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SEED RESERVE UTILIZATION AND HYDROLYTIC ENZYME ACTIVITIES IN GERMINATING SEEDS OF SWEET CORN

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Abstract

In this study, two *sh2* sweet corn cultivars (i.e., the initial seed dry weight for FT018 and TB010 was 0.16±0.02 g/grain and 0.09±0.01 g/grain, respectively) were used to determine the physiological characteristics of seed reserve utilization in germination. The data implied that the weight of mobilized seed reserve (WMSR) and seed reserve utilization efficiency (SRUE) increased with seed germination. FT018 exhibited higher SRUE than TB010 due to its sufficient energy production for growth. Sugar (sucrose and fructose) contents were at different levels in the germinating seed of *sh2* sweet corn. The protein content and number of protein species were highest in the early stage of germination. Enzyme activity in the germinating seed indicated that enzymes for starch and sugar hydrolysis were important and that enzyme activities significantly differed at each germination stage and between the cultivars under dark conditions. Succinate dehydrogenase, sucrose synthase, and glucose-6-phosphate dehydrogenase accumulated in the late germination stage. Thus, appropriate efforts should be focused on improving the seed reserve utilization in sweet corn by identifying the physiological mechanism of germinating seed.

Key words: Sweet corn, Seed reserve utilization efficiency, Hydrolytic enzyme activity, Germination.

Introduction

Sweet corn (*Zea mays* L.var. saccharata Bailey) is a form of maize that can't complete the conversions of the carbohydrate to starchand is a staple foodin the world (Tracy, 1994; Tracy *et al*., 2006). During the growth period, seed germination first involves the uptake of water, then radicle emergence, and finally stand establishment in sweet corn cropping systems (Wang *et al*., 2010). The starchy endosperm of sweet corn has a smaller proportion of grain than other corn varieties because the recessive genes prevent carbohydrate conversion. Therefore, cultivating a vigorous seedling is an important goal in sweet corn breeding to increase weed competitive ability.

Seedling establishment is an important process that is affected by genetic and environmental factors (Hodgkin *et al*., 2008). Germination begins with water uptake and ends in the protrusion of a radicle. During germination, the major seed storage reserve provide the required nutrients and energy to the seedling in germination (Bewley *et al*., 2013). The physiological processes are activated under the various enzymes during germination (Zeeman *et al*., 2010). The heterotrophic seedling growth can be influenced by the weight of mobilized seed reserve (WMSR) and seed reserve utilization efficiency (SRUE) (Mohammadi *et al*., 2011; Soltani *et al*., 2006). To date, phenotype studies of seed reserve utilization have been reported in many crops, such as soybean, wheat, rice, and chickpea (Mohammadi *et al*., 2011; Soltani *et al*., 2006; Cheng *et al*., 2013; Soltani *et al*., 2002). However, only few studies have been reported on the SRUE of sweet corn during germination.

SRUE is regulated by enzymatic hydrolysis of seed storage and formation of new seedling (Soltani *et al*., 2006). The mobilization of seed stored reserves is facilitated by enzyme activity. During seed germination, the seed storage reserve is mobilized and degraded into storage compounds, which include proteins, glucose, sucrose, fructose, and adenosine-5′-triphosphate (ATP) used in the development of autotrophy. Amylase (AMY) and sucrose synthase (SUS) are apparently synthesized in the scutellum and aleurone layers and then promoted the starch hydrolysis in the endosperm (Potokina *et al*., 2002; Hirose *et al*., 2008). This process has been investigated in rice, *Arabidopsis*, and pumpkin (He *et al*., 2011; Cheng *et al*., 2015; Holdsworth *et al*., 2008; Finch *et al*., 2006; Rout *et al*., 1999). However, the associations of glucose, sucrose, fructose, ATP, enzyme formation, and enzyme activity with SRUE varied in the stage of seedling growth. In this study, dynamic changes in the physiological characteristics of SRUE were investigated in germinating seed of sweet corn to further understand the initiation of plant germination through metabolism regulation.

