Theor Appl Genet (2015) 128:1893–1916
DOI 10.1007/s00122-015-2565-2

REVIEW

AGPase: its role in crop productivity with emphasis on heat tolerance in cereals

Gautam Saripalli[1] · Pushpendra Kumar Gupta[1]

Received: 26 February 2015 / Accepted: 16 June 2015 / Published online: 8 July 2015
© Springer-Verlag Berlin Heidelberg 2015

Abstract

Key message **AGPase, a key enzyme of starch biosynthetic pathway, has a significant role in crop productivity. Thermotolerant variants of AGPase in cereals may be used for developing cultivars, which may enhance productivity under heat stress.**

Abstract Improvement of crop productivity has always been the major goal of plant breeders to meet the global demand for food. However, crop productivity itself is influenced in a large measure by a number of abiotic stresses including heat, which causes major losses in crop productivity. In cereals, crop productivity in terms of grain yield mainly depends upon the seed starch content so that starch biosynthesis and the enzymes involved in this process have been a major area of investigation for plant physiologists and plant breeders alike. Considerable work has been done on AGPase and its role in crop productivity, particularly under heat stress, because this enzyme is one of the major enzymes, which catalyses the rate-limiting first committed key enzymatic step of starch biosynthesis. Keeping the above in view, this review focuses on the basic features of AGPase including its structure, regulatory mechanisms involving allosteric regulators, its sub-cellular localization and its genetics. Major emphasis, however, has been laid on the genetics of AGPases and its manipulation for developing high yielding cultivars that will have comparable productivity under heat stress. Some important thermotolerant variants of AGPase, which mainly involve specific amino acid substitutions, have been highlighted, and the prospects of using these thermotolerant variants of AGPase in developing cultivars for heat prone areas have been discussed. The review also includes a brief account on transgenics for AGPase, which have been developed for basic studies and crop improvement.

Communicated by R. K. Varshney.

✉ Pushpendra Kumar Gupta
pkgupta36@gmail.com

[1] Molecular Biology Laboratory, Department of Genetics and Plant Breeding, Ch.Charan Singh University, Meerut 250004, India

Introduction

Starch is the major component of seed endosperm, accounting for 56–74 % of the available carbohydrates in the grain of most food crops including cereals (Koehler and Weiser 2013). It is widely known that the following four enzymes play a key role in starch biosynthesis: (1) ADP glucose pyrophosphorylase (AGPase), (2) starch synthase (SS), (3) starch branching enzyme (SBE) and (4) starch debranching enzyme (SDBE). Using ATP, AGPase converts glucose-1P into ADP-glucose, which is converted into amylose starch using SS, and into amylopectin starch using starch branching enzyme (SBE). Therefore, the activity of each of these four enzymes attracted the attention of plant breeders, with the goal of enhancing the grain weight or grain number through increase in the content of synthesised starch. Of these four enzymes, AGPase and SS have attracted the major attention. AGPase catalyses the first key enzymatic step (synthesis of ADP-glucose) and has been described as a rate-limiting enzyme in the biosynthesis of starch. Major evidence suggesting AGPase to be the rate-limiting enzyme for starch synthesis has come from the results of transgenic cereals (e.g., maize, wheat and rice), where an overexpression of modified forms of AGPase resulted not only in an enhanced rate of starch synthesis, but also in increased final grain yield (Stark et al. 1992; Giroux

et al. 1996; Greene and Hannah 1998a; Smidansky et al. 2002, 2003; Sakulsingharoj et al. 2004; Obana et al. 2006; Wang et al. 2007; Li et al. 2011; Hannah et al. 2012). Null mutants with decreased starch content (Johnson et al. 2003) and deregulation of AGPase activity (in terms of insensitivity to Pi inhibition) leading to increase in seed yield (Giroux et al. 1996; Cross et al. 2004) gives further support to the idea that this enzyme is really a rate-limiting enzyme in starch synthesis (for further details and more references, see later). We, however, recognise that SS is also a major enzyme, which has a significant impact on starch biosynthesis and grain yield (Keeling et al. 1993; Hawker and Jenner 1993; Smith et al. 1995; Preiss 2004), but a discussion on SS is beyond the scope of this review.

Physiologically, crop yield depends on the relationship between the source and the sink, since starch stored in the seed is actually the result of conversion of photosynthate into the dry matter in the seed. The plastidial AGPase in the leaf and the cytosolic AGPase in the endosperm function independently to produce starch, which is then accumulated in the seed endosperm (Huang et al. 2014; for plastidial and cytosolic AGPase, see later). Thus AGPase plays a key role in the modulation of photosynthetic efficiency in source tissues (leaves) and also determines the level of starch in the storage organ, the sink (seed), which in turn determines the overall crop yield potential (Moss and Denyer 2009; Danishuddin et al. 2011). AGPase activity is also regulated by two key allosteric regulators, 3-PGA acting as an activator and Pi acting as an inhibitor. Since deregulation of AGPase activity (insensitivity to Pi inhibition) in the endosperm also leads to increase in seed yield, the activity of AGPase can also be manipulated through these allosteric regulators for the purpose of crop improvement (Giroux et al. 1996; Cross et al. 2004).

More recently, it has been shown that the activity of AGPase goes down under high temperature, so that it should be possible to develop thermotolerant crops through the use of thermotolerant variants of AGPase. For this purpose, the fate of this enzyme under heat stress has also been studied, but not fully understood. The grain weight is known to be significantly reduced under heat stress due to reduction not only in the rate and duration of starch synthesis (Viswanathan and Khanna-Chopra 2001) but also that in the number and size of starch granules (Kossmann and Lloyd 2000; Hurkman et al. 2003; Hannah et al. 2012). This information is important in view of the realisation that cereals including wheat (like several other crops) are going to experience a rise in temperature due to climate change. In view of this, major efforts are underway to produce thermotolerant genotypes in all major crops including cereals. This brief review is devoted to basic information about AGPase including its functionally important structural features (Table 1) and genetics (Table 2). Strategies that may be used to utilise this information for manipulating this enzyme for increasing cereal productivity (particularly under heat stress) are also discussed.

Structure, function and evolution of AGPases in higher plants

Structure of AGPase has been studied in some prokryotes and also in a number of plant systems including model plants like *Arabidopsis thaliana* and several crops including maize, rice, wheat, barley, banana, chickpea, broad bean (*Vicia faba*), rapeseed (*Brassica napus*), potato and tomato (Table 1). Genes for AGPase in a number of these plant systems have also been cloned, characterised and utilised for a study of the evolution of AGPase in higher plants. Details of these cloned genes are summarised in Table 2, and the salient features are briefly discussed.

AGPase in higher plants is a heterotetramer

The enzyme AGPase is a homotetramer (α_4) in bacteria, but heterotetramer in higher plants, where each molecule carries two smaller subunits (AGP-S or SS = α_2) and two slightly larger subunits (AGP-L or LS = β_2). Thus, AGPase in higher plants is an $\alpha_2\beta_2$ heterotetramer with a molecular weight within the range of ~200–240 kD (Morell et al. 1988; Lin et al. 1988; Okita et al. 1990; Preiss et al. 1990). The difference in the size (mol.wt.) between the SS and LS is rather small and ranges from a mere 1–5 kD (Ballicora et al. 2004), the molecular weight of LS in potato being 51 kD. The amino acid sequence of SS is more conserved relative to that of LS in different species and also in different tissues of the same species (Smith-White and Preiss 1992); this is also apparent from the fact that same SS can associate with any of the different LSs to form an active and functional hetrotetrameric AGPase.

AGPase is also known to undergo allosteric modification, which is regulated by an interaction between the two subunits. The active site of AGPase was found to consist of a long cleft bordered at each end with a sugar- or adenine-binding pocket and lined by a number of residues that are conserved in related nucleotidyltransferases. The entire molecule of ATP binds to the active site, although the conformation of the phosphate domains is completely different from that seen in other sugar-nucleotide PPase/NTP complexes (Jin et al. 2005). An intermolecular disulfide (S–S) bond that occurs between two Cys_{12} residues of the catalytic site in SS also regulates the activity of plant AGPase, as observed in the study of mutants lacking this S–S bond. This S–S bond enhances the activity of the enzyme (through higher affinity to activator 3-PGA and the substrate ATP) and maintains stability of the enzyme through maintenance

Table 1 Some recent studies involving AGPase and their characteristic features in different plant systems

Features	Plant species	Details of study	References
Subcellular location of AGPase (plastidial and cytosolic) and its significance	*Lycopersicon esculentum*	Most AGPase in developing tomato fruit in the plastid	Beckles et al. (2001a)
	Triticum aestivum	Expression of AGPase mostly cytosolic	Burton et al. (2002)
	Zea mays	Major form of AGPase activity is cytosolic	Denyer et al. (1996)
	Cereals and other grasses		Moss and Denyer (2009)
	Hordeum vulgare	Cytosolic AGPase is important in ADP-Glc synthesis in endosperms but not in other starch storing organs	Beckles et al. (2001b)
	Oryza sativa	Starch biosynthesis in endosperm is controlled predominantly by the cytoplasmic AGPase	Tuncel et al. (2014)
	Zea mays	Higher cytoplasmic AGPase activity in transgenics [15 % increase in 100-GW over the wild type (WT) and 9 % increase in starch content]	Li et al. (2011)
		Plastidial AGPase is also required for normal level of starch accumulation in cereal endosperm even when cytosolic form is fully functional	Huang et al. (2014)
	Triticum aestivum	Over-expression of cytoplasmic LS gives enhanced starch in wheat grains; improvement in grain yield related traits	Kang et al. (2013)
Tissue specific expression	*Zea mays*	Tissue specific expression for six genes encoding plastidial LS and SS to be differentially expressed in endosperm, leaves and embryo	Huang et al. (2014)
	Oryza sativa	Tissue specific expression of six AGPase genes (2 SS and 4 LS) each gene expressed specifically in seed, leaves, stem and roots; SS genes expressed in both seeds and leaves LS genes expressed in all tissues	Akihiro et al. (2005)
Evolution of AGPase subunits	*Hordeum vulgare*	Same gene (*Hv.AGP.S.1*) encodes for cytosolic SS in endosperm and plastidial SS in leaves	Rosti et al. (2006)
	Zea mays	SS exhibits more evolutionary constraints in planta than does the LS	Georgelis et al. (2007)
		Two genes SS (*Bt2* and *L2*) are paralogoues; Bt2 encodes only endosperm cytosolic SS, L2 only leaf AGPase	Rosti and Denyer (2007)
	Angiosperms	SS evolved under strong constraints in relative to LS; co-evolution among amino acid residues	Corbi et al. (2011)
Crsytal structure, homology modelling and SS–LS interaction	*Solanum tubersome*	Atomic resolution structure reported; structural basis for allosteric regulation described	Jin et al. (2005)
	Solanum tubersome	Homology modelling for LS and LS–SS subunit interactions studied	Tuncel et al. (2008)
	T. aestivum	3D structure based on crystal structure of potato tuber AGPase. Interaction between LS and SS	Danishuddin et al. (2011)
	Oryza sativa	Prediction of 3D structure of rice AGPase using comparative modelling	Dawar et al. (2013)

Table 1 continued

Features	Plant species	Details of study	References
Effect of 3PGA and Pi on AGPase activity	*Zea mays* and *Solanum tuberosome*	Maize endosperm AGPase is less dependent on 3PGA relative to potato tuber AGPase	Burger et al. (2003)
	Zea mays	3-PGA and Pi stabilise maize endosperm AGPase against thermal inactivation; glycerol phosphate and R-5-P also increase the activity like 3-PGA and Pi	Boehlein et al. (2008)
		In absence of 3-PGA, Pi stimulates enzyme activity at low substrate level lowering the Km values	Boehlein et al. (2010)
Mutants and their significance	*Hordeum vulgare*	Low starch mutant *Risø 16* lacking SS revealed the importance of cytosolic AGPase in starch synthesis and identity of plastidial AGPase	Johnson et al. (2003)
	Solanum tuberosum	Identified several variants of AGPase SS with enhanced affinity to activator and/or greater resistance to inhibition	Salamone et al. (2002)
	Zea mays	Mutant genes encoding plastidial AGPase demonstrated that cytosolic and plastidial enzymes both contribute to endosperm starch content	Huang et al. (2014)
	Hordeum vulgare	The low starch phenotype of the *lys5* mutants showed that the ADP-Glc transporter is needed for normal rates of starch synthesis	Patron et al. (2004)
	Oryza sativa	Identified mutant *Apl3*(LS) using tilling approach and the protein characterised by monoclonal antibody	Cook (2011)
	Arabidopsis thaliana	Substitution of Cys_{81} with serine prevented SS dimerisation; role of Cys_{81} in the regulation of AGPase turnover	Hadrich et al. (2012)
	Zea mays	Insertion of cysteine in the N terminus of the AGPase SS enhances the heat stability of endosperm	Linebarger et al. (2005)
		Variants with enhanced properties i.e., heat stability, enhanced activity at 37 °C, catalytic activity at 55 °C, reduced Ka in absence of 3-PGA identified and combined into a single gene Sh2-E	Boehlein et al. (2014)
		Heat stability variants with enhanced allosteric properties identified through iterative saturation mutagenesis	Boehlein et al. (2015)

Table 2 Details of cloned genes/cDNAs for AGPase subunits in cereals

Species	Gene	Encoding unit	Target tissue	References	Accession ID
Zea mays	*Sh2*	LS	Endosperm	Shaw and Hannah (1992)	M81603
	Agplemzm	LS	Embryo	Giroux et al. (1995)	Z38111
	Agpllzm	LS	Leaves	Corbi et al. (2012)	HM749614.1
	Bt2	SS	Leaves	Hannah et al. (2001)	AF334959
	Agpsemzm	SS	Embryo	Hannah et al. (2001)	AY032604
	Agpslzm	SS	Leaves	Hannah et al. (2001)	AY032604
	Agpl3	LS	ND	Huang et al. (2014)	–
Triticum aestivum	*Agpl1(Agpl1enta)*[a]	LS	Endosperm	Ainsworth et al. (1995)	–
	Agp2	LS	Leaves and developing endosperm	Ainsworth et al. (1995)	AJ563452
	Agpl2(Agpl2enta)	LS	Endosperm, leaves and embryo	Ainsworth et al. (1995)	–
	Agps1(Agps1enta)	SS	Endosperm	Ainsworth et al. (1993)	X66080
Oryza sativa	*OsApl1*	LS	Endosperm	Lee et al. (2007)	AK100910
	OsApl2(AGPL2ENOS)	LS	Endosperm	Lee et al. (2007)	AK071497
	OsApl3	LS	ND	Lee et al. (2007)	AK069296
	OsApl4	LS	ND	Lee et al. (2007)	AK121036
	OsAps1(AGPS1ENOS)	SS	Endosperm	Tiana et al. (2009)	GQ150840
	OsAps2	SS	ND	Tiana et al. (2009)	GQ150855
Hordeum vulgare	*Agps1(Agps1lhv)*	SS	Leaves	Rosti et al. (2007)	Z48578
	Agps2(Agps2enlemhv)	SS	Endosperm, leaves, embryo	Rosti et al. (2007)	AF537363
	BepsF1	SS	Endosperm	Thorbjornsen et al. (1996b)	X67151
	Blpl14	LS	Leaves		U66876

ND not determined

[a] Proposed standard nomenclature wherever not available in published literature is given in parenthesis

of ordered structure at high temperature. However, this S–S bond does not occur in SS of all AGPases and is certainly absent in AGPase of cereal endosperms. However, successful efforts have been made to introduce the S–S bond into maize endosperm AGPases leading to 70-fold increase in heat stability of the endosperm AGPase (for details see later).

