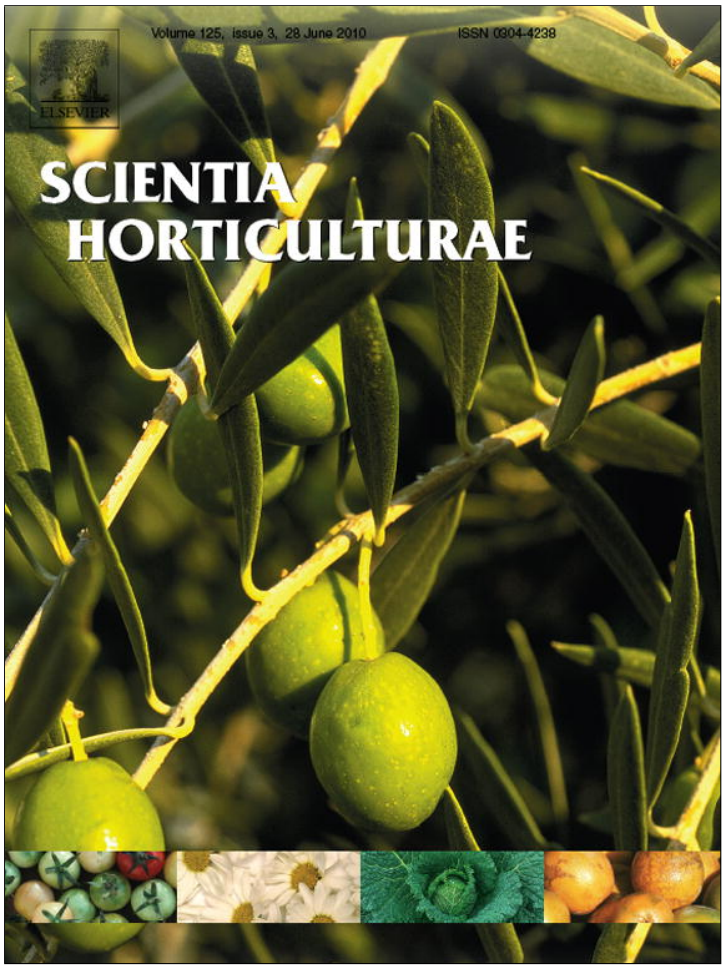

Scientia Horticulturae 125 (2010) 524–527

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Short communication

Soluble carbohydrates content in tomato pollen and its variations along and between blooming periods

Carolina Carrizo García[a,*], Massimo Guarnieri[b], Ettore Pacini[b]

[a] Instituto Multidisciplinario de Biología Vegetal (CONICET-UNC), Av. Velez Sarsfield 299, CC 495 CP 5000, Córdoba, Argentina
[b] Department of Environmental Sciences, Siena University, Via P.A. Mattioli 4, 53100, Siena, Italy

ARTICLE INFO

Article history:
Received 17 February 2010
Received in revised form 13 April 2010
Accepted 16 April 2010

Keywords:
Carbohydrates variations
Fructose
Glucose
Maltosaccharides
Tomato pollen

ABSTRACT

The soluble carbohydrates content in the mature (starchless) pollen of the tomato (*Solanum lycopersicum* L.) cv. Platense was studied at several moments of the blooming period in two consecutive years. The aim of the analysis was to evaluate if the content of soluble carbohydrates is relatively constant or if it can fluctuate along the blooming period. No significant variations in pollen viability were recorded along each season. The soluble carbohydrates found and their concentrations can change significantly among samples, but the fluctuations observed did not follow a strongly definite pattern in any season. Reducing sugars predominated; small quantities of a phosphorylated sugar, UDP-glucose, and maltosaccharides were also recorded. The constant presence of maltosaccharides is a novel record for pollen. Sucrose was absent in one season, but present in the other, in low percentages in contrast to reducing sugars. Changes in the soluble carbohydrates content have been usually related with alterations in pollen fertility. However, there may be some flexibility in the metabolism of the pollen studied this time, at least within a certain range, which may allow constant adjustments to maintain acceptable levels of viability despite the variations in the carbohydrates concentrations.

