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Physiological and genetic studies on early vigour of *Triticum aestivum* L. and *Triticum spelta* L.

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To my parents.

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List of abbreviations

a	the additive component of the QTL B allele effect
AA	the homozygote wheat allele
AB	the heterozygote of wheat and spelt crosses
BB	the homozygote spelt allele
bp	base pairs
cDNA	complementary DNA
cM	centi Morgan
d	the dominance effect of the heterozygote AB genotypes as compared to the midparent value
DNA	deoxyribonucleic acid
FAP	Swiss Federal Research Station for Agronomy Zürich-Reckenholz
F_x	the x^{th} filial generation
GA_3	gibberellic acid
gDNA	genomic DNA
GR 1	growth rate of the coleoptile from germination to emergence of the primary leaf
GR 2	growth rate of the primary leaf from emergence to a length of 12 to 15 cm
LOD	log-odds, the probability of existence of a QTL versus non-existence
PCR	polymerase chain reaction
PSR	characterises probes from the John Innes Institute of Plant Science Research, Norwich, UK
Q/q	spelt factor
QTL	quantitative trait loci
RFLP	restriction fragment length polymorphism
Rht	reduced height
RNA	ribonucleic acid
SGI	seedling growth index
SRS	stress resistance syndrome
X...	characterises a genetic locus

1. SUMMARY

In rainy and cold regions of central and northern Europe, *Triticum spelta* L. has been the traditional cereal crop for centuries. In the hilly regions of alemanic Switzerland, spelt is still grown for bread, but its agronomic quality has remained rather low. The aim of ongoing breeding programmes is to reduce lodging while maintaining good product quality and adaptation to marginal regions. Little is known about the physiological basis of adaptation to harsh conditions. This study focussed on early vigour, which differs for wheat (*T. aestivum* L.) and spelt (*T. spelta* L.), and on the main morphological differences between the two species, these being the spelt factor (*Q/q*) and the *Tenacious glume (Tg)* gene.

Two sets of cultivars of wheat (Arina, Bernina, Forno) and spelt (Altgold, Oberkulmer, Rouquin) were described morphologically and submitted to laboratory tests to determine the coleoptile growth rate under normoxia and hypoxia. The cultivars were characterised using RFLP. A cross of Arina x Oberkulmer was used to construct genetic maps and to map possible quantitative trait loci (QTL) for the coleoptile growth rate and the length of the ear internodes. Colouration of the pericarp and the culm by anthocyanin proved to be typical of spelt cultivars, but no linkage to the examined morphological and physiological traits was found.

The length of the ear internodes was identified as being a main morphological effect of *Q/q* on the ear which is the principal organ used to differentiate wheat and spelt.

The coleoptile growth rate of wheat cultivars was 77% of that of spelt cultivars on wet filterpaper in the dark at 22°C. Reciprocal F_1 crosses of wheat and spelt revealed a limited maternal effect on the coleoptile growth rate. The growth rate of the emerged primary leaf of wheat and spelt was not significantly different.

A laboratory test imitating germination and early growth in wet soils was developed by using a gas flow mixture of 1% O_2/99% N_2 in a closed system at 22°C in the dark. Spelt cultivars showed a markedly higher tolerance to the reduced oxygen supply (hypoxia) by developing 17% of their usual biomass as compared to normoxia, while wheat cultivars developed 10% of their usual biomass. Thus, modern wheat and old spelt cultivars were clearly different as far as hypoxia tolerance was concerned.

The RFLP screening of all the genomes of the two sets of cultivars showed that

24% of the loci were monomorphic within the cultivar sets but polymorphic between the sets.

Genetic maps of chromosomes 1BS, 2A, 2B, 2D, 3A, 3B, 3D, 4A, 5A, 6AS, 6BS, and 6D were constructed from the segregation data of 132 F_2 Arina x Oberkulmer plants.

Based on those RFLP maps, a QTL affecting the length of ear internodes was mapped on chromosome 5A and linked to the spelt factor *Q/q*. The modification of effects on the action of *Q/q* will be discussed.

A major QTL for the coleoptile growth rate was mapped on 2D. Linkage to *Tenacious glume* is postulated. At marginal locations, plants with fast growing coleoptiles of evolving populations were favoured independent of their linkage to *Tenacious glume*. The coleoptile growth rate correlated with the shape of the caryopses (elongation) and was used to some extent as a marker for the former trait in wheat and spelt crosses.

Hypoxia tolerance was inherited quantitatively in the F_2 population. A QTL distinct from the underlying fast coleoptile growth on 2D was mapped on chromosome 3AS. Hypoxia tolerance is not linked to known spelt characters and is also found in old wheat cultivars.

The results will be discussed in terms of agronomic importance and general stress resistance.

ZUSAMMENFASSUNG

In den regenreichen und kalten Regionen Mittel- und Nordeuropas war Dinkel (*T. spelta* L.) während Jahrhunderten die dominierende Brotfrucht. Zur Erhaltung dieser Kulturart müssen in den laufenden Zuchtprogrammen - unter Beibehaltung der typischen Produktequalität und der Robustheit - Fortschritte erzielt werden.
Über die physiologischen Ursachen der Anpassungsfähigkeit des Dinkels an solche Grenzlagen ist wenig bekannt. Die vorliegende Arbeit konzentriert sich auf die frühe Jugendentwicklung von Weizen und Dinkel und auf die hauptsächlichen morphologischen Unterschiede zwischen den Arten, dem Spelz-Faktor (*Q/q*) und dem Gen für Spelzenschluss, *Tenacious glume (Tg)*.
Eine Auswahl von je drei Weizen- (Arina, Bernina, Forno) und Dinkelsorten (Altgold, Oberkulmer, Rouquin) wurde morphologisch beschrieben.
Die Färbung des Perikarps und des Halmes durch Anthocyane wurde als dinkeltypisch erkannt, aber es konnte keine Verbindung zu den untersuchten morphologischen und physiologischen Eigenschaften hergestellt werden.
Die Länge der Ähreninternodien wurde als Mass zur Beschreibung der Wirkung von *Q/q* auf die Ähre, dem Organ mit den auffälligsten Unterschieden zwischen Weizen und Dinkel, herangezogen.
In Laborversuchen (22°C, Dunkelheit, Unterlage: nasses Filterpapier) war die Wachstumsrate der Koleoptilen der Weizensorten, unabhängig von der Temperatur, 77% von derjenigen der Dinkelsorten. Reziproke F_1-Kreuzungen von Weizen und Dinkel zeigten einen beschränkten maternalen Effekt für die Koleoptilenwachstumsrate. Die Wachstumsrate des Primärblattes war für Weizen und Dinkel vergleichbar.
Ein Labortest sollte die Keimung und das frühe Wachstum in durchnässten Böden imitieren. Dazu wurde ein Mischgasstrom von 1% O_2/99% N_2 durch ein geschlossenes System bei 22°C und Dunkelheit geblasen. Dinkelkeimlinge erreichten bei eingeschränkter Sauerstoffversorgung (Hypoxia) 17% der in der Umgebungsluft produzierten Biomasse, während Weizensorten 10% bildeten. Moderne Weizensorten und alte Dinkelsorten formten demnach zwei unterscheidbare Gruppen bezüglich ihrer Hypoxia-Toleranz.
Um die Weizen und Dinkel unterscheidenden Eigenschaften zu kartieren, wurden RFLPs, verteilt über das ganze Genom, gesucht. 24% der geprüften Sonden zeigten arttypische Polymorphismen, d.h. waren monomorph innerhalb der Art, aber

polymorph zwischen Weizen und Dinkel. Basierend auf 132 F_2 Arina x Oberkulmer Pflanzen wurden genetische Karten der Chromosomen 1BS, 2A, 2B, 2D, 3A, 3B, 3D, 4A, 5A, 6AS, 6BS und 6D konstruiert.

Ein QTL, der die Internodienlänge der Ähre beeinflusst, wurde auf 5A kartiert und in Zusammenhang mit *Q/q* gebracht. Modifizierende Einflüsse auf die Wirkung von *Q/q* wurden diskutiert. Auf 2D wurde ein bedeutender QTL der Wachstumsrate der Koleoptile kartiert; Koppelung zum Gen *Tenacious glume* wird postuliert.

In einer Grenzlage des Ackerbaues steigerte sich in Evolutionsstämmen der Anteil schnell wachsender Koleoptilen unabhängig vom Spelzenschluss *Tg*. Die Form der Karyopse (die Streckung im speziellen) wird als morphologischer Marker für die Koleoptilenwachstumsrate und den Spelzenschluss in Weizen x Dinkel-Kreuzungen vorgeschlagen.

Hypoxia-Toleranz wurde in einer F_2 Weizen x Dinkel-Population quantitativ vererbt und ein QTL, unterscheidbar von der überlagerten Koleoptilenwachstumsrate, wurde auf Chromosom 3A kartiert. Hypoxia-Toleranz konnte nicht mit bekannten Dinkeleigenschaften in Verbindung gebracht werden und wurde auch in alten Weizensorten gefunden.

Die Resultate werden bezüglich ihrer agronomischen Bedeutung vor dem Hintergrund allgemeiner Stressresistenz diskutiert.

2. INTRODUCTION

Spelt (*Triticum spelta* L.) is the cereal species best adapted to the marginal farming areas of the rainy and cold mountainous regions of central and northern Europe. What are the specific characteristics which make spelt so suitable for this environment? How can factors which contribute to a general robustness be defined, and how are they linked to the known spelt characteristics of the mature plant?
MacFadden's and Sears' (1946) theory that spelt, an ancestral hexaploid wheat, originated in southwestern Asia and came to central Europe via a northern route as a component of mixed crops together with Einkorn (*T. monococcum*) and Emmer (*T. dicoccum*) is generally accepted (Andrews, 1964; Kuckuck, 1964). In southwestern Germany and northern Switzerland conditions are particulary favourable to spelt which has become a major crop.
T.aestivum, common bread wheat, is regarded as being a descendant of the speltoid forms *T. spelta* and *T. macha* and, as such, acquired a genetic factor which suppressed the spelt characteristics (Kuckuck, 1982).
The classification and nomenclature of spelt in the genera *Triticum* is not yet clear as is true for the whole tribe of the *Triticeae*. In more recent classifications Jakubziner (1959) and Dorofeev and Korovina (1979) name the species *T. spelta* L., Bowden (1959) and Morris and Sears (1967) *T. x aestivum* (L.) Bowden *cv. group spelta* and *T. aestivum* L. em Thell. *cv. group spelta*. Mackey (1966) finally classified the spelt wheat as *T. aestivum* (L.) Thell. *ssp. spelta* (L.) Thell. We will use the classifications *T. spelta* L. and *T. aestivum* L. for the sake of convenience.
The principle differences among the hexaploid wheats are due to single genes. These are characterised by Swaminathan and Rao (1961) as follows:

chromosomal location	5AL	2DL	3DX
T. spelta	*qq*	*cc*	*SS*
T. macha	*qq*	*CC*	*SS*
T. compactum	*QQ*	*CC*	*SS*
T. aestivum	*QQ*	*cc*	*SS*
T. sphaerococcum	*QQ*	*cc*	*ss*

Q/q: q speltoid gene complex
Q free-threshing grain and tough rachis
C: compact ear
s: spherical grain

The agronomic characteristics of spelt wheat are the same as those of old bread wheat landraces: tall stand, lax leaves, late flowering, few but long grains with high protein concentrations, and low harvest index. The inheritance of agronomic characters interacting with different environments was investigated by Rimle (1995).

Spelt cultivars are very robust as compared to wheat (Balon, 1978; Jaquot et al., 1960; Koblet, 1965). This is due to a number of traits (Rüegger, 1988): good seed vigour expressed as high germination rate and fast coleoptile extension, good seedling emergence under wet, cool conditions, and good winter hardiness with, with increasing age, sprouting resistance and resistance to fungal diseases. Because of the complementary characters of wheat, it was an excellent crossing partner for investigating the genetics of these traits.

Little is known about the physiological background of spelt's resistance to harsh conditions during sowing and plant establishment. To elucidate the physiological and genetic bases of selected spelt traits for hardiness, we

- chose modern *Triticum aestivum* cultivars as crossing partners lacking the degree of robustness of the spelts,
- screened wheats and spelts with molecular markers for polymorphisms
- developed laboratory tests to identify factors contributing to the 'robustness complex' and studied the genetics of two possible components
- mapped QTL for the above mentioned traits.

We attempted to integrate the findings in a general concept of stress.

3. MATERIAL and METHODS

3.1 Description of genotypes

The cultivars Arina and Oberkulmer were selected as typical representatives of varieties adapted to the agronomic environment of central Europe which originated from the gene pools *Triticum aestivum* L. and *T. spelta* L.

Arina is a high quality bread wheat from the Swiss Federal Research Station at Zürich-Reckenholz (FAP). It is a typical, modern, high-yielding wheat combining good quality and disease resistance.

Oberkulmer was first released in Switzerland in 1948 and has been the most widely grown spelt wheat ever since. It shows typical spelt morphology (tall stand and lax ear) and generally withstands abiotic stresses. The quality of the grain is typical of spelts, and the yield is relatively low.

Reciprocal F_1 crosses of Arina and Oberkulmer, F_2 progenies of Arina x Oberkulmer (provided by R. Rimle), and their F_3 families were used in physiological studies. For morphological and molecular analysis, Arina and Oberkulmer and F_2 progenies of Arina x Oberkulmer were used.

The F_3 families were developed from caryopses of the F_2 plants. Yield components and general morphology of the grain were determined on caryopses of the main ear, and physiological studies of caryopses originating from tiller ears were conducted.

Further wheat and spelt genotypes used for genetic characterisation in different experiments were all of European origin and were chosen to represent both gene pools. Bernina, Forno, and Arina were treated as a set of wheat cultivars, Altgold, Rouquin, and Oberkulmer as a set of spelt cultivars.

Reciprocal F_1 crosses of Bernina and Oberkulmer, Bernina and Rouquin, and Forno and Rouquin were used to investigate possible maternal effects on shoot growth. Table 3.1.2 summarises the commercial genotypes used in the investigations.

Specific wheat genotypes were used in some of the investigations: April Bearded isogenic lines for *Rht* genes (*Rht1*, *Rht2*, *Rht3*, *Rht1+Rht2*, *Rht2+Rht3*; provided by Dr. A. Boerner, Gatersleben), and isogenic lines for *Red Coleoptile* (RVPM 21, RVPM 132) and *Rht8/Ppd* (Cappelle; provided by Dr. A.J. Worland, Norwich) were used to investigate the influence of those genes on shoot growth under two oxygen regimes. Chinese Spring and its substitution line Chinese Spring (*T. spelta*

5A) (provided by Dr. J. Snape, Norwich) were selected to study the influence of the *Q/q* loci on the investigated morphological and physiological traits in a different genetic background.

In order to study the impact of the environment on the allele frequency of specific seedling traits, two populations, derived from spelt x wheat crosses, were used. These populations were derived from a mixture of 10 lines, each resulting from complex crosses within a worldwide collection of wheat and spelt genotypes. In 1989 the two populations were initiated from the F_4 onwards by growing them for five years at Zürich-Reckenholz or Oberwallestalden (material provided by Dr. H. Winzeler, FAP). Table 3.1.1 gives a description of the climate at the two locations. After each harvest the two populations, one at each location, were artificially equilibrated again into a 50:50 mixture of hulled and naked kernels. Each population was multiplied on 27 m^2 with 10 g of spikelets and with 7.2 g of naked grains per m^2 respectively. Seedbed preparation, sowing, fertilization, and plant protection were according to usual agronomic practices. Tight glumes were removed by hand, if necessary, and only naked grains were used. All seeds of the cultivars came from untreated seed stocks of the Swiss Federal Research Station of Agronomy, FAP, Zürich-Reckenholz.

Table 3.1.1: Climatic and geographical description of the field locations. Numbers are means of the last decade.

	altitude [a.m.s.l]	mean rainfall	annual temp.
Zürich-Reckenholz	450 m	850 mm	8°C
Oberwalle-stalden	1000 m	1400 mm	4°C

Table 3.1.2: Cultivars used to form two sets of wheat and spelt and cultivars used in various experiments.

cultivar	year of release	pedigree	breeder
		set of winter wheat cultivars:	
Arina	1981	Moisson/Zenith	Swiss Federal Research Station of Agronomy (FAP)
Bernina	1983	Caribo/Hoeser 52	FAP
Forno	1986	NR 72.837/Kormoran	FAP
		set of winter spelt cultivars:	
Altgold Rotkorn	1952	*Oberkulmer 3/Sandmeier//* Oberkulm II	FAP und A. Bättig, Rickenbach (CH)
Oberkulmer Rotkorn	1948	selection from landraces	FAP
Rouquin	-	line 24/Ardenne// Altgold	Station d'Amélioration des Plantes, Gembloux (B)

cultivars used in various experiments:

wheat:		***spelt:***	
April Bearded	GB	Hercule	B
Probus	1948, CH	Ostro	1978, CH
Boval	1990, CH	Lueg	1990, CH
		Hubel	1992, CH

3.2 Selected characters of mature plants

Morphological data on commercial cultivars were provided by Rimle (1995) and are means of three field trials located in different climatic regions in Switzerland. All the experimental F_2 plants grew in pots (Ø 15 cm) containing standard soil, one plant per pot, in the glasshouse. Each pot was supplied automatically with a water/nutrient solution adapted for wheat cultivation using drip irrigation. The populations were treated twice for aphids and powdery mildew.

Plant **height** and **peduncle** length of the F_2 Arina x Oberkulmer population were measured on the main culm of each plant at harvest (Table 3.2.1). The lengths of the ears on the main culm of all plants were also measured, and the fully developed spikelets on these ears were counted. The ratio of ear length [mm]/ number of spikelets gave the **length of the ear internodes**.

The single ears were processed in a modified laboratory shaker built by Bleiker and Winzeler (1991) to score the characters '**brittleness of the rachis**' and '**tightness of the glumes**', using a scale from 1 (=wheat) to 9 (=spelt). The threshed grains were collected and, if not free, dehulled by hand. All the caryopses were dried at 40°C to a water content of approx. 12% and stored appropriately until use.

The **single grain weight** was calculated as the **main ear yield** divided by the grain number. Of 15 well-filled caryopses from the main shoot of each F_2 and of 30 caryposes of various cultivars, the shape, expressed as the ratio of length to diameter, was determined and used to characterise the grain **elongation** type. The length was measured from the brush to the attachment region. The diameter was defined as the distance from the most elevated point of the caryopses to the plane where it was laid flat on its ventral side. All measurements were obtained with a micrometer to within 1/10 mm.

The length of the brush was scored for 10 caryopses of each F_3 family using a scale from 1 to 4, where 1 was the short brush of Arina and 4 the long brush of Oberkulmer.

Table 3.2.1: Characters used to describe the morphological, pigment, and agronomical properties of F_2 plants, their F_3 families, and cultivars:

a) F_2 plants:

Plant morphology:	
plant height	[cm]
peduncle length	[cm]
General ear morphology:	
ear length	[mm]
length of ear internodes (=ear length/spikelet number)	[mm]
brittleness of the rachis	[1 to 9]
tightness of the glumes	[1 to 9]
Yield components of the ear:	
grain number	[-]
single grain weight	[mg]
main ear yield	[mg]

b) F_3 families, cultivars

Morphology and physiology of the grain:	
elongation= grain length / grain diameter	[-]
brush length	[1 to 4]
Pigmentation:	
purple pericarp	[red or amber]
purple culm	[red or green]
red coleoptile	[red or white]

3.3 Selected characters of caryopses, seedlings, and vegetative plants

3.3.1 Pigmentation

The expression of anthocyans in the coleoptiles (*Red Coleoptile)* and culms (*Purple Culm*) was scored visually on 30 seedlings of each cultivar and on 12 seedlings of each F_3 family: after soaking, grains were kept at 4 °C for 48 hours, grown for five days in the dark at 22 °C, and finally exposed to daylight for one day at room temperature.

The *Purple Pericarp* character was investigated on 30 caryopses of each cultivar and on 95 Arina x Oberkulmer F_3 caryopses immersed in 2 ml of 10% NaOH for one hour at 55 °C. The colour of the liquid after squashing the caryopses, red-brown or straw-yellow, indicated the dominant and the recessive allele status of *Purple Pericarp.*

Manually dehulled caryopses of tiller ears were used in all experiments to determine the shoot growth characters of F_3 families, whereas caryopses of seed stocks (provided by Dr. H. Winzeler, FAP) were chosen for cultivars.

