Flooding tolerance of spelt (*Triticum spelta* L.) compared to wheat (*Triticum aestivum* L.) – A physiological and genetic approach

M. St. Burgos[1], M.M. Messmer[2], P. Stamp[1] & J.E. Schmid[1,*]

[1]*Institute of Plant Sciences, Swiss Federal Institute of Technology, ETH-Zurich, Experimental Station, Eschikon 33, CH-8315 Lindau, Switzerland;* [2]*Swiss Federal Research Station for Agroecology and Agriculture (FAL) Zürich-Reckenholz, Reckenholzstr. 191, CH-8046 Zürich; Present address: Institute of Pharmaceutical biology, Univ. Basel, Benkenstr. 254, CH-4108 Witterswil, Switzerland; (*author for correspondence; e-mail: juerg.schmid@ipw.agrl.ethz.ch)*

Summary

In marginal, agroclimatic zones, yield is often affected by flooding, but the effect is much less for winter spelt (*Triticum spelta* L.) than for winter wheat (*Triticum aestivum* L.). This study evaluates the reaction of a wheat × spelt population (F_5 RILs of Forno × Oberkulmer) to flooding stress in the early phase of germination. Lines with greater tolerance to 48 h flooding just after imbibition showed less electrolyte leakage (r = –0.79) indicating greater membrane integrity and better survival. Five QTL explaining 40.6% of the phenotypic variance for survival to flooding were found, and localized on the chromosomes 2B, 3B, 5A, and 7S. The tolerance to 48 h flooding four days after sowing was best correlated with the mean germination time (r = 0.8), indicating that the plants with a fast coleoptile growth during flooding are less susceptible to flooding. Ten QTL were found for seedling growth index after flooding explaining 35.5% of the phenotypic variance. They were localized on chromosomes 2A, 2B, 2D, 3A, 4B, 5A, 5B, 6A, and 7S. Standard varieties of spelt and wheat showed the same tolerance characteristics. The possibility to use marker assisted selection for flooding tolerance is discussed.

Introduction

Flooding occurs worldwide and has a strong effect on natural vegetation and agricultural crops. Much of the world's wheat is grown in marginal, agroclimatic zones, where yield is often affected by excessive soil moisture (Blom, 1999). In cold mountainous areas in central Europe, high precipitation is often associated with heavy soil. These conditions are suboptimal for growing bread wheat. Excess water in the root environment blocks the transfer of oxygen between the soil and the atmosphere causing O_2 deficiency and decreasing redox potential (Kludze et al., 1993). This can be injurious to or even lethal for the plants (Drew, 1997). Most aerobic organisms are highly dependent on an adequate oxygen supply; numerous important processes, such as cell division and oxidation of carbohydrates for the supply of energy, cannot take place

in the absence of oxygen (Crawford, 1982). This lack of energy can lead to death of the apical part of the roots (Waters et al., 1991), modification of the root structure (Wiedenroth, 1993), decrease enzyme activity (Guglielminetti et al., 1995; Perata et al., 1993), damage to the lipids of the cell membranes and, thus, leakage of diverse products into the soil solution, and post anoxic stress when seedlings reach the air again (Braendle, 1996; Pfister-Sieber & Braendle, 1994).

Plants have developed several strategies for surviving such stress situations: 1. adaptation to oxygen deficiency by lowering the rate of metabolism, by changing metabolic pathways or by removal of toxic products (Crawford & Braendle, 1996); 2. avoidance of oxygen deficiency by morphological adaptation (formation of aerenchyma) or provision of oxygen (Drew, 1997; Rüegger et al., 1990).

Because wheat (*Triticum aestivum* L.) is one of the most important crops for human nutrition, selection for flooding tolerance is of great interest. Germination and early seedling establishment is probably the most vulnerable period in the life cycle of plants (Baskin & Baskin, 1998). Later at two to four weeks growth waterlogging decreases yield (Bousqué, 1992; Cannell et al., 1980; McKersie & Hunt, 1987; Musgrave, 1994), but genotypes show differences in tolerance (Davies & Hillman, 1988; Ueno & Takahashi, 1997; Thomson et al., 1992). However, the identification of genes for flooding tolerance and their localization on the chromosomes of wheat is poorly studied. Poysa (1984) localized genes for flooding tolerance on group 5 chromosomes, whereas Taeb et al. (1993) identified genes for flooding tolerance on the chromosome 4B and 4D. Rimle (1995) found that, in wet, cold conditions, spelt (*Triticum spelta* L.) established better than did wheat. Spelt cultivars have been described as being more robust during germination and early seedling development than bread wheat (Jaquot et al., 1960; Rimle, 1995): in general, winter spelt had a higher rate of growth and faster extension of the coleoptile, good seedling emergence under wet, cool conditions, and better winter hardiness compared to winter wheat (Riesen et al., 1986; Rüegger et al., 1990). Wheat can be easily crossed with spelt, which provides an opportunity to examine processes that are related to flooding tolerance and to locate the responsible genes.