1. Introduction

In plants reproduction, pollen grains carry the sperm cells to the pistil, ultimately to the ovules through the pollen tubes, to achieve fertilization. Successful fertilization is a key factor for many crop species. Among the different aspects related to pollen fertility and functioning, the relevance of the carbohydrates metabolism has been demonstrated by a number of studies (e.g. for tomatoes: Pressman et al., 2002; Firon et al., 2006; Nashilevitz et al., 2009). Although the male function can vary naturally along the plant life, especially in perennials (e.g. Bellani et al., 1985a,b; Dag et al., 2000), the studies of the carbohydrates metabolism in pollen have been generally done in standardized conditions, and eventual natural variations have been overlooked. Regarding soluble carbohydrates, even if they can be variable among species (e.g. Aloni et al., 2001; Castro and Clément, 2007), it has not yet been investigated if the soluble carbohydrates content in the pollen of a species is constant or if it can fluctuate along the blooming period. To address this question, pollen samples were taken along two blooming periods in a tomato cultivar to describe its soluble carbohydrates content and to define any degree of variation.

2. Materials and methods

The observations were made in the tomato (*Solanum lycopersicum* L.) cv. Platense. Plants were grown in the field in Siena Botanical Garden, Italy, in 2006. The plants flowered between May and September (spring–summer; mean temperatures from 17 to 32 °C). Seeds obtained from these plants were sown in 2007 in Cordoba, Argentina, and the plants were grown in pots in a partially heated greenhouse during winter–spring. The plants flowered from mid-July to the beginning of October (mean temperatures from 18 to 25 °C). The sampling was done during the peak of flowering (July–August in 2006, mid-August to mid-September in 2007), from the same group of plants within each year. A couple of samples from the beginning and the end of the season were also included in 2006. Each sample contained pollen from 1 to 2 days, obtained from several flowers (10–50). The quantifications will be referred to the pollen fresh weight.

The fluorochromatic test (Heslop-Harrison et al., 1984) was made regularly along each season to determine pollen viability. Mixed pollen from 10 to 15 flowers was used each time, counting stained pollen over 300 (3 × 100). The percentage of viable pollen was more or less constant along each season; therefore average values will be shown.

The pollen was homogenized in distilled water and the soluble fractions were separated by centrifugation at 4 °C. For the 2006 samples, soluble carbohydrates were determined in these fractions

* Corresponding author. Tel.: +54 351 4331056; fax: +54 351 4332104.
E-mail address: ccarrizo@imbiv.unc.edu.ar (C. Carrizo García).

0304-4238/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.scienta.2010.04.026

without further treatment through HPLC. A Sugar-Pack column (Waters Co.) was used, with water as mobile phase (flow rate 0.5 ml min^{-1}, at 90 °C), and refractometric detection. Each sample was analyzed three times. Most peaks were identified by comparison with the retention times of pure substances. A particular peak had an ambiguous identification, either fructose-6-phosphate (Fru-6P) or glucose-6-phosphate (Glc-6P), whose retention times coincided. The method proposed by Shen and Perreault (1998) was followed to solve this conflict. Glc-6P could not be detected; therefore the presence of Fru-6P was indirectly suggested. Nevertheless, this issue was left unsolved. An early peak could not be identified with standard sugars, then called 'unidentified peak' (UP), even though a number of carbohydrates were tested, i.e. maltoheptaose, stachyose, raffinose, melezitose, maltose, melibiose, lactose, xylose, galactose, rhamnose, mannose, and arabinose. None of these substances eluted as early as the UP. Two facts lead to support the hypothesis of a maltosaccharide of high polymerization degree. First, the UP appeared in the left end of the chromatogram, in an area where products of partial starch hydrolysis can be found (pers. obs.). Second, the polymerization degree of maltosaccharides decreased along the chromatogram, and the UP was found ca. 2 min before the retention time of maltoheptaose. Indirect evidence supported this hypothesis, since the UP, as well as the maltopentaose peak (the maltosaccharide present in the samples tested), disappeared after incubation of the samples with the enzyme amyloglucosidase. However, because the UP was present only in a few samples at the beginning of the blooming period in 2006, making more precise measurements and tests was not possible. The peak area was codified in the samples in which the UP was present in order to compare them.

