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Carbohydrate metabolism before and after dehiscence in the recalcitrant pollen of pumpkin (*Cucurbita pepo* L.)

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ABSTRACT

Pumpkin (*Cucurbita pepo* L.) pollen is starchy, sucrose-poor and recalcitrant, features opposite to those of several model species; therefore, some differences in carbohydrate metabolism could be expected in this species. By studying pumpkin recalcitrant pollen, the objective was to provide new biochemical evidence to improve understanding of how carbohydrate metabolism might be involved in pollen functioning in advanced stages. Four stages were analysed: immature pollen from 1 day before anthesis, mature pollen, mature pollen exposed to the environment for 7 h, and pollen rehydrated in a culture medium. Pollen viability, water and carbohydrate content and activity of enzymes involved in carbohydrate metabolism were quantified in each stage. Pollen viability and water content dropped quickly after dehiscence, as expected. The slight changes in carbohydrate concentration and enzyme activity during pollen maturation contrast with major changes recorded with ageing and rehydration. Pumpkin pollen seems highly active and closely related to its surrounding environment in all the stages analysed; the latter is particularly evident among insoluble sucrolytic enzymes, mainly wall-bound acid invertase, which would be the most relevant for sucrose cleavage. Each stage was characterised by a particular metabolic/enzymatic profile; some particular features, such as the minor changes during maturation, fast sucrolysis upon rehydration or sharp decrease in insoluble sucrolytic activity with ageing seem to be related to the lack of dormancy and recalcitrant nature of pumpkin pollen.

INTRODUCTION

In plant reproduction, pollen grains carry the sperm cells to the pistil in order to reach the embryo sac within the ovule and achieve double fertilisation. The relevance of carbohydrate metabolism for pollen fertility and functioning has been demonstrated in a number of studies (Goetz *et al.* 2001; Pressman *et al.* 2006; Zhang *et al.* 2010). Most research focused on pollen development, while the stages following anther opening (*e.g.* pollen survival or germination) have received much less attention (Singh *et al.* 1978; Ylstra *et al.* 1998; Carrizo García *et al.* 2013; Geng *et al.* 2013).

The carbohydrate content of ripe pollen is determined by events that occurred during development, *i.e.* quantity and quality of nutrients supplied by the sporophytic tissues and metabolism of the different molecules. The type of pollen carbohydrates is closely related to the water content, which can vary among species (Pacini *et al.* 2006). Moreover, both carbohydrate and water content affect, and may even determine, some vital functions of pollen grains, such as desiccation tolerance, longevity and/or speed of germination (Franchi *et al.* 2011). The pollen of pumpkin (*Cucurbita pepo* L.) is starchy, sucrose-poor (Nepi *et al.* 2010) and recalcitrant (in analogy with seeds; Franchi *et al.* 2011), features opposite to those of several model species (*e.g.* tobacco, petunia). Recalcitrant pollen is almost ready for dispersal when the locular fluid of the anther has disappeared (Firon *et al.* 2012), *i.e.* there is no dormancy period. Therefore, some differences in carbohydrate metabolism could be expected. In fact, the sensitivity to dehydration and trends in carbohydrate fluctuations in pollen subjected to hydric stress differ in pumpkin, in contrast to *Petunia*, whose pollen is sucrose-rich, starchless and orthodox (Nepi *et al.* 2010).

Stitt & Gibon (2014) have recently reviewed the importance of studying enzyme activity to integrate this information with other data, such as metabolite profiles, to identify patterns and networks. Therefore, using pumpkin pollen as a model of the little known recalcitrant pollen type, the aim of this study is to provide new biochemical evidence to improve understanding of how carbohydrate metabolism may be involved in pollen functioning in advanced stages.

MATERIAL AND METHODS

Pumpkin cv. Alberello di Sarzana was grown outdoors in the Botanical Garden of Siena (Italy) during summer. Pollen samples were taken from flowers and flower buds picked at 08:00 h.

Three processes were analysed at four stages: (i) pollen final maturation – in immature pollen from 1 day before anthesis (1 DBA) to mature pollen from recently open flowers; (ii) pollen ageing – mature pollen separated from the anthers and exposed to the environment (25 ± 2 °C) for 7 h after flower opening, called 7 HAA (7 h after anthesis) pollen; and (iii) pollen

rehydration – mature pollen (T0) rehydrated *in vitro* for 10 min (T1) in a culture medium (15% sucrose in water) at 25 ± 0.5 °C.

