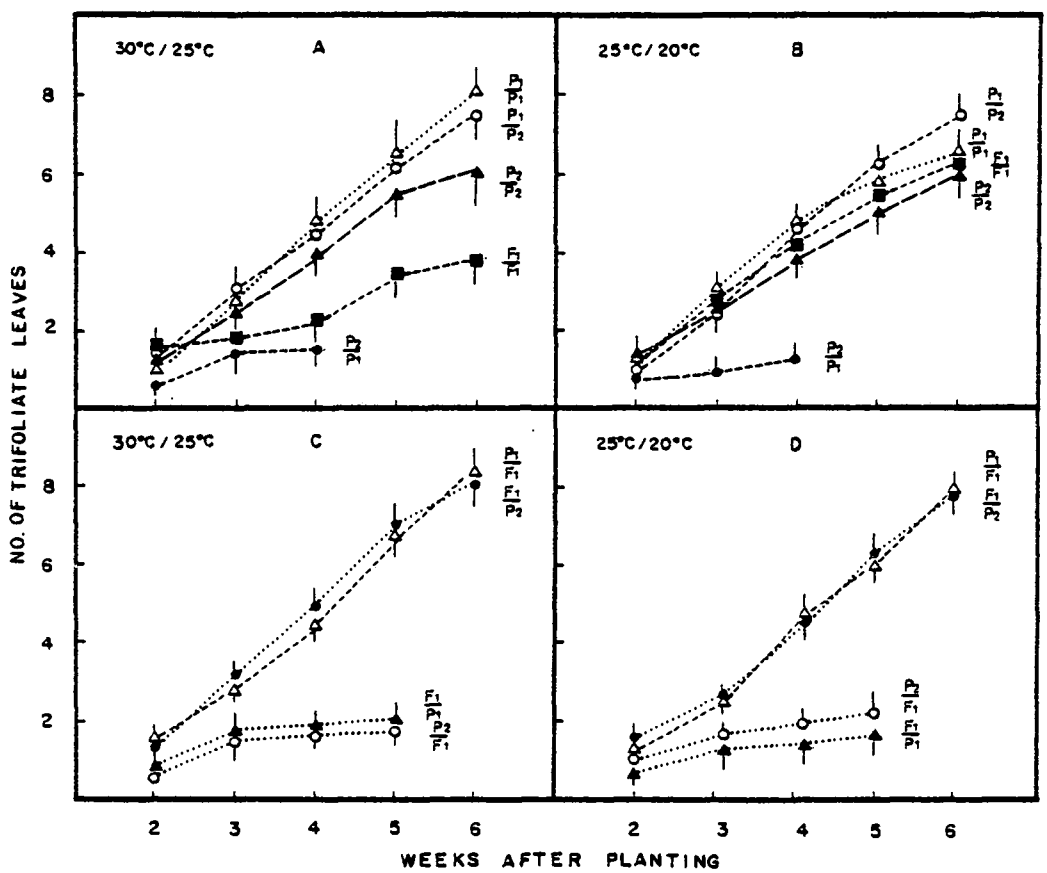

30°C / 25°C
A
25°C / 20°C
B
30°C / 25°C
C
25°C / 20°C
D
NO. OF TRIFOLIATE LEAVES
WEEKS AFTER PLANTING
2
3
4
5
6
8

Table 1. Number of adventitious roots on the hypocotyl and dry weights and standard deviations of roots and shoots of graft combinations measured at six weeks after planting.

Graft Combination	Temperature Regime					
	30°C/25°C			25°C/20°C		
	No. of Adv.	Dry Weight/Plant		No. of Adv.	Dry Weight/Plant	
Scion/Stock	Roots/Plant	Shoot (g)	Root (mg)	Roots/Plant	Shoot (g)	Roots (mg)
P_1/P_1	1	2.3 ± 0.4	455 ± 110	1	4.3 ± 0.5	893 ± 185
P_2/P_2	3	2.5 ± 0.3	482 ± 91	3	5.7 ± 1.9	835 ± 102
F_1/F_1	62	1.0 ± 0.5	90 ± 75	47	3.9 ± 2.1	369 ± 150
P_1/P_2	2	2.7 ± 0.4	402 ± 76	6	3.7 ± 0.4	837 ± 111
P_2/P_1*	7	0.2 ± 0.1	20 ± 3	56	0.3 ± 0.1	22 ± 5
P_1/F_1	4	2.2 ± 0.5	351 ± 140	1	4.6 ± 0.6	839 ± 191
F_1/P_1**	59	0.4 ± 0.1	34 ± 4	58	0.4 ± 0.1	28 ± 3
P_2/F_1**	52	0.3 ± 0.1	24 ± 3	57	0.5 ± 0.1	25 ± 4
F_1/P_2	4	2.3 ± 0.2	359 ± 51	3	5.0 ± 0.8	871 ± 168

* Measurements were taken at the fourth week as this combination did not survive beyond that time.