Crystal structure of AGPase

Since the extraction of enzyme in its pure state is difficult, several attempts to deduce the crystal structure initially failed until the atomic structure of homotetramer AGPase SS from potato tuber was resolved for the first time by Jin et al. (2005). In potato tuber, AGPase SS monomer consists of an N-terminal catalytic domain and a C-terminal β-helix domain. The catalytic domain mainly consists of a largely parallel but mixed seven-stranded β sheet covered by α helices, which represent a fold reminiscent of the dinucleotide-binding Rossmann fold. N-terminal catalytic domain was also reported to make strong hydrophobic interactions with the C-terminal β-helix domain via an α helix. This structure was used by Tuncel et al. (2008) to propose a model for the three-dimensional structure of potato LS, followed by a model for heterotetrameric structure of the entire potato AGPase and the interactions between the two subunits. This knowledge generated from potato AGPase was used as a base to deduce the crystal structure of AGPase in rice (Fig. 1; Dawar et al. 2013) and wheat (Danishuddin et al. 2011).

Modelling of three-dimensional structure of rice LS and SS was undertaken using the structure of potato SS as template, and the resulting models were evaluated and docked to obtain the stable heterodimer orientation (LS as receptor and SS as ligand). LS–SS interaction studies identified a total of 57 residues in SS and 63 residues in LS that may be involved in interactions. Also, the models thus generated when further visualised and examined depicted 19 β strands and 15 α helices in LS and 18 β strands and 15 α helices in the SS (Fig. 2). Dimer–dimer interface studies identified 31 hydrophobic and 31 hydrophilic residues at LS interface and 30 hydrophobic and 31 hydrophilic residues at SS interface (Dawar et al. 2013). Similarly, in wheat, interaction studies revealed the involvement of 15 amino acids of SS in hydrophobic contacts with 17 amino acids of LS, with 6 amino acids involved in hydrogen bonding (Danishuddin et al. 2011). In the same study, when multiple sequence alignment was undertaken, all residues at interface were found to be conserved in maize, rice and potato. The superimposition of rice AGPase heterotetramer

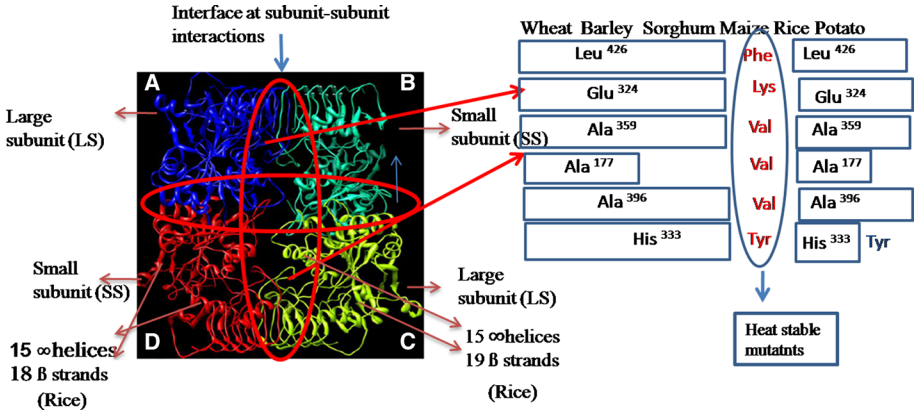

Fig. 1 Crystal structure of AGPase in rice (reproduced with permission from Dawar et al. 2013). The amino acid positions in *boxes* indicate the conserved residues, and the amino residues highlighted in *red* indicate those substituted in the heat-stable mutants due to substitutions in maize (Burger 2001; Boehlein et al. 2015) (color figure online)

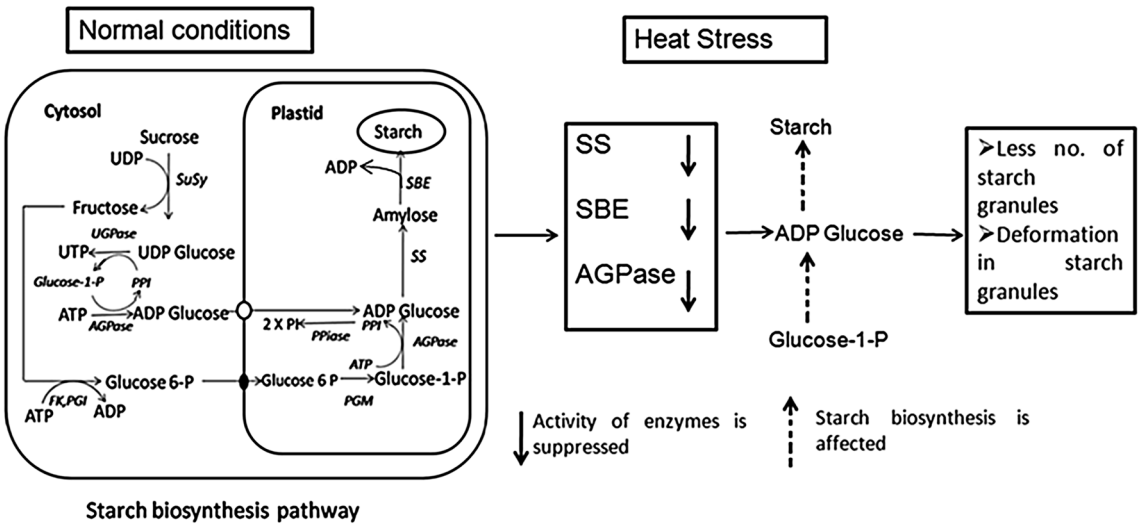

Fig. 2 Flow chart showing the significance of three important enzymes during heat stress. Abbreviations for enzymes (*italic*) are *SuSy* sucrose synthase, *UGPase* UDPglucose pyrophosphorylase, *PGM* phosphoglucomutase, *FK* fructokinase, *PGI* phosphoglucose isomerase, *PPiase* pyrophosphatase, *AGPase* ADPglucose pyrophosphorylase, *SS* starch synthase, *SBE* starch-branching enzyme. The transporters in the plastidial inner membrane are shown as *circles: white hollow circle*, ADPglucose/ADP transporter; *black solid circle*, glucose 6-phosphate/phosphate transporter. Other abbreviations are *PPi* pyrophosphate, *Pi* phosphate

and that of wheat AGP-L/AGP-S on to the potato SS homotetramer suggested that the structures were similar [root mean square deviation (RMSD) = 1.09, 1.08, 1.30 Å] (Dawar et al. 2013; Danishuddin et al. 2011).

Cytosolic and plastidial isoforms of AGPase

In plant tissues, AGPase generally occurs only in plastids within leaf cells in all non-cereal plants. However, in the seed endosperm of grasses including cereal crops, the predominant form of AGPase occurs in the cytosol and only a minor form resides in the amyloplasts (for reviews, see Moss and Denyer 2009; Hannah and Greene 2009; Geigenberger 2011). The presence of two AGPase isoforms (the plastidial and the cytosolic forms) has been demonstrated through biochemical studies (sometimes involving plastid isolation) in maize (Denyer et al. 1996; Shannon et al. 1998), barley (Thorbjornsen et al. 1996a), rice (Sikka et al. 2001) and wheat (Tetlow et al. 2003). These two forms of AGPase differ in structure and are encoded in different nuclear genes. However, no evidence is available for the presence of distinct plastidial and cytosolic forms in the storage tissues of non-graminaceous species like potato and tomato (Beckles et al. 2001b).

Major AGPase in cereals is cytosolic

Sub-cellular fractionation revealed that 85–95 % of total AGPase activity is cytosolic in endosperm cells of rice, maize, barley and wheat (Denyer et al. 1996; Thorbjornsen

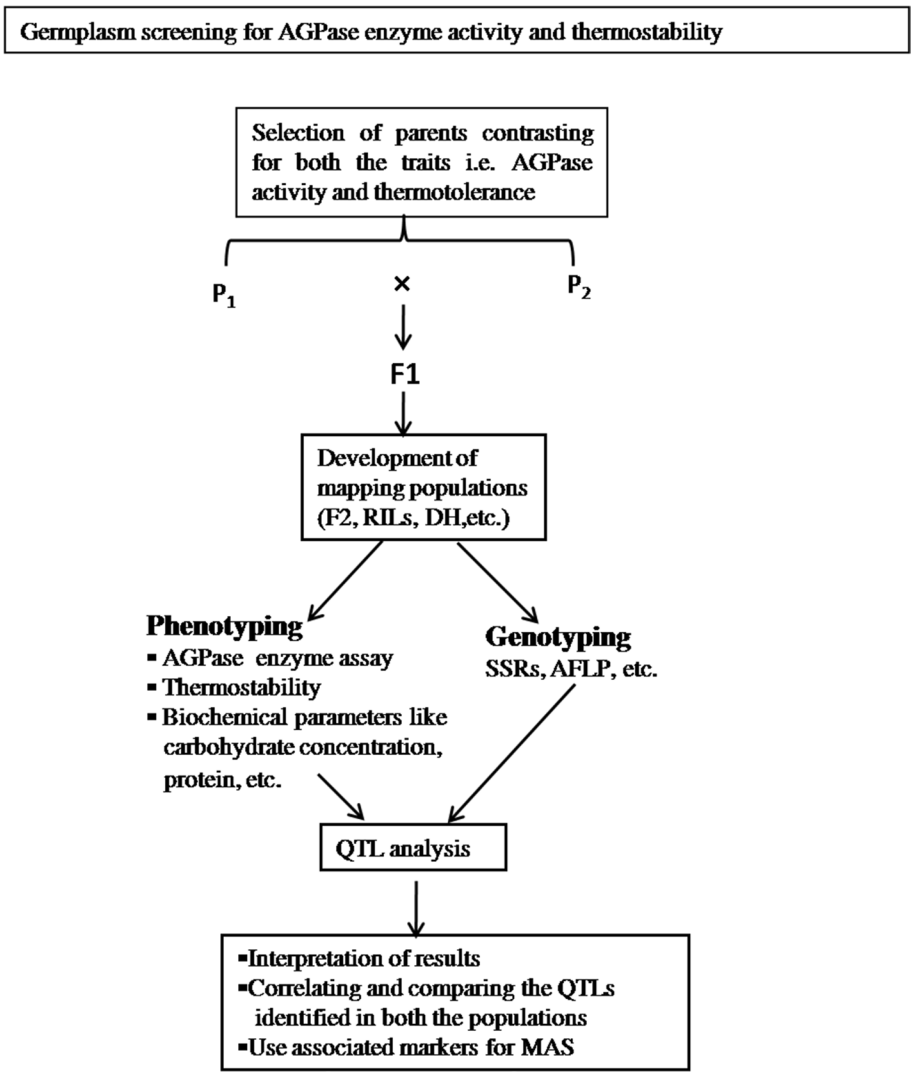

Fig. 3 Experimental strategy designed to study the QTLs for AGPase activity and thermostability

et al. 1996b; Shannon et al. 1998; Sikka et al. 2001; Tetlow et al. 2003; Rosti et al. 2006). Importance of cytosolic ADP-glucose for starch synthesis has also been demonstrated by low-starch mutants of maize and barley, which either lack the cytosolic form of AGPase (Tsai and Nelson 1966; Dickinson and Preiss 1969; Denyer et al. 1996; Johnson et al. 2003) or carry ADP-glucose transporter for the transport of ADP glucose from the cytosol to the plastids (Li et al. 1992; Shannon et al. 1998; Patron et al. 2004). The higher concentration of ADP-Glc and 1:1 ratio of ADP-Glc and UDP-Glc concentration in graminaceous endosperms also indicated the presence of major cytosolic AGPase activity in cereals (Beckles et al. 2001a). This is mainly due to the fact that the phosphorylation reactions involving both ADP-Glc and UDP-Glc make use of the same substrates (Pi and G-1-P) (Fig. 3) and hence would tend to be in equilibrium. Kang et al. (2013) observed that over-expression of the gene for wheat cytosolic AGPase LS (*TaLSU1*) enhanced starch biosynthesis in endosperm of wheat grains, which led to an increase of endosperm starch weight, grain no. per spike and single grain weight. Similarly, rice transgenics with cytosol targeted AGPase activity showed increase in seed weight as compared to transgenics with amyloplast-targeted AGPase activity. This also provided the evidence that the major form of AGPase in cereals is cytolosic (Sakulsingharoj et al. 2004). AGPase distribution has also been shown to differ in different cellular compartments (Tetlow et al. 2003; Denyer et al. 1996).