Pollen viability and water content

Pollen viability was tested for the mature and 7 HAA pollen using the fluorochromatic reaction test (Heslop-Harrison *et al.* 1984). Average values were calculated for 300 pollen grains (3 × 100).

Pollen water content was defined for the stages 1 DBA, mature and 7 HAA. Fresh samples were weighed (0.1 mg accuracy) and dried at 100 °C until constant dry weight (DW; 2 h, weighed every 30 min). The water content was indirectly calculated according to the weight loss. Measurements were done in triplicate for each stage.

Carbohydrate analysis

Pollen samples from stages 1 DBA, mature and 7 HAA were homogenised in distilled water with a PRO homogeniser (PRO Scientific, Oxford, CT, USA) at 4 °C. For the rehydration process, a fraction of the culture medium was separated by centrifugation at T1 (3500 × *g*, 4 °C for 5 min) and analysed for carbohydrate content without further treatment. The rest of the sample, *i.e.* rehydrated pollen and culture medium, was homogenised together as described. After homogenisation, every sample was heated at 100 °C for 10 min, and the supernatants and insoluble pellets separated by centrifugation (13,000 × *g*, 4 °C for 30 min) and stored at −80 °C (pellets were washed three times with distilled water before storage). Independent samples were prepared to quantify sucrose in the insoluble fraction; the pellets were washed five times in this case.

Soluble glucose, fructose and sucrose were identified and quantified using HPLC (Carrizo García *et al.* 2010). Sucrose in the insoluble fraction was quantified colorimetrically after hydrolysis with invertase (Carrizo García *et al.* 2010). The entire pellets were incubated with invertase and the amounts of glucose and fructose released quantified with the D-Fructose/D-Glucose assay kit (Megazyme, Bray, Co., Wicklow, Ireland). Starch was quantified after hydrolysis with amyloglucosidase (Carrizo García *et al.* 2013).

The absolute concentration of carbohydrates was calculated according to the DW of each sample (see 'water content'). For rehydrated pollen, DW was calculated from the fresh weight (FW) of the sample before rehydration, while soluble carbohydrate quantities were calculated as the difference between the total (cellular content mixed with culture medium) and the separated culture medium.

Glucose, fructose and sucrose were tested in the locular fluid at 1 DBA, which was absorbed on filter paper from anthers gently opened with a needle through the stomium. The pieces of paper were put into a tube with distilled water and, after vigorous vortexing, the solubilised substances were separated by centrifugation (13,000 × *g*, 4 °C for 5 min). Glucose, fructose and sucrose were quantified in the supernatant as described above.

Enzyme extraction and activity assays

The samples were homogenised in an extraction buffer containing 50 mM sodium phosphate, 1 mM DTT, 1 mM EDTA, 3 mM $MgCl_2$ and 2% glycerol (pH 7.4). For the rehydrated pollen, 2× extraction buffer was added in a 1:1 proportion to the culture medium. The samples were homogenised as described above and the supernatants were separated after centrifugation (12,000 × *g*, 4 °C for 25 min). The supernatants were dialysed in 12 mM potassium phosphate (pH 7.4) buffer at 4 °C, for 3 h for the 1 DBA, mature and 7 HAA pollen, and for 10 h for the rehydrated pollen. The soluble enzyme activity was assessed in these extracts. The insoluble residue was washed three times with distilled water and then incubated for 24 h at 4 °C in a solubilisation buffer containing 0.2 M NaCl citrate/phosphate (pH 5). The solubilised enzymes were separated by centrifugation (12,000 × *g*, 4 °C for 25 min). Activity of insoluble enzymes was assessed in these extracts.

Soluble acid (SAI) and neutral (SNI) invertases, sucrose synthase in the cleavage direction (SUSY) and amylase activity were assayed and quantified according to Carrizo García *et al.* (2013). Wall-bound acid invertase (WAI) activity was tested according to Kumar Bhowmik *et al.* (2001), with modifications, and quantified following the method of Castro & Clément (2007). Insoluble sucrose synthase activity in the cleavage direction (INSUSY) was analysed following the same conditions for soluble sucrose synthase (cleavage direction), but the activity was quantified as for wall-bound acid invertase. Activity of all the lytic enzymes was expressed as the amount of reducing sugar released $min^{-1} \cdot mg^{-1}$ total protein.

