



Differences in seedling emergence, plant morphology, soil moisture removal by cropping, yield and quality components and allelism of several Betzes barley (*Hordeum vulgare* L.) brachytic isotypes
by Matthew Norman Ries

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE
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Abstract:

To assist in breeding a lodging resistant, more efficient short barley with high yield and quality, I studied the characteristics of eight brachytic and one erectoides isotype. Isogenic analysis provided close evaluation of mutant alleles in the background variety, Betzes.

An early May planted dryland nursery in Amsterdam Silty clay loam of single row plots of derived and mutant isotypes of Aks,uz/4*Bz and ms3,Gwy,br 1/2*Titan//4*Bz, and an early June dryland planting in the same soil type of 4-row yield plots of Hly,br 1/4*Bz which has an early maturing derived normal, Cpn,br 1/4*Bz, Hnh,br 1/4*Bz, ms3,Gwy,br 1/ Vtg//4*Bz, Shl,br 2/4*Bz, Beebe,br,,/7*Bz and Bz Double Ert (Ert I and Ert II) were evaluated.

Isogenic analysis allowed evaluation of brachytic genes. The isotypes were developed through backcross breeding with Betzes as the recurrent parent. Bz Double Ert is the result of crossing two different spontaneous mutants. Diallele analysis determined br 1, br 2, uz, and a new br., gene were involved. Coleoptiles grown in a dark, moist, germinator at 22°C showed all reduced height types had significantly shorter coleoptiles than derived normals. No significant difference for seedling emergence was detected when isotypes were planted in sandy loam at 25 and 50 mm deeper than each mean isotype coleoptile length. Plant height, awn length and rachis internode length of main culm spikes of brachytic isotypes were significantly shorter than derived normals, except for awn length of Bz Double Ert. Soil moisture removal percent by weight, was significantly less at the 120-150 cm depth for all brachytics except Hly,br 1/4*Bz. Soil moisture samples were not taken for Aks,uz/4*Bz and ms3,Gwy,br 1/2*Titan//4*Bz.

Brachytic yields ranged from 56% - 96% of their derived normal. Yield reductions of brachytic types were due to fewer tillers for Cpn,br 1/ 4*Bz, Beebe/br,,/7*Bz and Bz Double Ert, while lower kernel weight accounted for the reduction in all other entries. No protein or lysine difference was detected between isotypes by use of Neotec GQA and microbiological assay.

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DIFFERENCES IN SEEDLING EMERGENCE, PLANT MORPHOLOGY, SOIL MOISTURE
REMOVAL BY CROPPING, YIELD AND QUALITY COMPONENTS AND ALLELISM OF
SEVERAL BETZES BARLEY (*Hordeum vulgare* L.) "BRACHYTIC" ISOTYPES

by

MATTHEW NORMAN RIES

A thesis submitted in partial fulfillment
of the requirements for the degree

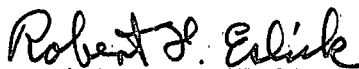
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ABSTRACT

To assist in breeding a lodging resistant, more efficient short barley with high yield and quality, I studied the characteristics of eight brachytic and one erectoides isotype. Isogenic analysis provided close evaluation of mutant alleles in the background variety, Betzes. An early May planted dryland nursery in Amsterdam Silty clay loam of single row plots of derived and mutant isotypes of Aks,uz/4*Bz and ms3,Gwy,br 1/2*Titan//4*Bz, and an early June dryland planting in the same soil type of 4-row yield plots of Hly,br 1/4*Bz which has an early maturing derived normal, Cpn,br 1/4*Bz, Hnh,br 1/4*Bz, ms3,Gwy,br 1/Vtg//4*Bz, Shl,br 2/4*Bz, Beebe,br,,/7*Bz and Bz Double Ert (Ert I and Ert II) were evaluated.