3-PGA, Pi and sugar as effectors of AGPase, and sensitivity of AGPase to these effectors

AGPase reaction takes place in the presence of divalent Mg^{2+} and is reversible in vitro (Ghosh and Preiss 1966; Ballicora et al. 2004). As mentioned earlier, the activity of AGPase is also regulated by two effectors (3-PGA as

Table 3 A summary of the results of studies showing sensitivity of AGPase to effectors (3-PGA and Pi) and sugars

Crop	Details of study	References
Pea	40-fold decrease in AGPase activity due to increased sensitivity to Pi and 3-PGA	Hylton (1992)
Bean	Insensitivity of AGPase towards 3-PGA in crude extracts of cotyledons; fivefold activity in leaves. Pi inhibition more in leaves than in cotyledons	Weber et al. (1995)
Maize	Enhanced enzyme activity and reduced sensitivity to 3-PGA and Pi due to proteolitic degradation of AGPase	Plaxton and Preiss (1987)
Maize	Pi more stable under heat stress as compared to 3-PGA. Glycerol phopshate and R-5-P as substitutes for 3-PGA	Boehlein et al. (2008)
Chlamydomonas	Reduced sensitivity to 3-PGA and Pi in some low starch mutants	Ball et al. (1991)
Rice	Endosperm AGPase show similar sensitivities to 3-PGA and Pi as that of corresponding chloroplast AGPase	Sikka et al. (2001)
Potato		Ballicora et al. (1995)
Wheat	Endosperm AGPase insensitive to 3-PGA activation but sensitivie to Pi and fructose-1,6,bi-phosphate inhibition	Gomez-casati et al. (2002)
Wheat	Plastidial isoform of wheat AGPase relatively more insensitive to 3-PGA and Pi compared to plastidial AGPase of other species	Tetlow et al. (2003)
Pea	Leaf AGPase redox activated by thioredoxins. Thioredoxin f increases AGPase activity by threefold increase	Geigenberger et al. (2005)
Potato	Detachment of tubers from mother plant reduces AGPase activity which is reversed back when incubated with high sucroce levels	Tiessen et al. (2002)
Arabidopsis	Significance of sucrose in enhancing the AGPase actvity in the leaves under dark conditions	Hendriks et al. (2003)
Arabidopsis	Trehalose activates AGPase via post translational redox modification in leaves	Kolbe et al. (2005)
Arabidopsis	Addition of sucrose increases trehalose-6-phosphate which ultimately enhances activity of AGPase in leaves	Lunn et al. (2006)

an activator and PPi as an inhibitor; see later for details). Therefore, not only a manipulation of this enzyme, but also that of its effectors can impact grain yield and grain quality. The ratio of these two allosteric effectors is believed to play a key role in the control of starch synthesis in photosynthetic tissues (Preiss 1991). The level of sensitivity of AGPase to 3-PGA and PPi also varies among different plant species (Table 3). In some crops like wheat, endosperm AGPase is insensitive to 3-PGA activation but sensitive to Pi inhibition when they act separately. But when 3-PGA interacts with Pi, it overcomes the inhibition caused by Pi (Gomez-casati and Iglesias 2002). In other crops like pea, endosperm AGPase exhibited increased sensitivity towards both the effectors. In still other crops like barley endosperm AGPase is completely insensitive towards both the effectors. When full-length cDNAs encoding both subunits of barley endosperm cytosolic AGPase were expressed in *E. coli* or baculovirus systems, the heterologous enzyme showed the same insensitivity to 3-PGA and Pi regulation as AGPase from endosperm extracts, indicating that insensitivity to effectors may represent an intrinsic property of endosperm AGPase (Villand and Kleczkowski 1994; Doan et al. 1999).

A higher sugar concentration is also found to enhance the AGPase activity in potato, Arabidopsis and also in maize (Table 3), suggesting that the supply of sugars leads to stimulation of starch synthesis. It is observed that sugar metabolites like glycerol phosphate (GP) and fructose 6 phosphate (F-6-P) increase the catalytic rate of maize endosperm AGPase to the same extent as 3-PGA, although the concentration of these metabolites varies in comparison to 3-PGA concentration required. Amount of GP required for one-half activation (time required for loss of one half of AGPase activity) of AGPase is approximately 15 fold to that of 3-PGA (Boehlein et al. 2008). In contrast, in potato, F-6-P stimulates the activity of AGPase to only 4 % to that of 3-PGA. Amount of F-6-P required for one-half activation in potato is approximately 25-fold that of 3-PGA (Iglesias et al. 1993). An understanding of the detailed mechanisms for sugar-induced activation of AGPase needs further investigation.

AGPase activity under abiotic stresses (heat, drought and cold)

Abiotic stress affects different cellular processes in plants such as growth, photosynthesis, carbon-partitioning, carbohydrate and lipid metabolism, osmotic homeostasis, protein synthesis and gene expression. Some of these processes are adversely affected due to reduced AGPase activity under abiotic stresses and will thus adversely influence supply of sugars to sink leading to reduced starch accumulation in the seed. This adverse effect due to abiotic stresses mainly occurs during grain filling period.

AGPase activity, starch synthesis and effect of PGA and Pi under heat stress

Under conditions of heat stress, biosynthesis of starch gets impaired largely due to inhibition of the activities of AGPase and SS (Fig. 2). However, there seems to be a lack of unanimity regarding the relative roles of these two enzymes for the reduced yield under heat stress. While significant reduction in the activity of AGPase under high temperature has been reported in several studies (Singletary et al. 1994; Duke and Doelhert 1996; Geigenberger et al. 1998; Wilhelm et al. 1999; Boehlein et al. 2008), there are also reports suggesting little or no reduction in the activity of AGPase under heat stress (Hawker and Jenner 1993; Lohot et al. 2010; Bansal et al. 2012). A comparison of the effect of heat on the above two major enzymes of starch synthesis (AGPase and SSS) also suggested reduced activity of both AGPase and soluble starch synthase (SSS) during heat stress (Hawker and Jenner 1993; Singletary et al. 1994). In a study conducted in wheat, when during the grain filling period (from 16 to 32 days after anthesis), the ears were subjected to heat stress (35 °C), significant reduction (35 %) was observed in AGPase activity in endosperm under continuous heat treatment, relative to that under interrupted heat treatments. However, in control (21 °C day/16 °C night), no decline in AGPase activity was observed, although UGPase and SS showed a significant decline in their activities during the grain filling period (Hawker and Jenner 1993). Also, it was observed in case of maize that although the decline in the activities of both the enzymes (AGPase and SS) was responsible for decreased starch synthesis under heat stress, AGPase showed greater reduction (87 %) in its activity as compared to SS (57 %), when the temperature was raised from 19 to 32 °C (Singletary et al. 1994).

It is widely known that activities of enzymes depend on several factors. In case of AGPase from cereal endosperm, the activity also depends on the concentrations of the two major effectors 3-PGA and Pi (although wheat endosperm AGPase has been reported to be generally insensitive to 3-PGA activation), whereas in case of AGPases from other crops (e.g., potato), the presence of the disulphide-bridges between small subunits of AGPase imparts increased heat stability so that there is no reduction in activity under heat stress. For instance, the presence of these disulphide bridges in the SS of potato endosperm is the major factor which enhances stable LS–SS interactions leading to increased heat stability. The cystein residue that is found in the 12th position of ten amino acid region of N-terminus in the potato SS is mainly responsible for these disulphide bridges (Linebarger et al. 2005). Since this cysteine residue is absent in the AGPase of cereal endosperms, (as mentioned earlier), it was introduced in the SS of maize endosperm using site-directed mutagenesis leading to stable LS–SS interactions. This mutant conferred increased heat stability of about 70-fold as compared to wild type when the whole cell extracts of mutant and wild-type maize endosperm were incubated at 58 °C for 6 min (Greene and Hannah 1998b; Burger 2001). Similarly, several other thermostable variants which conferred stable LS–SS interactions have been isolated in maize using different approaches. Efforts have also been made to overexpress the modified forms of maize endosperm AGPase into other important cereals (rice and maize) (for details see later section on thermostable variants).

Similar to disulphide bridge which provides heat stability to endosperm AGPase in non-cereal crops, concentrations of 3-PGA and Pi also help in providing heat protection not only to cereal endosperm but also to starch storage organs (like tubers) in other crops like potato. For instance, a reduced level of AGPase activity and consequently that of starch synthesis under high temperature has been reported in potato (Geigenberger et al. 1998). In this study, the decline in enzyme activity was shown to be due to increase in respiration rate under high temperature leading to decline in the level of 3-PGA (an activator of AGPase). This indicates that higher concentration of 3-PGA helps in protecting potato tubers against heat protection. In case of maize, purified maize endosperm AGPase protein was mixed with various concentrations of 3-PGA and incubated at 42 °C for 10 min. AGPase activity in heated and unheated samples indicated that 50 mM of 3-PGA provided about 35 % protection from high temperature to AGPase activity. Pi in the absence of 3-PGA also stabilised the endosperm AGPase leading to increased heat protection. In fact, the protection conferred by Pi was more (55 %) as compared to that conferred by 3-PGA (35 %) when the same concentration of Pi was used as that of 3-PGA, i.e., 50 mM. A change in ratio of the concentrations of 3-PGA (activator) and Pi (inhibitor) may also lead to change in enzyme activity (Boehlein et al. 2008). This was shown in a study, where wheat cultivars with difference in their sensitivity to these allosteric effectors were used (Lohot et al. 2010). In this study, under normal sowing conditions, significant correlation of AGPase activity with starch accumulation and grain development indicated that an efficient AGPase that is insensitive to regulation by PGA and Pi may lead to faster starch accumulation and early grain filling during extreme terminal temperature stress experienced during grain development.

Studies have also been conducted where the effect of heat on AGPase activity was compared with that on starch accumulation, although no consensus emerged. While there are reports, where the effect of heat on AGPase was correlated with reduced starch accumulation (Singletary et al. 1994; Duke and Doelhert 1996; Bansal et al. 2012), there

are also reports, where no such correlation was observed (Hawker and Jenner 1993; Lohot et al. 2010). The lack of correlation in the study conducted by Hawker and Jenner (1993) appears to be due to the use of in vitro conditions, where concentration of AGPase and not that of SS was tenfold. These conditions perhaps do not mimic the in vivo conditions, where several other factors like concentrations of the substrate and that of the effectors (3PGA and Pi) influence the enzyme activities under high temperature (Boehlein et al. 2008). A correlation was also reported between significant decrease in dry weights of mature maize kernels and reduction in AGPase activity, when the temperature was raised to 35 °C for 15 days, relative to normal condition of 25 °C (Duke and Doelhert 1996). Similarly, when the activities of 11 different enzymes related to starch biosynthesis were studied in seven different maize inbreds under heat stress (33.5/25 °C) and control (25/20 °C) conditions, apparently activities of four enzymes (AGPase, SS, glucokinase and sucrose synthase) were significantly reduced, but when the Q_{10} values were corrected to growth temperature, only AGPase activity was found to be greatly reduced leading to about 7 % reduction in mature dry weight of kernels (Wilhelm et al. 1999).

Effect of heat on AGPase activity was also compared in heat sensitive and heat-tolerant wheat cultivars. In one such study, AGPase activity declined more drastically in a sensitive variety relative to that in a tolerant variety (Bansal et al. 2012). Consequently, efforts have been made to select or develop thermostable variants of AGPase in important cereals (for details, see later). However, SS also plays an important role in starch biosynthesis under heat stress as evident from studies discussed above, but this aspect is beyond the scope of this review.

AGPase activity under drought and cold stress

Under drought also, cereals show significant alterations in the activity of enzymes of starch biosynthetic pathways (Thitisaksakul et al. 2012), due to which there is a drastic reduction in the production of grain starch, ultimately leading to reduction in grain size, yield, etc. AGPase activity also declines rapidly under water stress due to changes in osmotic pressure. Under extreme water deficit, the loss of AGPase LS activity can exceed that of SS leading to premature cessation of starch deposition (Ahmadi and Baker 2001; Caley et al. 1990). Mild stress during the period of 10–24 DAA causes a significant reduction in the activity of AGPase observed in the form of reduction in grain number and dry weight. This reduction is profound (80 and 86 %) under severe stress conditions (i.e., 32 DAA) (Ahmadi and Baker 2001).

Cold stress is also reported to have an impact on the activity of AGPase. Ahmed et al. (2008) investigated the effects of cold stress on key regulatory enzymes for starch biosynthesis in rice endosperm; they observed a reduction in the activities of all enzymes (including AGPase, but excluding GBSS) of starch biosynthesis under cold conditions (12 °C) as compared to normal conditions (22 °C) in rice endosperm. Surprisingly, there was no significant effect on grain weight due to reduction of enzyme activity, but 21 % increase was observed in amylose content in mature endosperm of hulled rice. Thus, it was concluded that the reduction in catalytic activities of all enzymes was associated with increase in activities of GBSS leading to increase in amylopectin content in the endosperm.

Genetics of AGPase in plants

The genetics of AGPase has been studied both in bacteria and higher plants. As a result, it is now known that the bacterial AGPases are encoded each in a single gene, while AGPases in plants are encoded each in multiple genes (Table 2). It is also known that in higher plants, independent genes encode the large subunits (LSs) and the small subunits (SSs), sometimes with more than one gene for the same subunit in an individual species. Also, there are more functional LS genes than SS genes as reported in the following cases: (1) in Arabidopsis, single SS gene and four LS genes; (2) in rice, two SS genes and four LS genes; (3) in poplar, one SS gene and six LS genes; (4) in tomato and potato each, one SS gene and three LS genes and (5) in barley, two SS genes and two LS genes. The LS genes are also known to be more tissue specific than SS genes as far as their expression level is concerned. Thus, the gene redundancy for LS is larger relative to SS (Georgelis et al. 2007, 2008). A comparison of the gene sequences for LS and SS also shows a very high degree of homology among both, the SSs (>70 %) and the LSs (>50 %), although significant homology occurs even between SS and LS (>40 %).

Genetics of AGPases in cereals

Genetics of small subunits (SS)

It is now known that in most cereals including barley, rice and wheat, two types of genes encode AGPase SS, Type 1 and Type 2 (Rosti and Denyer 2007). Type 1 genes through alternative splicing also give rise to two types of transcripts, Type1a and Type 1b. These two transcripts are similar at their 3′ ends, but differ at their 5′ ends (which were predicted to be transit peptides) (Kang et al. 2010). It is also known that Type 1a AGPase SS is cytosolic (endosperm), Type 1b is leaf plastidial and Type 2 is endosperm plastidial. This distribution of AGPases SSs is suggested by the following observations: (1) AGPase SS protein isolated

from endosperm plastids in wheat or barley matched the amino acid sequence predicted for Type 2 AGPase (Johnson et al. 2003), but did not match the sequence predicted for Type 1b gene meant for leaf plastidial AGPase (Burton et al. 2002; Johnson et al. 2003); Type 2 AGPase transcript is also known to occur in small amounts in embryo (reported in barley), but not in the leaf; (2) AGPase SS protein of Type 1b occurs abundantly in leaf plastids, as evident from the fact that a mutation in Type 1b gene in barley and rice caused major reduction in leaf enzyme activity (Rosti et al. 2006; Lee et al. 2007); it occurs only in minor amounts in the endosperm or embryo; also a loss of Type 1b in the embryo did not affect the total AGPase activity, even though its mRNA occurred in abundance, suggesting that some kind of post-transcriptional modification prevents the translation of mRNA in the embryo, but not in the leaf (Rosti et al. 2006).

