CHAPTER 3

Seg8 mutant analysis during seed development

3.1 INTRODUCTION

Developing caryopses of cereals accumulate starch in the endosperm as the major carbon and energy resource, and several mutants defective in starch accumulation have been isolated (Miller and Chourey, 1992; Cheng *et al.,* 1996; Tyynelä *et al.,* 1995; Harrison *et al.,* 1998; Maitz *et al.,* 2000). The majority of the described mutants are either defective in early stages of cell division and morphogenesis or fail to accumulate storage compounds such as starch during maturation. In the past, research has been focused on genetic and molecular physiological aspects of carbohydrate metabolism in miniature, shrunken and brittle mutants of maize. Miller and Chourey (1992) demonstrated that the miniature-1 seed mutation is connected to cell wall-bound invertase, and Cheng *et al.* (1996) identified miniature1 seed loci encoding isoforms of cell wall-bound invertase 2 in maize. *Shrunken* (*sh*) mutants are affecting sucrose synthase (Chourey and Nelson, 1976; Doehlert and Kuo, 1990; Tobias *et al.,* 1992) and *shrunken2* and *brittle2* affect the small and large subunit of ADP glucose pyrophosphorylase, respectively (Hannah and Nelson, 1976; Bhave *et al.,* 1990; Tobias *et al.,* 1992). Waxy mutants on the other hand lack granule bound starch synthase and amylose (McDonald *et al.,* 1991). Similar studies are scarce in barley.

Normal seed development in barley is significantly dependent on the maternal tissue. Sucrose and other nutrients are unloaded first into the maternal pericarp tissue through the vascular bundles, are further symplastically transported via the nucellar projection cells and finally released into the endosperm cavity. Despite the importance of the maternal tissues to supply sucrose and other nutrients to the developing filial tissues (endosperm and embryo), little is known about the maternal-filial interaction. The analysis of gene expression networks as well as generated molecular-physiology data from developing grains of shrunken endosperm mutants in barley during seed development will provide new knowledge about the role and the importance of maternal tissues.

In barley the shrunken endosperm mutants xenia (*sex*), non-xenia (*seg*) and the high lysine (*lys*) mutants appear to alter carbohydrate metabolism in the endosperm. Endosperm differentiation in *sex* mutants has been described by Bosnes *et al.* (1992). Further, a recessive shrunken endosperm mutant called *shx* has been isolated as spontaneous mutation. Schulman and Ahokas (1990) reported that the *shx* mutant has only 25% of the starch content found in grains of the wild type cv Bomi. To analyse the block of starch synthesis, enzyme activities of ADP glucose pyrophosphorylase and soluble starch synthase were measured and found to be reduced by about 20%. Northern blot analysis has shown that the transcript for ADP glucose pyrophosphorylase (small subunit) is less abundant in the mutant (Tyynelä *et al*, 1995). Although several other enzymes involved in starch synthesis are affected, the authors suggested that the soluble starch synthase might be the primary site of mutation in *shx*. Among *seg* mutants, eight different shrunken endosperm mutants (*seg1-8*) have been identified and designated as maternal effect mutants (Jarvi and Eslick, 1975; Ramage and Crandall, 1981). The *seg* mutants may become useful to study maternal-filial interaction during endosperm development. Felker *et al.* (1985) described them based on the anatomy of immature grains. Based on histological descriptions, the shrunken endosperm mutants were classified into two groups: the chalazal necrosis mutants (*seg1*, *seg3*, *seg6* and *seg7*) and the abnormal endosperm mutants (*seg2*, *seg4*, *seg5* and *seg8*). In the first category, death of the maternal chalaza and nucellar projection tissue during early seed development cuts off the supply of assimilates and causes the shrunken endosperm phenotype, while in the second one the maternal tissue develops normally and abnormality occurs in the endosperm tissue itself. Felker *et al.* (1985) speculate that the lack of factor(s) in the cytoplasm of the central cell causes the phenotype in the second category. Furthermore, they suggested the mutants as powerful source to study maternal effects on endosperm development.

Seg8 is one of the eight mono-factorial recessive shrunken endosperm mutants in barely that do not express xenia, *i.e.* the source of pollen does not affect the embryo or endosperm phenotype. Ramage and Crandall (1981) originally identified and characterised the *seg8* mutant from a field stock of the cultivar 'Klages'. *Seg8* seeds exhibit shrunken endosperm and weigh about 21% of the seeds produced by normal plants. When the seeds from mutants were sown in the green house, the resulting plants were normal having seven pairs of chromosomes. Pollen mother cell meiosis as well as pollen and ovule fertility were normal too. Moreover the researchers did not observe any change in the mutant due to variation in

environmental conditions and they have not encountered difficulties in establishing the stands of the mutant. The *seg8* mutant has been crossed with other shrunken mutants *seg1* to *seg7*, and in all crosses the F1 plants always produced plump seeds. F2 plants were grown from all of the allele crosses and all segregated in an approximately 9 plump seeded : 7 shrunken seeded ratio indicating that *seg8* is a distinct non-allelic *seg* mutant. Ramage (1983) reported that *seg8* is located on chromosome 1 (7H). Djarot and Peterson (1991) studied seed development in the *seg8* mutant. The anatomical description therein indicates that the mutant phenotype, *i.e.* the shrunken endosperm appeared at 4 DAF, and some biochemical estimates revealed that the mutant had lower starch content and higher sucrose concentration than the wild type. Though studies have addressed barley seed mutants at morphological, anatomical and biochemical levels, molecular physiological and genetic studies are lacking. Hence an attempt in this direction has been made in the present study to study expression profiling by macroarray analysis to look into the seed development of barley *seg8* mutant. A *seg8* mutation was used which had been transferred to the genetic background of "Bowman" (kindly provided by Prof. D. Frankowiak, NDSU, USA).