Isogenic analysis allowed evaluation of brachytic genes. The isotypes were developed through backcross breeding with Betzes as the recurrent parent. Bz Double Ert is the result of crossing two different spontaneous mutants. Diallele analysis determined br 1, br 2, uz, and a new br,, gene were involved. Coleoptiles grown in a dark, moist, germinator at 22°C showed all reduced height types had significantly shorter coleoptiles than derived normals. No significant difference for seedling emergence was detected when isotypes were planted in sandy loam at 25 and 50 mm deeper than each mean isotype coleoptile length. Plant height, awn length and rachis internode length of main culm spikes of brachytic isotypes were significantly shorter than derived normals, except for awn length of Bz Double Ert. Soil moisture removal percent by weight, was significantly less at the 120-150 cm depth for all brachytics except Hly,br 1/4*Bz. Soil moisture samples were not taken for Aks,uz/4*Bz and ms3,Gwy,br 1/2*Titan//4*Bz. Brachytic yields ranged from 56% - 96% of their derived normal. Yield reductions of brachytic types were due to fewer tillers for Cpn,br 1/4*Bz, Beebe/br,,/7*Bz and Bz Double Ert, while lower kernel weight accounted for the reduction in all other entries. No protein or lysine difference was detected between isotypes by use of Neotec GQA and microbiological assay.

INTRODUCTION

Many factors such as plant height, seed size, kernel weight, tiller number, rachis internode number, and water use efficiency limit the yield of short statured barley (*Hordeum* spp.), but some are more limiting than others. Identification of the most limiting factor or factors for a given environment would assist in directing future research emphasis.

Commercial production of a short statured barley could prove useful under several cropping systems and practices. The shorter plant type may reduce trash and facilitate planting under continuous cropping systems. They have improved lodging resistance in high rainfall areas and under irrigation. The short types may also be used to obtain taller winter types, but still maintaining much of the straw strength. The short types are also easily recognized and can be utilized as genetic markers in research programs.

Isotype analysis offers a reasonably effective means of evaluating a particular allele in a given genetic background. Nine Betzes reduced height isotypes were studied to: 1) determine the allelism of the height reducing genes; 2) evaluate isotype differences for plant height, awn length, rachis internode length, spike length, culm internode length, coleoptile length; 3) determine root system extent and soil water removal differences; and 4) determine yield and quality differences between isotypes.

LITERATURE REVIEW

Semi-brachytic and/or brachytic barley types have been described by many researchers. Three genes br 1 (brachytic), br 2 (brachytic), and uz (semi-brachytic) have been located and described.

The br 1 mutant plant type is described as brachytic or semi-dwarf with short leaves, short awns, short internodes, can be readily classified and is equal in viability to the normal (5,13,18). The br 1 gene is a monofactorial recessive located on chromosome 1. It was found as a spontaneous mutant in the cultivar Himalaya (5,13,18).

The br 2 mutant plant type is described as being shorter in height and having shorter stems, leaves, spikes, awns, kernels, glumes and glume awns, rachillas and coleoptiles than the normal. The auricles are well developed and are larger than normal (5). The br 2 gene is a monofactorial recessive located on chromosome 4. It was found as an X-ray induced mutant in the cultivar Svanhals (5).

The uz mutant plant type has shorter coleoptile length, leaves, spikes, awns, culms, empty glumes, axis of rachilla, and grain, than normal barley. There is to be no influence on the kernel weight or heading date (5). The coleoptile possesses a hook near the apex and often has a V-shaped notch on the opposite side of the hook, thus causing a double notch at the apex. The uz gene is a monofactorial recessive located on chromosome 3. It was a spontaneous mutant found in many Japanese cultivars (5,19,20,22).

The plants in barley populations segregating simultaneously for both the br 1 and uz gene were reported to be lethal or semi-lethal (8). Another experiment reported the double recessives to have an additive diminutive effect (8).

The uzu type barley is common to the warmer regions of Korea and Japan. It occupies 80% of Japan's barley acreage (22). Currently, nearly all Korean varieties are winter habit and uzu.

Plant Morphology (Coleoptile)

The coleoptile length of barley varies with growing conditions. The mean coleoptile length of a variety grown under specified conditions is stable and regarded as a heritable character peculiar to a variety (19,20).

The F_1 coleoptile lengths of uzu(uz)-lax(L) X normal (Uz)-dense (l) and (Uz-L) X (uz-l) crosses equal or exceed the length of the normal type parents (19,20). The frequency distribution of coleoptile lengths grown from seed harvested from F_1 plants is bimodal. In his study, coleoptiles less than 20 mm in length were uzu type, while those greater than 21 mm in length were normal type (20).

Dominance of the Uz or L genes over their recessive is not complete. Genotypes singly or double heterozygous for Uz and/or L have shorter coleoptiles than homozygous dominant genotypes (19,20).