3.3.2 Seedling shoot growth

3.3.2.1 Seedling shoot growth of wheat and spelt cultivars at different temperatures

Thirty caryopses of the wheat cultivars Arina, Bernina, Forno, Chinese Spring, and Chinese Spring (*T. spelta* 5A) and of the spelt cultivars Altgold, Oberkulmer, and Rouquin were soaked in ice-water for three hours and laid out in two blocks on wet filter paper on the bottom of a 32x22x5 cm Perspex box. The blocks consisted of eight rows and 15 caryopses of each genotype per row. The caryopses were placed at intervals of 1.5 cm with 2 cm between the rows.

After two days at 4 °C, the boxes were placed in the dark at 6, 12, 18, 24, and 30 °C. Similar preliminary experiments showed the approximate period of time required for the primary leaf to rupture the coleoptile. The plants were checked at the critical phases at intervals from 12 (30 °C) to 48 (6 °C) hours, depending on the temperature. The hours till the rupture of the coleoptile and the coleoptile length at that time were recorded for each temperature and cultivar. The length of the coleoptile from the seed to the tip of the coleoptile and the total shoot length from

the seed to the tip of the first leaf were measured.
The **growth rate 1 (GR 1)** was calculated for each plant as: coleoptile length at rupture of the first leaf divided by the hours of growth , i.e. the growth rate of the coleoptile itself. At a total shoot length of 12-15 cm, the hours of growth were recorded and the shoot lengths measured. The **growth rate 2 (GR 2)** was calculated for each plant as shoot length - coleoptile length / hours since coleoptile rupture, i.e. GR 2 is the growth rate of the visible primary leaf.
Plants that showed an abnormal growth rate or retarded germination were not used for the calculation of GR 1 and GR 2.
For the spelt x wheat populations, which had been developed at Zürich-Reckenholz and Oberwallestalden, the same trials were carried out as described above. Seedlings from 100 caryopses of each population were germinated, and the shoot length of each seedling was measured from the seed to the tip after 72 hours of growth at 22°C in the dark. These experiments were repeated three times, and the mean shoot length was calculated for each population as the average shoot length of all seedlings.

3.3.2.2 Shoot growth of wheat x spelt crosses

a) Reciprocal F_1 crosses of wheat and spelt
Because of the major differences found in the seed coat characters of spelt and wheat, possible maternal effects on the coleoptile growth and its degree of heterosis were analysed in a standard experiment at 22°C in the dark during a growth period of 72 hours. Thirty caryopses of reciprocal F_1 crosses of Arina and Oberkulmer, Bernina and Oberkulmer, Bernina and Rouquin, and Forno and Rouquin were grown on wet filter paper in Perspex boxes, described in section 3.4.3., and aerated with ambient air.

b) F_3 families of Arina x Oberkulmer
The coleoptile extension of F_3 families of Arina x Oberkulmer was measured on the control plants in hypoxia tolerance experiments. Four replications of four plants per family were analysed. See section 2.4.3 b for a detailed description of this procedure.

3.3.2.3 Seedling growth under hypoxia stress

a) Seedling growth index SGI of wheat and spelt and of reciprocal F_1 crosses of wheat and spelt

Eight caryopses of various wheat and spelt cultivars and reciprocal F_1 crosses between these cultivars were soaked in ice-water for three hours and then placed immediately on wet filter paper at the bottom of an airtight jar (Nalgene), surface of 28.3 cm^2, a volume of 250 ml, and fitted with two 0.7 x 30 mm gauges as in- and outlets. The jar was vacuumed with an air pump and a constant flow of approximately 1litre / hour of a 1% O2 / 99% N_2 gas mixture applied. The jars were placed in a growth chamber and kept in the dark for 96 hours at 22°C. The experiment was repeated three times. An identical arrangement, with a constant supply of air, was used as a control.

After 96 hours under hypoxia the plant material was immediately lyophilised for 48 hours. The seedlings were then separated from the endosperm and both were weighed separately as bulked seedlings and grains. The ratio of seedling weight / [seedling weight + grain weight] was calculated and termed the **seedling growth index (SGI)**. Hypoxia tolerance was expressed as SGI 1% O2 / SGI 21% O2.

Thirty caryopses of each reciprocal F_1 cross and 24 caryopses of each cultivar were tested under the same conditions as described above. The degree of heterosis was measured as the relative deviation of the F_1's SGI mean from the parents' mean (*mid parent heterosis*).

In two runs, 128 caryopses from free-threshing ears and 128 caryopses from manually dehulled spikelets of both populations (Zürich-Reckenholz and Oberwallestalden) were also tested for hypoxia tolerance in the same way.

b) F_3 families of Arina x Oberkulmer: coleoptile length

Four grains of each F_3 family, weighed individually to determine possible effects of grain size, were soaked for five hours in ice-water and then laid on wet filter paper for 48 hours at 4 °C in the dark. Thereafter, the filter paper was transferred to an airtight Perspex box (49,5 x 33,5 x 30 cm) with a siphoned in- and outlet tube placed in a growth chamber at a constant temperature of 22 °C. The box was evacuated by means of an air pump for 10 min, while a 1% O_2/ 99% N_2 gas mixture, with a flow rate of 500 Nl/h, was pumped into the box. The gas flow was then regulated to 30 Nl/h and the box left in the dark. The oxygen content in both

boxes was controlled visually with BBL® Dry Anaerobic Indicator Strips (Becton Dickinson and Co., Cockeysville, MD 21030, USA). After 96 hours the temperature was lowered to 4°C, and the coleoptile length was measured to the next millimeter within four hours.

An identical box was used for the control treatment which received a constant flow of 300 Nl / hour of ambient air. Because of the faster development of the control plants, the coleoptile length was measured after only 72 hours, and the shoot lengths of these seedlings were recorded after 120 hours (see 2.4.2 b).

At the end of the shoot growth experiment, the colour of the coleoptiles, red or green, was recorded for the seedlings of all families in the hypoxia treatment after exposing them to light for one day at room temperature.

The experiment was repeated three times (12 grains) and five times (20 grains) so as to test each family under hypoxic and ambient conditions.

The non-germinating kernels were checked for viability by a tetrazolium test (ISTA, 1993). Caryopses without physiological activity were not included in further investigations. Only families consisting of at least 11 or 12 plants were used for genetic studies of growth under hypoxia.

Hypoxia tolerance was calculated as the ratio of shoot length under hypoxia to shoot length under normoxia.

3.4 RFLP procedures

3.4.1. Preparation of probes

For the RFLP analysis, all the clones were supplied by Dr. M.D. Gale's group at Norwich, UK as plasmids containing the inserts which were to be transformed.

Bacterial strain DH5α was used in all transformation experiments, the genotype of which is DH5α: F^-endA1, hsdR17(r_k^-,m_{k+}), SupE44, thi-1, λ^- recA1, gyrA96, relA1, Δ(argF-lacZYA) U169, p80d, lacZΔM15.

3.4.2. Preparation of competent cells

The method used was a modification of the procedure of Cohen et al. (1972) reported in Sambrook et al. (1989). One hundred microliters of an overnight

culture of DH5α were added to 250 ml LB broth (Luria-Bertani Medium: 1% (w/v) bacto-tryptone, 0.5% (w/v) bacto-yeast extract, 1% (w/v) NaCl, pH at 7.0) in a 500 ml Erlenmeyer and incubated at 37°C on a rotary shaker (300 cycles/min). To monitor the growth of the culture, the OD_{550} was determined every 15-20 min. At OD_{550}= 0.3 (after approx. 4 1/2 h) the culture was transferred to sterile, disposable 50 ml polypropylene tubes and stored on ice for 10 min. After centrifugation in a swing out rotor at 4°C and 4000 rpm for 3 min, the media was decanted from the cell pellets and the tube inverted for 1 min to drain away all the media. The pellet was then resuspended in 10 ml of ice-cold 0.1 M $CaCl_2$ and the tube kept on ice. Centrifugation and draining, as described above, were repeated and the pellet resuspended in 2 ml of ice-cold 0.1 M $CaCl_2$. Ice-cold glycerin (> 99.5%) was added to a concentration of 20% and the competent cells stored in aliquots of 200 µl at -80°C until use.

3.4.3 Transformation of DH5α

Two hundred microliter aliquots of competent cells were slowly thawed on ice. One hundred microliters of a 1:100 diluton of plasmid DNA were added, the tube gently swirled, and then left on ice for 30 min. The sample was then placed in a circulating water bath at 42°C for exactly 90 sec. Special care was taken not to shake the tube. It was then chilled on ice for 2 to 3 min. Eight hundred microlitres of LB medium (Luria-Bertani medium: 1% (w/v) bacto-tryptone, 0.5% (w/v) bacto-yeast extract, 1% (w/v) NaCl; pH adjusted to 7.0) were added to the cells. To allow the cells to express the antibiotic resistance encoded by the plasmid, the tube was incubated on a shaker (225 cycles / min) at 37°C for 45 min. Thirty microliters of the cell culture were plated out onto LB agar (Luria-Bertani medium with 1.5% bacto-agar) containing ampicillin (60 µg/ml) on a 90 mm plate. The inverted plate was incubated overnight at 37°C.

3.4.4 Selection of transformed cells

The pUC plasmids used contain the ampicilline resistance gene *amp*^{r}.
Therefore, only transformed cells grew with the ampicillin in the medium.

Single colonies were selected and transferred with a sterile toothpick to 4 ml of LB medium containing 60 µg/ml of ampicillin. The inoculated media was incubated overnight on a shaker at 300 cycles / min at 37°C.

3.4.5 Mini preps of plasmid DNA

1.6 ml of an overnight culture of transformed DH5α were poured into a 1.5 ml Eppendorf and centrifuged at 5,000 rpm for 1 min. The supernatant was removed and the remaining pellet suspended by vortexing in 200 µl of STET buffer (8% (w/v) sucrose, 0.1% (w/v) Triton X-100, 50 mM EDTA, 50 mM Tris.Cl pH 8.0). The suspension was incubated for 10 min at room temperature with 2,300 units of lysozyme (Boehringer Mannheim GmbH); 1.6 units of RNase (Boehringer Mannheim GmbH) were added, vortexed, and incubated at 37°C for 5 min.
The tubes were then placed in a boiling water bath for 45 sec and then immediately chilled on ice for at least 3 min.
After centrifugation at 14,000 rpm for 10 min the pellet was removed with a sterile toothpick and the supernatant incubated with 8 µl CTAB (5% (w/v) hexadecyltrimethylammoniumbromide in 0.5 M NaCl) for 5 min at room temperature. Following centrifugation at 14,000 rpm for 1 min, the supernatant was removed and the pellet resuspended in 300 µl 1.2 M NaCl by gently flicking the tube.
750 µl of -20°C absolute ethanol were added, mixed by inversion, the tube left at -20°C for at least 30 min, and then centrifuged at 14,000 rpm for 20 min at 4°C. The pellet was washed with 70% ethanol and air-dried for several hours. Finally, the plasmid DNA was dissolved in 20 to 50 µl of 0.1 x TE buffer (10 mM Tris.Cl, 1 mM EDTA (pH 8.0)).

3.4.6 Testing the insert size

Five microlitres of plasmid DNA solution were restricted with two units of the appropriate excision enzyme(s) in a standard reaction of 1 x reaction buffer, being provided by the enzyme suppliers, in a total volume of 15 µl. The reaction was stopped by adding loading buffer (0.0025% (w/v) bromophenol blue, 25% (w/v)

Ficoll-400, 0.1 M EDTA, 1% SDS). The DNA was separated in a 1% agarose gel and the fragment size estimated by comparing it with a mixture of fragments of standard size (1 kb ladder, Gibco BRL, Life Technologies A.G., Basel, Switzerland).

3.4.7 Isolation of inserts from plasmids

Since the preparation of plasmid DNA was time consuming, the method of insert-amplification, Polymerase Chain Reaction (PCR), was chosen whenever possible. Commercially available M13 forward and reverse primers were used.

a) PCR amplification of inserts from plasmids

Insert DNA was amplified by adding 2 µl of a 100-fold dilution of plasmid mini-prep DNA to 48 µl of a PCR reaction mixture with PCR buffer (10 mM Tris.Cl pH 8.3, 3 mM $MgCl_2$, 50 mM KCl, 0.1 mg/ml gelatine), 100 µM of each dNTP, 50 nM M13 single stranded forward and reverse primers, and 1 U of Amplitaq. The samples were overlaid with a drop of paraffin oil to prevent evaporation and subjected to 30 cycles of 1 min at 94°C, 1 min at 60°C and 1 min 30 sec at 70°C in a DNA Thermal Cycler (Perkin-Elmer Cetus). Then, the samples were incubated for 5 min at 70°C and cleaned by spinning through a self packed Sephadex® G-50-80 (Fluka Chemie AG, Buchs, Switzerland) column to remove unincorporated nucleotides. A 3 µl aliquot of the amplified insert was checked for size and concentration on a 1-2 % gel, depending on the size of the inserts.

b) Isolation and purification of inserts from LMP agarose gels

Inserts which failed to amplify by PCR were isolated from their plasmids following digestion and separation on low melting point (LMP) agarose gels. Eighty microlitres of digested plasmid DNA was loaded on a 1% LMP agarose gel and separated by a constant voltage of 50 V at 0°C. The bands containing the insert were cut out and purified by using the Qiaex gel purification kit (Qiagen Inc., Chatsworth, CA 91311, USA). One hundred microlitres of the appropriate buffer per 100 µg of gel and 10 µl of Qiaex beads were added to the gel slice and

vortexed thoroughly. The sample was incubated for 20 min at 50°C and vortexed quickly every two minutes. After spinning down the beads, the supernatant was removed and the beads washed twice with Qiaex washing solutions and dried briefly in the air. The DNA was eluted by adding 30 µl of 1 x TE buffer and incubated for 10 min at 50°C.
The concentration of the insert was estimated by running 3 µl of the diluted insert on a gel with a number of weight standards ranging from 25 to 250 ng/µl of Lambda DNA. The gel was stained in a 2 mg/ml ethidium bromide solution and then photographed on a UV illuminator.

3.4.8 Isolation of DNA from wheat leaves

Wheat and spelt plants, grown in the glasshouse, were harvested between stages EC 20 and 30 by cutting young, healthy leaves into pieces approx. 2 cm in length. The plant material was placed in a plastic bag and frozen immediately in liquid nitrogen. After storing the samples at -80°C they were freeze-dried for five days and stored at -20°C in sealed plastic bags with silica gel as the desiccant.
The samples were ground in a modified ball mill (Retsch MM2, Retsch GmbH, 5657 Haan, Germany) to a fine powder and approx. 1 g of the powder was transferred to a 50 ml polypropylene tube (Falcon).
Twenty millilitres of Buffer 'S' were added (0.1 M Tris.Cl pH 8.5, 0.1 M NaCl, 0.05 M EDTA, 2% (w/v) SDS) and the powder was suspended by mixing gently; 100 µl of a 10 mg/ml Proteinase K (from *Tritirachium album*, Boehringer Mannheim GmbH) solution were added and the samples were incubated on a rotating platform in an oven at 65°C for two hours and stirred occasionally. The suspension was then extracted with an equal volume (20 ml) of phenol/chloroform/isoamylalcohol (25:24:1;v:v:v). The samples were gently mixed by inverting to form an emulsion and then separated by centrifugation at 2,000 rpm for 20 min at room temperature. The aqueous upper phase was transferred to another tube and 0.6 volume (approx. 15 ml) of isopropanol was added and the contents mixed carefully by inversion. The DNA was then spooled on a glass hook and rinsed three times in an excess of 70% ethanol. The DNA was briefly air-dried and dissolved in 5 ml of 1 x TE-buffer (10 mM Tris.Cl, 1 mM EDTA (pH 8.0)) for at least three days at 4°C. Ten microlitres of DNase-free RNase (10mg/ml)

(Boehringer Mannheim GmbH) were added and the solution incubated at 37°C for one hour and mixed occasionally. The samples were then extracted as before with an equal volume of phenol / chloroform / isoamylalcohol and a second time with chloroform / isoamylalcohol. The aqueous phase was transferred to a new tube and the DNA precipitated by adding 3 M sodium acetate to give a concentration of 0.3 M and two volumes of -20°C 96% ethanol; the samples were air-dried and redissolved in 0.5 -1 ml of 1x TE depending on the size of the pellet, and stored at 4°C.

3.4.9 Measurement of the DNA concentration

The concentrations of the samples were determined by a spectrophotometer (Ultrospec II, LKB Biochrom, Cambridge, UK) which measured the amount of UV irradiation absorbed by the bases since a DNA concentration of 50 µg/ml has an OD reading of 1 at a wavelength of 260 nm. The ratio 'absorbance at 260 nm/ absorbance at 280 nm' was determined. For pure DNA solutions it is ideally 1.8; lower values indicate the presence of proteins, and higher values are due to the presence of chloroform. For samples with values outside the range of 1.6 to 1.8, the extraction was repeated with phenol / chloroform / isoamylalcohol as described above.

3.4.10 Restriction digestion and separation of fragments of wheat DNA by agarose gel electrophoresis

Ten micrograms of total wheat genomic DNA were routinely digested with 20 units of the 6-base cutter enzymes *Eco*RI, *Eco*RV, *Dra*I and *Hin*dIII (Boehringer Mannheim GmbH) in a total volume of 30 µl at 37°C overnight; the appropriate reaction buffer was supplied by Boehringer. The reaction was terminated by adding 6 µl of 5x loading buffer (0.0025% (w/v) bromophenol blue, 25% (w/v) Ficoll-400, 0.1 M EDTA, 1% SDS).

To screen several probes, the enzymes *Bgl*II and *Bam*HI were used in the same way as described above.

Standard 0.8% agarose gels (24.1 cm x 20.4 cm x 0.8 cm) with 30 wells were

prepared in 1x TAE-buffer (0.04 M Tris-acetate, 0.001 M EDTA). Ten micrograms of restricted genomic DNA, in a volume of 35 µl, were loaded per well. The outside wells were loaded with 0.6 µg of marker DNA (lambda/*Hin*dIII). A constant voltage of 40 V was applied overnight in a submarine gel apparatus (Model HRH, International Biotechnologies, Inc., New Haven, CT 06535, USA).

3.4.11 Capillary transfer of DNA from agarose gels to Hybond® N⁺membranes

Electrophoresis was carried out until the dye had migrated approximately 13 cm. The gel was then stained in ethidium bromide (0.5 µg/ml) for 20 min. Photographed under UV light (256 nm), the DNA was blotted from the gel, using alkaline capillary blotting (modified from Southern, 1975), onto a Hybond N^+ membrane (Amersham International plc, Amersham, UK).

The gels were removed from the casting tray and trimmed to 12 x 18 cm for screening blots or 11 x 19 cm for F_2 segregation analysis blots.

The DNA was degraded by incubating the gel in 1 l of 0.25 M HCl (d 1.18) on a shaker for 15 min or until the dyes turned yellow. The HCl was poured off and the gel rinsed several times in tap water; then a small amount of 0.4 M NaOH was added to the gel.

A square petri dish (23 x 23 cm) was filled with 600 ml of 0.4 M NaOH and a glass bridge was used to support the gel.

Three layers of Whatman 3MM filterpaper were soaked in 0.4 M NaOH and placed over the bridge to form the wick. The gel was placed on the bridge and a Hybond N^+ membrane, cut to the size of the gel and presoaked in 0.4 NaOH, was laid on top of the gel. Three layers of Whatman 3MM filter paper, soaked in 0.4 NaOH, were placed on top of the nylon membrane. Care was taken to ensure that no air bubbles were trapped between the layers of the transfer set-up. Paper towels were placed on top of the 3MM paper and weighed down with a 500 g weight. The rest of the square dish was covered with Saran® wrap to stop any 0.4 M NaOH bypassing the gel to the paper towels. The transfer continued overnight after which the apparatus was dismantled, the membrane rinsed in 2 x SSC, wrapped in Saran®, and stored at 4 °C until use.

