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Genetic variability for feeding value of faba bean seeds (*Vicia faba*): Comparative chemical composition of isogenics involving zero-tannin and zero-vicine genes

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SUMMARY

In a preliminary experiment, 74 faba bean genotypes including winter genotypes (autumn-sown) and spring genotypes (spring-sown) and isogenic population pairs (tannin-containing *v.* tannin-free and vicine/convicine-high *v.* vicine/convicine-low), were analysed for the chemical composition of their seeds. A large variability was found for the main constituents (starch, protein and fibre). Autumn-sown genotypes contained 2·3% less proteins but 2·5% more starch in the seed dry matter (DM) than spring-sown genotypes. The *vc*$^-$ gene, which lowers the vicine and convicine contents, did not significantly modify the main seed components in the isogenic comparisons. The *zt1* and *zt2* genes, which eliminate condensed tannins in the seed coats, lowered by 2·1% the proportion of the seed coat in the DM. In the isogenic comparisons, the *zt2* gene had a stronger effect than *zt1* in reducing the total seed fibre and increasing the protein content.

In a second experiment, from the original 74 genotypes, 12 contrasted genotypes were selected and multiplied for animal nutrition trials. Their chemical analysis confirmed the variability between the faba bean categories observed in Expt 1, but detailed chemical analyses illustrated the variability in amino acid, fatty acid, amylose and oligosaccharide composition, trypsin inhibitory activity, condensed tannins, lectins and phytic phosphorus contents.

INTRODUCTION

Among seed legumes used as a protein source for animal feeds in Europe, the faba bean (*Vicia faba* L.) ranks second after pea, with around half a million tonnes being produced annually (UNIP–ITCF 1995).

The incorporation rate of faba bean seeds in monogastric feeds usually falls between 15 and 30% according to animal age, sex and species, with the exception of laying hens, which have a 7% limit due to the antinutritional factors vicine and convicine (Olaboro *et al.* 1981; Lacassagne 1988). With genetic improvements in faba bean seed quality, these incorporation rates could be increased.

The main antinutritional factors in faba bean seeds (Grosjean *et al.* 1995) are tannins and two pyrimidine glucopyranosides, vicine and convicine. Tannins can be removed by mutations in one of two independent genes named *zt1* (*Vicia faba* major L. origin) *zt2* (*Vicia faba* minor L. origin) (Picard 1976). Vicine and convicine can be lowered by 10 to 20-fold by mutation in the *vc*$^-$ gene (Duc *et al.* 1989). For each gene, the mutant allele reducing the antinutritional factor behaves as recessive against the wild allele.

Starch, protein and fibre are the major seed constituents in faba bean and these have shown great variation among genotypes in several studies (Bjerg *et al.* 1988).

In the present study, the first experiment evaluated the range of genetic variation for nutrition-related components in a collection of 74 faba bean genotypes of diverse origin and containing different concentrations of antinutritional factors. Isogenic faba bean populations differing only in genes controlling tannin, vicine and convicine contents were examined. From

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Table 1. *Characteristics of faba bean genotypes involved in Expt 2*

Code	Type	Genotype-Breeder	Zero-tannin [T^-] or tannin-containing [T^+]	Vicine-convicine low [V^-] or vicine-convicine high [V^+]	Isogenicity (*except for antinutritional factor genes*)
665	Spring population	EB-INRA	$[T_{(1)}^-]$	$[V^+]$	665 = 666 = 667 = 668
666	Spring population	EB-INRA	$[T_{(1)}^-]$	$[V^-]$	665 = 666 = 667 = 668
667	Spring population	EB-INRA	$[T^+]$	$[V^+]$	665 = 666 = 667 = 668
668	Spring population	EB-INRA	$[T^+]$	$[V^-]$	665 = 666 = 667 = 668
617	Winter population	Fabiola ZT-INRA	$[T_{(1)}^-]$	$[V^+]$	617 = 615
615	Winter cultivar	cv. Fabiola-INRA	$[T^+]$	$[V^+]$	617 = 615
629	Winter population	E3202-INRA	$[T_{(1)}^-]$	$[V^+]$	629 = 630
630	Winter Population	E3202-INRA	$[T^+]$	$[V^+]$	629 = 630
662	Spring population	EE-INRA	$[T_{(2)}^-]$	$[V^-]$	
611	Winter cultivar	cv. Glacier-PBI	$[T_{(1)}^-]$	$[V^+]$	
646	Spring cultivar	cv. Robin-PBI	$[T^+]$	$[V^+]$	
613	Winter cultivar	cv. Bourdon-PBI	$[T^+]$	$[V^+]$	

(1) Determined by zero-tannin gene no. 1.
(2) Determined by zero-tannin gene no. 2.

this collection, 12 genotypes were selected for a second experiment where they were grown in larger quantities for detailed chemical analyses and animal feeding trials. This paper deals with chemical composition of the faba beans. In another paper, the nutritional value of these genotypes in cockerels, laying hens and pigs is examined (Grosjean *et al.*, submitted).