Maize differs from other cereals with respect to the identity of transcripts that specify AGPase SS in the endosperm cytosol and the leaf plastids. Identification and cloning of the genes encoding cytosolic AGPase LS and SS have been facilitated by the study of mutations at the classical maize gene loci *shrunken2* (*sh2*) and *brittle2* (*bt2*) located on chromosomes 3 and 4 respectively (Bae et al. 1990; Bhave et al. 1990). Following three genes have been identified for SS (*Bt2*) loci: (1) The Type 1a gene *BT2* which generates two transcripts (Rosti and Denyer 2007), referred to as *BT2a* and *BT2b* operates in endosperm plastids. (2) The Type 2 gene encoding AGPase SS and named *AGPSEMZM* (formerly referred to as *agp2*), cloned from embryo tissue (Giroux and Hannah 1994; Giroux et al. 1995). (3) A third gene (Type 1b) encoding AGPase SS for leaf plastids, termed *AGPSLZM* (formerly referred to as *L2*), is present in maize owing to a duplication of the ancestral Type 1 gene (Prioul et al. 1994; Hannah et al. 2001; Rosti and Denyer 2007; Cossegal et al. 2008). All the three genes specifiy plastidial proteins (Rosti and Denyer 2007; Slewinski et al. 2008).

In rice, AGPase SS is encoded by two genes (*OsAPS1*, *OsAPS2*) and AGPase LS is encoded by four genes (*OsAPL1-4*). These genes were identified through mining of database of rice full-length cDNAs and were successfully cloned. Also, these genes were isolated from *Oryza sativa* ssp. japonica cv. Nipponbare, to which the cDNA library used for mining also belonged. It was also reported in this study that *OsAPS1* was mapped on chromosome 8 and differed only for one amino acid residue from *pVK1* that was previously isolated from ssp. japonica cv. Kinmaze (Sikka et al. 2001). Similarly, *OsAPS2* was mapped on chromosome 9 and differed for three amino acids from *RSc6*, which was isolated from ssp. japonica cv. Biggs M201 (Anderson et al. 1989). Since *OsAPS1* and *OsAPS2* were isolated from ssp. japonica cv. Nipponbare, it was assumed that *OsAPS1* and *OsAPS2* are alleles of *pVK1* (from cv Kinmaze) and *RSc6* (from cv Briggs M201), respectively.

Genetics of large subunit (LS)

The available information on genetics of AGPase LS in cereals is not as elaborate as that for the SS. Each in maize and rice, four genes are known to encode AGPase LS (Bae et al. 1990; Lee et al. 2007; Giroux and Hannah 1994; Giroux et al. 1995; Yan et al. 2009; Huang et al. 2010). In maize, the most widely known gene for LS is *Sh2*, which encodes cytosolic AGPase. Two other genes for AGPase LS in maize are *AGPLEMZM* (Giroux and Hannah 1994; Giroux et al. 1995) and *AGPLLZM* (Yan et al. 2009; Huang et al. 2010). *AGPLEMZM* is a major contributor of LS in embryo, although no genetic analysis using mutants for this gene has been conducted. However, neither *AGPLLZM* nor *Sh2 is* expressed in embryo, as apparent from the fact that the *agpllzm* null mutations did not affect the total embryo AGPase activity. But in leaf, both the transcripts (*AGPLEMZM* and *AGPLLZM*) are available and loss of *agpllzm* led to major reductions in AGPase activity. Apart from these three genes, transcripts of fourth gene *AGPL3* are found to be present in embryo, but the transcripts were present in very low concentration; consequently, this gene has not been studied in detail and apparently it does not play any major role in the synthesis of AGPase (Huang et al. 2014).

In rice, there are four independently encoded LS genes (*OsAPL1, OsAPL2, OsAPL3, OsAPL4*), which are located on chromosomes 3, 1, 5 and 7. They showed high homology with each other except their N-terminal regions. *OsAPL1* also showed significant homology with barley leaf type LS; similarly, *OsAPL2* and *OsAPL3* showed significant homology with wheat LS (Akihiro et al. 2005).

In wheat and barley, there are two transcripts for LS (LS1 and LS2). In wheat, LS1 gene is called *AGPL1* and LS2 gene is called *AGPL2* (Johnson et al. 2003; Kang et al. 2010). Similarly, in barley, LS1 gene is called *BEPL* (barley endosperm pyrophosphorylase large subunit), and LS2 gene is called *BLPL* (barley leaf pyrophosphorylase large subunit). Both in wheat and barley, LS1 codes for cytosolic isoform and LS2 codes for leaf isoform (Johnson et al. 2003; Burton et al. 2002; Doan et al. 1999).

Cloning, characterisation and expression of AGPase genes and their promoters

Genes encoding AGPase have also been cloned and characterised from a number of plant species including wheat (Table 2). The genes in the form of cDNAs for LS (*Sh2*) and SS (*Bt2*) were cloned for the first time from maize

(Bhave et al. 1990; Bae et al. 1990). For isolation of *Sh2*, 1.3 kb clone isolated earlier from maize endosperm (21 DPA) was used. The identity of *Sh2* cDNA was confirmed through RFLP using a series of maize stocks that differed only at *Sh2* locus. Further confirmation was also achieved through RNA gel blot assay (Bhave et al. 1990). Similarly, *Bt2* clones were also isolated and their identity was conformed through northern blot analysis of *Bt2* mutants which altered the size of transcripts but not necessarily the steady-state level of transcripts (Bae et al. 1990). In barley (*blpI14*) and wheat (*Agp2*), cDNAs for large subunit of AGPase (which corresponded to *shrunken 2* in maize) were identified and cloned (Eimert et al. 1997; Ainsworth et al. 1995). This was achieved through Northern blot screening of the cDNA library prepared from mRNA isolated from leaves in case of barley and from developing grains in case of wheat. A full-length genomic clone encoding large subunit of AGPase (*Agp2*) was also isolated and characterised from genomic library of etiolated leaves of hexaploid wheat, which was mapped to chromosome 1B using nullisomic-tetrasomic (NT) lines. The characterisation was done by screening the λ Gem12 genomic library with known radiolabelled *Agp2* cDNA clone (Ainsworth et al. 1995). Northern blotting and overexpression of the genes due to a promoter in transgenic wheat plants were also examined. Thus the large promoter region of *Agp2* gene encoding an endosperm specific plastidial AGPase was isolated. Its subsequent analysis through overexpression showed that the gene is differentially expressed in wheat (Thorneycroft et al. 2003). Similarly, 0.9 kb 5′flanking sequence that represented the maize *Bt2* promoter was cloned and its role in expression was studied via GUS assay in transgenic tobacco. *Bt*2P promoter involved in seed-specific expression and cis-regulatory elements for tissue-specific expression were also identified within the same 0.9-kb promoter region (Chen et al. 2007).

Relative roles of genes for two subunits of AGPase in starch biosynthesis and grain yield (overexpression of specific genes using transgenics)

In maize and rice, transgenics have been produced, which exhibit a tissue-specific overexpression (endosperm versus leaf) of genes encoding one or both AGPase subunits (either LS or SS or both). These transgenics in maize suggested that the relative roles of two subunits of AGPase (LS and SS) in starch biosynthesis, grain yield, biomass production and plant growth depend upon the location of AGPase [either cytosol (endosperm) or plastids (amyloplasts or leaf)]. In majority of studies, it is the cytosolic endosperm LS, which is targeted for overexpression, the results suggesting its importance in grain yield improvement in cereals (Sakulsingharoj et al. 2004; Wang et al. 2007; Kang et al. 2013; Hannah et al. 2012; Li et al. 2011). Similarly, in rice, transgenics with overexpression of leaf AGPase SS exhibited increment in plant growth in the form of higher biomass providing an evidence for significance of leaf AGPase activity in biomass production (Li et al. 2011; Schlossar et al. 2014). However, the precise nature of the metabolic and developmental changes that result due to genetic engineering of AGPase remains to be elucidated (Stark et al. 1992; Greene and Hannah 1998a; Smidansky et al. 2002, 2003, 2007; Hannah et al. 2012).

Intracellular location of AGPase also affects its activity and consequently its role in starch synthesis. AGPase activity is perhaps also regulated by intronic regulatory sequences, as shown in barley through a study of mutants, which had sequence alterations in the domain regions of the gene responsible for transport of ADP-Glucose (ADP-Glc), thus influencing AGPase activity. Transport of ADP-Glc across the plastid envelope also influences the role of AGPase in starch deposition. A summary of the results of studies examining the role of AGPase subunits in improving grain yield along with relevant references is presented in Table 4.

Spatial and temporal expression of AGPase genes

The genes encoding the SS and the LS of AGPase are differentially expressed not only in different plant organs, but also in the same organ at different stages of development. This means that the subunit composition of AGPase may vary in different parts of the same plant (there being more than one types of SS and LS), as shown in potato (La Cognata et al. 1995), rice (Nakamura and Kawaguchi 1992) and barley (Villand et al. 1992). For instance, in rice, the genes *OsAPS1a* and *OsAPL1* for leaf plastidial AGPase are expressed not only in leaves, but also in the early stages of endosperm/embryo development, while *OsAGPS2b* and *OsAGPL2* for cytosolic AGPase are expressed in the later stages of embryo/endosperm development (after 5 days of flowering) (Ohdan et al. 2005) (Table 1). In wheat as well as in barley, out of the two transcripts of SS (*AGPS1a* and *AGPS1b*), *AGPS1a* for cytosolic AGPase is expressed in the late stages of endosperm (rapid starch synthesis), while *AGPS1b* coding for putative plastidial AGPase is expressed in young endosperms, leaves and to a lesser extent in old stems. In case of LS, the gene *AGPL1* for cytosolic AGPase is expressed abundantly in the developing endosperm, but to a lesser extent in leaves and in roots. In contrast, *AGPL2* coding for plastidial leaf AGPase is expressed abundantly in leaves, but to a lesser extent in endosperms and embryos (Burton et al. 2002).

From the above results, it is apparent that although most of the genes coding for cytosolic AGPase are expressed in

Table 4 A summary of the results of studies involving transgenics and mutants for the study of the role of AGPase in improving grain yield in cereals

Crop and variety	Gene	Tissue	Results	References
Wheat	*TaLSU1*	Endosperm	Enhanced starch synthesis in grains	Kang et al. (2013)
	Sh2r6hs	Endosperm	38 % increase in seed no/plant; 31 % increase in total plant biomass	Smidansky et al. (2002)
	Sh2r6hs	Endosperm	Significant yield increase in space planted irrigated, relative to densely planted rainfed environments	Meyer et al. (2007)
Maize	*E. coli* mutant *glgC 16*	Endosperm	13–25 % increase in seed wt	Wang et al. (2007)
	Sh2r6hs	Endosperm	64 % increase in seed wt due to function of transgene in maternal tissue instead of endosperm	Hannah et al. (2012)
	Sh2hs33	Endosperm	Enhanced heat stability due to stable SS–LS interaction	Greene and Hannah (1998a)
	Sh2 and *Bt2*	Endosperm	Higher grain wt due to both overexpressed transgenes (Sh2 and Bt2) relative to single overexpressed gene (either Sh2 or Bt2)	Li et al. (2011)
Rice	*E. coli* mutant *glgC* 16	Endosperm	Increase in seed wt due to cytoplasmic AGPase relative to amyloplast AGPase	Sakulsingharoj et al. (2004)
Rice (Nipponbare cultiver)	*Sh2r6hs and Bt2*	Leaf	*Sh2r6hs* and *Bt2* transcript levels 20X higher; starch accumulation with increased AGPase activity; 29 % increase in plant biomass	Schlosser et al. (2014)
Rice (Tos17 insertion lines)	*Apl3*[a]	Embryo and endosperm	Reduced embryo starch content; no significant reduction in grain wt; starch reduction in culms	Cook (2011)
Barley (3 Lys5 mutants)	*Hv.Nst1*[a]	Endosperm	Increased levels of ADP-Glc; decreased starch content; reduced grain wt	Patron et al. (2004)

[a] Indicates studies invovling mutants. All others indicate studies involving transgenics/overexpression in grain yield

developing endosperm in cereals, they are not solely confined to endosperm. They also contribute for starch synthesis in leaves and other organs like root, though to a lesser extent. The plasitidial AGPase contributes to starch synthesis in younger endosperm as well as in leaf. However, the relative contributions of cytosolic and plastidial SS and LS in starch synthesis in cytosol and plastid need further examination. It would also be interesting to examine the relationship between the structure of different SS and LS subunits, their spatial and temporal expression and also the regulatory system, which controls this temporal and spatial expression.

Mutants for AGPase

In maize, site-specific mutagenesis was used to create short insertion mutations (e.g., insertion of DS element) in a region of the AGPase LS gene that is known to be responsible for its allosteric regulation. It was interesting to note that about one-third of the mutants gave revertants to produce wild type, but some of them had insertions of 3 or 6 bp involving codons, which coded for amino acids tyrosine or serine or both. Also, some of the mutants having an insertion showed 8–11 % increase in seed weight relative to wild-type revertants, which exhibited reduction in seed weight and AGPase activity. Reduced sensitivity to phosphate (AGPase inhibitor) was also observed in some of the mutants (Giroux et al. 1996).

Null mutations in genes encoding cytosolic AGPase were reported in maize, barley and rice. These mutants caused 20–70 % reduction in total endosperm starch content and 12–25 % reduction in AGPase activity (Tester et al. 1993; Johnson et al. 2003; Rosti et al. 2006; Lee et al. 2007; Dickinson and Preiss 1969; Johnson et al. 2003). However, mutations that affect plastidial AGPase in endosperm have not been described, and we still do not know if plastidial AGPase contributes to starch accumulation in the

Table 5 List of mutants for AGPase activity reported in maize, rice and potato

Crop and mutation	Function	References
I. Potato		
1. LS_{P52L}	Reduces the efficiency of 3-PGA by 45 fold	Green et al. 1998
2. LS_{P66L} 3. LS_{E38K} 4. LS_{G101N} 5. LS_{P66L}	Higher affinity for activator 3-PGA and increased resistance to inhibitor orthophosphate and also partially sensitive to heat	Green et al. 1998
6. SS_{L46F} 7. SS_{P112L} 8. SS_{R356K} 9. SS_{P308L}	Affect the allosteric regulatory properties of the enzyme when interact with LS	Kim et al. 2007
10. SS_{C12A}	Less enzyme activity and stability under high temperature conditions since disulphide bonds were removed	Kim et al. 2007
II. Maize		
1.LS_{T341D}	Enzyme activity lowered by 2.7 and 6 fold	Boehlein et al. 2014
2.LS_{G261H}	Enzyme activity lowered by 9.5 and 4 fold	Boehlein et al. 2014
3.LS_{T341R} 4.LS_{V416E}	Reduced efficiency towards 3-PGA (5-10 fold)	Boehlein et al. 2014
5.LS_{T142F}	30% activity even in the absence of 3-PGA	Giroux et al. 1996
6. *$LS_{YS494,495}$	11-18% increase in seed weigth	Giroux et al. 1996
III. Rice		
1. *Tos 17 insertion	Reduction in leaf starch content	Rosti et al. 2007
IV. Barley		
1. LS_{P228S} 2. LS_{V273E}	Reduced capacity for ADP	Patron et al. 2004

LS large subunit, SS small subunit
*Indicates the insertions whereas all the others are substitutions

endosperm. Some of the mutants caused due to amino acid substitutions are listed in Table 5.