3.4.12 Radiolabelling of insert DNA to high specific activity by random primed labelling

The 'Oligolabelling' method of Feinberg and Vogelstein (1983) was used to label the inserts. The DNA Labelling Kit, ver. 1.1 (MBI Fermentas, Vilnius, Lithuania) was used in a standard reaction: 25 ng for 1/2 filters and 50 ng for five filters of insert DNA in a volume of 30 µl were denatured at 100°C for seven minutes. The denatured DNA was chilled on ice for three minutes, spun down, and added to a tube containing the random hexamers (1.5 o.u./ml), dATP, dGTP, dTTp 0.02 mM each) and reaction buffer containing 0.025 Tris.Cl pH 8.0, 2.5 mM $MgCl_2$, 1 mM dithiotreitol, and three units of the Klenow fragment of *E. coli* DNA Polymerase I. The contents of the tube were mixed and spun down. Then 2 or 4 µl of [α-^{32}P]dCTP, with a specific activity of 110 TBq/mmole (Amersham International plc, Amersham, UK), were added to the contents of the tube, mixed briefly, and incubated at 37°C for one to two hours.

Radiolabelling of marker DNA

Ten nanograms of marker DNA (lamba/*Hin*d III) were labelled with [α-^{32}P]dCTP according to the method described above. The radiolabelled marker DNA was diluted with 1 x TE-buffer until an activity of 6000 cpm/µl was measured with a Geiger counter.

3.4.13 Hybridisation of radiolabelled probes to restricted genomic wheat DNA

Prehybridization

The membranes containing the digested DNA were soaked in 2 x SSC and put in a 4 x 25 cm glass tube (Hybaid Limited, Teddington, UK). Up to five filters were stacked alternately with fine nylon meshes (Hybaid). Ten millilitres for one and 20 millilitres for five filters of prehybridisation buffer were added to the tube. The filters were prehybridised in a hybridisation oven (Hybond Midi Dual 14, Hybaid Limited, Teddington, UK) at 65°C for at least five hours.

Prehybridisation solution: 1 x HSB, 10 % (v/v) Denhardt's III, 0.5 mg/ml denatured carrier DNA (Salmon tested DNA, type II, Sigma)

5 x HSB: 3 M NaCl, 0.1 M PIPES, 20 mM EDTA, pH 6.8
Denhardt's III: 2% (w/v) gelatin, 2% (w/v) Ficoll-400, 2% (w/v) PVP-360, 10 % (w/v) SDS, 5 % (w/v) $Na_4P_2O.10H_2O$

Hybridisation

The labelled probe and lambda/Hind III DNA were denatured by boiling for five minutes and then added to prewarmed 5 and 10 ml hybridisation buffer for 1 and up to 5 filters respectively.
The prehybridisation buffer was replaced by the hybridisation buffer containing the labelled probe and hybridised in the hybridisation oven overnight at 65°C.
The post-hybridisation washes were carried out at high stringency. Two 15 min washes at 65°C in 2 x SSC and 1% SDS were followed by two washes for 15 min in 0.2 x SSC and 1% SDS at 65°C. The filters were then wrapped in Saran® wrap and exposed to X-OMAT™ film (Eastman Kodak Company, Rochester, NY 14650, USA) between two intensifiying screens (Cronex® Lightning Plus, E.I. Du Pont de Nemours & Co. (Inc.), Wilmington, DE 19898, USA) at -80°C. The period of exposure depended on the strength of the hybridisation signal detected by the Geiger counter and was usually 10 to 15 days. The autoradiographs were then scored and the data used in further examinations.

Removal of the radiolabelled probe from the nylon membrane

The radiolabelled probe was removed from the nylon membrane by three five min washes in boiling 0.1 x SSC, 0.1% SDS. After the final wash the filters were soaked in 2x SSC, wrapped in Saran® foil, and stored at 4°C until needed.

3.5 Probes used to screen two sets of wheat and spelt cultivars

Table 3.5.1 summarises the probes used to screen two cultivars sets of wheat (*cvs.* Arina, Bernina, Forno) and spelt (*cvs.* Altgold, Oberkulmer, Rouquin).

Table 3.5.1: Probes used to screen sets of cultivars of wheat and spelt for RFLPs. All PSR probes were provided by Dr. M.D. Gale, Institute for Plant Science Research, Cambridge Laboratory, Norwich, UK.

Homeologous probes

Group 1
PSR162
PSR544
PSR596
PSR634

Group2
PSR102
PSR107
PSR126
PSR135
PSR131
PSR304
PSR388
PSR390
PSR609
PSR666
PSR900
PSR901
PSR932
PSR933
PSR934

Group3
PSR345
PSR454
PSR598
PSR689
PSR904
PSR907
PSR931
PSR1077
PSR1196

Group 4
PSR104
PSR144
PSR584
PSR573
PSR580
PSR1051

Group5
PSR14 (*α-amy*-3)
PSR120
PSR426
PSR628
PSR574
PSR906
PSR929
PSR1204

Group 6
PSR8 (*Cxp*3)
PSR10 (*Gli*-2)
PSR106
PSR142
PSR154
PSR167
PSR312
PSR546
PSR831
PSR964

Group 7
PSR129
PSR311
PSR547

Non-homeologous probes

PSR648	1, 4 , 7
PSR963	1, 5
PSR1201	1, 5
PSR903	2, 3
PSR133	2, 5
PSR899	2, 6
PSR540	2, 7
PSR386	3, 4, 5
PSR547	3, 7
PSR147	4, 5
PSR164	4, 5
PSR115	4, 5
PSR160	4, 7
PSR392	4, 7
PSR604	4, 7
pC-I-1-4 [1)]	5H

[1)] Probe provided by Prof. P.R. Shewry, Long Asthon Research Station, University of Bristol; for references see Pl. Mol. Biol. 10: 521-535, 1988.

3.6 Genetic analysis and statistics

Banding patterns which resulted from hybridisations of probes with Southern blots were checked for polymorphisms within and between the wheat and the spelt sets. Each of the F_2 plants was scored for its allele status at the selected molecular marker loci. The allele status of the Arina parent was scored as AA, the Oberkulmer parent as BB, and, consequently, the heterozygotes as AB.

Polymorphisms **within** wheat or spelt, were characterised according to the occurrence of at least one RFLP among the three cultivars. Polymorphisms **between** wheat and spelt were characterised by uniform allele status in one cultivar group and the absence of the allele in the other.

The molecular and the phenotypic data were used in statistical analyses performed with SAS (SAS Institute Inc., Cary, NC, USA).

The significance levels of correlations and T-tests were displayed using the following scheme:

*: $p<0.1$

**: $p<0.05$

***: $p<0.01$

****: $p<0.001$

Probes spread all over the genomes were selected for hybridisation to F_2 plants. The selected polymorphisms of each chromosome were grouped and ordered with a threshold of log-odds (LOD) > 1.5 using Mapmaker/EXP (Lander et al., 1987), i.e. the likelihood of linkage is $10^{1.5}$ larger than the likelihood of non-linkage. This order was used as a framework to place the markers of unknown location on the map by the maximum likelihood method of multipoint linkage analysis performed by Mapmaker/EXP 3.0. Once the maps were established, the phenotypic data were analysed with Mapmaker/QTL 1.1 (Paterson et al., 1988) to locate possible QTL by 'interval mapping'. The indication of QTL was based on LOD scores calculated every 2 cM between the mapped loci. A threshold of LOD = 3 was used, i.e. the probability of the existence of a QTL versus the likelihood of no QTL at that locus is 10^3. Indicated QTL were rejected when dominant markers only were involved.

Based on the assumption that

$$Trait_i = mean + (a \times \#B_i) + (d \times AB_i) + \text{'noise'},$$

where mean = average trait value for AA genotypes;
a = the additive component of the QTL B allele effect;
$\#B_i$ = number of B alleles carried by plant i, either 0, 1, or 2;
d = the dominance effect of the heterozygote AB genotypes compared to the midparent value;
AB_i = 1 if plant is AB heterozygote, and 0 otherwise,

the additive (a) and the dominance (d) component were estimated for each putative QTL every 2 cM.
In addition, the non-genetic 'noise' was quantified as the relative decrease in the estimated standard deviation when a QTL was allowed to control a trait, as opposed to when no QTL was assumed, i.e. all the variation was attributed to 'noise'.
If not stated otherwise, a 'free genetics' model was used, allowing each marker to have a dominant or recessive action on the trait.
A QTL 'confidence interval' was defined as the span for which the LOD score remains above a 10-fold decrease of the maximum LOD score (i.e. a log-likelihood drop of 1.0). This 'confidence interval' indicates the range of QTL positions which can best explain the observed data (see Mapmaker/QTL tutorial).

4. RESULTS and DISCUSSION

4.1. Morphological and agronomical characters

4.1.1 Morphological and agronomical characters of wheat and spelt cultivars

Rimle (1995) described extensively morphological and agronomical characters of the cultivars Arina, Bernina, Forno (*T. aestivum* L.), Oberkulmer, and Rouquin (*T. spelta* L.) all grown under field conditions (Tab. 4.1.1).

The shoots of spelt cultivars were longer, because all the parts of the shoot were longer, i.e. the culm below the flag leaf, the peduncle, and the ear. The greatest variation over years and locations was registered for the length of the peduncle (data not shown). All cultivars except Forno had comparable length ratios for the different organs. The short stand of Forno was due to the conspicuously short parts on the lower culm. Chromosome 5A of *T. spelta* caused the elongation of the upper parts of the shoot, the peduncle, and the ear in Chinese Spring by the same factor, while the lower part of the culm was, in comparison, shortened as in Forno.

Thus, the total shoot elongation clearly differentiated the two species, while no differences between Chinese Spring and Chinese Spring (*T. spelta* 5A) could be found for this trait.

On average the following ratios for the length of spelt : wheat were found for the different organs: 1.52 for plant height, 1.42 for peduncle length, 1.51 for ear length, 1.47 for length of ear internodes, and 1.33 for kernel elongation. The corresponding ratios for glasshouse grown Chinese Spring (*T. spelta* 5A) : Chinese Spring plants were 0.88, 1.41, 1.23, 1.35, and 1.06.

Two pigment characters, typical of the spelt group, were found (Tab. 4.1.1). While all tested genotypes of wheat and spelt were of the *Red grain* type, the *Purple Pericarp* was typical of the spelt group. None of a set of 25 European winter wheats showed the character as opposed to most of 15 spelt lines (data not shown). The *Purple Culm* and *Red Coleoptile* characters were also typical of the spelt group. These traits, however, were of limited use as markers, because the expression of the anthocyans depended strongly on light and temperature interactions. Moreover, Piech and Evans (1978) suggested a genetic relationship among these three pigment characters. Therefore, the information obtained would be identical if it were coded by the same genes.

4.1.2 Morphological and agronomical characters of F_2 Arina x Oberkulmer plants (and their F_3 families)

Nilsson-Leissner (1926) stated, "The degree of spelting of the [...] heterozygotes seems to be directly correlated with the length of the ear internodes in such a way that the laxer the ears the more spelt-like the plants, and vice versa". We also found the lengths of the ear internodes to be the best indicators of the "degree of spelting". Nevertheless, the linkage between the mapped traits close to *Q/q* (grain weight/ear, grain size, plant height, ear length, spikelet number/ear (expressed as length of ear internodes), and grain weight/plant; see Snape et al., 1985), was not tight in our population of 132 F_2 Arina x Oberkulmer crosses. Table 4.1.2 shows the main ear and grain characters of parents and their F_2 offspring from Arina x Oberkulmer. The population was sorted according to the ear internode lengths, and the means of the quartile are displayed.

The strong environmental effect on those traits was illustrated by a comparison of the plant height of the field-grown parents and the mean of the population grown in the glasshouse, which is lower than that of the shorter field-grown parent (Arina), although a positive heterotic inheritance exists for this trait (Rimle, 1995). Kernel elongation was also a good indicator of spelt characteristics in our cross. Its correlation coefficients with the characters 'length of ear internodes' ($r=0.58^{****}$), 'plant height' ($r=0.48^{****}$), 'peduncle length' ($r=0.48^{****}$), 'brush length' ($r=0.29^{****}$), 'tightness of the glumes' ($r=0.62^{****}$), and 'brittleness of the rachis' ($r=0.29^{****}$) were among the highest obtained.

The correlations 'single grain weight' to 'tightness of the glumes' and 'single grain weight' to 'brittleness of the rachis' were weak ($r=0.26^{***}$ and $r=0.27^{***}$ respectively).

The population as a whole did not tend morphologically towards wheat or spelt alleles. A surplus of long lax ears and brittle rachis contrasted with a surplus of free threshing and wheat-shaped grains as reported by Winzeler et al. (1991).

In accordance with other authors, all phenotypes, from *spelta* and speltiforme to *aestivum*, were found (Winzeler et al., 1994; Schmid and Winzeler, 1990). Unfortunately, compactoid forms were not classified separately, and it was impossible to identify those types later, because some ears were not affected on the

Table 4.1.1.: Morphological, agronomical, and pigment characters of the wheat cultivars Arina (Ari), Bernina (Ber), and Forno (For), the spelt cultivars Altgold (Alt), Oberkulmer (Obk), and Rouquin (Rou), the spring wheat cultivar Chinese Spring (CS) and its substitution line CS (*T. spelta* 5A).

cultivar:	Ari	Ber	For	wheat	Alt	Obk	Rou	spelt	CS	CS(*T. spelta* 5A)
morphological characters										
length of shoot [cm][1]	107	98	94	100	-	164	139	152	74	65
culm to first internode [cm]	54.8	50.6	40.3	48.9	-	83.2	72.9	78.5	46.6	28
peduncle [cm][1]	40.6	36.5	43.3	40.1	-	62	51.8	56.9	18.6	26.2
ear [mm][1]	116	109	104	110	-	188	143	166	88	108
ear internodes [mm][1]	5.66	4.36	4.41	4.81	-	7.9	6.27	7.09	3.7	5.0
elongation ratio of the kernel [ ]	2.0	2.0	2.3	2.1	2.8	2.9	2.7	2.8	1.8	1.9
agronomic characters										
tightness of the glumes [1 to 9]	1	1	1	1	9	9	9	9	1	8.9
brittleness of the rachis [1 to 9]	1	1	1	1	9	9	9	9	1.7	8.6
pigment characters										
purple pericarp	0	0	0		+	+	+		0	0
purple culm	0	0	0		+	+	+		0	0
red coleoptile	0	0	0		+	+	+		0	0

[1] data from field trials at three locations (Rimle, 1995); CS and CS(*T. spelta* 5A) grown in the glasshouse

whole length by the shortening of the *C/c* gene, i.e. those ears had segments of different ear types (see Rimle, 1995).

The first and the fourth quartile of the F_2 population, sorted according to length of the ear internodes, showed different means at the p=0.001 level for all characters (Table 4.1.2) .

The tightness of the glumes and the length of the ear internodes, which were regarded as the main spelta characters on chromosome 2D and 5AL respectively, were indeed inherited as independent loci (r=0.11, p=0.22). However, both characteristics had similar linkages to morphological traits.

Single plants with possible breakage of the Q/q-complex, e.g. lax ear combined with short stand, were not detected, but one plant, #108, combined very short ear internodes with a long culm.

Photo 4.1.1: The variation in the lengths of ear internodes results in different types of ears, from speltiforme to compactoid, in a F_2 population of Arina x Oberkulmer.

Table 4.1.2: Morphological and agronomical characters of 132 F_2 plants of Arina x Oberkulmer (Obk) grown in the glass-house; the F_2 plants were sorted in ascending order according to the length of their ear internodes. The character means and standard deviations for the population quartiles 1- 4 are shown.

		F_2 Arina x Oberkulmer in order of increasing lengths of ear internodes ---------- quartiles ----------							
trait	correlation to length of ear internode	1	2	3	4	range	mean	Arina	Obk
length of ear internode [mm]	------	4.46 ±0.9	6.01 ±0.3	6.92 ±0.2	8.08 ±0.7	2.86 to 10	6.37	5.36 ±0.4	7.90 ±0.7
plant height [cm]	0.47***	85 ±15.4	95 ±14.4	97 ±12.8	101 ±12.6	51 to 129	94	-	-
peduncle [cm]	0.62***	42.0 ±10.7	51.0 ±7.1	53.8 ±9.2	57.3 ±7.6	20 to 78	51	-	-
elongation	0.58***	2.11 ±0.1	2.18 ±0.2	2.27 ±0.2	2.39 ±0.2	1.9 to 2.8	2.24	2.0 ±0.2	2.9 ±0.2
brittleness of the rachis	0.30***	4.38 ±2.9	8.33 ±3.1	8.04 ±1.8	8.57 ±0.8	1 to 9	4.5	1	9
tightness of the glumes	0.42***	1.61 ±1.8	1.42 ±1.0	3.15 ±2.5	3.97 ±3.1	1 to 9	4.5	1	9

4.1.3 Discussion

A comparison of general morphology and architecture of wheat and spelt plants was made by Rimle (1995). The relatively constant spelt : wheat ratios of plant height (1.52), peduncle length (1.31), length of ear internodes (1.47 and its resultant ear length 1.51), as well as elongation of caryopses (1.33) (see 4.1.1) was explained by a dominant 'extension factor' in spelt. The substituted chromosome 5A of *T. spelta* in the winter wheat cultivar 'Hobbit' elongated whole plants and ears by factors of 1.12 and 1.32 respectively (Snape et al., 1985). The Chinese Spring line in comparison with Chinese Spring (*T. spelta* 5A) showed more variable ratios. The total plant height was significantly lower in the (*T. spelta* 5A) substituted line. The length of the parts of the upper culm, however, was affected as expected with the exception of kernel elongation (1.06). We suggest that this is evidence of the location of the 'extension factor' on 5A and suspect the Q locus to be its main cause. The elongated ear internodes and peduncles in Chinese Spring (*T. spelta* 5A) on the one hand and the shorter lower culm internodes on the other might be due to the influence of the potent vernalisation locus (*Vrnl*) on (*T.spelta* 5A) in a vernalised spring wheat genetic background. A similar mechanism which shortens the culm up to the flag leaf node, as in Forno, is possible. The elongation of caryopses must be determined by a different gene locus.

The length parameters discussed above were strongly influenced by environmental factors. The means of plant heights at three field locations of F_2 crosses of Arina x Oberkulmer differed by 10 cm from year to year. But, again, the ratio of plant height to ear length, and with it the general architecture of the plants, remained remarkably constant over years and locations (Rimle, 1995). Zeven and van Hintum (1992) also found a correlation of plant height to the length of the ear internodes of $r= 0.29$ ($p< 0.01$) in 183 North American hexaploid landraces and improved cultivars and outlined the association of dense ears with short plants.

We concluded above that the elongated shape of spelt caryopses was not determined by the same 'elongation factor' and now interpret the elongated shape to be a result of the strength of the tenacious glumes. The tight glumes do not allow the caryopses to expand easily along their transversal axes. The intensified elongation along the longitudinal axis is the response of the expanding caryopses, resulting in elongation as compared to those caryopses which developed between loose glumes. This is in good agreement with the reports of Millet (1986a,b) who

found that large floret cavities, arranged on long ear internodes, induce the growth of large grains. An indication of the strength of the glumes is given by Nilsson-Leissner (1926) who observed the imprint of the main vein of the glume on the dorsal side of caryopses from spelt plants. He suggested that the 'degree of spelting' could be estimated from the depth of this imprint, provided that development and ripening were regular. Based on these observations, the elongation of the caryopses would be nothing more than the phenotypical expression of the *Tenacious glume* locus on 2D. The elongation could be used as a marker for 'tightness of the glumes' ($r= 0.62^{****}$) and the growth rate of the coleoptile (GR 1; $r= 0.35^{****}$). The correlation of elongation to 'tightness of the glumes' and GR 1 (see below) is expected to be even higher in homozygous material and plants with regular grain filling.

The characters 'tightness of the glumes' and 'brittleness of the rachis' could not be analysed accurately, because the scale of the scoring used here was not precise enough. The development of methods for measuring the physical strength of the tightness of the glumes and the shearing force of the rachis would be very valuable in further studies.

4.2 Pigment characters

4.2.1 Pigment characters of wheat x spelt crosses

The *Purple Culm* and the *Purple Pericarp* characters, both of which were good markers for separating the wheat and spelt groups, were inherited differently. While the *Purple Culm* showed a type of dominant-recessive mode of inheritance, the *Purple Pericarp* was inherited maternally with additive gene action.