A plant designated Dxx, an X-ray mutant from the cultivar Domen, was shown to be allelic to br 1 and the coleoptiles of Dxx were significantly shorter than those of Domen (7).

Plant Morphology (Roots)

The importance of the root system for maintenance of water balance in the plant, and as a characteristic of drought hardy varieties has been emphasized repeatedly (15). Most researchers who have investigated cereal crops have found that the greater the depth of adequate moisture in the soil, the greater the root penetration. It was also found that drying of the upper soil layer increases growth of roots at deeper depths (15).

Experiments on barley plants that were grown in large pots indicated that maximum root development, as regards to weight of roots, is reached at the time of pollination (27). Nitrate fertilizer at any rate lessens root penetration but greatly increases root branching, whereas potassium salts and phosphates greatly promote root penetration (27). Depending upon environmental and soil conditions, barley roots penetrate to as little as 10 cm, to depths beyond 205 cm (27). Root hair elongation varies with temperature and temperature duration around the roots and also with water absorption rate of the roots (12). The development of secondary roots of barley is similar to that of wheat and oats (27).

There was no significant difference between two wheat genotypes, a single gene dwarf, Sonalike RR-21, and a three gene dwarf, Up 301, in respect to either the total water extracted or the extraction rate per unit root volume for the 12 day duration of the experiment, or in root volume (17). However, the dry weight of roots per unit root volume increased significantly with increasing soil water tension of 0.3-0.8, 2.0-2.5, and 4.0-4.5 bars, for both genotypes (17). Longer finer roots developed under drier soil conditions which gave more root surface area in a fixed soil volume.

Plant Morphology (Leaves)

Leaves from both normal and br 1 Himalaya barley contained longer, narrower terminal cells and shorter, wider basal cells, with middle region cells intermediate in length and width. Older leaves had longer, narrower cells than younger leaves for both types (18).

A less extensive study of leaf morphology compared an equal number of tall and uz short types. The short type seedling was thick-set with deeper green leaves, positioned at an acute angle to the culm. These leaves also have projections on both surfaces, a V-shaped notch at the leaf apex, and a counter-clockwise twist at the middle (19,25). The tall type seedlings were slender, and had paler green leaves that were devoid of the short type peculiarities. Leaf sheath length was 19-37 mm for the tall type and 9-10 mm for the short type.

The leaf shape index, width/length, for the tall and short types was 45-105 and 105-170, respectively. Data taken on length and width of upper most leaves of mature plants suggested a similar pattern (19,25).

Plant Morphology (Culm)

In 1929 a spontaneous dwarf barley mutant found in the cultivar Himalaya was described as "A shortened plant with short leaves, short awns and short internodes, something like brachytic corn, but far less extreme." It is easily distinguished, and is equally as viable as the normal (18). Normal and mutant Himalaya mean culm epidermal cell lengths were 118 μ and 115 μ , respectively, with mean culm parenchyma cell length 173 μ and 163 μ , respectively. A reduced number of cells contributed to the reduced brachytic height (18). This mutant gene was labeled br 1 (13).

A marked reduction in plant height is noted for the uzu type compared to the normal (19,21).

Kumar et al. (10) broadly classified height induced mutants into three groups: 1) shortening of ear bearing internode is the major path of height reduction; 2) length of each successive internode is reduced in proportion to their contribution to total culm length in the control; and 3) the length of all middle internodes are reduced to the same length irrespective of their relative lengths in the control. Class two is the typical brachytic type.

Plant Morphology (Spike)

Rachis internode number and length determine total spike length. Uzu type spike length is primarily determined by the Uz alleles, the L alleles, or a combined effect of both genes (20), although quantitative characters also affect spike length (6).

Takahashi (20) evaluated the relationship of spike length and rachis internode length to spike type and found them closely related. The normal-lax spike is the longest and the uzu-dense spike is the shortest. The normal-dense and uzu-lax ears are intermediate. The effects of the dominant alleles Uz and L on ear length and rachis internode length were almost equal. Ear length increased by a factor of approximately 1.5 when homozygous dominant genes, Uz or L, were present (20).

Barthakur and Poehlman (2) and Hoskins and Poehlman (9) were unable to distinguish between uzu-lax and uzu-dense.