Materials and Methods

Plant materials: Two *sh2* sweet corn cultivars (i.e., FT018 and TB010) were utilized in the experiment. All seeds were dried at the experimental station of Anhui Science and Technology University. The initial seed dry weight (ISDW) for FT018 and TB010 was 0.16±0.02 g/grain and 0.09±0.01 g/grain, respectively.

Sweet corn seed germination: Sweet corn seeds were sterilized with 0.1% $HgCl_2$ solution for 15min, rinsed with sterile distilled water three times, and used for the germination experiment. Seeds were germinated in the germinating box (diameter: 120mm) with three replications. The seeds were incubated at 30 ± 1°C under a dark condition to avoid photosynthesis and achieve uniform germination. A randomized complete block design was used in the experiment WMSR (mg/grain) and SRUE (mg/mg) were performed as the described method of Cheng (2013; 2015). First, the ISDW (mg/grain) of the seeds was determined. Three seed replicates were weighed (W_1) and the dried seed were reweighed (W_2) at

104°C for 24 h in an oven. Second, the ISDW in each replicate was then computed [$W_1 \times (1 - WC)$] (WC (seed water content) is [$(W_1 - W_2)/W_1$]). Finally, the WMSR (mg/grain) was estimated as the dry weight of seed remnant subtracted from the ISDW. SRUE(mg /mg) was calculated as the seedling dry weight (mg/grain) by the WMSR (Soltani *et al*., 2006).

Protein assay: The proteins and enzyme activity were investigated at 8h, 24h, 48h, 4d, and 6 d after germination in the dark. The imbibed seeds were harvested for protein and enzyme extraction.

Proteins were extracted from the germinated seeds according to the method described by Vasconcelos (2005). Seed protein was separated using SDS-PAGE (Jisha *et al*., 2011). Each sample was loaded onto the 12% SDS-PAGE (Laemmli *et al*., 1970). Proteins were stained with Coomassie Brilliant Blue R-250.

Enzyme activity assay: The assay of total AMY activity was performed as the described method of Braga (2009). The assay of starch-branching enzyme (SBE) activity was performed using the phosphorylase-stimulation method (Smith *et al*., 1990). The assay of succinate dehydrogenase (SDH) activity was performed as the methods of Nachlas (1957). The assay of SUS activity was performed as the described method of Choudhury (2010). Glucose-6-phosphate dehydrogenase (G-6-PD) was calculated using the methemoglobin reduction method.

ATP was determined using the phosphomolybdic acid colorimetric method (Rattledge *et al*., 1997). The enzyme activity was expressed as $U \cdot g^{-1}$fresh weight (FW).

Data analysis: The data from the experiment were analyzed using the Statistical Analysis System software. The traits were compared via Student's *t*-test at the 5% and 1% levels of probability.

Results

Dynamic change of seed reserve utilization: The results indicated that WMSR and SRUE increased in both varieties during the germination (Fig. 1). By comparison, WMSR significantly differed between cultivars FT018 and TB010 at the late germination stage (6 d). SRUE also significantly differed between the cultivars at 4d and 6d of germination. However, FT018 exhibited significantly higher WMSR and SRUE than TB010 during germination. WMSR and SRUE were 75.4mg/grain and 0.34mg/mg in FT018, whereas they were 51.4mg/grain and 0.26mg/mg in TB010 at the late germination stage, respectively.

Dynamic change of sugar content: Sugars were present at different levels in the germination of *sh*$_2$ sweet corn (Fig. 2). Sucrose and fructose started to accumulate in germination. However, sucrose mostly accumulated in the early germination and decreased significantly after 24h. Fructose accumulated during germination. Comparison indicated that the difference of sucrose and fructose contents was significant between the cultivars. FT018 exhibited a higher fructose content during germination and peaked at 24h and 48h. Sucrose content peaked at 24h in TB010 and reached the level of 84.87mmol/L FW (Fig. 2).This peak correlated well with the time point when the major part of the starch reserve in the seed was depleted.

Proteome profiling of germinating sweet corn seed: Protein content during germination started in the early germination stage. Nineteen different proteins were identified with high probability in this study (Fig. 3). The protein content and number of protein species in *sh*$_2$ sweet corn were highest at 24 h and 48 h of germination. Comparison indicated that protein content and species significantly differed between the cultivars. TB010 exhibited a higher protein content than FT018 and had 9 proteins in the late germination stage.

