Theor Appl Genet (2001) 102:630–634

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Trigonelline inheritance in the interspecific *Coffea pseudozanguebariae*×*C. liberica* var. dewevrei cross

Received: 2 May 2000 / Accepted: 19 June 2000

Abstract Trigonelline alkaloid is present in coffee beans, and during roasting it gives rise to the major coffee aroma compounds (several alkyl-pyridines and pyrroles). In this study we investigated the genetic inheritance of trigonelline accumulation in green beans in an interspecific cross between a wild east African species, *Coffea pseudozanguebariae* (PSE) and the west African species *C. liberica* var. dewevrei (DEW). Trigonelline content was measured by HPLC in both parental species, F_1 hybrids and the reciprocal backcross hybrids (BCDEW and BCPSE). The results showed that, on average, PSE accumulated twice as much trigonelline as DEW. No year effect or interaction (genotype×year) was recorded. Trigonelline showed high heritability (71%), which meant that the genotypic value could be easily estimated from the phenotypic value. However, the fact that this trait was not additive suggested the possibility of nucleo-cytoplasmic inheritance. This hypothesis was confirmed by: (1) similar levels of trigonelline content in the PSE, F_1, BCPSE and BCDEW groups, all having the same maternal cytoplasm, and (2) the location of one nuclear QTL on the G linkage group.

Keywords Trigonelline content · HPLC · Interspecific cross · *C. pseudozanguebariae* · *C. liberica* var. dewevrei · QTLs · Nucleo-cytoplasmic inheritance

Communicated by P.M.A. Tigerstedt

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Introduction

Commercial coffee cultivation mainly involves two species, i.e. *Coffea arabica* L. (2n=44) and *C. canephora* P. (2n=22) (commonly known as Robusta). While all green coffee beans have a high trigonelline alkaloid (1-methyl-pyridinium-3-carboxylate) content, *C. arabica* cultivars have a higher content [0.79–1.06% dry matter basis (dmb)] than *C. canephora* cultivars [0.66–0.68% dmb] (Stennert and Maier 1993 1994). At roasting temperatures (240°C), this alkaloid produces pyridines and pyrrole derivatives (Viani and Horman 1974 1975; De Maria et al. 1994 1996), which are important volatile coffee flavour components (Flament 1991). Trigonelline is also involved in the nicotinic acid formation (Czok 1965) and in the biological stored form of niacin (Taguchi 1988). In addition, it is considered to be important for both taste and nutrition (Adrian and Frangne 1991). Consequently, one objective in coffee breeding research programmes is to increase trigonelline content in Robusta green beans.

Interspecific crosses have been performed in coffee with the aim of investigating introgression possibilities – in cultivated varieties – of valuable traits found in wild species (Louarn 1992). Louarn (1992) focused on the following cross: *Coffea pseudozanguebariae* (PSE) (a wild species originated from Kenya and Tanzania)×*C. liberica* var. dewevrei (DEW) (a species originating from the Central African Republic). This interspecific cross has three major advantages: (1) the availability of F_1 hybrids and reciprocal backcross (BC) hybrids; (2) the marked differences between species with respect to numberous biochemical traits, such as caffeine and heteroside (Barre et al. 1998), chlorogenic acids (Ky et al. 1999) and sucrose (Ky et al. 2000b), and (3) the availability of an interspecific genetic linkage map (Ky et al. 2000a).

The objectives of the investigation reported here were: (1) to evaluate trigonelline content in green beans of parental species and hybrids using high-performance liquid chromatography (HPLC), (2) to assess between-

year variations, (3) to analyse the genetic inheritance of trigonelline content and (4) to map quantitative trait loci (QTLs) for trigonelline.

Materials and methods

Plant material

Plant material was maintained at the IRD (Institut de Recherche pour le Développement) agricultural station (Man, Ivory Coast).

