Seed development in shrunken endosperm, high lysine mutants of barley (Hordeum vulgare L.)
by Christine Elizabeth Fastnaught

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Crop and Soil Science
Montana State University

Abstract:
'Betzes1 seg1, 'Ingrid' seg6 and seg7, 'Compana' seg3 and sex1a, 'Hiproly' lys1, 'Bomi' sex1f (Risθ 13), sex3c (Risθ 1508), and Risθ 8, and 'Carlsberg II' Risθ 56, shrunken endosperm, high lysine mutants of barley, were compared to their normal isotypes for moisture percentage, dry matter accumulation, alpha-amylase activity, and total and reducing sugar at 3 or 6 day intervals during seed development. All mutants had a lower mean kernel weight (averaged over 8 to 10 sampling dates) than normal. This occurred in the seg mutants (seg1, seg3, seg6, and seg7) because dry matter accumulation stopped 6 to 18 days earlier than normal, and in the sex mutants (all other mutants) because it proceeded at a slower rate than normal. Differences were not detected between mutants and normals in alpha-amylase activity because of a strong isotype x sampling date interaction, but a significant correlation was detected between peak activity at six days after anthesis and kernel weight at harvest in the six normal genotypes. Moisture percentage was lower than normal in seg3 and higher in sexla, sex1f, sex3c, and Risθ 8. Total sugar was lower than normal in all seg mutants, and higher in sex1a, sex1f, and sex3c. The correlation between total sugar and moisture explains the plumpness of the sex mutants during seed development. Reducing sugar was lower than normal in seg1 and seg3, and higher in sex1a and sex1f. The decrease in sugars in the seg mutants was proportional to the kernel weight decrease. The lower moisture, kernel weight, total and reducing sugars, and higher alpha-amylase activity of the seg mutants compared to the sex mutants may be related to a nutrient translocation problem.

Correlations among sampling dates for total sugar of all genotypes indicated that the ranking of genotypes for total sugar content was consistent from 12 days after anthesis to harvest. The mutant-normal difference in total sugar at harvest was highly correlated to the mean mutant-normal difference during seed development (r=,98**).

The above mutants and Carlsberg II sex1d (Risθ 86) were compared to their normal isotypes for total sugar content of harvest ripe seed grown in 7 to 13 environments. The results confirmed the differences observed during seed development. The allelic mutants, sex1a, sex1d, and sex1f, responded similarly relative to their normal isotypes.

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Date November 22, 1982

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ABSTRACT

'Betzes' seg1, 'Ingrid' seg6 and seg7, 'Compana' seg3 and sex1a, 'Hiproly' lys1, 'Bomi' sex1f (Risø 13), sex3c (Risø 1508), and Risø 8, and 'Carlsberg II' Risø 56, shrunken endosperm, high lysine mutants of barley, were compared to their normal isotypes for moisture percentage, dry matter accumulation, alpha-amylase activity, and total and reducing sugar at 3 or 6 day intervals during seed development. All mutants had a lower mean kernel weight (averaged over 8 to 10 sampling dates) than normal. This occurred in the seg mutants (seg1, seg3, seg6, and seg7) because dry matter accumulation stopped 6 to 18 days earlier than normal, and in the sex mutants (all other mutants) because it proceeded at a slower rate than normal. Differences were not detected between mutants and normals in alpha-amylase activity because of a strong isotype x sampling date interaction, but a significant correlation was detected between peak activity at six days after anthesis and kernel weight at harvest in the six normal genotypes. Moisture percentage was lower than normal in seg3 and higher in sex1a, sex1f, sex3c, and Risø 8. Total sugar was lower than normal in all seg mutants, and higher in sex1a, sex1f, and sex3c. The correlation between total sugar and moisture explains the plumpness of the sex mutants during seed development. Reducing sugar was lower than normal in seg1 and seg3, and higher in sex1a and sex1f. The decrease in sugars in the seg mutants was proportional to the kernel weight decrease. The lower moisture, kernel weight, total and reducing sugars, and higher alpha-amylase activity of the seg mutants compared to the sex mutants may be related to a nutrient translocation problem.

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INTRODUCTION

The shrunken endosperm mutants of barley (Hordeum vulgare L.) have been of interest for two main reasons. The first involves an increase in the amino acid lysine, associated with the shrunken endosperm character, which increases the nutritional quality of such mutants. The quality of small grains is extremely important to the humans and livestock which rely almost solely upon them for food and feed. The increased lysine of the shrunken endosperm mutants is useful only if the lysine content of the mutants can be transferred to high yielding cultivars. Breeding programs around the world have approached this problem by attempting to break the association between the shrunken endosperm and the high lysine, i.e., increasing the plumpness of the mutant seed while maintaining the high lysine content. To date, complete success has not been reported.

A second reason for the interest in shrunken endosperm mutants is their potential usefulness in understanding the biochemical and developmental pathways of seed development. The actual mutant gene(s) function which results in a shrunken seed and high lysine content is unclear. Since a shrunken seed should contain less starch, an examination of starch precursors or degradation products along with the enzymes involved in such processes, may help explain the relationship between shrunken endosperm, high lysine, and the actual gene(s) function. A better understanding of these relationships should prove

useful to the breeder attempting to produce high yielding, high lysine cultivars.

The objective of this research was to compare starch associated characters (dry matter accumulation, alpha-amylase activity, total and reducing sugars) in shrunken endosperm, high lysine mutants and their normal isotypes during seed development.

LITERATURE REVIEW

Description of Mutants

Selection of shrunken endosperm, high lysine mutants has been based on both visual selection of the shrunken endosperm character and biochemical selection of the high lysine character. The mutants seg1, seg3, sex1a (Jarvi and Eslick, 1975), seg6, and seg7 (Ramage and Eslick, 1975), were selected on the basis of the shrunken endosperm character. These mutants were later classified as being high lysine (Eslick and Hockett, 1976b and Ullrich and Eslick, 1978c). The mutants 'Hiproly' (Munck et al., 1970), and Risø 8, 13, 56, 86, and 1508 (Doll et al., 1974), were selected on the basis of biochemical analysis for lysine, but all of them had an associated shrunken seed or reduced seed weight.

The shrunken endosperm and high lysine phenotype of all the mutants, except Risø 8, are inherited in a monofactotial, recessive manner (see Table 1 for authority on specific genes). The phenotype of Risø 8 is controlled by a dominant or semi-dominant gene (Jensen, 1979a). The shrunken endosperm (or reduced seed weight) and high lysine phenotype have not been separated in any of the mutants, indicating that either the two traits are controlled by the same gene and are pleiotropic (Oram et al., 1975; Ullrich and Eslick, 1978a; Jensen, 1979a; Nelson, 1979; and Olsen, 1980) or they are controlled by two tightly linked genes (Hagberg et al., 1970 and Eslick and

Table 1. Genetic and phenotypic classification of 11 shrunken endosperm, high lysine mutants of barley.

Normal Cultivar	Mutant Name	Type of Mutation	Type of Expression	Seed Phenotype	Chromosome Location	Authority
Betzes	seg1	spontaneous	non-xenia	thin	1	6, 8
Compana	seg3	spontaneous	non-xenia	thin	3	6, 9
Ingrid	seg6	spontaneous	non-xenia	thin	–	7, 11
Ingrid	seg7	spontaneous	non-xenia	thin	–	7, 11
Hiproly Normal	Hiproly	spontaneous	xenia	dorsal depression	7	1, 2
Compana	sex1a	spontaneous	xenia	dorsal depression	6	6, 10
Bomi	Risø 8	induced by ethyl methane sulfonate	xenia	dorsal depression	5	5, 14, 15, 19
Bomi	Risø 13	induced by ethyl methane sulfonate	xenia	dorsal depression	6	5, 14, 15, 18
Carlsberg II	Risø 56	induced by gamma-rays	xenia	dorsal depression	5	5, 14, 17
Carlsberg II	Risø 86	induced by ethyl methane sulfonate	xenia	dorsal depression	6	5, 14, 15
Bomi	Risø 1508	induced by ethyleneimine	xenia	dorsal depression	7	3, 4, 12, 13, 16

Authority

1. Munck et al., 1970
2. Karlsson, 1972
3. Doll, 1973
4. Ingversen et al., 1973
5. Doll et al., 1974
6. Jarvi and Eslick, 1975
7. Ramage and Eslick, 1975
8. Eslick, 1976a
9. Eslick, 1976b
10. Eslick and Hockett, 1976a
11. Ramage and Scheuring, 1976
12. Karlsson, 1977
13. Ullrich and Eslick, 1978a
14. Ullrich and Eslick, 1978b
15. Jensen, 1979a
16. Jensen, 1979b
17. Doll, 1980
18. Fastnaught et al., 1981
19. Jensen, 1981

Hockett, 1976b). The chromosome location of some of the mutant genes is found in Table 1. 'Compana', sex1a, 'Bomi', Risø 13, and 'Carlsberg II', Risø 86 are reported to be allelic (Jensen, 1979a and Fastnaught et al., 1981).

These mutants can be classified according to whether the inheritance of shrunken endosperm and high lysine exhibits xenia (Table 1). Those mutants which do not exhibit xenia (i.e., the F_2 segregates in a normal 3:1 ratio) have been classified as shrunken endosperm genetic (seg) mutants and those which do exhibit xenia (i.e., the F_2 segregates in a 1:2:1 ratio, with plump and shrunken seed produced on the same spike from the heterozygous Sex sex plant) have been classified as shrunken endosperm xenia (sex) mutants. The terms, seg and sex, have been used as gene symbols for those mutants visually selected for shrunken endosperm (Eslick and Hockett, 1976c).

To date, the general term, lys, has been used as a gene symbol for most of the mutants selected on the basis of high lysine (Jensen and Doll, 1979), regardless of whether or not they exhibited xenia. Based on the gene symbols suggested by Eslick and Hockett (1976c), the terms lyg and lyx would be used to symbolize the high lysine genes.

Confusion has arisen in recent years concerning the gene symbols for the shrunken endosperm, high lysine mutants. Some of the mutants have been assigned two symbols, seg or sex, and lys. One reason for doing so would be the assumption that the two characters are controlled by separate genes (Eslick and Hockett, 1976b). A second reason would be the point of view of the researcher. When selecting a mutant on the basis of a certain phenotype, a researcher would be

inclined to give that mutant a gene symbol related to that phenotype, regardless of other associated mutant characteristics (Ullrich and Eslick, 1978b and Jensen and Doll, 1979). Munck (1972b) suggested that the lys symbol should be revised once the basic gene action is understood. Jensen and Doll (1979) felt that was impractical. However, one of these mutants, Risø 56, was not assigned a gene symbol until recently when it was discovered that it caused a reduction in hordein-2 and was located at or near the previously described Hor locus. Thus, Risø 56 was assigned a completely different gene symbol, Hor2ca (Doll, 1980). The normal or dominant allele of this gene is found in Carlsberg II and designated Hor2Ca. A summary of the gene symbols assigned to seg1, seg3, seg6, seg7, sex1a, Hiproly, and Risø 8, 13, 56, 86, and 1508 are in Table 2. The symbols used in this manuscript are starred.

Mutant-normal comparisons of kernel weight, and protein and lysine content, were reported by Ullrich and Eslick (1978c and 1978e) over a range of Montana and Arizona environments. They indicated that all 11 mutants used in this study had significantly lower kernel weights (Table 3), significantly higher protein percentage (Table 3), significantly higher lysine in the grain percentage (Table 4), and significantly higher lysine in the protein percentage (Table 4) than their normal isotypes. Ullrich and Eslick (1978c and 1978e) postulated that a starch dilution effect could result in the higher protein and lysine contents of the smaller mutant seed. They tested this by adjusting the mutant kernel weight to the normal kernel weight and calculating adjusted percentages for protein and lysine in the grain

Table 2. Summary of gene symbols assigned to 11 of the shrunken endosperm, high lysine mutants of barley.

Symbol Proposed By	Mutant Name										
	seg1*	seg3*	seg6*	seg7*	Hiproly	sex1	Risø 8*	Risø 13	Risø 56*	Risø 86	Risø 1508
Munck, 1972a	–	–	–	–	lys1*	–	–	–	–	–	–
Eslick, 1976a	seg1a	–	–	–	–	–	–	–	–	–	–
1976b	–	seg3c	–	–	–	–	–	–	–	–	–
Eslick and Hockett, 1976a	–	–	–	–	–	sex1f	–	–	–	–	–
1976b	lys2b	–	–	–	–	–	–	–	–	–	–
Ramage and Scheuring, 1976	–	–	seg6g	seg7h	–	–	–	–	–	–	–
Ullrich and Eslick, 1978a	–	–	–	–	–	–	–	–	–	–	sex3c*
1978b	–	–	–	–	–	–	sex5g	sex4f	–	–	–
1978d	–	–	–	–	–	sex1a*	–	–	–	sex1d*	–
Jensen and Doll, 1979	–	–	–	–	–	lys5e	Lys4d	lys5f	–	lys5h	lys3a
Doll, 1980	–	–	–	–	–	–	–	–	Hor2ca	–	–
Fastnaught et al., 1981	–	–	–	–	–	–	–	sex1f*	–	–	–

*Mutant name or gene symbol used in this manuscript.

Table 3. Kernel weight and protein comparisons of 11 shrunken endosperm, high lysine mutants of barley and their normal isotypes. (Adapted, with permission, from Ullrich, 1978).

Normal Cultivar	Mutant Name	Mean Kernel Weight (mg)			Mean Protein (%)			
		No. of Comparisons	Mutant Isotype ($\bar{x}$)	Normal Isotype ($\bar{y}$)	$\bar{x} - \bar{y}$	No. of Comparisons	Mutant Isotype ($\bar{x}$)	Normal Isotype ($\bar{y}$)	$\bar{x} - \bar{y}$
Betzes	seg1	17	18.8	34.3	-15.5**	18	15.9	14.6	1.3**
Compana	seg3	8	26.2	46.9	-20.7**	12	16.6	14.4	2.2**
Ingrid	seg6	3	15.1	39.9	-24.8**	7	14.5	12.6	1.9**
Ingrid	seg7	8	26.8	36.7	-9.9**	11	15.0	12.8	2.2**
Hiproly Normal	Hiproly	26	38.4	49.2	-10.8**	26	18.4	17.2	1.2**
Compana	sex1	8	38.6	47.6	-9.0**	14	17.9	14.4	3.5**
Bomi	Risø 8	26	32.6	45.0	-12.4**	26	14.4	13.2	1.2**
Bomi	Risø 13	27	35.6	45.0	-9.4**	28	15.0	13.2	1.8**
Carlsberg II	Risø 56	25	35.9	39.7	-3.8**	28	14.7	12.9	1.8**
Carlsberg II	Risø 86	33	34.0	39.2	-5.2**	36	14.7	12.8	1.9**
Bomi	Risø 1508	44	34.3	43.4	-9.1**	47	13.8	13.2	0.6**

**Significant at the 0.01 level based on a paired t-test.

Table 4. Microbiological assay lysine comparisons of 11 shrunken endosperm, high lysine mutants of barley and their normal isotypes. (Adapted, with permission, from Ullrich, 1978).

Normal Cultivar	Mutant Name	No. of Comparisons	Mean Lysine in Grain (%)			Mean Lysine in Protein (%)		
			Mutant Isotype ($\bar{x}$)	Normal Isotype ($\bar{y}$)	$\bar{x} - \bar{y}$	Mutant Isotype ($\bar{x}$)	Normal Isotype ($\bar{y}$)	$\bar{x} - \bar{y}$
Betzes	seg1	18	0.518	0.436	0.082**	3.25	3.05	0.20**
Compana	seg3	12	0.534	0.391	0.143**	3.40	2.76	0.64**
Ingrid	seg6	7	0.514	0.387	0.127**	3.55	3.09	0.46*
Ingrid	seg7	11	0.488	0.407	0.081**	3.25	3.20	0.05
Hiproly Normal	Hiproly	26	0.581	0.448	0.133**	3.15	2.61	0.54**
Compana	sex1	14	0.598	0.379	0.219**	3.37	2.66	0.71**
Bomi	Risø 8	26	0.548	0.386	0.162**	3.80	2.92	0.88**
Bomi	Risø 13	28	0.544	0.384	0.160**	3.68	2.93	0.75**
Carlsberg II	Risø 56	28	0.538	0.379	0.159**	3.69	2.97	0.72**
Carlsberg II	Risø 86	36	0.514	0.390	0.124**	3.56	3.06	0.50**
Bomi	Risø 1508	47	0.607	0.386	0.221**	4.39	2.95	1.44**

*, **Significant at the 0.05 and 0.01 levels, respectively, based on a paired t-test.

using the following formula:

$$\text{adjusted mutant \% (protein or lysine)} = \frac{\text{observed mutant \% (protein or lysine)}}{\text{normal kernel weight/mutant kernel weight}}$$

They suggested that when adjusted mutant-normal differences in protein percentage were nonsignificant (Table 5), starch deposition in the mutant may be restricted more than protein deposition. When the adjusted protein percentage of the mutant was significantly lower than the normal, protein as well as starch deposition may have been affected in the mutant. They observed that after the adjustment, seg mutants had a significantly lower lysine in the grain percentage than their normal isotypes, and sex mutants were either the same or significantly higher than their normal isotypes (Table 5).

Protein in Shrunken Endosperm, High Lysine Mutants

Greater than 50% of the protein found in normal barley seed is storage material having a high content of glutamine and proline which are easily mobilized and utilized during germination (Cameron-Mills et al., 1980). This storage protein, prolamin, is low in lysine and other amino acids essential for human and animal nutrition. Prolamin, or hordein as it is called in barley, is synthesized in the developing barley seed between two and five weeks after anthesis (Shewry et al., 1979) following a pattern similar to dry weight production. It is deposited primarily in protein bodies in the endosperm and can be separated into four components, A, B, C, and D hordein (Shewry et al., 1982). The composition of the two main components, C and B hordein (also called hordein-1 and hordein-2, respectively), is determined by

Table 5. Theoretical comparisons of 11 shrunken endosperm, high lysine mutants adjusted to normal kernel weights and their normal isotypes for Kjeldahl protein and microbiological assay of lysine in the grain. (Adapted, with permission, from Ullrich, 1978).

Normal Cultivar	Mutant Name	No. of Comparisons	Mean Protein (%)			Mean Lysine in Grain (%)		
			Adjusted Mutant Isotype ($\bar{x}$)	Observed Normal Isotype ($\bar{y}$)	$\bar{x} - \bar{y}$	Adjusted Mutant Isotype ($\bar{x}$)	Observed Normal Isotype ($\bar{y}$)	$\bar{x} - \bar{y}$
Betzes	seg1	16	8.6	14.5	-5.9**	0.281	0.435	-0.154**
Compana	seg3	8	9.5	13.9	-4.4**	0.328	0.415	-0.087**
Ingrid	seg6	3	5.1	12.3	-7.2**	0.184	0.396	-0.212**
Ingrid	seg7	8	9.8	12.5	-2.7**	0.310	0.401	-0.091*
Hiproly Normal	Hiproly	26	14.4	17.2	-2.8*	0.456	0.448	0.008
Compana	sex1	9	14.6	14.1	0.5	0.568	0.412	0.156**
Bomi	Risø 8	25	10.3	13.1	-2.8**	0.388	0.386	0.002
Bomi	Riso 13	27	11.8	13.1	-1.3**	0.433	0.385	0.048**
Carlsberg II	Risø 56	25	13.3	13.0	0.3	0.494	0.386	0.108**
Carlsberg II	Risø 86	33	12.9	12.8	0.1	0.456	0.397	0.059**
Bomi	Risø 1508	44	11.0	13.3	-2.3**	0.481	0.386	0.095**

*, **Significant at the 0.05 and 0.01 levels, respectively, based on a paired t-test.

codominant alleles at each of two linked loci, Hor1 and Hor2, respectively, on chromosome 5 of barley (Oram et al., 1975 and Shewry et al., 1978).

Hiproly and the Risø mutants have been studied extensively to understand the mechanisms resulting in high lysine content in barley seed. These mutants have reduced amounts of hordein (Køie and Doll, 1979 and Munck, 1972b) compared to their normal isotypes, although in Hiproly the decrease is slight. The Risø mutants also have more free amino acids.

Specifically, Risø 56 has a drastically reduced content of hordein-2 (Køie and Doll, 1979) and Risø 1508 has a drastically reduced content of both hordein-1 and hordein-2 (Shewry et al., 1979). Since the gene controlling Risø 56 is located on chromosome 5, Doll (1980) suggested that the mutation is at or very near the Hor2 locus. This has not bee shown genetically. The mutant gene in Risø 1508, sex3, is located on chromosome 7, thus, it has no apparent connection to the known hordein genes. Shewry et al. (1980) suggested it should be loosely termed regulatory, and that the effect on hordein is probably secondary.

More important than the slight decrease in hordein observed in Hiproly is the 50% increase in the "metabolic" fraction (albumins and globulins) of protein (Munck, 1972a). Increased amounts of four lysine-rich proteins have been identified which account for over 50% of the increased lysine content of Hiproly (Hejgaard and Boisen, 1980). These four albumin proteins, beta-amylase, protein Z, and two chymotrypsin inhibitors, are unique in that they respond to nitrogen

fertilization just as the hordeins do. In general, barley seed protein increases with increased levels of nitrogen fertilization and most of this increase is reported to be in the hordein fraction of seed protein (Andersen and Køie, 1975). This increase in the lysine-poor hordein fraction results in a decrease in the lysine/protein ratio in the seed and a negative correlation between the lysine/protein percentage and the protein percentage. Normally the albumin fraction of protein would change very little with increasing nitrogen fertilization, but Hejgaard and Boisen (1980) report that in Hiproly a stable lysine/protein ratio is observed as a result of increases in the four albumin proteins named above. They suggest that breeding for increased content of lysine-rich proteins might be an approach less likely to affect starch production, and subsequently, yield.

Relationship Between Protein and Starch Synthesis

Bhatia and Rabson (1976) calculated that the change in protein composition caused by the high lysine genes could at most result in a decrease in grain weight of 2.5%. Thus, any reduction larger than 2.5% would have to be attributed to changes in other grain components (Hagberg et al., 1979). The mutants being used in this research have reductions in grain weight ranging from 9% to 62% (Table 3). Køie and Doll (1979) reported reduced levels of starch and increased levels of soluble sugars in ripe seed of the Risø mutants and Hiproly.

Shrunken endosperm, high lysine mutants of maize also have decreased starch content and increased soluble sugars (Nelson and Burr, 1973). Decreased amounts of all starch synthesizing enzymes has

been reported for the opaque-2 mutant of maize (Mehta et al., 1979) which Nelson (1979) suggested is similar to Risø 1508 in barley. Chourney and Nelson (1978) identified the sh sh mutant as a mutant of the structural gene for sucrose synthetase in maize endosperm. While there is still no evidence indicating that all changes observed in shrunken endosperm, high lysine mutants are pleiotropic effects of a single gene, if this were the situation, a mechanism would have to be found linking protein and starch synthesis.

Two hypotheses have been presented which are based on studies of starch and protein synthesis in maize. Mehta et al. (1979) suggest that a reduced rate of protein synthesis in opaque-2 results in reduced amounts of the starch synthesizing enzymes, thus, reduced starch synthesis. This hypothesis implies that the primary lesion in the opaque-2 mutant affects protein synthesis rather than starch synthesis.

The second hypothesis is more general in that it does not apply to a specific mutant, and it assumes that the primary lesion affects a starch related enzyme. Burr (1979) reported that zein (prolamin in maize) synthesis occurs on the outside of the protein body, and subsequently, the polypeptide is secreted through the membrane for storage. During the secretion process, two post-translational modifications occur 1) removal of a signal peptide, and 2) addition of one molecule of glucose from ADP-glucose. He suggested that if there were reduced levels of ADP-glucose, and assuming that glycosylation is a requirement for packing of the zein molecule into the protein body, then zein chains would accumulate on the outside of the protein body

and protein synthesis would stop. Cameron-Mills et al. (1980) report that the processes involved in hordein synthesis are similar to those reported for zein synthesis.

Hormonal Changes in Shrunken Endosperm, High Lysine Mutants

Recently researchers have speculated on the possible role of phytohormones in reducing seed size in high lysine barley mutants (Hagberg et al., 1979 and Mounla et al., 1980). Positive correlations have been reported between grain size or rate of grain growth and cytokinin content (Michael and Seiler-kelbitsch, 1972) and giberellin-like activity in the seed (Mounla, 1978). Thus, the genes which result in high lysine see may exert some effect on the hormone-based growth-regulating system of the seed (Hagberg et al., 1979).

Hiproly and Risø 1508 have been compared to their normal isotypes for differences in abscissic acid, gibberellin, and auxin content in the developing seed. Abscissic acid content was reported to be similar in normal and high lysine genotypes (Hagberg et al., 1979). Gibberellin-like activity was reported to be 2 to 4 times greater at its peak (18 to 22 days after anthesis) in the high lysine genotypes compared to the normal genotypes (Hagberg et al., 1979 and Mounla et al., 1980). This puzzled researchers since previously they had reported a positive correlation between rate of grain growth and gibberellin-like activity in normal barley. Indole-type auxin content was reported to be different in the two mutants (Mounla et al., 1980). Since other differences between Risø 1508 and Hiproly have been mentioned previously (e.g., protein composition), this should not be

considered unusual. Auxin content was similar in Hiproly and its normal isotype except the normal maintained peak activity for nine days longer than Hiproly. Risø 1508 had a very low level of auxin content throughout seed development compared to Bomi which had a peak content 10 times greater than Risø 1508. Although these studies do not provide conclusive evidence of hormonal changes resulting in shrunken endosperm, high lysine seed, they do provide evidence of additional differences between the mutants and normals. Regardless of how these multitudes of differences are explained, i.e., many linked genes or pleiotropy, any theory must account for all of them. It is probable that there are changes in these mutants which have not yet been discovered.