A colorimetric approach was followed in 2007 to quantify total reducing sugars, sucrose and maltosaccharides. The soluble fractions were boiled for 10 min and divided in three aliquots for the different assays; those for sucrose and maltosaccharides quantification were vacuum dried and resuspended in different buffers (0.2 M sodium acetate buffer, pH 4.5, and 50 mM sodium acetate buffer, pH 4.5, respectively). Total reducing sugars were directly tested using dinitrosalicylic acid (DNSA) reagent, reading the absorbance at 560 nm in a spectrophotometer (Castro and Clément, 2007). Sucrose and maltosaccharides were measured after specific enzymatic hydrolysis, as the equivalent of reducing sugars released, quantified with DNSA reagent, as described above. The samples were incubated in controlled conditions with the appropriate enzyme (30 units invertase mL^{-1} for sucrose, 5 units amyloglucosidase mL^{-1} for maltosaccharides). Each assay was done three times for each sample.

The degree of change of pollen viability and the variations in carbohydrates concentrations were evaluated with one-way ANOVA ($P = 0.05$).

3. Results and discussion

The average percentage of pollen viability in 2006 was slightly higher in the first part (74.50 ± 4.21 for June/July) than in the second one (71.75 ± 4.57 for August/September), but the difference was not significant. Pollen viability was slightly higher in the 2007 sampling, reaching a peak of 81.25 ± 3.50% in the period whose samples are reported here.

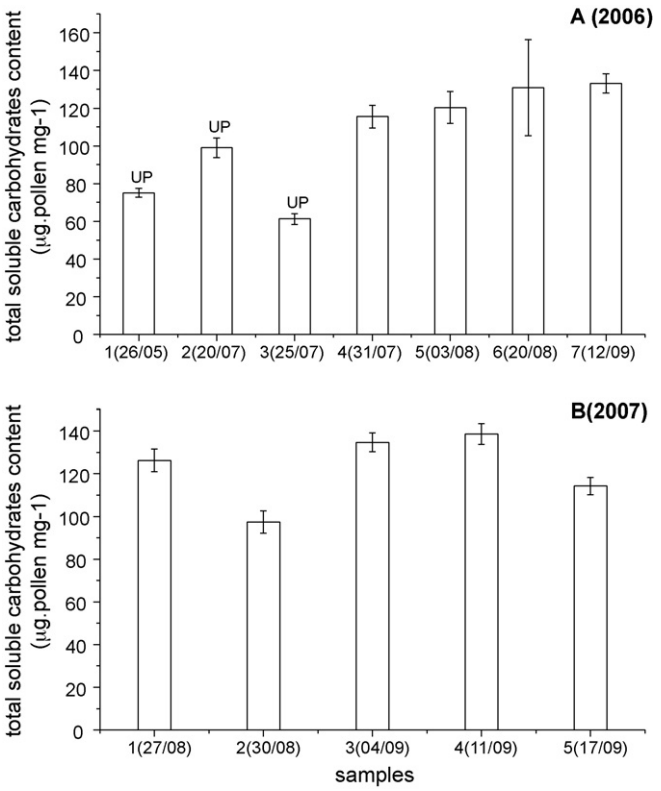

Fig. 1. Total soluble carbohydrates content in pollen from different dates (dd/mm) in two blooming periods sampled in tomato Platense. Mean values ± S.E. A: 2006 sampling. The bars marked with 'UP' do not include the 'unidentified peak' (possibly a maltosaccharide), absent in the other 2006 samples. B: 2007 sampling.

The content of soluble carbohydrates changed among samples collected at different times in both seasons. Some selected samples are reported here, which evidence the phenomenon observed. The total soluble carbohydrates content showed a certain tendency to increase as the time passed in 2006 (Fig. 1A), but the values might be underestimated in the samples 1–3 due to the presence of the UP (Fig. 1A and Table 1), which could not be quantified. The total amount of soluble sugars in 2007 varied without a definite trend in a range partially overlapped with that of 2006 (Fig. 1B).