The current study reports the genetic analyses of survival and growth under flooding stress between imbibition and emergence in a wheat × spelt population. The objectives of the study were i) to identify genes that are involved in flooding tolerance and ii) to evaluate the possibility of integrating such genes of spelt in a wheat breeding program using marker assisted selection.

Material and method

Plant material

In this study a population of 226 F_5-recombinant inbred lines (RILs) originating from a cross between the winter wheat Forno (FO) and the winter spelt Oberkulmer (OK), both standard varieties was used. This population is described in detail by Messmer et al. (1999). The parental lines and five other Swiss winter wheat varieties (Arina, Bernina, Danis, Runal, Tamaro) and eight winter spelt varieties which originated from Switzerland (Balmegg, Hubel, Lueg,

Oberkulmer, Ostro), Germany (Schwabenkorn), and Belgium (Redouté, Rouquin) were also tested as standard varieties. Dehulled grains were used.

Experimental conditions

Three different experiments were carried out to determine the flooding tolerance and related traits. The tests for growth speed and the imbibition in the other experiments were performed in closed plastic boxes (0.12 × 0.2 × 0.04 m), with three filter papers (Schleicher & Schuell, sort 0860) and 20 ml distilled water. All grains were sterilized for 5 min in 1% sodium hypochlorite and washed for 5 min with distilled water.

All experiments were performed in a growth chamber at 20 °C with 75% humidity and a light intensity of about 300 μmol photons m^{-2}s^{-1} during the 16 h day period.

Experiment 1: Growth speed without flooding

Ten grains of each RIL and the standard varieties were germinated and harvested when 80% of them showed a rupture of the coleoptile. For each germinated plant, the length of the shoot and of the total length of all roots was measured. Each batch of 10 seeds of a line was randomly located in the germination boxes with each time a parental line (FO or OK) as reference.

Experiment 2: Germination under flooded conditions in sand

Ten grains of each RIL and the standard varieties were sterilized, imbibed for 24 h on moist filter paper and afterwards sown in sand (quartz sand 0.08–0.2 mm) in randomly distributed quadratic pots (9 cm side and 12 cm deep) at a depth of 3 cm. The two treatments applied were: control with continuous water supply without flooding and flooding four days after sowing. The grains were flooded with tap water for 48 h to a level of 1 cm above the surface of the sand. After flooding, the pots were drained and watered regularly like the control plants. The experiment was repeated three times. The plants were harvested ten days after sowing. The plants were dissected into roots, shoot and rest of the endosperm from the grains, dried for 48 h at 65 °C, and dry weights determined. For each treatment, the seedling growth index was calculated as the sum of dry weight of the root and the shoot divided by the sum of the dry weight of root, shoot and rest of the endosperm. This ratio accounts for the size of the grain and gives a good appreciation of the vigor of

the seedling. Each day, the number of emerged plants was noted. A plant was considered as emerged if the coleoptile was visible above the soil surface. Each day (D), counted from the start of the sowing, the number of emerged plants during the period D-1 and D (dn) was noted. The mean germination time (MGT) was calculated as MGT = $\Sigma(dn/D)$.

Experiment 3: Germination under flooded conditions in Erlenmeyer flasks

Twenty grains of each RIL and the standard variety were weighed and imbibed on moist filter paper. The number of grains with a primary root length >1 mm was recorded at 24 h. When 75% of the grains had reached this stage the grains were put into an Erlenmeyer flask (120 mL) completely filled with distilled water and sealed with a rubber cap to avoid oxygen exchange with the air. Control flasks without grains were also used. This experiment was repeated three times. The oxygen concentration in the water was measured after 0, 24, 48 h with a gold electrode (WTW multiline p3, cellOX 325). The daily oxygen consumption was calculated. The conductivity of the water (in mS), representing the leakage of electrolytes, was measured at the beginning and at the end of the flooding period (conductometer E518, Metrohm Herisau); conductivity was calculated as the difference of conductivity between beginning and end minus the conductivity of the no-grain control. After a flooding period of 48h, the twenty grains were put again on moist filter paper and the percentage of grains which survived was recorded after three days. A grain was considered alive if it continued to grow and produced normal roots and shoot.

Statistical and QTL analysis

The genetic map used for QTL mapping comprised 182 marker loci (2469 cm) with an average marker density of 13.6 cm (Messmer et al., 1999). This covers about 2/3 of the wheat genome. The QTL analysis was performed with 204 genotypes by the softwarepackage PLABQTL (Utz & Melchinger, 1996) based on the composite interval mapping (CIM). Cofactors were assessed by the procedure cov SELECT. The threshold for detection of the QTL was fixed at a LOD value of 3.0. Explained phenotypic variance of each QTL and of all detected QTL were calculated by multiple regression. The squared partial correlation coefficient of individual QTL taking all other QTL as fixed effects were obtained (part. R^2).

Multiple regression models for survival to flooding at juvenile growth stage with all morphological traits as variables were calculated with the procedure REG, the test for normal distribution was performed by the Shapiro-Wilk statistic of the procedure UNIVARIATE using SAS / STAT 6.03 program (SAS Institute Inc. Cary, NC, USA). Broad sense heritability (h^2) was calculated according to Hallauer & Miranda (1981).

