Genetic and physiologic characterization of an agravitropic barley mutant
by Laura Ann Tagliani

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Agronomy
Montana State University

Abstract:
A chemically induced mutation in barley (Hordeum vulqare L.) which results in agravitropic roots was examined to determine genetic and physiologic controls of the trait. Crosses between wild type and mutant plants yielded three to one F2 segregation ratios, indicating monogenic control with gravitropism completely dominant over agravitropism. No linkage was found between agravitropism and seven barley translocation breakpoints, the V locus on chromosome two, or the Hor-1 and Hor-2 loci on chromosome five.

No evidence of gravicurvature was found in light or dark grown agravitropic roots, although shoots displayed complete negative gravitropism. Equivalent amounts of starch were found in root tips of agravitropic and gravitropic plants, indicating the presence of sufficient starch in the amyloplasts for gravitropic perception. Total root growth was similar for mutant and wild type roots, although the mutant had fewer roots per seed and greater elongation per root. The agravitrope's root growth was more tolerant of inhibitory levels of applied IAA than wild type roots. Agravitropic and gravitropic roots were equally sensitive to applications of NAA and 2,4-D. High pressure liquid chromotography determinations of root endogenous IAA levels showed no differences between gravitropes and agravitropes. The data from these experiments suggest that auxin controlled growth regulation may be altered in the mutant, particularly the ability of the tissue to transport, receive, or respond to IAA.

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ABSTRACT

A chemically induced mutation in barley (Hordeum vulgare L.) which results in agravitropic roots was examined to determine genetic and physiologic controls of the trait. Crosses between wild type and mutant plants yielded three to one F_2 segregation ratios, indicating monogenic control with gravitropism completely dominant over agravitropism. No linkage was found between agravitropism and seven barley translocation breakpoints, the V locus on chromosome two, or the Hor-1 and Hor-2 loci on chromosome five.

No evidence of gravicurvature was found in light or dark grown agravitropic roots, although shoots displayed complete negative gravitropism. Equivalent amounts of starch were found in root tips of agravitropic and gravitropic plants, indicating the presence of sufficient starch in the amyloplasts for gravitropic perception. Total root growth was similar for mutant and wild type roots, although the mutant had fewer roots per seed and greater elongation per root. The agravitrope's root growth was more tolerant of inhibitory levels of applied IAA than wild type roots. Agravitropic and gravitropic roots were equally sensitive to applications of NAA and 2,4-D. High pressure liquid chromotography determinations of root endogenous IAA levels showed no differences between gravitropes and agravitropes. The data from these experiments suggest that auxin controlled growth regulation may be altered in the mutant, particularly the ability of the tissue to transport, receive, or respond to IAA.

INTRODUCTION

Novel qualitative mutants are valuable research tools. Such mutants can be utilized as genetic markers in linkage studies and in the construction of genetic maps. Determinations of physiological mechanisms or biochemical pathways have also been facilitated by the use of mutants.

Mutations arise spontaneously or through induced mutagenesis, and are identified by a change in phenotype. Effective use of a mutant in genetic or physiologic research requires the establishment of the genotype and biochemical alteration of the mutant.

The purpose of this work was to characterize the genetic and physiologic mechanisms conferring barley (Hordeum vulgare L.) root agravitropism. The goal of the genetic studies was to identify the number of genes controlling agravitropism, the type of gene action, and the chromosome carrying this mutation. The physiological studies were designed to investigate mechanisms for the mutant's lack of root graviresponse. Results from this work can be utilized in general gravitropic physiology or barley genetics research.