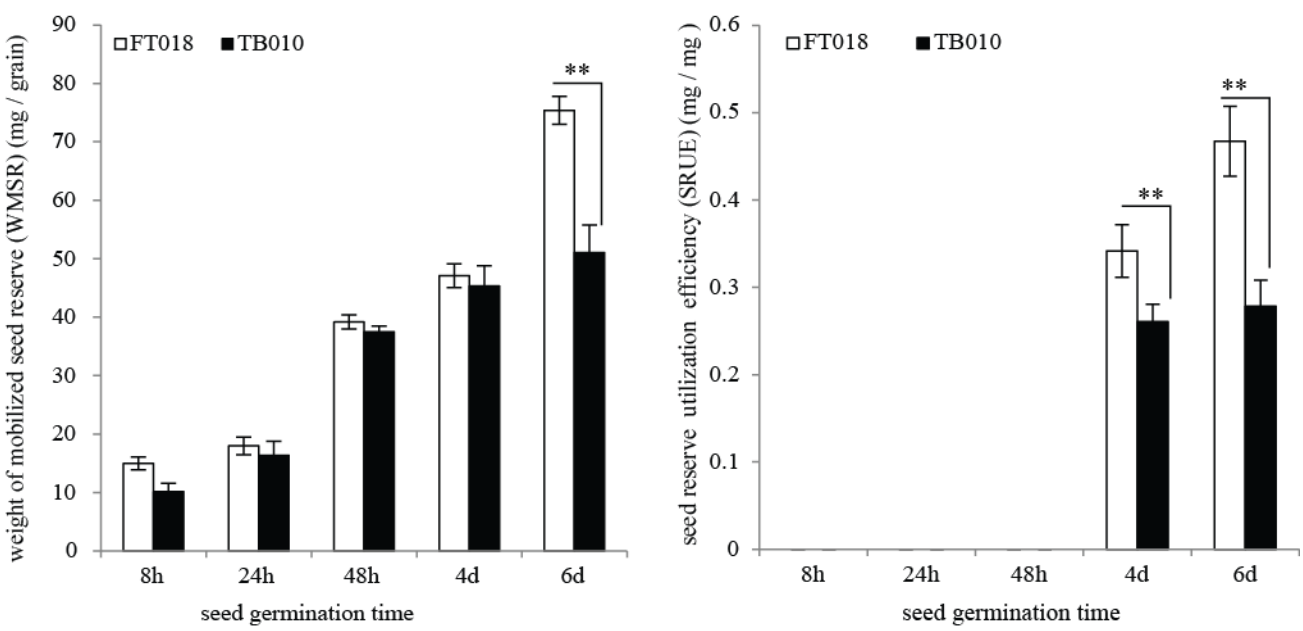

Fig.1 Dynamic changes of the WMSR and SRUE during seed germination in *sh*$_2$ sweet corn.
** Indicates1% level of significance according to Student's *t*-test