The results of expression profiling of the *seg8* mutant compared to wild type 'Bowman' are presented on both whole caryopses level and in the dissected maternal and filial fraction of developing caryopses during pre-storage and the ongoing storage-phase (0-14 DAF). Furthermore, an attempt was made to compare transcript and metabolite profiling data of the *seg8* mutant and the Bowman wild type.

3.2 RESULTS

3.2.1 Fresh weight of developing caryopses of *seg8* and wild type

The fresh weight of developing *seg8* mutant and Bowman wild type caryopses was measured (Fig. 17). The fresh weight of *seg8* and Bowman grains were similar until 8 DAF. From 10 DAF onwards Bowman grains were significantly heavier (Fig. 17A). A similar pattern is seen in the data for the filial part of the caryopses (embryo and endosperm) with a decrease in fresh weight content in the mutant as compared to wild type beginning 10 DAF (Fig. 17B). Fresh weight did not vary in the maternal pericarp fraction between mutant and wild type during caryopses development until 16 DAF. On the other hand the filial tissue fraction of mutant showed a drastic reduction in fresh weight from 10 DAF onwards (Fig. 17B). This seems to be due to accumulation of higher amounts of starch in the embryo sac fraction of the wild type caryopses. Further, we calculated fresh weight ratio of pericarp and embryo sac fractions of developing caryopses of mutant and wild type were measured, the prominent differences were observed from 12 DAF onwards (Fig. 17C).

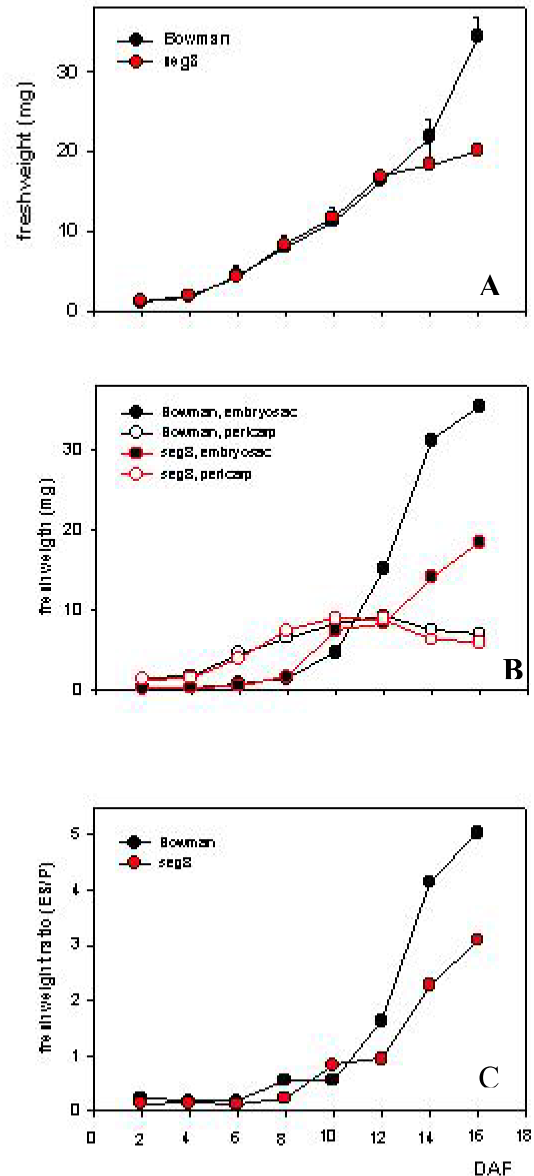

Fig. 17

After separation of maternal (pericarp) from the filial part (embryo sac), fresh weight of both fractions was estimated and the fresh weight ratio was calculated. On X-axis the developmental scale 2-14 DAF (Days After Flowering) is given in every two day intervals.

3.2.2 Starch content in caryopses of *seg8* and wild type

During development starch content of seg8 and wild type seeds was measured in two-day intervals. During 2 to 6 DAF of pre-storage phase the starch content remains similar in both *seg8* and wild type seeds. From 10 DAF onwards, the starch content in *seg8* seeds was significantly lower in mutant than in wild type (Fig. 18). During 12 to 14 DAF the starch accumulates to only 40% of wild type, resulting in reduced grain weight.

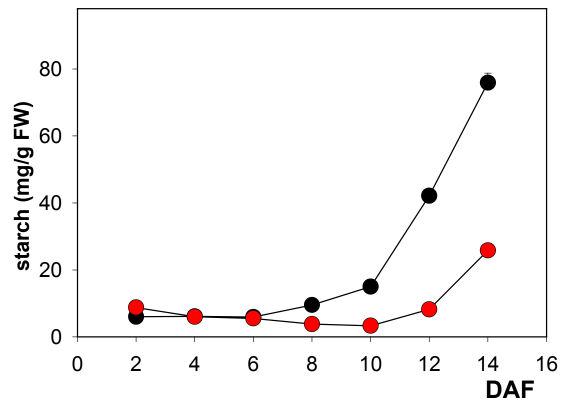

Fig. 18

Red colour filled circles- *seg8*
Black colour filled circles- Bowman
For further legend see Fig. 17

3.2.3 Characteristic changes in sugar and metabolite concentrations in *seg8* and Bowman during pre-storage and storage phase in pericarp and embryo sac fractions

Sugar and metabolite profiles of carbohydrate metabolism were determined in maternal pericarp and filial embryo sac fractions of *seg8* and Bowman (Fig. 19). Total sugar content and glucose content are comparatively lower in *seg8* mutant pericarp during 0-4 DAF and sugar and key metabolites such as UDP-glucose and ADP-glucose concentration increased in the mutant pericarp during later stages (12-14 DAF). Strikingly, but not surprisingly, sugar levels (glucose and fructose) and the metabolite ADP-glucose decreased in the embryo sac fraction of the mutant during the onset of storage process in comparison to wild type (Fig. 19). A further interesting observation is the increase of sucrose content and UDP-glucose in the mutant during initial storage phase (8-12 DAF).