Shrunken Endosperm and Alpha-Amylase

Shrivelled, low density seed has been one of the problems in the development of triticale as a crop (Klassen et al., 1971). Since triticale has very little post-harvest dormancy, Muntzing (1963) postulated that shrinkage and partial collapse of the endosperm might result from rapid conversion of starch to sugar prior to the onset of precocious germination. Significant negative correlations have been reported between alpha-amylase activity in mature triticale seed and seed density (Klassen et al., 1971 and Srivastava, 1978). Dedio et al. (1975) reported that shrivelled triticale lines had more alpha-amylase in the pericarp for a longer period of seed development and that as levels declined in the pericarp, they increased in the aleurone and endosperm. Thomas et al. (1980) have disagreed that alpha-

amylase is the major determinant of shrivelling in triticale seed, although it is certainly responsible for the high levels of reducing sugars and lack of dormancy observed in the seed at maturity. They suggested that shrivelling probably originates from mitotic division errors in the early development of the endosperm and is aggravated by other factors including the premature appearance of alpha-amylase.

Alpha-amylase activity in developing barley seed is well documented (MacGregor et al., 1971; MacGregor et al., 1972; Allison et al., 1974; and Riggs and Gothard, 1976). The primary isozyme of alpha-amylase found in developing seed is called green alpha-amylase or alpha-amylase I (MacGregor et al., 1972). It is easily distinguished from alpha-amylase II and III which are produced upon germination. Alpha-amylase I is mainly found in the pericarp and is probably present to hydrolyze pericarp starch to provide energy for the growing kernel (MacGregor et al., 1972).

Peak alpha-amylase I activity varies in occurrence and amount depending upon genotype and environment. The occurrence of peak activity varied from 10 to 16 days after anthesis in studies performed in Great Britain (Allison et al., 1974 and Riggs and Gothard, 1976) to only 2 to 10 days after anthesis in a study performed in Canada (MacGregor et al., 1972). Although the amount of activity is reported to vary with genotypes (Riggs and Gothard, 1976), no relationship was observed between this alpha-amylase activity and rate of grain growth nor final grain weight.

The synthesis of alpha-amylase II and III in the germinating barley seed is reported to be hormonally controlled by gibberellin-like

substances (Ho, 1979 and Jacobsen et al., 1979). It is not clear whether alpha-amylase I synthesis is under a similar type of control. Duffus (1969) reported that formation of alpha-amylase in immature barley seed could be prevented by applying chlorocholine chloride, an inhibitor of gibberellic acid synthesis, to the immature seed. MacGregor et al. (1972) could not induce alpha-amylase synthesis by the addition of gibberellic acid to isolated pericarps or whole immature barley seed. MacGregor (1976) later reported that the formation of a portion of alpha-amylase I appeared to be independent of both embryo and an exogenous supply of GA_3 in mature seed.

Alpha-amylase activity has been measured in the Risø mutants and Hiproly only after malting (similar to germination) (Allison, 1978). Hiproly and Risø 56 had extremely low malt alpha-amylase activity, while Risø 8, 13, and 1508 had higher than normal activity. As previously mentioned, the developing seed of Hiproly and Risø 1508 had significantly higher levels of gibberellin-like substances than their normal isotypes. The data reported by Allison (1978) would indicate that this high gibberellin-like activity had no consistent effect on the formation of alpha-amylase in malted barley.

MATERIALS AND METHODS

Experiment I

Barley Samples

Comparisons of starch associated characters during seed development were made between 10 shrunken endosperm, high lysine mutants and their normal isotypes. Four are classified as seg mutants, seg1, seg3, seg6, and seg7, and six are classified as sex mutants, lys1, sex1a, sex1f, sex3c, Risø 8, and Risø 56. A complete genetic description of the mutants is in Tables 1 and 2, and a comparison between the mutants and their normal isotypes for lysine and protein content is in Tables 3 and 4.

All mutant and normal isotypes were grown in pairs at Bozeman, Montana in 1979 in unreplicated, four row plots, 30 m long. These plots were planted at a commercial rate (3 gm/m) and irrigated once.

Dry Matter Accumulation and Moisture

Four hundred heads of each mutant and normal isotype were tagged upon reaching anthesis. All heads within a plot were tagged on the same day. Forty heads of each isotype were harvested approximately 6, 9, 12, 18, 24, 30, 36, 42, 48, and 54 days after anthesis. Heads were weighed to obtain the fresh weight, then placed in a drying oven at 50 C for 48 h after which the dry weights were recorded. Moisture

percentage was calculated as:

[fresh weight (g) - dry weight (g)]/[fresh weight (g)] x 100

Heads were threshed and kernel weights determined on at least 300 whole seed or 10 g of whole seed (this amount of seed was not available for a few of the earliest samples). Kernel weights were used as a measure of dry matter accumulation. Samples were ground on a Udy Cyclone Mill using a 0.5 mm mesh screen for chemical analysis.

Alpha-Amylase Activity

Alpha-amylase enzyme activity was determined on each of the ground samples using the Blue Plate Agar (BPA) assay described by Fox (1981). Each plate contained 32 samples and four standard checks. Activity was measured as the diameter of a clear ring (digestion ring) around each sample well and converted to area. The digestion ring area was converted to ug CBA dye released/mg/min at 65 C (units based on an assay using cibachrome blue amylose) using the measurements of the four checks to calculate a regression line and prediction equation for each plate. Activity was further converted and is reported as ug dye released/kernel/min at 65 C using the equation:

ug dye released/mg x mg/kernel = ug dye released/kernel

Sugar Content

Free sugars were extracted from each ground sample using Method 80-60 described in the AACC Methods Handbook (American Association of Cereal Chemists, 1969). This procedure was modified for 0.25 and 0.05 g samples.

A 0.25 g (or 0.05 g) subsample of each ground sample was weighed and placed into a 20 ml centrifuge tube. The sample was wetted with 1 ml (or 0.5 ml) of 95% ethyl alcohol. A 10 ml (or 5 ml) aliquot of an acid buffer solution (3 ml glacial acetic acid, 4.1 g anhydrous sodium acetate, and 4.5 ml H_2SO_4, diluted to 1 L with water) was added to each tube. The flour was stirred into suspension, 0.4 ml (or 0.2 ml) of a 12% sodium tungstate solution added to the tubes, and the entire contents mixed thoroughly. The suspension was centrifuged for 20 min at 4000 rpm and 5 C, and the pellet discarded.

The 3,5-dinitrosalicylic acid method of determining reducing value (Bruner, 1964) was used to estimate both reducing and total sugar in each extract. Reducing sugars were determined directly from the extract. In order to determine total sugars, it was necessary to hydrolyze nonreducing sugars (mainly sucrose) by boiling the extract prior to determination. Upon hydrolysis, sucrose is broken into its component monosaccharides which were measurable by the reducing sugar assay. Glucose was used as the reference sugar.

A 1 ml aliquot of each extract sample was placed into a 10 ml test tube sitting in an ice water bath. After adding 1 ml of 3,5-dinitrosalicylic acid reagent (Bruner, 1964), the tubes were capped and placed into boiling water for 5 min. Upon boiling, the solutions developed a color ranging from light yellow to orange to dark orange-brown, depending upon the reducing sugar content. The tubes were placed back into the ice water bath and the solutions diluted with 8 ml of distilled water. After shaking thoroughly, the relative absorb-

ance of each mixture was read on a spectrophotometer set at 540 nm. These readings were converted to produce the reducing sugar content of each sample.

A second 1 ml aliquot of each extract sample was placed into a test tube, capped, and boiled for 15 min to hydrolyze the nonreducing sugar, sucrose, into reducing sugar. After boiling, each sample was placed into an ice water bath, allowed to cool, and the total sugars determined using the procedure described for reducing sugars.

A standard curve was prepared using twelve glucose solutions ranging from 0.5 to 7.0 umol glucose/ml. The reducing sugar assay was performed on 1 ml of each standard as described previously. The absorbance reading at 540 nm for each standard was plotted against the known concentration of glucose (r=.99**) to produce the prediction equation:

$$\text{umol glucose/ml} = (\text{absorbance})\ 6.157 + 0.1521$$

Since other sugars besides glucose are measured by this assay, the units reported in this manuscript are referred to as glucose equivalents (equiv.). In order to make direct comparisons between samples, it was necessary to convert the above units to either % glucose equiv. or umol glucose equiv./kernel. The latter was obtained by the following calculations:

$$\text{umol glucose equiv./0.25 g flour} = \text{umol glucose equiv./ml} \times 11.4\ \text{ml/0.25 g flour},$$

or,

$$\text{umol glucose equiv./0.25 g flour} = \text{umol glucose equiv./ml} \times 5.7\ \text{ml/0.05 g flour} \times 5,$$

then,

umol glucose equiv./kernel = (umol glucose equiv./0.25 g)/
(no. kernels/0.25 g).

Percent glucose equivalents were obtained by the following calculations:

mg glucose equiv. = umol glucose equiv./0.25 g x
0.00018 g/umol glucose,

then,

% glucose equiv. = (mg glucose equiv./250 mg) x 100.

Germination Test

Bulk seed samples were harvested from all previously described plots grown in 1979 after the last sampling date. Each sample was cleaned and in February, 1980, three lots of 100 seed counted. Each lot was placed between two moistened blotters in a germination box. The seed were germinated in an incubator at 15 C and continuous darkness for 5 days. Germination is reported as the average germination percentage of the three replications.

Statistical Procedures

A paired t-test was used to compare each of the mutant isotypes with its normal isotype over 8 to 10 sampling dates for dry matter accumulation, alpha-amylase activity, total sugar, reducing sugar, and moisture percentage. Using this procedure, sampling dates were treated as replications.

An analysis of variance was calculated to evaluate the differences among the 10 shrunken endosperm, high lysine mutants in moisture percentage, dry matter accumulation, alpha-amylase activity, total

sugars, and reducing sugars. Sampling dates were used as replications. Since two of the dates were not available for all mutants, only eight sampling dates were included in this analysis. Three orthogonal comparisons were made. The first was to detect differences, if any, between seg and sex mutants. The second was to determine if Compana sex1a and Bomi sex1f were different. The third was to determine if any differences existed between the mean of the two sex1 mutants and the other four sex mutants. The percentage of genotype variation accounted for by each comparison was calculated using the sums of squares which are additive.

Differences in germination percentage among all of the genotypes, both mutant and normal, were evaluated by calculating an analysis of variance after the data were transformed by an arcsin transformation for skewed proportions (Snedecor and Cochran, 1967). An LSD was used to compare the mean germination percentage of each of the mutants with their corresponding normal isotype.

Experiment II

Barley Samples

The 10 shrunken endosperm, high lysine mutants and their normal isotypes which were grown at Bozeman, Montana in 1979 and used in Experiment I, were included in this experiment to determine the total sugar content relationship between developing and mature seed. The growing conditions and sampling methods were described in Experiment I.

The above mutant and normal isotypes, plus mutant Risø 86 (sex1d) in Carlsberg II, were grown in 7 to 12 environments in either Arizona

or Montana from 1974 to 1981. Grain samples were bulk harvested from these plots upon ripening. Kernel weights were determined on 15 g of seed and then samples were ground as described in Experiment I.

Sugar Content

Total sugar was extracted and determined on the samples from Experiment I as described in Experiment I. Similar procedures were used for the remaining samples, except that during the extraction 8 ml of buffer was used in place of 10 ml, and 1 ml of 12% sodium tungstate solution was used in place of 0.4 ml. Six glucose standards ranging from 0.0 to 5.56 umol glucose/ml were used to produce the prediction equation:

umol glucose/ml = (absorbance) 6.894 + 0.0387.

The calculations used to convert umol glucose equiv./ml to % glucose equiv. or umol glucose equiv./kernel are described in Experiment I.

Statistical Procedures

Analyses of variance were calculated using data from Experiment I to determine if differences in total sugar existed among the three isotypic classes (seg, sex, normal) on each of the 10 sampling dates. Individual mutant isotypes or normal cultivars were used as replications. Non-orthogonal comparisons were used to detect differences among classes. A correlation matrix for total sugar among sampling dates was calculated. The relationship between the mean mutant-normal difference in total sugar and the mutant-normal difference at harvest was determined.

A paired t-test was used to compare each of the 11 mutant isotypes with its normal isotype over 7 to 12 environments for total sugar content at harvest. An analysis of variance using a completely randomized design with environments as replications was used to detect differences among mutants. The normal isotypes were included in the analysis. A set of four orthogonal comparisons were computed and the percentage of genotype variation accounted for by each comparison calculated.

RESULTS AND DISCUSSION

Experiment I

Moisture Percentage

All of the seg mutants had a lower mean moisture percentage than their normal isotype and all of the sex mutants, except lys1, had a higher mean moisture percentage than their normal isotype. However, not all of these differences were significant (Table 6). No significant difference was detected in mean moisture percentage between three of the seg mutants, seg1, seg6, and seg7, and their normal isotype, but a highly significant difference was detected between seg3 and Compana. Of the sex mutants, neither lys1 nor Risø 56 were significantly different from their normal isotype. The remaining four sex mutants, sex1a, sex1f, sex3c, and Risø 8, had a significantly higher moisture percentage than their corresponding normal isotype. Figures 1 through 5 indicate that differences were generally observed throughout the development of the seed.

Significant differences in moisture percentage were detected among the shrunken endosperm, high lysine mutants (Table 7). Half of the genotype variation (52%) was accounted for by the highly significant difference between seg and sex mutants. Sex mutants had a higher mean moisture percentage than seg mutants, 38.1% vs. 31.3%, respectively. Almost one-fourth (24%) of the genotype variation was due to the highly significant difference between the mean of the two sex1

Table 6. Moisture percentage comparisons between barley shrunken endosperm, high lysine mutants and their normal isotypes from 6 to 54 days after anthesis.

Normal Cultivar	Mutant	Number of Comparisons	Moisture (%) Mutant Isotype (x) mean	Mutant Isotype (x) range	Normal Isotype (y) mean	Normal Isotype (y) range	Difference $\bar{x}-\bar{y}$
Seg Mutants							
Betzes	seg1	10	32.43	3.07-54.66	32.76	3.54-56.26	-0.33
Compana	seg3	8	25.33	5.09-43.12	34.48	4.68-56.71	-9.15**
Ingrid	seg6	10	32.44	3.27-55.71	32.60	3.30-56.57	-0.16
Ingrid	seg7	10	32.08	2.94-56.48	32.60	3.30-56.57	-0.52
Sex Mutants							
Hiproly Normal	lys1	10	34.29	3.04-58.07	34.88	2.34-59.61	-0.59
Compana	sex1a	9	37.57	2.79-61.80	31.17	4.68-56.71	6.40**
Bomi	sex1f	10	40.25	4.17-57.43	33.48	4.04-56.99	6.77**
Bomi	sex3c	10	34.81	5.45-57.32	33.48	4.04-56.99	1.33*
Bomi	Risø 8	10	36.02	3.07-61.18	33.48	4.04-56.99	2.54**
Carlsberg II	Risø 56	10	34.61	3.09-57.13	32.36	3.25-57.91	2.25

*, **Significant at the 0.05 and 0.01 levels, respectively.

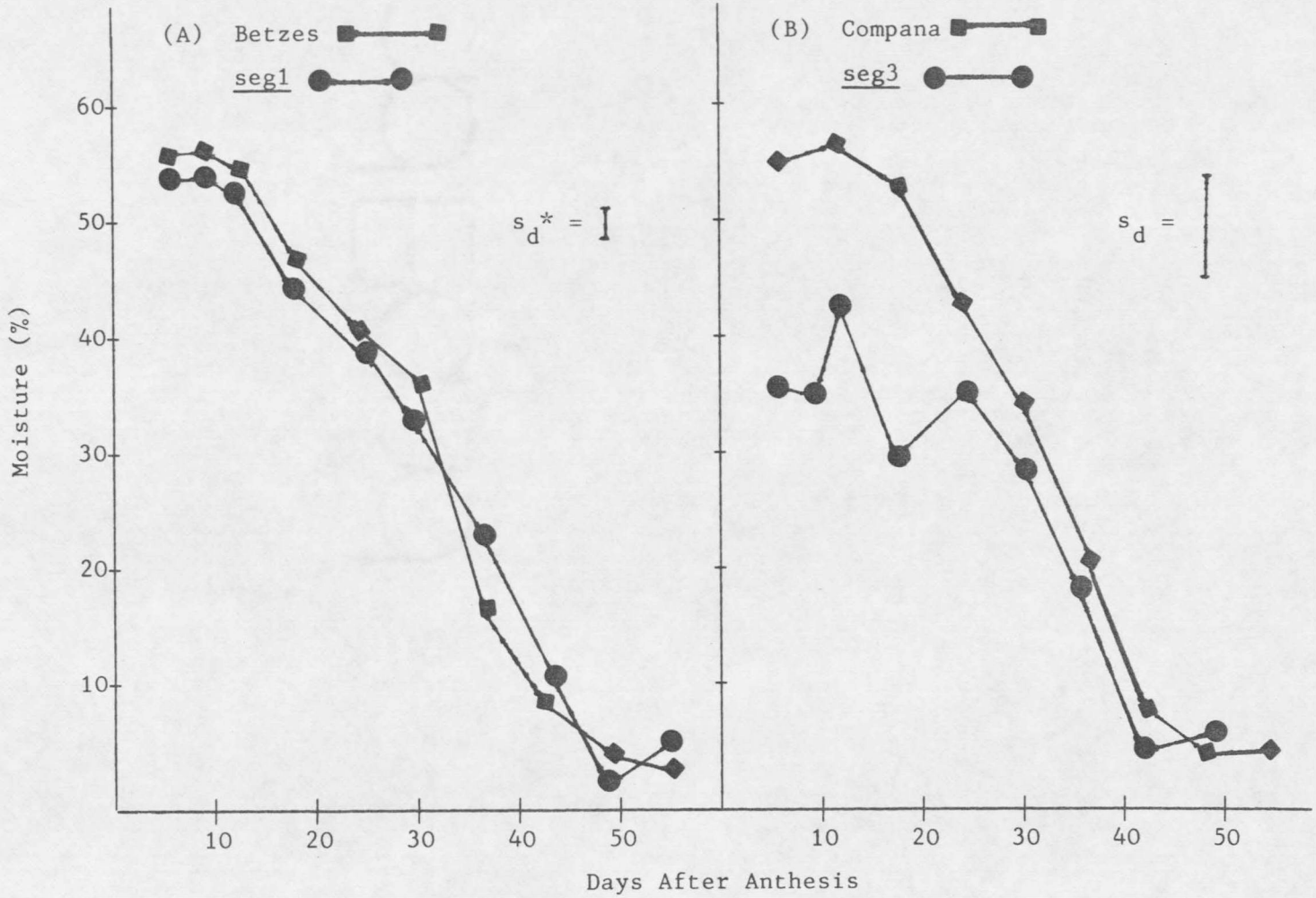

Figure 1. Moisture percentage of spikes harvested from 6 to 54 days after anthesis for A) Betzes and seg1, and B) Compana and seg3.
*s_d = standard error of the difference using a paired t-test.

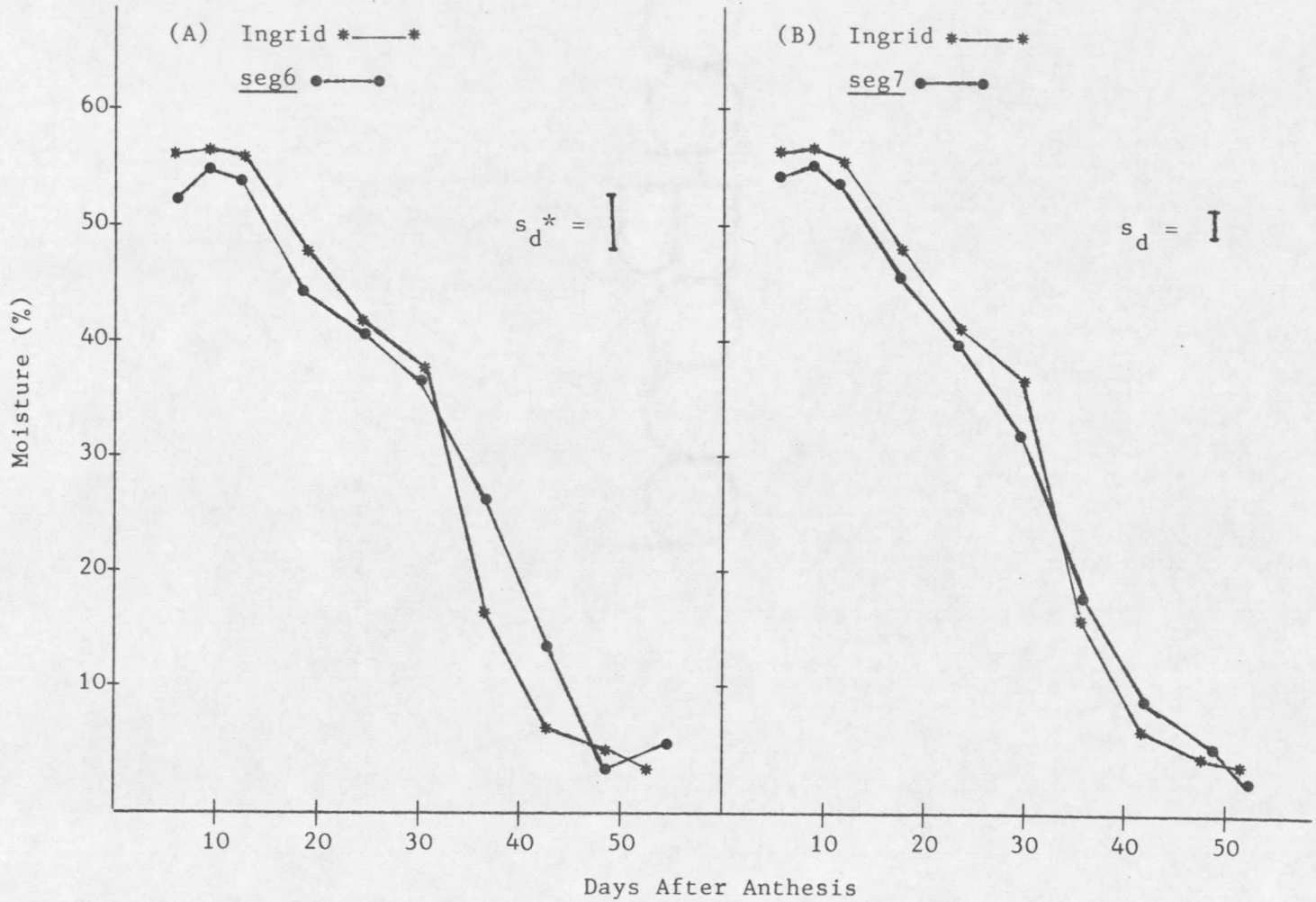

Figure 2. Moisture percentage of spikes harvested from 6 to 54 days after anthesis for A) Ingrid and seg6, and B) Ingrid and seg7.
*s_d = standard error of the difference using a paired t-test.

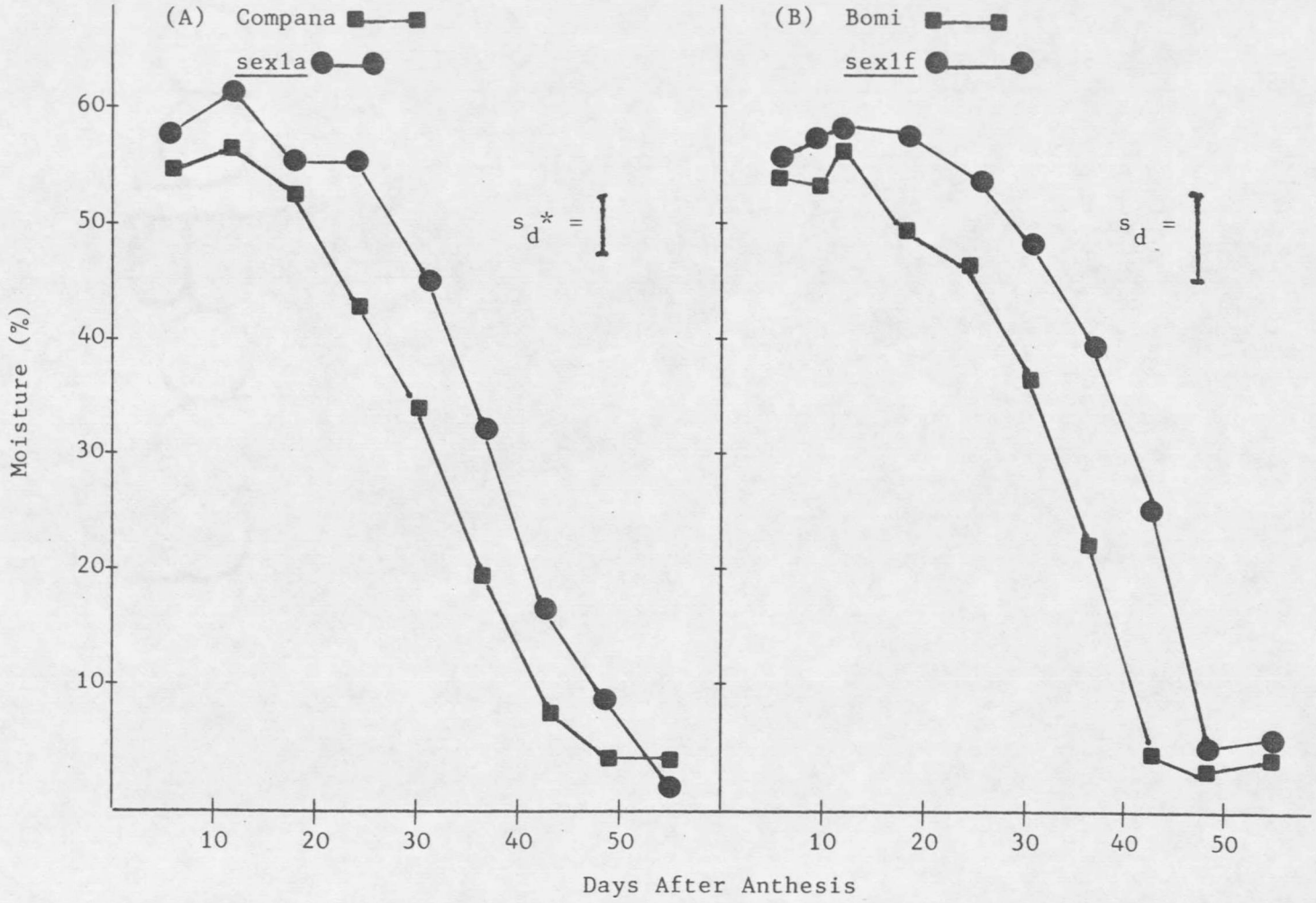

Figure 3. Moisture percentage of spikes harvested from 6 to 54 days after anthesis for A) Compana and sex1a, and B) Bomi and sex1f.
*s_d = standard error of the difference using a paired t-test.