LITERATURE REVIEW

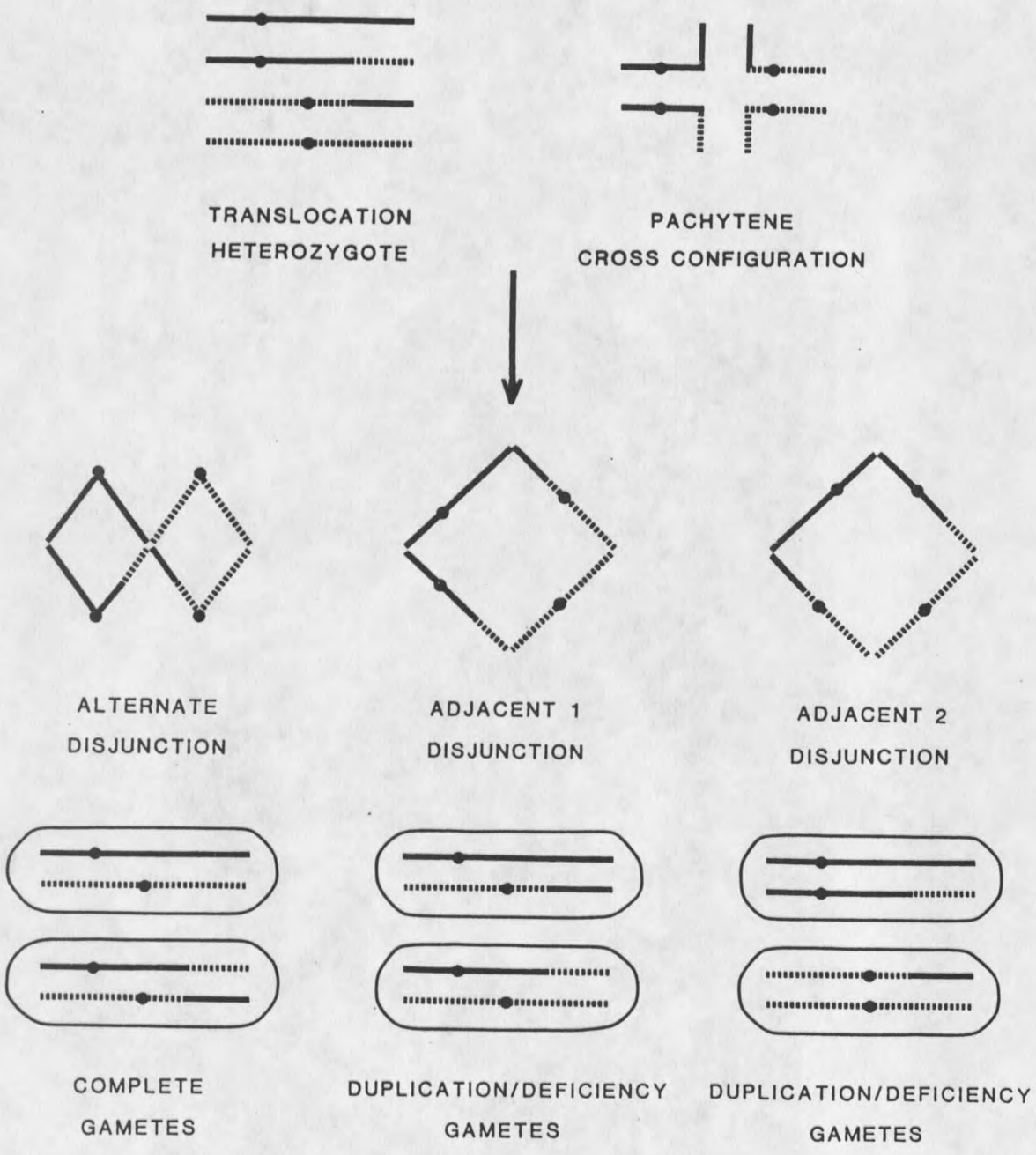
Genetic Analysis

Interchange Chromosome Behavior

Strickberger (1976) and Sybenga (1975) describe the behavior of translocated chromosomes. A reciprocal translocation, or interchange, is an exchange of segments of nonhomologous chromosomes. A translocation heterozygote is an individual that carries two normal and two translocated chromosomes, thereby retaining a full chromosome complement. In meiotic prophase, homologous segments of translocation heterozygotes pair, forming a cross configuration. At diakinesis, a ring of four is often formed due to terminalization of chiasmata. Separation of the quadrivalent can occur in three combinations; alternate, adjacent 1, or adjacent 2. Alternate disjunction appears as a folded ring, the two translocated chromosomes move to one pole and the two normal chromosomes move to the other pole, resulting in complete, viable gametes. Adjacent disjunction occurs in an open ring, with one translocated and one normal chromosome moving together. This results in duplication and deficiency gametes, which are usually lethal. In adjacent 1 separation, nonhomologous centromeres move to the same pole, and in adjacent 2, homologous centromeres move together (Figure 1).

Centromere disjunction types are expected to occur at a frequency of 50% alternate, 25% adjacent 1 and 25% adjacent 2 orientations

Figure 1. Diagrammatic representation of translocation heterozygote pairing and separation during meiosis.



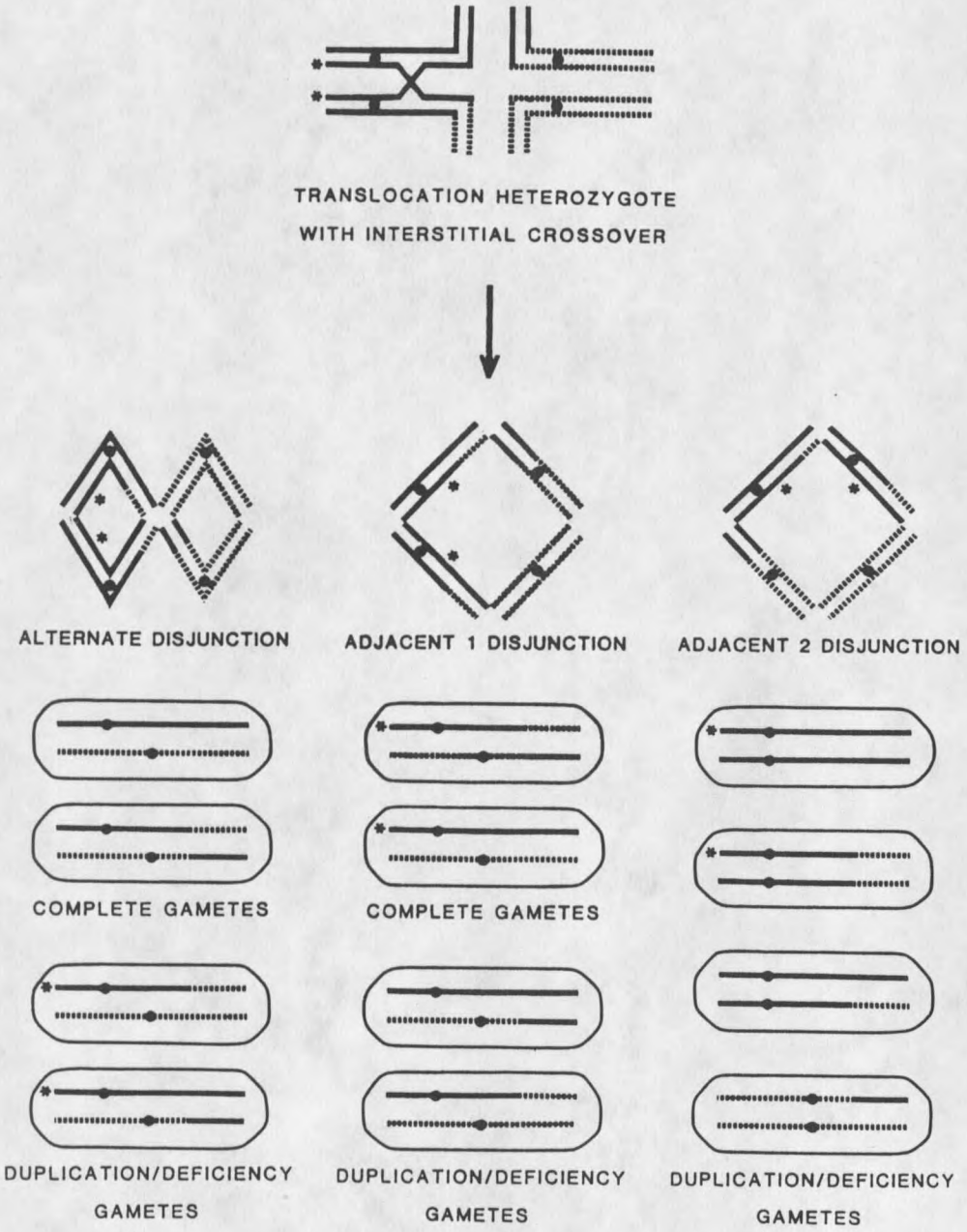
(Rickards 1983). Diploids cannot tolerate most chromosomal deficiencies (Burnham 1956), therefore in the absence of recombination, only those gametes resulting from alternate disjunction should be viable. Hence, one half of the eggs and pollen produced would be nonviable, yielding 50% seed sterility.

