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Rutin and Proanthocyanidin Contents in Buckwheat Grains: Combined Biosynthesis in Interspecific Hybrids between *Fagopyrum esculentum* Moench x *F. homotropicum* Ohnishi and their Progeny

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ABSTRACT

Hybrids between sporophytic self-incompatible common buckwheat *F. esculentum* and self-fertilizing *F. homotropicum* are promising with respect to an improvement of agronomic productivity. However, the nutritional potential of these hybrids regarding their flavonoid phenolics profiles exhibit wide variability which is based on the metabolic differences between the two species. In *F. homotropicum* the flavan 3-ol content is dominating whereas rutin – being the focus of interest for buckwheat consumers – was represented in higher amounts in *F. esculentum.* Four classes of phenolic compounds, namely benzoic acids, hydroxycinnamic acids, flavonols and flavan 3-ols, were quantified in the grains of breeding lines and hybrids. Based on these data it could be shown that the metabolic channeling within the flavonoid biosynthesis is inherited. Two main regulatory steps of the phenylpropanoid/flavonoid pathway are postulated which seems to be responsible for different flavonoid phenotypes, rutin- and flavan 3-ol-phenotypes, in particular. Metabolomic studies by using HPLC/DAD and HPLC/CRD methods allow the description of the flavonoid phenotypes for selection of appropriate parents and valuable hybrids as a basis for breeding of varieties with optimized phenolic profiles.

Keywords: benzoic acids, breeding, HPLC, hydroxycinnamic acids, flavanols, flavonols, metabolomics, propelargonidins

INTRODUCTION

The high nutritional value of common buckwheat (*Fagopyrum esculentum* Moench, Polygonaceae) is based on its content of favourable amino acid composition with high levels of lysine, arginine, aspartic acid and tryptophan as well as on its high contents of phenylpropanoids and flavonoids (Reichling and Horz 1993; Hagels 1996; Watanabe 1998). Phenolic metabolites are recognised as beneficial for humans by their multifunctional bioactivity (Kaur and Kapoor 2001; Auger *et al.* 2004). The most prominent buckwheat flavonoid is the flavonol rutin which is a well known plant antioxidant (Knekt *et al*. 1996; Oomah and Mazza 1996; Chao *et al.* 2002; Fabjan 2003; Kim *et al*. 2005; Jiang *et al.* 2007). In contrast, a high proanthocyanidin content also occurring in buckwheat is occasionally regarded as adverse because of its negative effect on protein digestibility (Butler 1988; Ikeda *et al.* 1991; Kolodziej 1994; McMahon *et al*. 1999). However, also for flavan 3-ols, including proanthocyanidins, beneficial health effects have been reported including free radical scavenging activities, anti-cancerogenic and anti-bacterial properties (Kada *et al.* 1985; Middleton and Kandaswami 1993; Mukoda *et al.* 2001; Carnesecchi *et al.* 2002; Natella *et al.* 2002; Sun and Ho 2005; Zern and Fernandez 2005; Faria *et al.* 2006). Watanabe *et al*. (1997) and Quettier-Deleu (2000) already pointed out that the proanthocyanidins of buckwheat grains contribute substantially to their antioxidant property. Polyphenolic phytochemicals of various structures also have indirect antioxidant effects through induction of endogenous protective enzymes and may exhibit gene modulatory activity (Stevenson and Hurst 2007; Wenzel and Daniel 2008).

Nevertheless, high rutin content is the focus of interest for buckwheat consumers (Jiang *et al.* 2007). With regards to the leaves, several studies show the influence of plant development, of environmental conditions and of agrotechniques on rutin accumulation (Ohsawa and Tsutsumi 1995; Kreft *et al.* 2002; Fabjan e*t al.* 2003; Germ 2004; Suzuki *et al.* 2005). Breeding is another promising tool to optimize the content of diverse phenolic compounds in buckwheat grain (Ölschläger *et al.* 2008).

One weakness of *F. esculentum* limiting its cultivation is its self-incompatibility leading to low grain yield (Zeller and Hsam 2004). The detected wild species *F. homotropicum* (Ohnishi and Asano 1998) possesses self-fertility which is inherited by two complementary dominant genes (Wang *et al*. 2005b). Hybrids between *F. esculentum* and *F. homotropicum* are promising with respect to an improvement of agronomic productivity (Chen *et al.* 2004; Wang *et al.* 2005a). Moreover, the nutritional potential of these hybrids with respect to their rutin content and general phenolics profiles exhibit a wide variability (Ölschläger *et al*. 2008). In particular, the quantities of rutin and flavanols differ markedly between the two species. In *F. homotropicum* the flavan 3-ol content is dominating whereas the rutin was represented in higher amounts in *F. esculentum* (Ölschläger *et al*. 2008).