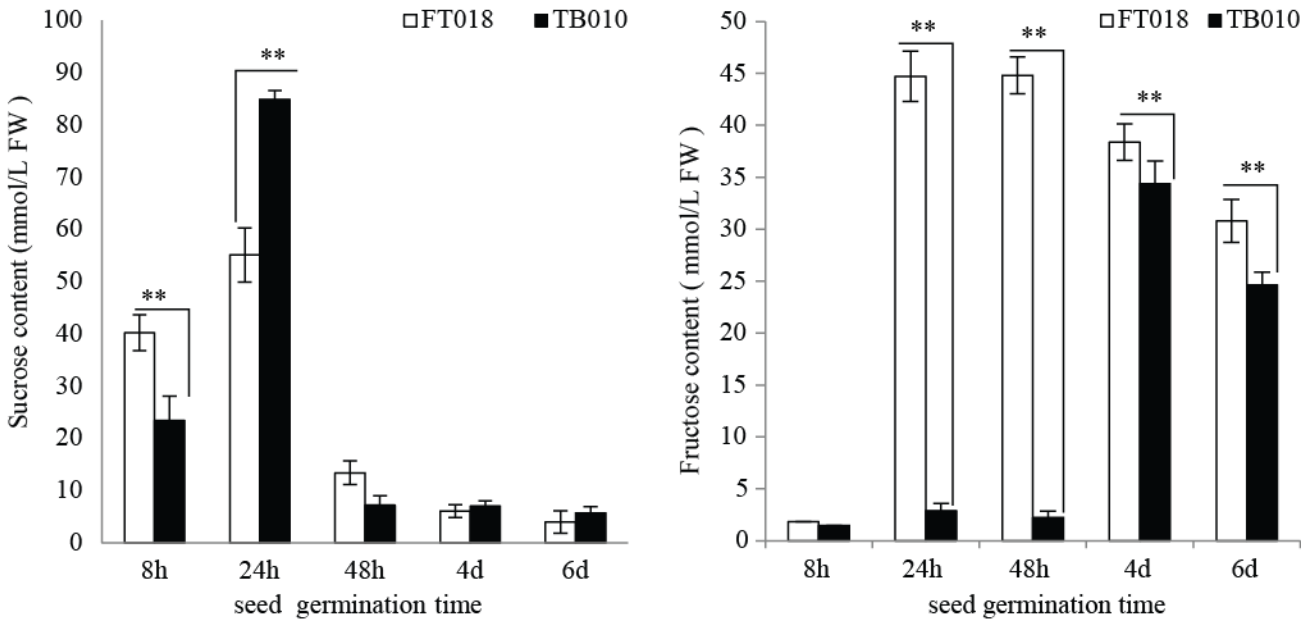

Fig. 2. Dynamic changes of the sugar content during seed germination in *sh2* sweet corn.
** Indicates1% level of significance according to Student's *t*-test

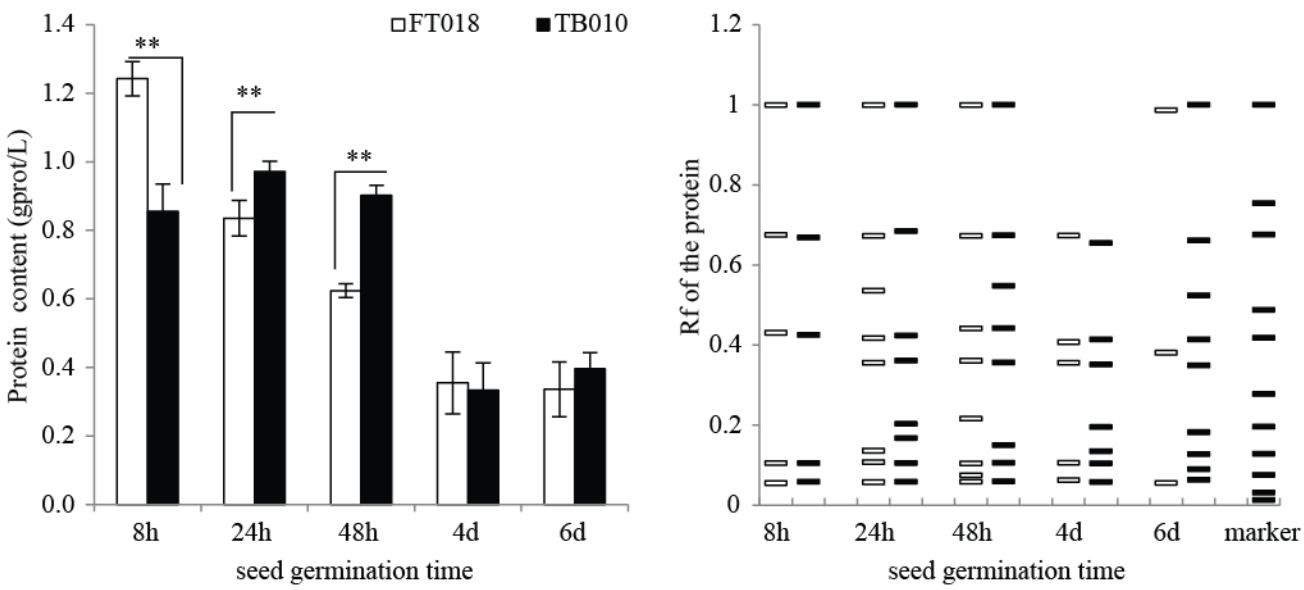

Fig. 3. Dynamic changes of proteome profiling during seed germination in *sh2* sweet corn.
**Indicates 1% level of significance according to Student's *t*-test