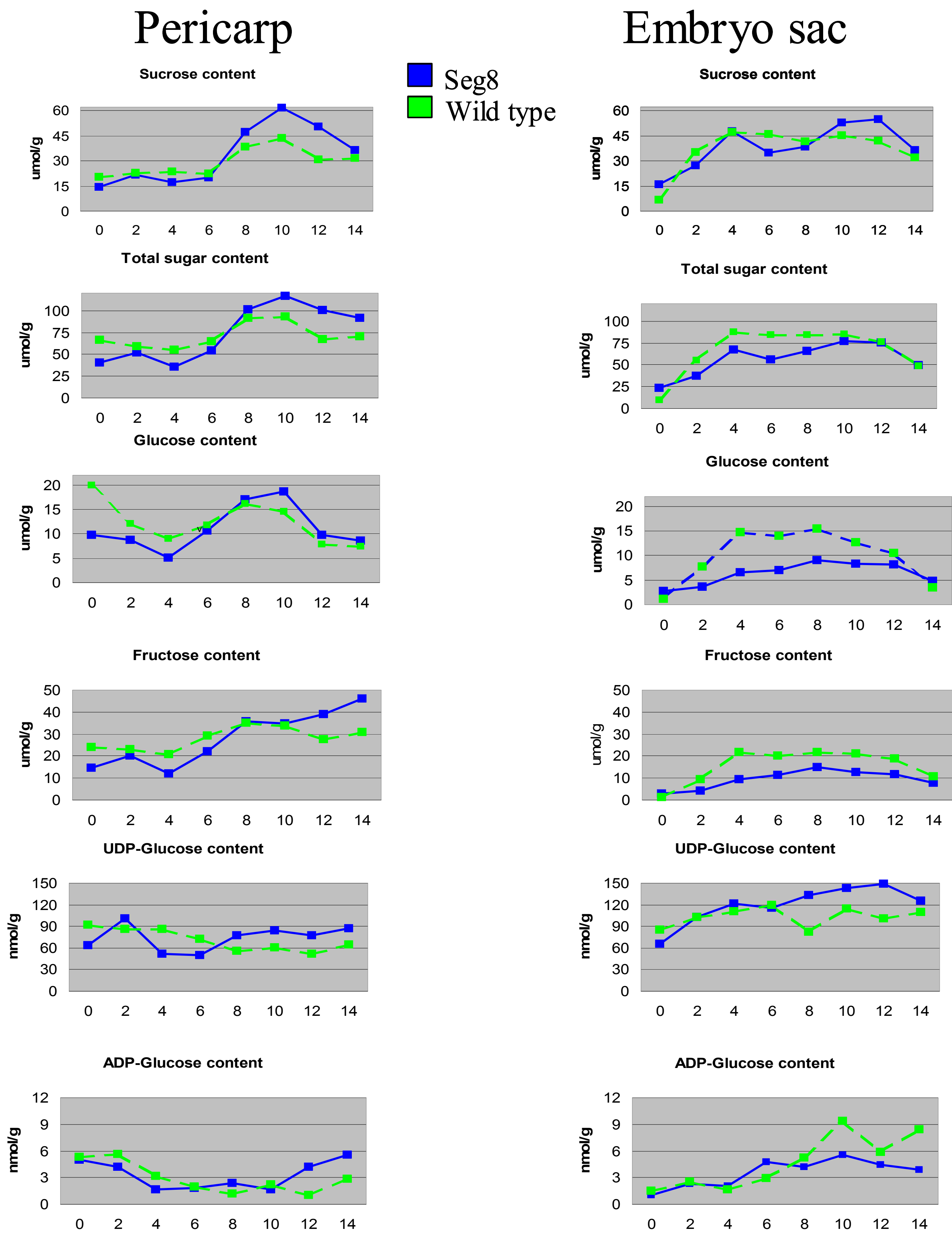

Fig. 19
Sugar and metabolite concentrations determined in maternal and filial fraction of *seg8* and wild type of developing caryopses (0-14 DAF) in two day intervals, scaled on X-axis) and measurement values represented on Y-axis.

3.2.4 Anatomy and starch distribution pattern in developing grains of *seg8* mutant and wild type

The *seg8* seeds show normal development of the pericarp tissue during caryopses development. However, the shrunken endosperm phenotype was noticed during the onset of starch accumulation. The endosperm develops as two separate lobes with the abnormal nucellar projection touching the dorsal crease of the caryopses. The starch distribution pattern was studied in developing grains of *seg8* mutant and wild type during onset of starch accumulation (8-12 DAF) by iodine staining (Fig. 20 A,B,C). Contrary to the wild type accumulating first starch grains in the wings of the starchy endosperm (20 G), starch accumulation in the mutant endosperm starts in the regions adjacent to the maternal-filial boundary during 8 DAF (20 A,D). The starch accumulation is drastically reduced in the two lobes of the endosperm mutant (20 E,F) in comparison to a well-filled starchy endosperm of wild type during 10 and 12 DAF (Fig. 20 I). Quantitative measurements of starch content (Fig. 19) carried out in developing caryopses of *seg8* and wild type developing caryopses confirmed the result visualised by Iodine staining.