Hayes (6) summarized information on barley rachis internode length: 1) it is an environmentally stable character; 2) segregation occurs in the F_2 generation where homozygous dense types are found; 3) in some crosses, densities differing from their parents cannot be isolated, while in other cases, lines of non parental densities may be isolated; and 4) unexplained minor factors result in homozygous intermediate densities with continuous variation between parent means.

Yield

Takahashi et al. (21) compared yield data for 24 lines isogenic for the uz gene. Isogenic pairs showed marked differences for stem length, grain yield, 1000 kernel weight, and number of spikes per plant. Grain yield and 1000 kernel weight for normal lines exceeded that of the uz lines in most instances. Differential interaction of Uz and uz of these isogenic pairs originated from their differing genetic background, especially in the alleles L and l.

Barthakur and Poehlman (2) compared a normal Minnesota winter barley variety, Mo. B-475, and a uzu type winter barley variety, C.I. 7439, in yield trials. Three fertilizer rates were applied to solid and space planted rows. The Mo. B-475 produced higher grain yield as a result of more spike-bearing tillers and heavier kernels. The uzu type had more kernels per spike. No significance was found for the fertilizer by variety interaction (2).

Pleiotropy and Associated Genes

Leonard et al. (11) reported a second uzu gene (uz 2). Garza-Falcon (4) reported a third uzu gene (uz 3). Plant height was the only determining criterion used in these studies. Tsuchiya (23, 24,25) determined that improper classification was the reason uz 2 and uz 3 were reported and argues that only one uz gene is known to exist.

The expression of some characters, particularly culm length, are affected by the Lk 2 lk 2 gene for awn length, the L 1 gene for rachis internode length and other quantitative factors (19,20). Spring and winter growth habit affects culm length under some greenhouse conditions (25).

GENERAL MATERIALS AND METHODS

Eight brachytic Betzes isotypes and one erectoides Betzes isotype were evaluated (Table 1). Physiological, morphological, and agronomic traits as well as components of the quality were evaluated (Table 2).

The data reported are from both field planted and greenhouse planted experiments. The field data are from two dryland plantings fallowed the previous year. Isotypes Aks,uz/4*Bz and ms3,Gwy/2*Titan//4*Bz were early planted in single rows of 50 seed per 3 m row. The other entries were planted as a yield trial in a split plot design with replicated four row plots 3 m long, 30 cm spacing between rows and with one gm of seed planted per 30 cm of row. The soil was an Amsterdam silty clay loam which receives an average annual precipitation of 38.73 cm (Appendix Tables 1 and 2). The greenhouse was temperature controlled with a sandy loam soil in the benches. A soluble fertilizer of 14.39% ammonical nitrogen (NH_4^+), 5.61% nitrate nitrogen (NO_3), 20% phosphoric acid (P_2O_5) and 20% potash (K_2O) is added to the greenhouse water.

Each mutant isotype was compared to its normal derived Betzes by means of the F-test. When applicable, the analysis of variance and individual degree of freedom F-test was utilized (Appendix Tables 3 through 9).

Table 1. Pedigrees of Betzes isotypes evaluated.

| Pedigree [#] | Seed Source | | Mutant Phenotype |
|--|----------------|------------|------------------|
| | Derived Normal | Mutant | |
| Hly, <u>br</u> 1/4*Bz, [†] (5) | 76BBIN 193 | 76BBIN 195 | br |
| Shl, <u>br</u> 2/4*Bz, (5) | 75BBIN 344 | 75BBIN 309 | br |
| ms3,Gwy/2*Titan//4*Bz, [†] (br 1) | 77BBIN 66 | 77BBIN 81 | br |
| ms3,Gwy/Vtg//4*Bz, [†] (br 1) | 75BBIN 392 | 75BBIN 375 | br |
| Cpn/4*Bz, [†] (br 1) | 75BBIN 709 | 75BBIN 746 | br |
| Hnh/4*Bz, [†] (br 1) | 75BBIN 420 | 75BBIN 464 | br |
| Aks, <u>uz</u> /4*Bz (5) | 77BBIN 1 | 77BBIN 14 | uzu |
| Beebe/7*Bz | MT4483 | MT44814 | br |
| Bz Double Ert,(<u>ert</u> I/ <u>ert</u> II) | CI6398 | MT87148 | ert |

[†] Allelic height reducing genes, (Table 3).