Starch hydrolytic enzyme activity of germinating sweet corn seed: Starch is hydrolyzed to different storage compounds used for seedling growth under the action of enzymes, and AMY, SBE, and SDH are important enzymes during germination. AMY, SBE, and SDH activity analysis showed that SDH activity was the highest and SBE activity was the lowest in germinated seed (Fig. 4). AMY activity accumulated in the early (8h) and late germination stages (4d and 6d). SBE activity peaked at 24h and reached the levels of 0.29-0.30 U/g FW. SDH activity accumulated in the late germination stage. By comparison, both cultivars had a slight difference.AMY activity in FT018 and TB010 ranged from 0.08 U/g FW to 1.59 U/g FW and from 0.16 U/g FW to 1.69 U/g FW, respectively. SDH activity in FT018 and TB010 ranged from 0.95U/g FW to 5.24 U/g FW and form 1.50U/g FW to 7.68 U/g FW, respectively.

Sugar hydrolytic enzyme activity of germinating sweet corn seed: The sugar hydrolytic enzyme activity of the germinating seed was initiated by water uptake. SUS and G-6-PD activity increased during germination and accumulated in the late germination stage (Fig. 5). However, ATP decreased in the germinated seed because of the seedling growth and consumption. ATP reached the highest level at the early germination stage. The sugar hydrolytic enzyme activity significantly differed between FT018 and TB010. SUS activity peaked at 4 d and reached the level of 4.67 U/g·prot FW in FT018 and 6.27U/g·prot FW in TB010, where as G-6-PD activity reached the highest level at 6 d of germination.

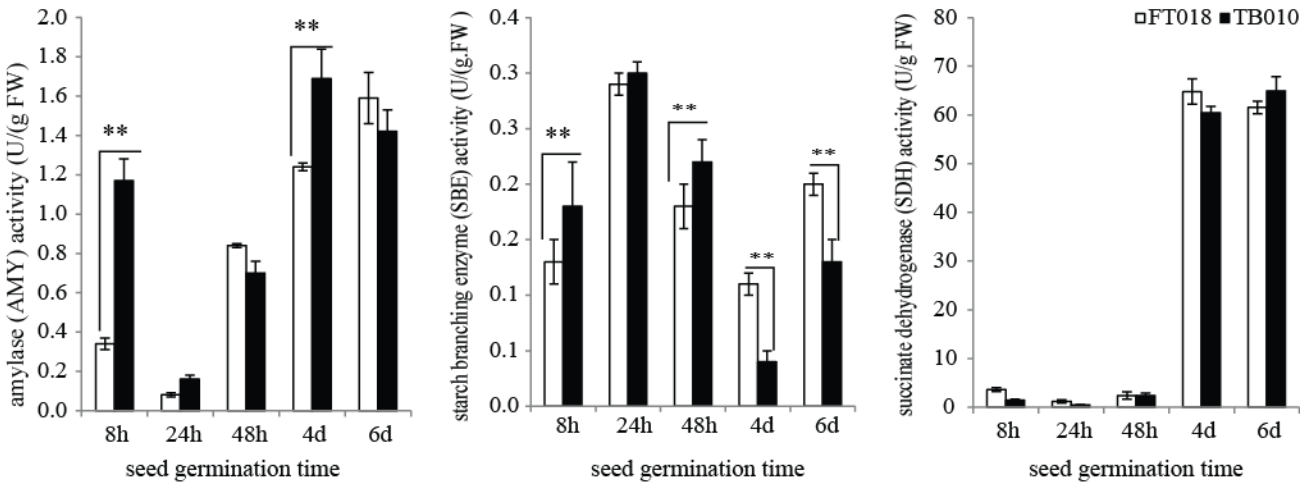

Fig. 4. Dynamic changes of the starch hydrolytic enzyme activities during seed germination in *sh2* sweet corn.
**Indicates1% level of significance according to Student's *t*-test

