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SHORT COMMUNICATION

Desiccation tolerance in relation to soluble sugar contents in seeds of ten coffee (*Coffea* L.) species

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Abstract

Large differences in seed desiccation sensitivity have been observed previously among ten coffee species (*Coffea arabica*, *C. brevipes*, *C. canephora*, *C. eugenioides*, *C. humilis*, *C. liberica*, *C. pocsii*, *C. pseudozanguebariae*, *C. sessiliflora* and *C. stenophylla*). Of these species, *C. liberica* and *C. humilis* were the most sensitive to desiccation and *C. pseudozanguebariae* the most tolerant. A study was carried out using the same seed lots to investigate if these differences in desiccation tolerance could be correlated with differences in soluble sugar content. Soluble sugars were extracted from dry seeds and analysed using high performance liquid chromatography. The seed monosaccharide (glucose and fructose) content was very low (1.5 to 2 mg g^{-1} dry weight [dw]) in all species studied. The sucrose content ranged from 33 mg g^{-1}dw in *C. liberica* seeds to 89 mg g^{-1}dw in seeds of *C. pocsii*. Raffinose was detected in the seeds of only five species (*C. arabica*, *C. brevipes*, *C. humilis*, *C. sessiliflora*, *C. stenophylla*), among which only three species (*C. arabica*, *C. sessiliflora* and *C. brevipes*) also contained stachyose. Both raffinose and stachyose were present in very low quantities (0.3–1.4 mg g^{-1}dw and 0.1–0.7 mg g^{-1}dw, respectively). Verbascose was never detected. No significant relationship was found between seed desiccation sensitivity and: (i) the sugar content; (ii) the presence/absence of oligosaccharides; and (iii) the oligosaccharide:sucrose ratio.

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Introduction

Soluble sugars have been proposed to play an important role in the desiccation tolerance and the high storability of orthodox seeds (see Leopold *et al.*, 1994; Vertucci and Farrant, 1995; Pammenter and Berjak, 1999 for a review). Two main hypotheses have been proposed to explain the potential beneficial effects of soluble sugar accumulation: the 'water replacement' hypothesis (Crowe *et al.*, 1992) and vitrification of the aqueous phase (Williams and Leopold, 1989; Leopold *et al.*, 1994).

The hypothesis that sugar composition might be used as an indicator for seed storage behaviour has been previously tested in two studies in which both recalcitrant and orthodox categories were comprehensively represented (Lin and Huang, 1994; Steadmann *et al.*, 1996). Sugar content was determined in the whole embryo in the study of Lin and Huang (1994) while it was determined separately in storage (cotyledons/endosperm) and embryonic (embryonic axis/embryo) tissues in Steadman *et al.* (1996). In both studies, the oligosaccharide:sucrose (O:S) ratio was found to be a good indicator of storage behaviour since it was significantly higher in orthodox seeds than in recalcitrant ones (Lin and Huang, 1994; Steadmann *et al.*, 1996). Within each of the orthodox and recalcitrant categories, Steadman *et al.* (1996) did not find any significant difference in the O:S ratio between reserve and embryonic tissues. For

seeds of oil palm and *Coffea arabica* which have an intermediate storage behaviour, these authors showed that embryos exhibited an O:S ratio equivalent to that of orthodox seeds, while the O:S ratio of the endosperm corresponded to values found in recalcitrant seeds. Thus, Steadman *et al.* (1996) concluded that the intermediate behaviour of oil and *C. arabica* seeds was more likely related to endosperm characteristics rather than to those of the embryo. In contrast, both the endosperm and embryo of intermediate seeds of papaya displayed an O:S ratio situated in the range of values found for orthodox seed tissues.

Intermediate species of the sub-genus *Coffea* display a broad variability in seed desiccation sensitivity (Hong and Ellis, 1995; Dussert *et al.*, 1999; Eira *et al.*, 1999a). In a previous study including nine coffee species, we have observed that the water content at which half of the initial viability was lost ranged from 0.05 g H_2O g^{-1} dry weight (dw) for *C. pseudozanguebariae* to 0.38 g H_2O g^{-1} dw for *C. humilis* (Dussert *et al.*, 1999). Intermediate seed species were little represented in the studies of of Lin and Huang (1994) and Steadman *et al.* (1996). In the present study, the possibility of using seed sugar composition as an indicator of seed behaviour in ten coffee species was tested.