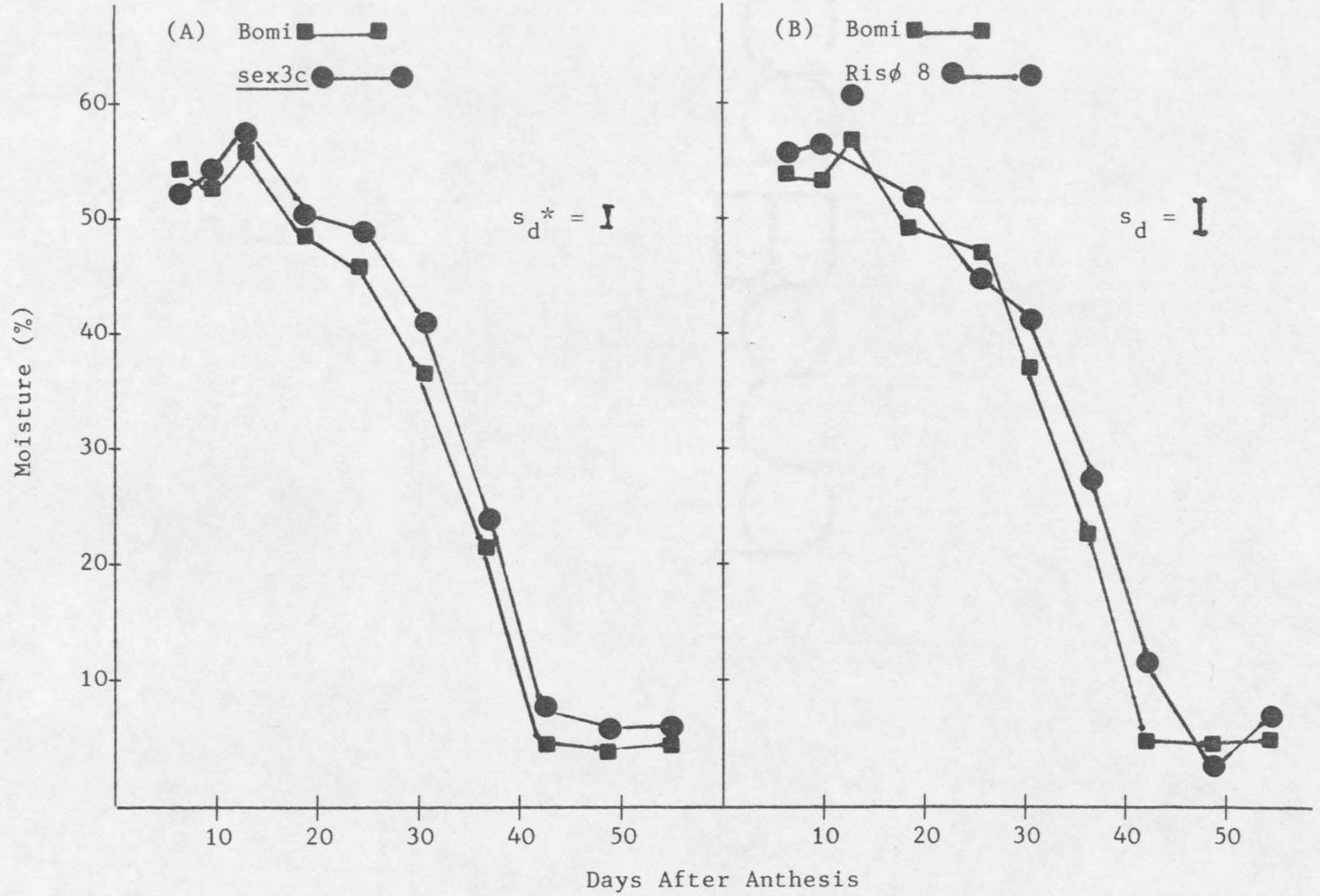

Figure 4. Moisture percentage of spikes harvested from 6 to 54 days after anthesis for A) Bomi and sex3c, and B) Bomi and Risø 8.
*s_d = standard error of the difference using a paired t-test.

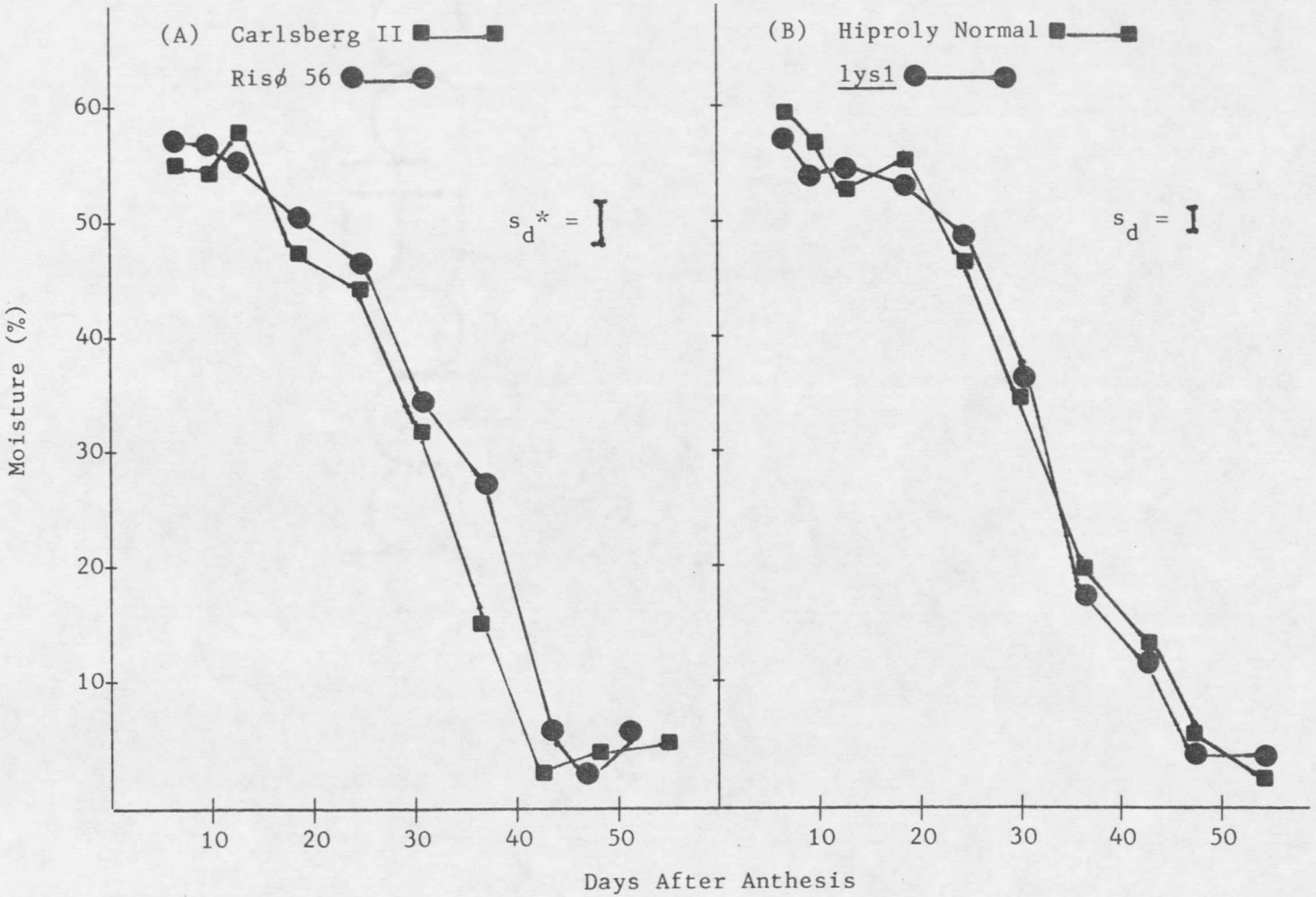

Figure 5. Moisture percentage of spikes harvested from 6 to 54 days after anthesis for A) Carlsberg II and Risø 56, and B) Hiproly Normal and lys1.
*s_d = standard error of the difference using a paired t-test.

Table 7. Analysis of variance for moisture percentage, kernel weight, alpha-amylase activity, total sugar, and reducing sugar for 10 shrunken endosperm, high lysine mutants of barley collected from 6 to 48 days after anthesis.

Source of Variance	DF	Mean Square: Percent Moisture	Mean Square: Kernel Weight (mg/kernel)	Mean Square: Alpha-Amylase Activity (ug dye released/kernel/min at 65 C)
Genotype (G)	9	189.36**	430.83**	1124.45
seg vs. *sex*	1	889.69**	2714.15**	6265.09**
sex1a vs. *sex1f*	1	1.62	171.61**	93.99
sex1 vs. other *sex*	1	406.27**	164.07**	90.27
remainder	6	67.78**	137.94**	611.78
Date (D)	7	3708.79**	1021.19**	12779.18**
Error (G x D)	63	15.33	20.90	878.76

Source of Variance	DF	Mean Square: Total Sugar (umol glucose equiv./kernel)	Mean Square: Reducing Sugar (umol glucose equiv./kernel)
Genotype	9	171.76**	2.984**
seg vs. *sex*	1	869.54**	13.811**
sex1a vs. *sex1f*	1	14.69	1.232*
sex1 vs. other *sex*	1	566.14**	7.611**
remainder	6	15.91	0.700*
Date (D)	7	73.37**	5.017**
Error (G x D)	63	7.39	0.260

*, **Significant at the 0.05 and 0.01 levels, respectively.

mutants and the mean of the other four sex mutants. Sex1 had a higher mean moisture percentage than the other mutants, 42.2% vs. 36.1%, respectively. No difference was detected between sex1a (41.9%) and sex1f (42.6%).

Highly significant differences in moisture percentage were detected among sampling dates (Table 7). As expected, the earliest dates had the highest moisture percentage and the later dates generally had the lowest moisture percentage (Figures 1 through 5). With one exception, all of the isotypes, both mutant and normal, had a moisture content higher than 54% on the first sampling date. The exception, seg3, had a low moisture percentage on the first date, 35.7%. Although the moisture percentage of seg3 did vary during the first five sampling dates (Figure 1 (B)), the tendency was for it to always be lower than Compana. This would indicate that this mutant should be studied much closer, since it has been reported (Coles, 1979) that starch synthesis is reduced or stopped when the seed gets below 40% moisture.

Dry Matter Accumulation

All of the shrunken endosperm, high lysine mutants had a significantly lower mean kernel weight (averaged over 8 to 10 sampling dates) than their corresponding normal isotype (Table 8). These differences were generally not observed during the first 2 to 3 sampling dates (Figures 6 through 10). The largest differences were observed between 18 and 54 days after anthesis, depending upon the mutant-normal pair. The smallest mean difference was observed between Carlsberg II and Risø 56.

Table 8. Kernel weight comparisons between barley shrunken endosperm, high lysine mutants and their normal isotypes from 6 to 54 days after anthesis.

Normal Cultivar	Mutant	Number of Comparisons	Kernel Weight (mg/kernel)				Difference $\bar{x}-\bar{y}$
			Mutant Isotype (x)		Normal Isotype (y)		
			mean	range	mean	range	
Seg Mutants							
Betzes	seg1	10	12.58	2.3-14.5	29.55	3.2-41.9	-16.97**
Compana	seg3	8	20.11	2.4-27.7	41.03	2.5-55.8	-20.92**
Ingrid	seg6	10	10.51	2.9-11.7	27.56	3.4-38.9	-17.05**
Ingrid	seg7	10	21.27	3.4-27.8	27.56	3.4-38.9	-6.29**
Sex Mutants							
Hiproly Normal	lys1	10	28.00	5.2-38.2	36.95	5.3-52.8	-8.95**
Compana	sex1a	9	32.01	8.3-44.7	38.21	2.5-53.9	-6.20**
Bomi	sex1f	10	26.80	2.9-37.8	31.94	3.3-44.8	-5.14**
Bomi	sex3c	10	26.65	2.5-36.7	31.94	3.3-44.8	-5.29**
Bomi	Risø 8	10	25.46	4.4-35.7	31.94	3.3-44.8	-6.48**
Carlsberg II	Risø 56	10	23.45	3.0-30.2	26.34	3.1-36.9	-2.89**

**Significant at the 0.01 level.

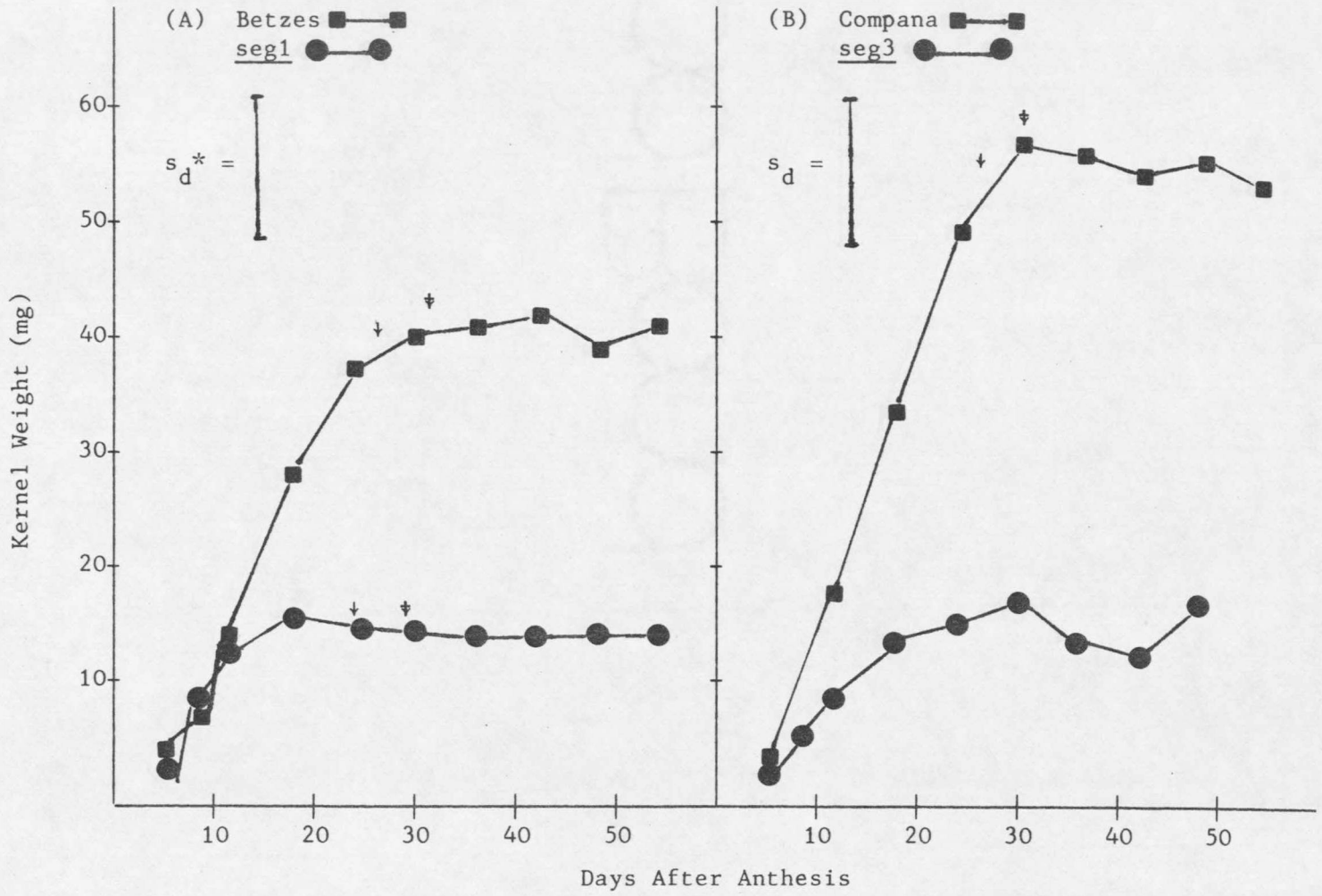

Figure 6. Kernel weight of seed harvested from 6 to 54 days after anthesis for A) Betzes and seg1, and B) Compana and seg3. Arrows indicate approximate day seed reached 40% (↓) and 35% (↓) moisture. Mutant seg3 was always 35% moisture or less except on day 12.
*s_d = standard error of the difference using a paired t-test.

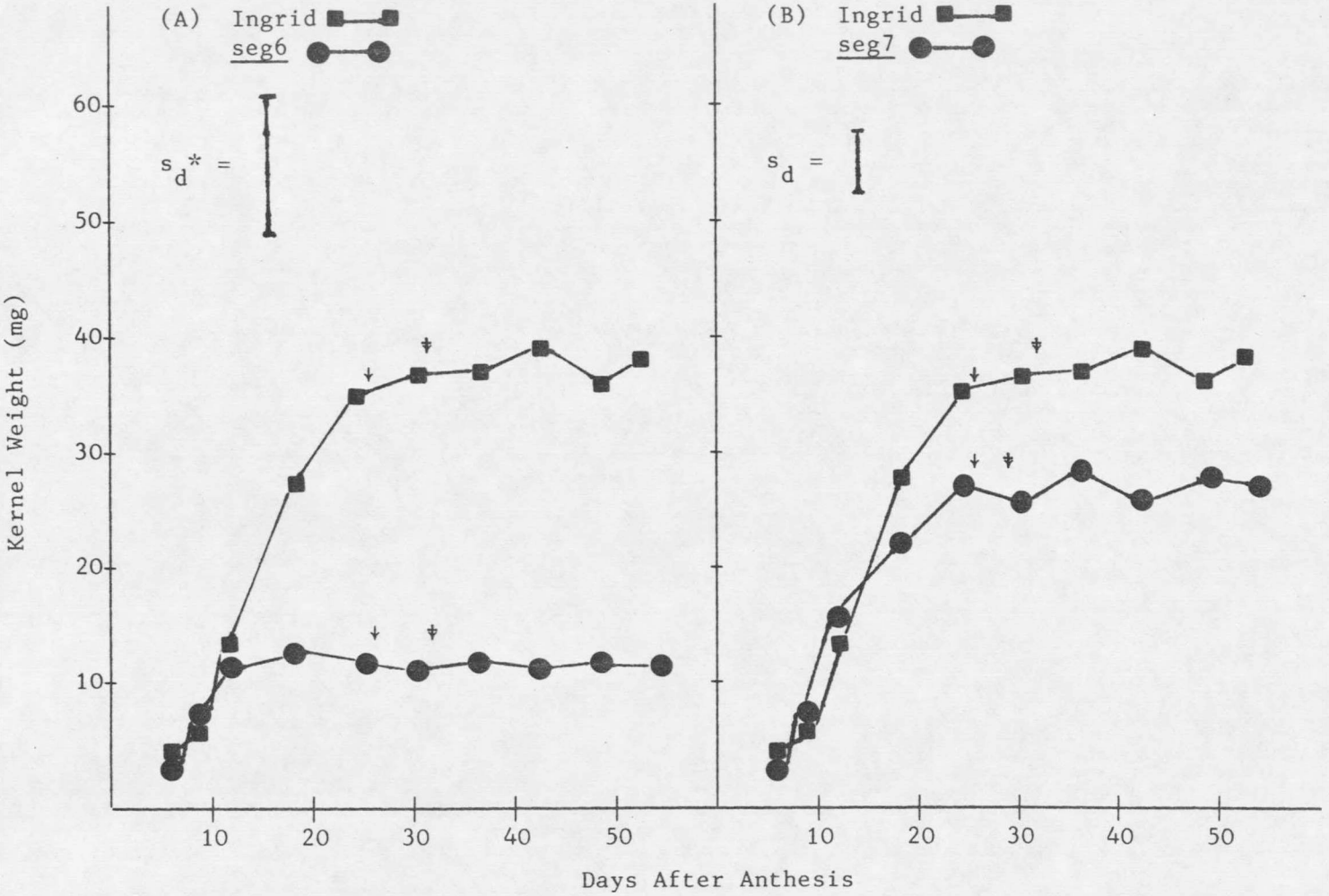

Figure 7. Kernel weight of seed harvested from 6 to 54 days after anthesis for A) Ingrid and seg6, and B) Ingrid and seg7. Arrows indicate approximate day seed reached 40% (↓) and 35% (↟) moisture.
*s_d = standard error of the difference using a paired t-test.

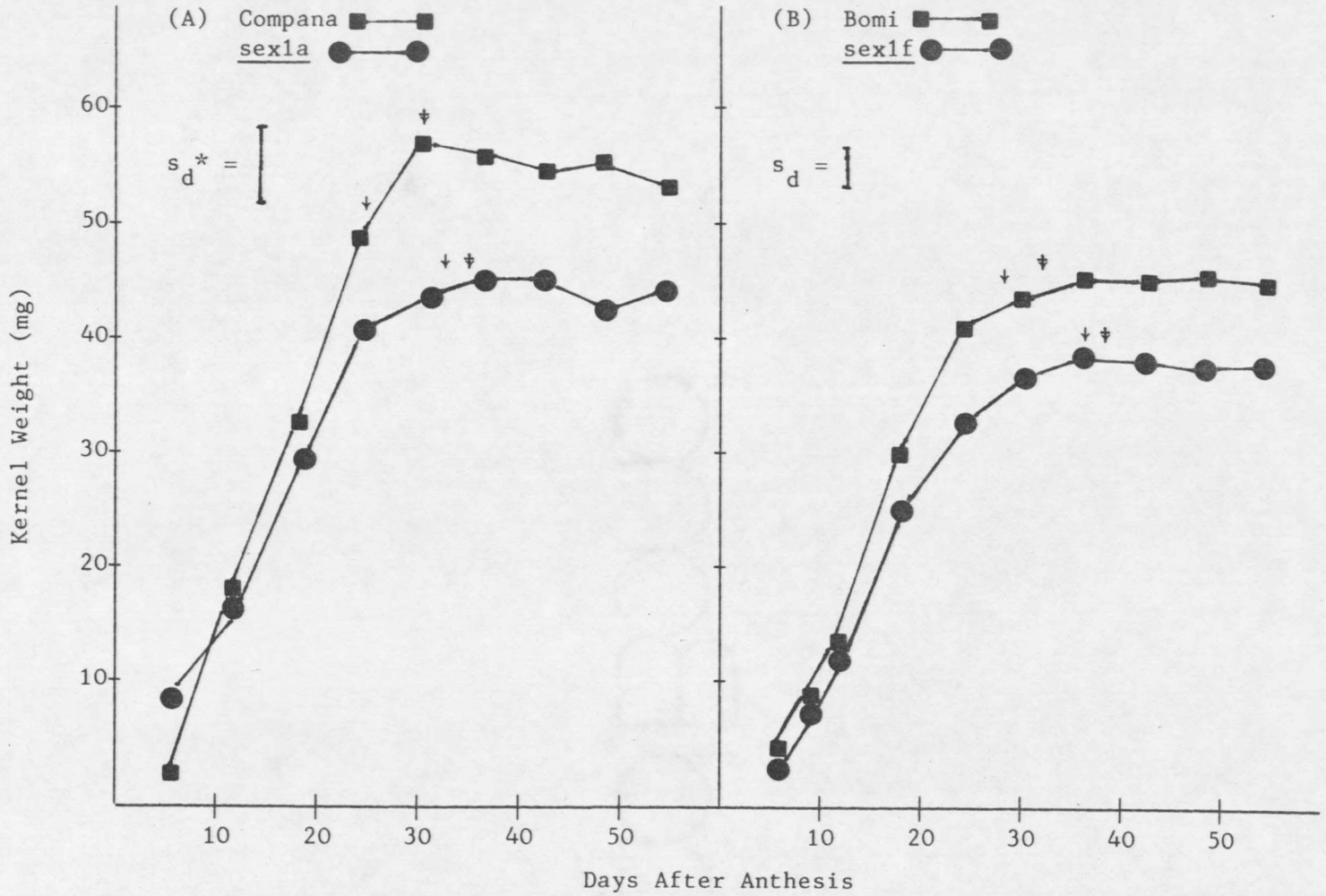

Figure 8. Kernel weight of seed harvested from 6 to 54 days after anthesis for A) Compana and sex1a, and B) Bomi and sex1f. Arrows indicate approximate day seed reached 40% (↓) and 35% (↟) moisture.
*s_d = standard error of the difference using a paired t-test.