Evolutionary relationships within and between SS and LS

Gene sequences for SS and LS from monocots and dicots have also been examined for evolutionary relationships. The LS and SS of AGPase in higher plants are closely related not only to each other, but also to those found in prokaryotes. Therefore, the AGPase subunits in higher plants are believed to have evolved from a common prokaryotic structure, through duplication and divergence. The sequences for SS genes from monocots are more closely related to each other than to the sequences of SS genes from dicots, suggesting that duplication of the small subunit gene occurred after the separation of monocots and dicots (Hannah et al. 2001; Johnson et al. 2003; Patron and Keeling 2005). The gene sequences for LS, however, form three distinct groups of plants, each with both monocot

and dicot members, suggest that the LS genes duplicated prior to the dicot–monocot split and that after this split, in some lineages, there were additional duplications (Patron and Keeling 2005). The level of diversity in the nucleotide sequences of genes encoding the subunits of AGPase is, however, low within the germplasm of an individual species (Tuncel et al. 2008). For instance, in a study involving 50 accessions of maize and teosinte, very few nucleotide polymorphisms were observed in *Sh2* gene (for LS) encoding AGPase, as compared to other maize genes indicating lack of its involvement in maize domestication (Maniacci et al. 2007). Similar results were also reported in rice *AGPL2* (AGPase LS) gene, suggesting strong selective constraints in these genes (Yu et al. 2011). It has also been observed that the overall rate of non-synonymous mutations for LS is 2.7-fold higher relative to that for the SS.

QTL analysis and association mapping for genetic dissection

Although major genes encoding AGPases are known in all major cereals (as discussed above), AGPase activity itself is basically a quantitative trait and may also be controlled by genomic regions other than the AGPase genes themselves. Therefore, QTL analysis and association mapping have been conducted for AGPase activity in different cereals (like maize and rice). These studies provide further insight into the genetics of AGPases, but studies involving genetical genomics and identification of eQTLs (expression QTLs) for AGPase activity have never been undertaken and would be rewarding, if undertaken in future.

QTL analysis

QTL mapping for activities of different enzymes involved in starch biosynthesis (including AGPase activity) has been attempted to identify QTLs involved in different steps of starch biosynthesis in cereals, particularly in maize (Prioul et al. 1999; Zhang et al. 2010) and rice (Umemoto et al. 2008). These studies involved initial screening of the available germplasm for enzyme activities and thereby cataloguing the germplasm for variability in enzyme activity. The genotypes having contrasting enzyme activities could be selected and used for developing mapping populations like RILs/DH/F_2 to be utilised for QTL mapping (Fig. 3).

In maize, QTL analysis was carried out not only for the activity for three important enzymes associated with starch biosynthesis (soluble invertase, sucrose synthase and AGPase), but also for the concentrations of carbohydrates including sucrose, fructose and glucose (Thevenot et al. 2005). Since the activity of the enzymes varied at different stages of grain filling, enzyme activity in 100 RILs was recorded at three different stages, i.e., 15, 25 and 35 days after pollination (DAP). Several QTLs each explaining up to 56 % of the phenotypic variability (PVE) were detected. The QTLs for either carbohydrate concentration or enzyme activities or both were found clustered. However, the QTL clusters for different stages differed and were located on different chromosomes. At 25 and 35 DAP, AGPase QTLs were found on chromosome 8 and chromosome 4, respectively, and were closely linked to *Bt2* loci, which encode AGPase SS. The candidate genes encoding vacuolar invertase (*Ivr1, Ivr2*), sucrose synthase (*Sus1, Sh1*) and AGPase (*Sh2, Bt2*) were found to be co-located with the identified QTLs that were spread over all the 10 chromosomes in maize. For instance, seven AGPase QTLs were found to be coincident with various candidate genes for AGPase and other enzymes mentioned above.

In addition to *Bt2* loci, *SPS* (sucrose phosphate synthase) genes were also found to be co-localised with AGPase QTL on chromosome 8. One of the possible explanations for colocalization of *Bt2* and *SPS* genes was also available from a study conducted in potato. In this study, the expression of AGPase B gene in potato which is homologous to *Bt2* gene for maize AGPase SS was inhibited using antisense technology. Significant reduction in the starch content of potato tubers was observed, but this was accompanied by large accumulation of soluble sugars (mainly sucrose) due to an increase in the mRNA transcripts for *SPS*, which is a major sucrose synthesising enzyme (Muller-Rober et al. 1992). This also indicated the possibility of a regulatory relationship between *BT2* and *SPS* so that the effect on the expression of *BT2* was reflected on the expression of *SPS*. It is known that *Bt2* is located on chromosome 4 (Teas and Teas 1953), but in the above reported study, it was found to colocalize with AGPase QTL on chromosome 8. It is possible that the *Bt2* locus on chromosome 8 may be a regulatory gene for AGPase activity and that on chromosome 4 is the structural *Bt2* gene, although this needs to be confirmed.

Although *Sh2* was not colocalized with AGPase QTLs in the above study, in a separate study, a QTL with large additive effects on protein and starch components was detected on chromosome 3L, the RFLP alleles of which were found to be linked to RFLP alleles at the *Sh2* locus (Goldman et al. 1993). In another report, a QTL linked to amylose content was found to be colocalized with *Sh2* locus on chromosome 3 (Sene et al. 2000). These reports indicate a significant linkage of QTLs for starch content to that of AGPase gene (*Sh2*) in maize. The implications of clustering of QTLs with candidate genes for AGPase in maize are not fully understood.

In barley, QTLs for β-amylase enzyme activity and its thermostability were reported using two different DH populations (Kaneko et al. 2001), although no such study for QTLs associated with AGPase activity and its thermostability is available concerning other cereals.

Association mapping

Association mapping for AGPase genes using candidate gene approach has been conducted in rice. SNPs and in-dels showing significant association with either grain quality traits or grain weight were identified. An in-del polymorphism was identified in *OsAGPL4* and an SNP was detected in *OsAGPL2*, both AGPase LS genes (Bao et al. 2012). In another association mapping study in rice, sequence variation was observed in AGPase LS and SS genes, which showed significant marker-trait associations with rice grain quality traits. For instance, one 12-bp in-del in the exonic region of *OsAGPL4*, another 8-bp indel in an intron of *OsAGPL4* and one SNP (A/G) transition in *OsAGPS1* gene were identified. These markers showed significant associations with different grain quality traits, when GLM and/or MLM models were used for association mapping (Lu and Park 2012). These results will facilitate the development of breeding strategies for marker assisted selection (see later for details).

Genotypic variability for AGPase and its use in breeding cereals

Thermostable variants for heat tolerance

As described earlier, a number of independent genes for AGPase SS and LS are known in each individual plant species. Allelic variation for one or more of these AGPase genes occurs in cereals including wheat, rice and maize and is associated with variation in seed starch content under normal as well as under heat stress conditions. Thermostable variants for some of these genes have also been reported. One of the most important studies for the identification and use of AGPase variants for imparting heat tolerance has been in progress under a National Science Foundation (NSF) project at the University of Florida, under the leadership of L. C. Hannah, a plant molecular biologist. It was shown by this group that AGPases are typically downregulated by heat stress, resulting in drastic reduction in grain yield. It was also shown that a loss of leaf AGPase SS is associated with reduced grain yield, when corn was grown in hot, stressful environments (www.reeis.usda.gov/web/crisprojectpages/224539.htm). Similarly, some of the gene variants for maize endosperm AGPase provided up to 64 % increase in yield depending on temperatures during early seed development (Hannah et al. 2012). Several heat-stable variants of AGPase were also identified in maize (Greene and Hannah 1998a; Georgelis et al. 2009a; Boehlein et al. 2015), which when overexpressed gave improved yield not only in maize, but also in wheat (Smidansky et al. 2002) and rice (Smidansky et al. 2003). In case of maize, increased yield was also observed when a thermostable variant was overexpressed under heat stress conditions, but in other cereals, yield improvement under heat stress was not examined. Some of these gene variants were also isolated using different approaches and used for further studies (Greene and Hannah 1998a; Georgelis et al. 2009b; Boehlein et al. 2015; for some details, see next section).

Production of thermostable variants using chemical mutagenesis

Chemical mutagenesis was used to isolate thermostable variants of AGPase LS in maize. Following are some examples: (1) Greene and Hannah (1998a) identified a heat-stable (*hs*) mutant *Sh2hs33*, which exhibited enhanced interactions between the two subunits of AGPase (i.e., *Sh2* and *Bt2*), leading to heat stability. Sequencing of *Sh2hs33* mutant alongwith several other mutants identified a mutation involving *his*-to-*tyr* substitution at amino acid position 333. Several other thermo-sensitive mutants that were identified, when combined with *Sh2hs33,* yielded almost threefold increase in enzyme activity under heat stress (Burger 2001). (2) Greene and Hannah (1998a) reported in maize another thermostable variant, *Sh2r6hs*, which contains two changes: the first, called *rev6*, involves a 6-bp insertion resulting in the addition of tyrosine and serine at amino acid positions 495 and 496 of the LS; this change renders AGPase less sensitive to Pi inhibitor; the other mutation, *hs33* was a point mutation causing *his*-to *tyr* substitution at amino acid position 333 (as in *Sh2hs33*); this change confers more stable LS–SS interactions (Giroux et al. 1996). Here, *rev6* was initially identified in maize using site-specific mutation caused due to transposable *Ds* elements. Hannah et al. (2012) also used *Sh2r6hs* and reported 64 % increase in yield under heat stress, i.e., when the temperature during the first 4 days after pollination was above 33 °C. A successful attempt was also made to test the overexpression of *Sh2r6hs* in transgenic rice, which resulted in 38 % increase in seed weight along with 31 % increase in total plant biomass. (3) Meyer et al. (2007) reported a *Sh2* variant in maize, which gave 42 % yield advantage in commercial plots. Some of the above AGPase thermotolerant variants will certainly be used in future not only in maize but also in other cereals (Giroux et al. 1996; Burger 2001; Meyer et al. 2004; Fig. 3).

Amino acid substitutions for heat tolerance through site-directed mutagenesis (SDM)

Individual amino acids having a possible role in providing thermotolerance were first identified, before site-directed mutagenesis could be used to generate thermotolerant variants of AGPase. Georgelis et al. (2009a) identified 29 key

amino acids, and Boehlein et al. (2015) identified 36 amino acids, which may be altered to get the desirable thermostable variants of AGPase. Site-directed mutagenesis (SDM) was used to change each of these amino acids. Later, each such variant was expressed in *E. coli* and changes in heat stability, allostery and kinetic parameters were recorded. Georgelis et al. (2009a) found that 6 out of the 9 amino acid residues located in SS and LS (*Sh2Bt2*) subunit interface exhibited altered allosteric properties indicating the importance of these interfaces in functional specialisation of AGPase. As many as 14 amino acid residues of the maize endosperm AGPase LS (*Sh2*) could also be altered and each altered LS was expressed with SS (*Bt2*). Only 11 amino acids were further explored for a study of allosteric properties. Out of these genetic variants, one variant, i.e., *BT2/S163F* (serine to phenylalanine substitution at position 163) was identified which showed increased heat stability. Similarly, when cysteine was replaced by valine at position 424, increased enzymatic activity was observed.

The effects of alterations in specific key amino acids (as above) leading to changes in enzyme kinetics and thermostability were also reported in a recent study (Boehlein et al. 2014). Several variants were identified which had fairly good enzyme activity and increased $T_{1/2}$ at 55 °C. Changes at three positions (160, 425 and 444) also conferred enhanced k_{cat} values. One mutant, *T142F,* had higher activity, lower 3-PGA K_a and elevated activity at 55 °C. All these variants were combined and pyramided into a single gene (*Sh2*), which was named *Sh2-E*. Seven important sites which were identified in this study included N131, T142, A160, Q261, A396, V416 and S444. *T142F* was found to be the most critical thermostable variant as evident from the fact that removal of this variation from *Sh2* resulted in the loss of 90 % enzyme activity and reduced 3-PGA activation at 55 °C.

Another heat-stable variant of AGPase that was identified in maize was *Bt2-T1*. This mutant involved a single amino acid change (threonine to isoleucine) at position 462 and was described as T1. This T1 mutation, when combined with another heat-stable SS variant, called MP (Mutant Polypeptide), exhibited enhancement in heat stability of MP, as shown through expression in *E. coli* (Georgelis and Hannah 2008). MP is a mutant polypeptide consisting of first 200 amino acids of *BT2* and last 275 amino acids of potato SS AGPase (mosaic of maize/potato SS) (Cross et al. 2004). Its further characterisation suggested the possibility of using it for increased starch yield in the endosperm under heat stress.

Alterations in sites for protein folding through iterative site saturation mutagenesis (ISM)

It is widely known that conformationally mobile residues are sites of protein unfolding and that elimination of these motions should make the protein less susceptible to heat denaturation. Keeping this in mind, in a recent study, 18 conformational mobile residues (9 in *Bt2* and 9 in *Sh2*) were first identified using atomic displacement parameters (B-factors) in protein X-ray crystal structures (Boehlein et al. 2015). Among these 18 residues, 9 residues corresponding to LS (*Sh2*) in maize were further explored for their allosteric properties and heat stability. Iterative saturation mutagenesis (ISM) was implemented for the nine selective amino acid residues, and the amino acid positions that were responsible for heat stability were identified. At the end of the first round of SIM, two variants, i.e., Q96R and Q96G were selected as anchors for further rounds of mutagenesis. Two other variants, namely D161G and D161Q exhibited increased AGPase activity at 55 °C. The double mutants (Q96G/D161G and Q96R/D161G) were eventually selected as promising anchors for further studies.

During the second round of mutagenesis, Q96R/D161G was used as a starting template plasmid and each of the remaining target amino acids was replaced. An amino acid substitution (A443R) was found to be present in 12 independent colonies and a triple mutant (Q96R/D161G/A443R) was selected for further protein engineering and enzyme kinetic studies. The two mutants, i.e., the single mutant D161G and the triple mutant Q96R/D161G/A443R exhibited stability at 55 °C and increased catalytic efficiency at 37 °C, retaining significant activity even in the absence of 3-PGA.