Semisterility of translocation heterozygotes in barley is usually less than the expected 50%, ranging from 31.6% (Hanson 1952) to 12% (Smith 1951). Reduced sterility probably indicates a greater occurance of alternate disjunction (Ramage 1963; Burnham et al. 1954; Burnham and Hagberg 1956). Sterility also varies with environment and specific interchanges (Nilan 1964).

When translocation heterozygote chromosomes are in the meiotic cross configuration, crossover can occur in paired regions (Sybenga 1975). Interstitial crossover, between the centromere and translocation break point, alters gamete viability (Hanson 1952). Alternate and adjacent 1 disjunction result in 50% duplication and deficiency gametes (Burnham 1956). McClintock (1945) and Burnham (1950) observed that chromosomes involved in interstitial crossover pass to opposite poles, essentially eliminating adjacent 2 segregation. Due to meiotic metaphase orientations, interstitial recombinants are recovered solely from adjacent 1 segregation. Viable gametes from alternate disjunction contain only parental chromosomes (Figure 2).

The observed amount of interstitial recombination in interchange heterozygotes depends on the frequency of centromere disjunction types and the expected crossover frequency for the two interstitial regions (Hanson 1952). An abundance of alternate disjunction increases the

Figure 2. Diagrammatic representation of meiotic pairing and separation of a translocation heterozygote with interstitial recombination (* indicates crossover chromatids).



recovery of parental gametes and decreases the observed amount of recombination. Pairing and crossover may also be reduced due to torsions around the break point or heterochromatin near the centromere (Schulz-Schaeffer 1980). DeVries (1983) concluded that in rye, the low recombinant recovery is due to disturbance in pairing and crossover, rather than an excess of alternate disjunction.

The amount of interstitial crossover in barley is masked by the increased frequency of alternate disjunction (Hanson and Kramer 1949; Burnham and Hagberg 1956; Ramage 1963). Kramer and Blander (1961) estimated that 16.7% was the maximum recombinant recovery in barley translocations. DeVries (1983) argues that on the basis of recombination and cytological data, alternate disjunction of chromosomes with interstitial chiasma should be assumed at 50%, not 75% as Kramer and Blander (1961) used. DeVries (1983) found that interstitial recombination was masked due to higher alternate disjunction of chromosomes without chiasma. Regardless of cause, decreased recovery of interstitial recombinants occurs in barley (Kasha and Burnham 1965; Ramage and Suneson 1961; Ramage 1966).

Linkage Analysis

Barley translocations have been used in linkage studies, and are based on the association of genetic traits and semisterility (Nilan 1964; Joachim 1947; Hanson and Kramer 1950; Kasha and Burnham 1965; Tuleen 1971). Self-pollinated translocation heterozygotes segregate one translocation homozygote: two translocation heterozygotes: one normal homozygote (Ramage 1963). The homozygotes have normal fertility, but the interchange heterozygotes exhibit semisterility, due