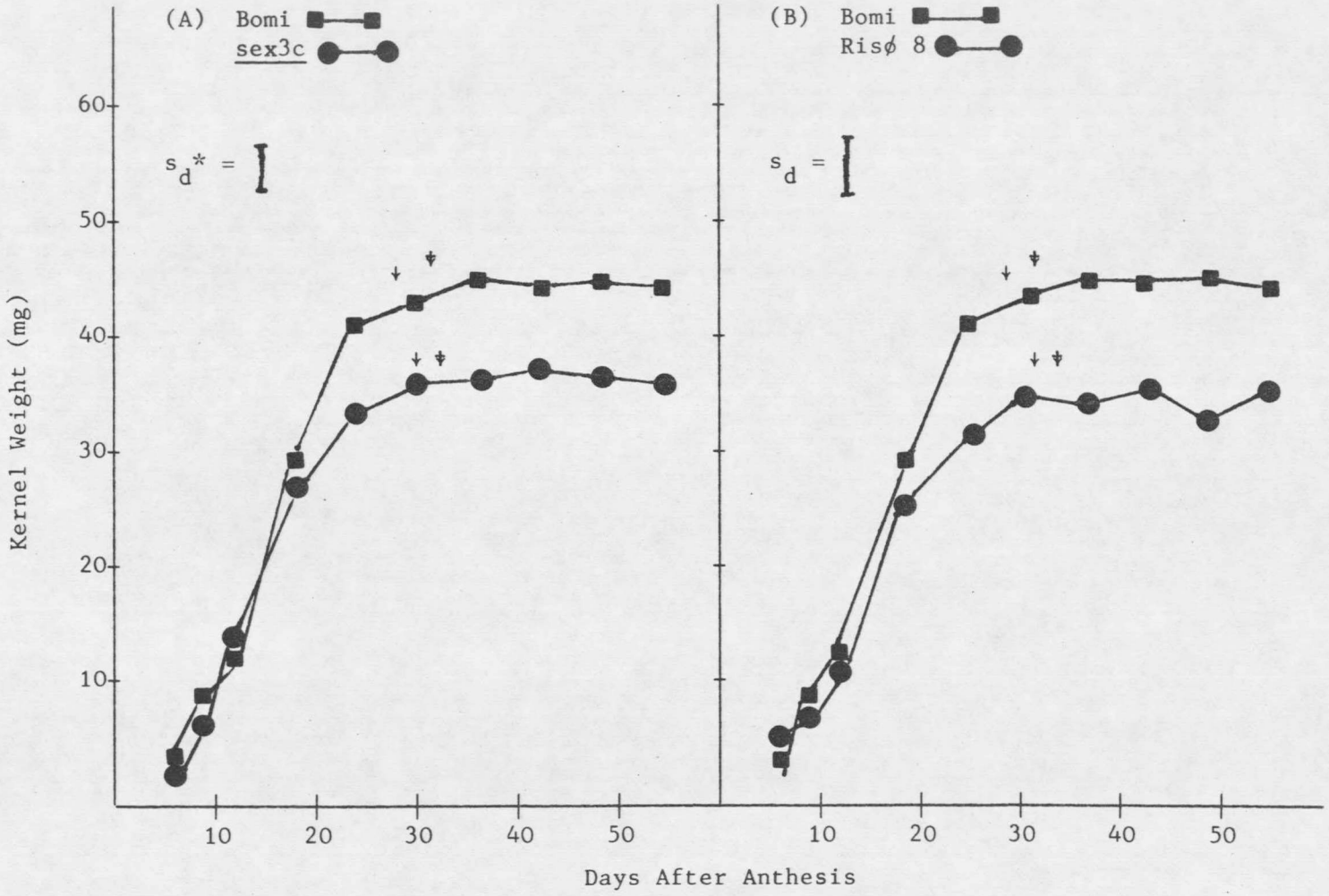

Figure 9. Kernel weight of seed harvested from 6 to 54 days after anthesis for A) Bomi and sex3c, and B) Bomi and Risø 8. Arrows indicate approximate day seed reached 40% (↓) and 35% (↡) moisture.
*s_d = standard error of the difference using a paired t-test.

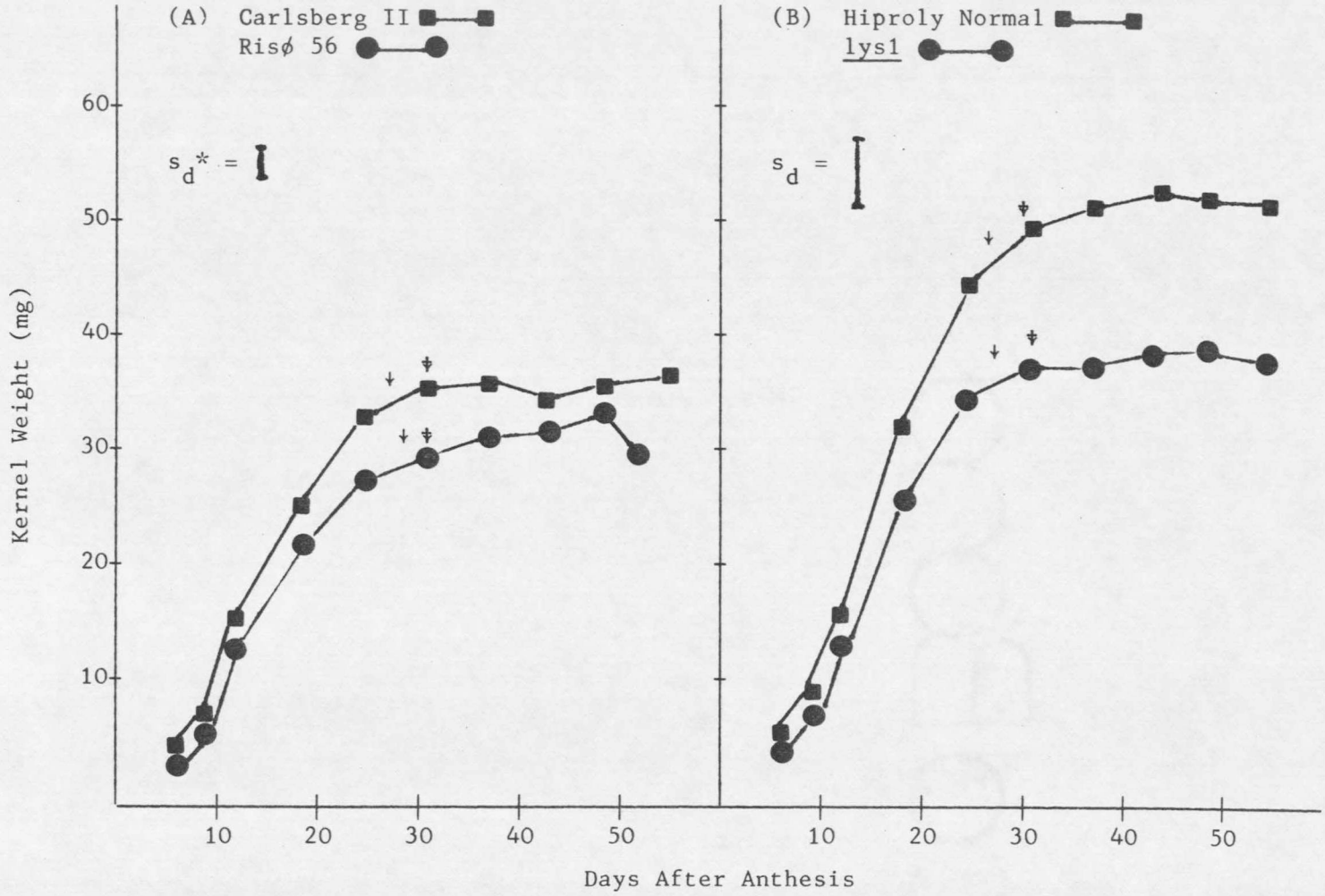

Figure 10. Kernel weight of seed harvested from 6 to 54 days after anthesis for A) Carlsberg II and Risø 56, and B) Hiproly Normal and lys1. Arrows indicate approximate day seed reached 40% (↓) and 35% (↟) moisture.
*s_d = standard error of the difference using a paired t-test.

A highly significant difference in kernel weight was observed among the shrunken endosperm, high lysine mutants (Table 7). Seventy percent of the variability among mutants was accounted for by their genetic classification, i.e., the mean kernel weight of the sex mutants (28.5 mg) was significantly higher than the mean kernel weight of the seg mutants (16.6 mg). All of the sex mutants had a higher mean kernel weight than any of the seg mutants.

A small amount (4%) of the variability among mutants was accounted for by the significant difference between the mean kernel weight of sex1 (31.2 mg) and the other four sex mutants (27.1 mg). This was due primarily to the large average kernel weight of sex1a (34.4 mg) in the cultivar Compana, which had a significantly higher mean kernel weight than sex1f (27.9 mg) in Bomi. This indicates a strong genotype effect. The importance of background genotype to the response of sex1 has been observed previously in comparisons between sex1f and sex1e (Risø 29) in the cultivar Carlsberg II (Køie and Doll, 1979). It is interesting to note that the kernel weight of sex1a at harvest was 44.7 mg. This was equal to or greater than many of the normal cultivars used in this study.

Highly significant dofferences in kernel weight were detected among dates of sampling (Table 7). As expected, the earliest dates had the lowest kernel weight and the latest dates generally had the highest kernel weight (Figures 6 through 10). The pattern of dry matter accumulation tended to differ depending upon whether a mutant was classed as sex or seg. Within the sex mutants (Figures 8 through 10), dry matter accumulation followed a pattern similar to the normal

isotypes only at a slower rate. Within the seg mutants (Figures 6 and 7), dry matter accumulation ceased earlier than in the normal isotype (12 to 24 days vs. 30 to 36 days after anthesis). In seg1 and seg6, the rate of accumulation was approximately equal to the normal isotype until it stopped (12 days after anthesis). The rate of accumulation appeared slower than the normal in seg3 and seg7 prior to its stopping (18 and 24 days after anthesis, respectively).

The moisture data previously reported indicated that four of the sex mutants had a higher mean moisture percentage than their normal isotype (Table 6). A strong relationship has been reported between cessation of dry matter accumulation and the point at which 35 to 40% moisture is reached (Coles, 1979). In Figures 8 and 9, arrows have been used to indicate the approximate date upon which the mutant and normal reached 40% (↓) and 35% (↓) moisture. A small amount of dry matter appeared to accumulate in both of the normal isotypes, Bomi and Compana, even after 35% moisture was reached. The allelic mutants, sex1a and sex1f, reached 40% moisture 7 to 8 days later than their normal isotype. But, dry matter accumulation did not appear to be prolonged. This suggests that the physiological processes causing the seed to reach maturity proceed regardless of the moisture content in these mutants.

It was mentioned previously that seg3 had a moisture percentage of 35% or less during three of the first four sampling dates. Figure 6 indicates that dry matter accumulation in this mutant appeared similar to Compana during this period.

Alpha-Amylase Activity

No significant differences in mean alpha-amylase activity were detected between any of the shrunken endosperm, high lysine mutants and their normal isotypes (Table 9). Differences which were observed (Figures 11 through 15) were generally during the 6 to 18 days after anthesis (the first 2 to 3 sampling dates). Later samples generally had very similar alpha-amylase activity, with one exception. An increase in activity was observed in sex3c (Figure 14) at harvest. This may be related to the enlarged embryo observed in sex3c (Tallberg, 1977).

No significant differences were detected among the 10 shrunken endosperm, high lysine mutants (Table 7). This was due primarily to the large interaction between genotype and sampling date which was used to test the genotype effect. A highly significant difference was detected between seg and sex mutants. The seg mutants with an activity of 30.9 ug dye released/kernel/min at 65 C had a higher mean alpha-amylase activity than the sex mutants with an activity of 12.8 ug dye released/kernel/min at 65 C. The allelic mutants, sex1a and sex1f, were not significantly different, nor was their mean significantly different from the other sex mutants.

Significant differences were detected among sampling dates (Table 7). The highest alpha-amylase activity was observed at 6 days after anthesis for each isotype. It has been reported previously that alpha-amylase activity in developing barley seed peaks 2 to 10 days after anthesis (MacGregor et al., 1972). The lowest activity was generally observed at harvest.

Table 9. Alpha-amylase activity comparisons between barley shrunken endosperm, high lysine mutants and their normal isotype from 6 to 54 days after anthesis.

Normal Cultivar	Mutant	Number of Comparisons	Alpha-Amylase Activity (ug dye released/kernel/min at 65 C)				Difference $\bar{x}-\bar{y}$
			Mutant Isotype (x)		Normal Isotype (y)		
			mean	range	mean	range	
Seg Mutants							
Betzes	seg1	10	25.90	0.70-148.49	15.12	1.05-74.33	10.78
Compana	seg3	8	21.39	2.72-91.79	17.77	0.90-97.46	3.62
Ingrid	seg6	10	25.74	1.76-112.44	11.85	0.80-49.92	13.89
Ingrid	seg7	10	45.05	0.56-318.49	11.85	0.80-49.92	33.20
Sex Mutants							
Hiproly Normal	lys1	10	8.94	0.59-63.08	19.87	0.78-108.44	-10.93
Compana	sex1a	9	12.32	2.58-47.10	15.96	0.90-97.46	-3.64
Bomi	sex1f	10	9.40	0.63-41.42	17.29	0.73-77.22	-7.89
Bomi	sex3c	10	17.55	1.71-87.70	17.29	0.73-77.22	0.26
Bomi	Risø 8	10	7.12	0.70-38.94	17.29	0.73-77.22	-10.17
Carlsberg II	Risø 56	10	25.63	1.02-116.96	12.41	0.55-71.14	13.22

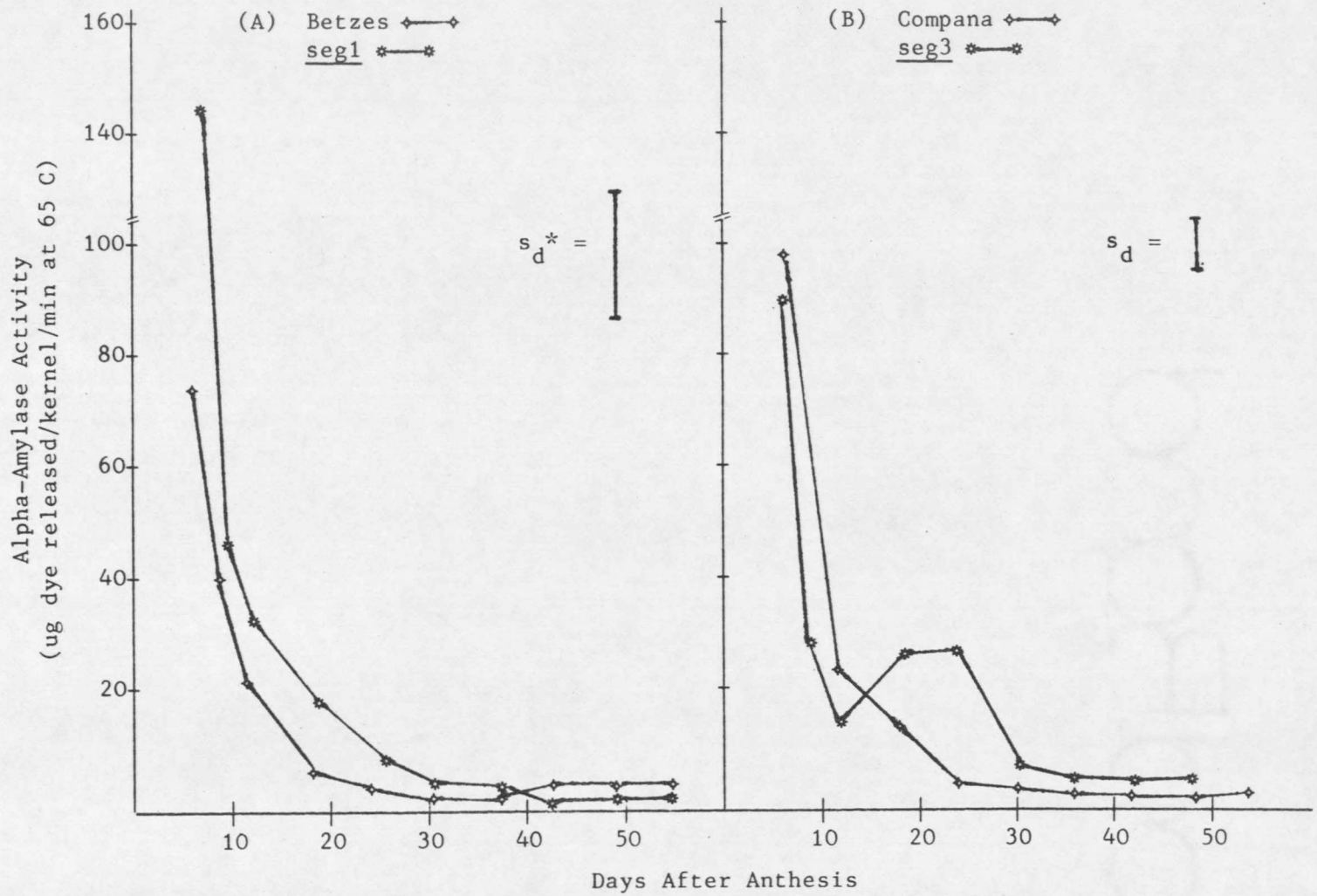

Figure 11. Alpha-amylase activity of seed harvested from 6 to 54 days after anthesis for A) Betzes and seg1, and B) Compana and seg3.
*s_d =standard error of the difference using a paired t-test.

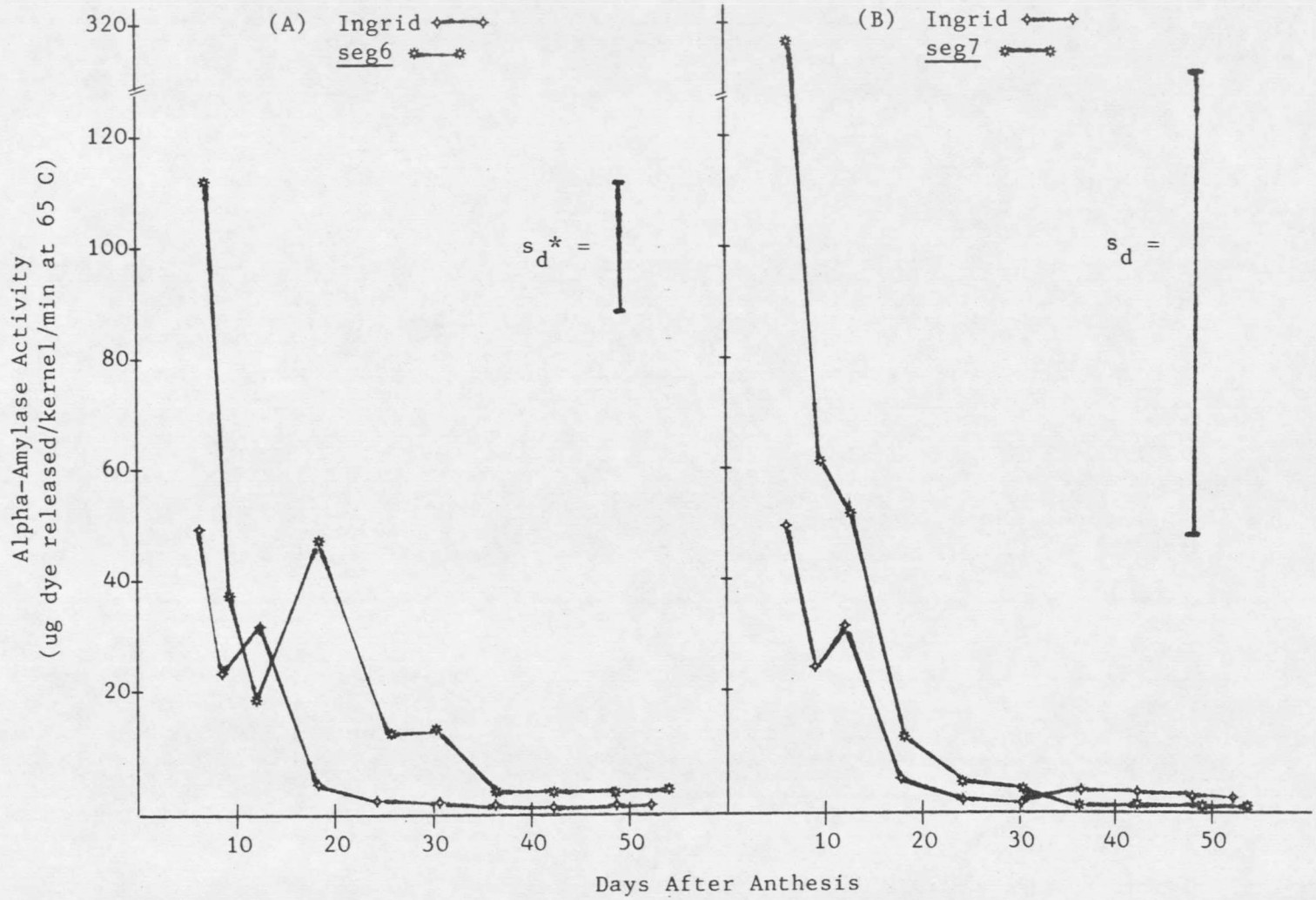

Figure 12. Alpha-amylase activity of seed harvested from 6 to 54 days after anthesis for A) Ingrid and seg6, and B) Ingrid and seg7.
*s_d = standard error of the difference using a paired t-test.

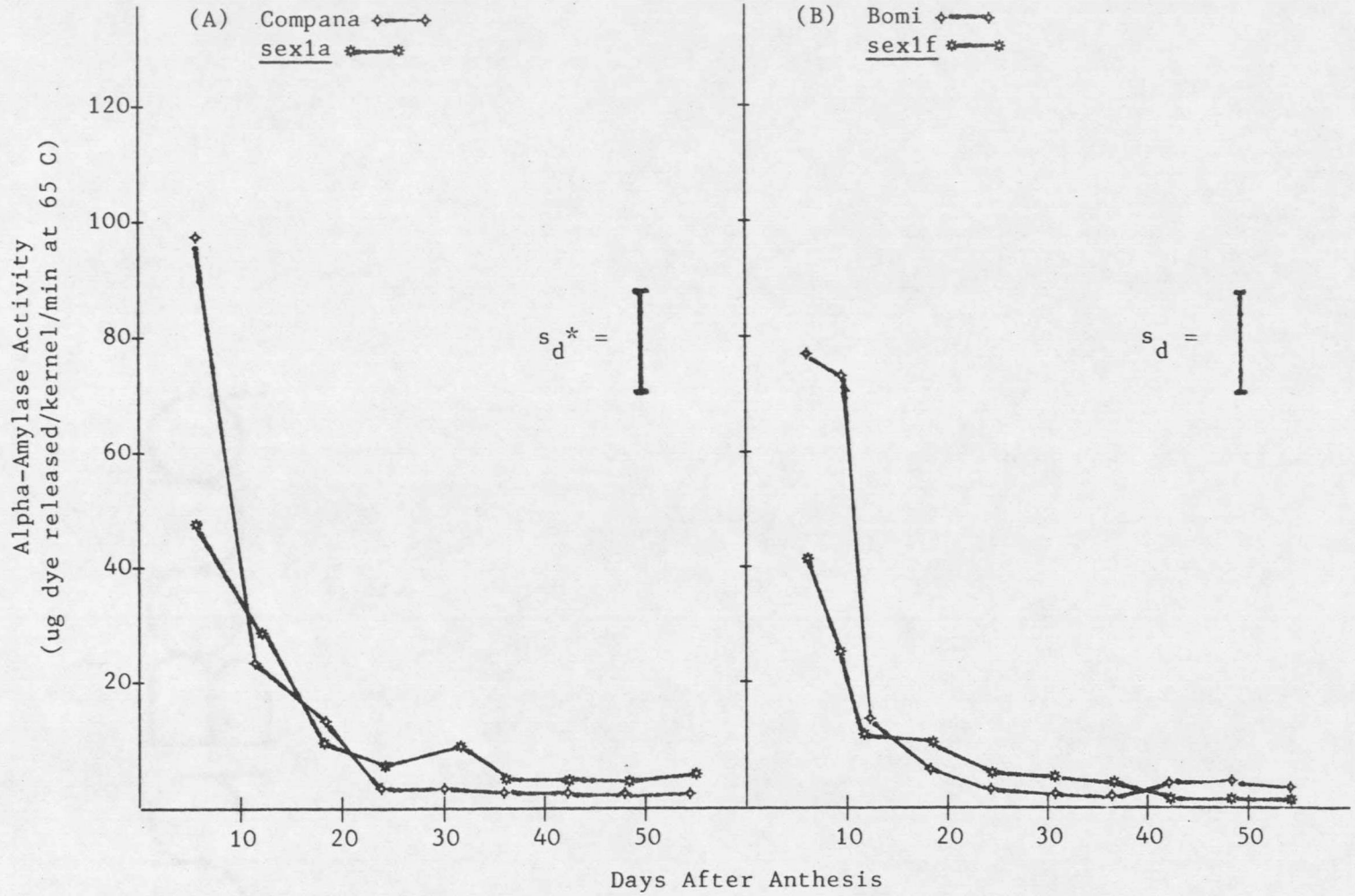

Figure 13. Alpha-amylase activity of seed harvested from 6 to 54 days after anthesis for A) Compana and sex1a, and B) Bomi and sex1f.
*s_d = standard error of the difference using a paired t-test.