Sucrose synthesis was tested in the soluble extracts. Sucrose phosphate synthase was assayed in a reaction medium containing 50 mM MOPS (pH 7.5), 25 mM glucose-6-phosphate, 25 mM fructose-6-phosphate and 25 mM UDP-glucose (Cheikh & Brenner 1992; Guy *et al.* 1992; Jones & Ort 1997). The reaction mixture was incubated for 60 min at 25 °C, 30 °C and 37 °C in independent assays. Sucrose synthase in the synthesis direction was assayed according to Pontis *et al.* (1981) in a reaction medium consisting of 100 mM Tris-HCl (pH 8), 15 mM fructose and 25 mM UDP-glucose, and incubated at 30 °C for 60 min. The activity of both enzymes was calculated according to changes in sucrose concentration $min^{-1} \cdot mg^{-1}$ total protein. Sucrose was identified and quantified through HPLC, as described above. Total protein concentrations were quantified according to Bradford (1976) separately in the soluble and insoluble fractions.

Statistics

Significant changes in carbohydrate concentrations and enzyme activities were determined by ANOVA ($P = 0.05$), comparing average values (n = 3 in all the cases) pair-wise in consecutive stages (pairs compared: 1 DBA–mature pollen, mature–7 HAA pollen and mature–rehydrated pollen).

RESULTS AND DISCUSSION

Pollen viability and water content

Pollen viability was *ca.* 80% in the mature pollen and decreased significantly (*ca.* 25%) during anthesis (Fig. 1A). The water content fell significantly from 1 DBA to 7 HAA pollen (from 62% to 22%; Fig. 1A). As reported previously for this species (Gay *et al.* 1987; Nepi & Pacini 1993; Nepi *et al.* 2010), the pollen of pumpkin cv. Arberello di Sarzana is recalcitrant and has

a short life. The trends in reduced pollen viability and water content with ageing in this cultivar are similar to those recorded in cv. Greyzini (Nepi & Pacini 1993). In contrast, both of these variables decreased faster in the cv. Tondo di Nizza (Nepi *et al.* 2010). In general, there is a threshold value of percentage water below which pollen viability is quickly lost (Firon *et al.* 2012), which was determined as 13% in pumpkin cv. Tondo di Nizza (Nepi *et al.* 2010). That value was not reached by the 7 HAA pollen analysed in this study, which may explain why viability did not fall below 50%. The higher amount of carbohydrate reserves (see below), in contrast to cv. Tondo di Nizza (cf. Nepi *et al.* 2010), may be related to increased resistance. Nevertheless, a deterioration in cellular functions can be expected as time proceeds after anther opening (see below) when the pollen loses water, together with a recorded drop in viability.

Soluble and insoluble carbohydrate utilisation and homeostasis

Sucrose, glucose and fructose were found in all the development stages examined. All three substances increased to different degrees from immature to mature pollen and during ageing (Table 1, Fig. 1B). The proportion of glucose, fructose and sucrose was, respectively, 41%, 24% and 35% in immature pollen, 39%, 24% and 37% in mature pollen, and 28%, 25% and 47% in 7 HAA pollen. Thus, the proportions remained almost unchanged during final maturation, while they were modified during ageing, when sucrose quantity reached the highest level (Table 1).

The locular fluid at 1 DBA was highly viscous and sticky. Glucose, fructose and sucrose were present in the locular fluid in similar proportions to the immature pollen at 1 DBA, *i.e.* 40%, 23% and 37%, respectively.

Starch was always abundant; starch amount increased significantly from 1 DBA to the mature pollen ($F = 37.33$, $P = 0.0036$) and then fell significantly in 7 HAA pollen (*ca.* 40%; $F = 285.426$, $P \leq 0.0001$; Table 1, Fig. 1B). Starch also increased significantly during rehydration ($F = 4.049$, $P = 0.1131$; Table 1, Fig. 1B).