Materials and methods

Seed desiccation sensitivity

Seeds of *Coffea brevipes* Hiern, *C. canephora* Pierre, *C. eugenioides* Moore, *C. humilis* Chevallier, *C. liberica* Hiern, *C. pocsii* Bridson, *C. pseudo-zanguebariae* Bridson, *C. sessiliflora* Bridson and *C. stenophylla* G. Don were provided by the stations of IRD-CNRA, Divo and IRD, Man, Côte d'Ivoire. Seeds of *C. arabica* L. were provided by CATIE, Turrialba, Costa-Rica. The seed desiccation sensitivity of nine of these ten coffee species has been studied previously (Dussert *et al.*, 1999) by measuring the production of normal seedlings after equilibration of seeds for 2 weeks at 25°C over 13 different saturated salt solutions ($BaCl_2$, K_2CO_3, K_2SO_4, KNO_3, KOH, $Mg(NO_3)_2$, $MgCl_2$, Na_2CO_3, $NaNO_3$, NaOH, NH_4Cl, NH_4NO_3, $(NH_4)_2SO_4$). Seeds were re-hydrated and cultured *in vitro* on a water gel under the conditions described by Dussert *et al.* (1997). Seed desiccation sensitivity has been quantified by the water content and the corresponding water activity at which half of the initial viability is lost, WC_{50} and a_{w50}, respectively. The desiccation sensitivity of *C. sessiliflora* seeds was studied and quantified using the same methods.

Sugar extraction and analysis

Sugar analysis was performed with the same seed lots as were used for desiccation sensitivity experiments. Seeds were dried for 2 weeks over silica gel before sugar extraction. Twenty to 40 seeds were ground in a ball mill (Dangoumill). Eighty milligrams of powder were used for extraction of sugars in 3 ml of ethyl alcohol 80% (v/v) containing 0.5 mg ml^{-1} lactose as an internal standard. Extracted samples were heated for 20 min at 80°C before centrifugation at 4000 rpm for 15 min. Each supernatant was collected and freeze-dried. Dried extracts were dissolved in 25 ml distilled water and filtered (0.22 μm pore diameter) before analysis. For each seed lot, sugar extraction was carried out five times. Sugar analysis was performed by anion exchange chromatography (Dionex Chromatography Co.). Sugar separation on a CarboPac PA-100 (Dionex) column was achieved using an isocratic eluant (NaOH, 150 mM). The sugar content was determined by comparison with retention times and calibration curves obtained with sugar standards (glucose, fructose, sucrose, raffinose and stachyose were obtained from Sigma and verbascose from Megazyme).

Results and discussion

The monosaccharide content was consistently very low in seeds of all the coffee species studied (Table 1); it ranged from 1.26 mg g^{-1} dw for *C. eugenioides* to 2.34 mg g^{-1} dw for *C. pseudozanguebariae*. Sucrose was the major soluble sugar in seeds of all the coffee species studied and its content was highly variable: it ranged from 33 mg g^{-1} dw for *C. liberica* to 89 mg g^{-1} dw for *C. pocsii*. The values obtained for *C. arabica*, *C. canephora*, *C. liberica* and *C. pseudozanguebariae* were equivalent to those mentioned in previous reports (Clifford, 1985; Trugo, 1988; Ky *et al.*, 2000). No oligosaccharides were found in five of the ten species studied. Raffinose was detected in seeds of *C. arabica*, *C. brevipes*, *C. humilis*, *C. sessiliflora* and *C. stenophylla*, and stachyose was present in the seeds of only three of these five species (*C. arabica*, *C. brevipes*, *C. sessiliflora*). Verbascose was never detected. When raffinose and stachyose were present in seeds, their content was always very low (0.3–1.4 mg g^{-1} dw and 0.1–0.7 mg g^{-1} dw, respectively). The stachyose content of *C. arabica* seeds found in the present study was identical to that measured by Steadman *et al.* (1996); in contrast, these authors did not detect any raffinose in the endosperm of *C. arabica* seeds, while about 0.3 mg g^{-1} dw of this compound was detected in the seeds studied. The absence of oligosaccharides in seeds of *C. liberica* and *C. pseudozanguebariae* has been reported previously by Ky *et al.* (2000) in a study

Table 1. Monosaccharide (glucose and fructose), sucrose, raffinose, and stachyose contents, oligosaccharides:sucrose (O:S) ratio and water content at which 50% of initial viability was lost, WC_{50}, and corresponding water activity, a_{w50}, in seeds of the ten coffee species. Results of one-way ANOVAs: *F* and *P*. Means followed by the same letter were not significantly different at the *P*=0.05 probability level as determined by the Newman–Keuls test.