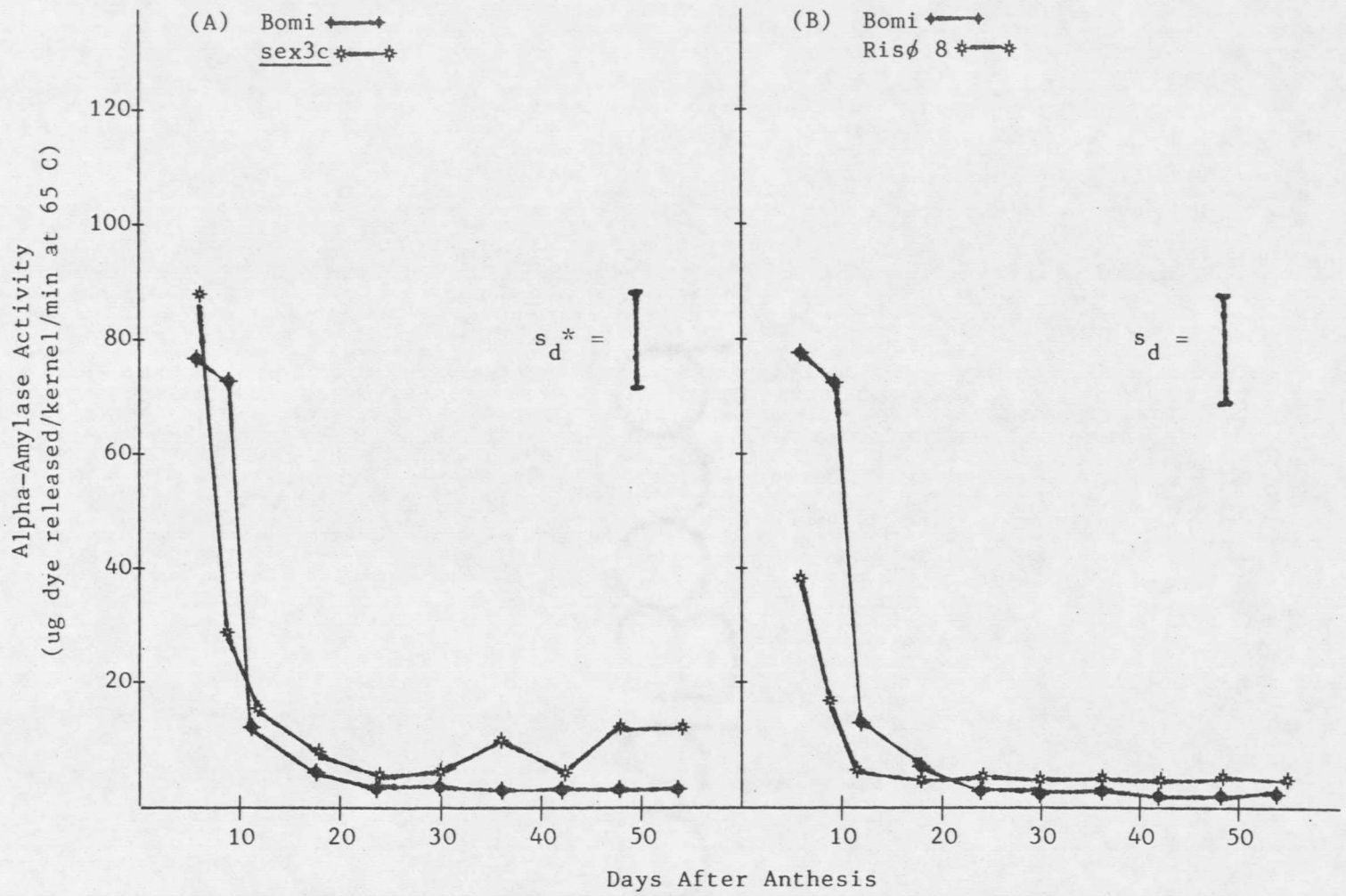

Figure 14. Alpha-amylase activity of seed harvested from 6 to 54 days after anthesis for A) Bomi and sex3c, and B) Bomi and Risø 8.
*s_d = standard error of the difference using a paired t-test.

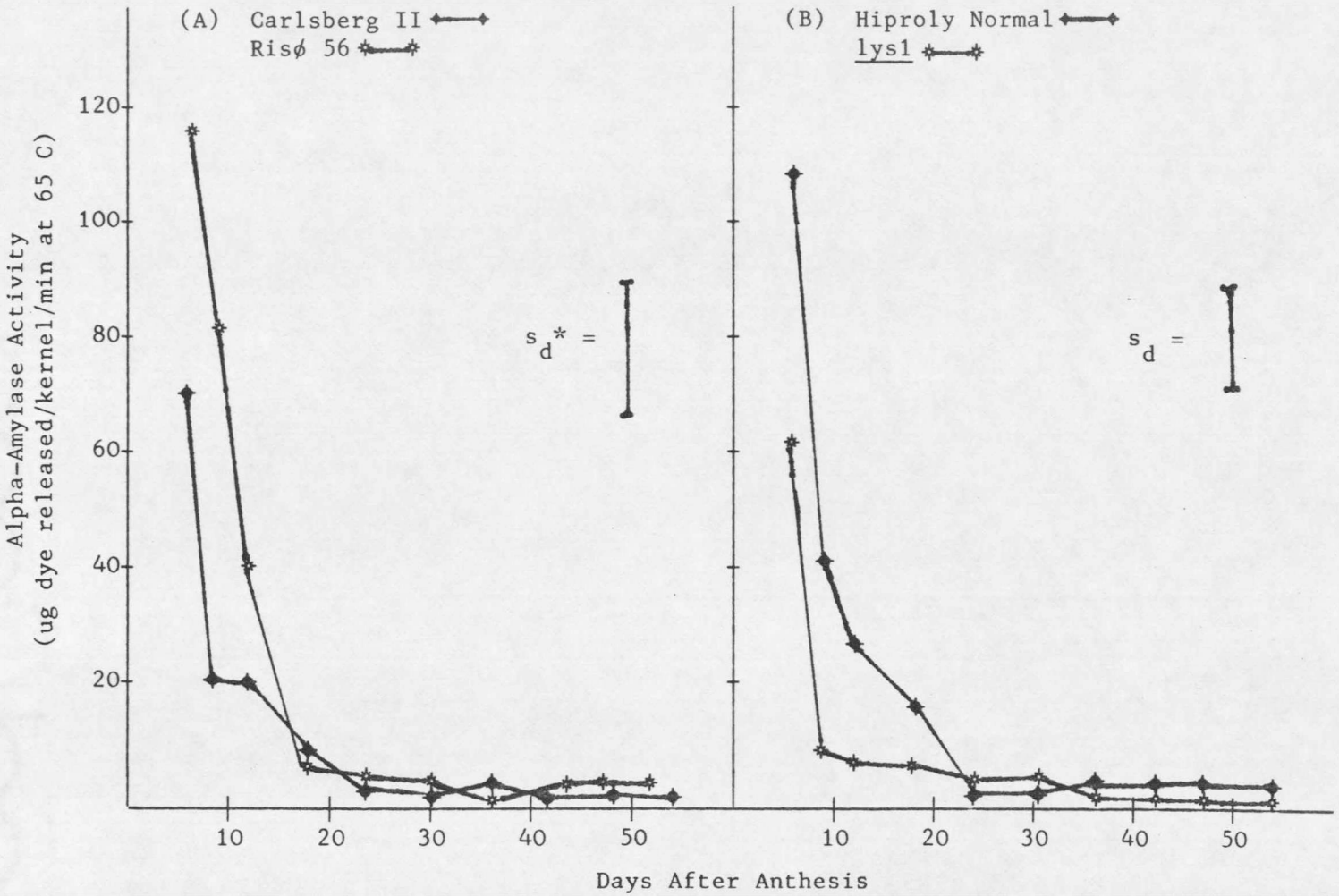

Figure 15. Alpha-amylase activity of seed harvested from 6 to 54 days after anthesis for A) Carlsberg II and Risø 56, and B) Hiproly Normal and lys1.
*s_d = standard error of the difference using a paired t-test.

The significant difference in mean activity between seg and sex mutants was largely due to unusually high activity at six days after anthesis observed in seg1, seg6, and seg7. Even though significant differences were not detected between these mutants and their normal isotypes, it is probable this was due to the design of the experiment rather than the lack of a difference. The high activity observed in these mutants has not been reported previously, thus more research is indicated.

This research was performed to determine if a relationship existed between shrunken seed and alpha-amylase activity. Unfortunately, it was difficult to detect difference in alpha-amylase activity. The largest differences among isotypes were among the peak activities at six days after anthesis. However, among the 10 shrunken endosperm, high lysine mutants, no relationship could be detected between peak alpha-amylase activity and kernel weight at six days after anthesis ($r=-.34$) or kernel weight at harvest ($r=-.55$). Among the six normal isotypes, a significant correlation ($r=.89^*$) was detected between peak alpha-amylase activity and kernel weight at harvest (Figure 16). No relationship was detected for these cultivars between peak alpha-amylase activity and kernel weight at six days after anthesis ($r=.43$), nor between the latter response and kernel weight at harvest ($r=.29$). The positive correlation which was detected was unexpected, especially since Riggs and Gothard (1976) found no such relationship among seven cultivars. No explanation can be provided at this time.

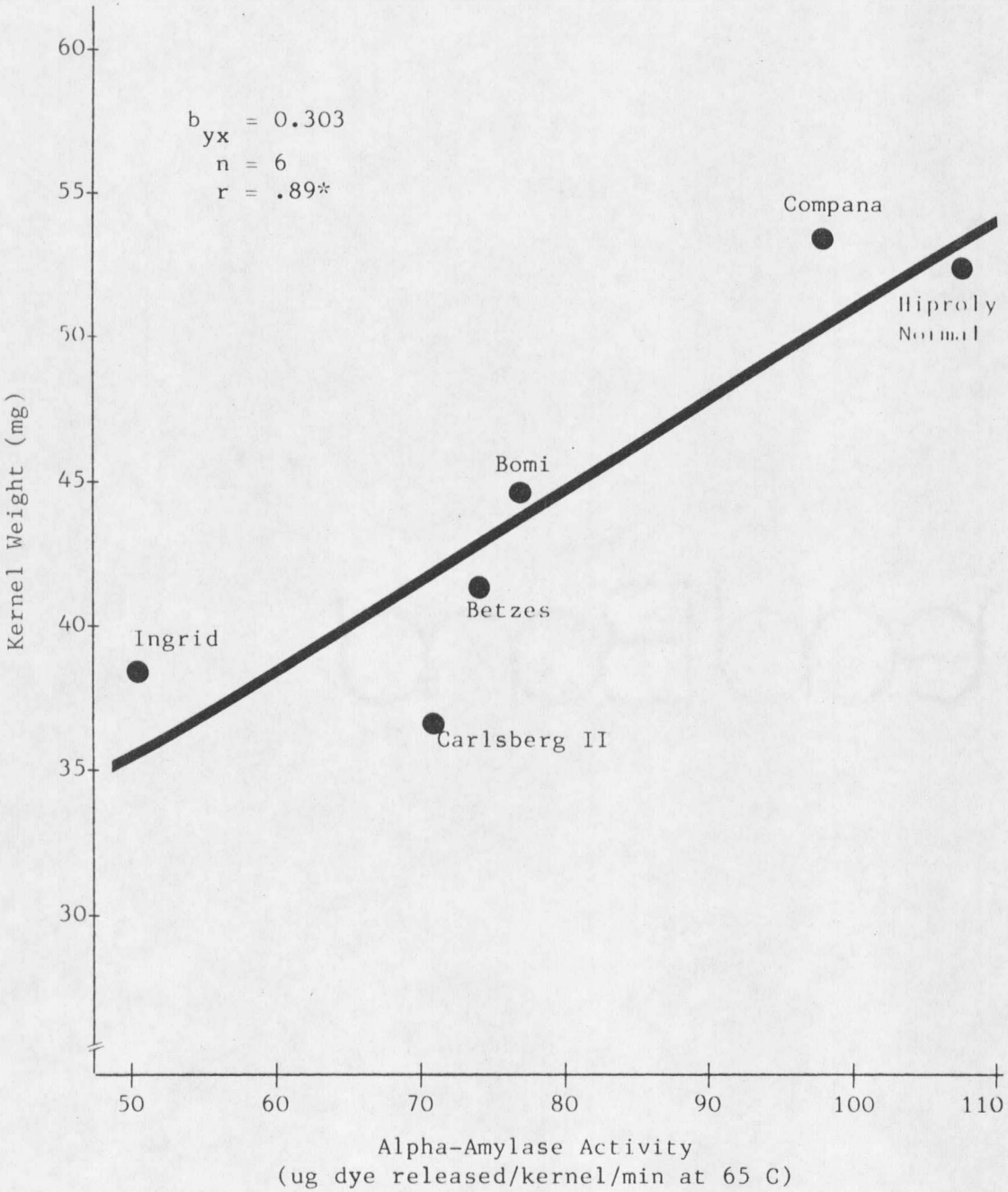

Figure 16. Relationship between alpha-amylase activity six days after anthesis and kernel weight at harvest in seed of six normal cultivars.
*Significant at the 0.05 level.

Total Sugar

Significant differences in total sugar were detected between all of the shrunken endosperm, high lysine mutants and their normal isotypes with the exception of lys1, Risø 8, and Risø 56 (Table 10). All of the seg mutants had a significantly lower mean total sugar than their normal isotype. Three of the sex mutants, sex1a, sex1f, and sex3c, had a significantly higher mean total sugar than their normal isotype. These differences were generally observed throughout seed development (Figures 17 through 21).

Total sugars have been reported as umol glucose equiv./kernel. These units were chosen (rather than umol glucose equiv./mg) because of the large difference in seed size between the mutants and normals. When comparing seed of different sizes, it might be expected that a smaller seed would have less of any component (when measured as units/kernel), assuming that most components decrease proportionately to the decrease in kernel weight. In the sex mutants, the levels of total sugars were equal to or greater than the normal isotypes. This contradicted the expectation and indicates that the level of total sugar in these mutants was independent of the kernel weight decrease. In the seg mutants, total sugars were decreased as expected. By converting the data from umol glucose equiv./kernel to % glucose equiv. (i.e., % of kernel), it was possible to determine if the decreases were proportional to the decreases in kernel weight. No significant differences were observed (Table 11) between seg1, seg3, and seg6 and their normal isotypes in glucose percentage, indicating that total sugars

Table 10. Total sugar comparisons between barley shrunken endosperm, high lysine mutants and their normal isotypes from 6 to 54 days after anthesis.

Normal Cultivar	Mutant	Number of Comparisons	Total Sugar (umol glucose equiv./kernel)				
			Mutant Isotype (x)		Normal Isotype (y)		Difference $\bar{x}-\bar{y}$
			mean	range	mean	range	
Seg Mutants							
Betzes	seg1	10	4.85	1.73–12.01	7.65	5.64–11.95	-2.80**
Compana	seg3	8	5.46	2.50–10.47	7.88	4.94–14.14	-2.42**
Ingrid	seg6	10	5.68	1.05–11.35	7.53	4.62–13.06	-1.85*
Ingrid	seg7	10	4.17	1.43–10.37	7.53	4.62–13.06	-3.36**
Sex Mutants							
Hiproly Normal	lys1	10	10.99	7.10–15.44	10.86	6.64–16.85	0.13
Compana	sex1a	9	17.30	7.80–28.46	7.55	4.94–14.14	9.75**
Bomi	sex1f	10	15.29	6.96–22.64	8.13	5.88–12.69	7.16**
Bomi	sex3c	10	10.23	4.82–13.85	8.13	5.88–12.69	2.10*
Bomi	Risø 8	10	9.10	4.94–12.45	8.13	5.88–12.69	0.97
Carlsberg II	Risø 56	10	6.72	4.04–14.42	5.61	3.84–11.22	1.11

*, **Significant at the 0.05 and 0.01 levels, respectively.

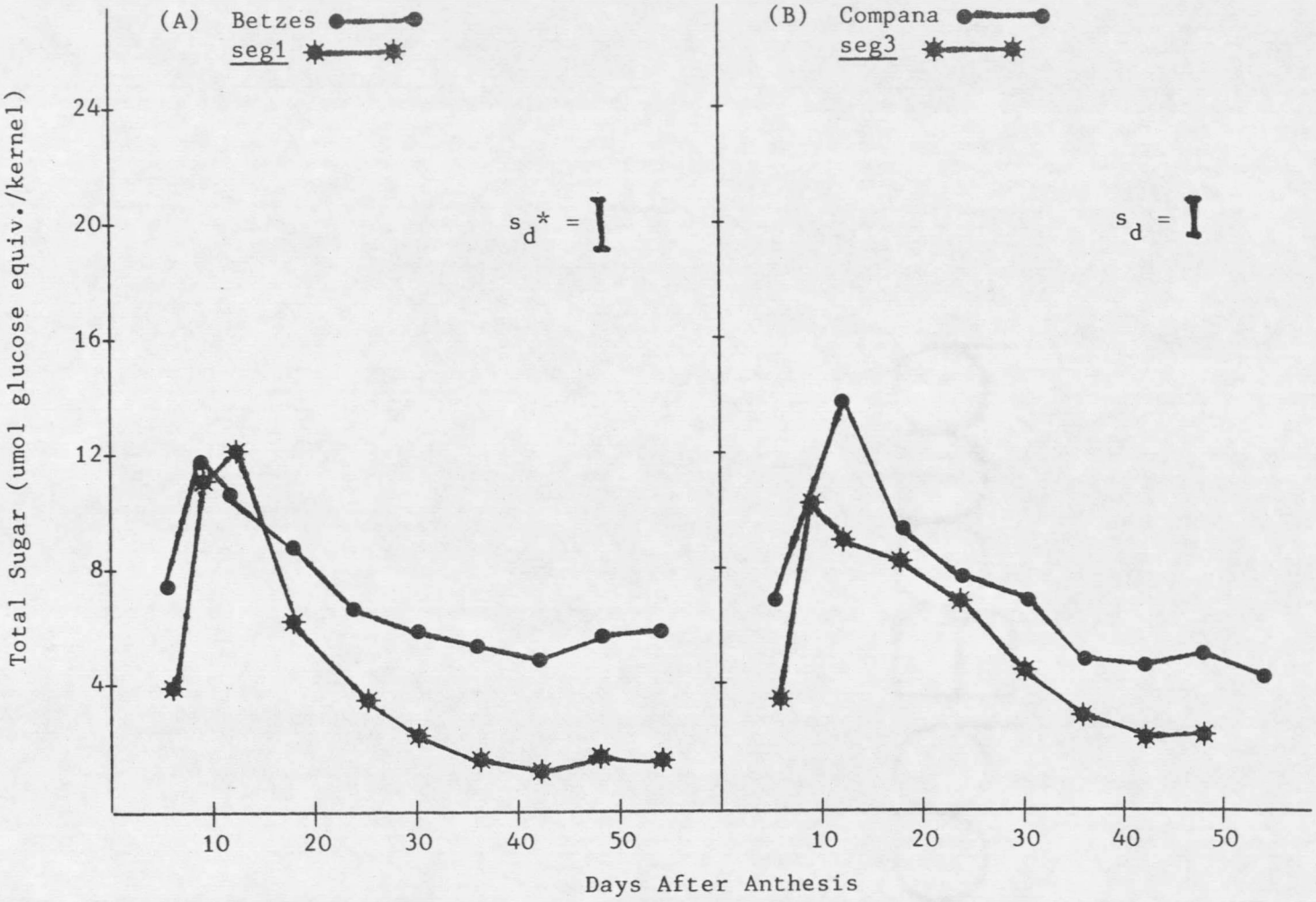

Figure 17. Total sugar of seed harvested from 6 to 54 days after anthesis for A) Betzes and seg1, and B) Compana and seg3.
*s_d = standard error of the difference using a paired t-test.

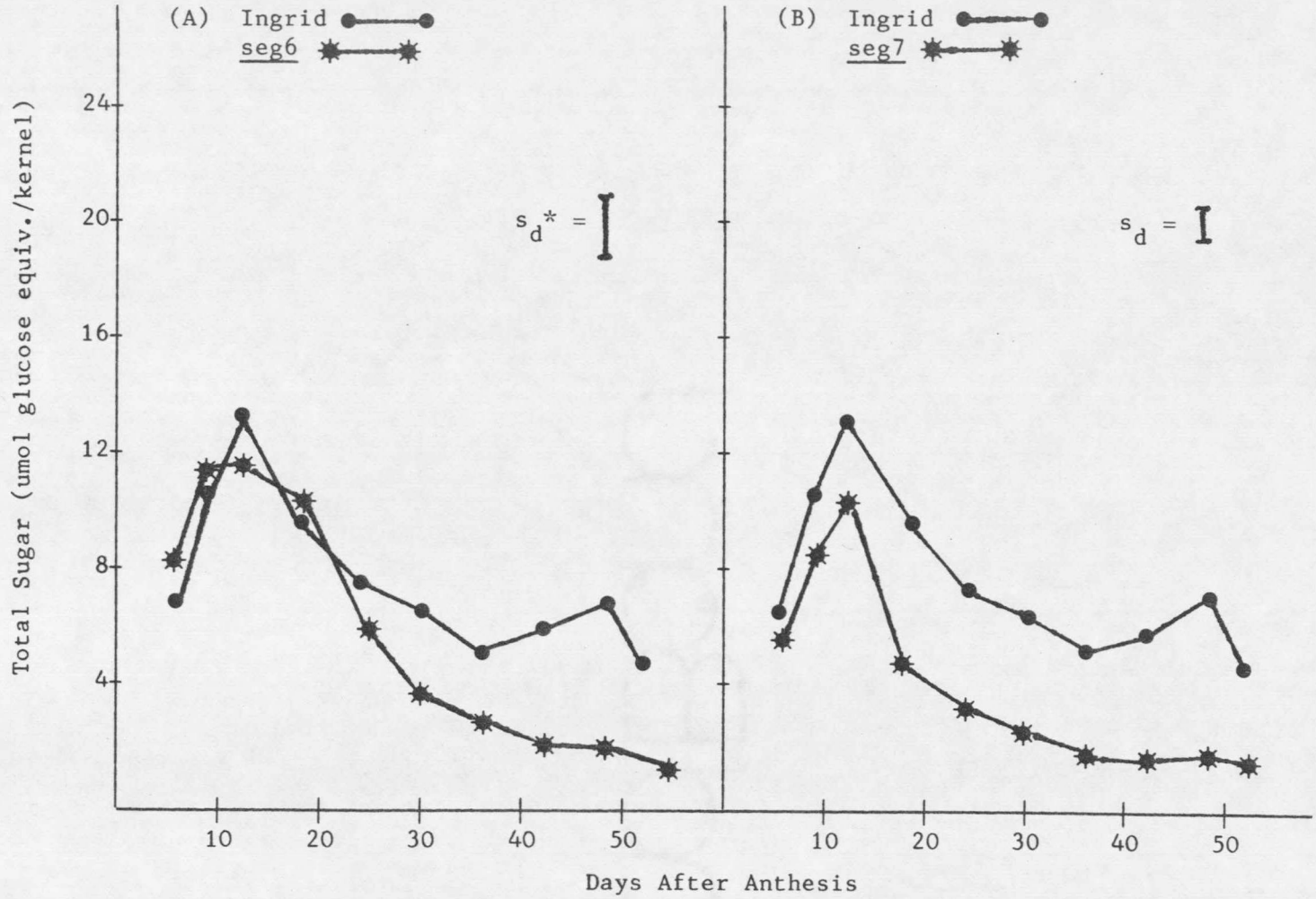

Figure 18. Total sugar of seed harvested from 6 to 54 days after anthesis for A) Ingrid and seg6, and B) Ingrid and seg7.
*s_d = standard error of the difference using a paired t-test.

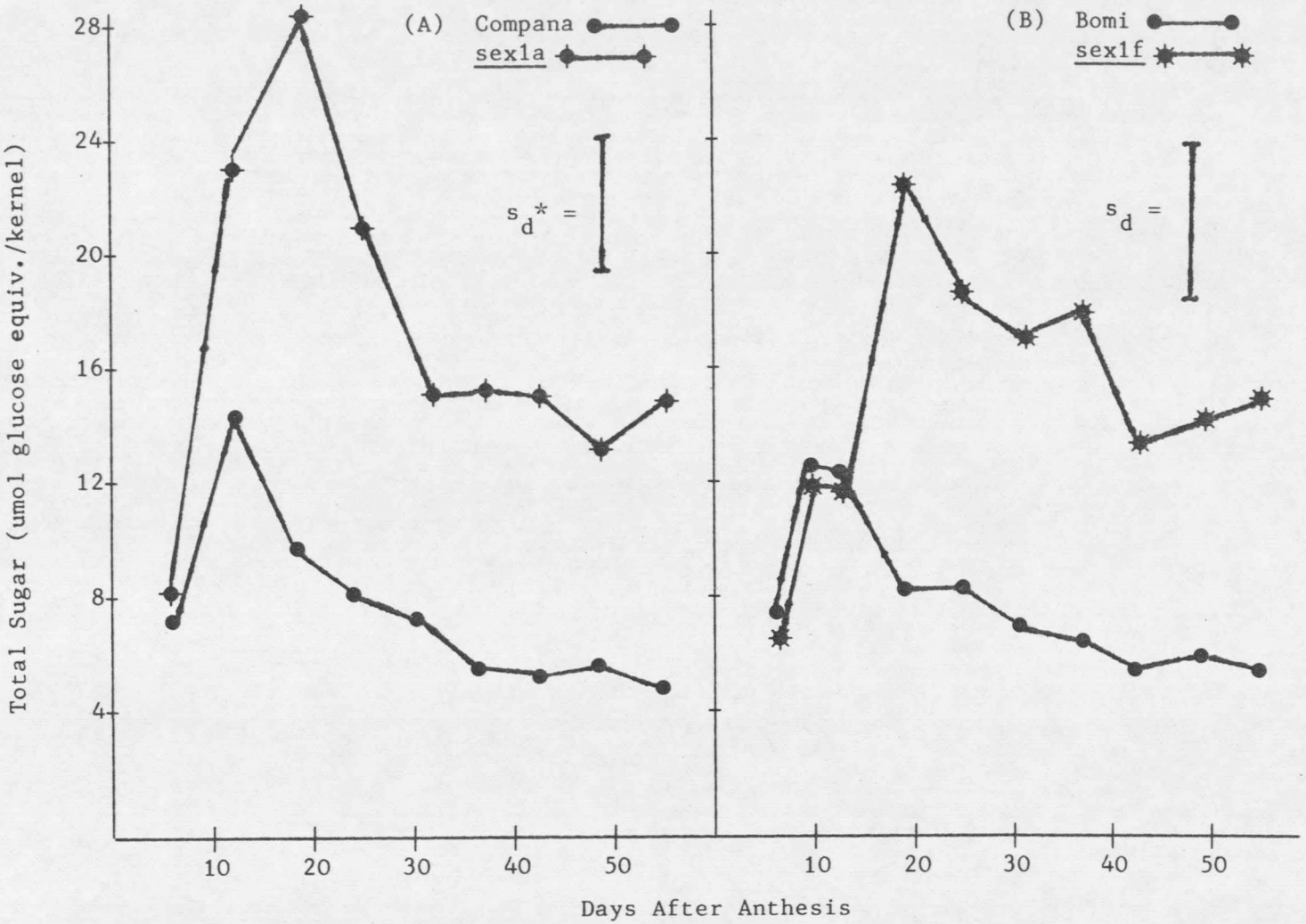

Figure 19. Total sugar of seed harvested from 6 to 54 days after anthesis for A) Compana and sex1a, and B) Bomi and sex1f.
*s_d = standard error of the difference using a paired t-test.

