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A Transcriptomic View of Barley Embryo Development

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ABSTRACT

Due to the agricultural interests in grain crops, there has been a substantial focus on molecular aspects of grain development, particularly related to grain filling. However, only little attention has been on gene regulation in the developing embryo. Recently, array analyses revealed that the transcriptome of the developing barley embryo is quite different from that of the surrounding tissues. In order to generate a general view of the processes involved in embryo development, transcriptome data from previous array analyses of developing embryos of barley, maize, wheat and *Arabidopsis* are discussed. An interesting aspect is the dual role of abscisic acid, involved in the regulation of storage product synthesis and in the desiccation tolerance of the embryo. The latter phenomenon might involve the transcription factors DREB2A and DREB2B, probably by up-regulating both late embryo abundant proteins and perhaps cysteine protease inhibitors to help the embryo tolerate desiccation and prevent initiation of programmed cell death. In addition, a detailed analysis of changes in the transcriptome of the developing barley embryo is presented with a particular interest in the embryo-specific initiation of at least two forms of developmental defense activations (DDAs), initiated at 21 and 37 days after flowering and termed early and late DDA, respectively. The initiation of both early and late DDA could help explain the protection of the developing embryo against disease and could provide valuable information on the regulation of potential allergens. Combined these data help to elucidate the regulatory networks involved in barley embryo development.

Keywords: gene regulation, desiccation tolerance, developmental defense activation
Abbreviations: **ABA**, abscisic acid; **ABRE**, ABA responsive element; **CPI**, cysteine proteinase inhibitor; **DAF**, days after flowering; **DAP**, days after pollination; **DDA**, developmental defense activation; **GA**, gibberellic acid; **JA**, jasmonic acid; **LEA**, late embryo abundant; **LOX**, lipoxygenase; **LTP**, lipid transfer protein; **PCD**, programmed cell death; **POX**, peroxidase; **PR**, pathogenesis related; **SA**, salicylic acid; **SAR**, systemically acquired resistance; **TLP**, thaumatin-like protein

INTRODUCTION

Plant seed development remains of practical and scientific interest, in particular because of its notable impact on human nutrition. Current models on the developmental scheme of seeds have their basis in studies of numerous species, including insights into the nature of cellular processes from the fertilized egg to the desiccated, mature seed. Recently, advances in various omics studies have substantially extended and broadened the understanding on molecular biological aspects of grain development and germination – the subject of the present report, with focus on the cereal crop barley.

The grain of barley is a typical starch (and to some degree, protein) storage sink organ, which consists to a large part of the maternal pericarp, the diploid embryo and the triploid endosperm. While much focus has been on the understanding of the metabolic pathways and molecular fluxes that determine the composition of the mature endosperm (Finnie *et al*. 2002; Sreenivasulu *et al*. 2004), only little attention has until recently been on the developing embryo.

Grain development is initiated with the double fertilisation, in which the egg cell is fertilised by one sperm nucleus, while the two polar nuclei fuse with the second sperm nucleus. Subsequently, the three main grain tissues are formed and undergo different developmental schemes – both regarding physiological and transcriptional changes. The latter property was recently addressed in a comparison of gene expression in 15 tissues sampled throughout barley development (Druka *et al*. 2006), revealing that the embryo transcriptome has a higher resemblance to those of the coleoptile and inflorescence than it has to those of the endosperm and pericarp. Such differences may reflect the specific initiation of programmed cell death (PCD) in pericarp and endosperm tissues (Young and Gallie 2000).

Embryo development

In terms of developmental timing, several ways can be used to follow how the cereal embryo develops under defined environmental conditions, for example indirectly by rating endosperm hardiness (Feekes-, Haun- and Zadok scale). However, this author finds that when using growth chambers to create consistent growth conditions, more reliable results are obtained by referring to days after flowering (DAF) or days after pollination (DAP).

0-9 DAF

While the primary endosperm nucleus has undergone its first division ~7 h after pollination, the first zygotic mitosis is first initiated after ~23 h in a process yielding a basal and an apical cell. A subsequent high rate of cell divisions combined with a modest increase in embryo mass makes the relative cell volume decrease during the early stages of its development. Later, however, the average embryo cell volume approaches that of other meristimatic cells (Bennet 1975). Two tissues initiate differentiation in the embryo: one develops into cotyledon(s), and one into the apical (or stem) meristem from which the leaves develop. In grasses, the single cotyledon is reduced to the scutellum that confers nutrient absorption from the endosperm during germination (Sopanen 1979), but may also serve as a storage organ in itself (Kisselbach 1945).