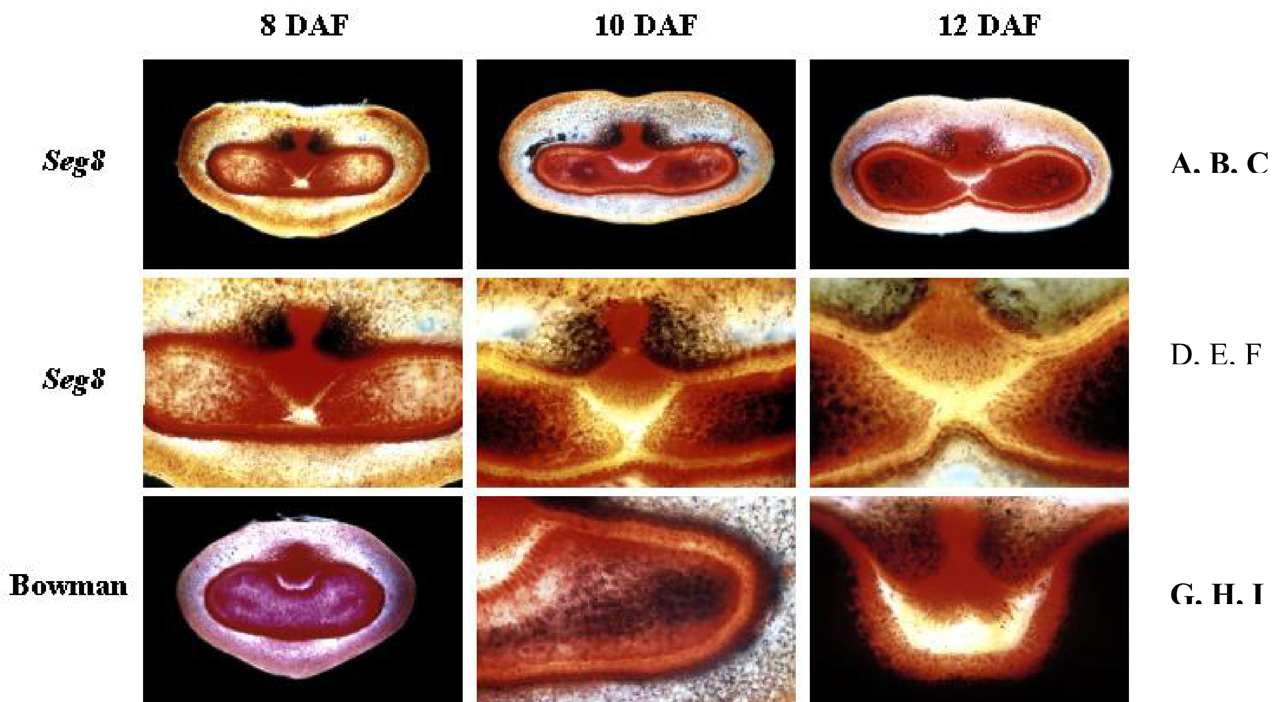

Fig. 20

Starch distribution pattern of *seg8* caryopses shown in median-transversal sections (8-12 DAF) by Iodine staining. Dark field images, scale 1mm. Images provided by Dr . R. Panitz

3.2.5 Characteristic changes of gene expression in developing caryopses of *seg8* and Bowman

To monitor difference in gene expression patterns between mutant and wild type by DNA macroarray analysis, we prepared ^{33}P-labelled cDNA probes from pooled whole caryopses (2-14 DAF) and hybridised them to the 1412 macroarray. The experiments were repeated at least twice with independent plant material. In expression analysis, 48 genes were found to be down-regulated in the mutant as compared to wild type by at least by two-fold or more (Fig. 21). The reproducibility of data generated by the two independent materials is shown in scatter-plot representation.

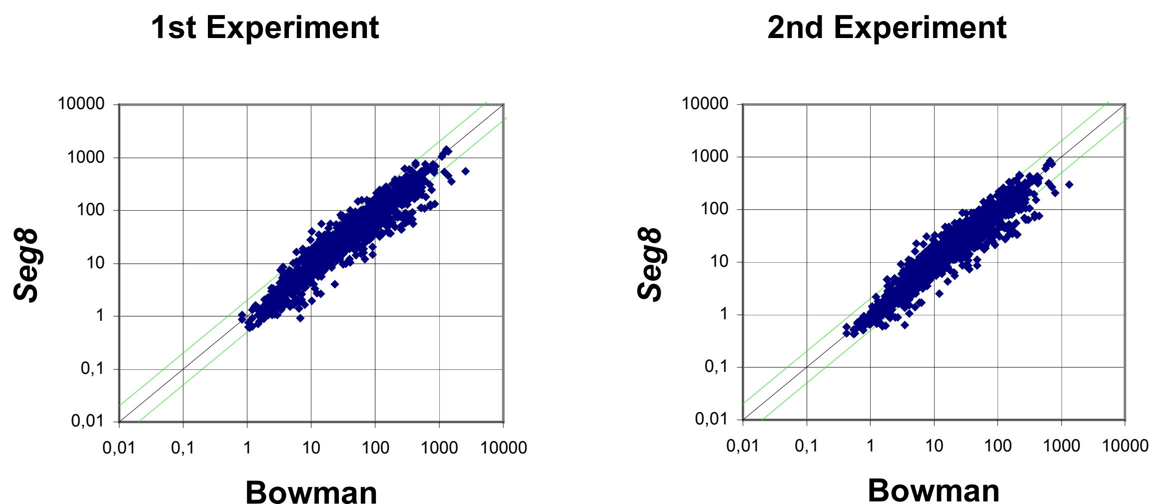

Fig. 21

Comparison of the normalized signal intensities obtained from two independent experiments (experiment 1 and 2). Two different arrays were hybridised with the labelled cDNA from *seg8* and, after successful stripping, with Bowman developing caryopses (pooled probe 2-14 DAF). Experiments were repeated with independent probe preparation from plant material and hybridization to independently spotted arrays. Signals outside the green lines differ by more than a factor of two between mutant and wild type.