Results

Experiment 1: Growth speed without flooding

The phenotypic distribution of the frequencies for the 226 RILs of the length of the roots and the shoot were normal and transgression on both sides was observed (Table 1). The RILs showed a wide range for the length of the roots and for the length of the shoot with means intermediate between parental values. For the length of the shoot there were significant differences ($p < 0.01$) between the parental lines Oberkulmer and Forno as well as between the spelt and the wheat varieties; spelt varieties having much longer shoots. The correlation between the length of the roots and the length of the shoot was 0.588 ($p < 0.01$).

Experiment 2: Germination under flooded conditions in sand

In the pot experiment, a similar distribution of the RILs was observed for the seedling growth index of the control. The mean of the RILs was identical to the parental spelt values. The mean of the seedling growth index for the spelt varieties was higher than that of the parental spelt line Oberkulmer and significantly higher than that of wheat varieties (Table 1). With regard to the mean germination time of the control plants, the RILs had a much broader range than the parental varieties with a mean closer to the spelt parent. Again, spelt and wheat varieties differed ($p = 0.061$) from each other, with a faster germination observed in spelt.

The seedling growth index of plants flooded four days after sowing was weakly correlated (r = 0.39, $p < 0.01$) with the seedling growth index of the control. As the tolerance for flooding is given by the ratio seedling growth index flooded / seedling growth index control and because this ratio very closely correlated with the seedling growth index of the plants flooded (r = 0.91), the QTL analysis was performed on this last parameter.

The distribution of the RILs was near to the normal distribution (Figure 1). The broad sense heritability for

Table 1. Mean and range of parental and recombinant inbred lines (RILs) of the cross Forno × Oberkulmer, and of standard spelt and wheat varieties of seedling germination traits. LROO_C = Length of the roots (mm), LSHO_C = Length of the shoot (mm), SGI_C = Seedling growth index of control, MGT_C = Mean germination time of the control plants, SGI_F = Seedling growth index of the flooded plants, MGT_F = Mean germination time of the flooded plants, FWG_C = Grain weight at 15% humidity (g), N24_C = % of plant with first root > 1mm after 24 h (%), SUR_F = Percentage of survival (%), LKG_F = Leakage on metabolites (mS), O2_F = Daily oxygen consumption (mg $grain^{-1}d^{-1}$)

	Experiment 1		Experiment 2				Experiment 3				
	LROO_C	LSHO_C	SGI_C	MGT_C	SGI_F	MGT_F	FWG_C	N24_C	SUR_F	LKG_F	O2_F
	(mm)	(mm)					(g)	(%)	(%)	(mS)	(mg $grain^{-1}d^{-1}$)
RILs											
range	59–238	19–46	0.54–0.87	0.85–2.59	0.19–0.72	0.0–2.33	0.95–1.40	2–90	8.3–98.5	17.1–46.6	3.99–7.67
mean	148.10	31.73	0.70	1.79	0.41	0.760	1.13	51	70.5	27.6	6.31
LSD[1]	33.39	6.21	0.146	1.02	0.16	0.94	0.068	16.6	6.3	6.09	1.92
Parental lines											
Oberkulmer	187	40	0.69	1.82	0.55	1.72	1.17	80	36.5	35.9	6.4
Forno	132	25	0.63	1.44	0.40	0.48	1.17	17	96.5	22.3	6.6
LSD[1]	58.1	3.13	0.23	1.89	0.10	1.22	0.07	9.0	10.0	4.32	0.85
Varieties[3]											
Spelt											
range	119–197	29–42	0.66–0.87	1.46–2.48	0.39–0.66	0.28–2.12	0.99–1.26	10–90	82–98	17.7–24.1	5.2–6.7
mean	153	38	0.76	1.94	0.55	1.22	1.17	63	92	21.5	6.24
wheat											
range	92–197	22–33	0.63–0.73	1.15–1.87	0.4–0.49	0.24–0.67	1.01–1.23	2–43	36.5–100	15.7–35.9	4.47–6.56
mean	129	26	0.67	1.5	0.44	0.42	1.11	30	82	22.7	5.52
LSD[2]	87.5	5.69	0.07	0.47	0.07	0.47	0.109	4.18	10.1	3.2	0.57

[1] least significant difference between genotypes ($p < 0.05$).

[2] least significant difference between group of genotypes ($p < 0.05$).