As previously reported for pumpkin pollen (Nepi *et al.* 2010), in all the stages examined starch was the dominant carbohydrate over sucrose, glucose and fructose. During final maturation, all these substances were stored, which would suggest a continuous uptake of resources from the locular fluid inside the anther (Fig. 1B), since pollen grains

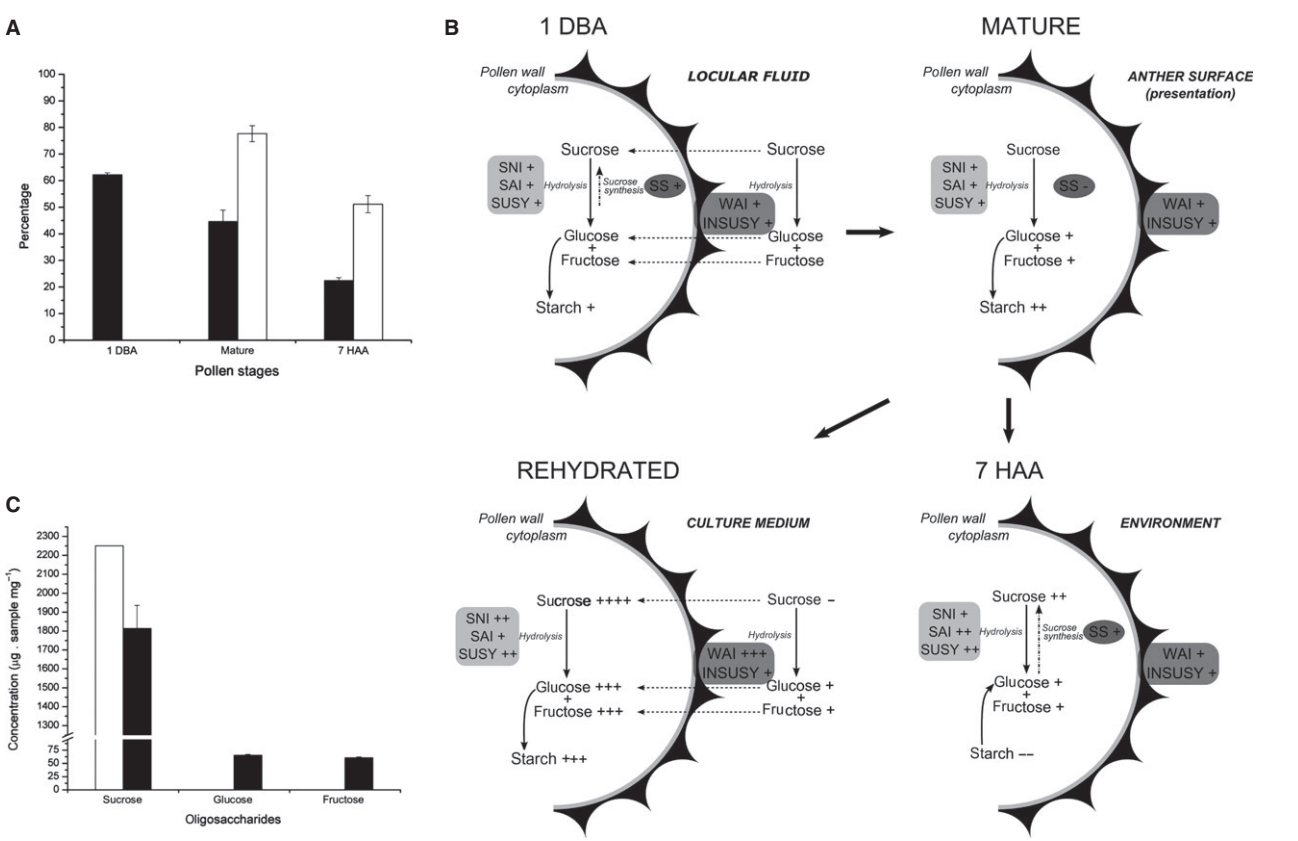

Fig. 1. Pollen features and carbohydrate metabolism in pumpkin pollen at different stages. (A) Pollen viability (white) and water content (black) fluctuations. (B) Patterns of carbohydrate metabolism (enzyme activity and carbohydrate content) and relation to the surrounding environment. Plus (+) and minus (–) indicate fluctuations in carbohydrate concentrations and enzyme activity, not comparable between substances and enzymes. (C) Mono- and disaccharide content of the culture medium used for pollen rehydration. White: start of the experiment (T0, original concentration); black: after 10 min of pollen culture (T1). 1 DBA, immature pollen 1 day before anthesis; 7 HAA, mature pollen exposed for 7 h after anthesis; SAI, soluble acid invertase; SNI, soluble neutral invertase; SUSY, soluble sucrose synthase (cleavage direction); WAI, wall-bound acid invertase; INSUSY, insoluble sucrose synthase (cleavage direction); SS, sucrose synthase (synthesis direction).