Inheritance analysis

The quantitative inheritance of trigonelline content was investigated using a balanced experimental design with seven genotypes for each of the five following groups: PSE and DEW (parental species), F_1 hybrids and BCDEW (originated from the F1×DEW) and BCPSE (F1×PSE) hybrids. Forty-two BCDEW trees were used for the QTL mapping. For parental species, DEW trees originated from seven different areas in the Central African Republic and PSE from four regions of Kenya. F_1 hybrids were obtained from a controlled cross between PSE (genotype 8044 used as female parent) and DEW (genotype 5851 used as male parent). Backcross hybrids were obtained using F_1 trees as the female parent. All of these plants were harvested in 1995. To test the effect of harvest year, we harvested 4 BCDEW and 3 BCPSE plants in both 1995 and 1997.

QTL analysis

The mapped progeny consisted of 62 BCDEW hybrids. The map and mapping strategies are described in Ky et al. (2000a). AFLP (Amplified length fragment polymorphism) markers used for mapping are specific – and monomorph within – PSE (but absent in DEW).

Trigonelline analysis

Sample preparation

Coffee cherries were harvested at complete maturity and depulped using the wet processing method. Beans were desiccated on silica gel to reduce their water content. Fifty beans per tree were crushed in a ball mill (Dangoumill) for 2 min to obtain a fine powder. The powder was splitted in six samples, three for estimating water content and three for trigonelline extraction and analysis.

Extraction procedure

Trigonelline is a strong polar hydrophilic compound. It was extracted in a basic aqueous medium according to Trugo et al. (1983). Each sample (50 mg of powder) was extracted in a 50-ml capped tube (Sarstedt) contained 500 mg of magnesium oxide (Merck) and 25 ml of distilled water. Tubes were heated for 20 min at 105°C under pressure in an autoclave. Extracts were filtered (0.2-µm pore size) and directly analysed by HPLC.

Analytical HPLC

Chromatography was carried out in a system consisting: (1) two Waters Associates Model 510 pumping units, (2) an automated sample injector (Waters 717 plus autosampler), (3) a variable-wavelength UV detector (Waters 996 Photodiode Array Detector), (4) a C_{18} pre-column, and (5) a 250-mm×4-mm Merck Superspher 100 RP 18 column (5-µm particle size). The elution programme used two solvents. Solvent A was an aqueous solution containing triethylamine and acetic acid (pH 5.3); solvent B was methanol (HPLC grade). These two mobile phases were filtered (0.2-µm pore size), degassed and sonicated (Ney, 300 ultrasonic) before use. Samples or standard (10 µl) were analysed at room temperature using the following elution programme: A-B mixture (70/30) of linear gradient for 15 minutes. Flow rate was 1.0 ml/min. UV detection was carried out at a wavelength of 263.3 nm, which corresponds to trigonelline maximum absorption.

Trigonelline quantification

Trigonelline quantification involved peak-area measurements and comparisons with a trigonelline standard (Sigma Chemical, T-5509). A calibration curve ($C=8.56\times10^{-5}\times$peak area) was generated using three replicate points for trigonelline at 10, 20, 30 and 40 mg/l.

Statistical analysis

Inheritance analysis

All results were analysed using Statistica software programme (Microsoft 5.1 version; 1997).

Differences between harvest years in the seven backcross hybrids were tested using two-way ANOVA: between hybrids (random effects) and between harvest years (fixed effects).

The relative importance of between-group (PSE, BCPSE, F1, BCDEW, and DEW) and between-tree (within-group) variation was analysed using the nested ANOVA model with two factors: group (fixed effects) and tree (random effect nested in groups).

QTL analysis

QTLs were identified using MAPMAKER/QTL software (Lincoln et al. 1992). A LOD score threshold value of 2.6 (equivalent to an α risk of 0.05%) was used.