Species	Monosaccharides (mg g^{-1} dw)	Sucrose (mg g^{-1} dw)	Raffinose (mg g^{-1} dw)	Stachyose (mg g^{-1} dw)	O:S ratio	WC_{50} (g H_2O g^{-1} dw)	a_{w50}
C. arabica	1.69	75.36^{d}	0.29^{ab}	0.68^{b}	0.013	0.109	0.444
C. brevipes	1.67	54.56^{b}	1.42^{d}	0.64^{b}	0.038	0.203	0.766
C. canephora	2.04	61.61^{bc}	0.00^{a}	0.00^{a}	–	0.170	0.692
C. eugenioides	1.26	73.60^{d}	0.00^{a}	0.00^{a}	–	0.110	0.409
C. humilis	2.15	71.32^{cd}	0.48^{b}	0.00^{a}	0.007	0.382	0.920
C. liberica	1.58	32.96^{a}	0.00^{a}	0.00^{a}	–	0.288	0.874
C. pocsii	1.79	89.29^{e}	0.00^{a}	0.00^{a}	–	0.153	0.744
C. pseudozanguebariae	2.34	76.21^{d}	0.00^{a}	0.00^{a}	–	0.056	0.293
C. sessiliflora	1.99	77.87^{d}	1.05^{c}	0.14^{a}	0.015	0.207	0.546
C. stenophylla	1.50	71.03^{d}	0.31^{ab}	0.00^{a}	0.004	0.158	0.670
F	1.1	30.3	26.6	35.1			
P	0.4280	0.0000	0.0000	0.0000			

performed with numerous genotypes of these two species. The O:S ratio was calculated for the five species in which seeds contained some oligosaccharides. It ranged from 0.004 for *C. stenophylla* to 0.038 for *C. brevipes*. The values of the O:S ratio observed for these five coffee species corresponded to the lowest limit of the range of values measured in recalcitrant species (Lin and Huang, 1994; Steadmann *et al.*, 1996).

The sucrose content, the total soluble sugar content and the O:S ratio were not correlated with seed desiccation sensitivity, as estimated by the water content and the water activity at which half of the initial viability was lost (Table 2). Moreover, no association was found between the presence/absence of oligosaccharides and the two parameters used for quantifying seed desiccation sensitivity (*P*=0.3772 and 0.2191, respectively, as determined by one-way ANOVA). Thus, in contrast to the results of Lin and Huang (1994) and Steadman *et al.* (1996), none of the variables resulting from seed sugar analysis constituted a good indicator of the intraspecific variability for seed desiccation sensitivity among coffee species. This result supports those reviewed by Vertucci and Farrant (1995) and Pammenter and Berjak (1999), which suggest that soluble sugars alone do not confer seed desiccation tolerance.

Table 2. Result of linear regression analysis between the two parameters used for quantifying seed desiccation sensitivity, a_{W50} and WC_{50}, and the sucrose content, the total sugar content, and the oligosaccharides:sucrose (O:S) mass ratio of seeds of the ten coffee species studied: proportion of variance explained, R^2, and probability, *P*.

		a_{W50}	WC_{50} (g H_2O g^{-1} dw)
Sucrose (mg g^{-1} dw)	R^2	0.2180	0.1856
	P	0.1736	0.2139
Total sugars (mg g^{-1} dw)	R^2	0.2138	0.1780
	P	0.1785	0.2246
O:S ratio	R^2	0.0200	0.0179
	P	0.6973	0.7128

Since the values of the O:S ratio found in the present work corresponded to the lowest values previously reported for recalcitrant seed species (Lin and Huang, 1994; Steadmann *et al.*, 1996), this ratio appears to be a poor indicator to distinguish between intermediate and recalcitrant species. In contrast to seeds in the recalcitrant and orthodox categories, it is possible that seeds of the intermediate category are characterised by differences in the O:S ratio between storage and embryonic tissues (Steadman *et al.*, 1996). In the present study, sugar content was measured in whole decoated seeds; it was not possible, therefore, to verify whether the variability for seed desiccation tolerance observed among coffee species was related to the sugar composition of the endosperm or of the embryo, or both. The endosperm represents more than 99% of the seed dry weight in coffee seeds (Eira *et al.*, 1999b), and the sugar composition measured in our study corresponded closely to that of the endosperm. Steadman *et al.* (1996) have suggested that the poor storability of some intermediate seeds could be linked to endosperm characteristics, e.g. the O:S ratio, rather than those of the embryo. The O:S ratios observed here for ten coffee species do not allow rejection of this hypothesis. Similarly, the sensitivity of *C. arabica* seeds to liquid nitrogen temperatures was shown to be related to endosperm sensitivity only (Dussert *et al.*, 1997). In contrast, Eira

et al. (1999b) have recently shown that water sorption properties of the endosperm of coffee seeds are very similar to those of reserve tissues of orthodox seeds, while coffee embryos exhibit water sorption properties intermediate between those of recalcitrant and orthodox embryonic tissues. This discrepancy between the conclusions drawn from different approaches, i.e. soluble sugars vs. water sorption properties, emphasises the complexity of the intermediate seed physiology.

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