** Measurements were taken at the fifth week as these combinations did not survive beyond that time.

Figure 2: Reciprocal and control graft combinations (scion/stock) between P_1 and P_2 after four weeks at 25°C/20°C. From left to right, P_1/P_1, P_1/P_2, P_2/P_1 (the lethal phenotype) and P_2/P_2.

roots and low dry weights of roots and shoots (Table 1). The fact that the P_2/P_1 grafts grown at high temperature had only a few adventitious roots on the hypocotyls was due to the premature death which necessitated measurement at the fourth week. These results indicate that the parental combination of P_1 as stock and P_2 as scion displays abnormal development similar to that of the lethal class of mutants (homozygous dominant at the DL_1 and DL_2 loci) whereas the reciprocal combination exhibits normal growth.

In order to examine the effects of P_1 and P_2 as scion or stock in conditioning other mutant phenotypes, such as the sub-lethals, combinations between parents and F_1s were examined. Grafts with the hybrids as the stock and P_1 as the scion exhibited normal growth at both temperature regimes (Figs. 1C, D). The dry weights of the roots and shoots of this combination were slightly higher than the controls (P_1/P_1 at both temperatures and F_1/F_1 at low temperature) and only a few adventitious roots were formed on the hypocotyls (Table 1). However, the reciprocal grafts (F_1/P_1, scion/stock) were phenotypically abnormal at both temperature regimes. The number of trifoliate leaves did not increase after grafting and pre-existing leaves did not expand. Adventitious roots appeared on the hypocotyls and growth ceased completely after five weeks. The pattern of development of the F_1/P_1 combinations resembled that of the sub-lethals.

Graft combinations between F_1s and P_2 gave results opposite of those obtained with F_1/P_1 combinations. F_1 as the scion with P_2 as the stock resulted in normal growth at both temperatures with few adventitious roots formed (Figs. 1C, D and Table 1). P_2/F_1 grafts exhibited phenotypes similar to those of F_1/P_1 combinations (Fig. 3). These results

Figure 3: Reciprocal graft combinations (scion/stock) between P_2 and F_1 after four weeks at 25°C/20°C. Left, F_1/P_2; right, P_2/F_1 (the sub-lethal phenotype).

suggest that a root factor of P_1 and a shoot factor of P_2 contribute to the occurrence of abnormal developments. In addition, the severity of the abnormalities seems to be dependent on the dosage of dominant alleles of DL_2 in the scion and of DL_1 in the stock.

The differential expression of DL_1 and DL_2 in scion and stock led to attempts to identify the physiological components involved. In order to closely examine shoot and root growth, plants were grown in hydroponic culture. The growth pattern of parents and F_1s as represented by dry weights of shoots and roots are presented in Fig. 4. At high temperature, the restricted root growth of the F_1s was apparent (Fig. 4C). The limited root development was accompanied by the formation of a large number of adventitious roots on the hypocotyls and chlorosis of the leaves. However, the reduction in growth of the shoots (Fig. 4A) of the F_1s as compared with the parents was less severe than previously observed when plants were grown in solid planting medium. At lower temperature, the growth of F_1 roots (Fig. 4D) was still significantly less than that of both parents particularly at later stages, but it was substantially improved compared with growth at the high temperature (Fig. 4C). The dry weights of the shoots (Fig. 4B) were similar to those of the parents with the exception of the final measurement (six weeks).

The effects of exogenously supplied cytokinins on the growth of parents and F_1s were tested by the addition of N^6-benzyladenine to the hydroponic solution at the beginning of the culture. The dry weights of roots and shoots and the number of adventitious roots on the hypocotyls (of the F_1s) were determined after six weeks (Figs. 5, 6). At high temperature, the presence of cytokinin increased the root and shoot growth of F_1s dramatically (Figs. 5A, C, 7, 8). At the optimal concentration

Figure 4: The dry weights of shoots and roots of P_1, P_2 and F_1 grown in hydroponic culture at high and low temperature regimes over a period of six weeks.