Empirical data on gene expression in the first develop-

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mental phase of the cereal embryo are sparse, primarily because of experimental difficulties in dissecting the small organ. When employing laser capture microdissection techniques, it should be possible to isolate cells from this complex tissue and resolve the corresponding transcriptome at high levels of detail (Casson *et al*. 2005).

10-19 DAF

Differentiation of the root primordium is initiated ~10 DAF, followed by the development of four seminal roots. Concomitantly, the developing coleoptile encloses three leaf primordia, which differentiate from the apical meristem. Although the embryo develops vascular bundles, there is no differentiation into phloem or xylem. The high level of cellular differentiation at this stage of embryo development coincides with elevated expression of genes involved in the regulation of cell division (Nielsen *et al*. 2006). This ensemble of genes continues to be highly expressed at 21 DAF, with marked down-regulation thereafter. Since cell expansions account for embryo weight gain up to ~40 DAF (King 1976), cell divisions are likely to arrest <37 DAF.

20-39 DAF

Following organ formation, the embryo's development is described as maturation. During this stage the embryo accumulates lipids and proteins (Murphy 1990; Aalent *et al*. 1994), and furthemore undergoes a general preparation for both desiccation and the first stages of normal germination (Bewley and Black 1994).

Molecular regulation of embryo maturation has been proposed to be partially controlled by gibberellic acids (GAs), as supported by the finding of three highly expressed orthologs of three rice proteins involved in GA signalling (manuscript in preparation; Khan *et al*. 2005). Likewise, the phytohormone abscisic acid (ABA) appears to induce the embryo maturation process – but also to suppress precocious germination (Koornneef *et al*. 2002; Seo and Koshiba 2002). Although ABA and GA are thought to act antagonistically in many aspects of plant development (Yazaki and Kikuchi. 2007), both hormomes accumulate during maturation in quantities that restrict germination (White *et al*. 2000). Elevated expression levels of the ABA–controlled biosynthetic genes were observed in the embryo, together with two genes encoding the ABA–related transcription factors DREB2A and DREB2B (Sreenivasulu *et al*. 2006). Homology searches to rice sequences revealed that the promoter region of the gene for DREB2B contains two ABA responsive elements (ABREs; Rogers and Rogers 1992), while none were found in the DREBA2–encoding gene. This points to two ways for control of desiccation-related genes: ABA-dependent and ABA-independent via DREB2B and DREB2A, respectively (Sreenivasulu *et al*. 2006).

The action of lipoxygenase (LOX) enzymes in plants is vital for the synthesis of oxylipins and the signaling compound jasmonic acid (JA), all compounds involved in plant developmental processes (Porta and Rocha-Sosa 2002), and also relevant in response to pathogen and insect attacks by acting either as signaling or antimicrobial molecules (Shah 2005). Analysis of LOX activity in developing barley grains was reported as being predominated by 13-LOX activity (derived from LOX2 action), with a distinct shift after 30 DAF to 9-LOX activity (LOX1 action; Schmitt and Mechelen 1997). 13-LOX–catalysed dioxygenation of linolenic acid, followed by the action of other 13-LOX pathway enzymes, leads to the production of JA, an important signalling molecule in a vide variety of plant species (Liechti and Farmer 2006). Although the two LOX pathways share several reaction characteristics, the role of the 9-LOX pathway is still unclear. Most of the knowledge on this pathway has been obtained from *Solanaceaus* plants, where 9-LOX activity is strongly induced in response to *Phythothora* as well as oomycete-derived elicitors (Weber *et al*. 1999; Göbel *et al*. 2001). Interestingly, genes encoding potential enzymes of the 9-LOX pathway are up-regulated in the developing embryo, whereas genes in the 13-LOX pathway remain un-regulated (Nielsen *et al*. 2006; Sreenivasulu *et al*. 2006). On top of that is the initiation of the first of at least two types of developmental defense activation (DDA), including up-regulation of several defense–related genes encoding thionins, peroxidases (POXs) and enzymes involved in phenylpropanoid phytoalexin generation (Nielsen *et al*. 2006). Based on these results, it is hypothesized that 9-LOX pathway enzymes contribute to early DDA in the developing embryo, such that that the corresponding oxylipins act as either signalling or antimicrobial compounds.