In the current paper we describe that the metabolic channeling within the flavonoid biosynthesis is inherited and we give an approach for selection of appropriate parents for breeding of varieties with optimized phenolic profiles.

Phenolic compounds in buckwheat grains

Members of five phenolic classes have been described in buckwheat grain so far. Simple benzoic acids were described by Durkee (1977). These are *p*-hydroxybenzoic acid

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Original Research Paper

Table 1 Description of the parental plants used in this study.

Short name	Description	Origin	Characteristics	No. back crosses	No. self crossings
954, 9510	Sobano x [Ballada x *F. homotropicum*]	Japan	homostyle flower	6	4, 10
615, 619	Sumchanka SUN	Russia	homostyle, determined growth	6	5, 9
852	Pulanska/PA054 x *F. homotropicum*	Japan	homostyle	3	4
15	Siva x *F. homotropicum*	Slovenia/Japan	homostyle	3	2
1556, 15514, 15517	Sun Rutin x [Sumchanka x *F. homotropicum*]	Korea/Russia	homostyle	1	6, 14, 17
158	Demetra x [Sumchanka x *F. homotropicum*]	Russia/Japan	homostyle, determined growth	5	3
26	Hruszowska x *F. homotropicum*	Poland/Japan	homostyle, determined growth	6	2

and the methylated ones vanillic acid and syringic acid (**Fig. 1**). Further phenolic acids belong to the class of hydroxycinnamates which occur as derivatives of *p*-coumaric acid (Durkee 1977; Ölschläger *et al.* 2008) and caffeic acid (Pomeranz 1985; Ölschäger *et al*. 2008). The flavonoids of buckwheat grain are flavones, flavonols and flavan 3-ols. All of them possess identical A-ring hydroxylation patterns at positions C-5 and C-7 whereas the B-ring is hydroxylated either in the *p*-position corresponding to *p*-coumaric acid or in positions C-3', 4' like caffeic acid (**Fig. 1**). The flavones identified so far are C-glucosides of apigenin (vitexin and isovitexin) and C-glucosides of luteolin (orientin and isoorientin) (Dietrych-Szostak and Oleszek 1999). The class of flavonols is represented by 3-*O*-glycosides of quercetin, namely the rhamnoside quercitrin, the galactoside hyperoside and the rhamno-glucoside rutin (Reichling and Horz 1993; Hagels 1996; Dietrych-Szostak and Oleszek 1999). The flavan 3-ols consist basically of catechin, epicatechin and epiafzelechin units. They form a series of oligomeric structures and several of them are acylated at position C-3 by forming ester linkages with benzoic acid derivatives (**Fig. 1**). The predominating flavan moiety is the epicatechin which also occurs as simple epicatechin itself as well as acylated with p-hydroxybenzoate (Watanabe 1998), with gallate (Quettier-Deleu *et al.* 2000; Ölschläger *et al.* 2008) and with 3,4-di-*O*-methylgallate (Watanabe 1998; Ölschläger *et al.* 2008). The dimeric procyanidins B2 and B5 (Quettier-Deleu *et al.* 2000; Ölschläger *et al.* 2008) are based on epicatechin units. Procyanidin B2 was also found acylated with either gallate (Quettier-Deleu *et al.* 2000; Ölschläger *et al.* 2008) or 3,4-dimethylgallate (Ölschläger *et al.* 2008). Epicatechin also forms the terminal unit of several propelargonidins (**Fig. 1**) with epiafzelechin as extension units. Some of them are linked to *p*-OH-benzoic acid or to methyl-gallates. A minor flavan 3-ol is catechin (Watanabe 1998; Ölschläger *et al.* 2008) which was also found as 7-*O*-glucoside (Watanabe 1998).

MATERIALS AND METHODS

Description of the plant material

The parental plants used in the study were inbred lines of interspecific hybrids between *F. esculentum* and *F. homotropicum* which had been back-crossed and selfed several times with common buckwheat. The description of parental plants is given in **Table 1**. The seeds were harvested after ripening on the individual plants during spring and summer.