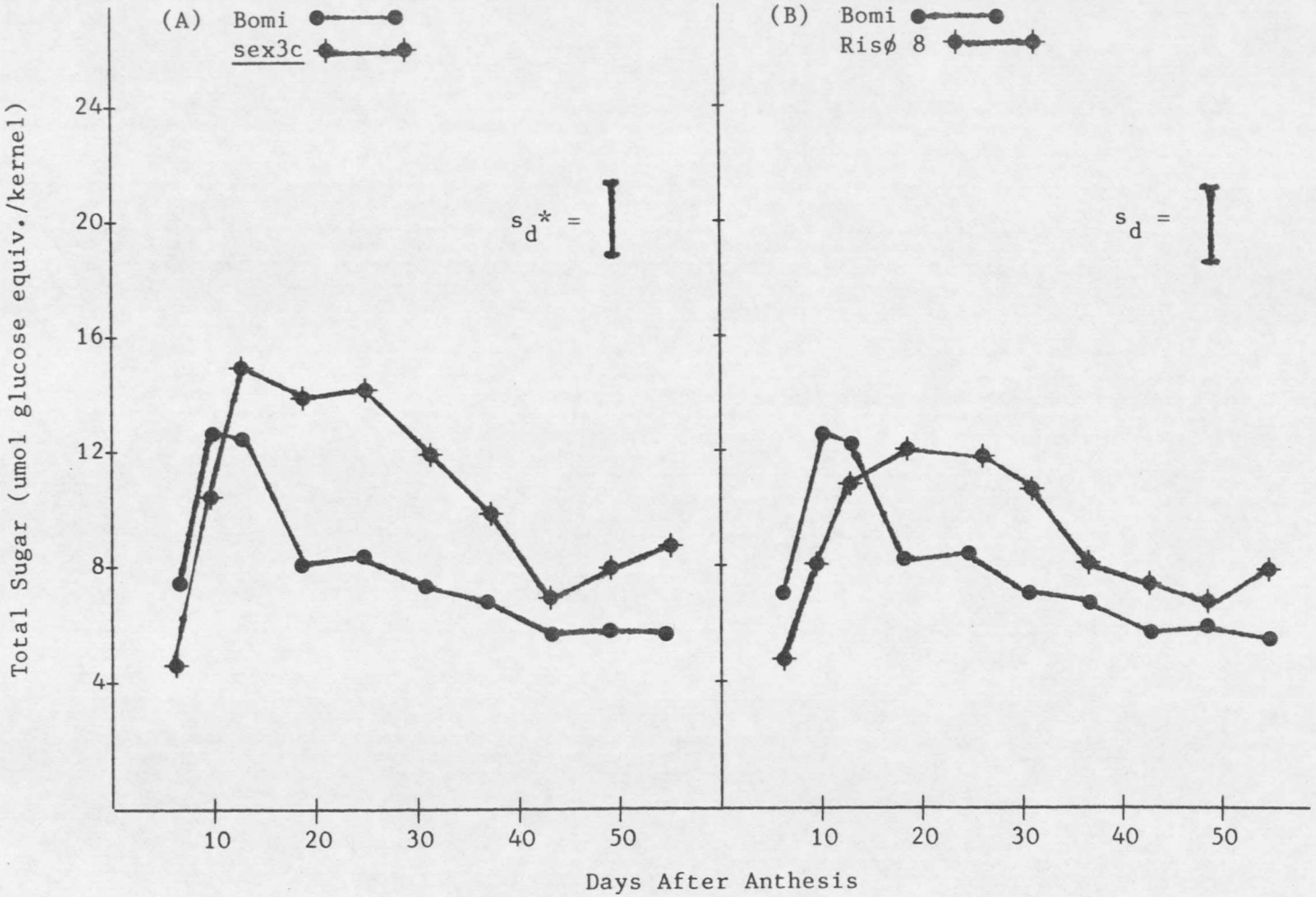

Figure 20. Total sugar of seed harvested from 6 to 54 days after anthesis for A) Bomi and sex3c, and B) Bomi and Risø 8.
*s_d = standard error of the difference using a paired t-test.

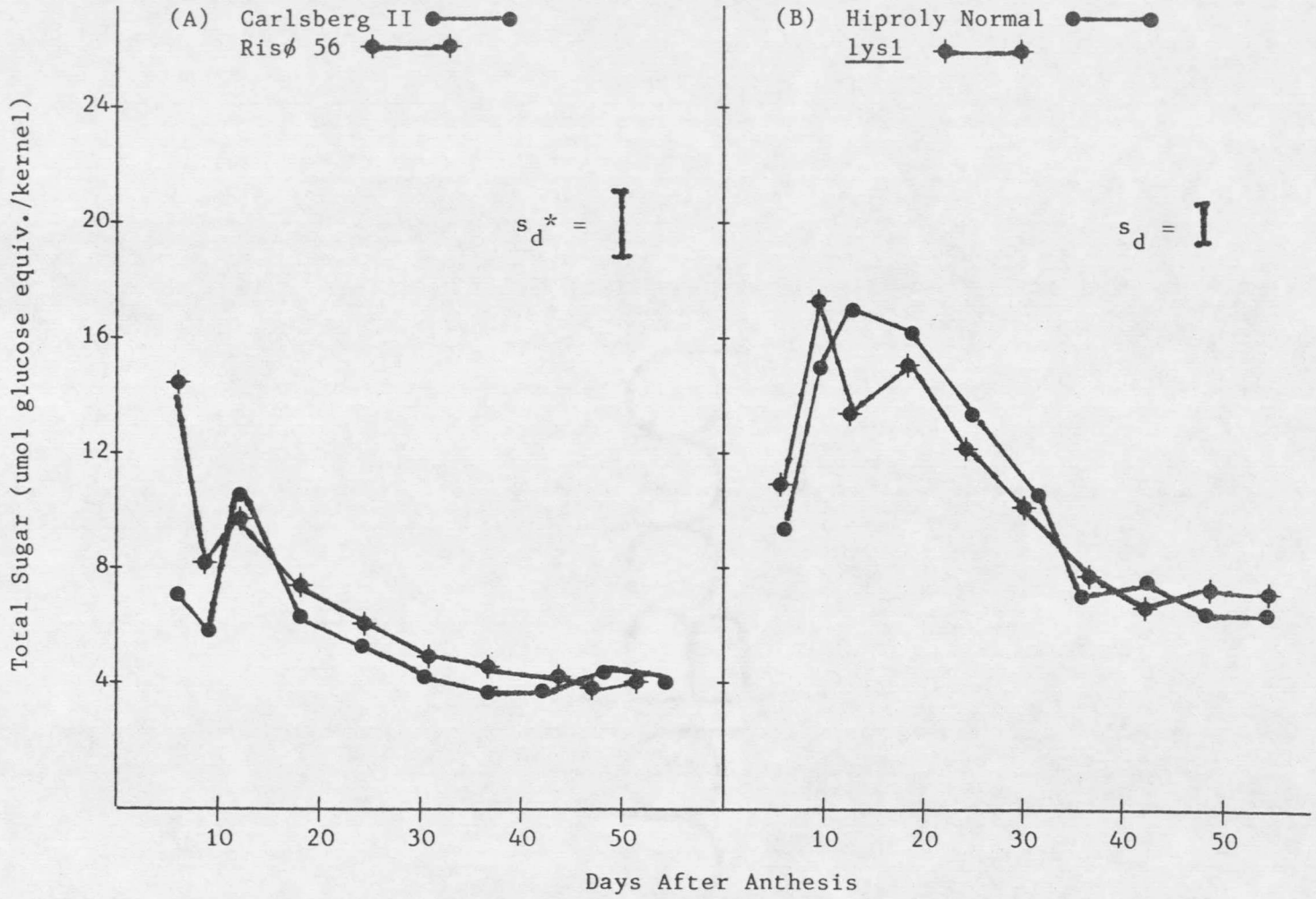

Figure 21. Total sugar of seed harvested from 6 to 54 days after anthesis for A) Carlsberg II and Risø 56, and B) Hiproly Normal and lys1.
*s_d = standard error of the difference using a paired t-test.

Table 11. Total sugar percentage comparisons between barley shrunken endosperm, high lysine mutants and their normal isotypes from 6 to 54 days after anthesis.

Normal Cultivar	Mutant	Number of Comparisons	Total Sugar (% glucose equiv.) Mutant Isotype (x) mean	range	Normal Isotype (y) mean	range	Difference $\bar{x}-\bar{y}$
Seg Mutants							
Betzes	seg1	10	10.10	2.17-31.89	10.98	2.40-42.97	-0.88
Compana	seg3	8	8.78	1.89-28.91	10.33	1.75-52.47	-1.55
Ingrid	seg6	10	13.71	1.62-49.90	10.56	2.14-34.77	3.15
Ingrid	seg7	10	7.99	0.93-31.99	10.56	2.14-34.77	-2.57**
Sex Mutants							
Hiproly Normal	lys1	10	13.29	3.28-40.39	10.75	2.27-31.58	2.54
Compana	sex1a	9	10.78	5.65-23.48	9.36	1.65-52.47	1.42
Bomi	sex1f	10	15.67	6.55-43.20	10.74	2.36-40.42	4.93**
Bomi	sex3c	10	12.17	3.43-34.70	10.74	2.36-40.42	1.43
Bomi	Risø 8	10	9.54	3.77-20.21	10.74	2.36-40.42	-1.20
Carlsberg II	Risø 56	10	14.69	2.21-86.52	8.78	1.90-40.41	5.91

**Significant at the 0.01 level.

were present in similar proportions in both mutant and normal. This suggests that the level of total sugar in these mutants was linked to kernel weight and as kernel weight decreased, so did total sugar, or vice versa. The remaining mutant, seg7, had a significantly lower total sugar percentage than its normal isotype, indicating that the decrease in total sugar was proportionately greater than the decrease in kernel weight.

Conversion of the data to % glucose equiv. did not provide additional information about the sex mutants (Table 11). Since all of the sex mutants were similar to or greater than their normal isotypes in absolute content of total sugar, it was expected that they would have a higher total sugar percentage because of their smaller seed size. This was not the case (Table 11). Although all of the sex mutants except Risø 8 had a higher mean % glucose equiv. than their normal isotypes, only sex1f was significantly higher. The lack of significant differences with lys1, sex3c, and Risø 56, was probably due to strong isotype x sampling date interactions which were not removed by the paired t-test. In the case of sex1a, the first sampling date had a total sugar percentage lower than the normal. By deleting this observation from the analysis, a highly significant difference was obtained with a mean difference of 6.04% glucose equiv. The latter is more consistent with the data for sex1f, thus, it seems likely that the sample from day 6 for sex1a was switched or contaminated.

Highly significant differences in mean total sugar were observed among the 10 shrunken endosperm, high lysine mutants (Table 7). More than half (56%) of the genotype variation was accounted for by the

difference between seg and sex mutants. The sex mutants had a significantly higher (11.73 umol glucose equiv./kernel) average total sugar than the seg mutants (5.00 umol glucose equiv./kernel). No difference was detected between the mean of sex1a (17.54 umol glucose equiv./kernel) and the mean of sex1f (15.63 umol glucose equiv./kernel), but the significantly higher mean of these two (16.59 umol glucose equiv./kernel) compared to the other sex mutants (9.30 umol glucose equiv./kernel) accounted for 37% of the variation among mutants.

Highly significant differences were observed among sampling dates (Table 7). The general pattern for all mutants and normals (Figures 17 through 21) began with a relatively low amount of total sugars six days after anthesis, increasing to a peak 12 to 30 days after anthesis. Total sugar declined quickly after reaching the peak in the seg isotypes and the normal isotypes. The high level of total sugar found at the peak in the sex isotypes was observed for a longer period of time before declining.

Total sugar in the normal isotypes ranged from a peak of 16.85 umol glucose equiv./kernel or 52.47% glucose equiv. to a low at harvest of 6.64 umol glucose equiv./kernel or 1.65% glucose equiv. These results are, in general, consistent with other published data. Total sugar in the developing barley seed has been reported to range from 5.55 to 16.67 umol glucose equiv./kernel or 2 to 50% glucose equiv. in 'Lami' (Coles, 1979), from 8.33 to 16.67 umol glucose equiv./kernel in 'Iris' (Cerning and Guilbot, 1973), from 5.55 to 11.00 umol glucose equiv./kernel in 'Conquest' (MacGregor et al., 1971), from 3 to 38% soluble sugar in 'Carlsberg' (Harris and MacWilliams, 1957),

and from 2 to 7% soluble sugar in Betzes (LaBerge et al., 1973). The peak sugar percentage reported by LaBerge et al. (1973) is inconsistent with other published data and the data reported here.

The two mutants which had the highest mean total sugar also had the highest mean moisture percentage. This prompted the calculation of the correlation between these two traits among all isotypes (mutant and normal). The relationship was highly significant both when all genotypes were used (r=.83**) (Figure 22), and when seg3 was excluded because of its unusually low moisture percentage (r=.94**). This suggests that the high sugar levels in sex1a and sex1f were strongly associated with the higher moisture levels in seed of these mutants. Higher sugar would result in a higher osmotic potential which would pull more water into the cell in order to maintain the overall water potential of the cell. This may also result in a higher turgor potential. The latter would explain the observed ability of an sex mutant seed to maintain plumpness until drydown even though less starch is filling the cells.

Reducing Sugar

Significant differences in mean reducing sugar were not detected between seg6, seg7, lys1, sex3c, Risø 8, and Risø 56, and their corresponding normal isotype (Table 12). Two seg mutants, seg1 and seg3, had a significantly lower mean reducing sugar than their normal isotype. Two of the sex mutants, sex1a and sex1f, had a significantly higher mean reducing sugar than their normal isotype. These differences were generally observed throughout seed development (Figures 23 through 27).

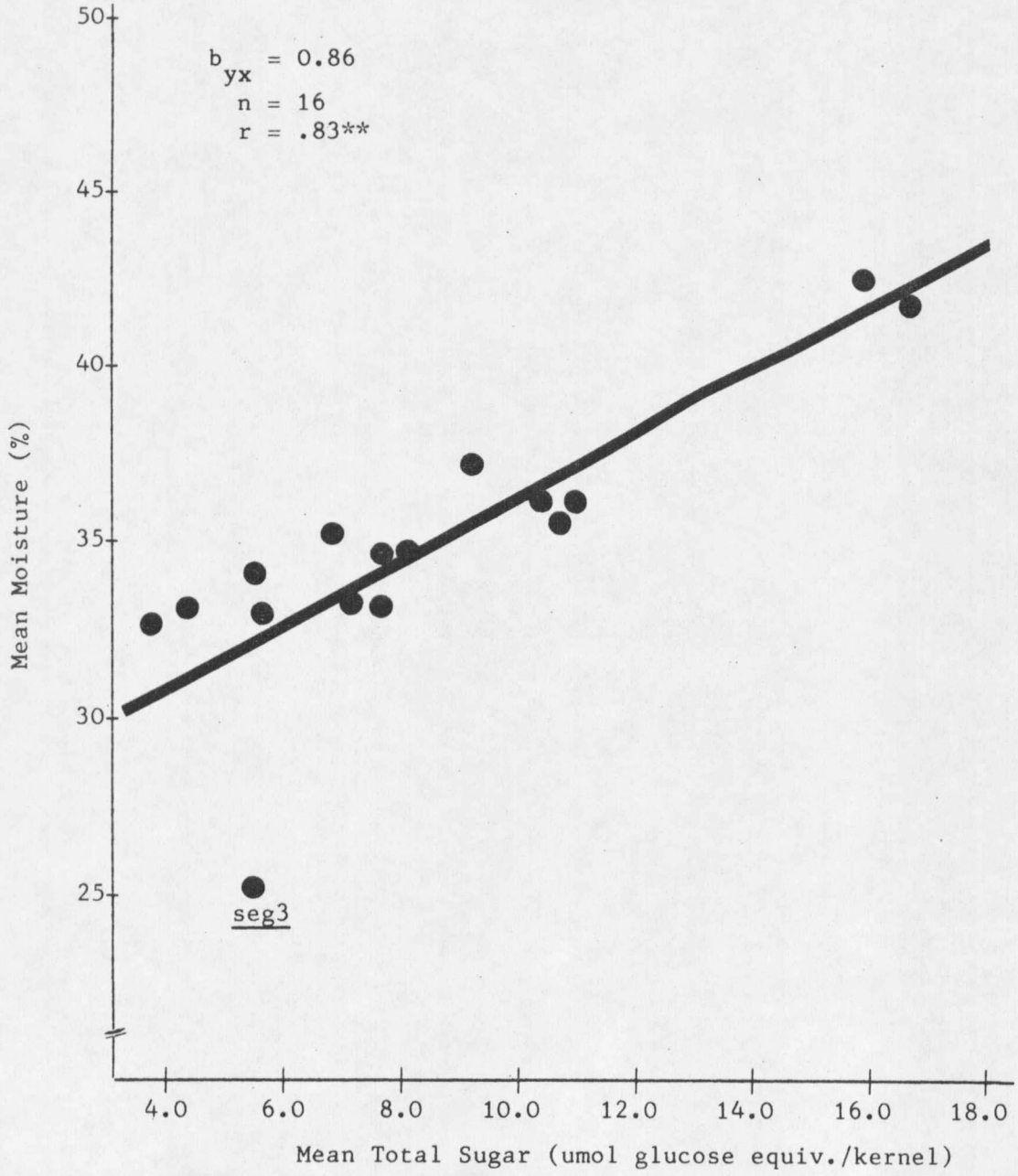

Figure 22. Relationship between mean total sugar and mean moisture percentage in 10 shrunken endosperm, high lysine mutants of barley and their normal isotypes.
**Significant at the 0.01 level.

Table 12. Reducing sugar comparisons between barley shrunken endosperm, high lysine mutants and their normal isotypes from 6 to 54 days after anthesis.

Normal Cultivar	Mutant	Number of Comparisons	Reducing Sugar (umol glucose equiv./kernel)				Difference $\bar{x}-\bar{y}$
			Mutant Isotype (x)		Normal Isotype (y)		
			mean	range	mean	range	
Seg Mutants							
Betzes	seg1	10	0.56	0.24-1.25	0.89	0.53-2.13	-0.33*
Compana	seg3	8	1.06	0.31-1.93	1.65	0.82-2.70	-0.59**
Ingrid	seg6	10	0.93	0.46-1.59	1.07	0.60-1.94	-0.14
Ingrid	seg7	10	0.99	0.43-1.73	1.07	0.60-1.94	-0.08
Sex Mutants							
Hiproly Normal	lys1	10	1.84	0.88-4.12	2.08	0.85-4.85	-0.24
Compana	sex1a	9	2.39	0.94-5.47	1.56	0.82-2.70	0.83*
Bomi	sex1f	10	1.91	1.07-4.55	1.23	0.70-2.25	0.68**
Bomi	sex3c	10	1.32	0.71-2.31	1.23	0.70-2.25	0.09
Bomi	Risø 8	10	1.30	0.77-2.49	1.23	0.70-2.25	0.07
Carlsberg II	Risø 56	10	1.14	0.59-1.68	1.03	0.56-1.89	0.11

*, **Significant at the 0.05 and 0.01 levels, respectively.

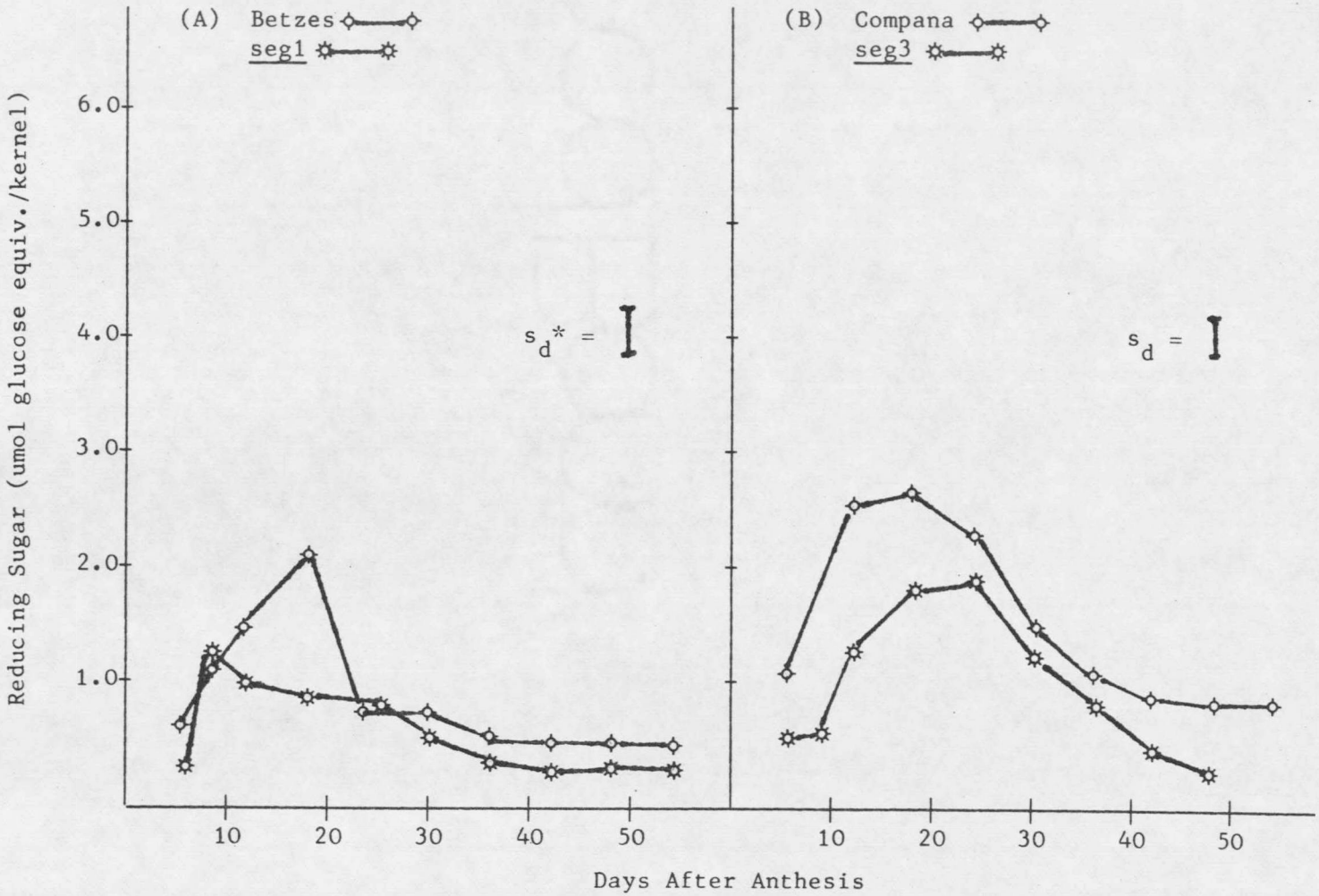

Figure 23. Reducing sugar of seed harvested from 6 to 54 days after anthesis for A) Betzes and seg1, and B) Compana and seg3.
*s_d = standard error of the difference using a paired t-test.