MATERIALS AND METHODS

Plant material

Seventy-four genotypes of *Vicia faba* L. were chosen to represent a wide genetic variability including 23 European cultivars of population type, 25 breeder's lines and 26 breeder's populations from different geographic origins in Europe. Of these genotypes, 37 were autumn-sown ('winter' genotypes) and 37 were spring-sown ('spring' genotypes). The collection included various genotypes low in antinutritional factors: 18 zero-tannin genotypes [$T_{(1)}$] determined by the *zt1* gene, five zero-tannin genotypes [$T_{(2)}$] determined by the *zt2* gene and five low-vicine and -convicine genotypes [V^-] determined by the vc^- gene (of which three were also zero-tannin).

The breeder's populations included 15 near isogenic pairs of populations, based on segregating F3 progenies from crosses involving the *zt1*, *zt2* and/or vc^- genes. Each population consisted of a mixture of 30 F3 progenies, all in the same homozygous state for the genes determining their tannin and vicine-convicine characters. These mixtures were bulked and multiplied for two generations before being entered in this work. Location for seed multiplication of each pair was always kept identical in order to eliminate location effect within pair comparisons.

Experiment 1

Genotypes were grown in field conditions on 2 m^2 plots and two replicates (25 plants/m^2) using standard agronomic practices for faba beans. The 'spring' genotypes were spring-sown and grown at Dijon, France (harvest 1991). The 'winter' genotypes were autumn-sown and grown either at Rennes, France or Cambridge, UK or Montauban, France (harvest 1991), cv. Bourdon being a common control in each of these situations. A 500 g sample was harvested on each plot, the two replicates mixed (1:1) and sent to laboratories for chemical analysis.

Experiment 2

After selection, 12 genotypes (Table 1, including six 'winter' and six 'spring' types, two isogenic pairs [T^+] *v.* [T^-] and one isogenic quadruplet with all combinations between [T^+] *v.* [T^-] and [V^+ *v.* [V^+]) were grown on 500 m^2 plots (40 plants/m^2) at Bersée, France for the 'spring' genotypes (harvest 1991) and

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Table 2. *Measurements performed on the samples of Expt 2*

Measurement (code)	Unit	Description	Reference
Proteins			
In vitro degradability of proteins (IVDP)	Percent	1 h assay with pronase at pH 8	Aufrère *et al*., 1989
Trypsin Inhibitors Activity (TIU)	Units/mg DM	Modified Kakade method	Valdebouze *et al*., 1980
Lectins	mg/g		Hamer *et al*., 1989
Amino acids	g/kg DM	Ion exchange chromatography	AFNOR NF 18-113 and Landry & Delhaye, 1993
Carbohydrates			
Amylose	Starch (%)	Iodine binding capacity comparatively to pure amylose and amylopectin	Larsson *et al*., 1953
Oligosaccharides (raffinose, stachyose, verbascose)	mg/g DM	Integration of HPLC peaks with reference to pure samples	Arentoft & Sorensen, 1992
Cotyledon cell-walls (CCW)	Cotyledon DM (%)	WICW measurement on dehulled samples	Brillouet & Carré, 1986 Carré & Brillouet, 1989
Other measurements			
Thousand seed weight (TSW)	g		Métayer *et al*., 1989
Proportion of integument (also named seed coat or testa) in the seed DM (INTEG)	(DM/DM) (%)	Hand decoating of 200 seeds after 6 h water soaking at +4 °C. DM determination of seed coats and cotyledons	Duc G., INRA, Dijon, Fr, Pers. Comm.
Lipid composition	Lipids (%)	Gas chromatography analysis of individual fatty acids	Coxon & Wright, 1985
Lipid content	g/kg DM	Summation of fatty acids	Coxon & Wright, 1985
Total phenolics	mg catechin equivalents/g	Absorbance at 725 nm after Prussian blue assay	Adapted by Brun, 1991
Condensed tannins (CT)	mg catechin equivalents/g	Absorbance at 500 nm after Vanillin-HCl test	Adapted by Brun, 1991
Tanning power of tannins (TP)	mg tannic acid equivalents/g	Haemanalysis test	From Bate-Smith, 1973 and adapted by Brun, 1991
Vicine-convicine	g/kg DM	HPLC determination	Duc *et al*., 1989
Total phosphorus	mg/g DM	Dry ashing at 530 °C and spectrophotometry	Gueguen L., INRA Jouy, Fr, Pers. comm.
Phytic phosphorus	Total P (%)	precipitation of phytate P with ferric chloride	Bagheri, 1983
Gross energy (GE)	kcal/kg DM	Calorimetry with an adiabatic bomb in triplicate	Rudeaux F., Sanders, Athis Mons, Fr, Pers. Comm.

at Rennes, France for the 'winter' genotypes using standard agronomic practices for faba beans. Around 2000 kg were produced from each genotype for use in animals trials (see Grosjean *et al*. 1999) and chemical analysis.