In several reciprocal F_1 crosses of wheat and spelt the colour of the culm was light purple. Twelve plants of 99 F_3 families were scored after five days of germination and growth at hypoxia

Tab. 4.2.1: Inheritance of *Purple culm* in F_2 Arina x Oberkulmer based on F_3 families.

purple culm		intermediate		green culm
2	:	83	:	14
2	:	97		

stress followed by one day at low light intensity (Tab. 4.2.1). Two families had dark purple culms (i.e. all 12 plants were scored as purple), 83 displayed intermediate colouration (i.e. the plant scores within the family ranged from purple to intermediate to green), and 14 were green (i.e. all plants of the family had green culms).

The test values of X^2=3.023 for 15:1 = (green+intermediate) : purple and X^2=0.135 for 63:1 = (green+intermediate) : purple fitted a two and three gene model respectively. The three gene model for *Purple Culm* of Gale and Flavell (1971) is confirmed by the better X^2 value. For intermediate inheritance we would expect one to two (1/64) green families. With complete dominance 41 to 42 green families (27/64) would be observed. We found 14 families with green coleoptiles and, therefore, suspect a partial dominant mode of inheritance for *Purple Culm*. The surplus of green culms could hardly be explained by the lack of a sufficient environmental stimulus inducing the colouration, because it needed 12 green F_3 plants to classify a F_2 genotype green. It is unlikely that all of those 12 plants, analysed in three replications, failed to express anthocyans in the culm and that such a rare constellation might have happened 12 times.

The character *Purple Culm* did not correlate with any other morphological character or with coleoptile length and growth (see below), neither at the single plant nor at the family level.

The *Purple Pericarp* showed a typical maternal inheritance in reciprocal crosses of Arina and Oberkulmer and of Bernina and Rouquin (see Fig. 2.1). Of 20 F_1 caryopses, i.e. caryopses from the mother plant, thus combining maternal tissue with a genetically F_1

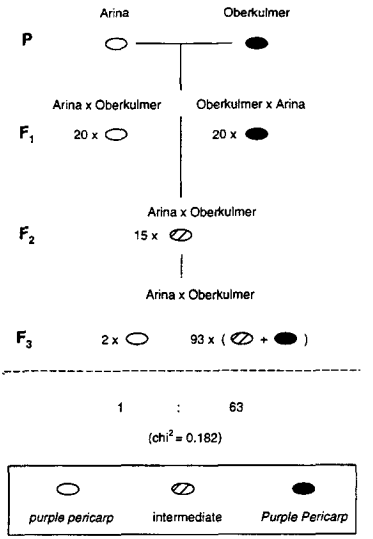

Fig. 4.2.1: The inheritance of *Purple pericarp* in F_1, F_2 and F_3 caryopses from crosses between Arina and Oberkulmer.

embryo, all showed the same colouration as the mother; 15 F_2 caryopses of each cross showed a light colouration of the NaOH extraction buffer. In a field grown F_3 population of Arina x Oberkulmer, two caryopses of 95 were colourless. This fitted the 1 : 63 ratio with X^2= 0.18. The variation in the intensity of the colouration could not be reliably attributed to different classes, and, therefore, only coloured and colourless groups were analysed. We take this infinite variation as further evidence of the location of the pigment in the maternally inherited pericarp, as has already been shown by Piech and Evans (1978) for a cross of the tetraploid wheat cultivar 'Nero' x Chinese Spring.

4.2.2 Discussion

The anthocyanin based characters did not prove to be reliable markers. Although histological location (Bradbury et al., 1956), biochemical composition (Dedio et al., 1972), and the genetics (Piech and Evans, 1978; Gale and Flavell, 1971) of both anthocyanins in the pericarp and the culm were known, no correlation with any of the other morphological or physiological traits were detected (see below). The physical properties of wheat and spelt pericarp layers do not seem to be altered by colouration. The *Purple culm* genes, too, could not be correlated directly with any of the characters tested. In young Oberkulmer plants, deep purple of the first leaves was induced by irrigating with 25 ppm Cu^{++}. The colouration segregated in a F_2 population of 124 Arina x Oberkulmer seedlings in a 1:3 ratio for green : coloured leaf blades ($p< 0.01$; data not shown). The anthocyanin colouration seems to be a typical stress reaction of spelt genotypes. But the genetic basis of the anthocyanin formation in different tissues and development stages is unclear, as are the stimuli which switch on the appropriate genes.
Nevertheless, anthocyanin formation, as a reaction to abiotic stress (exposure to high light intensity), must be advantageous for the plants, because it would not be very efficient to produce energy-rich metabolites with no ecological advantage. These characters will be discussed later. Investigations in our laboratory detected 84% (Zürich-Reckenholz) and 97% (Oberwallestalden) purple caryopses after two years of growth at the location.

4.3 Early shoot growth

4.3.1 Coleoptile length and growth rates of cultivars at different temperatures

Preliminary experiments demonstrated that, after three hours of soaking, the percentage of absorbed water was identical for wheat and spelt and sufficient for germination.

Table 4.3.1 shows the coleoptile length and the growth rates GR 1 and GR 2 of wheat and spelt cultivars at 6, 12, 18, 24, and 30°C. The coleoptiles of all cultivars were longest at 6°C and shorter at higher temperatures. A slight increase at 18°C corresponded with the optimal growth temperature for coleoptiles (Allan et al., 1962; Bhatt and Qualset, 1976; Huang and Taylor, 1993) of different genotypes (Fig. 4.3.1 a). The ranking for the coleoptile length of the cultivars was fairly constant for all temperatures used in our experiments with Forno having the longest coleoptiles and Arina the shortest.

The spelt coleoptiles of all cultivars grew faster than the wheat coleoptiles (GR 1), while the emerged primary leaves grew at approximately the same rates (GR 2) (see Fig. 4.3.1 b, c, and Tab. 4.3.1).

The standard deviation of growth rates was higher at all temperatures for wheat than for spelt due to an uneven and often retarded germination but never exceeded the 25.3% for GR 1 of Bernina at 12 °C. Considering the average standard deviation of growth rates of 12% (13,9% for GR 1, 10% for GR 2) for wheat at all temperatures and of 9% (9.6% for GR 1, 8.4% for GR 2) for spelt, the wide ratios GR 1_{wheat}/GR 1_{spelt} of 0.71 to 0.83 can be regarded as constant in the 6 to 30°C interval. However, at 6°C the wheat group had a lower GR 2 than a GR 1 relative to the spelt group, whereas at higher temperatures the ratio GR 2_{wheat}/GR 2_{spelt} was much higher and homogeneously close to 1 (Fig. 4.3.1). This indicates that the initial coleoptile growth of spelt was superior over the whole temperature range, whereas the elongation of the primary leaf was slightly higher only at low temperatures.

Between Chinese Spring and Chinese Spring (*T. spelta* 5A) no differences were found for coleoptile length and growth rates (data not shown). *Rht 1*, a GA_3-insensitive dwarfing gene, reduced the coleoptile length but not the growth rate of April Bearded.

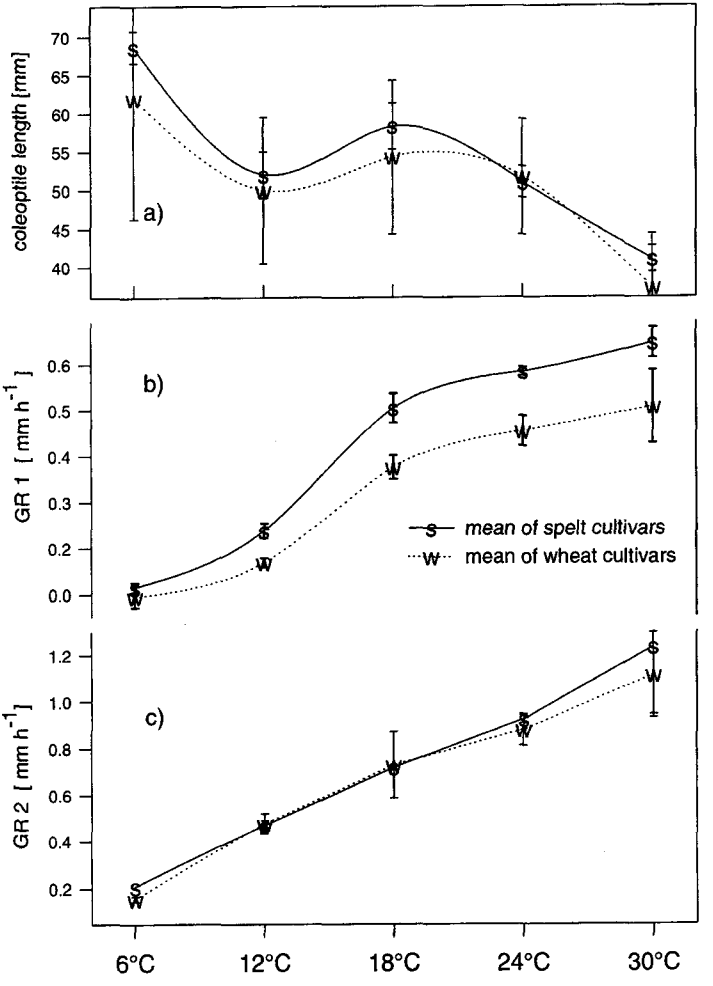

Fig. 4.3.1: Mean coleoptile lengths (a) and growth rates of coleoptiles GR 1 (b) and primary leaves GR 2 (c). The values are averages of sets of wheat (Arina, Bernina, Forno) and spelt cultivars (Altgold, Oberkulmer, Rouquin). Standard deviation is indicated by vertical lines.

Table 4.3.1: Coleoptile length and growth rates GR 1 and GR 2 under different temperatures for wheat cultivars Arina (Ari), Bernina (Ber), and Forno (For); and spelt cultivars Altgold (Alt), Oberkulmer (Obk), and Rouquin (Rou).

		Ari	Ber	For	wheat	Alt	Obk	Rou	spelt	GR 1_{wheat}/ GR 1_{spelt}	GR 2_{wheat}/ GR 2_{spelt}
	coleoptile length [mm]	44	69	73		69	66	71			
6°C	GR 1 [mm h^{-1}]	0.07	0.11	0.11	0.10	0.12	0.12	0.11	0.12	0.83	
	GR 2 [mm h^{-1}]	0.16	0.17	0.15	0.16	0.21	0.21	0.21	0.21		0.75
	coleoptile length [mm]	40	51	59		55	49	52			
12°C	GR 1 [mm h^{-1}]	0.16	0.17	0.18	0.17	0.22	0.25	0.24	0.24	0.71	
	GR 2 [mm h^{-1}]	0.46	0.45	0.53	0.48	0.49	0.47	0.45	0.47		1.02
	coleoptile length [mm]	44	55	64		61	55	59			
18°C	GR 1 [mm h^{-1}]	0.35	0.40	0.38	0.38	0.54	0.48	0.49	0.50	0.74	
	GR 2 [mm h^{-1}]	0.60	0.70	0.88	0.73	0.72	0.73	0.71	0.72		1.01
	coleoptile length [mm]	44	52	59		53	49	51			
24°C	GR 1 [mm h^{-1}]	0.44	0.43	0.49	0.45	0.59	0.59	0.58	0.58	0.78	
	GR 2 [mm h^{-1}]	0.80	0.90	0.93	0.88	0.93	0.90	0.93	0.92		0.95
	coleoptile length [mm]	30	38	44		40	40	43			
30°C	GR 1 [mm h^{-1}]	0.42	0.54	0.57	0.51	0.67	0.61	0.66	0.65	0.79	
	GR 2 [mm h^{-1}]	1.00	1.01	1.32	1.11	1.57	1.06	1.08	1.23		0.90
										0.77	0.93

4.3.2 Early shoot growth of wheat x spelt crosses

a) Early shoot growth of reciprocal F_1 crosses

Because principal differences in pericarp characters of wheat and spelt cultivars were found, we studied the possible influence of maternal effects on the coleoptile extension, and, in the same experiment, estimated the degree of heterosis. All F_1 crosses with the spelt mother showed *mid parent heterosis* in all crosses for coleoptile growth after 72 hours at 22°C in the dark (Tab. 4.3.2).

Table 4.3.2: Coleoptile length [mm] and *mid parent heterosis* of 30 caryopses of reciprocal F_1 crosses of wheat (Arina, Bernina, Forno) and spelt (Oberkulmer, Rouquin) cultivars after germination and growth for 72 hours at 22°C in the dark.

F_1 cross	coleoptile length [mm] (std. dev)	*mid parent heterosis* [%]
Arina x Oberkulmer	28.4 (6.2)	+7.5
Oberkulmer x Arina	32.7 (7.2)	+23.7
Bernina x Oberkulmer	23.0 (8.7)	-12.5
Oberkulmer x Bernina	28.1 (9.1)	+6.9
Bernina x Rouquin	21.6 (9.3)	-9.09
Rouquin x Bernina	30.2 (9.3)	+27.1
Forno x Rouquin	17.3 (8.5)	-28.39
Rouquin x Forno	30.5 (7.9)	+26.24
	mean of wheat x spelt	-10.6
	mean of spelt x wheat	+21.0

All crosses with the wheat mother developed shorter coleoptiles than crosses with the spelt mother. The crossing partner influenced the coleoptile length, but the difference was insignificant. F_1 crosses were always superior to the wheat parent (data not shown), and crosses where Rouquin was the mother had a *better parent heterosis*. Although the number of replications was just too low to detect

significant maternal effects for single combinations, the longer coleoptiles of all crosses with the spelt mother and their reciprocal counterpart indicate that, to some extent, the maternally predetermined tissue or the plasmon, determined the trait. With a longer growth period, 120 hours, the differences in the combined length of coleoptiles and primary leaves were less marked between the reciprocal crosses (data not shown).

b) Early shoot growth of F_3 families of Arina x Oberkulmer

Because the germination and growth of Arina caryopses was uneven in some series, each coleoptile length was divided by the mean of all plants in the series in order to minimise the effects of the series. Families with fewer than 15 and 18 measured plants contributing to the mean length after 72 and 120 hours respectively were not selected for further analysis. As a result, the segregation of 116 and 118 families was finally studied.

After 72 hours of growth under the conditions described above, the F_3 family means of the relative coleoptile lengths ranged from 0.494 to 1.576. The values for the parents, Arina and Oberkulmer, were 0.722 and 1.192 respectively. The F_3 population heterosis was +4.5%.

The population did not fit the normal distribution, and segregation into several distinct classes was investigated. Fig. 4.3.2 a shows the sorted family values and the class borders with their X^2 values. Class borders were considered to be between family #14 and #15 ($X^2 = 0.1525$ for a 7:57 segregation), families #100 and #101 ($X^2 = 0.9710$ for 57:7), and families #114 and #115 ($X^2 = 0.0137$ for 63:1). The clear cuts for the 7:57 and 57:7 ratios indicate the two flanking classes of (1:6):(15:20:15):(6:1) in a three gene model. The 63:1 cut is one homozygote versus the other homozygote and the heterozygotes in a three gene model. Other possible models from one to five genes were not significant.

Although a correlation of $r= 0.827^{****}$ between the shoot lengths of the first (coleoptiles) and second phase (primary leaves) was calculated, a clear classification on the basis of coleoptile length (Fig. 4.3.2 a) was no longer possible two days later (Fig. 4.3.2 b).

The even increase in family means in a newly sorted order (Fig. 4.3.2 c) and the more reliable fit to the normal distribution (98%, p=0.28) supported this observation.

The equalisation of shoot length is in accordance with the results in section 4.3.1 where the coleoptile growth rate (GR 1) differed to a much greater extent between the wheat and the spelt cultivar set than did the growth rate of primary leaves (GR 2). Therefore, a clear tendency exists towards shoots of equal length at later stages of growth.

The correlation of the shoot length of the F_3 families with the elongation ratio of the caryopses after 72 ($r=0.35^{****}$) and 120 hours of growth ($r=0.28^{****}$) became increasingly weaker.

A significant correlation of growth rates with grain weight was not found.

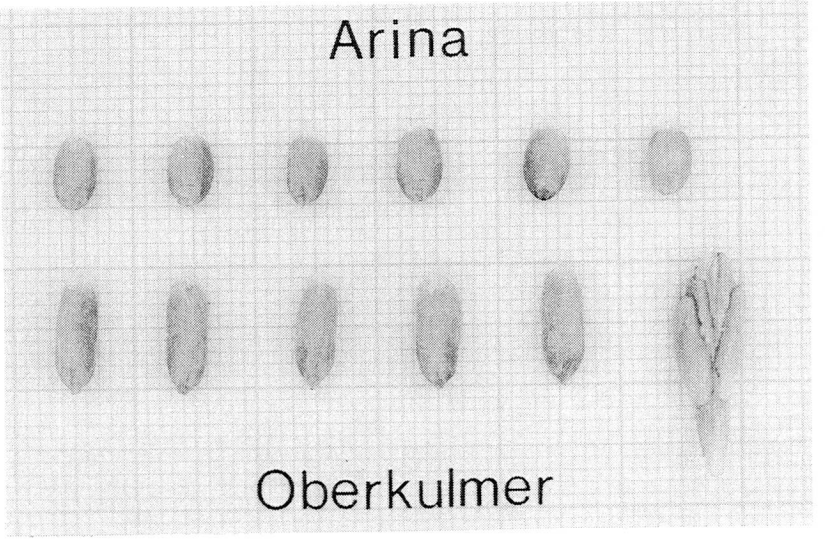

Photo 4.3.1: Caryopses of the wheat cultivar Arina and of the spelt cultivar Oberkulmer and a spikelet of Oberkulmer. The elongated shape of Oberkulmer can be clearly seen. For further information see sections 4.1, 4.3, and 5.

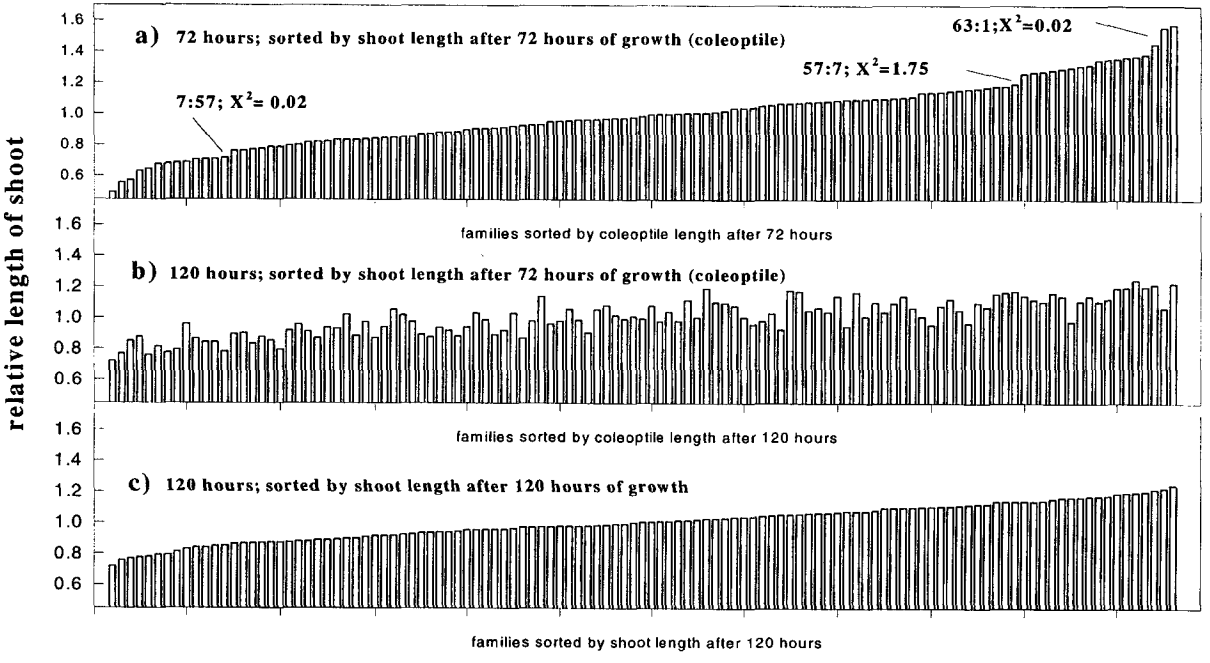

Fig. 4.3.2: Relative length of shoots (coleoptiles) (a) after 72 hours and of shoots (coleoptiles and primary leaves) (b,c) after 120 hours of growth of F_3 families of Arina x Oberkulmer, sorted according to the coleoptile length after 72 hours (a,b) and after 120 hours of growth at 22°C (c).