[3] Forno and Oberkulmer are included in these variety averages.
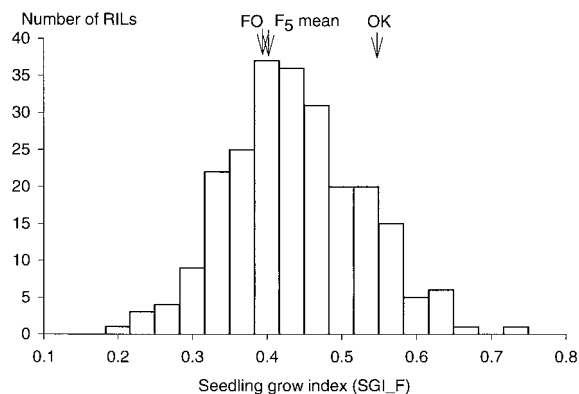

Figure 1. Frequency distribution of 226 RILs of Forno × Oberkulmer for seedling growth index (SGI_F) of plants exposed to 48 h flooding four days after sowing.

the seedling growth index of flooded plants (SGI_F) was $h^2 = 0.60$. The mean of the RILs was identical with that of the wheat parent, but more than 25% lower than in the spelt parent. The SGI_F of the spelt parent Oberkulmer and the mean of the spelt varieties were identical. The spelt and wheat varieties like the parental lines were significantly different ($p < 0.01$).

Using multiple regression, 78% of the phenotypic variation for SGI_F could be explained by seven significant parameters among them the mean germination time of flooded plants (64%), the dry weight of the rest endosperm of the control (5%), the consumption of oxygen between 24 h and 48 h (4%) and the mean germination time of the control plants (2%). Correlations between single traits are presented in Table 2.

The results of the QTL analysis of seedling growth index of the flooded plants are presented in Table 3. Ten QTL were found and explained together 35.5% of the phenotypic variance. Individual QTL explained between 0.1% (Chr 2B) and 20% (5A) of the phenotypic variance. At the QTL localized on the chromosomes 2A, 2B, 2D, 3A, 5A (206 cm), 6A, and 7S the positive allele was inherited from Oberkulmer and at the QTL on the chromosomes 4B, 5A (4 cm), and 5B the positive allele originated from Forno.

Table 2. Correlation between the different measured traits assessed in the three experiments for the 226 RILs and 13 standard varieties

Trait[1]	LROO_C	LSHO_C	SGI_C	MGT_C	SGI_F	MGT_F	FWG_C	N24_C	SUR_F	LKG_F	O2_F
Exp. 1:											
LROO_C	1.000										
LSHO_C	0.588**	1.000									
Exp. 2:											
SGI_C	0.067	0.131*	1.000								
MGT_C	0.127*	0.251**	0.709**	1.000							
SGI_F	0.184*	0.236**	0.394**	0.292**	1.000						
MGT_F	0.173*	0.343**	0.244**	0.378**	0.802**	1.000					
Exp. 3:											
FWG_C	–0.041	–0.014	–0.222	–0.049	–0.216**	–0.019	1.000				
N24_C	0.116	0.384**	0.147*	0.254**	0.221**	0.367**	0.153*	1.000			
SUR_F	0.226**	0.348**	0.346**	0.303**	0.372**	0.258**	–0.458**	0.161*	1.000		
LKG_F	–0.108	–0.259**	–0.298**	–0.242**	–0.367**	–0.235**	0.583**	–0.107	–0.794**	1.000	
O2_F	–0.015	0.072	0.162*	0.225**	–0.135*	0.000	0.111	–0.003	–0.076	0.158*	1.000

[1] LROO_C = Length of the roots (mm), LSHO_C = Length of the shoot (mm), SGI_C = Seedling growth index of control, MGT_C = Mean germination time of the control plants, SGI_F = Seedling growth index of the flooded plants, MGT_F = Mean germination time of the flooded plants, FWG_C = Grain weight at 15% humidity (g), N24_C =% of plant with first root > 1mm after 24 h (%), SUR_F = Percentage of survival (%), LKG_F = Leakage on metabolites (mS), O2_F = Daily oxygen consumption (mg grain^{-1}d^{-1}).
*, ** significant at probability level of $p < 0.05$ and $p < 0.01$, respectively.

For the mean germination time of flooded plants the distribution of the RILs was not normal and shifted to the side of the wheat parent. Wheat and spelt varieties were significantly ($p < 0.01$) different. The parental varieties also differed significantly ($p < 0.05$). Five QTL were found for the mean germination time of flooded plants explaining 21.5% of the phenotypic variance. Three of them coincided with QTL for seedling growth index of the flooded plants on the chromosomes 2A, 2D and 5A (Table 3).

Experiment 3: Germination under flooded conditions in Erlenmeyer flasks

Among the RILs broad variation was observed for the fresh weight of the grains. The mean of the RILs was identical with the parental mean. The wheat and spelt varieties showed no significant difference.

The RILs showed a broad variation for the number of grains which had primary roots longer than 1 mm (stage I) after 24 h (N24_C) (Table 1). The average N24_C of spelt varieties was significantly ($p < 0.01$) higher than for the wheat varieties. The range of the wheat varieties was smaller than that of the spelt varieties. For N24_C, transgression was present on both sides. The parental lines Oberkulmer and Forno were also significantly different ($p < 0.01$).