Extraction for HPLC-analysis

Single de-hulled buckwheat grains of the plants described in **Table 1** were ground in a mortar. The extraction of phenolic compounds was performed by adding 500 µl aqueous methanol, gradient grade (Merck) ($MeOH/H_2O$, 80/20, v/v), containing flavone (Roth Karlsruhe, Germany) (c = 0.02 mg/ml) as an internal standard, for 30 min in a cooled ultrasound water bath (7°C). After centrifugation, the supernatant was evaporated, the residue re-dissolved in 100 µl methanol (80%) and 10 µl were injected for HPLC analysis.

High-Performance Liquid Chromatography (HPLC)

The HPLC equipment used consists of an autosampler (Gilson Abimed Modell 231), of two pumps (Kontron Modell 422) and a diode array detector (Bio Tek Kontron 540) for UV/Vis detection (HPLC/DAD). For chemical reaction detection (HPLC/CRD) a further analytical HPLC pump (Gynkotek Modell 300 C) and a VIS-detector (640 nm, Kontron Detektor 432) were used.

The phenolic compounds were separated on a column (250 × 4 mm I.D.) prepacked with Hypersil ODS, 3 µm particle size (Shandon), following a stepwise gradient using mixtures of solvent A (formic acid (Merck), 5% in water) and solvent B (methanol, gradient grade, Merck) from 95: 5, v/v to 10:90, v/v with a flow rate of 0.5 ml/min (Treutter *et al.* 1994). The gradient profile (% B in A) used was: 0-5 min, isocratic, 5% B; 5-15 min, 5-10% B; 15-30 min, isocratic, 10% B; 30-50 min, 10-15% B; 50-70 min, isocratic, 15% B; 70-85 min, 15-20% B; 85-95 min, isocratic, 20% B; 95-110 min, 20-25% B; 110-140 min, 25-30% B; 140-160 min, 30-40% B; 160-175 min, 40-50% B, 175-190 min, 50-90% B.

Rutin, simple phenolics and hydroxycinnamic acids were detected at 360, 280 and 320 nm (HPLC/DAD), respectively, whereas the flavanols were estimated at 640 nm after post-column derivatization (HPLC/CRD) with *p*-dimethyl-aminocinnamic aldehyde (DMACA (Merck); Treutter 1989; Treutter *et al.* 1994).

Quantitative analysis

The known compounds were quantified based on response factors obtained from their calibration curves using the internal standard method. For that, flavone was added to the extraction solvent at a concentration of 0.1 mg/ml. Flavonols were estimated as rutin, hydroxycinnamic acids and simple phenolics as chlorogenic acid. The flavanols epiafzelechin-[4-8]-epiafzelechin-[4-8]-epicatechin, epiafzelechin-[4-6]-epicatechin, epicatechin-[4-8]-epicatechin-*O*-3,4-dimethylgallate, epiafzelechin-[4-8]-epicatechin-*O*-(3,4-dimethyl)-gallate, epiafzelechin-[4-8]-epiafzelechin-[4-8]-epicatechin-*O*-(3,4-dimethyl)-gallate, epiafzelechin-[4-8]-epicatechin-methylgallate and epiafzelechin-[4-8]-epicatechin-benzoate were quantified as epicatechin.

Peak identification

The peaks were identified according to their UV-absorbance, their chromatographic behaviour on reversed phase chromatography (HPLC) and thin layer chromatography in comparison to authentic standards, enzymatic hydrolysis by tannase and LC-MS/MS data.

The following standards were available from Roth (Karlsruhe, Germany): chlorogenic acid, catechin, epicatechin, rutin. The procyanidin B2 was previously isolated from service tree (*Sorbus domestica* L.) fruit (Ölschläger *et al.* 2004). Procyanidin B5 was isolated from apple leaves (Treutter *et al.* 1994).

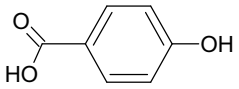
Description of the metabolic channelling

The strengths of the metabolic channels corresponding to the main junctions (**Fig. 5**) are defined as follows:

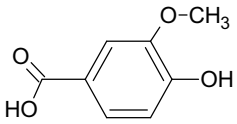
A: strong general phenolic pathway with a total phenol content of ≥ 200 mg/DW

a: weak general phenolic pathway with a total phenol content of < 200 mg/DW

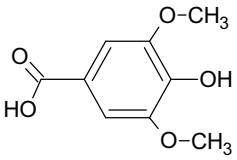
Benzoic acids



p-OH-Benzoic acid
(Durkee 1977)

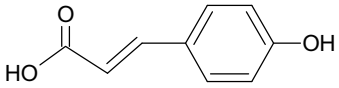


Vanillic acid
(Durkee 1977)