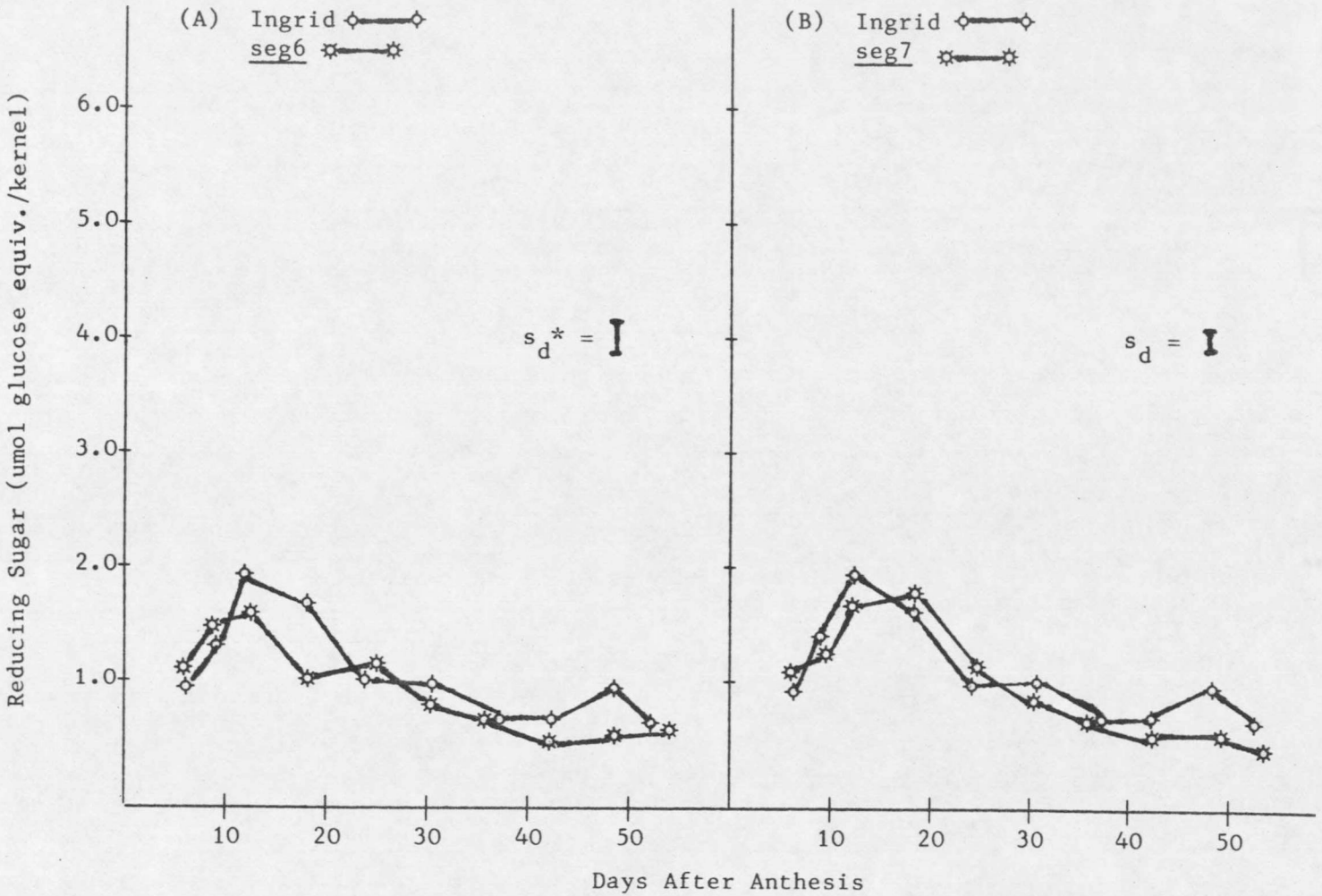

Figure 24. Reducing sugar of seed harvested from 6 to 54 days after anthesis for A) Ingrid and seg6, and B) Ingrid and seg7.
*s_d = standard error of the difference using a paired t-test.

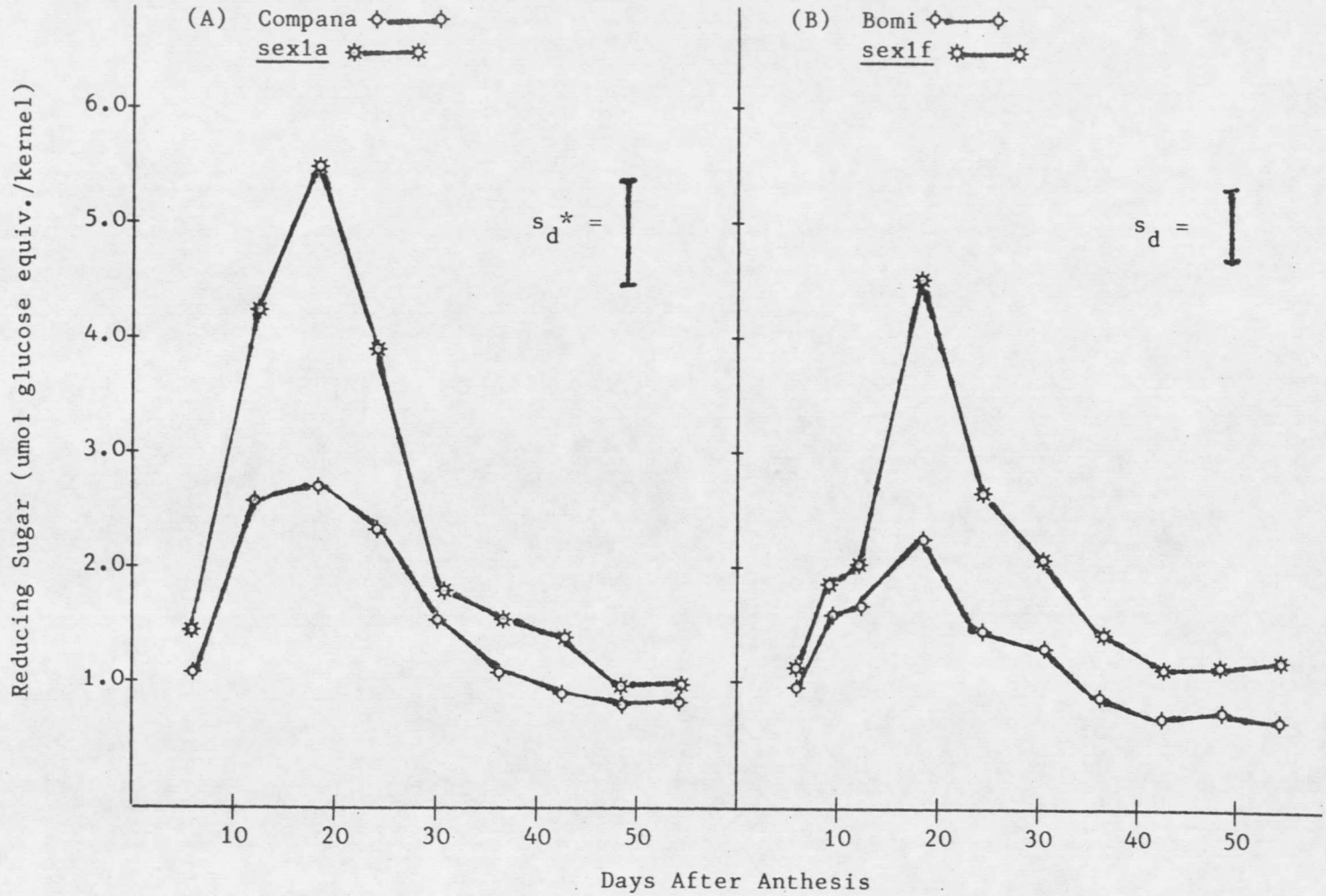

Figure 25. Reducing sugar of seed harvested from 6 to 54 days after anthesis for A) Compana and sex1a, and B) Bomi and sex1f.
*s = standard error of the difference using a paired t-test.

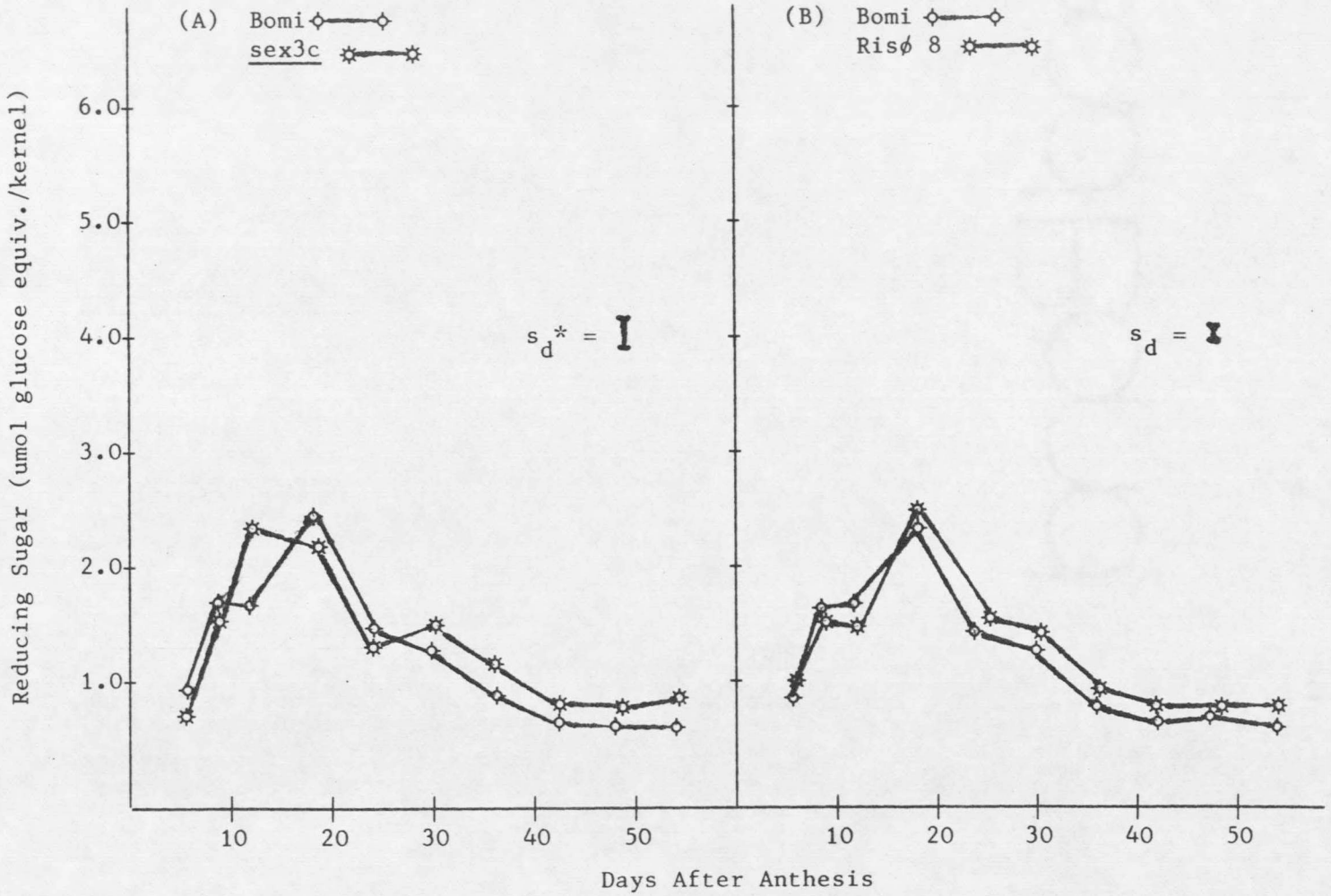

Figure 26. Reducing sugar of seed harvested from 6 to 54 days after anthesis for A) Bomi and sex3c, and B) Bomi and Risø 8.
*s_d = standard error of the difference using a paired t-test.

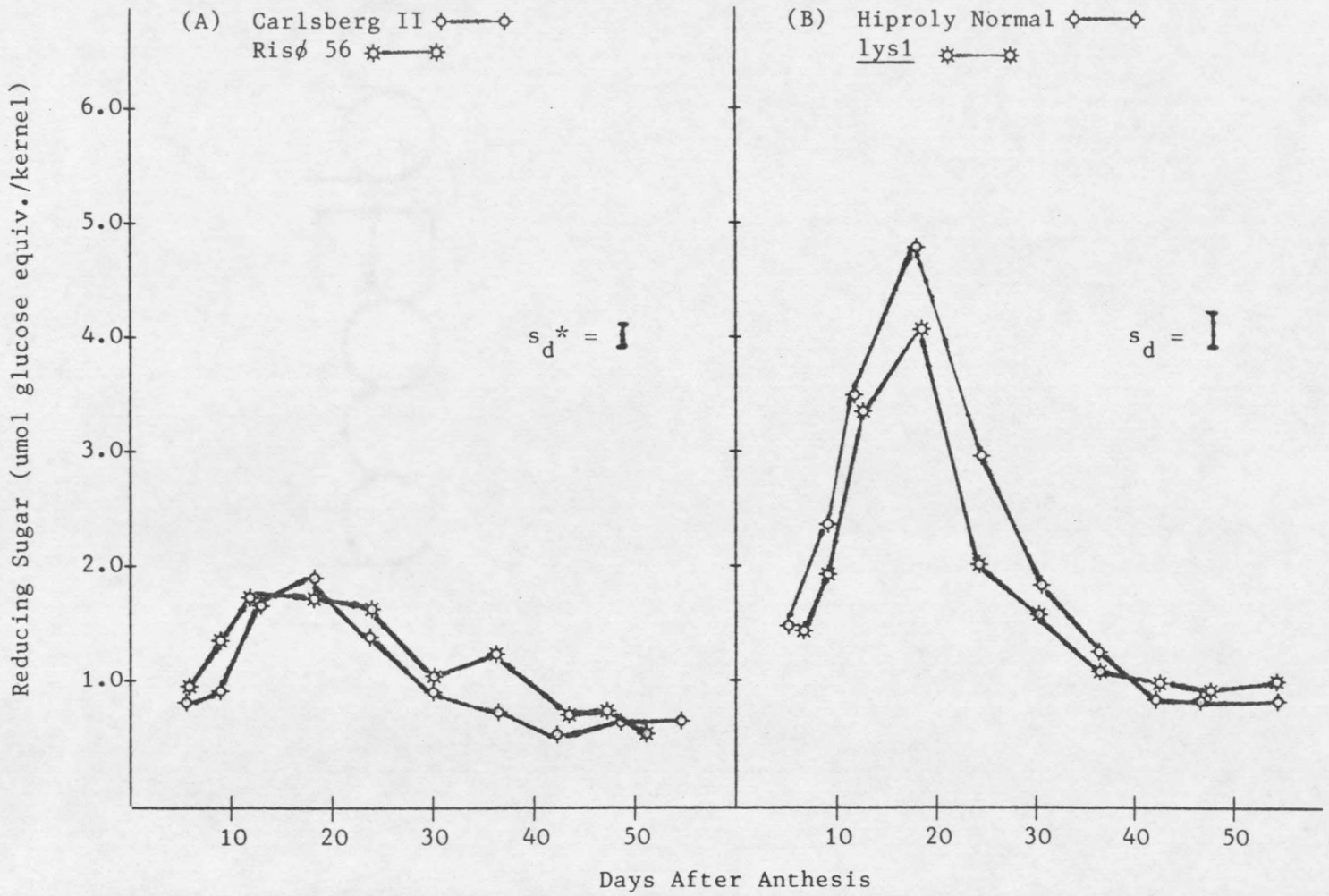

Figure 27. Reducing sugar of seed harvested from 6 to 54 days after anthesis for A) Carlsberg II and Risø 56, and B) Hiproly Normal and lys1.
*s_d = standard error of the difference using a paired t-test.

As with total sugar, it was necessary to determine whether the decreases in reducing sugar in the seg mutants were proportional to the decreases in kernel weight (Table 13). Both seg1 and seg3 had levels of reducing sugars proportional to their normal isotypes. This indicates that, as with total sugars, the levels of reducing sugar decreased in these mutants only to the extent that kernel weight decreased. As expected, seg6 had a higher reducing sugar percentage than its normal isotype, but seg7 did not. These results suggest that in both of these mutants, it is not the reducing sugar component of the total sugars which is decreased proportionate to the kernel weight decrease.

Because of their smaller seed size, it was expected that all of the sex mutants would have a higher reducing sugar percentage than their normal isotypes since they were similar to or greater than the normal in absolute sugar content (Table 12). This was the case only with sex1f and sex3c (Table 13). The lack of significantly higher reducing sugar percentage in lys1, sex1a, Risø 8, and Risø 56 cannot be explained.

Significant differences were detected among the 10 shrunken endosperm, high lysine mutants (Table 7). Approximately half of the genotype variation was explained by the difference between seg and sex mutants. The seg mutants had a significantly lower (0.88 umol glucose equiv./kernel) mean reducing sugar content than the sex mutants (1.73 umol glucose equiv./kernel). Another 28% of the genotype variation was explained by the significantly higher mean reducing sugar content of sex1 (2.29 umol glucose equiv./kernel) compared to the other sex

Table 13. Reducing sugar percentage comparisons between barley shrunken endosperm, high lysine mutants and their normal isotypes from 6 to 54 days after anthesis.

Normal Cultivar	Mutant	Number of Comparisons	Reducing Sugar (% glucose equiv.) Mutant Isotype (x) mean	Mutant Isotype (x) range	Normal Isotype (y) mean	Normal Isotype (y) range	Difference $\bar{x}-\bar{y}$
Seg Mutants							
Betzes	seg1	10	1.01	0.30-2.93	1.11	0.23-3.37	-0.10
Compana	seg3	8	1.46	0.20-4.21	1.86	0.27-8.51	-0.40
Ingrid	seg6	10	2.11	0.73-6.96	1.48	0.28-4.98	0.63**
Ingrid	seg7	10	1.56	0.28-5.97	1.48	0.28-4.98	0.08
Sex Mutants							
Hiproly Normal	lys1	10	2.04	0.40-4.92	1.95	0.29-4.92	0.09
Compana	sex1a	9	1.67	0.38-4.34	1.68	0.26-8.50	-0.01
Bomi	sex1f	10	2.22	0.54-6.64	1.47	0.28-5.13	0.75**
Bomi	sex3c	10	1.73	0.39-5.11	1.47	0.28-5.13	0.26*
Bomi	Risø 8	10	1.54	0.42-4.05	1.47	0.28-5.13	0.07
Carlsberg II	Risø 56	10	1.34	0.35-5.22	1.32	0.29-4.82	0.02

*, **Significant at the 0.05 and 0.01 levels, respectively.

mutants (1.45 umol glucose equiv./kernel). The mutant, sex1a, had a significantly higher (2.57 umol glucose equiv./kernel) mean reducing sugar content than sex1f (2.01 umol glucose equiv./kernel).

Significant differences were detected among sampling dates (Table 7). In general, reducing sugar content followed the same pattern observed for total sugar, beginning at some low point, increasing to reach a peak 9 to 24 days after anthesis, and finally decreasing to the lowest point at harvest.

Reducing sugar did not appear to be the most important component of the total sugars. They represented a maximum of approximately 50% of the total sugar content, and then, in only a couple of samples at harvest. In general, they were 6 to 20% of the total sugar content. In a few samples, high alpha-amylase activity might have been expected to result in an increase in reducing sugar content. An example would be the elevated alpha-amylase activity in sex3c at harvest. However, reducing sugar content did not appear abnormally high as a result of alpha-amylase activity in this nor any other sample.

Germination

A germination test was performed on all isotypes to be certain that the samples being studied throughout this research were developing into healthy, viable seed. The mean germination of all isotypes was 97.4%, with a range from 87.7% to 99.7%. This indicates that all samples had a high percentage of viable seed.

Significant differences were detected among isotypes (Table 14). No differences were detected among germination replications. The mean

Table 14. Analysis of variance in angles for germination percentage of 10 shrunken endosperm, high lysine mutants of barley and their normal isotypes.

Source of Variance	DF	Sum of Squares	Mean Square	F
Isotype	15	1231.82	82.12	6.52**
Replication	2	1.63	0.82	0.07
Error	30	377.87	12.60	

**Significant at the 0.01 level.

germination percentage of each isotype can be seen in Figure 28. Only three of the mutants differed significantly from their normal isotype. A lower germination percentage was observed in sex3c compared to Bomi. This difference may be related to the enlarged embryo observed in sex3c at harvest (Tallberg, 1977).

Compana isotypes had the lowest germination percentage. Both mutants in Compana, sex1a and seg3, had significantly higher germination than Compana. The reduced germination of Compana was probably the result of post-harvest dormancy not being completely broken even though the test was performed five months after harvest. The mutants sex1a and seg3 may provide the means of understanding the dormancy in this cultivar.

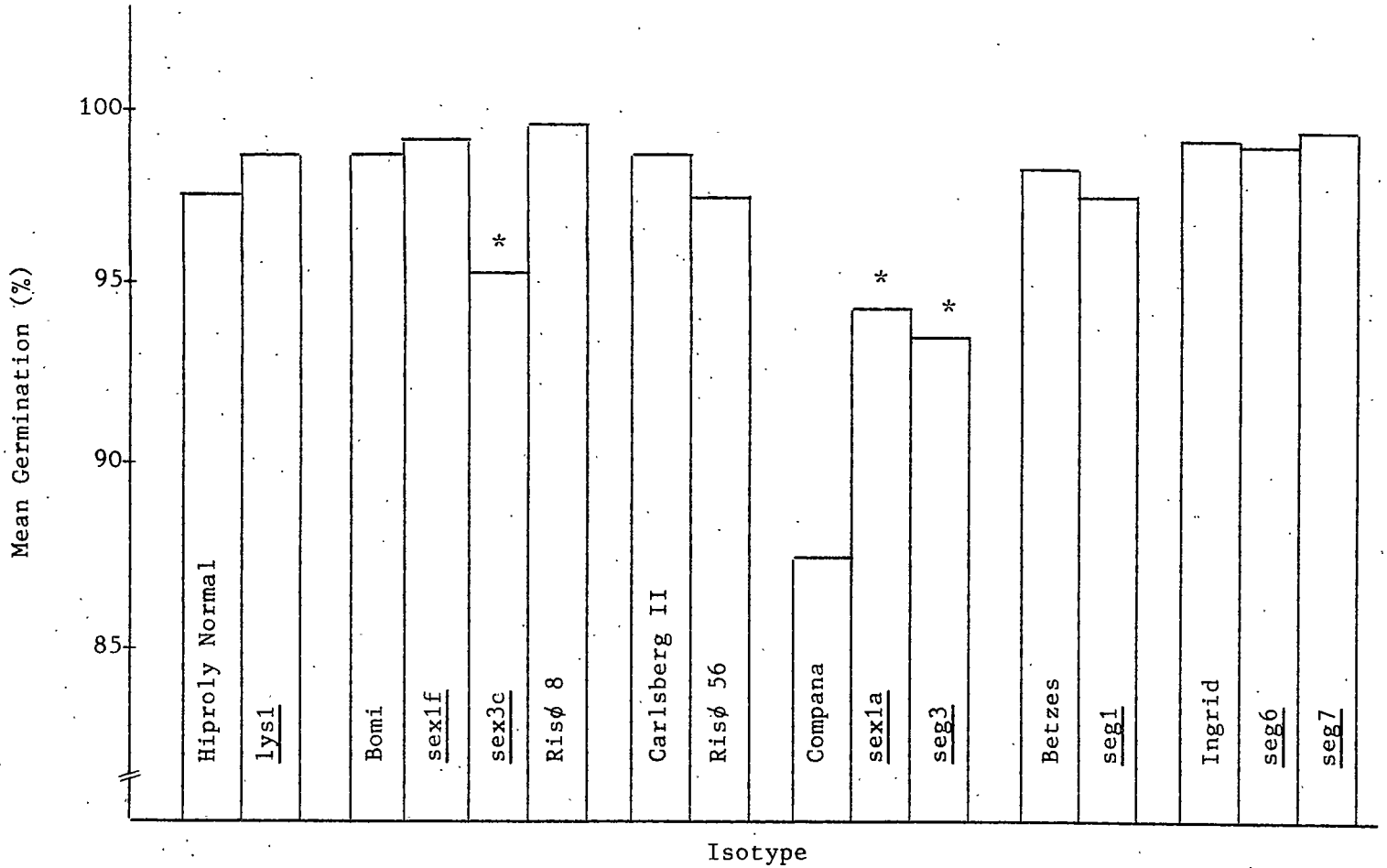

Figure 28. Mean germination percentage of 10 shrunken endosperm, high lysine mutants of barley and their normal isotypes.
*Significantly different from the normal isotype at the 0.05 level.

Experiment II

Total Sugar Content Relationship Between Developing and Mature Seed

In Experiment I, the sex and seg shrunken endosperm, high lysine mutants had higher and lower, respectively, total sugar/kernel than their normal isotypes when averaged over 10 sampling dates from anthesis to harvest. However, these differences were detected in only a single environment. It would be useful to know if these differences exist in other environments, but the design of Experiment I would make such an endeavor extremely time consuming. The purpose of this study was to determine if total sugar in harvest ripe seed could be used as an indication of total sugar throughout the development of the seed.

Significant differences in total sugar/kernel were detected among the three isotypes (seg, sex, normal) on seven of the ten sampling dates, 18, 24, 30, 36, 42, 48, and 54 days after anthesis (Table 15). The two comparisons shown in the analysis of variance table (Table 15) and the means in Table 16 were used to determine which differences among isotypes were significant. On all sampling dates, the seg isotype had the lowest total sugar/kernel, the sex isotype had the highest, and the normal isotype was between the two mutant isotypes. Thus, when a significant difference was detected between the seg and normal isotypes or between the sex and normal isotypes, it was possible to infer that a significant difference existed between the seg and sex isotypes. A significant difference in total sugar/kernel was detected between the seg and normal isotypes only on day 48. A significant difference in total sugar/kernel was detected between the sex

Table 15. Analyses of variance for total sugar (umol glucose equiv./kernel) of 16 genotypes classified as either sex, seg, or normal isotypes on 10 dates after anthesis.

Source of Variance	Days After Anthesis									
	Day 6		Day 9		Day 12		Day 18		Day 24	
	df	MS	df	MS	df	MS	df	MS	df	MS
Isotype	2	10.58	2	0.88	2	12.12	2	115.72*	2	103.89**
seg vs. normal	1	10.74	1	0.99	1	12.47	1	12.31	1	23.86
normal vs. sex	1	2.02	1	0.09	1	2.22	1	136.22*	1	98.90*
Error	13	6.65	11	9.80	13	10.25	13	28.07	13	14.77

Source of Variance	Days After Anthesis									
	Day 30		Day 36		Day 42		Day 48		Day 54	
	df	MS	df	MS	df	MS	df	MS	df	MS
Isotype	2	90.50**	2	89.33**	2	63.71**	2	59.74**	2	76.81**
seg vs. normal	1	30.45	1	25.55	1	33.23	1	34.81*	1	31.79
normal vs. sex	1	72.37*	1	77.57*	1	37.24	1	31.27	1	60.44*
Error	13	9.14	13	11.36	13	8.10	13	6.80	12	9.28

*,**Significant at the 0.05 and 0.01 levels, respectively.