[#] Variety abbreviations are as follows: Himalaya (Hly), Betzes (Bz), Svanhals (Shl), Gateway (Gwy), Vantage (Vtg), Compana (Cpn), Hannchen (Hnh), Akasiniriki (Aks), Beebe and Titan are not abbreviated (3). Gene symbols are as follows: brachytic (br), semi-brachytic (uz), and erectoides (ert), adapted from Craddock (3). After 4 and 7 backcrosses the mutant types are theoretically 93.75% and 99.21% isogenic respectively for the chromosomes not containing the mutant gene being backcrossed into the recurrent parent.

Table 2. Components of yield and other quality and agronomic traits measured.

| Characteristics |
|--|
| 1. coleoptile length, (mm) |
| 2. coleoptile reaction to Gibberellic acid |
| 3. seedling emergence depth, (%) |
| 4. number of roots originating from crown, (no.) |
| 5. removal of soil moisture by depths, (%) |
| 6. plant height, (cm) |
| 7. culm internode length by internode number, (mm) |
| 8. tiller number, (no.) |
| 9. awn length, (mm) |
| 10. spike length, (mm) |
| 11. rachis internode length, (mm per 10 internodes) |
| 12. seed per spike (no.) |
| 13. kernel weight, (mg per kernel) |
| 14. sieve size assortment (on 2.381×19.05 mm and thru 2.182×19.05 mm slotted sieves) |
| 15. yield, (quintals per hectare) |
| 16. protein, (%) |
| 17. lysine in grain, (%) |

ALLELE TEST

Materials and Methods

Single, space planted rows 3 m in length, 30 cm apart were field planted under dryland conditions, using segregating male sterile seed for each brachytic isotype (Table 1). The ms3,Gwy/2*Titan//4*Bz isotype was not included in this planting. The male sterility was introduced earlier for the development of the isotypes. All the brachytic isotypes were inter-crossed by hand pollination, and the crossed seed and one selfed spike of each brachytic isotype harvested.

A greenhouse planting followed the field harvest. A border row of the variety Betzes was planted first in each bench 5 cm from the edge and 15 cm between plants. Each experimental row in the bench began with two derived normal seed, followed by as many as 10 F₁ seed and ending with two brachytic seed. Rows were 15 cm apart with seed 5 cm apart within the row planted at a depth of 2.5 cm. A constant 22°C temperature was maintained throughout germination and seedling establishment. Alternating 13°C day and 8°C night temperatures were maintained for one month for tillering purposes. After the tillering period, temperatures were alternated 22°C during the day and 13°C during the night. No supplemental lighting was provided. Plant type was recorded, and all plants were harvested.

A field planting using the same row length and spacing followed the greenhouse harvest. A single row of derived normal, a row of F₁,

two rows of F_2 and a single row of brachytic were planted. Each iso-type was planted in the same manner. Plant type was recorded for each row and plant counts were made within segregating F_2 rows and recorded.

Results and Discussion

The expected F_1 plant type is brachytic for the allelic mutants (genes) and normal for the independent mutants. The Hly,br 1/ $4*Bz$, ms3,Gwy/Vtg// $4*Bz$, Hnh/ $4*Bz$ and Cpn/ $4*Bz$ were allelic (Table 3). The ms3,Gwy/2*Titan// $4*Bz$ originated from the same initial cross as ms3,Gwy/Vtg// $4*Bz$ and is assumed to be allelic to the above group. Crosses with br 1 are planned to verify this assumption. The expected F_2 ratio of the independent mutants is 3 normal:1 brachytic, and 0 normal:1 brachytic for the allelic mutants (Table 4).

The Beebe/ $7*Bz$ brachytic was not allelic to br 1 or br 2. It might well be a new brachytic gene. Until it is allelic tested with the newly designated brachytic genes, br 3 through br 7 (24), this is unknown.