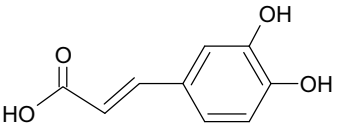


Syringic acid -3,5-dimethylgallate
(Durkee 1977)

Hydroxycinnamic acids (derivatives as esters and glycosides)

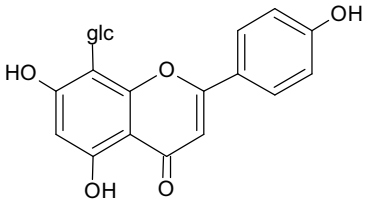


p-Coumaric acid
(Durkee 1977; Ölschläger *et al.* 2008)

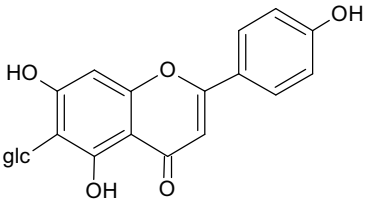


Caffeic acid
(Pomeranz 1985; Ölschäger *et al.* 2008)

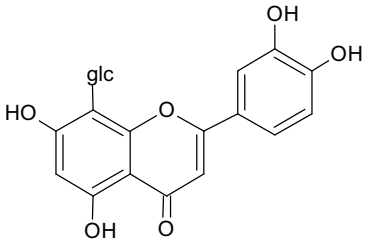
Flavones



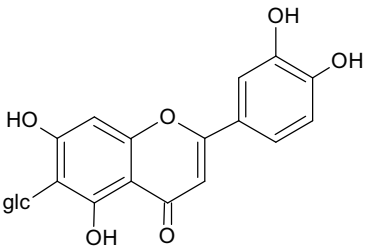
Vitexin
(Dietrych-Szostak and Oleszek 1999)



Isovitexin
(Dietrych-Szostak and Oleszek 1999

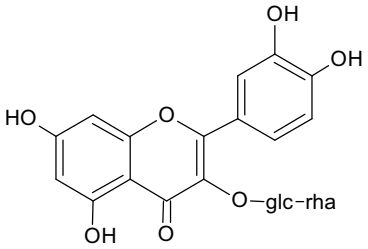


Orientin
(Dietrych-Szostak and Oleszek 1999

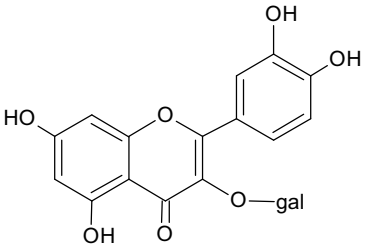


Isoorientin
(Dietrych-Szostak and Oleszek 1999

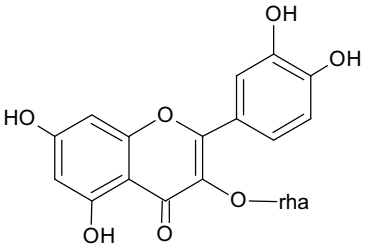
Flavonols



Rutin
(Reichling and Horz 1993; Hagels 1996; Dietrych-Szostak and Oleszek 1999)



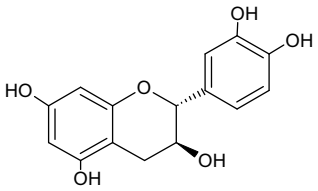
Hyperoside
(Hagels 1996)



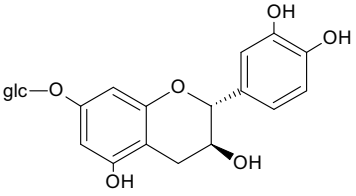
Quercitrin
(Hagels 1996)

Flavan 3-ols

Catechins



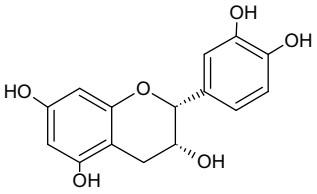
Catechin
(Watanabe 1998; Ölschläger *et al.* 2008)



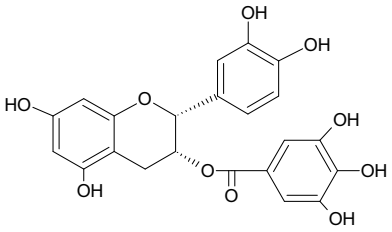
Catechin 7-*O*-glc
(Watanabe 1998)

Fig. 1 Structures of phenolic compounds occurring in buckwheat grains.