Examination of the EST clones showed that differences in expression between *seg8* and Bowman caryopses (pooled 2-14 DAF) belong to the starch biosynthetic pathway. As shown in Table12, 25 ESTs belonging to the starch biosynthetic pathway displayed at least two-fold or more differential expression. Among them 10 clones represent unique candidates (alpha-amylase tetrameric inhibitor, alpha-amylase/trypsin inhibitor, alpha-amylase/subtilisin inhibitor, alpha-hordothionin precursor, sucrose synthase1, sucrose synthase2, ADP-glucose

pyrophosphorylase small subunit, UDP-glucose pyrophosphorylase and UDP-glucose-6-dehydrogenase). Those transcripts were expressed at a lower level in the mutants as compared to wild type. The other classes of genes down-regulated in the mutant represent amino acid metabolism (aspartate aminotransferase, glycyl-tRna synthetase), oxidative phosphorylation mainly involved with energy production ($H^{(+)}$-transporting ATPase, inorganic pyrophosphatase, 5'-amp-activated protein kinase, beta-1 subunit), stress related and hypothetical proteins (data not shown).

Table 12: ESTs belongs to the sugar to starch pathway are preferentially down regulated in developing caryopses of seg8 mutant

			1st experiment			2nd experiment			2ndexperiment_rehybridized		
EST-ID	**EC nr**	**Putative gene identity**	**Signal intensity**		**Ratio**	**Signal intensity**		**Ratio**	**Signal intensity**		**Ratio**
			Bowmann	**Seg8**	**Bow/Seg8**	**Bowmann**	**Seg8**	**Bow/Seg8**	**Bowmann**	**Seg8**	**Bow/Seg8**
		Carbohydrate metabolism									
HY06K18	0	alpha-amylase tetrameric inhibitor	397,75	33,97	**11,71**	324,54	62,63	**5,2**	59,28	21,23	**2,8**
HY06J10	0	alpha-amylase/subtilisin inhibitor	53,95	10,99	**4,91**	66,18	18,68	**3,5**	21,48	5,77	**3,7**
HY09N22	0	alpha amylase/trypsin inhibitor BTI-CME3	653,26	151,75	**4,30**	764,48	245,32	**3,1**	144,94	65,15	**2,2**
HY03M02	0	alpha-hordothionin precursor	2995,67	301,52	**9,94**	2570,44	543,94	**4,7**	566,32	99,47	**5,7**
HY09N15	2.4.1.13	sucrose synthase 2	283,68	33,29	**8,52**	390,27	55,06	**7,1**	50,81	14,21	**3,6**
HY04I11	2.4.1.13	sucrose synthase 2	411,00	49,47	**8,31**	60,41	14,90	**4,1**	7,46	1,34	**5,6**
HY07C09	2.4.1.13	sucrose synthase 2	495,55	81,01	**6,12**	731,06	128,86	**5,7**	147,27	33,23	**4,4**
HY09L14	2.4.1.13	sucrose synthase 2	556,67	106,29	**5,24**	531,92	196,64	**2,7**	213,93	24,35	**8,8**
HY06C06	2.4.1.13	sucrose synthase 2	109,77	21,17	**5,18**	174,95	37,08	**4,7**	26,30	8,22	**3,2**
HY09N16	2.4.1.13	sucrose synthase 2	171,25	41,28	**4,15**	11,48	3,31	**3,5**	0,72	0,51	**1,4**
HY04L06	2.4.1.13	sucrose synthase 2	37,43	12,06	**3,10**	61,68	18,42	**3,3**	10,37	3,77	**2,8**
HY08H21	2.4.1.13	sucrose synthase 2	123,40	41,41	**2,98**	165,11	73,01	**2,3**	41,99	8,76	**4,8**
HY09D18	2.4.1.13	sucrose synthase 1	153,50	33,84	**4,54**	382,39	67,90	**5,6**	73,65	32,48	**2,3**
HW08D05	2.4.1.13	sucrose synthase 1	2,69	0,62	**4,31**	94,75	34,21	**2,8**	34,49	8,08	**4,3**
HY03P12	2.4.1.13	sucrose synthase 1	46,06	11,30	**4,08**	91,35	20,54	**4,4**	9,57	5,67	**1,7**
HW08D05	2.4.1.13	sucrose synthase 1	78,89	20,08	**3,93**	94,75	34,21	**2,8**	34,49	8,08	**4,3**
HY05O13	2.4.1.13	sucrose synthase 1	131,46	33,65	**3,91**	294,56	56,67	**5,2**	61,63	28,70	**2,1**
HY10D02	2.4.1.13	sucrose synthase 1	79,00	21,72	**3,64**	126,53	37,26	**3,4**	22,98	9,32	**2,5**
HY10G10	2.4.1.13	sucrose synthase 1	111,07	32,29	**3,44**	150,29	54,95	**2,7**	47,22	17,05	**2,8**
HY07L04	2.4.1.13	sucrose synthase 1	55,57	18,57	**2,99**	81,63	27,92	**2,9**	19,97	6,52	**3,1**
HY08O12	2.7.1.90	6-phosphofructokinase (pyrophosphate)	19,06	2,54	**7,51**	23,52	3,99	**5,9**	6,33	1,33	**4,8**
HY10G16	2.7.7.27	ADP-glucose pyrophosphorylase, small chai	228,50	49,07	**4,66**	133,65	77,89	**2,8**	55,59	15,70	**3,5**
HY08N11	2.7.7.27	ADP-glucose pyrophosphorylase, small chai	77,09	24,24	**3,18**	161,39	38,17	**4,2**	30,87	7,36	**4,2**
HY03B24	1.1.1.22	UDP-glucose6-dehydrogenase	109,91	31,56	**3,48**	48,36	53,00	**1,1**	17,81	4,07	**4,4**
HY01E15	2.7.7.9	UDP-glucose pyrophosphorylase	321,57	106,89	**3,01**	142,46	185,18	**1,3**	52,31	9,51	**5,5**

ESTs that are preferentially down regulated in the *seg8* mutant are defined as those giving Bowman/*seg8* signal intensity ratios larger than 2 and absolute signal intensities greater than 5 au in three array experiments. Normalized signal intensities for Bowman and *seg8* pooled caryopses probes (2-14 DAF), as well as the corresponding Bowman/*seg8* ratios are listed for three experiments involving two independent probe syntheses and a 2nd experiment probe rehybridized onto a new membrane. The BlastX2 search against the protein database SwissProt provides secure functions for all EST clones represented in the Table.