>40 DAF

As mentioned previously, the desiccation process is initiated at ~40 DAF, eventually yielding a mature grain that contains 5-10% water (Romagosa *et al*. 1999). Interestingly, desiccation tolerance, i.e. the grain's ability to survive dehydration, is established in the embryo as early as 16 DAF (Bartels *et al*. 1988). Although its regulation mechanism remains unclear, ABA was reported to confer desiccation tolerance by inducing synthesis of late embryo abundant (LEA) proteins (Ooms *et al*. 1993), possibly via action of the mentioned DREB transcription factors (Sreenivasulu *et al*. 2006). Transgenic approaches demonstrated that the LEA proteins function in desiccation tolerance (Bahieldin *et al*. 2005), most likely by inducing conformational changes in proteins that prevent corresponding aggregate damage during water stress (Goyal *et al*. 2005). Typical DDA is active during this stage of embryo development, including up-regulation of several pathogenesis related (PR)-1 and PR-4 genes, as well as genes encoding chitinases and β-1,3-glucanases (Nielsen *et al*. 2006).

MATERIALS AND METHODS

Plant material and RNA

Barley (*Hordeum vulgare* L., cv. Barke) plants were propagated at 16°C in growth chambers with a light/dark regime of 16 h/8 h. Embryo material was harvested at 15, 18, 24, 27, 30, 33, 41, 45, 49 and 53 DAF. RNA extraction, hybridization and analyses were done according to the instructions provided by the supplier (http://www.affymetrix.com).

Microarray analysis

Raw intensity data was normalized using R implementation of qspline (Workman *et al*. 2002; Gautier *et al*. 2004). A list of significantly differentially expressed genes was obtained from previous microarray analyses of the same time interval (Nielsen *et al*. 2006). Gene expression profiles from significantly differentially expressed genes, were clustered by k-means clustering.

Functional categorization

The MIPS functional classification applied to *Arabidopsis* genes (Schoof *et al*. 2004) was adapted for barley by BlastX analysis and using custom PERL scripts.

RESULTS

Discovery of early and late DDA was based on the annotation of the most significantly, differentially regulated genes during barley embryo development, followed by analysis of the expression profiles at 12, 21 and 37 DAF (Nielsen *et al*. 2006). The findings prompted additional examination of especially the *PR* genes involved.

In the experiments, a total of 10 Affymetrix Barley1 GeneChip arrays were hybridised with mRNA extracted from developing barley embryos after 15, 18, 24, 27, 30, 33, 41, 45, 49 and 53 DAF (hybridisation data of the 24-DAF mRNA sample were experimentally unacceptable, and ac-

cordingly not included in the analyses). Because each time point in this data set was represented by one replicate only, the statistical significance of the changes in expression levels could not be calculated. Instead, the list of significantly regulated genes was obtained from an overlapping, previously obtained array data set (Nielsen *et al*. 2006). Data validation was obtained by comparing the expression profiles of several individual and co-regulated genes with those derived form the previously described array data set. Analysed genes included, in particular, the putative members encoding LOX pathway enzymes, Krebs cycle and genes involved in cell division as identified by FunCat annotation and similar expression profiles were determined for both data sets (data not shown). The agreement in expression profiles between the two data sets serve as a kind of validation, but none of the mentioned expression profiles have been validated by QRT-PCR analyses. Accordingly, the following analyses and discussions are only suggestive.

Regulation of cell division

Because of the shorter interval at which the time-points were dispersed, the distinct down-regulation of genes involved in cell division, was noticed to occur already before 27 DAF (data not shown). As discussed above, genes involved in regulating cell division are highly expressed at 21 DAF, indicating that a common developmental cue is involved in down-regulating this group of genes between 21- and 27 DAF. This observation is well in line with recent investigations of gene regulation in the developing *Arabidopsis* embryos (Spencer *et al*. 2007) and suggests that cell division and differentiation mainly takes place in early stages of embryo development.