Chemical methods

Experiment 1

All the faba beans in Expt 1 were analysed for their composition in a single laboratory, in one series, according to the standard methods used in the feed industry. Dry matter (DM) was determined after drying at 104 °C for 4 h; crude protein (CP) was calculated according to the Kjeldahl method using 6·25 as the protein : nitrogen ratio (Jones 1931); starch was measured using a polarimetric method (Ewers, CEE 3.72/199 modified JOCE 27.11.1980); crude fat (often referred to as ether extract) was extracted with petroleum ether after acid hydrolysis; total mineral content (Ash) was determined after ashing in an oven at 550 °C overnight; crude fibre (CF) was determined after acid and alkaline treatment (Weende 1977) on

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Table 3. *Chemical analysis of different categories of faba beans, 'spring' v. 'winter' and tannin-containing [T⁺] v. tannin-free [T⁻], in Expt 1. Each parameter is expressed in g/kg DM. For each subpopulation, the number of faba bean genotypes (nb) is given and standard deviation (S.D.) calculated*

	Spring [T⁺]					Spring [T⁻]					Winter [T⁺]					Winter [T⁻]				
	nb	Mean	S.D.	Min	Max	nb	Mean	S.D.	Min	Max	nb	Mean	S.D.	Min	Max	nb	Mean	S.D.	Min	Max
Protein	24	318	24	261	380	13	328	23	284	372	25	293	25	247	336	12	309	17	272	336
Starch	24	413	17	370	446	13	415	24	377	456	25	439	32	375	494	12	439	30	398	505
Crude fat	24	18	4	11	25	13	19	3	13	25	25	24	8	14	47	12	21	2	16	23
Sugars	24	36	4	35	37	13	43	4	37	50	25	43	4	37	52	12	44	6	37	63
Ash	24	41	2	38	47	13	42	2	38	47	25	38	4	30	46	12	40	5	35	55
Cellulose	24	86	8	71	103	13	87	6	74	94	25	88	11	66	118	12	89	13	65	107
ADF	24	108	14	75	131	13	96	9	76	109	25	109	23	87	128	12	101	14	77	129
NDF	24	213	25	173	264	13	187	25	134	237	25	206	51	141	259	12	186	27	137	222
WICW	24	178	16	153	222	13	160	18	127	198	25	176	11	156	206	12	172	16	150	212

an apparatus equivalent to Fibertec; neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to a procedure adapted from Goering & Van Soest (1970); water-insoluble cell walls (WICW) were determined according to Carré & Brillouet (1986); soluble sugars were measured by the Luff-Schoorl (1971) method.

In addition, on the isogenic material the *in vitro* degradability of proteins (IVDP) was measured according to Aufrère *et al*. (1989) and the condensed tannins were assayed using a modified vanillin-HCl method (Brun 1991).

Experiment 2

In Expt 2, the faba beans were analysed for the same components as in Expt 1. In addition, after a careful sampling on an automatic circular rotative batch divider, they were analysed for the components and using the methods listed in Table 2. Average seed size, expressed as 1000 seed weight (TSW), was determined just after harvest by weighing *c*. 500 seeds.

RESULTS

Phenotypic variation for seed chemical composition in Expt 1

The 74 seed batches showed wide variations in their main constituents (% DM basis) (Table 3): with starch from 37·0 to 50·5%, protein from 24·7 to 37·2%, cell-walls from 12·7 to 22·2% and NDF from 13·4 to 26·4%. The range of variation is smaller for the minor nutrients: sugars 3·5–6·3% of DM, ash 3·0–5·5% of DM and crude fat 1·1–4·7% of DM.

The most significant phenotypic correlations observed between these constituents were the strong negative correlation between protein and starch contents ($P < 0{\cdot}01$, $r = -0{\cdot}67$, 72 D.F., [starch %] = $-0{\cdot}74 \times$ [protein %] + 65·7) and the positive relationship between NDF and ADF ($P < 0{\cdot}01$, $r = 0{\cdot}68$, 72 D.F., [NDF %] = 0·23 [ADF %] + 5·8).

Since winter and spring groups were grown in different locations and climates, the phenotypic differences between these two groups incorporate possible environmental effects on composition which cannot be separated from genotypic ones. Such a comparison (*t*-test, 5% probability level) indicates that the seed DM of autumn-sown genotypes contain 2·5% more starch and 2·3% less protein than that of spring types. Within the tannin-containing group, the autumn sown genotypes contained significantly more sugar and fat than the spring ones.