Table 1. Carbohydrate content in pumpkin pollen at different stages (μg sample mg^{-1}).

	carbohydrates				
pollen stages	glucose	fructose	sucrose (soluble fraction)	sucrose (insoluble fraction)	starch
1 DBA	6.441 ± 0.480^{a}	3.841 ± 0.015^{a}	5.44 ± 0.402^{a}	0	133.653 ± 4.051^{a}
mature	11.160 ± 0.689^{ab}	7.631 ± 0.723^{abc}	9.718 ± 1.092^{abc}	0.416 ± 0.103^{a}	162.291 ± 0.035^{abc}
7 HAA	11.910 ± 0.227	10.959 ± 0.253^{b}	20.559 ± 1.768^{b}	1.167 ± 0.404^{a}	92.762 ± 1.148^{b}
rehydrated	67.673 ± 1.022^{b}	63.801 ± 0.212^{b}	191.585 ± 23.02^{c}	0	192.530 ± 10.63^{c}

1 DBA = immature pollen from 1 day before anthesis; 7 HAA = mature pollen exposed for 7 h after anthesis.
Values sharing the same letter in each column are different at the 95% level (ANOVA; pairs of stages compared: 1 DBA *versus* mature, mature *versus* 7 HAA, mature *versus* rehydrated).

are a sink during microsporogenesis (Karni & Aloni 2002). Although Hoekstra & van Roekel (1988) suggested that pollen grains would already be independent of the rest of the plant at 1 DBA, this is not the case for pumpkin pollen, likely because of the lack of a dormancy period. The presence of some locular fluid at 1 DBA, containing mono- and disaccharides, reinforces the result.

Since pollen grains were isolated during ageing, starch must have been the source of carbohydrates throughout this period, leading to its reduction (Fig. 1B). However, a higher quantity of starch was digested compared to the accumulated mono- and disaccharides, so that this must have been actively consumed. The decline in pollen viability could be caused mainly by high nutrient consumption due to high-intensity respiration (Geng *et al.* 2013), which may be the case for pumpkin pollen during exposure because it does not have a dormancy period. Furthermore, sucrose is needed to protect membranes and preserve protein structure during desiccation (Hoekstra *et al.* 1989), since pollen grains lose water quickly following exposure. This may explain the sucrose increment during ageing. However, although sucrose was synthesised, soluble sucrolytic activity increased (see below; Fig. 1B), which must have led to sucrose loss. A futile cycle may have been established where sucrose was digested because resources were needed to maintain cytoplasm activity, while sucrose was synthesised at the same time, consuming the hexoses for protective functions (Fig. 1B). Starch may have an important role by providing resources to sustain the opposing pathways working together. Eventually, perhaps the prevalence of one pathway over the other, leading to loss of homeostasis, determines the drop in pollen vitality over time.

Mature pollen reacted immediately in the culture medium. Incipient cytoplasmic protrusions from the pollen grains appeared after 10 min of incubation, while carbohydrates showed important changes. Sucrose amount decreased *ca*. 20% in the culture medium from T0 to T1, while low amounts of glucose and fructose were present (Fig. 1C). Both mono- and disaccharide concentrations increased significantly in the rehydrated pollen in contrast to the mature pollen (fivefold glucose, $F = 20775.7$, $P \leq 0.0001$; sevenfold fructose, $F = 1108.01$, $P \leq 0.0001$; 13-fold sucrose, $F = 186.855$, $P = 0.0002$; Table 1). The fluctuations in carbohydrate concentrations inside the pollen and in the culture medium suggest that exogenous sucrose was hydrolysed, mostly outside the pollen grains, while the hexoses released were imported into the pollen grains (Fig. 1B). Moreover, because there was no detectable sucrose synthesis during rehydration (see below), the high increment of endogenous soluble sucrose suggests the import of sucrose, regardless of possible extracellular hydrolysis. As in the immature pollen, it seems that both hexoses and sucrose are imported by the mature pollen from the surrounding environment, in this case, from the culture medium (Fig. 1B).