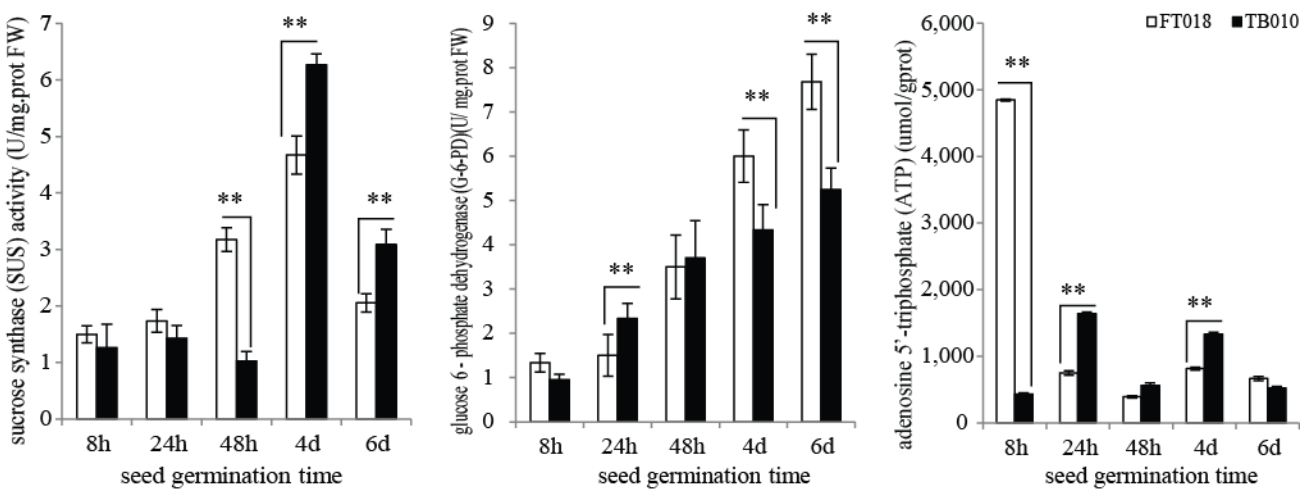

Fig. 5. Dynamic changes of the sugar hydrolytic enzyme activities during seed germination in *sh2* sweet corn.
**Indicates1% level of significance according to Student's *t*-test

Discussion

During seed germination, the dry weight of the growing seedling is always lower than that of the mobilized substrates due to respiration (Bewley *et al*., 1982; Soltani *et al*., 2006). To date, seed reserve utilization in *Hordeum vulgare*, *Brassica napus*, *Z. mays*, *Medicago sativa*, and *M. scutellata* is still affected by salinity and drought stresses, and SRUE is higher in corn than other seeds in salinity treatments (Saeed *et al*., 2012). Knowledge about the mobilization of sweet corn during germination is limited. In this study, FT018 (single-grain weight 0.16±0.02 g) exhibited higher WMSR and SRUE. SRUE is an important indicator of the conversion efficiency of the WMSR in germination. Seeds with high SRUE have low respiration and high energy usage for seedling growth during germination. In this study, FT018 exhibited a higher SRUE than TB010 due to its sufficient energy production for growth. This result is in agreement with previous studies (Douglas *et al*., 1994; Bockous *et al*., 1996; Soltani *et al*., 2006), where larger seeds were found to have a higher seed vigor and stronger growth potential than smaller seeds.

Starch is the major energy reserve in mature cereal seeds. The mobilization of seed reserve in sweet corn starts on the first day of germination. During seed germination, stored substrates are broken down into small molecular compounds, such as protein and sugar (sucrose and fructose), in imbibed seeds. This hydrolysis stimulates the synthesis of starch hydrolytic enzymes (e.g., AMY, SBE, and SDH), sugar hydrolytic enzymes (e.g., SUS and G-6-PD), and ATP, which are utilized in new tissues synthesis (Soltani *et al*., 2006). Svetlana (2010) found that sucrose and fructose start to accumulate after a few days of germination in oat. In this study, the major soluble sugars stored in seed were sucrose, which mostly accumulated in the early germination stage, and fructose. Comparison indicated that the difference in sucrose and fructose content was significant between the *sh2* sweet corn cultivars.