3.2.6 Different expression profiles of genes encoding enzymes of the sugar-to-starch pathway monitored in maternal and filial fractions of developing *seg8* mutant and Bowman wild type grains

As shown before (cf. 3.2.3.), starch filling phase of *seg8* grains is characterised by higher levels of total sugar as well as hexoses and sucrose in the maternal tissues, as compared to the wild type “Bowman”. In the filial tissue fraction, both the total sugar content and hexose levels of the mutant caryopses are lower. These findings, together with the remarkably lower starch content of the embryo sac fraction (cf. Fig. 20 and 18) hint to *seg 8*-specific disturbances in the starch accumulation process. To identify differences in gene expression possibly causing the diminished starch content of the mutant grain, expression profiles of genes encoding enzymes of the sucrose-to-starch pathway were identified on the cDNA array and compared between *seg 8* and Bowman (see Fig. 22). In the maternal tissues, no remarkable differences in the abundance of the respective mRNAs were observed. However, this is true only from 2 DAF onwards. In the moment of pollination (0 DAF), mRNAs of sucrose synthase (SUS) 1 and 2, CWINV1, hexokinases 1cyt and 2 as well as fructokinase and UGP glucose pyrophosphorylase are clearly down regulated in the *seg8* maternal fraction. In the early development of the filial fraction, down regulation of the hexose-producing enzymes as well as two hexokinases (GK, Hkcyt1) was registered, too. During the ongoing filling phase of the starchy endosperm, SUS1 and 2 as well as the small and the large subunit of AGP glucose pyrophosphorylase and the granule bound starch synthase (SS1), i.e. the key enzymes of the starch biosynthetic pathway, are down regulated on mRNA level in the filial fraction of *seg8* caryopses.

Fig. 22
Expression data of EST clones with homology to genes coding sugar to starch pathway were selected. The expression intensities were normalized by setting the highest intensity measured either in mutant or wild type to 100% in maternal and filial tissue fraction depicted at the left-hand (maternal) or the right hand-side (filial fraction) of the figure 22. The intensities of mRNA expression obtained after the normalization procedure are plotted on the Y-axis. On the X-axis the developmental time scale 0-14 DAF (Days After Flowering) is given in two day intervals. AGP-L, large subunit of ADP-glucose pyrophosphorylase; AGP-S, small subunit of ADP-glucose pyrophosphorylase; CWINV, cell wall-bound invertase; FK, fructokinase, GK, glucokinase; Hkcyt, hexokinase cytosolic; HKchl, hexokinase chloroplastic; PGM, phosphoglucomutase; SUS, sucrose synthase; SS, starch synthase; UGP, UDP-glucose pyrophosphorylase; VCINV, vacuolar invertase.