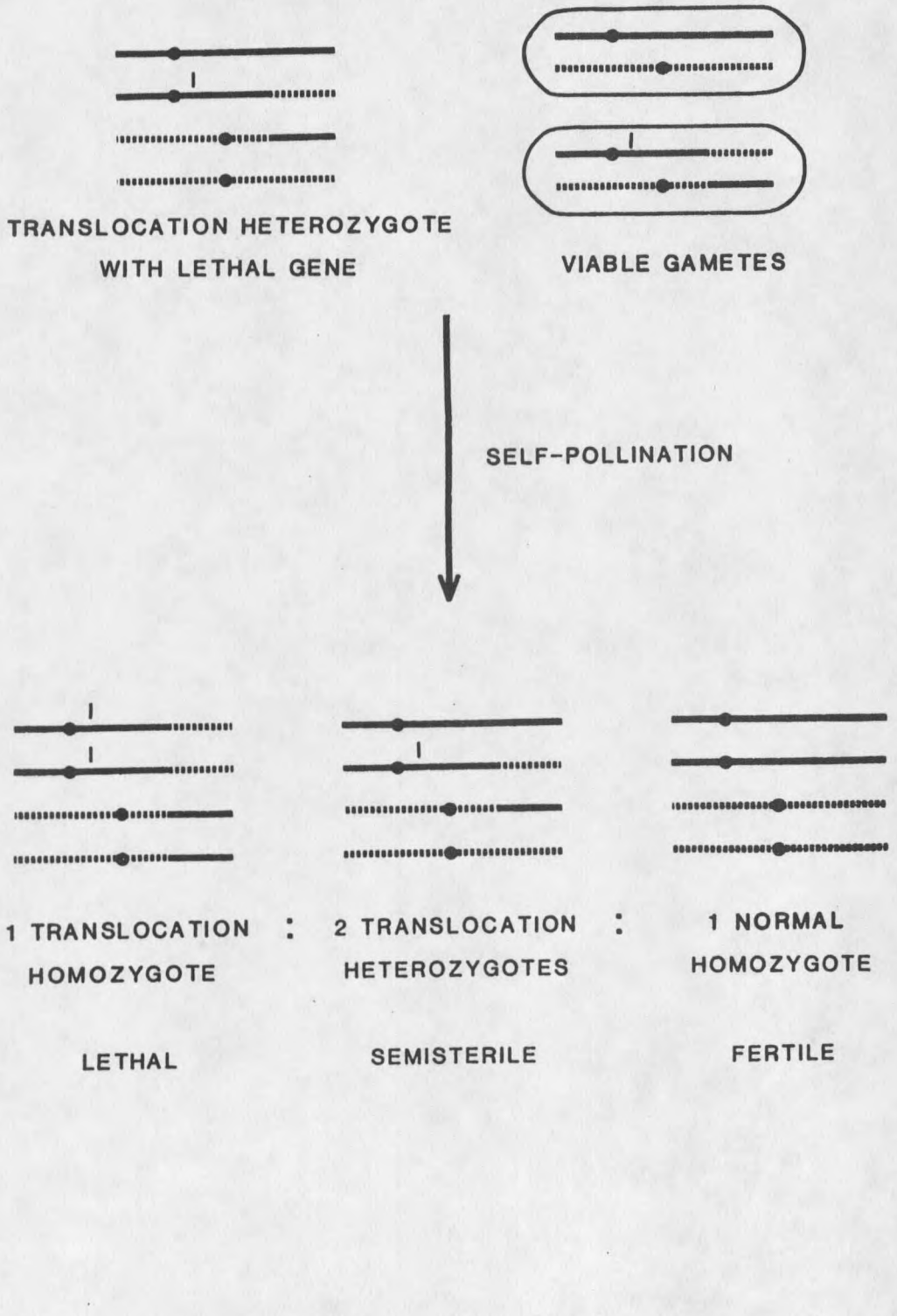
to adjacent disjunction in meiosis. Therefore, the phenotypic segregation ratio is one semisterile: one fertile.

Lethal translocation mutants express an aberrant segregation ratio of zero translocation homozygotes: two translocation heterozygotes: one normal homozygote, indicating the presence of a recessive lethal gene linked to the translocation breakpoint (Biggerstaff 1981) (Figure 3). Eliminating the translocation homozygote class increases linkage intensity information available per F_2 plant, thereby increasing precision in linkage studies (Biggerstaff 1981). The phenotypic segregation ratio of these lethal translocations is two semisterile: one fertile. Linkage is determined by lack of independent segregation in the F_2 generation in crosses between translocation heterozygotes and mutants to be mapped (Tuleen 1971).

Mutations linked to specific marker traits can similarly be examined. The B and C hordein storage proteins of barley are encoded by the Hor-2 and Hor-1 loci (Doll and Brown 1979; Shewry et al. 1980), and are located on the short arm of chromosome five (Jensen 1984). Techniques for characterization of these proteins by polyacrylamide gel electrophoresis are well described (Blake et al. 1982; Doll and Andersen 1981). The Hor-2 and Hor-1 loci are linked (Shewry et al. 1980) and have been used to characterize linkage with the Ml-a locus (Hash and Blake 1981; Jensen et al. 1980), and the Hor-3 locus (Blake et al. 1982; Shewry et al. 1983) of chromosome five.

A morphological trait for barley linkage analysis is the number of kernel rows per spike. This character is controlled by the V locus on the long arm of chromosome two, with two-row head types dominant over

Figure 3. Diagrammatic representation of the segregation of a self-pollinated lethal translocation heterozygote (l denotes recessive lethal allele).



six-row (Nilan 1964). The I locus on chromosome four controls the morphology of the lateral florets, but the expression of six-row spikes is not affected by this gene (Haus 1975).

Root Gravitropism

Perception

Primary plant roots growing towards gravity exhibit positive gravitropism. The first step in a tropic response is perception of the stimulus. The starch statolith theory was formulated in 1900 independently by Haberlandt and Nemec (see Audus 1975) and remains the most viable explanation of plant graviperception (Audus 1979; Juniper 1976). Movement with gravity of starch statoliths, within the statocyte cells, constitutes perception. Amyloplast movement is highly correlated with graviresponse (Shen-Miller and Hinchman 1974), and they are regarded as the functional statoliths in plants (Audus 1979; Moore 1984). Root statocyte cells have been identified as the columella or central cylinder of the root cap (Juniper and French 1970; Wilkins 1975).