In Expt 1, all spring genotypes were grown in the same environment and therefore the variability within this group reflects genotypic effects only. When comparing tannin-containing [T⁺] with tannin-free [T⁻] groups of these spring types, the only significant effects (*t*-test, 5% probability level) concerned ADF,

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Table 4. *Seed chemical composition of isogenic pairs made with the zt1 or the zt2 gene (g/kg of DM for all characters except for* in vitro *degradability of proteins [IVDP] expressed in %). P = probability for null difference $[T^+]-[T^-]$ in pair wise comparisons after Student's t-test*

	Gene *zt1* 10 isogenic pairs			Gene *zt2* 5 isogenic pairs		
Constituent	$[T^-]$	$[T^+]$	*P*(%)	$[T^-]$	$[T^+]$	*P*(%)
Protein	315	308	10	327	302	1
Starch	425	420	27	424	446	8
Fat	19	21	8	19	16	6
Sugars	37	36	8	37	36	29
Ash	39	40	35	38	40	12
Cellulose	89	95	6	85	86	39
ADF	104	107	30	92	107	3
NDF	184	191	23	185	214	1
WICW	174	181	11	157	176	0
Condensed tannins	0	10	0	0	9	0
Vicine	5	5	56	5	5	52
Convicine	2	2	45	2	2	70
Integuments	125	143	2	115	135	4
IVDP (%)	66	66	48	65	59	0

NDF and WICW, which are reduced in the zero-tannin forms. To compensate this fibre content reduction, the largest increases were on protein and sugar contents in DM which however were not significant individually.

Winter genotypes were grown at three locations, and although composition of the seed of the common control cv. Bourdon was constant over the three places (variation coefficients $< 4\%$ for all components), the genotypic and environmental effects cannot be separated in their contribution to the variability within this group. Therefore, this variability is not discussed.

Comparison of isogenic pairs made with the zero vicine-convicine gene in Expt 1

Each of the four isogenic pairs were grown in the same site and pair-wise comparisons (*t*-test, 5% probability level) provides an accurate measurement of the effect of glucopyranoside pyrimidine removal on other seed components.

The mean content of (vicine + convicine) in the seed DM was significantly reduced from 0·75% in the $[V^+]$ forms to 0·06% in the $[V^-]$ isogenics. For all the chemical characters measured in Expt 1 and also for *in vitro* degradability of proteins, there was no significant difference between the $[V^+]$ and $[V^-]$ groups (data not shown).

Comparison of isogenic pairs made with the zero tannin genes in Expt 1

For each gene (*zt1* and *zt2*) ten and five isogenic $[T^+]/[T^-]$ pairs respectively were each grown at the same site. Therefore, pair wise comparison on contrasted types enables us to assess the secondary effects on seed composition of removing tannins by genetics (Table 4).

The mean condensed tannin content in the seed DM was significantly reduced from nearly 1% (DM) in the $[T^+]$ forms to 0·01% in the $[T^-]$ isogenics of both genes. A sample of five isogenic pairs $[T^+]/[T^-]$ was taken for each gene and showed that a reduction of mean seed coat proportion by $2{\cdot}1 \pm 0{\cdot}3\%$ of DM accompanied tannin removal, with no significant difference between the two genes' effects. The *zt2* gene showed the most significant effect on ADF, NDF and WICW, which were significantly reduced and compensated for by a significant increase of 2·5% protein. Simultaneously, a significant improvement in IVDP (*in vitro* degradability of proteins) of 7% was observed after *zt2* incorporation. In comparison, the *zt1* gene resulted in non-significant fibre decrease and protein increase and no effect on IVDP.

Phenotypic variability for seed chemical composition in Expt 2

The number of samples (12) was much lower in this second experiment where genotypes were chosen to represent a large part of the variability detected in Expt 1 and were aimed at animal feeding trials. Chemical composition (Table 5) indicates that a broad range of variation was obtained for contents of the main components (DM basis) with protein (26·2 – 32·2%), starch (39·0 – 44·4%) and WICW (12·7 – 20·6%). As observed in Expt 1, the DM of the winter group had lower protein contents which was

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Table 5. *Chemical composition of faba bean seeds produced in Expt 2* (*g/kg of seed dry matter except, as described in Table 2 for TSW, IVDP and GE*). [$T_{(1)}^{+}$] iso *and* [$T_{(1)}^{-}$] iso *are the mean for each version in the four isogenic pairs made with gene* zt1. *Mean and standard deviation* (*s.d.*) *are given for the 12 genotypes*