(A) Dry weight (g/plant) of shoots of parents and F_1 at 30°C/25°C.

(B) Dry weight (g/plant) of shoots of parents and F_1 at 25°C/20°C.

(C) Dry weight (mg/plant) of roots of parents and F_1 at 30°C/25°C.

(D) Dry weight (mg/plant) of roots of parents and F_1 at 25°C/20°C.

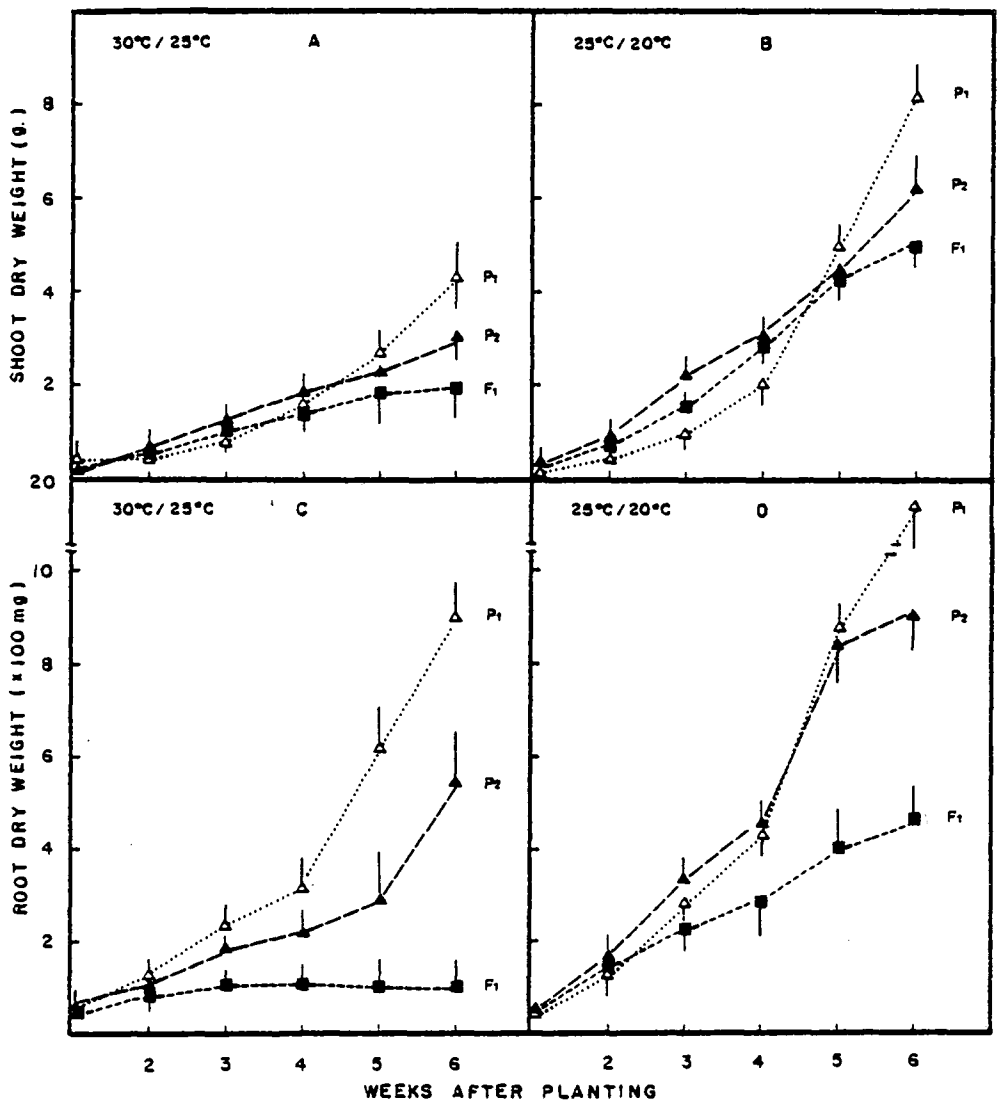

30°C / 25°C
A
25°C / 20°C
B
30°C / 25°C
C
25°C / 20°C
D
P1
P2
F1
SHOOT DRY WEIGHT (g.)
ROOT DRY WEIGHT (×100 mg)
WEEKS AFTER PLANTING
2
3
4
5
6
8
10
20

Figure 5: The dry weights of shoots and roots, measured after six weeks, of P_1, P_2 and F_1 grown in hydroponic culture at five concentrations of N^6-benzyladenine.