Disulphide bridge in AGPase SS imparts thermotolerance

In potato, the *Cys* residue at the N-terminal position of the SS and its involvement in the formation of disulphide bridge imparting thermotolerance are also widely known. A reduction in the frequency of these disulphide bridges has been shown to result in lack of thermostability of AGPase (Ballicora et al. 1995). Since this di-sulphide bridge is generally absent in cereals, an introduction of this disulphide bridge was shown to enhance thermostability of AGPase in maize endosperm (Linebarger et al. 2005).

Mechanism involved in increased yield due to thermostable gene variants under heat stress

It has been mentioned in previous sections that the overexpression of thermostable AGPase gene variants leads to increase in grain yield in maize. Similar results are likely to be obtained in other cereals also, when tests are conducted. The underlying mechanism for increased yield under heat stress due to thermostable gene variants is still not fully understood. Following are the two possible suggested mechanisms.

Seed number versus seed size

It has been shown that the plants giving higher yields due to AGPase variants produce more seeds, rather than larger seeds packed with more starch (Smidansky et al. 2002; Hannah et al. 2012). Data from the experiments conducted by L.C. Hannah and his group at Florida show that under normal conditions, only half of the ovaries on maize ears eventually become viable kernels (for references see earlier in this review). Therefore, the gene variants are simply blocking some mechanism that would normally lead to abortion of some developing seeds before they mature (http://www.livescience.com/3527-heat-tolerant-corn-prevent-future-starvation.html) One of the possible suggested reasons for increase in seed number may be the stimulation of the developmental activity of the apical meristem due to elevated starch accumulation, which results in increase in the number of axillary meristems that eventually form the reproductive organs and florets (Tuncel and Okita 2013).

Maternal effect (plant genotype versus seed genotype)

It has also been observed that the effect of AGPase gene variants is stronger, when these are derived maternally, but not when they are introduced via pollination. It has also been shown that sometimes all kernels on the plants with higher yield do not carry the desired gene variant (plant being heterozygote) so that the reason for the increase lies somewhere within the plant's mechanism for channelizing resources to the seeds, rather than in the seeds themselves (maternal effect) (Hannah et al. 2012).

Opportunities for breeding using AGPase variants

As described earlier, AGPase is an important enzyme for starch synthesis in cereal endosperms and undergoes drastic reduction in its activity under heat stress. Therefore, efforts have been made to identify and isolate genes for several thermostable AGPase variants, which provided increased yield under heat stress on over-expression in transgenic plants (Hannah et al. 2012). Using available gene sequences, markers (e.g., SSR, SNP, STS markers) showing significant association with genes for starch synthesis and also with grain quality and grain weight have been developed in cereals. For instance, in rice, markers developed from AGPase gene sequences show significant association with grain quality and grain weight (see below). However, no effort has been made so far to utilise these thermostable AGPase variants through marker-assisted selection (MAS) with an aim to develop heat-tolerant genotypes with enhanced yield. This certainly needs to be attempted so that both approaches including transgenics and MAS utilising thermostable variants of starch synthesis enzymes including AGPase may be used for developing heat tolerant genotypes in cereals.

Development of gene markers and their possible use in MAS

Markers associated with QTLs for activities of several enzymes involved in starch synthesis are now available. Similarly, perfect markers are available for a number of genes for AGPases and other known genes involved in starch synthesis (Tables 6, 7). None of these markers has

Table 6 List of reported QTLs linked to AGPase genes in maize

QTL type/name	Flanking markers[a]	Chromosome and location (cM)
MQTL/AGPase (15 DAP)	gsy54b_RPL7; Umc6	2 (2.0)
MQTL/AGPase (15 DAP)	gsy168_GTPB; gsy154_MDH5	5 (56.2)
MQTL/AGPase (15 DAP)	umc30; gsy224b_SPS	8 (125.7)
MQTL/AGPase (15 DAP)	gsy66_SH1; gsy89	9 (10.8)
MQTL/AGPase (15 DAP)	bnl510 and umc114	9 (52.8)
IQTL/AGPase (25 DAP)	gsy172b_EFLA; gsy404_UBIE2	8 (71.0) and 8 (121.0)
IQTL/AGPase (25 DAP)	gsy224b_SPS and umc36a	8 (121.0) and 8 (71.0)
IQTL/AGPase (25 DAP)	gsy147b_BSPT and gsy111a_TRF	8 (193) and 3 (93)
IQTL/AGPase (35 DAP)	gsy59c_BT2 and gsy156_csu84	4 (104) and 5 (80)
IQTL/AGPase (35 DAP)	gsy154_MDH5 and umc43	5 (80) and 4 (104)
Starch[b]	P10080 and Umc63	3
AM94a (amylose)[b]	gsy58_sh2 and gsy38_Al	3

MQTL main effect QTL, *IQTL* interaction QTL, *DAP* days after pollination

[a] Markers associated with all QTLs are RFLP markers, the details of which are reported in Causse et al. (1996)

[b] QTLs for starch and AM94a were reported by Goldman et al. (1993) and Sene et al. (2000) respectively, all other QTLs were reported by Thevenot et al. (2005)

Table 7 List of reported markers for AGPase and other starch synthesising genes in rice

Markers	Gene	Associated trait	References
In-Del (AGGAC at 505 position)	*OsAGPL4*	Grain weight	Bao et al. 2012
SNP(T/C at position 94)	*OsAGPL2*		
SNP (A/G)	*OsAGPS1*	Amylose content	Lu et al. 2012
Del (TCCGCCGCCGCC at 106-117th position)	*OsAGPL4*	Decreased breakdown viscosity	Bao et al. 2006
SSRs (CT)n	*Wx*	Different starch physicochemical properties	Bao et al. 2006
SNP (T/G)	*Wx*		
SSRs	*SBE1*		
SSRs	*SS1*		
STS	*SBE1*		
SNP (G/C)	*SBE3*		
SNPs and In-Del	*OsAGPS2*	Grain filling duration and AGPase activity	Kato et al. 2010

been utilised so far for marker assisted selection. Also, several markers like SSRs and STS (Table 7) have been identified for SSRGs in rice, but still such markers are not reported for sequences coding for AGPase subunit genes in cereals. Thus, there is ample scope to identify these markers from the genes coding for AGPase in cereals.

The markers either linked or lying within the AGPase genes can be used for MAS for transferring the AGPase variants to cereals leading to increased grain weight and improved grain quality. Marker-assisted backcrossing (MABC) as well as marker-assisted recurrent selection (MARS) could be implemented for the transfer of one or more than one of these genes (Fig. 3). In maize, it was found that when both *Sh2* and *Bt2* genes are combined, AGPase activity and yield improvement was better relative to the condition when only a single gene (either *Sh2* or *Bt2*) was used and overexpressed through endosperm specific promoters (Li et al. 2011).

Transgenic for improvement of yield and its components

The transgenes introduced in cereals like wheat (Smidansky et al. 2002, 2007), rice (Sakulsingharoj et al. 2004) and maize (Greene and Hannah 1998a; Wang et al. 2007; Hannah et al. 2012) included an altered AGPase LS with decreased sensitivity to allosteric inhibition by Pi and increased heat tolerance and higher AGPase activity. In these transgenic plants, significant increase was observed either in the yield or in the total biomass; (1) yield was measured as seed weight or seed number per plant, as also done in case of maize, where increase in seed number was observed in transgenics carrying *Sh2r6hs* (Hannah et al. 2012; Smidansky et al. 2002, 2007; Meyer et al. 2007), (2) increase in total plant biomass Li et al. (2011) reported yield enhancement in the form of 15 % increase in 100 grain weight and 74 % increase in starch biosynthesis as compared to wild type, when the unmodified (non-heat tolerant) versions of AGPase, i.e. *Sh2* and *Bt2* were overexpressed in the elite maize inbreds. Endosperm-specific promoters cloned from maize genes encoding 27-kD zein and 22-kD zein from inbred line DH4866 were used for this purpose. Gene variants imparting heat tolerance have perhaps been never used for the production of transgenic crops, but in future these variants will certainly be used.

Conclusion and perspectives

Grain yield and grain quality are important traits in cereals so that reduced yield and reduced grain quality in cereals, particularly under heat stress, is a major concern worldwide. In this connection, the enzyme AGPase has attracted the attention of cereal breeders, because AGPase is an important enzyme and has been shown to be the rate-limiting enzyme in starch biosynthesis. Another important enzyme for starch synthesis pathway is SS, which has not been discussed in this review. In several studies, AGPase activity has been shown to be affected by heat stress and thermostable variants have been shown to be available. Several factors, which influence the activity of AGPase include its structure, intracellular location and concentrations of major effectors 3-PGA and Pi along with that of other effectors like sugar. Complete crystal structure of AGPase heterotetrameric enzyme using X-ray crystallography in any species is still lacking due to which structure–function relationship of this enzyme is not fully understood. 3-D structure of AGPase has been studied in important cereals like wheat and rice using in silico approaches that were facilitated due to the availability of crystal structure of SS in potato, which was used as a template in these studies. Nevertheless, further efforts are needed to deduce the complete crystal structure of this enzyme in cereals.

Although the role of AGPase and its thermostable variants has been investigated in maize, there is very little information available for other cereals like rice and wheat. The transgenics were developed in maize and other cereals, where AGPase and its variants were overexpressed showing enhanced AGPase activity and grain yield; however, these were not tested for yield improvement under high temperature in cereals except in maize. Marker Assisted Selection (MAS) is yet to be tried for transfer of desirable thermostable variants (alleles) of genes coding for AGPase subunits.

Thus looking at the importance of AGPase in cereal productivity and the limited literature available describing its role and importance under heat stress conditions, there is an immediate need to explore this enzyme for its use in breeding for heat tolerance in cereals. A standard nomenclature for the genes coding SS and LS in cereals also needs to be implemented, since different names have been used in different studies.

Author contribution statement PKG conceived, outlined, edited and wrote a part of the review. SG wrote the first draft of the manuscript and assisted PKG in finalising and revising the manuscript.

Acknowledgments National Academy of Science India (NASI) and Indian National Science Academy (INSA), New Delhi, India, awarded Senior Scientist Fellowships to PKG, during the tenures of which this review was written. SG is thankful to Department of Biotechnology, Government of India, New Delhi, for the award of a Senior Research Fellowship. Authors are also thankful to Professor L.C.Hannah, University of Florida, for his comments and suggestions which helped in improving the manuscript.

Conflict of interest No conflict of interest declared.

References

Ahmadi A, Baker DA (2001) The effect of water stress on the activities of key regulatory enzymes of the sucrose to starch pathway in wheat. Plant Growth Regul 35:81–91

Ahmed N, Maekawa M, Tetlow IJ (2008) Effects of low temperature on grain filling, amylose content, and activity of starch biosynthesis enzymes in endosperm of basmati rice. Aust J Agric Res 59:599–604

Ainsworth C, Tarvis M, Clark J (1993) Isolation and analysis of cDNA clone encoding small subunit of Adp-glucose pyrophosphorylase from wheat. Plant Mol Biol 23:23–33

Ainsworth C, Hosein F, Tarvis M, Weir F, Burrell MM, Devos KM, Gale MD (1995) Adenosine diphosphate glucose pyrophosphorylase genes in wheat: differential expression and gene mapping. Planta 197:1–10

Akihiro T, Mizuno K, Fujimura T (2005) Gene expression of ADP-glucose pyrophosphorylase and starch contents in rice cultured cells are cooperatively regulated by sucrose and ABA. Plant Cell Physiol 46:937–946

Anderson JM, Hnllo J, Raymond L, Oklta TW, Morell M, Preiss J (1989) The encoded primary sequence of a rice seed ADP-glucose pyrophosphorylase subunit and its homology to the bacterial enzyme. J Biol Chem 264:12238–12242

Bae JM, Giroux M, Hannah LC (1990) Cloning and characterization of the *brittle-2* gene of maize. Maydica 35:317–322

Ball S, Marianne T, Dirick L, Fresnoy M, Delrue B, Decq A (1991) A *Chlamydomonas reinhardtii* low-starch mutant is defective for 3-phosphoglycerate activation and orthophosphate inhibition of ADP-glucose pyrophosphorylase. Planta 185:17–26

Ballicora MA, Laughlin MJ, Fu Y, Okita TW, Barry GF, Preiss J (1995) Adenosine 5′-diphosphate-glucose pyrophosphorylase from potato tuber. Significance of the N-terminus of the small subunit for catalytic properties and heat stability. Plant Physiol 109:245–251

Ballicora MA, Iglesias AA, Preiss J (2004) ADP-glucose pyrophosphorylase: a regulatory enzyme for plant starch synthesis. Photosynth Res 79:1–24

Bansal K, Munjal R, Madan S, Arora V (2012) Influence of high temperature stress on starch metabolism in two durum wheat varieties differing in heat tolerance. J Wheat Res 4(1):43–48

Bao JS, Corke H, Sun M (2006) Microsatellites, single nucleotide polymorphisms and a sequence tagged site in starch-synthesizing genes in relation to starch physicochemical properties in nonwaxy rice (*Oryza sativa* L.). Theor Appl Genet 113:1185–1196

Bao J, Lu Y, Yang F, Zhang G, Shao Y, Corke H, Sun M (2012) Nucleotide polymorphisms in OsAGP genes and their possible association with grain weight in rice. J Cereal Sci 55:312–317

Beckles DM, Craig J, Smith AM (2001a) ADP-glucose pyrophosphorylase is located in the plastid in developing tomato fruit. Plant Physiol 126(1):261–266

Beckles DM, Smith AM, Rees T (2001b) A cytosolic ADP-glucose pyrophosphorylase is a feature of graminaceous endosperms, but not of other starch-storing organs. Plant Physiol 125(2):818–827

Bhave MR, Lawrence S, Barton C, Hannah LC (1990) Identification and molecular characterization of shrunken-2 cDNA clones of maize. Plant Cell 2:581–588

Boehlein SK, Shaw JR, Stewart JD, Hannah C (2008) Heat stability and allosteric properties of the maize endosperm ADP-glucose pyrophosphorylase are intimately intertwined. Plant Physiol 146:289–299

Boehlein SK, Shaw JR, Stewart JD, Hannah CL (2010) Studies of the kinetic mechanism of maize endosperm Adp-glucose pyrophosphorylase uncovered complex regulatory properties. Plant Physiol 152:1056–1064

Boehlein SK, Shaw JR, Georgelis N, Sullivan B, Hannah LC (2014) Enhancing the heat stability and kinetic parameters of the maize endosperm ADP-glucose pyrophosphorylase using iterative saturation mutagenesis. Arch Biochem Biophys 543:1–9

Boehlein SK, Shaw JR, Stewart JD, Sullivan B, Hannah LC (2015) Enhancing the heat stability and kinetic parameters of the maize endosperm ADP-glucose pyrophosphorylase using iterative saturation mutagenesis. Arch Biochem Biophys 568:28–37