Despite the high increment of endogenous glucose and fructose, both substances must also be consumed during rehydration, either for glycolysis, since metabolism was clearly active, or for starch storage. Starch may function as an ultimate reserve for substances used by pollen tubes, a feature already observed in other species (Kessler *et al.* 1960; Dickinson 1968; Singh *et al.* 1978; Bellani *et al.* 1985).

A very low amount of sucrose was quantified in the insoluble fraction only in mature and 7 HAA pollen (Table 1), despite the extensive washing undertaken. The presence of sucrose attached somehow to cell membranes/walls was a surprising feature. A possible connection between sucrose and the membranes can be presumed, bearing in mind the protective role of sucrose against desiccation (Hoekstra *et al.* 1989).

Enzymes for inter-conversion of carbohydrates

The activity of all the sucrolytic enzymes, both soluble and insoluble, was almost constant during maturation (Table 2, Fig. 1B). The proportion of sucrolysis accomplished by the insoluble enzymes was 86–87% of the total in these stages. The minor changes in enzyme activity might be related to the recalcitrant nature of pumpkin pollen and the absence of a dormancy period, which contrasts with trends observed in orthodox pollen (Goetz *et al.* 2001; Pressman *et al.* 2006, 2012; Carrizo García *et al.* 2013). The activity of soluble sucrolytic enzymes increased to different degrees in the 7 HAA pollen (significantly for SAI: $F = 14.101$, $P = 0.0199$; and SUSY: $F = 39.446$, $P = 0.0033$), whereas insoluble sucrolytic activity decreased dramatically by this stage (*ca*. fourfold for WAI: $F = 103.253$, $P = 0.002$; and 12-fold for INSUSY: $F = 182.157$, $P = 0.0009$; Table 2, Fig. 1B). The proportion of sucrolysis accomplished by the soluble enzymes increased to *ca*. 56% from mature to 7 HAA pollen, slightly more than for the insoluble enzymes. Most sucrolytic activity increased significantly during rehydration (SNI: $F = 2.761$, $P = 0.172$; SAI: $F = 0.763$, $P = 0.431$; SUSY: $F = 27.87$, $P = 0.006$), except for soluble acid invertase and insoluble sucrose synthase (cleavage direction) (Table 2, Fig. 1B). The largest increase, of *ca*. 50%, was recorded for wall-bound acid invertase ($F = 41.961$, $P = 0.0029$; Table 2). Sucrose synthase (synthesis direction) was active in the synthesis direction, although at very low levels, during final

Table 2. Enzyme activity in pumpkin pollen at different stages (μg reducing sugars released $min^{-1} \cdot mg^{-1}$ total protein; average ± SD).

	enzymes						
pollen stages	SAI	SNI	SUSY	WAI	INSUSY	SS	amylases
1 DBA	11.536 ± 1.940	10.008 ± 0.233	10.933 ± 1.607	124.536 ± 9.443	92.475 ± 2.055	0.143 ± 0.014[a]	1.425 ± 0.144[a]
mature	10.998 ± 0.774[a]	11.376 ± 0.837[a]	13.197 ± 0.459[ab]	124.696 ± 12.37[ab]	95.331 ± 4.675[a]	0.032 ± 0.008[ab]	0.629 ± 0.109[ab]
7 HAA	14.249 ± 1.301[a]	13.287 ± 1.041	19.862 ± 1.785[a]	29.713 ± 2.887[a]	8.069 ± 0.741[a]	0.103 ± 0.009[b]	0.578 ± 0.020
rehydrated	12.028 ± 1.900	19.54 ± 1.297[a]	16.907 ± 1.13[b]	191.715 ± 12.96[b]	94.594 ± 12.47	0	1.187 ± 0.283[b]

1 DBA = immature pollen from 1 day before anthesis; 7 HAA = mature pollen exposed for 7 h after anthesis; SAI = soluble acid invertase; SNI = soluble neutral invertase; SUSY = soluble sucrose synthase (cleavage direction); WAI = wall-bound acid invertase; INSUSY = insoluble sucrose synthase (cleavage direction); SS = sucrose synthase (synthesis direction).
Values sharing the same letter in each column are different at the 95% level (ANOVA; pairs of stages compared: 1 DBA *versus* mature, mature *versus* 7 HAA, mature *versus* rehydrated).

maturation and throughout ageing, but not during rehydration (Table 2, Fig. 1B). Sucrose phosphate synthase was not active under any condition applied in any stage/process analysed (Table 2).