Among the 226 RILs, there was wide variation in percentage of survival to flooding imposed shortly
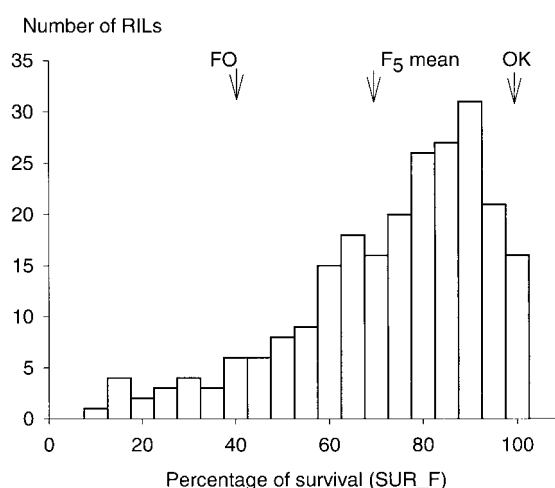

Figure 2. Frequency distribution of survival (SUR) of 226 RILs of Forno × Oberkulmer exposed to 48 h flooding after imbibition.

after imbibition. Flooding tolerance as measured by percent of survival ranged from 2 to 100% among the RILs with a mean of 70% (Figure 2). The RILs showed a continuous non-normal distribution shifted towards the spelt parent. The broad sense heritability was $h^2 = 0.69$. The survival of both spelt and wheat varieties was quite high. The lower average of wheat varieties is due to the remarkably lower value of the wheat parent Forno which was 37%. The survival was highly

Table 3. QTL for phenological traits: seedling growth index of control (SGI_C), mean germination time of the control plants (MGT_C), seedling growth index of the flooded plants (SGI_F), mean germination time of the flooded plants (MGT_F), % of plant with first root > 1mm after 24 h (%) (N24_C), percentage of survival (%) (SUR_F), leakage on metabolites (mS) (LKG_F). Averaged over three repetitions for 204 RILs from the cross Forno × Oberkulmer with the position on chromosomes, additive effects (add.), explained phenotypic variance (part. R^2) and total explained variance in a simultaneous fit

Chromosome	Experiment 2				Experiment 3									
	SGI_C		MGT_C		SGI_F		MGT_F		N24_C		SUR_F		LKG_F	
	a [1]	Part R^2	a	Part R^2	a	Part R^2	a	Part R^2	a	Part R^2	a	Part R^2	a	Part R^2
2A	0.015	8.5	0.089	4.5										
	84-**84**–90[2]		82-**86**–90											
2A					0.018	4.5	0.132	6.9	1.902	18.5				
					122-**126**–132		116-**126**–130		130-**132**–136					
2B											0.904	6.8		
											82-**84**–96			
2B					0.002	0.1								
					122-**126**–128									
2B									1.280	4.5				
									154-**158**–160					
2B							–0.103	4.1	–2.023	8.4	–1.070	8.3		
							174-**184**–184		172-**176**–182		174-**182**–184			
2D					0.025	8.8	0.077	2.5						
					0-**0**–6		0-**0**–6							
3A							0.130	5.7	1.204	7.6				
							52-**60**–64		64-**64**–70					
3A					0.016	3.5								
					86-**92**–96									
3B	0.008	2.6									1.221	10.9	–1.578	8.0
	34-**34**–38										34-**40**–44		38-**40**–44	
3D	0.019	11.0												
	0-**2**–4													
4A	0.014	6.2												
	172-**174**–178													
4B					–0.020	5.3								
					68-**82**–88									
5A					–0.019	5.5								
					0-**4**–16									
5A	–0.012	4.0							2.348	20.9				
	58-**60**–68								54-**60**–70					
5A	0.030	17.9	0.085	6.9	0.044	20.0	0.212	12.9			1.851	20.4	–3.230	26.3
	208-**212**–216		202-**210**–220		200-**206**–212		206-**208**–212				202-**206**–212		204-**206**–210	
5B									–1.281	7.7				
									2-**4**–8					
5B					–0.025	7.0								
					138-**144**–144									
6A					0.020	5.3			1.875	16.3				
					0-**4**–12				0-**2**–18					
7S					0.008	1.1			1.238	9.5	0.717	4.7		
					6-**8**–10				8-**10**–20		8-**10**–26			
R^2 (simultaneous fit)	**33.7%**		**21.2%**		**35.5%**		**21.5%**		**48.6%**		**40.6%**		**29.9%**	

[1] Additive effects were estimated from the simultaneous fit of all QTL. positive effects indicate that the Oberkulmer allele contributed to a higher value, i.e. higher seedling growth index (SGI_C, SGI_F), higher mean germination time (MGT_C, MGT_F), earlier visible germination (N24_C), higher survival (SUR_F) and lower leakage of metabolites (LKG_F).

[2] the first and the third number indicate the support interval, the number printed in bold indicates the position of the highest LOD score of the QTL.

significantly ($p < 0.01$) correlated with the leakage of metabolites ($r = -0.79$) representing the leakage of electrolytes in the solution (Table 2).