Genotype code	TSW (g)	Protein	Starch	Fat	Sugars	Ash	Cellulose	ADF	NDF	WICW	CCW	INTEG	IVDP (%)	GE (kcal/kg DM)
665	599	311	425	20	41	41	92	102	134	127	71	121	67·2	4428
666	580	302	441	18	42	39	94	108	167	172	71	121	67·3	4439
667	657	308	434	18	36	44	91	109	174	174	66	145	68·2	4506
668	626	304	420	18	36	39	91	109	173	185	81	139	65·7	4501
617	415	302	419	16	40	40	93	111	177	184	65	135	58·3	4383
615	483	304	403	17	37	43	118	121	217	189	76	156	68·2	4405
629	443	305	431	21	37	40	99	114	181	184	79	126	59·0	4441
630	694	322	390	22	42	42	96	128	179	188	65	148	66·2	4521
662	517	316	444	17	47	41	78	86	167	167	80	110	69·2	4455
611	533	278	437	18	54	48	88	112	192	184	74	125	67·6	4363
646	376	317	404	18	37	40	93	98	174	170	88	137	66·7	4444
613	577	262	427	20	38	46	98	117	207	206	70	143	54·4	4349
Mean	542	303	423	19	41	42	94	110	179	178	75	134	64·0	4436
s.d.	99	17	17	2	5	3	9	11	21	19	72	13	5·1	55
[$T_{(1)}^{+}$] iso	615	310	412	19	38	42	99	117	186	184	72	147	67·1	4483
[$T_{(1)}^{-}$] iso	509	305	429	19	40	40	95	109	165	167	72	126	63·0	4423
Spring mean	559	311	428	18	40	41	90	102	165	166	76	129	67·4	4462
Winter mean	525	295	418	20	42	43	98	118	193	190	74	139	60·6	4410

Table 6. *Amino acid profile* (*g/kg DM*) *of faba bean seeds produced in Expt 2* (*aspartic acid*, *threonine*, *serine*, *glutamic acid*, *proline*, *glycine*, *alanine*, *cysteine*, *valine*, *methionine*, *isoleucine*, *leucine*, *tyrosine*, *phenylalanine*, *lysine*, *arginine*, *tryptophane*, *histidine*). [$T_{(1)}^{+}$] iso *and* [$T_{(1)}^{-}$] iso *are the mean for each version in the four isogenic pairs made with the gene* zt1. *Mean and standard deviation* (*S.D.*) *are given for the 12 genotypes*

Genotype code	ASN	THR	SER	GLN	PRO	GLY	ALA	CYS	VAL	MET	ILE	LEU	TYR	PHE	LYS	ARG	TRY	HIS
665	33·6	12·0	15·1	56·7	12·9	13·8	13·3	3·9	16·0	2·4	13·4	23·3	8·4	13·3	20·6	29·6	3·1	8·2
666	33·0	11·6	14·7	55·5	12·7	13·4	12·9	3·9	15·3	2·5	13·2	23·1	7·1	12·5	19·7	29·9	3·2	8·1
667	32·6	11·7	14·5	55·9	12·7	13·7	13·3	4·2	15·8	2·5	13·0	22·6	5·5	12·4	20·0	28·7	3·0	8·1
668	34·9	12·3	15·0	60·2	13·8	14·4	14·1	4·3	16·8	2·7	13·0	24·1	5·8	13·4	21·2	27·8	2·8	8·1
617	29·8	11·5	14·2	48·4	9·4	12·5	12·2	3·1	14·4	2·7	13·0	21·8	7·3	12·5	19·1	26·9	2·3	7·7
615	31·2	11·5	14·2	49·3	11·8	13·0	12·6	3·5	14·7	2·5	13·2	21·7	6·9	12·2	19·3	26·9	2·6	7·6
629	30·5	11·5	14·1	49·0	11·9	12·9	12·6	3·3	14·5	2·7	12·7	21·7	7·6	12·1	18·8	28·0	2·3	7·5
630	34·6	12·8	16·3	53·9	13·7	14·1	13·7	3·8	15·6	2·9	13·9	24·1	7·8	13·7	20·9	30·3	2·5	8·3
662	35·4	12·4	15·4	59·0	13·6	14·2	13·7	4·1	16·5	2·5	14·0	24·4	5·8	13·4	21·6	29·7	3·0	8·3
611	30·0	11·3	14·1	47·2	11·8	12·6	12·1	3·3	13·9	2·7	12·6	21·1	6·7	12·1	19·2	23·3	2·1	7·2
646	34·1	12·1	14·9	56·8	13·4	14·1	13·4	4·0	16·3	2·4	13·8	23·4	5·2	13·0	20·1	30·8	3·0	8·2
613	26·0	10·2	12·6	41·6	9·6	11·1	10·7	2·9	12·8	2·3	11·9	19·4	6·4	11·4	17·3	20·0	2·0	6·7
Mean	32·1	11·7	14·6	52·8	12·3	13·3	12·9	3·7	15·2	2·6	13·1	22·6	6·7	12·7	19·8	27·7	2·7	7·8
S.D.	2·7	0·7	0·9	5·6	1·5	1·0	0·9	0·5	1·2	0·2	0·6	1·5	1·0	0·7	1·2	3·2	0·4	0·5
[$T_{(1)}^{+}$] iso	33·3	12·1	15·0	54·6	13·0	13·8	13·4	3·9	15·7	2·7	13·3	23·1	6·5	12·9	20·3	28·4	2·7	8·0
[$T_{(1)}^{-}$] iso	31·7	11·7	14·5	52·4	11·7	13·2	12·7	3·6	15·0	2·6	13·1	22·5	7·6	12·6	19·5	28·6	2·7	7·8
Spring mean	33·9	12·0	14·9	57·3	13·2	13·9	13·4	4·1	16·1	2·5	13·4	23·5	6·3	12·9	20·5	29·4	3·0	8·2
Winter mean	30·4	11·5	14·2	48·3	11·4	12·4	12·3	3·3	14·3	2·6	12·9	21·6	7·0	12·1	19·1	25·9	2·3	7·5