Expression of *PR* genes in developing barley embryos

Analysis of significantly regulated *PR* genes revealed an apparent ordering into three different expression profiles (**Fig. 1**). As described below, cluster A represented *PR* genes involved in early DDA, with expression initiation 18–27 DAF and peaking at ~30 DAF, followed by a decline to basal levels (**Fig. 1A**). Although clusters B and C represented *PR* genes involved in late DDA, they displayed distinct differences in gene expression profiles. Genes in cluster B remained unregulated until ~33 DAF, after which the expression increased until peaking at ~49 DAF (**Fig. 1B**), followed by a marked decrease at 53 DAF. In contrast, genes in cluster C exhibited a steady increase in expression throughout embryo development, reaching a maximum value at ~53 DAF (**Fig. 1C**). Finding that the expression profiles in cluster C correlates with the level of dehydration could indicate that this cluster of genes are regulated by dehydration responsive transcription factors like the two DREB transcription factors mentioned above (Liu *et al*. 1998).

Considering that this study has enabled clustering of developmentally regulated *PR* genes, the aim of the following analysis was to examine whether up-regulated members of the gene family could be assigned into specific clusters (**Table 1**).

Cluster A

Of highly expressed *PR* genes, 6 were found to encode POXs (PR-9). Although members of this large protein family have been proposed to be involved in cell wall lignification, auxin metabolism etc., POX action is important in responses to wounding and in the defense against pathogen infection (Hiraga *et al*. 2001), but biochemical details will await further analyses.

The same cluster also contains genes coding for thionins, members of a protein family of small, cysteine-rich, anti-microbial proteins including plant defensins and lipid transfer proteins, generally induced by fungal attack, and also in response to application of JA and SA (Kogel 1995; Gfeller and Farmer 2004). Thionins are known to inhibit growth of pathogenic fungi, a property which is synergistically enhanced 2-55 fold by the presence of Bowman-Birk type trypsin inhibitors (Terras *et al*. 1993). Finding that two such trypsin inhibitors are co-regulated with the three thionins supports the idea that the developing embryo generates a general, unspecific defense barrier. It is anticipated that this includes the action of POXs, because the corresponding genes share expression profiles with those of thionins and two Bowman-Birk type trypsin inhibitors.

Cluster B

Members of the predominant gene class in this cluster encode PR-1 proteins with antifungal activity. The *PR-1* genes are typically induced following SA application, and are often used as markers for systemically acquired resistance (SAR). In addition to *PR-1* gene induction, plants that exhibit SAR also induce genes for PR-2 and PR-5 proteins, as well as some chitinases (PR-3; Uknes *et al*. 1992; Busam *et al*. 1997). In this respect, it is interesting that the cluster contains two genes encoding β-1,3-glucanases (PR-2), three genes encoding chitinases and three genes encoding thaumatin-like proteins (TLPs; PR-5), in addition to six genes encoding PR-1 proteins. The chitinases cleave the β-1,4-glycoside bonds in chitin, a cell wall component in insects and fungi, but not in plants (Kasprzewska 2003). Similarly, β-1,3-glucanase enzymes hydrolyse the fungal cell wall β-1,3-glucan polymers and thus inhibit the growth of the microbes, especially when acting synergistically with chitina-