In the analysis of survival five QTL were found and they together explained 40.6% of the phenotypic variance with a range from 4.7 (Chr 7S) to 20.4% (Chr 5A). The QTL were localized on the chromosome 2B (2 QTL), 3B, 5A, and 7S. Four positive alleles came from Oberkulmer and one (Chr 2B, 182 cm) from Forno.

The analysis of electrolyte leakage gave two QTL, explaining 30% of the phenotypic variance and localized at the same place as for the survival on chromosome 3B and 5A (Table 3).

The daily oxygen consumption varied in a wide spectrum for the analyzed RILs. The means of the RILs was similar to parental mean and to the spelt varieties. On average the wheat varieties consumed less oxygen.

Discussion

Multiple regression analysis showed that for flooding shortly after starting germination, the survival of the grains was best correlated ($r = 0.79$) with the electrolyte leakage. Leakage of ions as a consequence of oxygen deprivation was described previously for wheat (Chirkova et al., 1991) and potatoes (Blom, 1999). It occurs when the cells suffer from a lack of energy, and the ATP-active ion carrier-proteins cannot function. As a consequence the membranes become depolarized and leaky and the cells loose their electrolytes and ions (Pfister-Sieber & Braendle, 1994; Buwalda et al., 1988). Therefore, plants with enough energy (ATP) available during flooding or with a low energy requirement can better survive a period of temporary flooding (Guglielminetti et al., 1995). The grain weight was negatively correlated ($r = -0.45$) with the survival of the seedlings and positively with the leakage ($r = 0.58$), it means that small grains can better preserve their membrane integrity. Because stress symptoms are typical for oxygen shortage, it is possible that the surviving lines required less energy or consumed less oxygen. However, factors additional to grain weight played a role in the survival since the two parents (OK and FO) showed the same grain weight but differed strongly in survival. Of the five QTL found for the survival, the two QTL with the major impact were located on chromosomes 5A (208 cm) and 3B (40 cm), respectively. The same genomic regions are involved in the expression of electrolyte leakage and grain weight at 15% humidity. On chromosome 5A we also found a QTL for grain weight (data not shown). Thus, part of flooding tolerance during germination seems to be controlled by a few major genes. This could explain the deviation from normal distribution (Becker, 1993). The involvement of a small number of major genes in flooding tolerance in wheat was also suggested by McKersie & Hunt (1987). For submergence tolerance of rice, a major gene and a few minor QTL were found (Nandi et al., 1997).

According to findings of experiment 2 in sand, the capacity to form a well developed seedling when flooding occurs four days after sowing showed a continuous and normal distribution as expected for a polygenic trait. The high positive correlation between the seedling growth index and the mean germination time of flooded plants indicated that the lines which were able to reach the top of the soil before the period of flooding, were advantaged. This shows clearly an avoidance strategy. However, the mean germination time of the control was only weakly correlated with that of the flooded plants ($r = 0.244$). Another interesting factor according to the regression analysis, is the consumption of oxygen between 24 and 48 hour of flooding. The slight but significant positive correlation ($r = 0.18$) indicated that the lines which consume more oxygen during this period might survive better. Similar to the survival rate in experiment 3, one major QTL on chromosome 5A (206 cm) was found to have a strong effect on the phenotypic expression of the seedling growth index after flooding and other QTL of that parameter coincided with QTL for germination rate.

The results of the two experiments showed two different aspects of flooding tolerance. The RILs which survived flooding well germinated early, emerged fast (avoidance), and were able to preserve their membrane integrity (avoidance or adaptation). Big grains germinate more slowly but ultimately produce longer shoots and roots (Guberac et al., 1999). Thus the biggest can suffer more from flooding. Therefore an optimal size seems to exist for flooding tolerance.

In order to use these characteristics in a breeding program and to verify that the reaction of the parental lines Forno and Oberkulmer is representative for wheat and spelt, the reaction of other standard varieties was studied. The low growth of the coleoptile of wheat standard lines diminished flooding survival after four days growth, but the effect of grain size helped to avoid some membrane damage as indicated

by reduced leakage. Forno, in contrast, showed more leakage and a similar grain weight as spelt varieties which might explain its sensitivity to both types of flooding stress.

Among the RILs some very interesting genotypes were found with good tolerance to both flooding treatments. First of all, an important role of the chromosome 5A must be underlined. Sixteen of the measured parameters showed a QTL at the location 210 cm. This is the position of the gene q responsible for the long lax ear of spelt and its hulled kernels. Other loci as well influencing traits such plant height and flowering time have been located in this chromosomal region (Keller et al., 1999; Kato et al., 1999). The importance of chromosome 5A was cited for flooding tolerance (Poysa, 1984), cold tolerance and vernalization requirement (Roberts, 1990), and absicic acid level (Quarrie et al., 1997). Because the flooding tolerance of spelt in the second experiment was partly based on the speed of the growth, it is reasonable to find a QTL for this trait near to the one for greater plant height. Thus, selection for this QTL could lead to undesirably tall plants with spelt ear type. For this reason it is important to look on other chromosomes for QTL which are not obviously linked to such undesirable traits. Among the chromosomes found for flooding tolerance, chromosomes 2B, 3B, and 6A are known to influence grain yield, seed weight and plant height of the variety Cheyenne and Wichita under normal conditions (Berke et al., 1992). The chromosomes of group 2 also have a positive effect on flooding tolerance by *Thinopyrum elongatum* L. (Taeb et al., 1993), and chromosome 3B influences winter hardiness (Zemetra & Morris, 1988). Unfortunately those studies did not provide precise information for the chromosomal location of these traits. The QTL on the chromosome 6A also influences plant height (Keller et al., 1999).