Glucose and fructose/total reducing sugars were the main soluble carbohydrates (Tables 1 and 2). Glucose and fructose were in similar concentrations in 2006, but variable among samples (Table 1). The UP, which might be a maltosaccharide, was present only in the samples 1–3, which had significant lower quantities of glucose and fructose than the samples 4–7 (Table 1). The total reducing sugars varied among the 2007 samples, following signif-

Table 1
Soluble carbohydrates in pollen grains from different dates (dd/mm) along the 2006 blooming period in tomato Platense. Mean values ± S.E. Identical letters within each column represent pair of data with significant differences (ANOVA, $P = 0.05$). The values in the 'unidentified peak' (UP) correspond to a codification of the peak area (1 = 7.50 mV s^{-1}).

Pollen samples	µg soluble carbohydrates pollen mg^{-1}						
	Glucose	Fructose	UP	Fru-6P/Glc-6P	UDP-Glc	Maltopentaose	Maltohexaose
1 (26/05)	37.67 ± 1.40^{a,b,c}	36.17 ± 1.30^{a,b,c}	1.00 ± 0.20	–	–	1.25 ± 0.30^{a}	–
2 (20/07)	43.67 ± 0.31^{d,e}	44.89 ± 1.41	46.09 ± 1.20	–	–	7.84 ± 2.46^{a,b}	–
3 (25/07)	28.78 ± 1.09^{f,g,h}	27.93 ± 1.17^{d,e,f,g}	12.26 ± 0.50	–	1.21 ± 0.50	1.08 ± 0.40	1.12 ± 0.40^{a}
4 (31/07)	51.68 ± 4.09^{f}	59.55 ± 0.72^{a,d}	–	3.32 ± 0.25	–	0.99 ± 0.28^{b}	2.89 ± 0.15^{a}
5 (03/08)	59.82 ± 6.02^{a}	56.51 ± 0.72^{e}	–	2.95 ± 0.14	–	4.06 ± 2.04	–
6 (20/08)	63.21 ± 8.22^{b,d,g}	63.81 ± 14.75^{b,f}	–	2.59 ± 1.12	–	3.85 ± 2.46	–
7 (12/09)	61.25 ± 1.06^{c,e,h}	65.00 ± 2.67^{c,g}	–	–	2.33 ± 0.32	6.93 ± 1.34	–

Table 2
Soluble carbohydrates in pollen grains from different dates (dd/mm) along the 2007 blooming period in tomato Platense. Mean values ± S.E. Identical letters within each column represent pair of data with significant differences (ANOVA, $P = 0.05$).

Pollen samples	μg soluble carbohydrates pollen mg^{-1}		
	Reducing sugars	Sucrose	Maltosaccharides
1 (27/08)	112.33 ± 1.86^{a,b}	2.89 ± 0.50^{a,b,c}	10.96 ± 2.99^{a,b}
2 (30/08)	86.65 ± 2.86^{a,c,d,e}	3.21 ± 0.92^{d,e,f}	7.45 ± 1.42
3 (04/09)	113.06 ± 3.15^{c,f}	16.13 ± 0.96^{a,d,g}	5.49 ± 0.30
4 (11/09)	107.46 ± 1.46^{d,g}	29.65 ± 2.83^{b,e,g}	1.46 ± 0.60^{a}
5 (17/09)	95.99 ± 1.68^{b,e,f,g}	15.88 ± 1.92^{c,f}	2.38 ± 0.50^{b}

icant changes between most samples (Table 2). Sucrose was not detected in the 2006 sampling, while it was found in all the samples analyzed in 2007 (Table 2). Sucrose concentration was highly variable, representing from ca. 2 to 21% of the total amount of soluble carbohydrates (cf. samples 1 and 2 vs. 3 to 5, Table 2). The trait of presence/concentration changes of sucrose is striking since it was the main soluble sugar in other tomato cultivars (Pressman et al., 2002; Firon et al., 2006). Besides, sucrose is important for pollen survival because it protects the membranes during desiccation, while monosaccharides are less effective in that function (Hoekstra et al., 1992). Sucrose is absent or scarce in short living pollen grains, which germinate rapidly and would need less protection against desiccation; some of these cases coincide with partially hydrated pollen grains (e.g. Cucurbitaceae – Franchi et al., 1996; Nepi et al., 2010). Conversely, according to our previous observations, the pollen studied here is partially dehydrated (water content ca. 20%), and its viability is over 60% during the three days of the flower life. Then, the pollen homeostasis has to be maintained at least during those days. Regarding the membranes protection, maybe the action of glucose and fructose could be enough, since the pollen remains inside the anthers during presentation. However, the degree of fatty acid unsaturation in the membranes also plays an important role (Hoekstra et al., 1992).