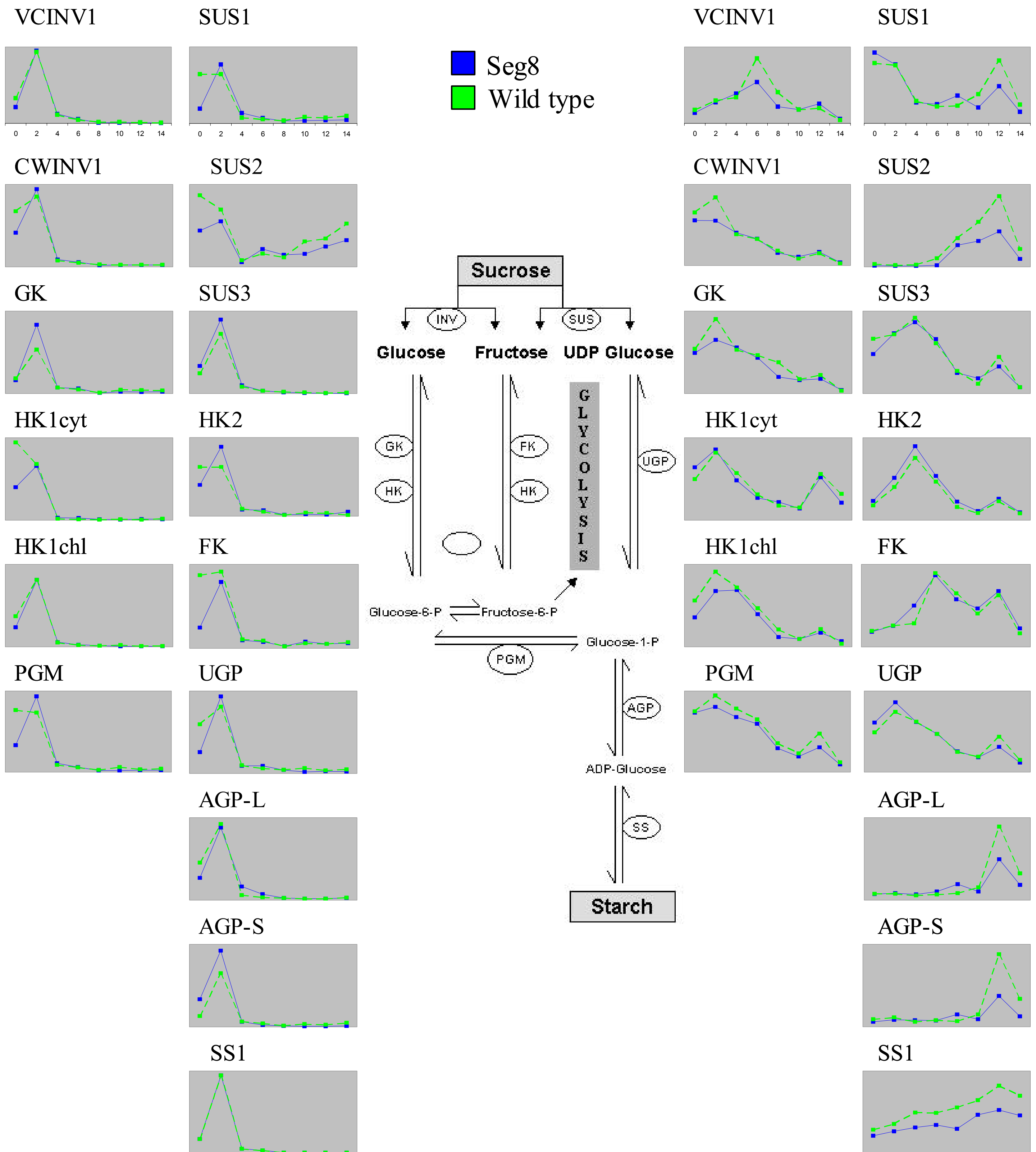

Fig. 22 For legend please see page number 76

3.2.7. mRNA expression of some transporter genes is drastically reduced in the filial fraction of developing *seg 8* grains

On the 1412 cDNA macro array filter, 12 cDNA fragments are spotted encoding putative transport proteins. Among them, three showed clear differences in their mRNA expression during the analyzed developmental period (0-14 DAF). As shown in Fig. 24, especially the potassium transporter expression is strongly influenced. In early development of the filial fraction, this transporter has a higher expression level in the mutant than in the wild type, whereas later in development expression decreases strongly in the mutant fraction, but increases to relatively high levels in the wild type. The ABC transporter, on the other hand, shows a general reduction of its mRNA expression level in the mutant filial fraction as compared to the wild type. Contrary to these two transporters that should be localised within the plasmamembrane of the starchy endosperm transfer cells, the ATP/ADP transporter is integrated in plastid membranes. Nevertheless, its expression is drastically reduced in the mutant, too (cf. Fig. 23).

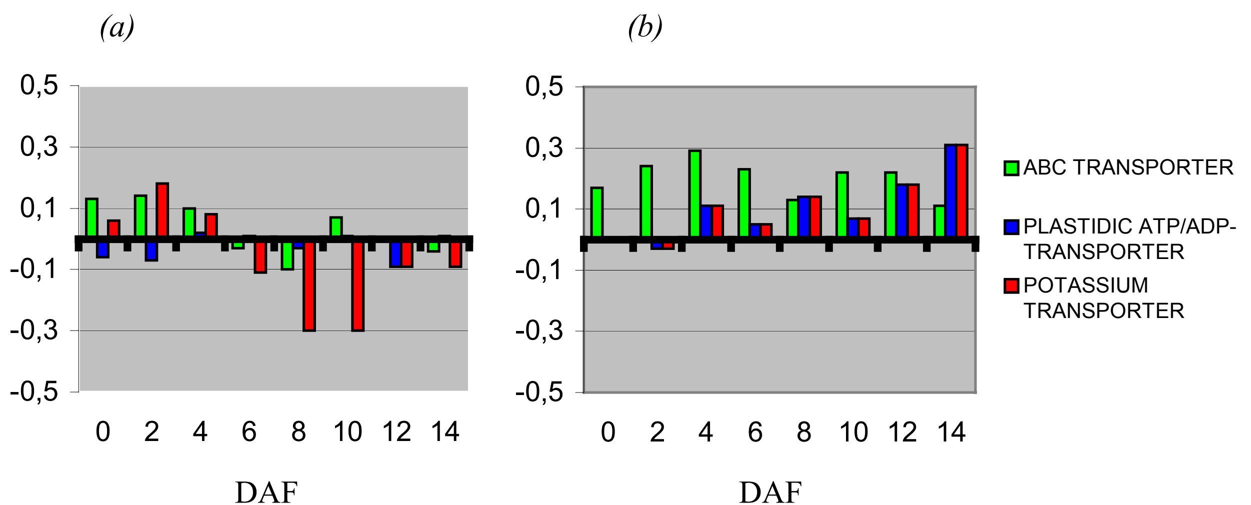

Fig. 23

Expression profiles of transporter genes in filial fraction of mutant (a) and wild type (b) during early and mid caryopses development. Putative function of the selected cDNA is provided on the right-hand side. The normalized signal intensity values are presented in Log 2 scale on the Y-axis. On the X-axis the developmental time scale 0-14 DAF (Days After Flowering) is given.

3.3 DISCUSSION

3.3.1 The *seg8* genomic environment integrated in "Bowman" displays the same features described for the original mutant identified in "Klages".

As reported by Felker et al. 1985, the main characteristic of mutant seg8 is development of the starchy endosperm in two lobes clearly separated from each other by the non-occurrence of endosperm cells in front of the grains maternal-filial boundary. Based on iodine staining of median-transversal sections of developing seg8 caryopses, this finding was reproduced for grains expressing seg8 in the "Bowman" background (see Fig. 20). Further on, as shown in Fig. 18, our analyses confirm the lower starch content measured by Djarod and Peterson (1991) in developing seg8/Klages grains. However, the overall reduction of the sucrose concentration estimated by Djarod and Peterson in the original mutant has been observed for the seg 8/Bowman caryopses only during the ongoing starch accumulation period (10-12 DAF), and the total sugar content as well as hexoses levels are higher or lower in the maternal or filial part of the mutant grain, respectively, as compared to the wild type. Nevertheless, the reduction of starch content shown in Fig. 18 is exclusively due to the filial tissues as shown by the reduction of fresh weight only in the embryo sac fraction of seg8 grains. This finding underlines the description of the mutant as maternally influenced but phenotypically expressed in the starchy endosperm.

3.3.2 Low mRNA expression of genes encoding key enzymes in the starch biosynthesis pathway may cause the reduced starch content of the mutant grain.