Hospital et al. (1997) stated that marker assisted selection (MAS) can be useful for characters with a low heritability. It is possible to use MAS to complement character assisted selection if the number of repetitions is limited or if the genetic effect of the characters explains less than 50% of the total genetic variance. In the present study the measured traits explained 66 to 78% of the phenotypic variance for survival and seedling growth index of flooded plants respectively. Both traits were influenced by a major gene at a position 208 cm along chromosome 5A. Unfortunately, as mentioned above, unfavorable other traits are located in this region. Therefore, it is not possible to select on these phenotypic parameters without altering plant height and ear type. A promising alternative would be to concentrate MAS on QTL found on chromosome 3A, or 2B, where no negative traits were found.

In conclusion this work has shown that some spelt varieties could be used as genetic resources to improve flooding tolerance in wheat screening. The tests and selection criteria are simple and the use of MAS could avoid the transmission of undesirable traits.

Acknowledgements

References

Baskin, C.C. & J.M. Baskin, 1998. Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, San Diego, Ill., 666 pp.

Becker, H., 1993. Pflanzenzüchtung, Verlag Eugen Ulmer, cop. Stuttgart, Germany, 327 pp.

Berke, T.G., P.S. Baenziger & R. Morris, 1992. Chromosomal location of wheat quantitative trait loci affecting stability of six traits, using reciprocal chromosome substitutions. Crop Sci 32: 628–633.

Blom, C.W.P.M., 1999. Adaptations to flooding stress: from plant Community to Molecule. Plant Biol 1: 261–273.

Bousqué, I., 1992. Les mécanismes et les conséquences de l'asphyxie racinaire. Persp Agr 175: 116–122.

Braendle, R., 1996. Ueberflutung und Sauerstoffmangel. In: C. Brunold, A. Rüegsegger & R. Braendle (Eds.), Stress bei Pflanzen, UTB für Wissenschaft, Haupt, Bern, pp. 133–148.

Buwalda, F., C.J. Thomson, W. Steigner, E.G. Barrett-Lennard, J. Gibbs, & H. Greenway, 1988. Hypoxia induces membrane depolarization and potassium loss from wheat roots but does not increase their permeability to sorbitol. J Exp Bot 39: 1169–1183.

Cannell, R.Q., R.K. Belford, K. Gales, C.W. Dennis & R.D. Prew, 1980. Effects of waterlogging at different stages of development on the growth and yield of winter wheat. J Sci Food Agric 31: 117–132.

Chirkova, T.V., T.M. Zhukova & N.N. Goncharova, 1991. Method of determining plant resistance to oxygen deficiency. Sov Plant Physiol 38: 265–269.

Crawford, R.M.M., 1982. The anaerobic retreat as a survival strategy for aerobic plants and animals. Trans Bot Soc Edinb 44: 57–63.

Crawford, R.M.M. & R. Braendle, 1996. Oxygen deprivation stress in a changing environment. J Exp Bot 47: 145–159.

Davies, M.S. & G.C. Hillman, 1988. Effect of soil flooding on growth and grain yield of populations of tetraploid and hexaploid species of wheat. Ann Bot 62: 597–604.

Drew, M.C., 1997. Oxygen deficiency and root metabolism: injury and acclimation under hypoxia and anoxia. Ann Rev Plant Pysiol Plant Mol Biol 48: 223–250.

Guberac, V., J. Martincic & D. Banaj, 1999. Influence of cereal seed size on shoot and root length. Bodenkultur 50: 39–43.

Guglielminetti, L., J. Yamaguchi, P. Perata & A. Alpi, 1995. Amylolytic activities in cereal seeds under aerobic and anaerobic conditions. Plant Physiol 109: 1069–1076.

Hallauer, A.R. & Fo.J.B. Miranda, 1981. Quantitative Genetics in Maize Breeding. The Iowa University Press, Ames, Iowa, USA, 468 pp.

Hospital, F., L. Moreau, F. Lacoudre, A. Charcosset & A. Gallais, 1997. More on the efficiency of marker-assisted selection. Theor Appl Genet 95: 1181–1189.

Jaquot, R., J. Adrian & A. Rerat, 1960. Eine vergessene Getreideart: der Speltz (Dinkel). Z Acker- und Pflanzenbau 111: 279–288.

Kato, K., H. Miura & S. Sawada, 1999. QTL mapping of genes controlling ear emergence time and plant height on chromosome 5A of wheat. Theor Appl Genet 98: 472–477.