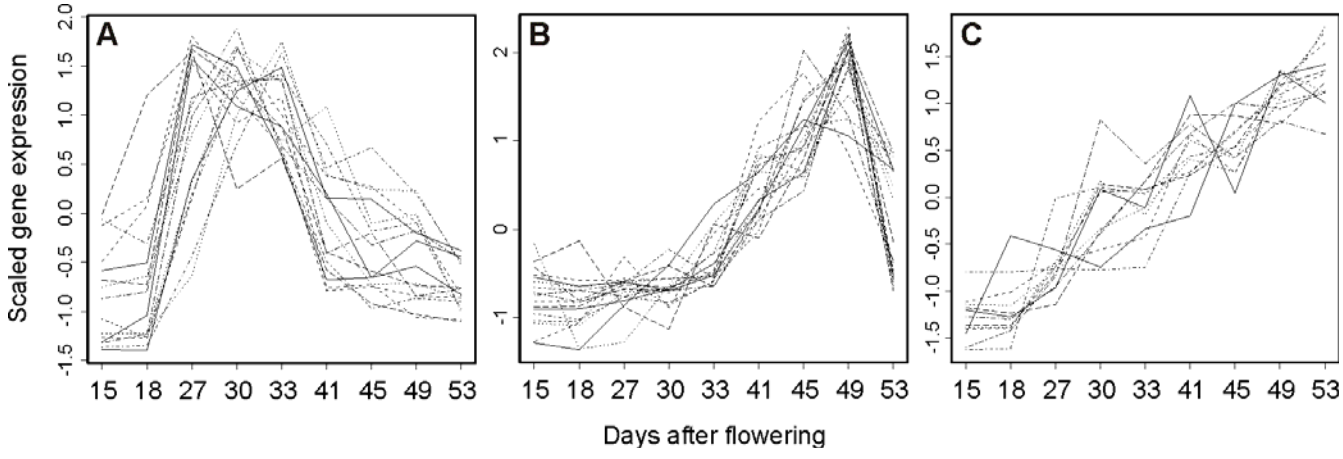

Fig. 1 Clustering of putative barley *PR* genes significantly regulated during embryo development. (**A**) Genes involved in early DDA, (**B**) genes involved in late DDA with a marked down-regulation at 53 DAF and, (**C**) genes involved in late DDA with no down-regulation.

Table 1 Putative PR genes significantly regulated during embryo development.

Cluster	GeneChip ID	BlastX hit description	Class	E-value
A	Contig17074_at	Putative pathogenesis-related protein PRB1-3 [*Oryza sativa*]	PR-1	4,00E-49
	Contig4324_s_at	Chitinase IV precursor [*Triticum aestivum*]	PR-3	9,00E-89
	Contig4324_at	Chitinase IV precursor [*Triticum aestivum*]	PR-3	9,00E-89
	Contig10686_s_at	Putative thaumatin-protein [*Oryza sativa*]	PR-5	1,00E-109
	Contig2088_s_at	Bowman-Birk type Trypsin inhibitor	PR-5	8,00E-76
	Contig2087_s_at	Bowman-Birk type Trypsin inhibitor	PR-5	6,00E-68
	Contig12498_at	Putative cystatin [*Zea mays*]	PR-6	8,00E-09
	Contig19834_at	Putative peroxidase [*Oryza sativa*]	PR-9	5,00E-36
	Contig6515_at	Class III peroxidase 130 precursor [*Oryza sativa*]	PR-9	1,00E-131
	Contig20678_at	Putative peroxidase isozyme 38K precursor [*Oryza sativa*]	PR-9	3,00E-74
	HVSMEf0009A08r2_s_at	Peroxidase	PR-9	3,00E-07
	Contig1858_at	Putative peroxidase [*Oryza sativa*]	PR-9	1,00E-126
	Contig19644_at	Peroxidase	PR-9	5,00E-21
	Contig1567_x_at	Thionin	PR-13	8,00E-64
	Contig1568_x_at	Thionin	PR-13	4,00E-62
	Contig1570_s_at	Thionin	PR-13	6,00E-70
	Contig2043_s_at	Type 1 non-specific lipid transfer protein precursor [*Triticum aestivum*]	PR-14	4,00E-41
B	Contig2209_at	PR-1a pathogenesis related protein (Hv-1a)	PR-1	1,00E-80
	Contig2214_s_at	PR-1a pathogenesis related protein (Hv-1a)	PR-1	1,00E-80
	Contig2210_at	Pathogenesis-related protein 1	PR-1	6,00E-80
	Contig12046_at	Pathogenesis-related 1a [*Triticum monococcum*]	PR-1	4,00E-73
	Contig2212_s_at	Pathogenesis-related protein	PR-1	1,00E-87
	Contig1637_s_at	1,3-β glucan endohydrolase precursor	PR-2	1,00E-155
	HVSMEm0003C15r2_s_at	B Chain B, 1,3-β-Glucanase (E.C.3.2.1.39)	PR-2	8,00E-48
	Contig7001_at	Endochitinase [*Triticum aestivum*]	PR-3	1,00E-154
	Contig2992_s_at	Chitinase	PR-3	1,00E-132
	Contig2990_at	Chitinase	PR-3	1,00E-133
	Contig2550_x_at	Pathogenesis-related protein 4	PR-4	7,00E-73
	Contig639_at	Pathogenesis-related protein 4	PR-4	2,00E-10
	Contig2790_s_at	Thaumatin-like protein TLP7	PR-5	1,00E-73
	EBem10_SQ002_I10_s_at	Thaumatin-like protein TLP8	PR-5	3,00E-03
	Contig3947_s_at	Thaumatin-like protein TLP4	PR-5	5,00E-72
	Contig9114_at	Putative cystatin [*Zea mays*]	PR-6	9,00E-31
	Contig2115_at	Peroxidase 4 [*Triticum monococcum*]	PR-9	1,00E-159
C	Contig6735_at	Putative cystatin [*Zea mays*]	PR-6	2,00E-38
	Contig4298_at	Cysteine proteinase inhibitor [*Triticum aestivum*]	PR-6	3,00E-60
	Contig5434_at	Cysteine proteinase inhibitor Scb-like protein [*Oryza sativa*]	PR-6	2,00E-21
	HT08H03u_s_at	Cystatin	PR-6	1,00E-33
	Contig1864_at	Class III peroxidase 90 precursor [*Oryza sativa*]	PR-9	2,00E-76
	HZ62K09r_at	Class III peroxidase 123 precursor [*Oryza sativa*]	PR-9	2,00E-07
	HT11E22u_x_at	γ-thionin	PR-12	5,00E-11
	Contig375_s_at	γ-thionin	PR-12	2,00E-32
	Contig1763_s_at	Hordothionin gamma	PR-12	4,00E-15
	Contig371_x_at	γ-thionin	PR-12	1,00E-26
	Contig1188_s_at	LTP 1	PR-14	1,00E-56
	Contig3622_s_at	Lipid transfer protein	PR-14	2,00E-35
	Contig1186_s_at	Lipid Transfer Protein	PR-14	7,00E-23
	X68656_s_at	Cw-19 peptide, non-specific lipid transfer protein	PR-14	1,00E-47
	Contig7855_s_at	Bowman-Birk type Trypsin inhibitor	PR-5	8,00E-41