Table 7. *Detailed chemical composition of faba bean seeds produced in Expt 2* (*g/kg DM*, *except as described in Table 2 for starch and lipid composition*, *TIU and Phytic P*). [$T_{(1)}^{+}$] iso *and* [$T_{(1)}^{-}$] iso *are the mean for each version in the four isogenic pairs made with the gene* zt1. *Mean and standard deviation* (*S.D.*) *are given for the 12 genotypes*

Genotype code	Amylose (%)	Lipid	C16:0 (%)	C18:0 (%)	C18:1 (%)	C18:2 (%)	C18:3 (%)	Sucrose	Raffinose	Stachyose	Verbascose	Oligosaccharides	Phenolics	CT	TP	TIU	Lectins	Total P	Phytic P (%)	Vicine	Convicine
665	25·9	16	15·7	2·6	26·6	51·9	3·1	18·8	14·5	5·9	12·4	32·9	5·7	0·1	1·7	5·3	0·6	4·8	49·6	3·4	2·5
666	25·5	15	15·1	2·4	27·0	52·7	2·8	20·7	12·1	4·9	9·6	26·7	6·5	0·1	2·0	4·2	0·6	4·7	51·3	0·5	0·2
667	29·9	15	15·1	2·4	24·9	54·4	3·2	16·1	2·2	4·2	10·5	17·0	12·4	6·0	12·4	4·7	0·6	5·2	49·6	3·8	2·6
668	27·7	13	15·0	2·3	26·4	53·4	3·0	8·4	8·4	4·7	15·5	28·6	12·9	5·5	11·2	4·9	0·8	4·5	45·3	0·2	0·1
617	—	20	14·7	2·4	28·9	49·9	4·4	5·6	4·3	7·2	24·4	35·9	6·5	0·2	2·3	1·1	1·7	3·8	80·6	10·4	1·8
615	—	21	14·8	2·1	25·2	54·1	3·8	8·8	3·9	7·5	32·9	44·3	17·1	10·4	14·1	0·5	1·8	5·9	58·3	10·1	4·3
629	—	20	14·2	2·4	28·7	50·7	4·1	4·2	2·3	5·9	20·3	28·5	6·4	0·1	1·9	0·8	1·6	4·1	52·3	9·6	1·7
630	—	22	14·1	1·7	27·8	52·7	3·7	15·0	0·6	7·6	32·7	40·9	11·9	4·5	9·8	1·3	1·9	4·2	70·0	9·7	2·3
662	28·0	14	16·8	2·3	27·2	51·0	2·7	23·2	2·4	2·8	9·3	14·5	5·3	0·1	1·5	4·1	0·5	4·7	55·9	0·6	0·1
611	—	19	15·2	2·4	26·7	51·7	3·5	10·5	4·9	9·6	36·0	50·5	4·7	0·2	1·9	0·3	1·8	4·6	82·0	10·0	2·6
646	31·2	17	12·6	2·1	36·5	45·6	3·2	3·7	2·8	2·7	8·3	13·9	15·2	5·6	13·6	5·1	1·0	5·6	67·8	5·1	2·6
613	—	20	14·4	2·2	27·8	52·4	3·2	7·9	5·1	14·9	42·4	62·3	11·6	8·0	11·7	0·9	1·5	4·8	72·5	9·7	2·5
Mean	28·0	18	14·8	2·3	27·8	51·7	3·4	11·9	5·3	6·5	21·2	33·0	9·7	3·4	7·0	2·8	1·2	4·7	61·3	6·1	1·9
S.D.	2·2	3	1·0	0·2	3·0	2·3	0·5	6·7	4·5	3·3	12·1	14·8	4·3	3·7	5·5	2·1	0·6	0·6	12·8	4·2	1·3
[$T_{(1)}^{+}$] iso	—	18	14·8	2·1	26·1	53·6	3·4	12·1	3·8	6·0	22·9	32·7	13·6	6·6	11·9	2·9	1·3	5·0	55·8	6·0	2·3
[$T_{(1)}^{-}$] iso	—	18	14·9	2·5	27·8	51·3	3·6	12·3	8·3	6·0	16·7	31·0	6·3	0·1	2·0	2·9	1·1	4·4	58·5	6·0	1·6
Spring mean	—	15	15·1	2·4	28·1	51·5	3·0	15·2	7·1	4·2	10·9	22·3	9·7	2·9	7·1	4·7	0·7	4·9	53·3	2·3	1·4
Winter mean	—	21	14·5	2·2	27·5	51·9	3·8	8·6	3·5	8·8	31·5	43·7	9·7	3·9	6·9	0·8	1·7	4·5	69·3	9·9	2·4