Table 3. Results of inter-crossing Betzes brachytic isotypes to determine alleles.

| Female Pedigree | Male Pedigree | | | | | | |
|-------------------------------------|---|----------------------------|--------------------------|----------------|---------------------------|--------------|--------------|
| | Hly, <u>br 1</u> / 4*Bz | Sh1, <u>br 2</u> / 4*Bz | Aks, <u>uz</u> / 4*Bz | Beebe/ 7*Bz | ms3,Gwy/ Vtg// 4*Bz | Hnh/ 4*Bz | Cpn/ 4*Bz |
| | Number of plants and F ₁ phenotypes [†] | | | | | | |
| Hly, <u>br 1</u> /4*Bz [#] | 10,br | | | | | | |
| Sh1, <u>br 2</u> /4*Bz | 5,N | 10,br | | | | | |
| Aks, <u>uz</u> /4*Bz | 12,N | 10,N | 10,br | | | | |
| Beebe/7*Bz | 11,N | 20,N | 12,N | 10,br | | | |
| ms3,Gwy/Vtg//4*Bz [#] | -- | 12,N | 12,N | 12,N | 12,br | | |
| Hnh/4*Bz [#] | 6,br | 12,N | 12,N | 10,N | 12,br | 10,br | |
| Cpn/4*Bz [#] | 10,br | 7,N | 12,N | 10,N | 22,br | 12,br | 10,br |

[†] br = brachytic, N = normal.

[#] Determined to be allelic.

Table 4. Results of inter-crossing Betzes brachytic isotypes to determine alleles.

| Female Pedigree | Male Pedigree | | | | | | |
|--|----------------------------|----------------------------|--------------------------|----------------|---------------------------|--------------|--------------|
| | Hly, <u>br 1</u> / 4*Bz | Shl, <u>br 2</u> / 4*Bz | Aks, <u>uz</u> / 4*Bz | Beebe/ 7*Bz | ms3,Gwy/ Vtg// 4*Bz | Hnh/ 4*Bz | Cpn/ 4*Bz |
| Number of plants, F ₂ ratio N:br [†] | | | | | | | |
| Hly, <u>br 1</u> /4*Bz [#] | br | | | | | | |
| Shl, <u>br 2</u> /4*Bz | 28:25 | br | | | | | |
| Aks, <u>uz</u> /4*Bz | 28:22 | 25:30 | br | | | | |
| Beebe/7*Bz | 10:13 | 25:31 | 18:14 | br | | | |
| ms3,Gwy/Vtg//4*Bz [#] | br | 34:24 | 30:10 | 40:17 | br | | |
| Hnh/4*Bz [#] | br | 31:20 | -- | -- | br | br | |
| Cpn/4*Bz [#] | | 29:21 | 22:27 | 27:23 | br | br | |

[†] br = brachytic. uz = semi brachytic, expected ratio for non-allelic genes is 9 normal:7 brachytic. By goodness of fit χ^2 test, all ratios will fit a 9:7 ratio at P .01.

[#] Determined to be allelic.

COLEOPTILE LENGTH AND SEEDLING EMERGENCE
COMPARISONS BETWEEN ISOTYPES

Materials and Methods

The nine plant height isotypes (Table 1), Hulless Compa (Sermo/7*Cpn, C.I. 16185) and Hulless Compa (Stamm/7*Cpn, C.I. 16183), were included in a coleoptile length and a seedling emergence experiment. The Sermo/7*Cpn mutant isotype has a normal gibberellic acid level in the seed (1) and has a normal coleoptile length (16). The Stamm/7*Cpn mutant isotype has a higher gibberellic acid level in the seed than the derived normal (1), and a shorter than normal coleoptile length (16).

Twenty seeds of each isotype were placed, embryo end down, between two pieces of 4 × 15 cm moist blotter paper. The seed apices were at the top edge of the blotter paper. The two pieces of blotter paper were then fastened together with paper clips at the bottom edge while firmly pressing the top edge, indenting the blotter to help hold the seed in place. The blotters with seeds at the top were placed vertically into slotted racks, submerging the bottom edge of the blotter 15 mm into distilled water. The isotypes were paired, arranged in a randomized complete block design with four replications, and germinated in a moist dark germination chamber at 22°C. After seven days, coleoptile length (mm) was measured from the seed apex to the tip of the coleoptile.

A coleoptile length pilot experiment using a distilled water check and 200, 400, and 600 ppm gibberellic acid solution for growing the seedlings was set up in the same manner as above, but using only one replication.