Several forms of evidence link amyloplasts with gravitropic response. Due to their size and density, amyloplasts are the only organelles which sediment rapidly enough to be correlated with gravicurvature (Audus 1979; Shen-Miller and Hinchman 1974). Root cap removal stops gravitropic response, and response return is correlated with amyloplast (Hillman and Wilkins 1982) and root cap (Barlow 1974; Juniper et al. 1966; Cercek 1970) regeneration. Gravitropic response is lost with starch removal from statocytes and response returns with

starch reformation (Iversen 1969; Grisafi et al. 1984; Kaufman et al. 1984).

Amyloplasts may interact with other cellular components in gravi-perception. Moore (1983) reported that gravistimulated, maize (Zea mays L.) root statocyte organelles were distributed asymmetrically and that amyloplast movement alone did not account for that distribution. Dictyosome activity and distribution is altered with gravistimulation (Shen-Miller and Hinchman 1974). Juniper and French (1970) found endoplasmic reticulum (ER) in maize root statocytes running parallel to the nuclear membrane and cell walls. With gravistimulation, the ER aggregates within the cell, and following reorientation towards gravity, the ER returns to its normal distribution relative to the cell walls (Juniper and French 1973). In cress (Lepidium sativum L.) root statocytes, the ER forms a cup-shaped aggregration at the lower side of the cell that does not move with a change in orientation (Volkmann and Sievers 1979). Amyloplasts resting on the ER may exert a pressure which changes with reorientation and amyloplast movement. This pressure change may be a form of perception. Yet, there are also reports of amyloplasts in statocytes which do not contact the ER (Ransom and Moore 1983; Moore and McClelen 1983), and statocytes that do not exhibit a particular ER distribution (Juniper 1976).

How the physical stimulus is transmitted into a physiological response is unknown (Moore 1984). Numerous theories have been reviewed in the literature (Perbal 1978; Juniper 1976; Audus 1975; Moore 1984; Volkmann and Sievers 1979) but little experimental evidence is available to support those theories.

Hormonal Response

The result of gravistimulus is curvature towards gravity in the elongation zone of the root. In 1926, Cholodny and Went independently proposed a hypothesis to explain gravitropic response (see Audus 1979). They suggested that in a horizontal root, auxin is displaced laterally to the lower side in supraoptimal concentrations, inhibiting cell elongation and resulting in downward curvature. Since then, there have been numerous investigations to determine if auxins or other growth inhibitors are functioning in root graviresponse (see reviews; Audus 1975; Juniper 1976; Wilkins 1979).

Root curvature is associated with a general inhibition of growth (Audus and Brownbridge 1957). Gibbons and Wilkins (1970) provide evidence of a growth inhibitor moving basipetally from the root cap in graviresponse. Removal of one half of the root cap in maize seedlings causes curvature towards the side with the intact cap, regardless of gravity. Mechanical barriers placed between the apex and elongation zone of maize and pea (Pisum sativum L.) roots provided similar results (Shaw and Wilkins 1973). Curvature always occured towards the untreated side, indicating that a growth inhibitor in the root cap moves basipetally to the elongation zone. In a similar experiment, Pilet (1973) showed that a growth inhibitor in the root cap moves laterally downward in horizontal roots, thereby inducing curvature.

Indoleacetic acid (IAA), a naturally occuring plant auxin, is present in root tips (Bridges et al. 1973) and root caps (Rivier and Pilet 1974). Its movement within the root is primarily acropetal (Iversen et al. 1971; Batra et al. 1975; Juniper 1976). Application of

IAA and two synthetic auxins, naphthalene acetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D), inhibits root growth (Andreae 1967). Applied exogenously, IAA redistributes laterally to the lower side of horizontally stimulated roots (Konings 1967). Yet, measurements of endogenous IAA distribution in maize roots showed no lateral asymmetry with gravistimulation (Mertens and Weiler 1983). Root graviresponse can be inhibited by application of 2,3,5-triiodobenzoic acid (TIBA), a polar auxin transport inhibitor (Konings 1969). Audus (1975) suggests that IAA is necessary for gravicurvature, but the regulation of graviresponse may be due to another inhibitor which moves basipetally from the root cap.

Auxin applied to roots inhibits growth by stimulating ethylene production (Chadwick and Burg 1967). Ethylene biosynthesis inhibitors have been used to demonstrate the role of ethylene as a growth inhibitor in auxin treated tissue (Mulkey et al. 1982a, 1982b). Low levels of applied auxins cause no alteration of root growth. Yet, in the presence of ethylene biosynthesis inhibitors, root growth is stimulated by the auxins. This auxin-ethylene growth regulating interaction has been suggested as a factor in graviresponse (Chadwick and Burg 1967; Wheeler and Salisbury 1980).

Abscisic acid (ABA) has been identified in maize root caps (Kundu and Audus 1974; Rivier et al. 1977) and its movement in roots is basipetal (Pilet 1975). Root elongation is inhibited by ABA (Milborrow 1974; Iversen et al. 1977). Pilet (1975) detected a lateral redistribution of ABA downward in horizontal roots, but Mertens and Weiler (1983) found that the asymmetry was slight, transient, and too

late to induce graviresponse. Exogenous ABA application (Wilkins and Wain 1975), or exposure to white light to induce ABA production (Wilkins and Wain 1974), is required for root graviresponse in the 'LG11' maize mutant. Yet, Smith et al. (1985) found normal gravitropic response in ABA depleted roots of other maize cultivars. Xanthoxin, an ABA analogue with similar properties as ABA (Taylor and Burden 1970), may also be a growth inhibitor in graviresponse (Audus 1975; Iversen et al. 1977; Kundu and Audus 1974).