4.3.3 Early shoot growth under hypoxia

In all hypoxia tolerance experiments, with the exception of the F_3 families, the seedling growth index, SGI, was used to describe the growth of the seedlings. In preliminary experiments with cultivars, SGI and shoot length were correlated at $r=0.90^{****}$ for the control and $r=0.78^{****}$ under hypoxia stress.

4.3.3.1 Early shoot growth under hypoxia of cultivars and lines

Table 4.3.3 summarises the SGI of wheat and spelt cultivars under hypoxia stress.

Table 4.3.3: Seedling growth index SGI of wheat and spelt cultivars at 21% O_2 (control) and 1% O_2 (stress) after 120 hours of growth at 22°C in the dark.

	SGI		
cultivar	control (std. dev.)	stress (std. dev.)	stress/control (tolerance)
Arina	0.175 ±0.092	0.0161 ±0.0128	0.091
Bernina	0.177 ±0.061	0.0202 ±0.0076	0.113
Forno	0.127 ±0.028	0.01267 ±0.0066	0.099
wheat	**0.160**	**0.016**	**0.098**
Altgold	0.317 ±0.027	0.0517 ±0.0080	0.162
Oberkulmer	0.297 ±0.032	0.0655 ±0.0086	0.220
Rouquin	0.327 ±0.043	0.0434 ±0.0048	0.132
spelt	**0.313**	**0.0535**	**0.171**
wheat /spelt	**0.51**	**0.30**	**0.57**

The SGI of wheat was already 49% lower than that of spelt under normal conditions and as much as 70% lower under hypoxia (1% O_2). Even when the generally low SGI of wheat was taken into account, the hypoxia tolerance of wheat was 43% less than that of spelt. In both sets of cultivars, the SGI under hypoxia stress was more variable than for the control. Generally, the SGI separated the wheat and the spelt cultivars into two distinct groups for ambient and hypoxic conditions. However, variations in tolerance existed within the species. Rouquin, for example, clearly belonged to the spelt species with regard to its SGI at hypoxia but was closest to wheat with regard to its tolerance.

The common trend in breeding wheat and spelt leads to short plants. If this process is achieved by including dwarfing genes, it will have no influence on the SGI at 1% O_2, as was found when isogenic lines of the winter wheat 'April Bearded' with *Rht*1, *Rht*2, *Rht*3, *Rht*1+2, and *Rht*2+3 genes and their respective *rht*-lines were compared (data not shown). Tab. 4.3.4 presents additional data on SGI and hypoxia tolerance for old wheat and new spelt cultivars. It is clear from this table that relatively low SGI at 1% O_2 are possible in new spelt cultivars, whereas values for old wheat cultivars can be close to those of old spelt cultivars (compare Tab. 4.3.4). This indicates that hypoxia tolerance is not related to the morphology of the spelt ear.

Tab. 4.3.4: Mean seedling growth index (SGI) and hypoxia tolerance of two new spelt cultivars, with wheat introgressions, and two old wheat cultivars.

	SGI		
cultivar	control	stress	stress/control (tolerance)
spelt			
Lueg	0.247	0.015	0.06
Ostro	0.248	0.035	0.14
wheat			
April Bearded	0.357	0.041	0.12
Probus	0.267	0.026	0.10

4.3.3.2 Early shoot growth under hypoxia of wheat x spelt crosses

a) Reciprocal F_1 crosses

Table 4.3.5 summarises the SGI of reciprocal wheat and spelt crosses. The SGI under hypoxia of F_1 crosses was not significantly influenced by the mother. All crosses showed a positive *mid parent heterosis* between 17.4 and 53.1%. No consistent positive maternal effect on wheat or spelt was found, and the difference between reciprocal crosses never exceeded 10.6%.

Table 4.3.5: Seedling growth index SGI and *mid parent heterosis*, calculated for 25 seedlings of reciprocal F_1 crosses of wheat (Arina, Bernina, Forno) and spelt (Oberkulmer, Rouquin) cultivars after germination and growth for 120 hours at 22°C and 1% O_2 in the dark.

F_1 cross	SGI [ ]	mid parent heterosis [%]
Arina x Oberkulmer	0.075	+53.1
Oberkulmer x Arina	0.074	+51.0
Bernina x Oberkulmer	0.065	+36.8
Oberkulmer x Bernina	0.070	+47.4
Bernina x Rouquin	0.064	+17.4
Rouquin x Bernina	0.068	+24.8
Forno x Rouquin	0.077	+36.3
Rouquin x Forno	0.072	+27.4
	mean of wheat x spelt	+35.9
	mean of spelt x wheat	+37.7

One combination, Arina and Oberkulmer, reached a positive *better parent heterosis* for both crosses (data not shown).

b) F_3 families of Arina x Oberkulmer

For technical reasons it was impossible to completely standardise the gas flow. Even slight fluctuations in the flow rate (30 Nl/h) can result in remarkable differences in the total amount of oxygen supplied in an experiment lasting 120 hours. For this reason, the relative length of each plant was calculated to the mean of the series, and the average relative shoot length of each plant from all series was used as the F_3 family mean. The data is not shown in detail. A relative shoot length of 1.0 corresponded to shoot lengths of 20, 23, and 18 mm for the series one to three at 1% O_2. The relative shoot length under hypoxia (1% O_2) ranged from 0.592 to 1.622 in the 116 families analysed, whereas the relative lengths of the coleoptile of the parents, Arina and Oberkulmer, were 0.364 and 1.212 respectively. The mean of the population was 21.2% higher than the mean of the parents (0.788), thus indicating a positive heterosis. For the control (21% O_2), the relative shoot length ranged from 0.718 to 1.288. The relative shoot length of 1.0 corresponded to an absolute shoot length ranging from 70 to 79 mm for the individual series. The normal distribution of F_3 family means was fitted with 97% at a confidence level of p=0.06.

The relative shoot length of the F_3 families under hypoxia was correlated to the control ($r= 0.41^{****}$) and to the relative coleoptile length after 72 hours of growth ($r= 0.50^{****}$).The correlation of hypoxia tolerance (=shoot length hypoxia/shoot length normoxia) to the shoot length under hypoxia was much stronger than to the shoot length under normoxia ($r= 0.78^{****}$ and $r= -0.21^{**}$ respectively), thus indicating the dominant role of the resulting shoot length under hypoxia for 'hypoxia tolerance'.

The seedlings grown under hypoxia did not differ morphologically to any extent from seedlings grown under normoxia, and the growth process of all seedlings continued normally after the completion of the experiment.

4.3.4 Correlations between characters of mature plants and seedling shoot growth under two oxygen regimes in a wheat x spelt cross

Correlations between selected morphological characters and shoot length at different stages and under reduced oxygen supply (hypoxia) are shown in Table 4.3.6. The experimental conditions were described earlier for the different

treatments. Significant but still rather low correlations existed for the elongation of the caryopses to the relative shoot lengths for all treatments. The highest correlation was found for shoot length after 72 hours of growth, thus representing the coleoptile growth rate. The correlation to the tightness of the glumes was weaker and less significant.

Tab. 4.3.6: Correlations for selected morphological characters of 116 F_2 Arina x Oberkulmer plants to the relative seedling shoot length of their corresponding F_3 families after 120 hours under hypoxia stress (1% O_2), and after 72 and 120 hours of growth at 21% O_2 (control) and 22°C.

	stress	control	
F_3 character:	under hypoxia	after 72 hrs.	after 120 hrs.
F_2 character:			
length of ear internodes	-0.05	0.10	0.09
plant height	-0.04	0.06	0.02
peduncle	-0.04	0.07	0.01
ear length	-0.07	0.14	0.08
elongation	0.23***	0.35****	0.28***
brittleness of the rachis	0.06	-0.10	-0.04
tightness of the glumes	0.16*	0.19**	0.11
single grain weight	-0.11	-0.29***	-0.21

4.3.5 Seedling traits of spelt x wheat populations after five cycles of growth in two environments

Nine years after their initiation and after five years of growth at climatically different locations, seedlings of spelt x wheat populations differed in mean shoot lengths after 72 hours of growth at 22°C (Table 4.3.7). The genetic diversity of the populations resulted in large standard deviations of up to 62% of the mean.

Tab. 4.3.6: Mean shoot length of different numbers of seedlings (n) after growth for 72 hours at 22°C in the dark of wheat/spelt populations evolved at Zürich-Reckenholz or Oberwallestalden. These two main populations were subdivided according to the tightness of the glumes of the mother ear. Means with the same letter are not different (p=0.05). F values of ANOVA for the field location and the tightness of the glumes and their interaction are displayed with their significance levels.

	Zürich-Reckenholz	Oberwallestalden	Total:
	n / length [mm]		
tight glumes:	298 / 33.6	302 / 36.8	600 / **35.2 B**
free-threshing:	271 / 22.9	281 / 33.4	552 / **28.2 A**
Total:	569 / **28.3 A**	583 / **35.1 B**	

Analyses of variance:	degree of freedom	mean square	F
location	1	13430	39.2****
tightness of the glumes	1	14131	41.2****
location*tightness of the glumes	1	3868	11.3****

Nevertheless, the free-threshing seeds, evolved at the favourable location Zürich-Reckenholz, had, on average, the shortest shoots.
Seedlings from the hulled caryopses at Zürich-Reckenholz did not differ from free-threshing seeds from Oberwallestalden ($p<0.05$). Analyses of variance accordingly showed significant effects for ($p<0.001$) and strong interaction between the location and the tightness of the glume ($p<0.001$). This indicated that tight glumes are involved in the reaction to the environment with regard to coleoptile extension. Tight glumes can, for this reason, be regarded as an indirect selection trait for fast growing coleoptiles under field conditions.

Tab. 4.3.7: Mean shoot length of seedlings of different wheat/spelt populations after growth in the dark for 120 hours at 22 °C and 1% O_2. For further information see Tab. 4.3.6. Percentage values for hypoxia tolerance are shown in brackets.

	Zürich-Reckenholz	Oberwallestalden	Total:
	n / length [mm]		
tight glumes:	118 / 20.3 (60%)	113 / 17.2 (47%)	221 / **18.8A**
free-threshing:	94 / 18.0 (79%)	90 / 15.2 (46%)	184/**16.6 A**
Total:	212 / **19.2 A**	203 / **16.2 B**	

Analyses of variance:	degree of freedom	mean square	F
location	1	901	5.1**
tightness of the glumes	1	449	2.5
location*tightness of the glumes	1	4	0.0

Hypoxia tolerance was less affected than the length of the coleoptile by selection pressure through field location and glume tightness . Although the trait was very variable (std. dev. up to 110%), significant effects were detected. The dehulled seeds from Zürich-Reckenholz and the free-threshing seeds from Oberwallestalden

were significantly different only as far as mean seedling shoot length was concerned ($p<0.05$). Only the selection pressure of the location had a slight significant effect on the lengths of the coleoptiles under hypoxia at Zürich-Reckenholz; these seedlings showed a greater tolerance to hypoxia and the coleoptiles were longer.

4.3.6 Discussion

Most investigations documented in the literature are concerned with the absolute coleoptile length. Coleoptile growth, which we defined as elongation over time, is rarely analysed, though it can be an important component of seedling vigour. It sometimes plays an important role in the complex trait 'time to emergence' which is used in standard seed vigour tests (ISTA, 1987). Our results reflect the early shoot growth rate as an integral component of these complex phenomena under two oxygen regimes, without effects of pathogens or soil compaction.

Coleoptile growth

A temperature optimum for coleoptile length at about 16°C as reported by Burleigh et al. (1964) was confirmed again, but a second increase in coleoptile length was found at 6°C (Fig. 4.3.1). We did not find a relationship between plant height and coleoptile length, as was the case for semidwarf wheats (Allan et al., 1961; Allan et al., 1962b; Chowdhry and Allen, 1963) and, to some extent, for standard cultivars (Sunderman, 1964; Allan et al., 1962b). Marais and Botma (1987) and Whan (1976) found that parentage and regional origin had a significant effect on the length of the coleoptile of a large set of Australian wheats.

Those findings, together with equal growth rates and lengths of Chinese Spring and Chinese Spring (5A *T. spelta*) are good evidence that coleoptile growth is largely independent of morphological spelt ear characters.

The positive correlation of coleoptile growth to elongation of the caryopses ($r=0.23$) may, hypothetically, be explained by a greater surface area : volume ratio for elongated seeds, thus providing better water flow to the endosperm during germination. This seems, however, rather unlikely, because the transport of water and solubles to the scutellum is purely osmotic (Bewley and Black, 1994) and,

therefore, is limited by the sink capacity of the embryo. This positive but weak correlation might indicate a genetic linkage for the *Tenacious glume* gene and other unknown factors contributing in some way to variation in coleoptile growth.

Histological differences in the pericarp were reported for wheat genotypes (Nilsson-Ehle, 1914; Bradbury et al., 1956) and are assumed to influence the water absorption and loss. With our method, we excluded differences in water imbibition which effect coleoptile growth (Masle and Passioura, 1987). Because significant correlations between grain weight and growth characters were missing, we concluded that physiological differences must have been the main causes of the different growth rates. This is in accordance with findings of Addae and Pearson (1992).

It was shown that a limited maternal effect existed, which could not be explained by pericarp and seed coat, inherited from the maternal parent. If enzyme activity depended on the number of copies of a structural gene in the endosperm, as Carlson (1972) demonstrated in trisomic barley, one would expect such a maternal effect. Because the endosperm is triploid tissue, the corresponding genotypes of this tissue would be *www* for wheat, *sss* for spelt, *wws* for wheat x spelt, and *wss* for spelt x wheat crosses. Fick and Qualset (1975) found correlations of amylase activity with coleoptile length ($r=0.82^{***}$) and mature plant height ($r=0.75^{***}$) for a standard wheat x dwarf wheat cross. They found one locus controlling the amylase activity and suspected blockage of GA_3 rather than inadequate biosynthesis to be the reason for dwarfism. All wheats do not have the same physiological bases of dwarfism (Allan et al.,1962b; Börner and Mettin, 1989); differences in our crosses, therefore, do not necessarily depend on the same structural gene for amylase activity. Other physiological mechanisms can be triggered by single structural genes; auxin, for example, is translocated from the endosperm of germinating maize to the tip of the coleoptile from where it regulates the growth of the cells of the elongating zone of that organ (Bewley and Black, 1994). Thus, a definite proof of cause cannot be based on the present data alone. Interestingly, we found different copy numbers between wheat and spelt for the gene *Carboxypeptidase 3* (*Cxp3*) (see section 4.4) which was formed in the GA_3-treated aleurone layers of de-embryonated seeds of wheat as well as in immature grains (Baulcombe et al, 1987b; Baulcombe and Buffard, 1983). PSR8, a cDNA clone of the *Cxp3* gene, revealed polymorphisms on all homoeologous arms of group 6 for a large set of wheat and spelt lines; this leads to speculation about the role of this

enzyme during the heterotrophic growth stages. The polymorphisms were based on the presence or absence of bands and thus different copy numbers could be postulated. This was in good agreement with findings of Baulcombe et al. (1987b) on the multigene familiy character of *Cxp 3*. Considering that, for the expression of a gene product at different stages of development, different members of a multigene family were recruited (Cullimore et al., 1984) and that *Carboxypeptidase 3* is also expressed in immature grains (Baulcombe et al., 1987b), these polymorphisms do not necessarily explain differences in coleoptile growth rates. Still, if the copy number of the GA_3-induced structural gene *Carboxypeptidase 3* is associated with a higher amylase activity, thus providing more substrate for metabolic processes, its role in coleoptile growth should be investigated further.
Allan et al. (1962b) associated the short coleoptiles of semidwarfs with fewer parenchyma cells. The same authors also stated that different genetic mechanisms govern the length of coleoptile parenchyma cells in different short wheats. If the variation in coleoptile length is due to fewer cells, the energy supply described above would become very important, because the mitosis in cell duplication is very energy consuming. If, as a second hypothesis, the variation in coleoptile length is due to the different lengths of the parenchyma cells, the energy supply would not be crucial, because the elongation of cells is based mainly on water uptake which does not consume metabolic energy.

Early shoot growth under hypoxia

The experimental stress conditions had a considerable impact on the seedlings. Al-Ani et al. (1985) reported very slow germination for wheat (*cv.* Capitole) at O_2 concentrations as low as 0.1%. They could not correlate this with the sensitivity of respiration to O_2 and concluded that fermentation processes yielded enough energy to compensate for the limited oxygen supply during germination. This was in accordance with our data: all cultivars germinated but at different rates. In hypoxia-stressed wheat seedlings (*cv.* Flavio), shoot elongation was reduced most between 5.0 and 2.5% O_2. The threshold of growth was fixed at 0.3% O_2 (Reggiani and Bertani, 1989). The viability of the shoots was also maintained at these hypoxic stress levels. Heichel and Day (1972) observed a small reduction in the germination rate but an important reduction in the coleoptile length of the wheat cultivar Stewart grown under hypoxia. All these experiments were carried out in gas flow

systems which removed the volatile anaerobic metabolites, e.g. cytotoxic ethanol or CO_2 (see Perata and Alpi, 1993). Crawford et al. (1987) stated that, under anoxia in static systems, seedling survival is generally markedly lower as compared to gas flow systems.

The effect of hormones produced in the root system as a response to mechanical stress (bonsai effect proposed by Masle and Passioura, 1987; see also Whitely and Dexter, 1982) could be excluded, since only hypoxia stress had an effect on the roots. Several environmental changes, brought about by flooding or waterlogging a soil (accumulation of CO_2, pH change, reduction of nutritive elements, products from anaerobic decomposition of organic matter, etc.), could also be excluded.

From these literature reports we concluded that the results of our experiments, conducted at 1% O_2, were (a) representative of a broad spectrum of wheat, (b) reflected differences in the ability of the shoot to elongate but not in germination itself, and (c) excluded shoot growth reduction as being a reaction to mechanical or other chemical stress.

One methodological problem is difficult to solve. Hypoxia tolerance, defined as shoot length hypoxia / shoot length normoxia compares physiological processes at different growth stages. While only the coleoptile growth was measured in 'shoot length hypoxia', the trait 'shoot length normoxia' normally includes the growth of the primary leaf. The latter process was similar for wheat and spelt in our experiments. However, comparing coleoptile growth at different times (coleoptile length hypoxia / coleoptile length normoxia) did not produce principally different values. Hypoxia tolerance values, calculated on these two bases, were correlated at $r= 0.63^{****}$. We preferred to use hypoxia tolerance based on the shoot growth, i.e. the control treatment, because it reflects the tolerance *sensu strictu,* and it is more suitable for the agronomic conditions in marginal regions where physical rather than physiological time sets the limits of growth.

Since shoot growth under hypoxia was measured on seedlings from naked spelt caryopses, the hypoxia stress tolerance must be predominantly physiologically determined (see also discussion above). Thus, the differences in hypoxia tolerance were explained by: (i) the expression of the coleoptile growth rate under hypoxia stress (see above) or (ii) genetic differences resulting either in an advantageous combination of many alleles or in a change in a physiological key position. (i) The correlation of $r=0.41^{****}$ between stressed and unstressed plants would be expected to be higher if only the same physiological processes were to influence coleoptile

extension under normoxic and hypoxic conditions. A maternal effect would have been detected if the same mechanism were responsible for variation in coleoptile growth under both oxygen regimes. Since no such effect was found, a locus overlying the one for shoot growth described before is postulated.
(ii) The ranking of shoot length within the stressed population as compared to the control, indicated that families with fast-growing coleoptiles do not necessarily produce long shoots under hypoxia. Indeed, families with good coleoptile growth and poor hypoxia tolerance, and vice versa, existed. Generally, much evidence exists for major genetic differences determining hypoxia tolerance. So far, no clear statements can be made about the genetic and physiological basis of the postulated 'hypoxia tolerance factor' of spelt. The results of section 4.3.5 will be discussed later.

4.4 Genetic mapping of wheat x spelt crosses using RFLP

4.4.1 Screening wheat and spelt cultivars and mapping a F_2 Arina x Oberkulmer population

4.4.1.1 General observations

With 76 probes and four restriction enzymes, the genomic DNA of three wheat (Arina, Bernina, Forno) and three spelt (Altgold, Oberkulmer, Rouquin) cultivars was screened for polymorphisms. Fifteen (19.7%) probes were monomorphic with all four enzymes. From the screening, 198 polymorphisms resulted which were suitable for proper identification and further analysis due to their known genetic location, their signal strength, or their copy number (Tab. 4.4.1). Twenty-seven percent and 16% of the screened loci were polymorphic with at least one enzyme within wheat and within spelt. Twenty-four percent of the loci were monomorphic within the cultivar sets but polymorphic between the two sets. The B genome proved to be the most polymorphic within and between the sets (20% polymorphic loci), while the A and D genomes were at lower levels (14% and 11.7% respectively) (data not shown).