Two planting depths, 25 and 50 mm deeper than the mean coleoptile lengths for each isotype were selected for a greenhouse depth of emergence experiment. Four replications of 25 seed per replication for each isotype were planted at their calculated depths spaced 5 cm between seed within rows and 15 cm between the rows in greenhouse benches filled with sandy loam. The isotypes were paired and planted in a randomized complete block design. The temperature was maintained at 22°C with no supplemental lighting for the entire experiment. Total emergence was recorded after 14 days and adjusted by dividing the total percent emerged by the germination percent, as determined by official germination tests.

Results and Discussion

The brachytic types have shorter coleoptiles than their derived normals (Table 5). This reduction in coleoptile length is associated with the reduced plant height of the brachytics (Table 7). The Bz Double Ert and Beebe/7*Bz brachytic type appear to have the longest coleoptiles of the reduced height types (Table 5), which may give them

Table 5. Mean coleoptile lengths and differences between ten Betzes isotypes after being grown in the dark for 7 days

| Pedigree | Coleoptile length (mm) | | difference Derived - Mutant |
|-------------------------------------|------------------------|--------|-----------------------------------|
| | Derived Normal | Mutant | |
| Aks, <u>uz</u> /4*Bz | 63 | 33 | 30 ** |
| Hnh/4*Bz [#] | 69 | 42 | 27 ** |
| Shl, <u>br 2</u> /4*Bz | 65 | 41 | 24 ** |
| Hly, <u>br 1</u> /4*Bz [#] | 63 | 40 | 23 ** |
| ms3,Gwy/Vtg//4*Bz [#] | 65 | 42 | 23 ** |
| ms3,Gwy/2*Titan//4*Bz [#] | 64 | 43 | 21 ** |
| Cpn/4*Bz [#] | 61 | 40 | 21 ** |
| Beebe/7*Bz | 60 | 48 | 18 ** |
| Bz Double Ert | 66 | 53 | 13 ** |
| Hulless Cpn [†] | 57 | 58 | -1 NS |
| Mean | 63 | 44 | 19 ** |

[†]Normal = Sermo donor for hulless, mutant = Stamm donor for hulless.

** Significant at P .01 level.

[#] Determined to be allelic.

an emergence advantage in heavy textured dry soils where deep planting is necessary to place the seed in contact with moisture for germination.

The coleoptile length pilot experiment showed no response of coleoptiles to the gibberellic acid treatment. These findings are not in agreement with Schneiter (16) where the Hulless Compa (Stamm/7*Cpn) isotype had a shortened coleoptile when treated with indol-acetic acid. Barr's analysis (1) of gibberellic acid levels in the seed indicates that the Hulless Compa (Stamm/7*Cpn) has a higher gibberellic acid level. The higher gibberellic acid level may be restricting cell elongation, thus giving the shortened coleoptile observed by Schneiter. The lack of response to gibberellic acid by both isotypes in this experiment indicates further investigation of the gibberellic acid levels of the seed is necessary, along with testing a wider range of gibberellic acid solutions.

Significant differences for percent emergence were detected among genotypes for both planting depths, but significance was not detected between isotypes within genotypes (Table 6 and Appendix Table 3). The shorter, wider, possibly stronger plumule of the brachytics was hypothesized to give a greater percent emergence over their derived normal at the deeper depths. This was determined to be a false assumption, when all brachytic isotypes were compared collectively with their respective normal isotypes.

Table 6. Adjusted percent emergence^{††} from planting depths 25 mm and 50 mm deeper than each isotype's mean coleoptile length, and isotype differences.

| Pedigree | Percent Emergence | | | | | |
|------------------------------------|-------------------|--------|--------------------------|----------------|--------|--------------------------|
| | 25 mm deeper | | | 50 mm deeper | | |
| | Derived Normal | Mutant | Difference Normal-Mutant | Derived Normal | Mutant | Difference Normal-Mutant |
| Cpn/4*Bz [#] | 84 | 72 | 12 | 72 | 50 | 22 |
| Shl,br 2/4*Bz | 79 | 67 | 12 | 57 | 43 | 14 |
| ms3,Gwy/Vtg//4*Bz [#] | 80 | 74 | 6 | 75 | 63 | 12 |
| ms3,Gwy/2*Titan//4*Bz [#] | 68 | 63 | 5 | 57 | 48 | 9 |
| Beebe/7*Bz | 68 | 63 | 5 | 61 | 61 | 0 |
| Aks,uz/4*Bz | 89 | 86 | 3 | 84 | 69 | 15 |
| Hly,br 1/4*Bz [#] | 80 | 78 | 2 | 64 | 49 | 15 |
| Bz Double Ert | 77 | 76 | 1 | 83 | 67 | 16 |
| Hnh/4*Bz [#] | 73 | 78 | -5 | 65 | 61 | 4 |
| Cpn Hulless | 46 | 51 | -5 | 26 | 34 | -8 |
| Mean | 74 | 71 | 3 | 64 | 54 | 10 |

[†]Normal = Sermo donor for hulless, mutant = Stamm donor for hulless.