compensated for by higher fibre rather than starch as in Expt 1; the zero-tannin group showed lower seed coat proportions (TEG) and the only one genotype carrying the *zt2* gene (code 662) was remarkable through its high protein and starch contents, its low fibre and seed coat proportions and its high IVDP.

In this Expt 2, the cotyledon cell-walls (CCW) were measured separately from the whole seed cell-walls (WICW). This cotyledon character varied from 6·5 to 8·8% of the DM. Three genotypes (617, 630, 667) appeared to have a low CCW content without being of a specific faba bean category. NDF correlated positively with ADF ($P < 0{\cdot}05$, $r = 0{\cdot}58$, 10 D.F., [NDF %] = 1·06 [ADF %] + 6·2) as in Expt 2 and NDF was related to WICW ($P < 0{\cdot}01$, $r = 0{\cdot}71$, 10 D.F., [WICW %] = 0·79 [NDF %] + 3·6). The seed coat proportion was positively correlated with cellulose ($P < 0{\cdot}01$, $r = 0{\cdot}71$, 10 D.F., [INTEG %] = 1·03 [Cellulose %] + 3·7). Gross energy measured on the 12 genotypes showed an extremely low coefficient of variation (1·2%) and this criterion could not be used to distinguish between them.

Detailed chemical analysis in Expt 2

Amino acid composition

The range of variation in amino acid composition was generally small and could not be used to discriminate between the genotypes (Table 6). Amino acid contents (seed DM basis) were positively correlated with total protein contents and this relationship was significant ($P = 0{\cdot}05$) except for glutamine, methionine, phenylalanine and histidine where the correlation was not significant (data not shown).

Starch composition

Mean amylose content of starch was 28% for the spring genotypes only.

Lipids composition

Total lipid content determined by summing GC measures of individual fatty acids contents (Table 7) or by ether extract procedure (Table 5) gave similar mean results although correlation between the two methods was not significant ($r = 0{\cdot}38$, 10 D.F.). Relative to total lipids, prominent fatty acids were C18:2 (52%) and C18:1 (28%). Even if a small tendency to higher total lipid contents was observed in the winter genotypes, the lipid characteristics measured did not discriminate between the genotypes of this experiment.

Oligosaccharides

Verbascose represented the major part (64%) of oligosaccharides. Winter genotypes were significantly distinct from spring genotypes through higher verbascose and total oligosaccharides contents (Table 7).

Antinutritional factors

While total phenolics were not significantly different between the faba bean groups (Table 7), the content of condensed tannins clearly distinguished the [T^-] from the [T^+] genotypes.

Trypsin inhibiting activity appeared higher in the spring (4·7 units/mg DM) than in the winter material (0·8 units/mg DM) (Table 7).

Mean content for lectins and phytic phosphorus were 0·12% and 0·29% of seed DM, respectively, without any clear differences between faba bean groups (Table 7).

Since the genotypes carrying *vc*$^-$ gene were only present in the spring faba beans, the winter/spring difference was due to this bias. However, it should be noted that the highest vicine contents (nearly 1% of seed DM) were always found in winter genotypes (Table 7).

DISCUSSION

The collection of faba bean genotypes considered in this work consisted mainly of cultivars or breeder's populations which had been adapted to European farming practices. Large seeded broadbeans (*Vicia faba* L. major) or exotic material were not included in the study, therefore the investigations do not cover the existing variability in this species. On chemical composition, the means and variances on the main seed constituents were close to data published by Eden (1968) and Bjerg *et al.* (1988) who considered several faba bean genotypes. The higher protein content in the spring-sown than in the autumn-sown faba beans is in agreement with the data of Eden (1968) and Grosjean (1984). Although our experimental scheme did not permit the separation of environmental effects from genetic ones, it has been shown by Bond & Toynbee-Clarke (1968) that this difference has a partial genetic basis, since it still exists when winter and spring genotypes are grown together in the spring. The variations in crude fat were small in general, although a few winter lines exhibited values of 4·7% of seed DM, which may offer a source of variability that could be of interest to breeders in achieving higher energy values.