As shown in Fig. 18, starch content is drastically reduced in *seg8* grains. Starch biosynthesis in barley grains starts with increasing sucrose levels of the filial tissues (Weschke *et al.,* 2000), and more general, with increasing sucrose/hexoses ratios in the storage organs of the seed (Weber and Wobus, 1999). A steep sucrose gradient, build up in the cell rows adjacent to the transfer tissues of developing *Vicia faba* seeds, regulates the activity of the sucrose utilising enzymes, for instance sucrose synthase (SUS) (Borisjuk *et al.,* 2002a). In a reversible reaction, SUS cleaves sucrose to fructose and UDP glucose. A general reduction in the mRNA expression of different isoforms of this enzyme as shown in the filial fraction of the *seg8* grain (cf. Fig. 22) may reduce the amount of active enzymes molecules and, therefore, sucrose cleavage, leaving back the higher sucrose content measured in the mutant's starchy endosperm (cf. Fig. 19). However, fructose as well as UDP glucose levels are higher in the filial fraction of *seg8* grains. Possibly, this finding can be explained by the reduced expression

of the two AGP glucose pyrophosphorylase subunits. If this lower expression is indicative for a general reduction of the enzyme activity, lower amounts of glucose-1-phosphate will be utilised to ADP glucose (compare the lower ADP glucose level in the *seg8* endosperm, Fig. 19) which may influence in turn the equilibrium between glucose-1-phosphate and UDP glucose resulting in the higher UDP glucose level measured. The low ADP glucose content together with the reduced amount of starch synthase (SS) (cf. Fig. 22) is indicative for the low starch content measured in *seg8* grains.

3.3.3 High sucrose levels in the maternal and filial fraction during storage phase hint to a delay in sugar utilisation and reduced starch accumulation in the mutant's endosperm.
The ongoing starch accumulation in the filial tissues causes higher sucrose demand of the filial endosperm, which will be supplied by maternal tissues. The sucrose imported via the vascular bundles into the maternal tissue will be supplied to the filial endosperm through the maternal-filial boundary. The establishment of sucrose gradients in the maternal tissues and in the maternal-filial boundary is nearly independent from disturbances in the development of the filial part in the pea *E2748* mutant (Borisjuk *et al.,* 2002b). In *seg8* mutant the drastic reduction of starch content is sensed in filial tissues. To accumulate lower amounts of starch in mutant, lower amounts of sucrose are expected. However, the maternal as well as filial tissues of mutant accumulate comparatively higher sucrose levels. Since there is a reduced expression in key enzymes of starch biosynthesis (sucrose synthase, ADP-glucose pyrophoshporylase and starch synthase) higher sucrose concentration must be expected, which is noticed in the *seg8* maternal pericarp as well filial fraction shown in Fig. 19. Important explanatory clues may come from a detailed study of the distribution of sucrose in wild type and *seg8* mutant and the possible detection of a sucrose concentration gradient between maternal and filial tissues.

On the other hand, detailed histological analyses comparing the structure of the maternal-filial boundary between *seg8* and Bowman have clearly shown an abnormal development of both, nucellar projection and endospermal transfer cells in *seg8* grains (S. Gubatz, personal communication). As shown in Fig. 23, mRNA expression of some of the genes encoding transporter proteins such as the potassium transporter, the ABC transporter and the plastidic ATP/ADP transporter is down regulated in the filial fraction of *seg8*, which could be correlated to abnormal development of endospermal transfer cells. Since a significant difference in mRNA levels of sucrose transporter between mutant and wild type was not

found and, more over, due to presence of higher concentration of sucrose in filial tissues of mutant, we prefer the interpretation that possibly the abnormal structure of the transfer tissues negatively influences the sucrose gradient in *seg8* grains.

3.3.4 A defect in starch accumulation can be expected for the developing gynoecium.

The style tissue, dominating the female gynoecium immediately before pollination, accumulates a lot of storage compounds, especially starch. At 0 DAF, sugar and UDP glucose content as well as the mRNA level of major enzymes of the sucrose-to-starch pathway are down regulated in the maternal fraction of *seg8* grains (cf. Figs. 19, 22). Similar to the down regulation of starch biosynthetic transcripts observed in starchy endosperm of the mutant during storage phase, the decreased levels of transcripts of starch-biosynthetic pathway is noticed during 0 DAF in maternal tissue. Despite the fact, that only the last moment in the development of the female gynoecium (0 DAF) was analysed, a slight decrease in starch accumulation of the maternal tissue (0 DAF) can be concluded. Thus, possibly not only the filial parts of the seed but also the maternal tissues prior to anthesis are influenced by decrease in starch accumulation. We speculate that the *seg8* mutant may have a defect in starch accumulation in all tissues of the seed before as well as after pollination.

3.4 SUMMARY

The *seg8* mutant with impaired starch accumulation provides an ideal and unique experimental system to analyze the gene expression networks in relation to the sugar-to-starch pathway and to carry out metabolite profiling. Expression analysis results provide an eclectic overview of gene expression networks in the starch metabolism and sucrose/hexose distribution pattern in maternal and filial tissues during early and mid caryopsis development between mutant and wild type. Although, the *seg8* mutant has been described as maternal mutant, the analysis revealed that the mutant phenotype "shrunken endosperm" and disturbances at the maternal-filial boundary determine the observed seed phenotype. Although we see defects in transfer cells, sucrose flow into endosperm cells seems to be not the major limiting factor of lower starch accumulation because of higher sucrose levels and lower levels of glucose, fructose and starch content. Thus, a deficiency in maternal tissues causing a limitation in the supply of sucrose to the endosperm can be ruled out and the failure of hexose utilization appears to be the resulting cause for the low content of starch in *seg8* seeds. In addition, it is also clear that transcripts of key enzymes in starch biosynthesis are affected mainly in the filial fraction of the mutant during the storage phase and in pericarp during early stages of grain development (0 DAF).