^{††}Percent emergence/germination percent × 100.

[#]Determined to be allelic.

PLANT HEIGHT, CULM INTERNODE LENGTH AND DISTRIBUTION
AMONG INTERNODES, RACHIS INTERNODE LENGTH, SPIKE
LENGTH AND AWN LENGTH

Materials and Methods

Data reported are for all isotypes from the two field plantings described in the General Materials and Methods section. Sixteen plant height measurements for each isotype were made after the seed reached the hard dough stage of maturity. Plant height was recorded as the distance from the ground to the apex of the uppermost kernel of the spikes in a handful of culms.

Sixteen mature dry main culms for each isotype were measured for internode length and number. The first culm headed was considered the main culm. The spike bearing internode or peduncle was designated as internode n and each consecutive internode below it as $n-1$, $n-2$, $n-3$, and $n-4$. Culm internode length of n is the distance from the base of the spike to the bottom of the first culm node. Internode length of $n-1$ is the distance from the bottom of the first culm node to the bottom of the second culm node and each consecutive internode is measured in the same manner.

Rachis internode length was measured for each isotype for 16 main and 16 random culm spikes as the length of rachis internodes 6 through 15, beginning the count at the spike base. The first several rachis internodes are considered too variable for a meaningful measurement. Dividing this length by 10 gives the mean rachis internode

lengths. Spike length was calculated by multiplying mean rachis internode length by rachis internode number.

Awn length of 16 main culm and 16 random culm spikes were measured as the distance from the apex of the uppermost kernel of the spike, vertically to the tip of the longest awn (7).

Results and Discussion

Plant height, culm internode length, rachis internode length, spike length, and awn length for the brachytic isotypes are significantly shorter than their derived normal isotypes except for awn length and peduncle length of Bz Double Ert, n-4 culm internode length of Aks,uz/4*Bz and spike length of Hly,br 1/4*Bz (Tables 7, 8, and 9 and Appendix Tables 4 and 5).

The n-4 culm internode of Aks,uz/4*Bz is significantly longer than its normal isotype (Table 8 and Figure 1). The tendency for less reduction is also evident for the n-3 internode. There is a marked tendency for the uz genotype to result in culm internodes of approximately equal length, which is not observable for other genotypes (Figure 1). The allelic isotypes are remarkably similar for all plant parts (Figure 1).

Main culm measurements are more reliable for detecting genetic differences because they are the first to develop and are less subject

Table 7. Mean plant height (cm) and differences between isotypes.

| Pedigree | Plant height (cm) | | difference Normal- Mutant |
|--------------------------------------|-------------------|--------|---------------------------------|
| | Derived Normal | Mutant | |
| Sh1, <u>br 2</u> /4*Bz | 68.3 | 42.5 | 25.8 ** |
| Aks, <u>uz</u> /4*Bz [†] | 83.7 | 59.7 | 24.0 ** |
| ms3, Gwy/2*Titan//4*Bz ^{†#} | 80.3 | 59.0 | 21.3 ** |
| Cpn/4*Bz [#] | 71.7 | 50.9 | 20.8 ** |
| Hnh/4*Bz [#] | 71.0 | 53.2 | 17.8 ** |
| Bz Double Ert | 69.1 | 51.9 | 17.2 ** |
| ms3, Gwy/Vtg//4*Bz [#] | 70.8 | 55.1 | 15.7 ** |
| Hly, <u>br 1</u> /4*Bz [#] | 67.1 | 52.2 | 14.9 ** |
| Beebe/7*Bz | 70.3 | 56.7 | 13.6 ** |

**Significant at P .01 level.

[†] Entries not grown in same nursery as others.

[#] Determined to be allelic.