The presence of more glucose and fructose than sucrose could mean instant energy for germination. Fru-6P and Glc-6P are important during germination, since Fru-6P is at the beginning of the glycolysis process (Dennis and Blakeley, 2000), while Glc-6P, through UDP-Glc, is involved in synthesis of the pollen tube wall components (Dennis and Blakeley, 2000). Small amounts of Fru-6P/Glc-6P and UDP-Glc could be quantified in some 2006 samples (Table 1). Putting these facts together, it could be wondered whether this pollen is somehow active or ready to be activated, i.e. ready for a fast germination, a feature that could also vary according to the quantifiable amounts of Fru-6P/Glc-6P and UDP-Glc.

Disregarding the UP, maltosaccharides were present in all the samples of both seasons (Tables 1 and 2). In 2006, small quantities of maltopentaose and maltohexaose were found, simultaneously in some samples, showing irregular variations (Table 1). The total amount of maltosaccharides in 2007 also varied erratically, reaching similar levels to those registered in 2006 (Table 2). The constant presence of maltosaccharides is a remarkable feature, since they have not been recorded in pollen so far. Maltosaccharides, maltose and glucose are products of starch hydrolysis (Dennis and Blakeley, 2000). In the pollen of tomato, starch is accumulated during its development and hydrolyzed just before anthesis; mature pollen has only a low amount of starch (Polowick and Sawhney, 1993; Pressman et al., 2002); the variety Platense shares this feature (Carrizo García et al., 2009). A final dehydration causes the arrest of pollen development when the anthers open (Pacini et al., 2006), and the degree of starch hydrolysis reached at that moment could be variable, producing different types and quantities of maltosaccharides along both seasons. Eventual oscillations in the conditions that influence pollen dehydration may be one cause for this trend.

Although the total amount of soluble carbohydrates fluctuated within a certain range in both seasons analyzed, the content varied among the samples taken in subsequent days, as well as between seasons. Because the changes observed involved not only the quantity of each carbohydrate but also the type of substances found (e.g. sucrose), this fact did not seem irrelevant. However, it is worth to remember that the pollen viability did not vary significantly during each flowering season; therefore the differences observed in the carbohydrates did not imply critical changes in pollen fertility, as it has been observed in other tomato varieties under stress (e.g. Pressman et al., 2002; Firon et al., 2006). The variations registered here could be related to metabolic changes that can naturally occur during the plants life, and/or they may be linked to slight changes in the environmental conditions that did not reach stressful levels (within and between seasons). Even so, it is remarkable that, despite the differences in the growing conditions between years and the carbohydrate content fluctuations, some general features were maintained (e.g. prevalence of reducing sugars, presence of maltosaccharides); thus the basic pollen physiology did not vary considerably. The present observations evidence some flexibility in the pollen metabolism, at least within a certain range, which may allow adjustments to maintain acceptable levels of viability despite eventual variations in the carbohydrates content, to continue successful reproduction.

Acknowledgments

CCG thanks the scholarship awarded by the Ministero degli Affari Esteri (Italia) and to Consejo Nacional de Investigaciones Científicas y Técnicas for the financial support. Italian authors are indebted to Piano di Ricerca dell'Ateneo for funding pollen research. The valuable help of the Botanical Garden of Siena's staff is also appreciated.

References

Aloni, B., Peet, M., Pharr, M., Karni, L., 2001. The effect of high temperature and high atmospheric CO_2 on carbohydrate changes in bell pepper (*Capsicum annuum*) pollen in relation to its germination. Physiol. Plant. 112, 505–512.