(A) Dry weight (g/plant) of shoots of parents and F_1 at 30°C/25°C.

(B) Dry weight (g/plant) of shoots of parents and F_1 at 25°C/20°C.

(C) Dry weight (mg/plant) of roots of parents and F_1 at 30°C/25°C.

(D) Dry weight (mg/plant) of roots of parents and F_1 at 25°C/20°C.

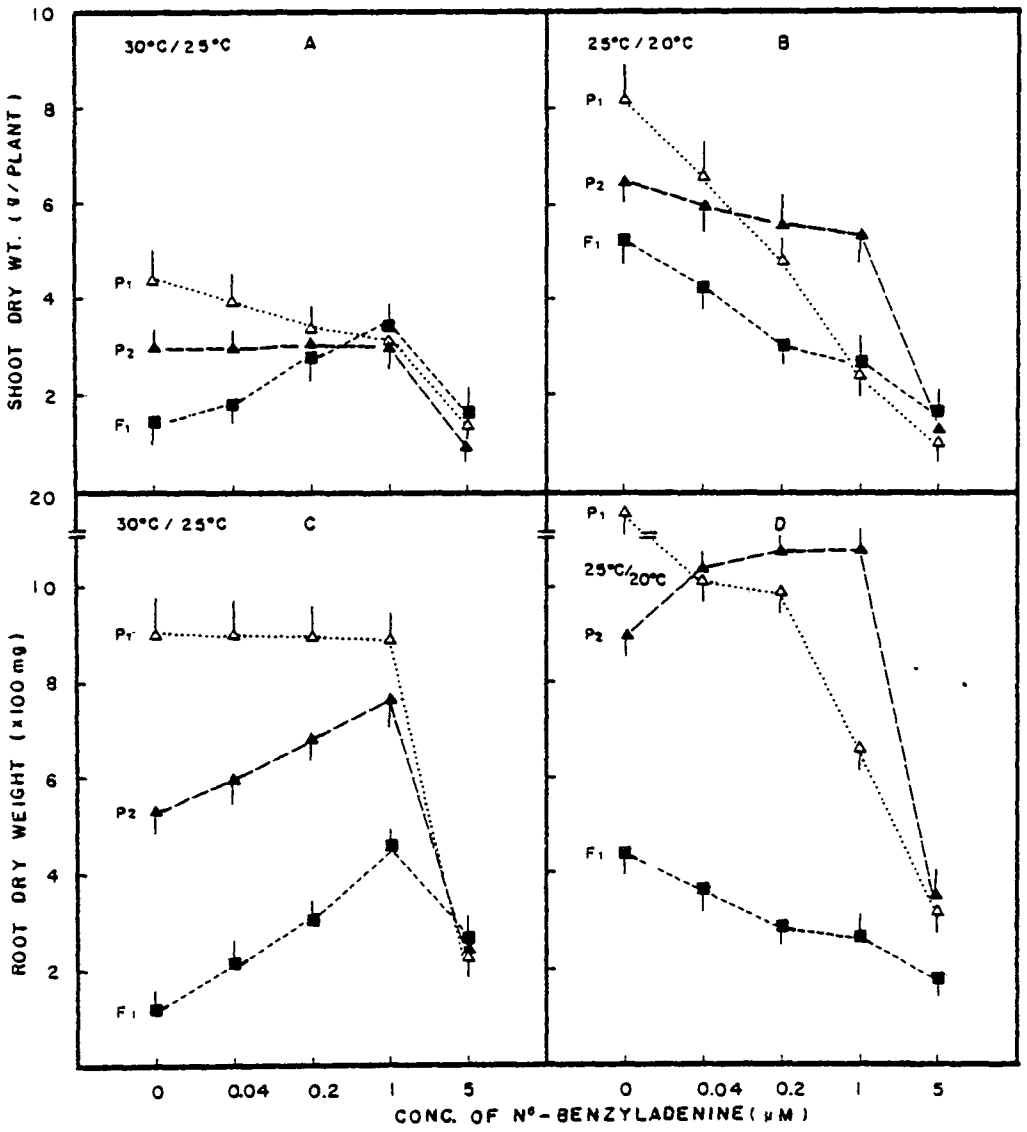
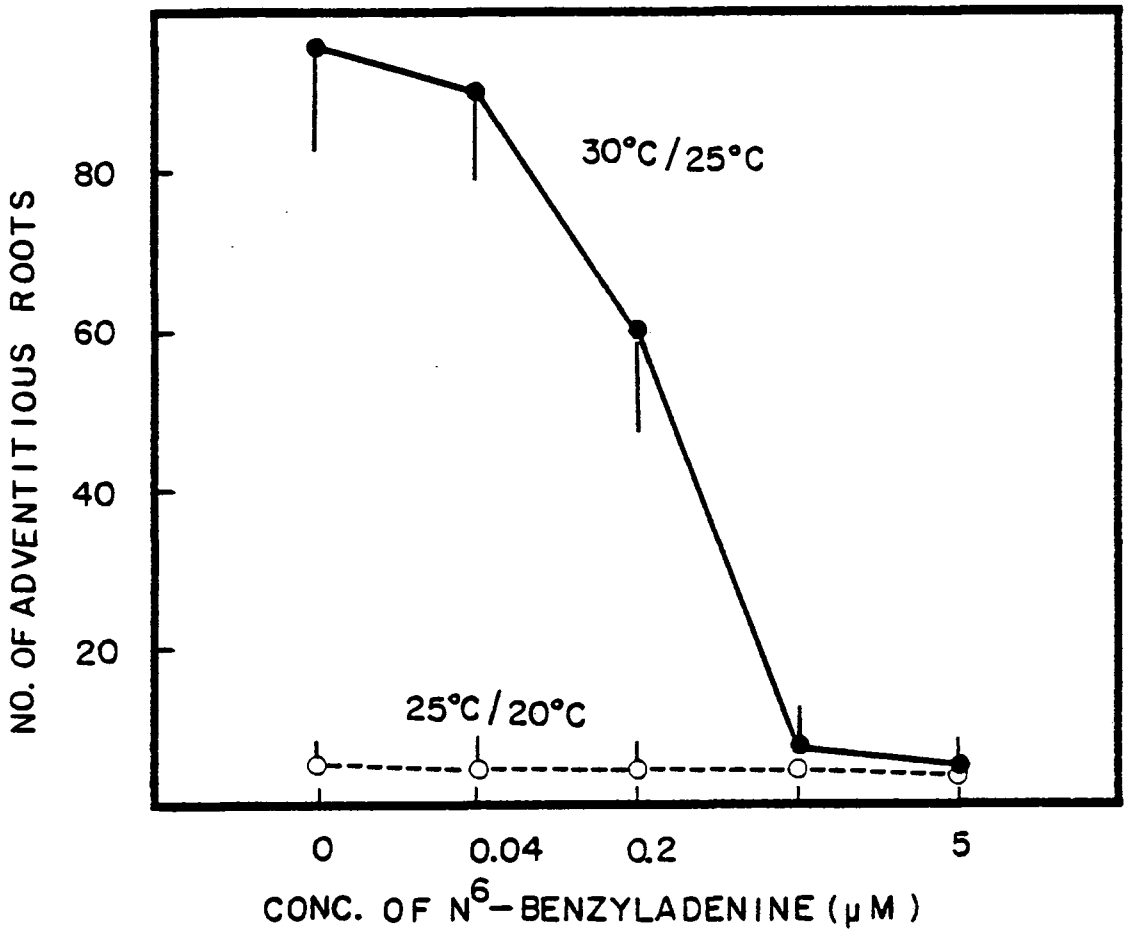