ses (Mauch *et al*. 1988). Like the cell wall depolymerases, PR-1 proteins and TLPs may inhibit fungal growth (Roberts and Selitrennikoff 1990; Niderman *et al*. 1995). Transcription of genes in cluster B may represent a common regulatory mechanism during embryo development – for example similar to that regulating *PR* gene expression in plants exhibiting SAR (Durrant and Dong 2004). The distinct down-regulation of genes in cluster B after ~53 DAF provides a clue on the prior up-regulation, namely that it does not represent an unspecific response in the overall desiccation of the grain.

Cluster C

In the last cluster, several genes encoding putative cysteine proteinase inhibitors (CPIs) were found. Such proteins accumulate to 2-3 mg kg^{-1} of mature rice grains and are thought to prevent precocious germination (Abe *et al*. 1987; Kondo *et al*. 1990). Interestingly, addition of the CPI oryzacystatin I to the diet of the coleopteran *Callosobruchus chinensis* and the hemiptera *Riptortus clavatus*, caused severe growth retardation and even lethality, suggesting that CPIs could function as potent inhibitors of invertebrate attack (Kuroda *et al*. 1996). Notably, a balanced action of cysteine proteases and CPIs can regulate PCD in plants and animals (Solomon *et al*. 1999). Therefore, besides preventing precocious germination and insect attack, CPI induction in the late stage of embryo maturation could be to reduce uncontrolled proteolytic activity and spontaneous cell death.

The two second-largest gene families in cluster C encode γ-thionins and lipid transfer proteins (LTPs). Key actions of γ-thionins include antifungal activity and inhibition of digestive enzymes in insects (Pelegrini and Franco 2005),

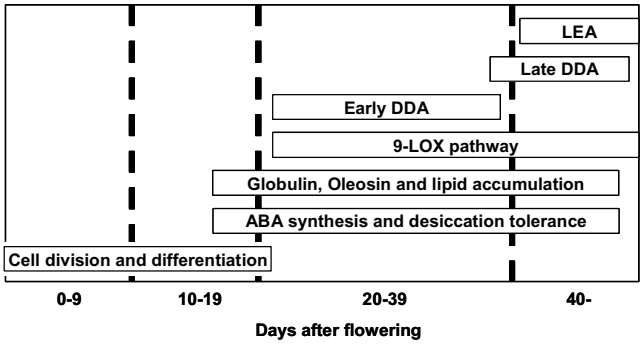

Fig. 1 Model representing key processes operating during barley embryo development. Based on time-series transcriptome changes of the developing embryos of barley, maize, wheat and *Arabidopsis*, the processes involved in desiccation tolerance, storage product accumulation and overall protection of the embryo against biotic and abiotic stress was identified.