Extracellular sucrolysis was the most important pathway for sucrose hydrolysis in pumpkin pollen. As regards final maturation, hexoses found in the locular fluid may be by-products of sucrolysis accomplished by wall-bound acid invertase in the developing pollen grains (Fig. 1B), since sucrose is the main nutrient provided by the sporophyte tissue. The key role of wall-bound acid invertase during pollen development has been recorded in several species (*e.g.* Goetz *et al.* 2001; Pressman *et al.* 2006, 2012; Proels *et al.* 2006; Jain *et al.* 2007). Because levels of endogenous sucrose cleavage and synthesis were low, import of carbohydrates from the locular fluid will be critical for pollen ripening, suggesting a key role for the insoluble sucrolytic enzymes.

A dramatic reduction in activity of both insoluble sucrolytic enzymes, which were not in contact with the pollen reserves, was observed during ageing. This trend may be related to the absence of exogenous nutrients. Pollen grains were exposed isolated, in conditions not favourable for germination, and therefore these enzymes would be unnecessary. Moreover, the behaviour of these enzymes may also be a consequence of the loss of pollen vitality.

During pollen rehydration there was a strong tendency for cleavage of exogenous sucrose. The absence of sucrose synthesis during rehydration and the lack of sucrose in the pollen insoluble fraction suggest the readiness to hydrolyse it. Total exogenous sucrose consumption from the medium was reported in developing *Petunia* pollen tubes (Ylstra *et al.* 1998) and attributed to wall-bound acid invertase activity. However, the most important changes in pollen tubes were observed in the soluble invertases in other cases, while wall-bound acid invertase activity was lower and/or remained constant (Singh *et al.* 1978; Nakamura *et al.* 1980). In the pumpkin pollen, both wall-bound acid invertase and insoluble sucrose synthase (cleavage direction) were most active during rehydration, with wall-bound acid invertase by far the most important. Insoluble sucrose synthase (cleavage direction) is an enzyme that is usually neglected, although some forms have been reported in *Nicotiana* pollen tubes (Persia *et al.* 2008). The steady level of insoluble sucrose synthase (cleavage direction) activity across the stages analysed, except for 7 HAA pollen, may be due to the absence of considerable cell growth, since it is related to cell wall construction (Persia *et al.* 2008). Nevertheless, insoluble sucrose synthase (cleavage direction) activity was high in contrast to the soluble enzymes and thus can be considered an important enzyme in sucrose catabolism in pumpkin pollen.

Amylase activity was always very low, decreasing gradually towards pollen maturation and with age, and increasing during rehydration (Table 2). Strikingly, amylolysis was higher at 1 DBA and during rehydration, when there was significant starch storage, while it was lower in 7 HAA pollen, when a significant amount of starch was hydrolysed. These data indicate a major role for amylogenesis (not analysed), which might exceed by far the rates of amylolysis, except during ageing, thus determining the availability of starch.

CONCLUSIONS

Pumpkin pollen seems to be highly active and closely related to its surrounding environment at all the stages analysed; effects of environment are particularly evident among the insoluble sucrolytic enzymes. Each stage was characterised by a specific metabolic and enzymatic profile (Fig. 1B), *i.e.* reserve storage and sustaining cellular activity in the late maturation and mature pollen; carbohydrate reserve consumption and water loss during ageing; and exogenous nutrient catabolism and further starch storage during rehydration. Some processes seem to be common to pollen grains in general, although the reserves stored and the main enzyme activities vary among species and probably among varieties. However, the rapid metabolic changes in pumpkin pollen are remarkable. Moreover, important differences from orthodox pollen are observed during maturation and ageing, features related to the lack of dormancy and the recalcitrant nature of pumpkin pollen.

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