Table 4.4.1: Degree of polymorphisms [%] within and between sets of wheat (Arina, Bernina, Forno) and spelt (Altgold, Oberkulmer, Rouquin) cultivars, screened at 198 selected loci dispersed over the genome.

homoeologous group	number of loci	within wheat	within spelt	between wheat and spelt only
1	15	45	5	10
2	51	20	21	21
3	27	14	19	42
4	32	38	37	33
5	38	19	11	13
6	27	32	11	31
7	18	20	8	20
total/mean	198	27	16	24

The markers which separated the three wheat and the three spelt cultivars were dominant in many cases. In 15% of the polymorphisms of spelt and in 21% of the polymorphisms of wheat, the absence of bands was characteristic. Those dominant polymorphisms were localized all over the genomes.

Of 93 loci scored for their segregation in 132 F_2 Arina x Oberkulmer plants, 83 loci showed a 1:2:1 or 1:3 segregation ($p<0.05$). Nine of the remaining 10 loci showing distorted segregation were located on chromosomes of groups 2 and 6. Table 4.4.2 lists the loci showing distorted segregation in our F_2 Arina x Oberkulmer cross.

The greatest distortion ratios were found for loci on chromosome 2D (*Xpsr304*, *Xpsr131*, *Xpsr932*) and group 6 (*Xpsr312*). A complete region of distorted segregation was found on chromosome 2D, where an interval of at least 56 cM (*Xpsr131* - *Xpsr304*) was affected.

Since many polymorphisms were dominant, the occurrence and frequency of such polymorphisms were studied for each group and genome. We could not, however, explane or detect any regularity in the occurrence of such events.

A total of 49 loci was used to construct the RFLP maps.

Table 4.4.2: RFLP loci showing distorted segregation in the F_2 generation. AA, BB, and AB indicate the homozygous allele of Arina, Oberkulmer, and the heterozygous allele respectively.

	frequency [1] of			
	AA	AB	BB	X^2 (1:2:1 or 1:3)
loci:				
Xpsr131-2A	93		16	6.2**
Xpsr933-2A	21	66	12	12.6***
Xpsr304-2B	16	65	25	7.0**
Xpsr304-2D	56	49		45.0****
Xpsr131-2D	65	49		62.3****
Xpsr932-2D	16	31	41	21.9****
Xpsr933-2(A or D)	10	61	12	18.4****
Xpsr312-6B	90		6	18.0****
Xpsr312-6X	6	78	30	18.2****
Xpsr150-7A	77		47	11.0****

[1] Numbers between the colums indicate dominant markers, for which only one homozygous allele, either AA or BB, could be identified, while the other homozygous allele and the heterozygotes were classed together.

4.4.1.2 RFLP screening of wheat and spelt cultivars and mapping in a cross of Arina x Oberkulmer

Homoeologous group 1

Only the short arm of chromosome 1B was polymorphic in this group (Fig. 4.4.1). The hybridisation patterns of digested DNA with PSR596, described by Siedler et al. (1994) as being characteristic of spelt lines in European wheat and spelt breeding material, were confirmed. Of a total of four polymorphims only one (PSR544) was restricted to one enzyme and was not polymorphic between the species. The other three detected polymorphisms with all four enzymes, and all were polymorphic between the sets, thus indicating a major change in the DNA

sequence of the wheat and spelt cultivars tested.

Homoeologous group 2

Several polymorphisms between wheat and spelt were located on the long arms of chromosomes 2A and 2B (probes PSR901, PSR934, PSR609, PSR304) whereas no probe / enzyme combinations revealed a polymorphism between wheat and spelt on 2DL.

A region of distorted segregation was identified on chromosome 2D. The telomeric region of 2DS has two markers with normal segregation (*Xpsr566*, *Xpsr933*; $p<0.05$). Towards the centromere, *Xpsr131* inherited too many wheat alleles and, on the long arm, *Xpsr 304* and *Xpsr901* both had too many wheat alleles. *Xpsr540* segregated normally in a 1:2:1 ratio ($X^2=1.723$), but *Xpsr932* had a surplus of spelt alleles, whereas *Xpsr934* fulfilled the expected segregation ratio (see Fig. 4.4.1). Although the loci of *Xpsr540* and *Xpsr932* were linked (44.4 cM) and were previously mapped on 2DL between *Xpsr131* and *Xpsr934* (Devos and Gale, 1993), they could not be integrated in this map (see below).

Homeologous group 3

PSR1196, PSR907, PSR903, and PSR598, on short arms of group 3 chromosomes, all produced monomorphic bands within the wheat and spelt sets and polymorphic bands btween the two sets. With four restriction enzymes, PSR904 detected only two species specific polymorphisms on 3AL and 3DL. We therefore classified this as minor polymorphisms, contrary to the polymorphisms on the short arms.

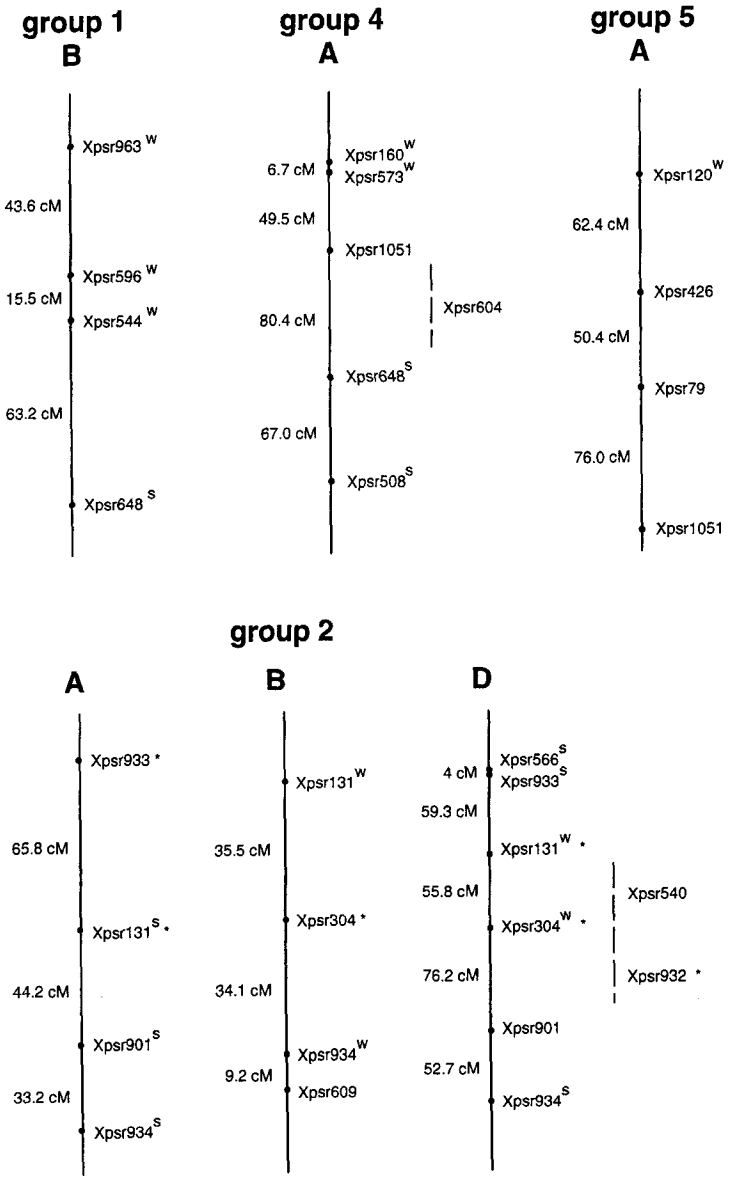
group 1
B
Xpsr963 W
43.6 cM
Xpsr596 W
15.5 cM
Xpsr544 W
63.2 cM
Xpsr648 S
group 4
A
6.7 cM
Xpsr160 W
Xpsr573 W
49.5 cM
Xpsr1051
80.4 cM
Xpsr604
Xpsr648 S
67.0 cM
Xpsr508 S
group 5
A
Xpsr120 W
62.4 cM
Xpsr426
50.4 cM
Xpsr79
76.0 cM
Xpsr1051
group 2
A
Xpsr933 *
65.8 cM
Xpsr131 S *
44.2 cM
Xpsr901 S
33.2 cM
Xpsr934 S
B
Xpsr131 W
35.5 cM
Xpsr304 *
34.1 cM
Xpsr934 W
9.2 cM
Xpsr609
D
4 cM
Xpsr566 S
Xpsr933 S
59.3 cM
Xpsr131 W *
55.8 cM
Xpsr304 W *
Xpsr540
76.2 cM
Xpsr932 *
Xpsr901
52.7 cM
Xpsr934 S

Fig. 4.4.1: Genetic maps of chromosomes 1BS, 2A, 2B, 2D, 3A, 3B, 3D, 4A, 5A, 6A, 6B, and 6D constructed with RFLP markers in 132 plants of a wheat x spelt cross.(s) and(*) indicate dominant markers with an additional band in wheat or spelt respectively. (*) indicates the loci with distorted segregation.

Homoeologous group 4

Chromosome 4A proved to be highly polymorphic in our sets of cultivars, but we found only a few interspecific polymorphisms (PSR1051, PSR604, PSR115). Xpsr604, around the breakpoint 4AL.7BS and previously mapped in this region (Devos and Gale, 1993), could not be integrated into our maps.

Homoeologous group 5

No polymorphic loci between the wheat and spelt sets and few within the species were found on chromosome 5A. α-*Amy3*, an α-amylase gene expressed only in immature grains (Baulcombe et al., 1987a), was monomorphic in all cultivars.

Homoeologous group 6

PSR8, a functional probe for the enzyme *Carboxypeptidase 3* (Baulcombe et al., 1987b) and located on the short arms of the group 6 chromosomes, was polymorphic between wheat and spelt for all six enzymes tested (*Eco* RI, *Eco* RV, *Dra* I, *Hin*d III, *Bam* HI, *Bgl* II).

Interestingly, for probe PSR10 (*Gli-2*), Arina had the major bands in common with the monomorphic spelt. Only one polymorphism could be detected on the long arms of group 6 (*Xpsr142-6DL*).

Homoeologous group 7

Group 7 chromosomes were almost non-polymorphic within the spelt cultivars but showed good polymorphisms between the sets.

Table 4.4.4: Alleles discriminating sets of wheat (Arina, Bernina, Forno) and spelt (Altgold, Oberkulmer, Rouquin) cultivars.

probe	restriction enzyme(s)[1]	location	max. band number Arina/Oberkulmer	type of polymorphism[2]
PSR1201	1,2,4	1AL, 5AL?	3/4	cod.
PSR596	1-4	1BS	5/5	cod., do.^{s}
PSR963	1-4	1XS,5AL	18/16	do.^{s}
PSR901	2,3,4	2AL, 2DL	5/3	cod., do.^{s}
PSR304	1	2DL	3/4	do.^{s}
PSR131	2,3	2XS	3/4	do.^{s}, do.^{w}
PSR934	1-4	2AL,2BL,2DL	5/4	cod., do.^{s}, do.^{w}
PSR133	4	2XS	5/5	cod.
PSR609	2,3,4	2AL,2BL,2DL	5/11	cod.
PSR907	4	3BS	5/3	cod.
PSR903	1-3	3DS	6/6	cod., do.^{w}
PSR689	3	3XS	3/3	do.^{w}
PSR115	2,4	4AL	3/2	cod.
PSR1051	1,2	4AL	3/3	cod.
PSR133	2	5AL	5/5	cod.
PSR1051	4	5AL	3/3	cod.
XCxp3	1,3,4	6AS,6BS,6DS	6/9	do.^{s}
XCxp3	1,2	6BS	6/9	cod.
PSR547	1	7BL	3/3	do.^{s}
PSR547	3	7BL	3/3	do.^{w}

[1] restriction enzymes: 1 = *Eco* RI, 2 = *Eco* RV; 3 = *Dra* I; 4 = *Hin*d III

[2] cod., do.^{s}, or do.^{w} indicate codominant, dominant for spelt, and dominant for wheat respectively of the corresponding bands in hybridisation patterns of the F_2 Arina x Oberkulmer plants.

4.4.2 Discussion

General observations

The relatively large number (36%) of dominant polymorphisms, which was reported for other inter- and intraspecific crosses of wheat and spelt (Liu and Tsunewaki, 1991; pers. communication M. Messmer), has to be carefully analysed because of its importance in the construction of genetic maps.

Dominant polymorphisms indicated the absence of detectable hybridisation and were interpreted as being (i) a major sequence rearrangement that resulted in insufficient homology for hybridisation to occur at a detectable level, (ii) an extra copy of the homologous sequence or, vice versa, a complete deletion, or (iii) polymorphisms with one allele co-migrating with a non-polymorphic band.

(i) Major sequence rearrangements could result in losses of homology for hybridisation. This would have had to have happened often and with a certain regularity on all chromosomes of a homoeologous group in order to explain our results. The cDNA clones, and probably many of the single and low copy gDNA clones used for hybridisation, were sequences of coding regions. Major sequence rearrangements, as required to loosen the binding affinity in our protocols, would therefore result in major changes in those genes and, consequently, in their direct products. Although an hexaploid organism, like wheat, tolerates different gene doses well (Gale et al., 1990), genetic changes at 20% of the loci would affect the plants more than the actual differences observed between wheat and spelt.

(ii) Therefore, the loss (deletion) or gain (insertion) of homologous copies of the probe sequence seem to explain the majority of dominant polymorphisms.

Insertion and deletion are recognised as the main sources of RFLPs in wheat (Gale, 1990). Still, the complete loss or gain of fragments, interpreted as null alleles, is rare, but reports on plants such as potato (Gebhardt et al, 1989), rice (McCouch et al., 1988), lettuce (Landry et al., 1987), and *Brassica* species (Slocum et al., 1990) exist. Sequence duplications, as reported for maize (Helentjaris et al., 1988) and to a lesser extent for tomato (Bernatzky and Tanksley, 1986), potato (Gebhardt et al, 1989), and rice (McCouch et al., 1988), are also possible in wheat and spelt, but no general rule could be formulated from our data.

It is obvious from Table 4.4.4 that, for the probes differentiating the two sets, no general rule for the sequence duplication number could be found. Many of the probes hybridised to a single homoeologous locus in Chinese Spring (three fragments) but

revealed an uneven number of bands in our genotypes. The copy number was seldom a multiple of three. The probes selected in Chinese Spring as single copy loci demonstrate that sequence duplication in our cultivars did not take place in all homoeologous loci. Contrary to *Brassica* (Slocum et al., 1990) and maize (Helentjaris et al., 1988), the distribution of duplicate loci in wheat and spelt was, apparently, random.

Because many genes in wheat are members of small families (Gale et al., 1990), it is suggested that the dominant polymorphisms were missing (null alleles) or were extra copies of gene families. This would not affect hexaploid organisms like wheat and spelt phenotypically. A minimum number of two fragments was found in every cultivar for all polymorphic probes. Therefore, the genetic information is present in the plant; effects of dose and regulation may play a role.

Reciprocal translocations involving very short segments, as reported by Baier et al. (1974), may also explain the great number of dominant polymorphisms. It is, however, rather unlikely that they occurred on all homoeologous arms as seems to be the case for group 2 or 6. However, considering the comparative mapping results that show a high conservation of genome structure between the *Triticeae*, it does seem unlikely that many deletions differentiate the *T. aestivum* and *T. spelta* genomes (Devos and Gale, 1993; Moore et al., 1993; Kurata et al., 1994).

(iii) Polymorphisms described as dominant in the parents are not necessarily true null alleles. If the allelic counterpart migrates with another band, this allele will also co-segregate with this band and thus never appear as segregating because there will always be at least one band present. In wheat, where homoeologous loci are well preserved on the genomes, a RFLP on just one homoeologous chromosome could well be interpreted as a null allele in segregating populations, since one allele will always co-segregate with the monomorphs of the two other chromosomes. To overcome these difficulties Wu et al. (1991) proposed a strategy based on single-dose restriction fragments.

Some of the dominant polymorphisms were consistent but not exclusive in a set of European wheat and spelt lines, as was shown by Siedler et al (1994). Liu and Tsunewaki (1991) also found a very high frequency of dominant polymorphims. For example, 100%, 66.7%, and 56.3% were hemizygous on three chromosomes, 1D, 6D and 6A respectively. The authors explain the occurrence of hemizygous alleles by insertion / deletion events, resulting from translocations and transpositions. We agree with this but assume that co-segregating bands also have an effect, because the cereal

genomes are very well conserved and no translocations or transpositions were ever reported that could explain the great number of presence / absence- polymorphisms. The deviating copy number of both species as compared to Chinese Spring still needs further explanation.

Evolutionary translocations

No dominant markers were found in the well-known evolutionary translocations 4AS.4AL-7BS, 5AS.5AL-4AL, and 7BL.7BS-5AL (Naranjo et al., 1987; Liu et al., 1992). Liu et al (1992) located PSR164 close to the breakpoint of 4AL-5AL, but PSR164 was not polymorphic between our wheat and spelt sets, whereas probes around the breakpoints of 7BS-4AL and 5AL-4AL translocations (PSR160 and PSR580 respectively) were highly polymorphic for all the cultivars. The 2BS/6BS translocation, proposed by Devos et al. (1993), was confirmed in our cross because of the presence of *Xpsr899* on 2BS and its absence on 6BS.

We conclude that the evolutionary translocations, previously characterised physically (Naranjo et al, 1987) and genetically (Liu et al., 1992), are not typical differences between the genomes and that, therefore, the separation of the gene pools of European spelt and wheat must have been a relatively recent event (Mac Key, 1954; Tsunewaki, 1968).

Mapping of RFLPs in a F_2 Arina x Oberkulmer cross

The presence of duplicated loci, as discussed above, would cause enormous problems in mapping if they were to be intrachromosomally duplicated. If the dominant markers are due to differential gene family structures of tightly linked genes, the influence on mapping procedures could be neglected because of the 'genetical neighbourhood' of such events.

The number of chromosomal interchanges in crosses between modern wheat cultivars and European spelt lines is greater than between Chinese Spring and spelt lines (Riley et al., 1966). We, therefore, might expect more regions of interchromosomal irregularities than in other mapping projects (Liu and Tsunewaki, 1991; Devos and Gale, 1993) which used Chinese Spring as the wheat parent. Because chromosomal interchanges often occur in wheat (Schlegel and Schlegel, 1989), such events may serve to explain the distorted segregation in some genomic

regions of our cross (see Gale et al., 1990). The repeated occurrence of such regions on the same chromosome, in different crosses (see 2D of Liu and Tsunewaki (1991) and our results) in a region of morphologically crucial importance, gave rise to the assumption of a stable chromosomal translocation in naturally evolved *T. spelta*. If all evolutionary translocations had occurred at the tetraploid level (AABB) before hybridisation with *Ae. squarrosa* (DD), as suggested by Naranjo et al. (1987), our hypothesised translocation would be relatively recent with regard to the theory of the origin of spelt. The translocation does not have to be restricted to spelt, since a 7B/2D translocation seems to be common in European wheat varieties (Schlegel and Schlegel, 1989). Future mapping projects based on wheat x spelt crosses should be accompanied by cytological studies to analyse the karyotype of crucial chromosomes.

Devos and Gale (1993) and Liu and Tsunewaki (1991) used comparably wide crosses to construct their maps. Devos et al. (1993) mentioned difficulties in handling null alleles when constructing maps. We support this and advise interpreting such results very cautiously, as we did. Software must be adapted to the special genetic features of wheat (large marker intervals, duplicated loci, hexaploid status, translocations). The problems occurring with mapping in polyploids were reviewed by Sorrells (1992) and, specifically for wheat, by Gale et al. (1990).