as well as antibacterial activity (Iwai *et al*. 2002; Vila-Perello *et al*. 2003). The γ-thionins exhibit a highly conserved structure and are thought to cause changes in membrane permeability in the invading pathogen, but the actual modes of action remain unclear (Pelegrini and Fanco 2005).

The LTPs represent a family of proteins that facilitate lipid transport, for example in the biosynthesis of the cutin layer of plants (Sterk *et al*. 1991; Pató *et al*. 2002). In addition, expression of genes encoding LPTs is induced in response to application with a wide variety of pathogen, drought, wounding and saline stress (Jung *et al*. 2003; Jang *et al*. 2004). Recent investigations of LTPs in whole barley grains revealed an adduction of LTP1b to a 9-LOX derived oxylipin, in a process that depends on the embryo (Bakan *et al*. 2006). Whether LTPs protect plants against microbial infections by mediating a signal that induce relevant plant defense responses, as previously discussed (Molina *et al*. 1993; Cammue *et al*. 1995; Park *et al*. 2002), could be a matter of further experimentation. Also, the discovery of an LTP-oxylipin adduct opens new perspectives on the direct involvement of LTPs in signalling events.

DISCUSSION

The recent examinations of gene regulation during development of the barley embryo, has revealed new information on desiccation tolerance and production of storage compounds, in addition to the initiation of a likely protection against potential pathogens in the developing embryo. Although conclusions drawn from the analyses of gene expression presented here would need further experimental validation, the combined information from studies in barley, but also wheat, maize and *Arabidopsis* embryos (Lee *et al*. 2002; Wilson *et al*. 2005; Nielsen *et al*. 2006; Sreenivasulu et al. 2006; Spencer *et al*. 2007), could be incorporated into a general model of some of the regulatory processes in embryo development (**Fig. 2**). As illustrated, the two earliest stages are predominated by genes involved in cell division and differentiation followed by an up-regulation of the ABA biosynthesis. This increase in ABA is likely to directly regulate the production of storage proteins and lipids as well as the overall desiccation tolerance in the embryo. During maturation, a pronounced up-regulation of genes in the 9-LOX pathway appears a long side an up-regulation of *PR* genes consisting mainly of POXs and thionins, entitled early DDA. Due to the similarities to the 13-LOX pathway which synthesises JA and regulating a large number of *PR* genes, the 9-LOX pathway could be involved in regulating early DDA. Although there are examples of regulation of the 9-LOX pathway in response to pathogens Weber *et al*. 1999; Göbel *et al*. 2001), the regulatory effects of the 9-LOX pathway on *PR* gene expression during embryo development remain to be experimentally verified. In the final stage of embryo development, late DDA is initiated together with an up-regulation of genes encoding LEA. In addition to the processes mentioned above, genes involved in amino acid metabolism, glycolysis and, transcription and translation, are also regulated (Lee *et al*. 2002; Wilson *et al*. 2005) adding to the complexity of the transcriptome of the developing embryo.

Although complex, the increasing knowledge on regulatory processes during embryo development might serve as a basis for quality crops with novel properties. An example could be reduced levels of potential allergens like α-amylase inhibitors, POXs, heat shock proteins and LTPs (Sanchez-Monge *et al*. 1992; Sanchez-Monge *et al*. 1997; Chiung *et al*. 2000; Pastorello *et al*. 2000; Garcia-Casado *et al*. 2001). Since such proteins also play roles in the defense against various pathogens and insects, their synthesis must be highly controlled. That several of the corresponding genes are highly regulated during embryo development, suggests that these genes are controlled by developmental switch factors and not only in response to microbial attack (Nielsen *et al*. 2006; Sreenivasulu *et al*. 2006). Further knowledge and understanding of how potential allergens are regulated might provide ways to change their temporal expression, such that the nutritional qualities of grains are improved without affecting the overall disease resistance of the plant.

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