Table 1 Trigonelline contents (% dmb) in parental species (DEW and PSE), F_1 hybrids and backcross hybrids (BCDEW and BCPSE). Between-tree differences are given by *F*-test results

	DEW	BCDEW	F1	BCPSE	PSE
Average	0.57[a]	1.08[b]	1.13[b]	1.21[b]	1.02[b]
Min-Max	0.51–0.66	0.74–1.38	0.87–1.44	1.03–1.52	0.86–1.19
Range	0.15	0.64	0.57	0.49	0.33
$F_{6, 21}$	17.8***	6.43***	22.3***	18.0***	17.3***

*** $P<0.001$ (very significant)
[a] Newmann and Keuls test results are indexed with a and b letters

Results

Trigonelline content inheritance

Parental species diversity

In DEW green beans, trigonelline content was 0.57% dmb on average and in PSE green beans, 1.02% dmb. The interspecific difference was very highly significant ($F_{1, 12}=59.4$; $P<0.001$) and corresponds to 71 % of the total variance. There was no overlap in the trigonelline content range between DEW (0.51–0.66% dmb) and PSE (0.86–1.19% dmb) (Table 1). Between-tree variations

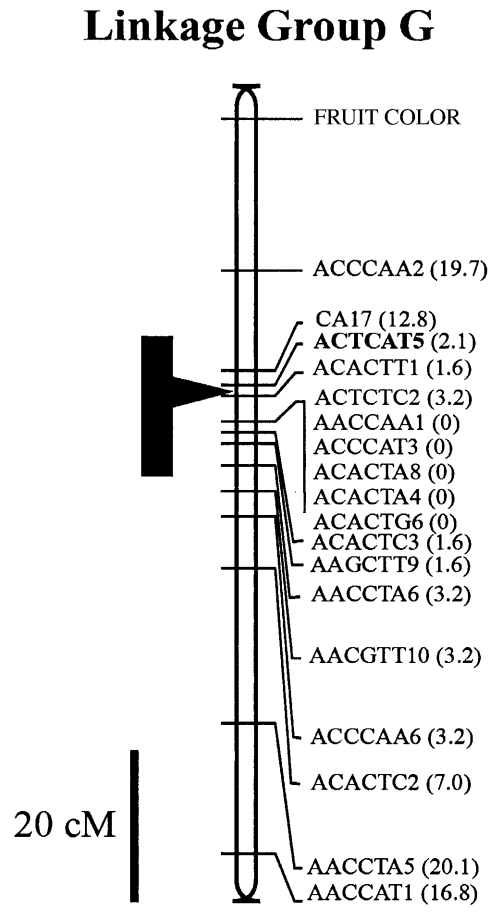

Fig. 1 Trigonelline QTL location on the linkage group G of the genetic map constructed in Ky et al. (2000a). AFLP markers names are symbolised on the *right side* of the linkage group. *Number in brakets* correspond to genetic distance (centiMorgans, cM) between markers. *Bars* to the *left* of the linkage group correspond to the 2.0 LOD support intervals for the QTL locations. *Arrows* indicates the most likely position of the trigonelline QTL (highest LOD peak: 3.56) estimated with MAPMAKER/QTL

(within-species) were also very highly significant ($F_{12, 42}$= 17.4; P<0.001), but the contribution of this factor to the total variance was only 8% (residual contribution represented 21% of the total variance).

Relative importance of between-tree and between-year variations

Variations in trigonelline content were mainly due to between-tree differences ($F_{6, 42}$=45.7; P<0.001). Between-year variations ($F_{1,42}$=1.06; P=0.58) and harvest×hybrid interactions ($F_{6,42}$=1.17; P=0.34) were not significant.

Hybrid diversity and absence of additivity

Trigonelline content in F_1 hybrids was 1.13% dmb on average (Table 1). Between-hybrid differences were highly significant ($F_{6,21}$=22.3; P<0.001). ANOVA ($F_{2,18}$= 28.5; P<0.001) and Newman-Keuls test results showed that the F_1 hybrid average content was similar to that of PSE, but differed from that of DEW, implying that trigonelline is not an additive trait. The trigonelline content of the F_1 hybrids was 42% higher than the mid-parental value (0.80% dmb).