The strong negative correlation measured between the two main faba bean seed constituents, protein and starch, has also been observed in peas ($P < 0{\cdot}01$, $r = -0{\cdot}64$, 171 D.F.) by Bastianelli *et al.* (1998). The positive correlation of cellulose content with seed coat proportion identified, is due to the fact that most of the cellulose is contained in the seed coat as is also

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found in peas (Brillouet and Carré, 1986; Grosjean *et al.* 1992). The negative correlation we found between *in vitro* degradability of proteins and NDF may be explained by a reduced enzymatic accessibility to proteins due to the cell walls. In peas, the cell wall fraction is considered as a possible factor reducing digestibility *in vivo* (UNIP–ITCF 1995).

The isogenic comparisons showed that reducing vicine and convicine by the *vc*$^-$ gene had no secondary effect on major seed components. No plant disease susceptibility has been detected in connection with this genetic change (Cubero & Duc 1995). However, varietal selection through the reduction of the vicine and convicine contents of the seeds can favour the colonization of *V. faba* by phytophagous insects, as demonstrated for *Callosobruchus maculatus* F. by Desroches *et al.* (1995). This problem may be overcome in a first step by the use of adequate insecticides. Therefore the breeding of zero-vicine cultivars of faba bean seems achievable.

Isogenic comparisons between [T$^+$] and [T$^-$] genotypes identified differences in seed composition indicating a theoretical nutritional superiority of the *zt2* gene over the *zt1* gene, based on fibre reduction and protein increase. This effect is in addition to the positive nutritional traits associated with both genes resulting in a substantial reduction of the tannins. It is also in addition to the reduction of the seed coat proportion in zero-tannin genotypes which accounted for 2·1% of DM in Expt 1 and 2, for 2·8% of DM in results of Helsper *et al.* (1993) and for 4% of DM in the study of large seeded faba beans by Cabrera & Martin (1986). Our results did not confirm the 14% higher vicine and convicine contents of tannin-free over tannin-containing genotypes found in cotyledons by Helsper *et al.* (1993). A mean higher susceptibility to soil borne pathogens has been observed in zero-tannin genotypes, which can be reduced by a selection pressure or seed dressing (Bond & Duc 1994; Cubero & Duc 1995). Therefore the breeding of zero-tannin types is feasible as confirmed by successful cultivar registrations in Europe over the last 10 years. Most of the zero-tannin cultivars presently released in Europe contain the *zt1* gene and our data suggest that the next improvement step of faba bean feeding value, may result from the use of *zt2* gene. However, this recommendation needs to be confirmed by further feeding trials.

The amino acid composition data are in good agreement with values reported in the literature by Palmer & Thompson (1975), Mossé & Baudet (1977) and Bjerg *et al.* (1988). In reference to essential amino acids needed in poultry feeds (Leclercq 1996), the faba bean protein is rich in lysine and low in methionine, cysteine and tryptophane. The high correlations between different amino acid contents (% of DM) and the total protein content published for faba bean (Mossé & Baudet 1977; Sjödin *et al.* 1981*a*) or pea (Bastianelli *et al.* 1998) holds true on the faba bean seed batches analysed in Expt 2.

The mean contents or activities of the main antinutritional factors in genotypes analysed fall within the range in the literature data for faba bean seeds. The zero values for tannin content in white-flowered cultivars we recorded are in agreement with those obtained by Picard (1976), Martin-Tanguy *et al.* (1977), Cabrera & Martin (1986) and Duc *et al.* (1995). Much higher maximum values for seed tannin content (3·5% of DM) were reported for some coloured-flowered genotypes by Cabrera & Martin (1986). Sjödin *et al.* (1981*b*) reported condensed tannin percentages from 0·1 to 1·4% of seed DM and trypsin inhibitor activities from 1·7 to 3·6 units/mg of DM. Even if values from 4·1 to 5·3 units of TI activity/mg were found in the spring material of Expt 2, these fall within the category of low antinutritional risk in comparison with some winter peas and soyabeans which can reach 15 and 50 units of TI activity, respectively. For the vicine and convicine contents, the [V$^+$] genotypes are within the limits previously found for different collections of faba beans (Gardiner *et al.* 1982; Duc *et al.* 1989; Wang & Ueberschär 1990) and the strong reduction in glucosides content induced by the *vc*$^-$ gene (12-fold in this experiment) is in agreement with previous experiments by Duc *et al.* (1989). Phytate phosphorus contents agree with the values of 0·21–0·31% of seed DM obtained by Griffiths & Thomas (1981). Stachyose, verbascose and oligosaccharides contents also fall within the range reported by Dini *et al.* (1989).

The database produced in this work is, to our knowledge, the largest composition database realized on diversified genotypes of faba beans, excluding broadbeans and exotic types. These composition data confirmed that faba bean seeds represent an interesting protein source in animal feeds. Breeders can improve the quality of this production through the reduction of antinutritional factors by using the zero-tannin gene *zt2* and the low vicine-convicine gene *vc*$^-$.

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