Tab. 4.4.5: Comparison of marker distances [cM] between the genetic maps constructed in this study (IPW) and the maps published by Devos and Gale (1993) (IPSR).

genome:	A	B	D
IPSR	471	296	272
IPW	599	375	545
IPW/IPSR	1.27	1.27	2

Intervals were greater in our maps than in maps of the Institute of Plant Science Research (IPSR) published by Devos and Gale (1993). Identical interval lengths were found for chromosomes 2B and 4A. The biggest difference was noted for chromosome 2D where our map spanned a distance 2.44 times greater than the same markers on Devos' and Gale's (1993) map. Table 4.4.5 compares the map sizes for the A, B, and D chromosomes. While our map of the A and B chromosomes spans distances 1.27 greater than on the map of Devos and Gale (1993), the distances for the D chromosomes were twice as great on our maps. Thus, the D chromosomes of

Arina and Oberkulmer recombined more often than those of the Synthetic x Chinese Spring cross used by Devos and Gale (1993).
As outlined below, the D genomes recombined more frequently than the A and the B chromosomes. The more frequent recombination in our cross is explained by the greater homology between Arina and Oberkulmer chromosomes as compared to the cross used by Devos and Gale (1993). The greater homology of the D genomes of our crossing partners might, once again, confirm the relatively recent origin of the two species.

Polymorphisms in homoeologous groups

Homoeologous group 1

All but one of the polymorphisms were on the short arms of chromosomes 1, where the highly variable ω-gliadins were coded (Lafiandra et al., 1989). PAGE analysis of gliadins detected at least two different allelic bands between the two cultivar sets (unpublished data). Flavell and Smith (1974) reported differences in copy number between Chinese Spring and *T. spelta* for rRNA genes on 1A and 1B.

Homoeologous group 2

The high proportion of polymorphisms revealed in group 2 is in good agreement with the findings of Liu and Tsunewaki (1991) who reported this group to reveal the second highest number of polymorphisms. Of special interest in this group were the *C/c* gene for *Compact ear* and the *Tg/tg* gene for *Tenacious glumes* (Milne and McIntosh, 1989), because they affect ear morphology as described later (section 4.5.1.2).
Chromosome 2D recombined more frequently than its homoeologous chromosomes 2A and 2B; this is expressed by greater interval spans between homoeologous loci (Devos et al., 1993). For example, the distance between *Xpsr131* and *Xpsr934* was 77.4 cM on 2A, 66.6 cM on 2B, and 184.7 cM on 2D. This indicated that the higher recombination frequency of the D genome was not restricted to the cultivated D (Chinese Spring) x wild D (*Ae. squarrosa*) in 'Synthetic' (McFadden and Sears, 1946) used by Devos et al. (1993). A comparable distance existed in our cross, therefore, due to its high recombination rate with the cultivated D genome of Arina,

we concluded the D genome in Oberkulmer was either well conserved or very young. In any case a phylogenetic difference to Arina is assumed.
The region of distorted segregation on 2DL and the impossibility of integrating some markers in this region was also reported for another wheat x spelt cross (Liu and Tsunewaki, 1991). The authors found two regions of distorted segregation in a cross of Chinese Spring x *T. spelta* var. *duhamelianum* on chromosomes 2D and 4A respectively. They suspected an 'unknown factor' of being responsible for the disturbance in segregation, while we think it is due to translocation or insertion/deletion events (see above).

Homoeologous group 3

Xpsr1196-3A and *Xpsr1196-3B*, whose alleles were proven to be typical of European wheat and spelt gene pools (Siedler, 1994), together with PSR903 and PSR907 were all polymorphic between wheat and spelt sets on short arms of group 3 chromosomes. The occurrence of two polymorphic loci between wheat and spelt on each short arm indicate a homoeologous sequential difference over a large distance for those regions.
On the long arms, no polymorphisms between the sets were found and thus no major difference was assumed. This is supported by the fact that the morphological marker *Red Grain Color (R/r)* on 3AL, 3BL, and 3DL (Milne and MacIntosh, 1989) is monomorphic in all six cultivars.

Homoeologous group 4

The highly polymorphic probes on 4AL showed this arm to be very variable for all cultivars. The loci *Xpsr115-4AL* and *Xpsr1051-4AL* were polymorphic for the sets. *Xpsr115-4AL*, which is located in a translocated fragment that originates from 7BS, could not be integrated in the map. Because all other translocations seemed to be older than the separation of European wheat and spelt pools, it is unlikely that only the 7BS fragment dated from an evolutionarily younger translocation, despite the uniform character of chromosome 7B in spelt.

Homoeologous group 5

We expected a large number of polymorphisms between the square-headed wheats (*QQ*) and the lax-eared spelts (*qq*). Additionally, the chromosomes of groups 4 and 5 host many genes favouring abiotic stress resistance, against, for example, low temperature and flooding (Forster, 1992), which is typical for spelt lines. Because only few polymorphisms were found, and none of them were consistent between the wheat and the spelt sets, we concluded that the *Q*-complex was not an important translocation / insertion event but had an effect only on the terminal area of the chromosome arm (Tsujimoto and Noda, 1989 and 1990).

Homoeologous group 6

The short arms of this group were highly polymorphic, while only one polymorphism was detected on the long arms. Since at least three enzymes detected several polymorphisms with probes PSR8(*Cxp3*), PSR312, and PSR899, there must be a major sequence rearrangement on this arm of all three genomes. *Xpsr312-6BS* and the same probe with an unknown genome location both showed a distorted segregation. PSR106 was previously mapped 15 cM distal from PSR312 (Devos and Gale, 1993) but could not be integrated in our group 6 maps despite its regular codominant segregation. *Xglk705*, located in the same region on 6B on the map of Liu and Tsunewakis (1991), showed the greatest distortion ratio ($X^2 = 21.12$) of all their markers. They speculated this marker to be linked to the pollen killer gene (*Ki*) of CS (Loegering and Sears, 1963) which could be responsible for the distorted segregation of *Xglk705*. This possibility can now be excluded because of the parallel occurrence of such regions in both wheat x spelt crosses, whereas no *Ki* genes have been reported for Arina or Oberkulmer.

Our data cannot explain the polymorphisms on the short arms of group 6 of wheat and spelt cultivars. Further evidence does, however, suggest that the telomeric region of 6DS is polymorphic at the species level (unpublished data).

Homoeologous group 7

Group 7 was almost uniform within the spelt set and revealed good polymorphisms between wheat and spelt. Siedler et al. (1994) found three loci in group 7 with a band frequency of at least 0.5 between 52 wheats and 20 spelt lines (*Xpsr129-7B*,

Xpsr547-7B, *Xpsr303-7A*).
The *Purple Culm* and *Red Coleoptile* markers were also mapped in this group, and we therefore suspect that these chromosomes carry species specific genes which were well conserved in spelt cultivars during the breeding process..

4.5 Modelling phenotypical differences with markers

4.5.1 Morphological characters

On 5AL, 17.1 cM distal from *Xpsr79-5A*, one QTL was located with a LOD score of 4.22; this fact alone explained 31.7% of the variation in the length of the ear internode (Fig 4.5.1).
The plants showing the homozygous allele of Oberkulmer (BB) of *Xpsr79-5A* had an average ear internode length of 7.2 mm, while the average length of the ear internodes of the homozygous Arina allele (AA) was, on average, only 5.7 mm, i.e. the spelt allele B increased the value by 11.6% as compared with the mean of the homozygotes (a = 0.75 mm). The average length of the ear internode of the heterozygotes was 1.9% greater than the mean of the homozygotes (d = 0.12 mm). Fig. 4.5.2 shows a histogram of the three subpopulations formed by the marker classes of *Xpsr79-5A*.
Interestingly, one plant showed the BB allele for *Xpsr79-5A,* but the length of the ear internodes were only 3 mm. This plant (#108) combined very short ear internodes with a relatively long culm, resulting in the clearly lowest ratio of ear internode length : height and ear length : height, i.e. the general architecture, previously described as very stable, was affected in this plant (see also 3.1.2). We could not find a marker or a set of markers that explained the short ear internodes of this plant. We conclude that at least one other factor must control the internode length as is proposed by Rimle (1995).

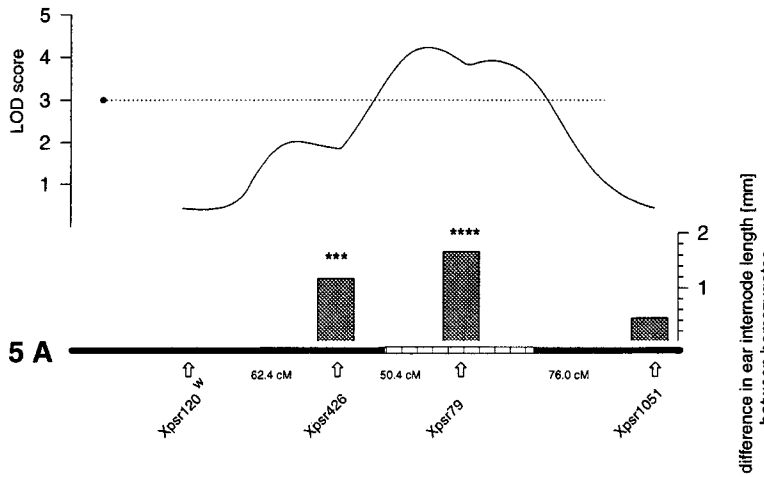

Fig. 4.5.1: LOD scores and differences in the lengths of the ear internodes between the homozygotes and the distinctive classes of codominant or dominant markers. QTL 'confidence interval' is shown on the chromosome around *Xpsr79*.

Means of the marker classes of *Xpsr904-3D* were 5.7 mm (AA), 6.2 mm (BB), and 7.1 mm (AB). The allele classes of *Xpsr904-3D* had a significant effect on the length of the ear internodes in a one-way ANOVA ($p<0.003$). At this locus, over-dominance was observed, but this has not been observed phenotypically. Due to the large marker intervals in this region it was not possible to reliably map a possible QTL, though LOD scores above the threshold were calculated close to this marker.

4.5.1.2 Discussion

The QTL on 5A supports the findings and conclusions drawn above (section 4.1.3) for the *Q/q* locus. The only plant with short ear internodes and the BB allele of

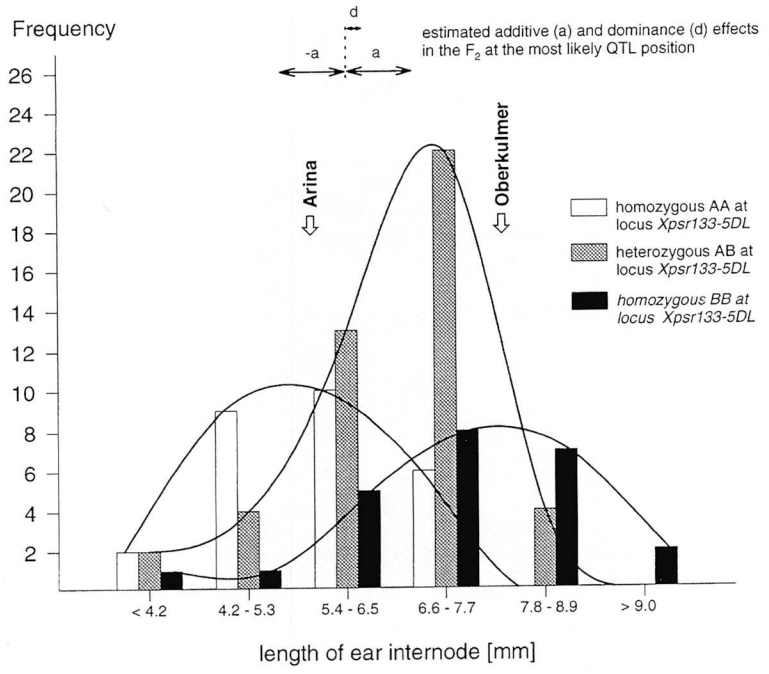

Fig. 4.5.2: Phenotypic distribution of ear internode length of the subgroups formed by the allele classes of *Xpsr79-5A* in a F_2 population of Arina x Oberkulmer.

Xpsr79-5A (#108) might be explained by a genetic model proposed by Rimle (1995). According to this, the QTL would act as a main determinant of the ear internode length and of the *Compact ear* (C/c) gene as its strong modifier. Our data support this model, but the following must be taken into consideration: the *Compact ear* gene is expressed in the ear internodes only and not in all phases of ear development. The many ears which were only partially affected by the jolting *C/c* support this proposed extension of the model. The shortening effect of *Q/q* on the lower culm internodes

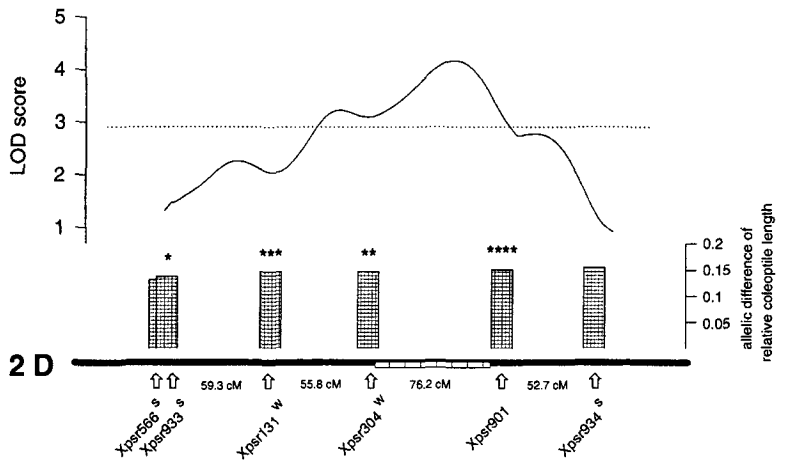

Fig. 4.5.3: LOD scores and allelic differences for the coleoptile length along chromosome 2D. The significance of mean differences for the marker classes are shown by * (see 3.6).

of Chinese Spring are interpreted as being the result of the different combined actions of *Q/q* and *C/c* as compared to our sets of wheat and spelt.
Because the QTL on 5A explains only 31.7% of the trait variation we assume that loci other than *Q/q* influence the type of the ear which is formed. One single gene or a family of tightly linked genes would result in a narrower QTL, and a single dominant locus may explain a higher proportion of the trait variation.

4.5.2 Coleoptile growth

4.5.2.1 QTL mapping of early shoot growth (coleoptiles)

A total of 21.2% of the variation in shoot length after three days of growth of F_3 families could be explained for their F_2 ancestors by a QTL in the interval *Xpsr304-Xpsr901*, 32 cM distal of *Xpsr901* on 2D (Fig. 4.5.3). The existence of this QTL is supported by a LOD of 4.2. The homozygous BB allele made the coleoptiles 18.6% longer (a= 5.2 mm) than the mean of AA and BB homozygotes, and heterozygotes

were shorted by -6% (d= -1.7 mm).
Strong indications of possible QTL between the two dominant markers, *Xpsr131* and *Xpsr304,* on the same chromosome had to be rejected due to insufficient LOD

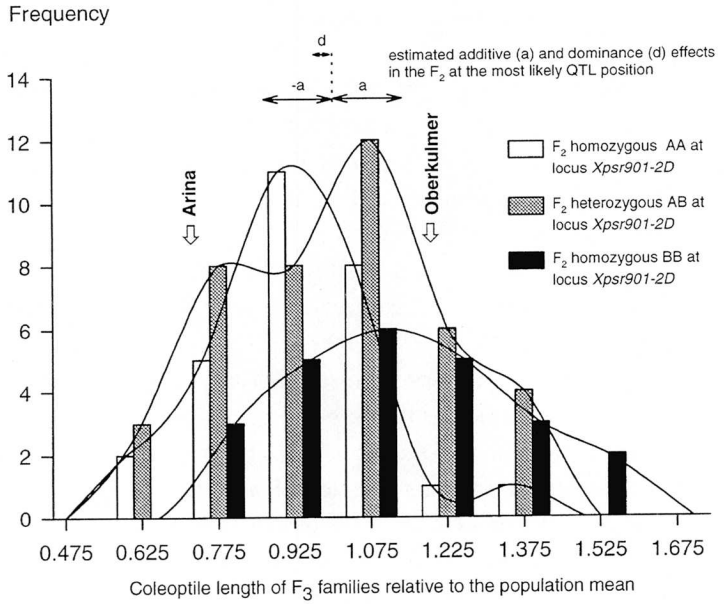

Fig. 4.5.4: Phenotypic distribution of the relative coleoptile length of F_3 families. The subgroups were formed by the allele classes of *Xpsr901-2D* of the F_2 progenitor.

scores (2.73) in an additive gene model. We assumed that the poor resolution power of the dominant markers did not allow us to map possible QTL in this region.
The distribution of the relative coleoptile lengths of the F_3 families divided into AA-, AB-, and BB-subgroups of their F_2 progenitors for *Xpsr901-2D* on 2DL are shown in Fig. 4.5.4. The dominance (d) components were reduced to one half, because the phenotypic data were collected on F_3 genotypes. Therefore, the estimated means of the F_2 allele classes were closer than would be expected from the F_3 data (see Fig.

4.5.4).
At the locus *Xpsr142-6D*, the means of relative coleoptile lengths of the homozygous allele classes were significantly different. The large distance to the flanking marker and its dominant character hindered us from mapping a possible QTL on the chromosome arm 6DS.

4.5.2.2 QTL mapping of shoot growth under hypoxia

For the resulting shoot length after growth under hypoxia, a QTL was detected in the interval *Xpsr304-Xpsr901* on 2D with a LOD score of 3.64. A second peak in the interval *Xpsr901-Xpsr934* (see Fig. 3.5.5) is due to the algorithms used by Mapmaker, leading to a drop in the LOD score around the marker and thus suggesting, in our case, a second QTL.
The interval *Xpsr304-Xpsr901* explained 19.3% of the trait variation. Dominance effects of d= -6.7% (-1.4 mm) and additive effects of a= 13.3% (2.7 mm) were found for the QTL locus (see Fig. 4.5.5).
On chromosomes 3A and 3B a QTL was localised at distances of 24.9 and 26.1 cM from *Xpsr1196* respectively. While a LOD score of 3.68 on 3A was found, the QTL on 3B was accepted, despite its LOD score of 2.65 because of the codominant character of its flanking markers and the relatively short distance between them.
A QTL with sufficient LOD scores could not be localised on 3D. *Xpsr1196-3D* was a dominant marker and, therefore, the resolution over wide intervals was low.
The means for the homozygous allele classes for neighbouring *Xpsr903*, however, were different at the p=0.07 level. At QTL on 3A and 3B, the additive effect (a) of BB alleles was 18% (3.7 mm) and 10.5% (2.1 mm) respectively, while the dominance (d) was negative (-4.8% (-1 mm) and -5.2% (-1.1 mm) respectively). The QTL mapping along group 3 chromosomes is shown in Fig. 4.5.6.
In a simultaneous fit, the three QTL together explained 29.9% (LOD 6.13) of the variation in the coleoptile length after five days of growth under hypoxia.

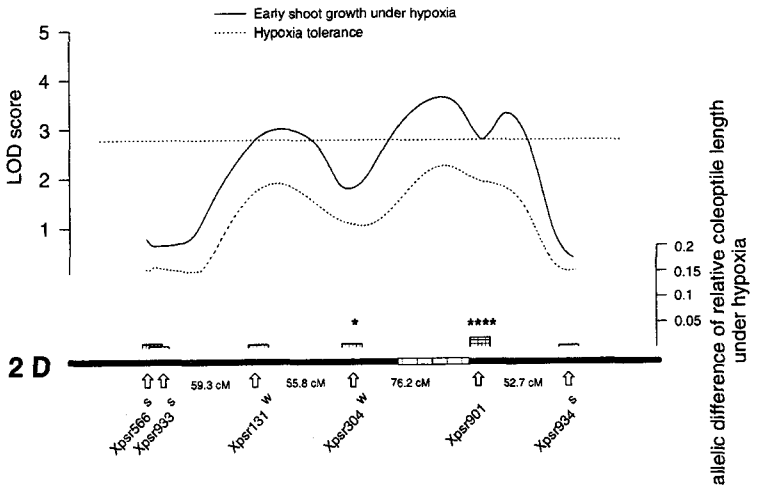

Fig 4.5.5: LOD scores and allelic differences for the relative shoot length at 1% O_2 of markers along chromosome 2D. Significance levels are indicated by * (see section 3.6).

4.5.2.3 QTL mapping of shoot growth (control treatment)

No region could be clearly identified as being responsible for the variation in shoot length after 120 hours of growth. The intervals on 5A of *Xpsr120-Xpsr426* and *Xpsr79-Xpsr1051* both indicated regions where shoot growth may be affected (LOD scores up to 3.2). Considering the large marker distance and the dominance of *Xpsr120*, the variation in shoot length after 120 hours of growth could not be explained conclusively.