Table 2 Trigonelline content and frequencies of the genotypic classes defined by the *t* (DEW) and *T* (PSE) alleles of the QTL (near ACTCAT5 locus)

	t t	*T t*
Average (% dmb)	0.83	1.20
Frequency	(28)	(19)

Average trigonelline contents in BCDEW and BCPSE hybrids were 1.08% dmb and 1.21% dmb, respectively (Table 1). The averages of the PSE, F_1, BCDEW and BCPSE groups were therefore similar and differed from that of DEW (Table 1).

As noted for the two parental species and F_1 hybrids, between-tree variations (within backcross) were highly significant.

Trigonelline QTLs analysis

The MAPMAKER/QTL results revealed the existence of one QTL for variations in trigonelline content within the BCDEW hybrids. This QTL was located on the G genetic linkage group of the map presented in Ky et al. (2000a), near the ACTCAT5 AFLP locus (LOD score=3.56), i.e. at 34.9 cM from the first marker of G linkage group (Fig. 1).

In BCDEW hybrids, there were two allelic combinations, [*t t*] and [*T t*], where *T* and *t* are the two alleles of the ACTCAT5 locus for PSE and DEW, respectively. Their frequencies were 54% and 46%, respectively, and the phenotypic values of [*t t*] (0.86% dmb) and [*T t*] (1.11% dmb) were significantly different ($F_{1, 45}$=22.3; P<0.001).

A distribution study highlighted the existence of recombinant individuals within the [*t t*] and [*T t*] classes (recombination events between ACTCAT5 locus and the corresponding QTL). This led us to define the *T* and *t* as alleles of the QTL, respectively, for PSE and DEW. Table 2 shows the average trigonelline contents in genotypes [*t t*] and [*T t*], which were significantly different ($F_{1, 45}$=125; P<0.001). Figure 2 illustrated the trigonelline content distributions of the [*t t*] (group 1) and [*T t*] (group 2) genotypes in BCDEW hybrids. We observed that the DEW phenotypic value was significantly lower than the [*t t*] genotype values, whereas those of the F_1 hybrids were similar to [*T t*] on average.