Gibberellic acid redistributes laterally to the upper side of horizontal roots (Webster and Wilkins 1974; El-Antably and Larson 1974). Gibberellins stimulate root elongation (El Hinnawy 1973) and inhibit elongation at high concentrations (El-Antably and Larson 1974). A role for gibberellic acid in georesponse has yet to be established (Wilkins 1979).

Ionic Response

Auxins stimulate proton extrusion, leading to cell wall loosening and cell enlargement (Rayle and Cleland 1977). In plant shoots, there is evidence that with gravistimulation, auxins redistribute laterally followed by asymmetric proton efflux and gravicurvature (Wright and Rayle 1983; Migliaccio and Rayle 1984). In roots the relationship is not as clear. Excess auxin causes growth inhibition and an apparent inward movement of protons in maize roots (Evans et al. 1980). Vertically growing roots show symmetric acid efflux along the elongation zone, while horizontal roots exhibit enhanced efflux on the upper surface and reduced efflux along the lower side (Mulkey and Evans 1981; Mulkey et al. 1981; Behrens et al. 1982). Conversely, Weisenseel

et al. (1979) found protons moving into the root tip and elongation zone, and out of the root hair zone.

Calcium redistributes in gravistimulated tissues (Arslan-Cerim 1966; Goswami and Audus 1976). In horizontally stimulated roots, calcium redistributes downward, moving laterally across the root tip (Lee et al. 1983a). Root cap removal inhibits lateral calcium movement (Lee et al. 1983a). Exogenous calcium application to roots causes curvature towards the calcium (Lee et al. 1983b). Root graviresponse is inhibited by calcium chelating agents and restored by application of calcium chloride. Calcium enhances lateral auxin movement across gravistimulated root tips (Lee and Evans 1985). Amyloplasts in the root cap cells of maize, pea, and lettuce (Lactuca sativa L.) contain calcium (Chandra et al. 1982). Lee et al. (1983a) suggest that calcium may have a role linking gravistimulation to graviresponse in roots.

Agravitropic Mutants

Plant mutants may be used to study gravitropic response mechanisms (Volkmann and Sievers 1979). An amylomaize mutant exhibits reduced coleoptile graviresponse apparently due to smaller amyloplasts in the statocytes (Hertel et al. 1969). This mutant has been used to examine auxin redistribution (Hertel et al. 1969; Ouitrakul and Hertel 1969) and gravitropic curvature (Hild and Hertel 1972). The 'LG11' maize mutant requires white light for ABA production and positive root graviresponse (Wilkins and Wain 1974), and has created interest in ABA as a root cap growth inhibitor in gravitropic response (Pilet 1983; Chanson and Pilet 1981). An agravitropic pea mutant described by Olsen and Iversen (1980a, 1980b) has ER distributed throughout the root cap

columella cells, rather than the cup-shaped distribution in normal statocytes. This altered distribution may disrupt transmission of the gravistimulus. Agravitropic Arabidopsis thaliana mutants were isolated by screening for auxin resistance (Maher and Martindale 1980). The mutants' roots display less growth inhibition due to applied IAA and 2,4-D than control roots. One mutation is recessive, another is an unlinked dominant gene which is lethal when homozygous (Mirza et al. 1984). Root anatomy studies yielded no differences in statocyte ultrastructure, but the recessive mutant exhibited decreased amyloplast sedimentation rates (Olsen et al. 1984).