Nevertheless, markers on 1B, 3B, 4A, and 5A displayed different means for the marker classes. At all of these loci, the identifiable homozygote AA or BB allele were shorter or longer than their allelic counterparts. The most reliable differences were found for the dominant markers PSR160 and PSR573 on 4A and PSR120 on 5A.

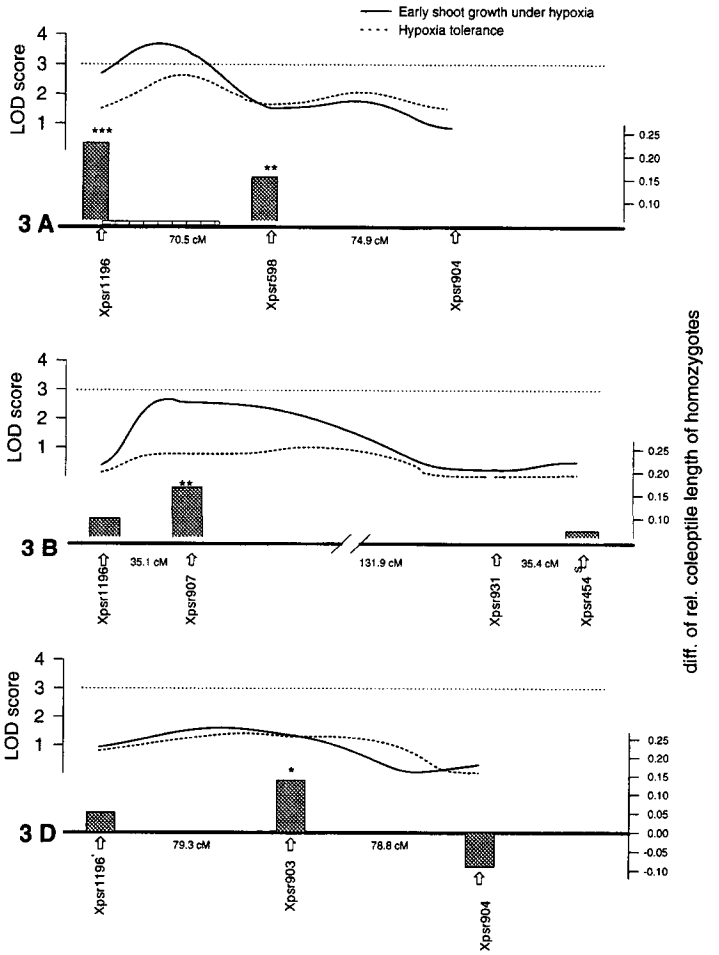

Fig. 4.5.6: LOD scores and allelic differences for the relative shoot length at 1% O_2 of markers of chromosomes 3A, 3B, and 3D respectively. Significance levels are indicated by * (see section 3.6).

4.5.2.4 QTL mapping of hypoxia tolerance

For the hypoxia tolerance (=coleoptile length hypoxia/ coleoptile length normoxia), the QTL positions for coleoptile length under hypoxia on 2D and 3A were confirmed by LOD scores of 2.25 and 2.64 respectively. The interval Xpsr304-Xpsr901 explained 12.3%, and the interval Xpsr1196-Xpsr598 10% of the variation in hypoxia tolerance. In a simultaneous fit both intervals explained 18.7% of the trait variation at LOD scores of 3.55. In figures 4.5.5. and 4.5.6, the LOD scores along chromosomes 2D and 3A are depicted by a dotted line.

4.5.2.5 Discussion

Coleoptile growth

The maternal effect on the coleoptile growth rate found in section 3.3.1.2 does not lead to clear differences in the analysed F_3 generation. Under the assumption of just one segregating genomic QTL besides the maternal effect, nine genetically different aleurone / genome combinations would occur. This are far too many to distinguish phenotypically. Since the maternal effect only partially determined the coleoptile growth, its influence on germination and early growth rate (Fick and Qualset, 1975) cannot be analysed further with our data. It is, however, not of major importance in determining the trait. The identical growth rates and coleoptile lengths of Chinese Spring and Chinese Spring (*T. spelta* 5A) show that the *Q/q*-'elongation factor' does not play a role.

The QTL for the coleoptile extension located on 2DL was clear and was confirmed by the significantly different means for the homozygotes of *Xpsr901-2D*. The significant differences between the classes of dominant markers, *Xpsr304-2D* and *Xpsr131-2D*, and the continuously high LOD scores between those markers may indicate that a large proportion of the chromosome determines coleoptile growth. The short distance to phenotypically important loci, *Compact ear* and *Tenacious glume* (Milne and McIntosh, 1989), was confirmed by the correlation coefficient for elongation, which was shown above to be a marker for the *Tenacious glume*, and the coleoptile growth rate ($r= 0.35^{****}$).

Fletcher (in Addae and Pearson, 1992) suggests that at least two genes influence coleoptile growth, whereas we conclude from our results (Fig. 4.3.2 a) that a few

major loci control the coleoptile growth and favour the hypothesis of three major loci. Since there is no evidence of a homoeologous locus, we assume that there are other QTL on the unmapped chromosomes.

Early shoot growth under hypoxia and hypoxia tolerance

The QTL found on 2D was located at the same 'confidence interval' as the one for coleoptile extension, but the differences in the marker classes were much less pronounced. We interpret this QTL as being the QTL responsible for coleoptile extension (see above), while the QTLs on 3AS and 3BS probably determine the effect of reduced oxygen on early shoot growth. The high proportion of the variance explained by marker intervals on 3A and 3B indicate the importance of those chromosomes in the phenotypic expression of hypoxia tolerance.

It is unclear whether the loci are homoeologous or not, because our map of 3D is not dense enough to give a conclusive answer.

The mapping of 'hypoxia tolerance' confirmed the QTL on 2D and 3A for growth under hypoxia. This is strong evidence that the QTL on 3A effectively governs a 'hypoxia tolerance factor'.

The fact that we found a QTL in the large hexaploid genome of wheat to be associated with hypoxia tolerance is strong evidence for some genetic changes governing this trait rather than being evidence of an advantageous combination of many favourable alleles, as was speculated in section 4.3.6. Those changes must determine physiologically key positions and could well be termed 'hypoxia tolerance factor'. Based on the present data, its physiological effects cannot be explained.

The QTL probably covered coding regions that were only expressed under anoxia, as was shown for maize (Sachs et al., 1980) and wheat (Bertani and Brambilla, 1982), thus representing a 'hypoxia response factor'. The occurrence of the QTL on 3A in the hypoxia stressed treatment confirmed this hypothesis.

Since all the hypoxic seedlings were morphologically similar to those at normoxia, a different genetic programme of spelt under hypoxia, as is reported for rice under anoxia where only the coleoptile grows (Perata and Alpi, 1993; Guglielminetti et al., 1995), can be excluded. We, therefore, suspect that differences in physiological efficiency or tolerance to cytotoxins explain the variation in hypoxia tolerance of anoxia intolerant wheat and spelt rather than a species specific hypoxia tolerance strategy. Nevertheless, many gene products built under anaerobiosis have been

shown to be glycolytic enzymes (reviewed by Bailey-Serres et al., 1988). On the corresponding chromosomes, several loci coding for glycolytic enzymes were mapped (Milne and McIntosh, 1989), e.g. *Triphosphate isomerase* and *Phosphodiesterase*. Further information is provided by Erdmann et al. (1988) who correlated the hypoxia-lowered extension rate of wheat roots to a retardation of mitosis. Lopez-Saez et al. (1969) demonstrated that the short shoot of hypoxia stressed *Vicia faba* is due mainly to a decrease in cell division rather than cell elongation. Jackson and Drew (1984) concluded that anaerobic metabolism did not provide sufficient energy for the initiation of the S-phase (DNA - synthesis phase of mitosis) in the root meristem. The action of anoxia on wheat was shown by Perata et al. (1992) to be based on the absence of α-amylase. The authors explained this absence by disturbed transcription of the enzyme (Perata et al., 1993). This would result in the failure of starch breakdown, and thus a reduction in the amount of energy provided to the seedling would occur. An isozyme facilitating those processes under hypoxia would be a good explanation of the variation between wheat and spelt and the way in which inheritance occurs as described above.

The use of a flow system and the complete recovery of all seedlings do not indicate serious damage as a consequence of cytotoxins.

Hypoxia-induced imbalances of hormones were also discussed in the literature (see review by Jackson and Pearce, 1991) and might coincide with our suggestion that even a few genetic changes may result in great variation in hypoxia tolerance.

So far, we have not detected genetic differences which determine the hypoxia tolerance of wheat and spelt. Hypoxia tolerance is normally inherited and gradually expressed but is not linked to any other known marker for wheat and spelt.

5. General discussion

5.1 Does a genetically distinct spelt species exist?

The classifications of *T. spelta* in the *Triticeae* described previously indicate that a clear discrimination can be made between wheat and spelt exclusively on the basis of a few genes such as *Q* and *Tenacious glume*. Siedler et al. (1994) extended the search for typical characters of wheat and spelt to RFLPs. With 58 probe / enzyme combinations they separated 20 spelt lines from 52 winter wheat lines in a principal component analysis and estimated the genetic distance, on the basis of common RFLP bands between wheat and spelt, to be 0.132. No single marker could differentiate the species (spring and winter wheat against spelt), and just a few markers revealed bands that occurred exclusively in winter wheat or spelt. We found a genetic distance of 0.162, based on RFLP banding patterns, between our wheat and spelt sets (data not shown). In our screening of two limited sets of cultivars of the two species many polymorphisms proved to be typical of one of the sets. This is expected when comparing two gene pools of this Swiss spelt (due to its pedigree, we also include the Belgian cultivar Rouquin) and modern Swiss bread wheat varieties. Different recombination frequencies occurred for chromosomes of the wheat and spelt A and B genomes and the D genome, thus indicating different origins of the D genome (Devos et al., 1993). The old English cultivar April Bearded and the primitive wheat Chinese Spring performed comparably well in our early vigour experiments. At least the fast coleoptile growth and the hypoxia tolerance were not spelt species specific traits and could not, therefore, support the existence of physiological traits unique to spelt species. This is supported by the fact that we did not find one polymorphism between wheat and spelt on 2D, the chromsome where a QTL for fast coleoptile growth was mapped. Morphology, stress tolerance strategies, yield structure, and harvest index were the same for spelt (Rimle, 1995) as for old wheat landraces (described by Feil, 1987 and Keller, 1990).

We believe that the European spelts have to be treated as geographically and thus genetically isolated gene pools. This explains the genetic distance to modern wheat, which is just slightly greater than the distances between European wheat gene pools (Siedler et al., 1994). Too little work in breeding has caused this crop to become restricted in recent times to areas where intensification was made impossible by the climate or by the lack of economic development.

5.2 Early vigour

Some arguments from the literature support the agronomical importance of our experiments:

-The growth rate of the coleoptile and the length of the coleoptile are good indicators of the penetration force of seedlings through compacted soil and the emergence of the plants in the field (Chowdhry and Allan, 1963; Kolp et al., 1967; Sunderman, 1964; Livers in Kolp et al., 1967; Addae and Pearson, 1992; Allan et al., 1962a).

-Effects on sprouting, to which spelt is known to be resistant (Koblet, 1965), may be due to differences in the basic physiological processes of germinating grains.

-Waterlogged soil could occur any time during the sowing period of spelt and wheat, thus rendering the soil almost free of O_2 and reducing the molecular diffusion by a factor of approx. 10^4 (Grable, 1966). This would faciliate hypoxia from germination to emergence. Such conditions occurred a few times during the last decade of trials carried out at marginal locations.

The principal difference between our experiments and agricultural practice is the use of naked spelt caryopses instead of spikelets. The impact of tight glumes on the early vigour of wheat and spelt was analysed by Rüegger (1988). He found that the glumes protect the caryopses during periods of flooding and low temperatures and explained this by the glume which protects against soil-borne fungi (Riesen et al., 1986). Germination was, however, slowed down by the glumes as a result of either their action as an oxygen barrier or as a result of their role as a carrier of microorganisms competing for oxygen. Rüegger (1988) stressed caryopses for 30 days (submersed at 8/6 °C) and let them recover for 17 days at 18/13 °C. Arina's shoot dry weight decreased under stress to a greater extent than that of the spelt cultivar Ostro; after the stress phase both cultivars recovered completely. Therefore, the agronomical importance of hypoxia tolerance seems to be restricted to the early growth stages, because lasting effects of the specific hypoxia tolerance of Ostro were not observed after 17 days of recovery. It might be that hypoxia tolerance, similar to fast shoot growth, is an absolute necessity if germination is to occur in an atmosphere created by tight glumes (limited O_2 supply; enrichment of gaseous metabolites). If so, the existence of two unlinked traits, *Tenacious glume* and 'hypoxia tolerance', would create genotypes with a reduced 'spikelet vigour' in wheat x spelt offsprings with tenacious glumes. Our experiments, however, demonstrated that 'hypoxia tolerance'

was also effective at later stages and not just at the breakthrough of tight glumes. In agreement with Rüegger (1988), we extrapolate and hypothesise that the tight glumes of spelt would have eliminated the superiority of naked spelt caryopses as compared to wheat caryopses. In agronomic practice, the better seed vigour, expressed as shoot growth rate, and possibly hypoxia tolerance is linked in a spelt cultivar in order to compensate for the hinderance of the glumes under normal conditions. Glumes would, however, be clearly advantageous in a very wet seedbed where fast emergence is not required. In fact, agricultural practice tends to late sowing dates which delay emergence until a snow cover can prevent frost damage (H. Winzeler, pers. communication). Since a QTL for seed vigour was, to some extent, related to the shape of caryopses, which is regarded as the phenotypic expression of *Tenacious glumes*, breeding in this direction is facilitated. Nevertheless, the mean coleoptile length of the naturally evolving populations tended to increase in the harsh environment of Oberwallestalden as well within the group of free-threshing seeds and within the group of seeds with tight glumes. This indicates that the advantage of fast growing shoots goes beyond the linkage to *Tenacious glume*. At the favourable location Zürich-Reckenholz, it is expected that caryopses with slowly elongating coleoptiles will emerge one to two days later than those with rapidly elongating coleoptiles when sown at a depth of 2 to 3 cm and soil temperatures of around 11 °C. This does not seem to be critical for survival and propagation during mild autumns and winters at Zürich-Reckenholz. The harsh environment at Oberwallestalden clearly favoured faster growing coleoptiles even from free-threshing ears. The fast coleoptile growth must, therefore, be advantageous under such harsh conditions *per se* in contrast to mild conditions.

The elongation of the caryopses is correlated to the coleoptile growth rate and to typical morphological spelt characters (see 4.1.2) and is, therefore, considered to be a cost effective and an easily scorable morphological marker for the coleoptile growth rate and for morphological characters of wheat and spelt. Furthermore, digital image analysis can provide fast and accurate recordings of morphological grain characters as has been described for wheat caryopses (Sapirstein et al., 1987; Travis and Draper, 1985; Zayas et al., 1986). The rather low correlation coefficient may prove limiting, so that fast progress would occur only in combination with other selection criteria. These correlation coefficients still have to be tested in field grown plants before they can be implemented in the selection process.

Hypoxia tolerance is easily lost in breeding because it is not linked to phenotypical

markers, and it is hardly recognised even in harsh environments. The great variation in hypoxia tolerance of newly bred spelt cultivars illustrates this. Indeed, hypoxia tolerance was greater after five years of selection at a favourable location, but the differences were small. In this context, the combined fast shoot growth and tight glumes are well integrated in a 'general stress concept' for plants in cold and wet climates. This might be even more important in systems of sustainable agriculture where sowing takes place in late autumn for reasons of phytosanitary.

5.3 Evolutionary population genetics

Hoffmann et al. (1993) reviewed the impact of oxygen deficiency on the basis of a general stress concept for plants (Levine et al., 1989; Beck and Lüttge, 1990; Chapin, 1991; Chapin et al., 1993). Taylor (1989) identified investment processes as being the most sensitive to stress and, consequently, defined stress tolerance as the 'ability of a genotype to use resources normally applied to sponsor growth and productivity to master stress effects'. The price paid for being able to keep the homoeostasis constant under a wide range of environmental conditions, i.e. to be stress tolerant, is a loss of efficiency and lower biomass production (Chapin, 1991). Chapin et al. (1993) suggest that many of the general adaptations to environmental stress result from relatively simple changes involving hormone physiology or other regulatory processes. These few changes would induce a series of seemingly unrelated physiological traits ('stress resistance syndrome SRS') resulting in better fitness for the appropriate environment. We have strong evidence that, for both coleoptile growth rate and hypoxia tolerance, single loci play important roles. As hypothesised in section 4.3, these traits may be physiological bottlenecks in the process of energy supply for heterotrophic growth. The mechanisms previously described for those traits may be part of a cascade of physiological processes resulting in better fitness, i.e. a SRS in the sense of Chapin et al. (1993). If agricultural selection for well defined traits (see below) succeeds without making use of such a SRS it would disappear. The disappearance of a single gene in cultivation has been demonstrated by Tsunewaki (1968) for *Hairy glumes* (*Hg*). This single dominant gene is present in **all** the progenitors of the hexaploid wheats and in about **half** of primitive hexaploids from Afghanistan and is almost **extinct** in modern US and Japanese hexaploids. The geographical distribution of the occurrence of *Hg* shows that

intensive breeding and temperate climates lead to the elimination of this gene. The selection pressure of the habitat on single loci of *Gramineae* was demonstrated by several authors (see Nevo, 1993; Allard, 1988).

A possible SRS 'hypoxia tolerance' would be advantageous in wet soils and should, therefore, be present in cultivars adapted to frequently waterlogged soils after sowing. In general, the more recent the cultivars of both groups were, i.e. the less similar they were to locally adapted landraces, the lower their hypoxia tolerance. The ranking in hypoxia tolerance was influenced more by yield potential than by phenotypic characters. Interestingly, the marginal location of Oberwallestalden favoured less hypoxia tolerant genotypes in evolving populations. Generally, the hypoxia tolerance must have been selected over a long period of time. The selection period in our experimental population was too short for such a trait that is seldom of vital importance and was probably selected when the climate was different.

The breeding for an ideotype adapted to the current agronomic production system may, therefore, result in less specific but strong tolerances to general stress. This was shown, for example, for semidwarfism which drastically reduced the stand establishment in some genotypes (Allen, 1980). Such decreases in stress tolerance have to be partly compensated by an increase in technical energy to avoid stress (plant protection, seedbed preparation, nutrients, irrigation, drainage, etc.).

The agronomic 'yield' in wheat is the product of the evolutionary necessity to propagate for the survival of the species. The chance that a biannual species will occupy a habitat, reproduce itself, and even expand its habitat is highest if its individuals regularly produce seeds at the end of each life cycle (Barrett and Husband, 1990). Good stress tolerance within a given habitat is necessary for this. Genotypes, like old wheat and spelt cultivars, which have been adapted to marginal environments with often unsatisfactory seedbeds have a broader stress reaction potential, illustrated by hypoxia tolerance or the anthocyan response. The former is rarely demanded but, if so, vital for survival. This might have been of great importance for old landraces which were multiplied by the farmer each year. The anthocyan response is energy intensive but its use is still unclear. Some indications exist that leaves with insufficiently developed chloroplasts (because of phosphorus deficiency, cold, or hypoxia) suffer less under high light intensity. Genotypes with such stress reaction patterns should be regarded as better adapted to an old-fashioned agricultural production system. Rimle (1995) showed that the yields of spelt cultivars were less influenced by environmental stress due to their yield structure.

What conclusions can be reached for wheat and spelt breeding in marginal regions as described in the introduction? The harsh environment is very variable, an uncontrollable factor, so that the genotypes adapted to marginal regions must be able to respond to a number of possible stresses. The breeding goal is, therefore, maximum yield stability (at the cost of maximum yield as shown above). The spelt gene pool offers a range of genotypes which are well adapted to marginal conditions. Crosses with wheat, which were selected according to agronomic criteria as defined for modern wheats in a modern production system at favourable locations, will necessarily result in genotypes less tolerant to stress. The correct strategy for maintaining stress tolerance is the selection of newly created germplasm at the appropriate location over a long period thus imitating the natural selection process. Preferably, composite crosses of wheat and spelt should be evolved from the early generations over many life cycles at marginal sites, as is already the case in breeding programmes of FAP.

6. LITERATURE

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