Burger BT (2001) Thermotolerant variants of maize endosperm Adenosine diphosphate glucose pyrophosphorylase. A thesis submitted to graduate school of University of Florida

Burger BT, Cross JM, Shaw JR, Caren JR, Greene TW, Okita TW, Hannah LC (2003) Relative turnover numbers of maize endosperm and potato tuber ADP-glucose pyrophosphorylases in the absence and presence of 3-phosphoglyceric acid. Planta 217:449–456

Burton RA, Johnson PE, Beckles DM, Fincher GB, Jenner HL, Naldrett MJ, Denyer K (2002) Characterization of the genes encoding the cytosolic and plastidial forms of ADPglucose pyrophosphorylase in wheat endosperm. Plant Physiol 130:1464–1475

Caley CY, Duffus CM, Jeffcoat B (1990) Effects of elevated temperature and reduced water uptake on enzymes of starch synthesis in developing wheat grains. Aust J Plant Physiol 17:431–439

Causse M, Santoni S, Damerwal C, Maurice A, Charcosset A, Deatric J, Vienne D (1996) A composite map of expressed sequences in maize. Genome 39(2):418–432

Chen X, Wang Z, Wang J, Wang M, Zhao L, Wang G (2007) Isolation and characterization of Brittle2 promoter from *Zea mays* and its comparison with Ze19 promoter in transgenic tobacco plants. Plant Cell Tiss Organ Cult 88(1):11–20

Cook FR (2011) Control of the size and composition of the embryo in cereals. A thesis submitted to University of East Anglia

Corbi J, Debieu M, Rousselet A, Montalent P, Le Guilloux M, Manicacci D, Tenaillon MI (2011) Contrasted patterns of selection since maize domestication on duplicated genes encoding a starch pathway enzyme. Theor Appl Genet 122(4):705–722

Corbi J, Duthiel JY, Damerval C, Tennailon MI, Manicacci D (2012) Accelerated evolution and coevolution drove the evolutionary history of AGPase sub-units during angiosperm radiation. Ann Bot 1:6

Cossegal M, Chambrier P, Mbelo S, Balzergue S, Martin-Magniette ML, Moing A, Deborde C, Guyon V, Perez P, Rogowsky P (2008) Transcriptional and metabolic adjustments in ADP-glucose pyrophosphorylase-deficient *bt2* maize kernels. Plant Physiol 146:1553–1570

Cross JM, Clancy M, Shaw JR, Greene TW, Schmidt RR, Okita TW, Hannah LC (2004) Both subunits of ADP-glucose pyrophosphorylase are regulatory. Plant Physiol 135:137–144

Danishuddin M, Chatrath R, Singh R (2011) Insights of interaction between small and large subunits of ADP-glucose pyrophosphorylase from bread wheat (*Triticum aestivum* L.). Bioinformation 6(4):144–148

Dawar C, Jain S, Kumar S (2013) Insight into the 3D structure of ADP-glucose pyrophosphorylase from rice (*Oryza sativa* L.). J Mol Model 19(8):3351–3367

Denyer K, Dunlap F, Thorbjornsen T, Keeling PL, Smith AM (1996) The major form of ADP-glucose pyrophosphorylase in maize endosperm is extra-plastidial. Plant Physiol 112:779–785

Dickinson DB, Preiss J (1969) ADP glucose pyrophosphorylase from maize endosperm. Arch Biochem Biophys 130:119–128

Doan DNP, Rudy H, Olsen OA (1999) The allosterically unregulated isoform of ADP-glucose pyrophosphorylase from barley endosperm is the most likely source of ADP-glucose incorporated into endosperm starch. Plant Physiol 121(3):965–975

Duke ER, Doelhert DC (1996) Effects of heat stress on enzyme activities and transcript levels in developing maize kernels grown in culture. Environ Exp Bot 36:199–208

Eimert K, Luo C, Dejardin A, Villand P, Thorbjornsen T, Kleczkowski LA (1997) Molecular cloning and expression of the large subunit of ADP-glucose pyrophosphorylase from barley (*Hordeum vulgare*) leaves. Gene 189(1):79–82

Geigenberger P (2011) Regulation of starch biosynthesis in response to a fluctuating environment. Plant Physiol 155:1566–1577

Geigenberger P, Geiger M, Stitt M (1998) High-temperature perturbation of starch synthesis is attributable to inhibition of ADP-glucose pyrophosphorylase by decreased levels of glycerate-3-phosphate in growing potato tubers. Plant Physiol 117:1307–1316

Geigenberger P, Kolbe A, Tiessen A (2005) Redox regulation of carbon storage and partitioning in response to light and sugars. J Exp Bot 56:1469–1479

Georgelis N, Hannah CL (2008) Isolation of a heat-stable maize endosperm Adp-glucose pyrophosphorylase variant. Plant Sci 175:247–254

Georgelis N, Braun EL, Shaw JR, Hannah CL (2007) The two AGPase subunits evolve at different rates in angiosperms, yet they are equally sensitive to activity-altering amino acid changes when expressed in bacteria. Plant Cell 19(5):1458–1472

Georgelis N, Braun EL, Hannah CL (2008) Duplications and functional divergence of ADP-glucose pyrophosphorylase genes in plants. BMC Evol Biol 8:1–17

Georgelis N, Shaw JR, Hannah CL (2009a) Phylogenetic analysis of ADP-glucose pyrophosphorylase subunits reveals a role of subunit interfaces in the allosteric properties of the enzyme. Plant Physiol 151:67–77

Georgelis N, Shaw JR, Hannah CL (2009b) Phylogenetic analysis of ADP-glucose pyrophosphorylase subunits reveals a role of subunit interfaces in the allosteric properties of the enzyme. Plant Physiol 151:67–77

Ghosh HP, Preiss J (1966) Adenosine diphosphate glucose pyrophosphorylase: a regulatory enzyme in the biosynthesis of starch in spinach leaf chloroplasts. J Biol Chem 241:4491–4504

Giroux MJ, Hannah LC (1994) ADP-glucose pyrophosphorylase in *shrunken-2* and *brittle-2* mutants of maize. Mol Gen Genet 243:400–408

Giroux M, Smith-White B, Gilmore V, Hannah LC, Preiss J (1995) The large subunit of the embryo isoform of ADP glucose pyrophosphorylase from maize. Plant Physiol 108:1333–1334

Giroux MJ, Shaw J, Barry G, Cobb BG, Greene T, Okita T, Hannah LC (1996) A single mutation that increases maize seed weight. Proc Natl Acad Sci USA 93:5824–5829

Goldman IL, Rocheford TR, Dudley JW (1993) Quantitative trait loci influencing protein and starch concentration in the Illinois long term selection maize strains. Theor Appl Genet 87:217–224

Gomez-Casati DF, Iglesias AA (2002) ADP-glucose pyrophosphorylase from wheat endosperm. Purification and characterization of an enzyme with novel regulatory properties. Planta 214:428–434

Greene TW, Hannah LC (1998a) Enhanced stability of maize endosperm ADP-glucose pyrophosphorylase is gained through mutants that alter subunit interactions. Proc Natl Acad Sci USA 95:13342–13347

Greene TW, Hannah LC (1998b) Adenosine diphosphate glucose pyrophosphorylase, a rate limiting step in starch biosynthesis. Physiol Plant 103:574–580

Greene TW, Kavakli IH, Kahn ML, Okita TW (1998) Generation of up-regulated allosteric variants of potato ADP-glucose pyrophosphorylase by reversion genetics. Proc Natl Acad Sci USA 95:10322–10327

Hadrich N, Hendriks JHM, Kotting O, Arrivault S, Feil R, Zeeman SC, Gibon Y, Schulze WX, Stitt M, Lunn JE (2012) Mutagenesis of cysteine 81 prevents dimerization of the APS1 subunit of Adp-glucose pyrophosphorylase and alters diurnal starch turnover in *Arabidopsis thaliana* leaves. Plant J 70:231–242

Hannah LC, Greene T (2009) The complexities of starch biosynthesis in cereal endosperms. In: AL Kriz, BA Larkins, eds, Molecular genetic approaches to maize improvement. Springer-Verlag, Berlin, pp 287–301

Hannah LC, Shaw JR, Giroux MJ, Reyss A, Prioul JL, Bae JM, Lee JY (2001) Maize genes encoding the small subunit of ADP-glucose pyrophosphorylase. Plant Physiol 127:173–183

Hannah CL, Futch B, Bing J, Shaw JR, Boehlein S, Stewart JD, Beiriger R, Georgelis N, Greene T (2012) A shrunken-2 transgene increases maize yield by acting in maternal tissues to increase the frequency of seed development. Plant Cell 24(6):2352–2363

Hawker JS, Jenner CJ (1993) High temperature affects the activity of enzymes in the committed pathway of starch synthesis in developing wheat endosperm. Aust J Plant Physiol 20:197–209

Hendriks JHM, Kolbe A, Gibon Y, Stitt M, Geigenberger P (2003) ADP-glucose pyrophosphorylase is activated by posttranslational redox-modification in response to light and to sugars in leaves of *Arabidopsis* and other plant species. Plant Physiol 133:838–849

Huang B, Chen J, Zhang J, Liu H, Tian M, Gu Y, Hu Y, Li Y, Liu Y, Huang Y (2010) Characterization of ADP-glucose pyrophosphorylase encoding genes in source and sink organs of maize. Plant Mol Biol Rep 29:563–572

Huang B, Hennen-Bierwagen TA, Myers AM (2014) Functions of multiple genes encoding Adp-glucose pyrophosphorylase subunits in maize endosperm, embryo, and leaf. Plant Physiol 164:596–611

Hurkman WJ, McCue KF, Altenbach SB, Korn A, Tanaka CK, Kotharia KM, Johnson EL, Bechtel DB, Wilson JD, Anderson OD, DuPont FM (2003) Effect of temperature on expression of genes encoding enzymes for starch biosynthesis in developing wheat endosperm. Plant Sci 164:873–881

HyltonC Smith AM (1992) The rb mutation of peas causes structural and regulatory changes in ADP-glucose pyrophosphorylase from developing embryos. Plant Physiol 99:1626–1634

Iglesias AA, Barry GF, Meyer C, Bloksberg L, Nakata PA, Greene T, Laughlin MJ, Okita TW, Kishore GM, Preiss J (1993) Expression of the potato tuber ADP-glucose pyrophosphorylase in *Escherichia coli*. J Biol Chem 268:1081–1086

Jin X, Ballicora MA, Preiss J, Geiger JH (2005) Crystal structure of potato tuber ADP-Glc pyrophosphorylase. EMBO J 24:694–704

Johnson PE, Patron NJ, Bottrill AR, Dinges JR, Fahy BF, Parker ML, Waite DN, Denyer K (2003) A low-starch barley mutant, *risø 16*, lacking the cytosolic small subunit of ADP-glucose pyrophosphorylase, reveals the importance of the cytosolic isoform and the identity of the plastidial small subunit. Plant Physiol 131(2):684–696

Kaneko T, Zhang W, Takahashi H, Ito K, Takedo K (2001) QTL mapping for enzyme assay and thermostability of B-amylase in barley (*Hordeum vulgare*). Breed Sci 51:99–105

Kang GZ, Wang YH, Liu C, Shen BQ, Zheng BB, Feng W, Guo TC (2010) Difference in AGPase subunits could be associated with starch accumulation in grains between two wheat cultivars. Plant Growth Regul 61:61–66

Kang G, Liu G, Peng X, Wei L, Wang C, Zhu Y, Ying M, Yumei J, Tiancai G (2013) Increasing the starch content and grain weight of common wheat by overexpression of the cytosolic AGPase large subunit gene. Plant Physiol Biochem 73:93–98

Kato T, Taniguchi A, Horibata A (2010) Effects of the alleles at *OsAGPS2* and *OsSUT1* on the grain filling in extra-heavy panicle type of rice. Crop Sci 50:2448–2456

Keeling PL, Bacon PJ, Holt DC (1993) Elevated temperature reduces starch deposition in wheat endosperm by reducing the activity of soluble starch synthase. Planta 191:342–348

Kim D, Hwang SK, Okita TW (2007) Subunit interactions specify the allosteric regulatory properties of the potato tuber ADP-glucose pyrophosphorylase. Biochem Biophys Res Commun 362:301–306

Koehler P, Weiser H (2013) Chapter 2: Chemistry of cereal grains. In: M. Gobbetti and M. Ganzle (eds.), Handbook on Sourdough Biotechnology, Springer Science + Business Media, New York, pp 11–45

Kolbe A, Tiessen A, Schluepmann H, Paul M, Ulrich S, Geigenberger P (2005) Trehalose 6-phosphate regulates starch synthesis via posttranslational redox activation of ADP-glucose pyrophosphorylase. Proc Natl Acad Sci USA 102:11118–11123

Kossmann J, Lloyd J (2000) Understanding and influencing starch biochemistry. Crit Rev Biochem Mol Biol 35:141–196

La Cognata U, Willmitzer L, Muller-Rober B (1995) Molecular cloning and characterization of novel isoforms of potato ADP-glucose pyrophosphorylase. Mol Gen Genet 246:538–548

Lee SK, Hwang SK, Han M, Eom JS, Kang HG, Han Y, Choi SB, Cho MH, Bhoo SH, An G, Hahn TR, Okita TW, Jeon JS (2007) Identification of the ADP-glucose pyrophosphorylase isoforms essential for starch synthesis in the leaf and seed endosperm of rice (*Oryza sativa* L.). Plant Mol Biol 65:531–546

Li HM, Sullivan TD, Keegstra K (1992) Information for targeting to the chloroplastic inner envelope membrane is contained in the mature region of the maize *Bt1*-encoded protein. J Biol Chem 267:18999–19004

Li N, Zhang S, Zhao Y, Li B, Zhang J (2011) Over-expression of AGPase genes enhances seed weight and starch content in transgenic maize. Planta 233:241–250

Lin TP, Caspar T, Somerville CR, Preiss J (1988) A starch deficient mutant of *Arabidopsis thaliana* with low ADPglucose pyrophosphorylase activity lacks one of the two subunits of the enzyme. Plant Physiol 88:1175–1181

Linebarger CR, Boehlein SK, Sewell AK, Shaw J, Hannah LC (2005) Heat stability of maize endosperm ADP-glucose pyrophosphorylase is enhanced by insertion of a cysteine in the N terminus of the small subunit. Plant Physiol 139(4):1625–1634

Lohot VD, Sharma-Natu P, Pandey R, Ghildiyal MC (2010) ADP-glucose pyrophosphorylase activity in relation to starch accumulation and grain growth in wheat cultivars. Curr Sci 98(3):426–430