GENETIC ANALYSIS

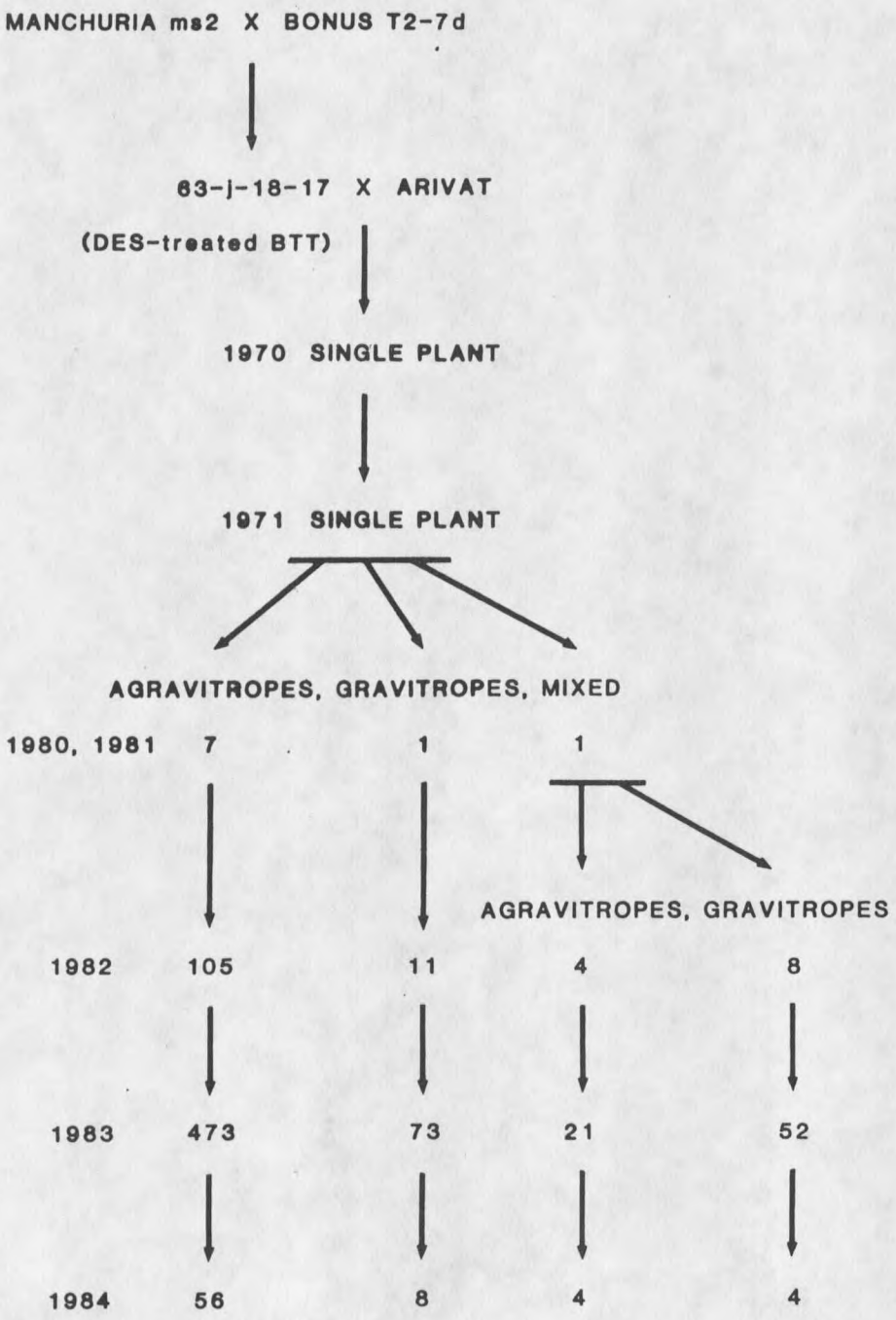
Materials and Methods

Genetic Stock

The agravitropic barley mutant arose from chemical mutagenesis of balanced tertiary trisomic (BTT) seed (Ramage 1965) (Figure 4). The diethyl sulfate (DES) treated '63-j-18-17' seed was planted in the field as the female rows in a crossing block, with 'Arivat' as the male parent. Diploid female plants were harvested and grown out two selfed generations in 1970 and 1971. Germination tests revealed root growth unresponsive to gravity in the progeny of a 1971 random single plant selection. That seed was grown in the field at Bozeman in 1980 and 1981. Nine single plants were selfed and harvested; the progeny of seven were completely agravitropic, one was gravitropic, and one was mixed. This seed was field grown and harvested as single plants in three selfed generations, in 1982, 1983, and 1984.

All crosses were made in the spring of 1984, in the Bozeman greenhouses, utilizing seed from 1983 harvested plants. To evaluate the type of inheritance, reciprocal crosses were made between selected agravitropic plants and gravitropes, specifically Arivat, 'Betzes', and gravitropic plants from the agravitropic population. All greenhouse produced seed was germinated in 2.89 mM gibberellic acid to overcome dormancy. F_1 seed was germinated to observe root graviresponse, then transplanted to pots in the greenhouse. F_2 seed was similarly

Figure 4. Origin of the agravitropic barley mutant. The phenotype and number of plants grown in self-pollinated field populations are shown for 1980 through 1984.



germinated and scored for root graviresponse. A chi-square test of deviation (Little and Hills 1978) was used in analysis of these crosses as well as the other genetics studies. Probability levels less than 0.10 were considered significant.

Population Analysis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to characterize the B and C hordein banding patterns of the agravitropic population. The hordein extraction and electrophoresis methods were a modification of that of Blake et al. (1982). The distal half of seeds were crushed with pliers and placed in 1.5 ml tubes with 300 ul 55% (v/v) propanol, 0.37 M Tris-Cl pH 8.8, and 2% (v/v) 2-mercaptoethanol. Samples were incubated at 60C for one hour, then centrifuged at 12,800 'g' for one minute. A 100 ul sample of supernatant was placed in clean tubes with 300 ul double distilled water, and refrigerated overnight. Precipitated proteins were pelleted by centrifugation at 12,800 'g' for two minutes and then dissolved in 200 ul 20% (v/v) glycerol, 0.124 M Tris-Cl pH 6.8, 2% (v/v) 2-mercaptoethanol, and 2% (w/v) SDS in a boiling water bath. The samples were electrophoresed in 11.1% (w/v) acrylamide, 0.09% (w/v) bisacrylamide gels at 15 mA per gel. Gels were stained with 0.1% (w/v) coomassie blue, 30% (v/v) methanol, 10% (v/v) acetic acid and destained in 25% (v/v) methanol, 8% (v/v) acetic acid.

The 1980/1981 and 1982 agravitropic populations were characterized for B and C hordein banding patterns. Four seeds per plant were analyzed and each plant was scored as a homozygote (four samples of one parental banding pattern) or a heterozygote (a heterozygous banding

pattern or both parental types). Percentage homozygosity due to inbreeding was tested against expected values for the population.

Linkage Analysis

Crosses were made between selected agravitropic plants and a lethal translocation tester set (Table 1). At least two crosses were made with each translocation line and translocation lines served as male and female parents. All F_1 seed was germinated to observe root graviresponse and transplanted to pots in the greenhouse. At maturity, plants without completely fertile heads were harvested and classified as semisterile.