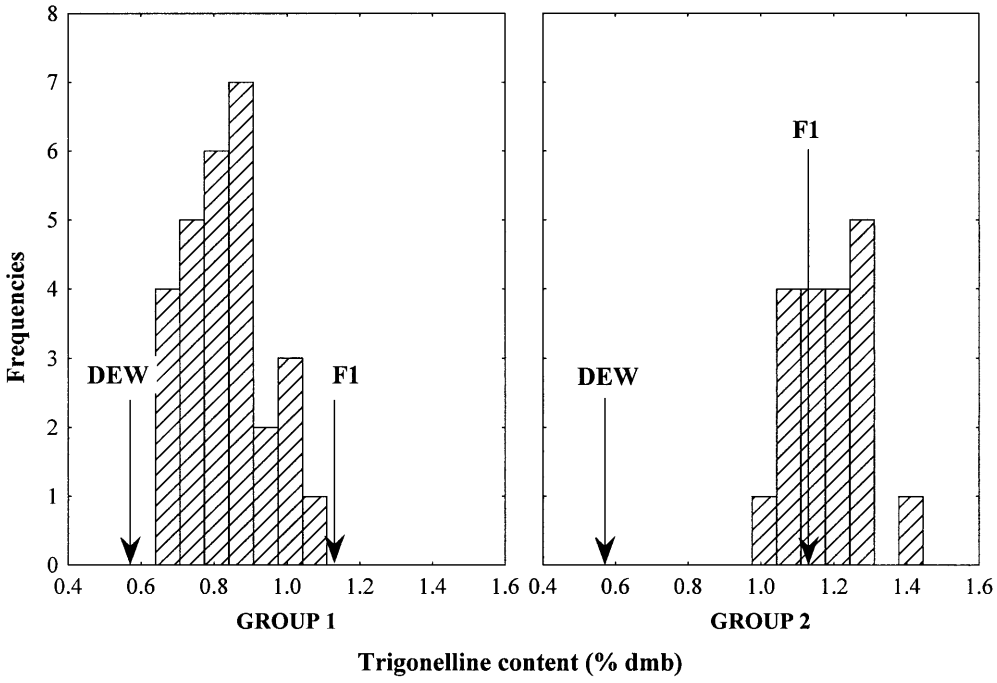

Fig. 2 Trigonelline content distributions in BCDEW hybrids showing the existence of group 1 [(*t t*) genotype] and group 2 [(*T t*) genotype] for the trigonelline QTL

Discussion

Relative importance of environmental, genetic and interaction effects

No environmental effect or interaction was recorded. Between-year climatic variations, differences in bean maturity at harvest and post-harvest variation in bean-drying conditions did not modify trigonelline content. In contrast, there were marked between-tree differences. The high genotypic contribution implies that for this trait clonal selection should be efficient for varietal multiplication by grafting or cuttings.

Trigonelline content inheritance

Trigonelline accumulation could be genetically determined by the cytoplasmic maternal heredity type (involvement of mitochondrial or chloroplastic genes). This hypothesis is supported by the fact that similar trigonelline content values were obtained for the PSE, F_1, BCPSE and BCDEW groups, which share the same maternal or grand-maternal origin (the 8044 PSE tree), whereas the values differed from these obtained for DEW (Table 1). This hypothesis could be tested by estimating trigonelline content in hybrids using DEW as the female parent. This reciprocal cross was attempted, unfortunately without success, and to date no hybrid with DEW as female has been obtained.

Within cytoplasmic group, nuclear effects were highlighted at two levels: (1) at the interspecific level with evidence of a QTL close to a nuclear AFLP marker (ACTCAT5) located on G genetic linkage group (Fig. 1); the *t* QTL allele increased trigonelline content by 0.37% dmb; and (2) at the intraspecific level, differences were recorded between trees within-species. They were also noted between F_1 hybrids. In this case, the allogamy and heterozygoty of the parental species were implied, as for caffeine (Barre et al. 1998), chlorogenic acid (Ky et al. 1999) and sucrose contents (Ky et al. 2000b), three biochemical traits showing nuclear inheritance. Nuclear intraspecific effects were also noted in *C. canephora* intraspecific crosses through a weak heritability (38%) (Montagnon et al. 1998). This weak heritability could be interpreted as being due to an intraspecific nucleo-cytoplasmic interaction and epistatic effects.

A comparison between [*t t*] genotypes in DEW and BCDEW showed that the cytoplasmic contribution of PSE to trigonelline content could be estimated as 0.26% dmb on average. We also noted that the effect diminished in F_1 hybrids.

Conclusions and prospects

This is the first study designed to evaluate trigonelline content in the wild species *C. pseudozanguebariae* and its hybrids with *C. liberica* var. dewevrei. The results showed that this trait shows: (1) a twofold higher content in PSE than in DEW, (2) strong heritability (71%), and (3) nonadditive inheritance. Similar content values observed in PSE, F_1 hybrids and the two reciprocal backcrosses suggest a cytoplasmic maternal (PSE) inheritance. Molecular marker analyses have also revealed an important interspecific nuclear effect, with evidence of a QTL on genetic linkage group G.

The importance of these results with respect to breeding is threefold. (1) The cytoplasmic inheritance of trigonelline

content means that trigonelline content can be increased in cultivated coffee using PSE as female parent and its cytoplasm in the next generations. (2) The evidence of a QTL that could explain the interspecific difference implies that marker-assisted selection could be used in further backcross generations. Such selection, at the plantlet stage, would avoid having to install large progeny populations in the field. (3) The existence of a small environmental effect, in addition to between-tree variations within [*t t*] and [*T t*] groups, similar to the within-species and within-F_1 variations, should facilitate clonal selection of hybrids throught vegetative multiplication. We now plan to investigate QTLs that could account for within-species variations.

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