30°C/25°C
A
25°C/20°C
B
30°C/25°C
C
25°C/20°C
D
P1
P2
F1
SHOOT DRY WT. (g/PLANT)
ROOT DRY WEIGHT (×100 mg)
0
0.04
0.2
1
5
CONC. OF N⁶-BENZYLADENINE (μM)

Figure 6: The effects of N^6-benzyladenine on the formation of adventitious roots (number/plant, after three weeks) of F_1s at 30°C/25°C and 25°C/20°C.


NO. OF ADVENTITIOUS ROOTS
80
60
40
20
30°C / 25°C
25°C / 20°C
0
0.04
0.2
5
CONC. OF N6-BENZYLADENINE (μM)

of 1 μM N^6-benzyadenine, the dry weight of shoots was similar to that of P_2 grown in the absence of added cytokinin. In addition, the appearance of morphological abnormalities such as the formation of adventitious roots was greatly repressed by increasing concentration of N^6-benzyladenine (Fig. 6). The presence of cytokinin was also beneficial to the root growth of P_2 at high temperature (Fig. 5C). However, the shoot dry weight (Fig. 5A) was not different from plants grown in the absence of cytokinin. Higher concentration of cytokinin (5 μM) reduced growth of all genotypes. At lower temperature (Fig. 5B, D), growth of parents and hybrids decreased with the addition of N^6-benzyladenine. The only exception was the increase in root dry weights of P_2 at 0.04, 0.2 and 1 μM of N^6-benzyladenine which was due to the formation of thick tap roots and pseudonodules.

DISCUSSION

The results of grafting experiments demonstrate that a root factor of P_1 and a shoot factor of P_2 contribute to the developmental abnormalities. This interpretation is substantiated by the mimicking of mutant phenotypes such as the lethals (P_2/P_1 combination) and sublethals (P_2/F_1 and F_1/P_2) through grafting. As the reciprocal combinations (P_1/P_2, F_1/P_2 and P_1/F_1) were all normal, it appears that the genotype of the scion and that of the stock individually determine the normal vs. abnormal development. These results indicate that in the whole plants, the loci DL_1 and DL_2 are expressed in the root and the shoot systems respectively.

As far as we know, this is the first report of abnormal development in plants as the result of interaction between independent root and shoot

Figure 7: The effects of N^6-benzyladenine on the shoot growth of F_1s at 30°C/25°C, showed after six weeks (from left to right, the concentrations of N^6-benzyladenine are 0, 0.04, 0.2, 1 and 5 μM).

Figure 8: The effects of N^6-benzyladenine on the root growth of F_1s at 30°C/25°C, showed after six weeks (from left to right, the concentrations of N^6-benzyladenine are 0, 0.04, 0.2, 1 and 5 μM).

factors. Developmental mutants such as the knotted mutant in maize (3) and the diageotropica and brush root mutants of the tomato (4, 5, 6) involve primary lesions of either shoot or root development, although in the brush root mutant the shoot morphology is affected as a consequence of abnormal root growth. Therefore, the DL system may be particularly useful in determining the relationship between developmental controls of the roots and the shoots.

One of the abnormal developmental events in the F_1 is the restricted root growth at high temperature which can be alleviated by the addition of cytokinin. Based on the interpretation derived from results of the grafting experiments, the slower root growth must be a primary consequence of the interaction between the root and the shoot factors. The effects of cytokinins in restoring normal root growth may suggest either a reduction of the levels of endogenous cytokinins, or an increase in the cytokinin requirement of the F_1s at high temperature. As roots are generally considered to be the primary site of cytokinin biosynthesis (7), it is possible that the root factor derived from P_1 decreases the level of cytokinins of F_1s at high temperature. This interpretation alone appears to be insufficient since the presence of cytokinin was inhibitory to the root growth of P_1. Thus increased cytokinin requirement of the F_1s as the result of the shoot factor derived from P_2 needs to be considered. As normal plant development is maintained by the balance between cytokinins and other growth regulators (8, 9, 10), an increase auxin biosynthesis by the shoot factor at high temperature could account for the increase cytokinin requirement of the F_1s. Higher endogenous auxin levels would also be compatible with the observations of adventitious root formation on the hypocotyls and the improved root growth of P_2 in

the presence of N^6-benzyladenine at high temperature. Based on interpretation of the results, the root factor of P_1 and the shoot factor of P_2 may be related to lower level of cytokinins and higher level of auxins respectively. Alternatively, the shoot factor may condition elevated auxin levels and the root factor auxin sensitivity. However, experiments involving exogenous application of auxins to parents and F_1s gave ambiguous results (unpublished). Thus the suggestion that the effects of cytokinins in restoring root growth of F_1s may in part be related to altered auxin biosynthesis remains to be tested.

As the DL system affects a wide range of developmental events, it was speculated earlier (1) that DL_1 and DL_2 may be related to the regulation of certain key processes of normal development, such as hormone biosynthesis or metabolism. Experimental results reported in the present paper are in agreement with this hypothesis. The task of identifying biochemical lesions associated with the mutations should be further facilitated by the finding of differential gene expression in the shoot and the root, and the ability to duplicate phenotypic conditions of different mutants by interchanging plant parts.

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