During the past decades, a number of proteomic studies related to seed germination have been conducted and indicated that the main storage protein in grains, such as *Arabidopsis* (Gallardo *et al*., 2001), rice (Yang *et al*., 2007), wheat (Mak *et al*., 2009), mungbean (Suparna *et al*., 2012), barley (Finnie *et al*., 2004) and soybean (Cheng *et al*., 2010), accumulate in stages I and II of

germination. However, the protein content and number of protein species in *sh2* sweet corn were highest at the early stage of germination and significantly differed between the cultivars. This result is in agreement with previous studies that found that the main storage protein in grains accumulate in stages of germination (Gallardo *et al*., 2001; Yang *et al*., 2007; Mak *et al*., 2009; Finnie *et al*., 2004; Suparna *et al*., 2012; Cheng *et al*., 2010).

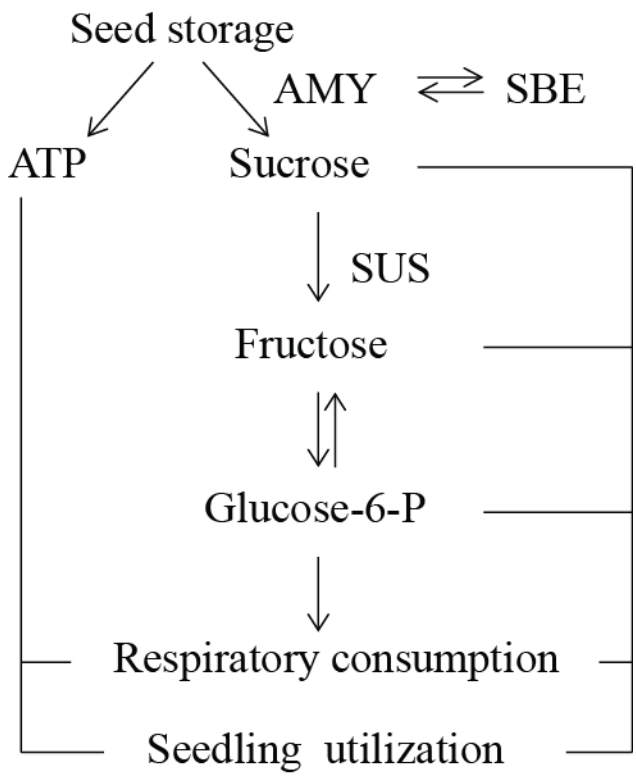

Fig. 6. Changes of seed storage in germinating stages.

Hydrolytic enzymes are apparently synthesized in the scutellum and aleurone layers during seed germination (Potokina *et al*., 2002; Hirose *et al*., 2008; Cheng *et al*., 2015). Previous studies have indicated that amylolytic activities decrease continuously during dark incubation and in subsequent germination stages (Koshiba *et al*., 1984). In the current study, results showed that starch and sugar hydrolytic enzymes were important enzymes in the germinating seed. The enzyme activities significantly differed at various germination stages and between the cultivars under dark conditions. SDH activity was the highest and SBE activity was the lowest among the starch hydrolyticenzymes.SUS and G-6-PD activities increased and accumulated in the late germination stage. However, ATP peaked at 8h and decreased in subsequent germination stages. Large grain of sweet corn had the higher WMSR and SRUE because of sufficient ATP and carbohydrate substances. So we speculate that the seed storage began to decompose ATP、protein and carbohydrate substances that mainly include the sucrose、fructose and glucose-6-p in early germination under the action of AMY and SBE enzymes is in the dynamic changes (Fig 6). ATP and carbohydrate substances are mainly used for respiratory consumption and seedling utilization. Under the action of the SUS enzyme the sucrose would convert to the fructose and glucose-6-p.

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