Bellani, L.M., Pacini, E., Franchi, G.G., 1985a. *In vitro* pollen grain germination and starch content in species with different reproductive cycle. I. *Lycopersicon peruvianum* Mill. Acta Bot. Neerl. 34, 59–64.

Bellani, L.M., Pacini, E., Franchi, G.G., 1985b. *In vitro* pollen grain germination and starch content in species with different reproductive cycle. I. *Malus domestica* Borkh. Cultivars Starkrimson and Golden Delicious. Acta Bot. Neerl. 34, 65–71.

Carrizo García, C., Guarnieri, M., Pacini, C., 2009. Particularities of the carbohydrate content in pollen and pollen tubes of a tomato variety, Annual Meeting of the Working Groups 'Cellular and Molecular Biology' and 'Biotechnology and Differentiation'. Italian Botanical Society, p. 53.

Castro, A.J., Clément, C., 2007. Sucrose and starch catabolism in the anther of *Lilium* during its development: a comparative study among the anther wall, locular fluid and microspore/pollen fractions. Planta 225, 1573–1582.

Dag, A., Eisenstein, D., Gazit, S., 2000. Effect of temperature regime on pollen and the effective pollination of 'Kent' mango in Israel. Sci. Hortic. 86, 1–11.

Dennis, D.T., Blakeley, S.D., 2000. Carbohydrate metabolism. In: Buchanan, B., Gruissem, W., Jones, R. (Eds.), Biochemistry and Molecular Biology of Plants. American Society of Plant Physiologist, Rockville, MA, pp. 630–675.

Firon, N., Shaked, R., Peet, M.M., Pharr, D.M., Zamski, E., Rosenfeld, K., Althan, L., Pressman, E., 2006. Pollen grains of heat tolerant tomato cultivars retain higher carbohydrate concentration under heat stress conditions. Sci. Hortic. 109, 212–217.

Franchi, G.G., Bellani, L., Nepi, M., Pacini, E., 1996. Types of carbohydrate reserves in pollen: localization, systematic distribution and ecophysiological significance. Flora 191, 143–159.

Heslop-Harrison, J.S., Heslop-Harrison, Y., Shivanna, K.R., 1984. The evaluation of pollen quality and a further appraisal of the fluorochromatic (FCR) test procedure. Theor. Appl. Genet. 67, 367–375.

Hoekstra, F.A., Crowe, J.H., Crowe, L.M., van Bilsen, D.G.J.L., 1992. Membrane behaviour and stress tolerance in pollen. In: Ottaviano, E., Mulcahy, D.L., Sari Gorla, M., Bergamini Mulcahy, G. (Eds.), Angiosperm Pollen and Ovules. Springer Verlag, New York, pp. 177–186.

Nashilevitz, S., Melamed-Bessudo, C., Aharoni, A., Kossmann, J., Wolf, S., Levy, A., 2009. The *legwd* mutant uncovers the role of starch phosphorylation in pollen development and germination in tomato. Plant J. 57, 1–13.

Nepi, M., Cresti, L., Guarnieri, M., Pacini, E., 2010. Effect of relative humidity on water content, viability and carbohydrate profile of *Petunia hybrida* and *Cucurbita pepo* pollen. Plant Syst. Evol. 284, 57–64.

Pacini, E., Guarnieri, M., Nepi, M., 2006. Pollen carbohydrates and water content during development, presentation, and dispersal: a short review. Protoplasma 228, 73–77.

Polowick, P.L., Sawhney, V.K., 1993. An ultrastructural study of pollen development in tomato (*Lycopersicon esculentum* Mill.). II. Pollen maturation. Can. J. Bot. 71, 1048–1055.

Pressman, E., Peet, M.E., Pharr, D.M., 2002. The effect of heat stress on tomato pollen characteristics is associated with changes in carbohydrates concentration in the developing anthers. Ann. Bot. 90, 631–636.

Shen, X., Perreault, H., 1998. Characterization of carbohydrates using a combination of derivatization, high performance liquid chromatography and mass spectrometry. J. Chromatogr. A 811, 47–59.