Table 1. Translocations, chromosomal breakpoints, and authorities for the barley lethal translocation tester set used to determine linkage to agravitropism.

Translocation	Break Point[a]	Authority
T1-3e,d	L, L	Persson (1970)
T1-5f,f	S, L	Ramage et al. (1961)
T1-6a,c	S, Sat	Ramage et al. (1961)
T1-6e,a	L, S?	Persson (1970)
T1-7k,c	L, L	Persson (1970)
T2-3a,b	S?, S	Kasha and Burnham (1965)
T2-4a,c	-, -	
T2-4d,q	S?, L	Ramage et al. (1961)
T2-5a,v	L?, S?	Ramage et al. (1961)
T3-4b,e	-, S	Nilan (1964)
T3-7c,3-7d,c	S, L, L, S	Kasha and Burnham (1965)
T4-7b,b	S, Sat	Ramage et al. (1961)
T5-6b,l	L, L	Hagberg et al. (1975)
T6-7c,l	S, S	Ramage et al. (1961)

[a]S=short arm, L=long arm, Sat=satellite, ?=probably in that arm, -=position not determined.

Whenever possible, five agravitropic and eleven gravitropic F_2 seedlings from each semisterile F_1 plant were transplanted to

greenhouse benches. These population sizes were determined from the following equation (Sedcole 1977).

$$n = \frac{\log(1-p)}{\log(1-q)}$$

The variable p is the probability of finding at least one individual with the desired trait, q is the probability of the occurance of the trait, and n is the sample size required by the chosen values for p and q. A q value of 0.67 was used for the probability of obtaining a semisterile individual in the fertile agravitropic class and 0.33 for a fertile individual in the semisterile gravitropic class. The probability, p, was set at 0.99.

At maturity, the F_2 plants were scored as fertile or semisterile. At least 16 seeds from each F_2 plant were germinated to determine the genotype of that plant. F_2 segregation ratios were tested for linkage between agravitropism and translocation breakpoints.

The translocation lines used in this study have been backcrossed to 'Scashabet', a cultivar with the dominant two-row head type. The agravitropic population expresses the recessive six-row head type. F_2 plants, from the translocation crosses described above, were scored for kernel rows per spike and evaluated for linkage between the V locus on chromosome two and agravitropism. Crosses involving a translocation on chromosome two were not used in the analysis due to aberrant ratios resulting from linkage between the translocation breakpoints and the V locus.

Plants from the 1980/1981 population that were identified as heterozygous for hordein banding patterns were utilized as F_1's in a linkage study. The 1982 progeny of those plants would be expected to

segregate one homozygous parental type: two heterozygotes: one homozygous parental type. Independence of segregation was tested for the Hor-1 and Hor-2 loci on chromosome five and agravitropism.

Results and Discussion

Genetic Stock

Reciprocal crosses between agravitropes and gravitropes yielded completely gravitropic F_1 progeny. F_2 segregation fits a ratio of three gravitropes: one agravitrope, indicating monogenic control, with gravitropism completely dominant over agravitropism (Table 2).

Table 2. F_2 segregation data and chi-square values for crosses between the agravitropic mutant and wild type barley plants.

Cross	No. of F2 Plants			X^2 for 3:1	P-Value
	Grav.	Agrav.	Total		
Betzes X 90-2[a]	77	24	101	0.030	0.863
67-1 X Betzes	76	23	99	0.842	0.772
Arivat X 116-1	41	15	56	0.024	0.877
77-1 X Arivat	71	31	102	1.310	0.252
22-1[b] X 92-6	48	21	69	0.816	0.366
Total	313	114	427	0.569	0.451

[a]Numbers refer to single plants from the 1983 agravitropic population.
[b]Gravitrope from the agravitropic population.

Population Analysis

Figure 4 shows the appearance of apparently homozygous gravitropic plants in 1980/1981 and later generations. Foreign seed or pollen contamination was considered as a possible origin of these plants. To determine the origin of the gravitropic plants in the agravitropic population, SDS-PAGE characterization of B and C hordein banding patterns was used. The Hor-2 and Hor-1 loci encoding these proteins

are highly variable and may be used as marker genes in evolutionary studies (Doll and Brown 1979). Distinct banding patterns were identified for both parents, 63-j-18-17 and Arivat, and their heterozygous progeny. No other banding patterns were found in the 516 seeds examined. The hordein banding patterns indicate a similar origin of gravitropes and agravitropes.

If the 1971 parent plant was heterozygous at the Hor-1 and Hor-2 loci, the following selfed generation would be expected to be one-half homozygotes and one-half heterozygotes. The next generation should consist of three-forths homozygotes and one-forth heterozygotes. Chi-square data for segregation at both loci in the 1980/1981 and 1982 generations agree with those expectations (Table 3). Hordein banding patterns of these generations show segregation indicative of a population inbred from a single heterozygous plant.

Table 3. Ratio of homozygotes to heterozygotes for B and C barley hordein banding patterns in two inbred generations of the agravitropic population.

	No. of Plants			X^2 for		
	Homo.	Hetero.	Total	1:1	3:1	P-Value
1980/1981						
B Hordeins	4	4	8	0	-	1.000
C Hordeins	4	5	9	0	-	1.000
1982						
B Hordeins	77	21	98	-	0.489	0.484
C Hordeins	96	33	129	-	0	1.000

Gravitropic plants are morphologically similar to agravitropes. With no unaccountable variation in banding patterns, and percent homozygosity due to inbreeding as expected, there is no evidence of a seed mixture or contamination in the agravitropic population. Thus,