Lu Fh, Park YJ (2012) Sequence variations in *OsAGPase* significantly associated with amylase content and viscosity properties in rice. Genet Res 94(4):179–189

Lunn JE, Feil R, Hendriks JHM, Gibon Y, Morcuende R, Osuna D, Scheible WR, Carillo P, Hajirezaei MR, Stitt M (2006) Sugar-induced increases in trehalose 6-phosphate are correlated with redox activation of ADPglucose pyrophosphorylase and higher rates of starch synthesis in *Arabidopsis thaliana*. Biochem J 397(1):139–148

Maniacci D, Falque M, Le Guillou S, Piegu B, Henry AM, Le Guilloux M, Damerval C, De Vienne D (2007) Maize Sh2 gene is constrained by natural selection but escaped domestication. J Evol Biol 20:503–516

Meyer FD, Smidansky ED, Beecher B, Greene TW, Giroux MJ (2004) The maize *Sh2r6hs* ADP-glucose pyrophosphorylase (AGP) large subunit confers enhanced AGP properties in transgenic wheat (*Triticum aestivum*). Plant Sci 167(4):899–911

Meyer RC, Steinfath M, Lisec J, Becher M, Witucka Wall H, Torjek O, Fiehn O, Eckardt A, Willmitzer L, Selbig J, Altmann T (2007) The metabolic signature related to high plant growth rate in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 104:4759–4764

Morell M, Bloom M, Preiss J (1988) Affinity labeling of the allosteric activator sites of spinach leaf. J Biol Chem 263:633–637

Moss SC, Denyer K (2009) The evolution of the starch biosynthetic pathway in cereals and other grasses. J Exp Bot 60(9):2481–2492

Muller-Rober B, Sonnewald U, Willmitzer L (1992) Inhibition of the ADP-glucose pyrophosphorylase in transgenic potatoes leads to sugar-storing tubers and influences tuber formation and expression of tuber storage protein genes. The EMBO Journal 11(4):1229–1238

Nakamura Y, Kawaguchi K (1992) Multiple forms of ADP glucose pyrophosphorylase of rice endosperm. Physiol Plant 84:336–342

Obana Y, Omoto D, Kato C, Matsumoto K, Nagai Y, Kavakli IH, Hamada S, Edwards GE, Okita TW, Matsui H, Ito H (2006) Enhanced turnover of transitory starch by expression of up-regulated ADP-glucose pyrophosphorylases in *Arabidopsis thaliana*. Plant Sci 170:1–11

Ohdan T, Francisco PB Jr, Sawada T, Hirose T, Terao T, Satoh H, Nakamura Y (2005) Expression profiling of genes involved in starch synthesis in sink and source organs of rice. J Exp Bot 56(422):3229–3244

Okita T, Nakata P, Anderson J, Sowokinos J, Morell M, Preiss J (1990) The subunit structure of potato tuber ADP-glucose pyrophosphorylase. Plant Physiol 93:785–790

Patron NJ, Keeling PJ (2005) Common evolutionary origin of starch biosynthetic enzymes in green and red algae. J Phycol 41:1131–1141

Patron NJ, Greber B, Fahy BF, Laurie DA, Parker ML, Denyer K (2004) The lys5 mutations of barley reveal the nature and importance of plastidial ADP-Glc transporters for starch synthesis in cereal endosperm. Plant Physiol 135:2088–2097

Plaxton WC, Preiss J (1987) Purification and properties of non-proteolytic degraded ADP-glucose pyrophosphorylase from maize endosperm. Plant Physiol 83:105–112

Preiss J (1991) Biology and molecular biology of starch synthesis and its regulation. In: Miflin BJ (ed) Oxford surveys of cellular and molecular biology, vol 7. Oxford University Press, Oxford, pp 59–114

Preiss J (2004) Plant starch synthesis. In: Eliasson AC (ed) Starch in food :Structire, function and applications. CRC Press, USA, pp 3–56

Preiss J, Danner S, Summers PS, Morell M, Barton CR, Yang L, Nieder M (1990) Molecular characterization of the *brittle-2* gene effect on maize endosperm ADP-glucose pyrophosphorylase subunits. Plant Physiol 92:881–885

Prioul JL, Jeannette E, Reyss A, Gregory N, Giroux M, Hannah LC, Causse M (1994) Expression of ADP-glucose pyrophosphorylase in maize (*Zea mays* L.) grain and source leaf during grain filling. Plant Physiol 104:179–187

Prioul JL, Pelleschi S, Sene M, Thevenot C, Causse M, de Vienne D, Leonardi A (1999) From QTLs for enzyme activity to candidate genes in maize. J Exp Bot 50:1281–1288

Rosti S, Denyer K (2007) Two paralogous genes encoding small subunits of ADP-glucose pyrophosphorylase in maize, *Bt2* and *L2*, replace the single alternatively spliced gene found in other cereal species. J Mol Evol 65:316–327

Rosti S, Rudi H, Rudi K, Opsahl-Sortberg HG, Fahy B, Denyer K (2006) The gene encoding the cytosolic small subunit of ADP-glucose pyrophosphorylase in barley endosperm also encodes the major plastidial small subunit in the leaves. J Exp Bot 57:3619–3626

Rosti S, Fahy B, Denyer K (2007) A mutant of rice lacking the leaf large subunit of ADP-glucose pyrophosphorylase has drastically reduced leaf starch content but grows normally. Funct Plant Biol 34:480–489

Sakulsingharoj C, Choi SB, Hwang SK, Edwards GE, Bork J, Meyer CR, Preiss J, Okita TW (2004) Engineering starch biosynthesis for increasing rice seed weight: the role of the cytoplasmic ADP glucose pyrophosphorylase. Plant Sci 167:1323–1333

Salamone PR, Kavakli IH, Slattery CJ, Okita TW (2002) Directed molecular evolution of ADP-glucose pyrophosphorylase. Proc Natl Acad Sci USA 99:1070–1075

Schlossar AJ, Martin JM, Beecher BS, Giroux MJ (2014) Enhanced rice growth is conferred by increased leaf Adp-glucose pyrophosphorylase activity. J Plant Physiol Pathol 2(4):2. doi:10.4172/2329-955X.1000136

Sene M, Causse M, Damerval C, Thevenot C, Prioul J (2000) Quantitative trait loci affecting amylase, amylopectin and starch content in maize recombinant inbred lines. Plant Physiol Biochem 38(6):459–472

Shannon JC, Pien FM, Cao H, Liu KC (1998) *Brittle-1*, an adenylate translocator, facilitates transfer of extraplastidial synthesized ADP-glucose into amyloplasts of maize endosperms. Plant Physiol 117(4):1235–1252

Shaw JR, Hannah LC (1992) Genomic nucleotide sequence of a wild-type shrunken-2 allele of *Zea mays*. Plant Physiol 98:1214–1216

Sikka VK, Choi S, Kavakli IH, Sakulsingharoj C, Gupta S, Ito H, Okita TW (2001) Subcellular compartmentation and allosteric regulation of the rice endosperm ADP glucose pyrophosphorylase. Plant Sci 161:461–468

Singletary GW, Banisadr R, Keeling PL (1994) Heat stress during grain filling in maize: effects on carbohydrate storage and metabolism. Aust J Plant Physiol 21:829–841

Slewinski TL, Ma Y, Baker RF, Huang M, Meeley R, Braun DM (2008) Determining the role of Tie-dyed1 in starch metabolism: epistasis analysis with a maize ADP-glucose pyrophosphorylase mutant lacking leaf starch. J Hered 99:661–666

Smidansky ED, Clancy M, Meyer FD, Lanning SP, Blake NK, Talbert LE, Giroux MJ (2002) Enhanced ADP-glucose pyrophosphorylase activity in wheat endosperm increases seed yield. Proc Natl Acad Sci USA 99:1724–1729

Smidansky ED, Martin JM, Hannah LC, Fischer AM, Giroux MJ (2003) Seed yield and plant biomass increases in rice are conferred by deregulation of endosperm ADP-glucose pyrophosphorylase. Planta 216:656–664

Smidansky ED, Meyer FD, Blakeslee B, Weglarz TE, Greene TW, Giroux MJ (2007) Expression of a modified ADP-glucose pyrophosphorylase large subunit in wheat seeds stimulates photosynthesis and carbon metabolism. Planta 225(4):965–976

Smith AM, Denyer K, Martin CR (1995) What controls the amount and structure of starch in storage organs? Plant Physiol 107:673–677

Smith-White BJ, Preiss J (1992) Comparison of proteins of ADP-glucose pyrophosphorylase from diverse sources. J Mol Evol 34:449–464

Stark DM, Timmerman KP, Barry GF, Preiss J, Kishore GM (1992) Regulation of the amount of starch in plant tissues by ADP glucose pyrophosphorylase. Science 258:287–292

Teas HJ, Teas AN (1953) Heritable characters in maize. Description and linkage of brittle endosperm-2. J Hered 44:156–158

Tester RF, Morrison WR, Schulman AH (1993) Swelling and gelatinization of cereal starches: V. Risø mutants of Bomi and Carlsberg II barley cultivars. J Cereal Sci 17:1–9

Tetlow IJ, Davies EJ, Vardy KA, Bowsher CG, Burrell MM, Emes MJ (2003) Subcellular localization of ADPglucose pyrophosphorylase in developing wheat endosperm and analysis of theproperties of a plastidial isoform. J Exp Bot 54(383):715–725

Thevenot C, Simond-Cote E, Reyss A, Manicacci D, Trouverie J, Guilloux ML, Ginhoux V, Sidicina F, Priou L (2005) QTLs for enzyme activities and soluble carbohydrates involved in starch accumulation during grain filling in maize. J Exp Bot 56(413):945–958

Thitisaksakul M, Jimenez RC, Arias MC, Beckles DM (2012) Effects of environmental factors on cereal starch biosynthesis and composition. J Cereal Sci 56:67–80

Thorbjornsen T, Villand P, Kleczkowski LA, Olsen OA (1996a) A single gene encodes two different transcripts for the ADP-glucose pyrophosphorylase small subunit from barley (*Hordeum vulgare*). Biochem J 313(1):149–154

Thorbjornsen T, Villand P, Denyer K, Olsen OA, Smith A (1996b) Distinct isoforms of ADP glucose pyrophosphorylase occur inside and outside the amyloplasts in barley endosperm. Plant J 10:243–250

Thorneycroft D, Hosein F, Thangavelu M, Clark J, Vizir L, Burrell M, Ainsworth C (2003) Characterization of a gene from chromosome 1B encoding the large subunit of ADP glucose pyrophosphorylase from wheat: evolutionary divergence and differential expression of *Agp2* genes between leaves and developing endosperm. Plant Biotech J 1:259–270

Tiana Z, Qianb Q, Liuc Q, Yanb M, Liua X, Yanc C, Liua G, Gaob Z, Tangc S, Zengb D, Wanga Y, Yud J, Guc M, Lia J (2009) Allelic diversities in rice starch biosynthesis lead to a diverse array

of rice eating and cooking qualities. Pros Natl Acad Sci USA 106(51):21760–21765

Tiessen A, Hendriks JHM, Stitt M, Branscheid A, Gibon Y, Farre EM, Geigenberger P (2002) Starch synthesis in potato tubers is regulated by post-translational redox-modification of ADP-glucose pyrophosphorylase: a novel regulatory mechanism linking starch synthesis to the sucrose supply. Plant Cell 14:2191–2213

Tsai CY, Nelson OE (1966) Starch-deficient maize mutants lacking adenosine diphosphate glucose pyrophosphorylase activity. Science 151:341–343

Tuncel A, Okita TW (2013) Improving starch yield in cereals by overexpression of ADP glucose pyrophosphorylase: expectations and unanticipated outcomes. Plant Sci 211:52–60

Tuncel A, Kavakli IH, Keskin O (2008) Insights into subunit interactions in the heterotetrameric structure of potato ADP glucose pyrophosphorylase. Biophys J 95:3628–3639

Tuncel A, Kawaguchi J, Ihara Y, Matsusaka H, Nishi A, Nakamura T, Kuhara S, Hirakawa H, Nakamura Y, Cakir B, Nagamine A, Okita TW, Hwang SK, Satoh H (2014) The rice endosperm ADP-glucose pyrophosphorylase large subunit is essential for optimal catalysis and allosteric regulation of the heterotetrameric enzyme. Plant Cell Physiol 55(6):1169–1183

Umemoto T, Horibata T, Aoki N, Hiratsuka M, Yano M, Inouchi N (2008) Effects of variation in starch synthase on starch properties and eating quality of rice. Plant Prod Sci 11(4):472–480

Villand P, Kleczkowski LA (1994) Is there an alternative pathway for starch biosynthesis in cereal seeds? Zeitschrift für Naturforschung C 49:215–219

Villand P, Aalen R, Olsen O-A, Lüthi E, Lonneborg A, Kleckowski LA (1992) PCR amplification and sequences of cDNA clones for the small and LS of ADP-glucose pyrophosphorylase from barley tissues. Plant Mol Biol 19:381–389

Viswanathan C, Khanna-Chopra R (2001) Effect of heat stress on grain growth, starch synthesis and protein synthesis in grains of wheat (*Triticum aestivum* L.) varieties differing in grain weight stability. J Agron Crop Sci 186:1–7

Wang Z, Chen X, Wang J, Liu T, Liu Y, Zhao L, Wang G (2007) Increasing maize seed weight by enhancing the cytoplasmic ADP-glucose pyrophosphorylase activity in transgenic plants. Plant Cell Tiss Organ Cult 88:83–92

Weber H, Heim U, Borisjuk L, Wobus U (1995) Cell-type specific, coordinate expression of two ADPglucose pyrophosphorylase genes in relation to starch biosynthesis during seed development in *Vicia faba* L. Planta 195:352–361

Wilhelm EP, Mullen RE, Keeling PL, Singletary GW (1999) Heat stress during grain filling in maize: effects on kernel growth and metabolism. Crop Sci 39:1733–1741

Yan HB, Pan XX, Jiang HW, Wu GJ (2009) Comparison of the starch synthesis genes between maize and rice: copies, chromosome location and expression divergence. Theor Appl Genet 119:815–825

Yu G, Olsen KM, Schaal BA (2011) Molecular evolution of the endosperm starch synthesis pathway genes in rice (*Oryza sativa* L.) and its wild ancestor, *O. rufipogon* L. Mol Biol Evol 28(1):659–671

Zhang N, Gibon Y, Gur A, Chen C, Lepak N, Hohne M, Zhang Z, Kroon D, Tschoep H, Stitt M, Edward B (2010) Fine quantitative trait loci mapping of carbon and nitrogen metabolism enzyme activities and seedling biomass in the maize IBM mapping population. Plant Physiol 154(1753):1765