Keller, M., Ch. Karutz, J.E. Schmid, P. Stamp, M. Winzeler, B. Keller & M.M. Messmer, 1999. Quantitative trait loci for lodging resistance in a segregating wheat × spelt population. Theor Appl Genet 98: 1171–1182.

Kludze, H.K., R.D. DeLaune & W.H.Jr. Patrick, 1993. Aerenchyma formation and methane and oxygen exchange in rice. Soil Sci Soc Am j 57: 386–391.

Lande, R. & R. Thompson, 1990. Efficiency of marker-assisted selection in the improvement of quantiative traits. Genetics 124: 743–756.

McKersie, B.D. & L.A. Hunt, 1987. Genotypic differences in tolerance of ice encasement, low temperature flooding, and freezing in winter wheat. Crop Sci 27: 860–863.

Messmer, M.M., M. Keller, S. Zanetti & B. Keller, 1999. Genetic linkage map of a wheat × spelt cross. Theor Appl Genet 98: 1163–1170.

Musgrave, M.E., 1994. Waterlogging effects on yield and photosynthesis in eight winter wheat cultivars. Crop Sci 34: 1314–1318.

Nandi, S., P.K. Subudhi, D. Senadhira, N.L. Manigbas, S. Sen Mandi & N. Huang, 1997. Mapping QTLs for submergence tolerance in rice by AFLP analysis and selective genotyping. Molecular and General Genetics 255: 1–8.

Perata, P., N. Geshi, J. Yamaguchi & T. Akazawa, 1993. Effect of anoxia on the induction of alpha-amylase in cereal seeds. Planta 191: 402–408.

Pfister-Sieber, M. & R. Braendle, 1994. Aspects of plant behaviour under anoxia and post anoxia. Proc Royal Soc Edinb 102B: 313–324.

Poysa, V.W., 1984. The genetic control of low temperature, ice-encasement, and flooding tolerances by chromosomes 5A, 5B, and 5D in wheat. Cereal Res Commun 12: 135–141.

Quarrie, S.A., D.A. Laurie, J. Zhu, C. Lebreton, A. Semikhodskii, A. Steed, H. Witsenboer & C. Calestani, 1997. QTL analysis to study the association between leaf size and abscisic acid accumulation in droughted rice leaves and comparisons across cereals. Plant Mol Biol 35: 155–165.

Riesen, T., H. Winzeler, A. Rüegger & P.M. Fried, 1986. The effect of glumes on fungal infection of germinating seed of spelt (*Triticum spelta L.*) in comparison to wheat (*Triticum aestivum L.*). J Phyt 115: 318–324.

Rimle, R., 1995. Agronomische und morphologische Charakterisierung von Weizen (Tritcum aestivum L.) und Dinkel (Triticum spelta L.) sowie von spezifischen Weizen/Dinkel-F_1-Hybriden und deren Folgegenerationen von der F_2 bis zur F_5. Diss ETH Nr. 11242, Swiss Federal Institute of Technology (ETHZ), Zürich.

Roberts, D.W.A., 1990. Identification of loci on chromosome 5A of wheat involved in control of cold hardiness, vernalization, leaf length, rosette growth habit, and height of hardened plants. Genome 33: 247–259.

Rüegger, A., H. Winzeler & J. Nösberger, 1990. Die Ertragsbildungs von Dinkel (Triticum spelta L.) und Weizen (Triticum aestivum L.) unter verschidene Umweltbedingung im Freiland. J Agr Crop Sci 164: 145–162.

Taeb, M., R.M.D. Koebner & B.P. Forster, 1993. Genetic variation for waterlogging tolerance in the Triticeae and the chromosomal location of genes conferring waterlogging tolerance in Thinopyrum elongatum. Genome 36: 825–830.

Thomson, C.J., T.D. Colmer, E.L.J. Watkin & H. Greenway, 1992. Tolerance of wheat (Triticum aestivum cvs. Gamenya and Kite) and triticale (Triticosecale cv. Muir) to waterlogging. New Phytol 120: 335–344.

Ueno, K. & H. Takahashi, 1997. Varietal variation and physiological basis for inhibition of wheat seed germination after excessive water treatment. Euphytica 94: 169–173.

Utz, H.F. & A.E. Melchinger, 1996. PLABQTL- a computer program to map QTL. Version 1.1. Institut für Pflanzenzüchtung, Saatgutforschung und Populationsgenetik, Universität Hohenheim, Stuttgart, Germany, 9 pp.

Waters, I., P.J.C. Kuiper, E. Watkin & H. Greenway, 1991. Effects of anoxia on wheat seedlings. I. Interaction between anoxia and other environmental factors. J Exp Bot 42: 1427–1435.

Wiedenroth, E.M., 1993. Responses of roots to hypoxia: their structural and energy relations with the whole plant. Env Exp Bot 33: 41–51.

Zemetra, R.S. & R. Morris, 1988. Effects of an intercultivaral chromosome substitution on winterhardiness and vernalization in wheat. Genetics 119: 453–456.