Table 16. Mean total sugar of seg, sex, and normal isotypes on 10 dates after anthesis.

Days After Anthesis	Isotype Mean Total Sugar (umol glucose equiv./kernel)		
	seg	normal	sex
6	5.41	7.52	8.34
9	10.45	11.14	11.30
12	10.81	13.09	13.95
18	7.53	9.80	16.53*
24	5.11	8.26	14.01*
30	3.36	6.92	11.83*
36	2.46	5.72	10.81*
42	1.94	5.66	9.19
48	2.12*	5.92	9.15
54	1.47	5.46	9.95*

*Significantly different from the normal isotype at the 0.05 level.

and normal isotypes on days 18, 24, 30, 36, 48, and 54. On day 42 neither of the two comparisons were significant, but since a significant isotype difference was observed, it must be assumed that the difference was between the sex and seg isotypes.

Simple correlation coefficients for total sugar of the 16 genotypes among the 10 sampling dates are shown in Table 17. The total sugar of the 16 genotypes on days 12, 18, 24, 30, 36, 42, 48, and 54, were significantly correlated. This indicates that the relative ranking of the genotypes for total sugar content was consistent from 12 days after anthesis to harvest.

These data indicate that the mean difference in total sugar between seg and sex isotypes which was shown in Experiment I, was ob-

Table 17. Simple correlation coefficients among sampling dates for total sugar measured in 16 genotypes.

Days After Anthesis	Days After Anthesis									
	6	9	12	18	24	30	36	42	48	54
6	–	0.22 (14)†	0.11	0.11	0.06	0.06	0.06	0.12	0.09	-0.002
9		–	0.63* (14)	0.55* (14)	0.45 (14)	0.40 (14)	0.31 (14)	0.36 (14)	0.32 (14)	0.29 (13)
12			–	0.78**	0.71**	0.60*	0.57*	0.70**	0.62*	0.60* (15)
18				–	0.96**	0.91**	0.91**	0.94**	0.89**	0.91** (15)
24					–	0.98**	0.95**	0.96**	0.94**	0.96** (15)
30						–	0.97**	0.95**	0.96**	0.97** (15)
36							–	0.97**	0.98**	0.99** (15)
42								–	0.98**	0.99** (15)
48									–	0.98** (15)
54										–

*, **Significant at the 0.05 and 0.01 levels, respectively.
†All 16 genotypes were not available for all sampling dates. N is indicated in parentheses when less than 16.

served at all but the first three sampling dates. This includes the last sampling date which would be considered mature or harvest ripe seed. The differences between the seg and normal isotypes and between the sex and normal isotypes which were observed in Experiment I, were not observed on all of the sampling dates, but in many cases, this could have been due to the small number of experimental units. The data suggest that the differences in total sugar/kernel observed between mutant and normal isotypes at harvest could be used as an indication of differences throughout seed development starting at day 12. Figure 29 shows that the mutant-normal difference in total sugar/kernel at harvest is an excellent indicator of the mean mutant-normal difference averaged over the development of the seed (r=.98**).

Total Sugar in Mature Seed
Grown in 7 to 13 Environments

Significant differences in total sugar/kernel were detected between all the shrunken endosperm, high lysine mutants and their normal isotypes except lys1, sex3c, and Risø 56, when grown in 7 to 12 environments (Table 18). The seg mutants, seg1, seg3, seg6, and seg7, had significantly less total sugar/kernel than their normal isotypes, and the sex mutants, sex1a, sex1d, sex1f, and Risø 8, had significantly more.

All of the sex mutants had a significantly higher total sugar percentage than their normal isotypes (Table 19). This was expected since they were all similar to or greater than their normal isotypes in total sugar/kernel.

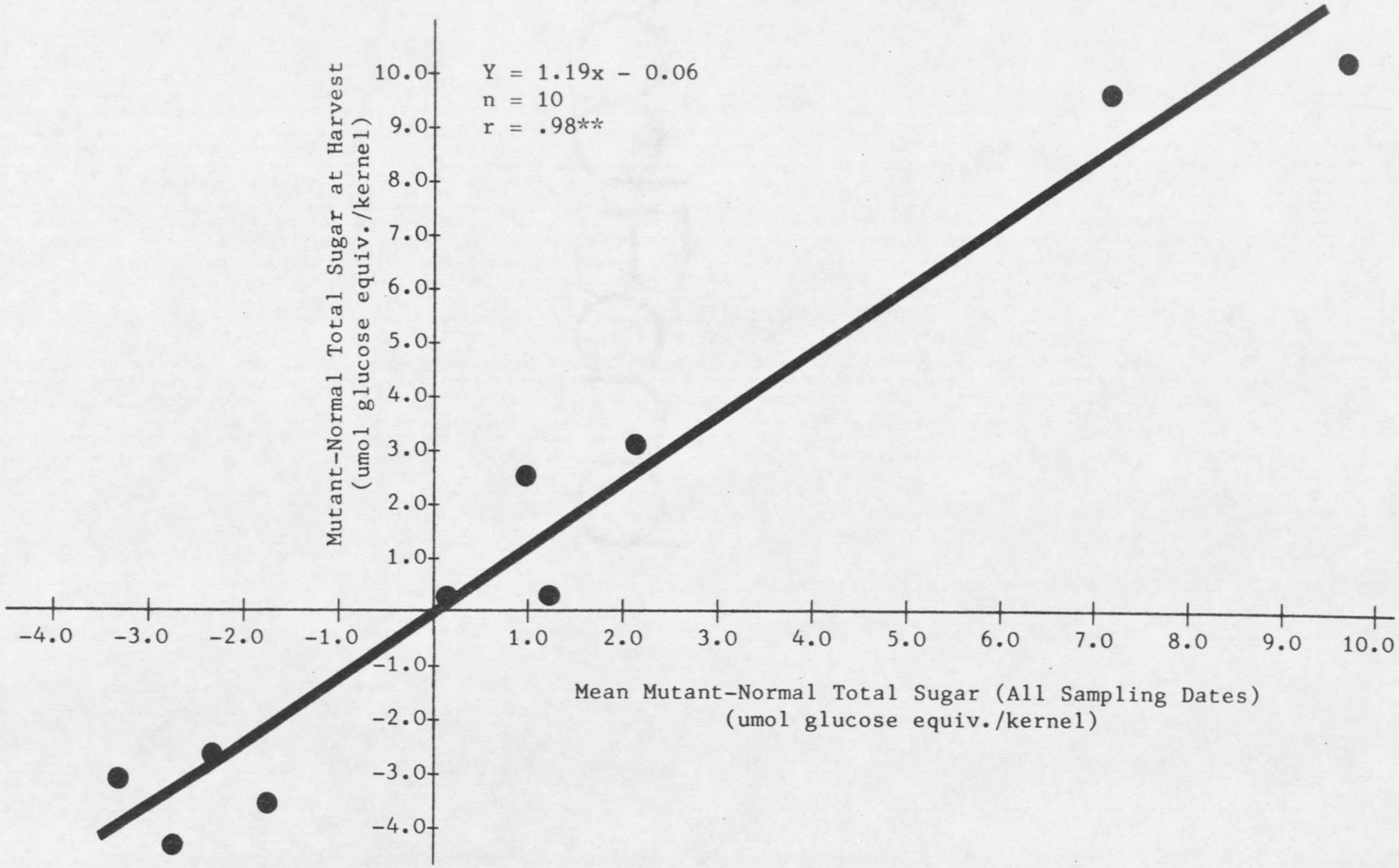

Figure 29. Relationship between the mean mutant-normal difference in total sugar and the mutant-normal difference in total sugar at harvest.

Table 18. Total sugar comparisons in ripe barley seed of 11 shrunken endosperm, high lysine mutants and their normal isotypes grown in 7 to 12 environments.

Normal Cultivar	Mutant	Comparisons	Total Sugar (umol glucose equiv./kernel)				
			Mutant Isotype (x)		Normal Isotype (y)		Difference $\bar{x}-\bar{y}$
			mean	range	mean	range	
Seg Mutants							
Betzes	seg1	9	2.00	0.92-4.29	3.28	1.47-5.13	-1.28**
Compana	seg3	8	1.98	1.54-3.02	3.37	1.86-4.77	-1.39**
Ingrid	seg6	8	1.72	1.32-2.09	3.33	1.45-5.26	-1.61**
Ingrid	seg7	8	2.01	1.15-2.66	3.18	1.45-5.26	-1.17**
Sex Mutants							
Hiproly Normal	lys1	12	3.93	2.06-5.73	4.04	2.02-5.73	-0.11
Compana	sex1a	7	7.35	2.32-11.28	3.17	1.86-4.01	4.18**
Carlsberg II	sex1d	9	6.52	4.17-9.22	3.81	2.71-4.91	2.71**
Bomi	sex1f	8	7.56	5.29-13.93	3.87	2.51-5.52	3.69**
Bomi	sex3c	10	3.70	2.51-7.30	3.72	2.51-5.52	-0.02
Bomi	Risø 8	8	4.45	2.33-6.51	3.87	2.51-5.52	0.58*
Carlsberg II	Risø 56	9	3.83	2.24-5.00	3.81	2.71-4.91	0.02

*, **Significant at the 0.05 and 0.01 levels, respectively.

Table 19. Total sugar percentage comparisons in ripe barley seed of 11 shrunken endosperm, high lysine mutants and their normal isotypes grown in 7 to 12 environments.

Normal Cultivar	Mutant	Number of Comparisons	Total Sugar (% glucose equiv.)				Difference $\bar{x}-\bar{y}$
			Mutant Isotype (x)		Normal Isotype (y)		
			mean	range	mean	range	
Seg Mutants							
Betzes	seg1	9	1.65	0.62-3.84	1.45	0.72-2.26	0.20
Compana	seg3	8	1.18	0.92-1.66	1.14	0.67-1.66	0.04
Ingrid	seg6	8	2.36	1.61-3.40	1.52	0.72-2.36	0.84**
Ingrid	seg7	8	1.33	1.06-1.96	1.43	0.72-2.36	-0.10
Sex Mutants							
Hiproly Normal	lys1	12	1.81	0.96-2.65	1.40	0.77-1.96	0.41**
Compana	sex1a	7	2.96	1.06-4.51	1.06	0.67-1.36	1.90**
Carlsberg II	sex1d	9	3.67	1.96-4.91	1.67	1.21-2.16	2.00**
Bomi	sex1f	8	3.52	2.70-6.40	1.39	0.96-1.81	2.13**
Bomi	sex3c	10	1.81	1.26-3.20	1.36	0.96-1.81	0.45**
Bomi	Risø 8	8	2.31	1.26-2.95	1.39	0.96-1.81	0.92**
Carlsberg II	Risø 56	9	1.91	1.16-2.36	1.67	1.21-2.16	0.24**

*, **Significant at the 0.05 and 0.01 levels, respectively.

Three of the seg mutants, seg1, seg3, and seg7, were similar to their normal isotypes in total sugar percentage indicating that the decrease in total sugar/kernel was proportional to the decrease in kernel weight. The fourth seg mutant, seg6, had a significantly higher total sugar percentage than Ingrid indicating that the decrease in total sugar/kernel was less, proportionately, than the decrease in kernel weight.

Highly significant differences were observed among the 17 genotypes (mutants and normals) (Table 20). Most of the variation among genotypes (76%) was due to the significant difference between the mean of the sex1 mutants (sex1a, sex1d, and sex1f) (7.25 umol glucose equiv./kernel) and the mean of the other mutants (sex and seg) (2.95 umol glucose equiv./kernel). Among the remaining mutants, the four sex mutants, lys1, sex3c, Risø 8, and Risø 56, had a significantly higher mean total sugar/kernel (3.97 umol glucose equiv./kernel) than the seg mutants (1.93 umol glucose equiv./kernel). These results are consistent with the data reported in Experiment I (Table 7).

The availability of sex1 in three different genotypes allowed conclusions to be made concerning the importance of background genotype. No significant difference in total sugar/kernel was detected between sex1a in Compana (7.35 umol glucose equiv./kernel) and sex1f in Bomi (7.88 umol glucose equiv./kernel). This is consistent with the results shown in Experiment I (Table 7). However, the mean of sex1f and sex1a (7.61 umol glucose equiv./kernel) was significantly higher than sex1d in Carlsberg II (6.52 umol glucose equiv./kernel). This difference is probably related to the smaller seed size of Carls-

Table 20. Analysis of variance for total sugar (umol glucose equiv./kernel) content of 11 barley shrunken endosperm, high lysine mutants and their normal isotypes grown in 7 to 13 environments.

Source of Variance	DF	Sum of Squares	Mean Square	F
Genotype	16	431.60	26.98	15.07**
sex1 vs. other mutants	1	329.76	329.76	184.22**
other sex vs. seg	1	73.37	73.37	40.99**
sex1d vs. sex1a & sex1f	1	7.29	7.29	4.07*
sex1a vs. sex1f	1	1.12	1.12	0.63
remaining comparisons	12	20.06	1.67	0.93
Error	141	252.95	1.79	

*, **Significant at the 0.05 and 0.01 levels, respectively.

berg II. This is supported by the observation that sex1d had a higher mean total sugar percentage (3.67% glucose equiv.) than either sex1a (2.96% glucose equiv.) or sex1f (3.52% glucose equiv.).

The differences in total sugar/kernel found in ripe seed grown in many environments of mutant and normal isotypes (Table 18) were, in general, consistent with the mean differences observed when averaged over seed development (Table 10). In both cases, the seg mutants had significantly less total sugar/kernel than their normal isotypes and, sex1a and sex1f had significantly more. Two inconsistencies were observed. The mutant sex3c had a significantly higher total sugar/kernel than Bomi when averaged over seed development, but not in mature seed grown in 10 environments. The opposite occurred with Risø 8. It had a significantly higher total sugar/kernel than Bomi when measured in mature seed grown in eight environments, but not when averaged over

seed development. The cause of these inconsistencies is not clear and further research would be necessary to understand them.

Mutant sex1f was the only mutant which had a significantly higher total sugar percentage than its normal isotype when averaged over seed development (Table 11). In contrast, all of the sex mutants and one seg mutant, seg6, had a significantly higher total sugar percentage than their normals when measured in mature seed over a number of environments (Table 19). This supports the suggestion that a strong isotype x sampling date interaction caused the lack of significance in the former measurements. This type of interaction would not be found in the latter type of data. Thus, the results here confirm what could not be shown statistically in Experiment I.

Mutants seg1 and seg3 had a total sugar percentage similar to their normal isotypes. This confirmed the results from Experiment I. The lower than normal total sugar percentage in seg7 reported in Experiment I was not confirmed by the results in Table 19. As with sex3c and Risø 8, the reason for the inconsistency is not clear.

General Discussion

Differences between seg and sex mutants have been noted previously. Table 1 shows that the seg mutants have a thin seed phenotype while sex mutants have a dorsal depression resulting in the shrunken seed phenotype. From field observations, it was noted that the thin seed of an seg mutant was observed throughout development, while the seed of an sex mutant was plump until drydown at which time the dorsal depression appeared. Ullrich and Eslick (1978c and 1978e) reported

that upon adjusting the lysine in the grain percentage of the mutant for a normal kernel weight (Table 5), the seg mutants had significantly less lysine than the normal and sex mutants were either equal to or significantly higher than the normal in lysine content in the grain.

The data presented from this research indicate other differences between seg and sex mutants, and suggest that not only are these genetic classes of mutants, but physiological classes as well. They also indicate that there is variability within a class of mutants, so that, while distinct differences between seg and sex mutants exist, not all seg or sex mutants behave identically.

The seg mutants had a significantly lower mean moisture percentage, kernel weight, total sugar, and reducing sugar, and a significantly higher mean alpha-amylase activity than the sex mutants. All seg mutants had significantly less total sugar than their normal isotypes and all sex mutants were equal to or significantly higher in total sugar than their normal isotypes. A higher concentration of sucrose (nonreducing sugar) has been reported (Kreis and Doll, 1980) for some of the sex mutants and it was suggested that these higher levels indicate a block in the pathway from sucrose to starch. Burr (1979) and Mehta et al. (1979) presented hypotheses linking starch and protein synthesis through an intermediate or end-product of one pathway being required by the other. Although not proven, it is probably not a coincidence that the sex mutants with higher sugar contents also had higher adjusted lysine in the grain percentage than normal, and the seg mutants with lower sugar contents had lower adjusted lysine in

the grain percentage than normal (Ullrich and Eslick, 1978c and 1978e). If protein and starch synthesis are closely related, then excesses of the components of both would be expected if a block occurred in one or both pathways (as with the sex mutants).

Although the seg mutants had a significantly lower total sugar on a kernel basis than their normal isotypes, they were similar to or greater than their normal isotypes in sugar percentage. This indicated that the decrease in total sugar in these mutants was proportionate to the decrease in kernel weight observed in them and suggests that a single mechanism could result in both of these decreases and the lower adjusted lysine in the grain percentage as reported by Ullrich and Eslick (1978c). Decreases in components of both starch and protein possibly indicate the inability of the plant to translocate them to the developing seed.

A difference was observed between seg and sex mutants in the pattern of dry matter accumulation. In the sex mutants, dry matter accumulation appeared to be slower than in their normal isotypes, but it proceeded for the same length of time (30 to 36 days after anthesis). In the seg mutants, the rate was similar to or slower than in the normal isotypes and it terminated earlier than in the normal isotypes (12 to 24 days after anthesis). Regardless of the cause of early termination of dry matter accumulation, this does explain the significantly lower mean kernel weight observed in the seg mutants compared to the sex mutants.

No significant differences in alpha-amylase activity were detected between mutant and normal isotypes by the methods used. Al-

though large differences were observed between some mutant-normal isotypes, their lack of significance was probably due to the design of the experiment, i.e., the lack of sampling date replications making it impossible to separate the sampling date x isotype interaction from the error term used in the paired t-test. Evidently the interaction was too large to detect differences between mutants and normals.

No relationship was detected between shrunken seed and high alpha-amylase activity in mature seed in this material. A significant relationship between the two was observed in triticale (Klassen et al., 1971 and Srivastava, 1978), and it was postulated that a similar relationship might be observed in barley, especially since a higher level of alpha-amylase activity in mature seed of sex3c was observed prior to these investigations (unpublished data) and higher than normal levels of gibberellin-like activity were reported for lys1 and sex3c (Hagberg et al., 1979 and Mounla et al., 1980). Very little alpha-amylase activity was observed in mature seed of any mutant or normal isotype with the exception of sex3c, and even this did not result in an increase in reducing sugar as expected. Since a similar increase was not observed in lys1, it may indicate that the high gibberellin-like activity reported for both mutants was not related to the increased alpha-amylase activity in sex3c at harvest. In fact, the high gibberellin-like activity appeared to have no effect on alpha-amylase activity in either of these mutants anytime during seed development. Naturally, it is possible that it is environmentally influenced, thus, not found in these particular samples.

Very high levels of alpha-amylase activity were observed in two of the seg mutants, seg1 and seg7, at six days after anthesis. MacGregor et al. (1972) have suggested that alpha-amylase is present in the seed at this time to hydrolyze pericarp starch to provide energy for the growing kernel. It is possible in the seg mutants that such high levels of alpha-amylase activity are required to supplement the decreased levels of sucrose being translocated to the developing seed. This is supported by the data from seg7. This mutant had the highest alpha-amylase activity at six days after anthesis (Table 10) and had a significantly smaller proportion of total sugar/kernel than its normal isotype in that one environment (Table 14).

SUMMARY AND CONCLUSIONS

Ten shrunken endosperm, high lysine mutants of barley, classified as *seg* and *sex* mutants, were compared to their normal isotypes and to each other for moisture percentage, dry matter accumulation (kernel weight), alpha-amylase activity, total sugar, and reducing sugar at regular intervals during seed development. All mutants had a significantly lower mean kernel weight (averaged over 8 to 10 sampling dates) than their normal isotypes, but a germination test of mature seed of all isotypes indicated that all mutants had a high percentage of viable seed (93.7% to 99.7% germination).

Significant differences in alpha-amylase activity were not detected between any of the mutants and their normal isotypes because of a strong isotype x sampling date interaction. A relationship between shrunken endosperm and alpha-amylase activity was not detected at any time during seed development, but a positive correlation was observed among the six normal isotypes between alpha-amylase activity at six days after anthesis and kernel weight at harvest (r=.89*). It would be interesting to know if this relationship is present among other genotypes and environments.

The *seg* mutants, *seg1*, *seg3*, *seg6*, and *seg7*, had significantly lower mean total sugar than their normal isotypes, and the *sex* mutants, *sex1a*, *sex1f*, *sex3c*, *lys1*, Risø 8, and Risø 56, were equal to or significantly higher than their normal isotypes in total sugar. The *seg*

mutants were equal to or significantly lower in reducing sugar than their normal isotypes and the sex mutants were equal to or significantly higher than their normal isotypes in reducing sugar. The decrease in total and reducing sugar in the seg mutants compared to their normal isotypes was proportional to the decrease in kernel weight with one exception. The mutants seg7 had a larger decrease proportionately, in total sugar than in kernel weight.

One of the seg mutants, seg3, had a significantly lower mean moisture percentage than its normal isotype, and three of the sex mutants, sex1a, sex1f, and sex3c, had a significantly higher mean moisture percentage than their normal isotypes. A significant positive correlation (r=.83**) was observed among all genotypes between mean total sugar/kernel and mean moisture percentage, suggesting that the higher than normal moisture percentage in sex1a, sex1f, and sex3c was a result of the high total sugar content. This would also explain the ability of an sex type seed to appear as plump as a normal seed until drydown.

Significant differences were detected between seg and sex mutants for all traits measured. The seg mutants had a significantly lower mean moisture percentage, kernel weight, total sugar, and reducing sugar, and a significantly higher mean alpha-amylase activity than the sex mutants. The pattern of dry matter accumulation in the sex mutants was similar to their normal isotypes, but at a slower rate. Dry matter accumulation stopped in the seg mutants 12 to 24 days after anthesis compared to 30 to 36 days after anthesis in the normal iso-

types. This would explain the lower kernel weights of the seg mutants compared to the sex mutants.

Differences in total sugar were detected among the three isotypes, seg, sex, and normal, on all sampling dates from 12 days after anthesis to harvest. Significant positive correlations for total sugar of mutants and normals among sampling dates indicated that the relative ranking of genotypes for total sugar content was consistent throughout most of seed development. The mutant-normal difference in total sugar/kernel at harvest was significantly correlated to the mean mutant-normal difference averaged over seed development (r=.98**), indicating that samples of harvest ripe barley seed could be used to measure the total sugar response of the shrunken endosperm, high lysine mutants and their normal isotypes in many environments.

Eleven shrunken endosperm, high lysine mutants (the ten previously mentioned and sex1d) were compared to their normal isotypes and each other for total sugar content of harvest ripe seed grown in 7 to 13 environments in Montana and Arizona. The results confirmed that the seg mutants had a significantly lower mean total sugar content than normal, and the sex mutants, including sex1d, were similar to or significantly higher than normal.

Three of the sex mutants, sex1a, sex1d, and sex1f, are allelic. Mutants sex1a and sex1f were similar in all traits except mean kernel weight and reducing sugar content. Mutant sex1a had a significantly higher mean kernel weight and reducing sugar content than sex1f. This provides evidence of the importance of background genotype for this mutant since sex1a is in Compana, a large seeded genotype (53.9 mg/

kernel at harvest), and sex1f is in Bomi, a medium sized seed genotype (44.8 mg/kernel at harvest). The mean of these two mutants was significantly higher than the mean of the other sex mutants for all traits except alpha-amylase activity. This indicates that while all sex mutants had certain similarities, they are by no means the same. These two mutants also had a significantly higher mean total sugar than sex1d. This difference was probably a function of the smaller seed of Carlsberg II, the background genotype of sex1d.

The differences between these mutants indicate that not only are seg and sex genetic classes, but they appear to be physiological classes as well. The most consistent difference, in total sugar, prompted suggestions concerning the mechanisms resulting in higher than normal (sex mutants) or lower than normal (seg mutants) total sugar content. The consistently excessive amounts of total sugar in sex1a, sex1d, and sex1f, indicate a possible block in the pathway from sucrose to starch. The high lysine content of these mutants would be a result of the two pathways, starch and protein synthesis, being linked by an intermediate or end-product of one pathway being required by the other. The result would probably be the same if the block were in the pathway for protein synthesis.

The decreased levels of total sugar in the seg mutants suggests an inability of the plant to translocate sucrose to the developing seed. The high lysine content of these mutants would be the result of starch dilution, not a real increase on a per kernel basis.

The higher mean alpha-amylase activity of the seg mutants compared to the sex mutants was due to high levels of activity in the

seed at six days after anthesis. Very little activity was observed in any of the mutants after that time. It was suggested that this higher activity may be the result of the lower total sugar content, i.e., a feedback mechanism. If less sucrose was translocated to the developing seed, higher levels of alpha-amylase activity would result in a faster degradation of pericarp starch to sucrose for use in the endosperm.

The data presented here suggest that sex mutants will probably prove to be more useful than seg mutants to the plant breeder attempting to produce plump seeded, high lysine cultivars. If translocation of all nutrients is a problem with the seg mutants, it is highly unlikely that seed size could be increased without simultaneously decreasing the lysine content. In the sex mutants, it is possible that alternate pathways of starch synthesis could be found which would result in a plump seed with high lysine content.

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