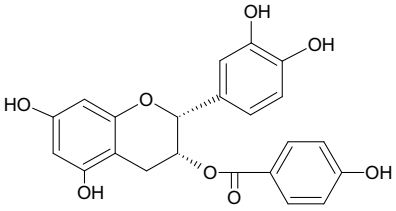
Flavan 3-ols (cont.)



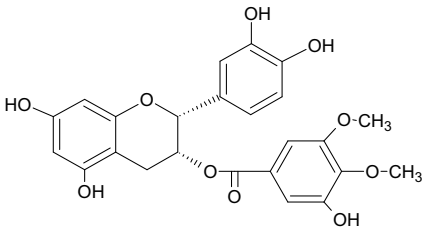
Epicatechin
(Watanabe 1998; Quettier-Deleu *et al.* 2000; Ölschläger *et al.* 2008)



Epicatechin 3-*O*-gallate
(Quettier-Deleu *et al.* 2000; Ölschläger *et al.* 2008)

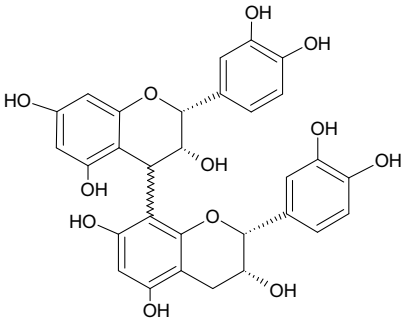


Epicatechin 3-*O*-*p*-OH-benzoate
(Watanabe 1998)

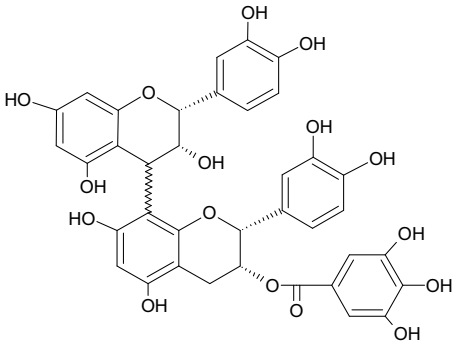


Epicatechin 3-*O*-(3,4-di-*O*-methyl)gallate
(Watanabe 1998; Ölschläger *et al.* 2008)

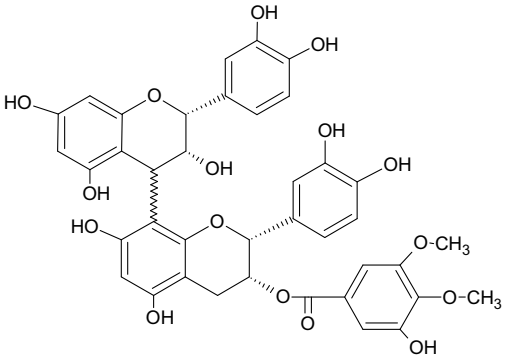
Procyanidins



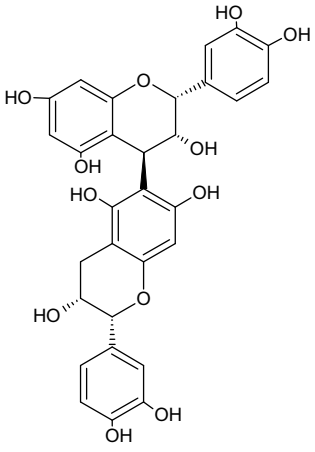
Procyanidin B2
(Quettier-Deleu *et al.* 2000; Ölschläger *et al.* 2008)



B2 3-*O*-gallate
(Quettier-Deleu *et al.* 2000; Ölschläger *et al.* 2008)



Procyanidin B2 3-*O*-(3,4-dimethylgallate) (Ölschläger *et al.* 2008)



Procyanidin B5
(Quettier-Deleu *et al.* 2000; Ölschläger *et al.* 2008)

B: strong path towards simple phenolics with a percentage of ≥ 25% of total phenolics

b: weak path towards simple phenolics with a percentage of < 25% of total phenolics

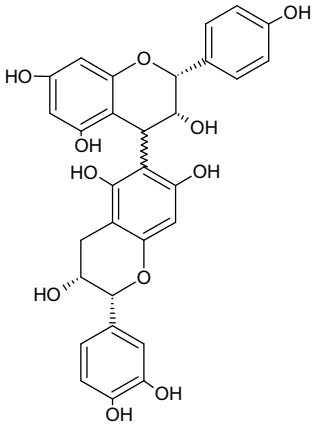
In our studies, all grains showed values for simple phenolics below 25%.

C: strong path towards hydroxycinnamic acids with a percentage of ≥ 30% of ([total phenolics] – [simple phenolics])

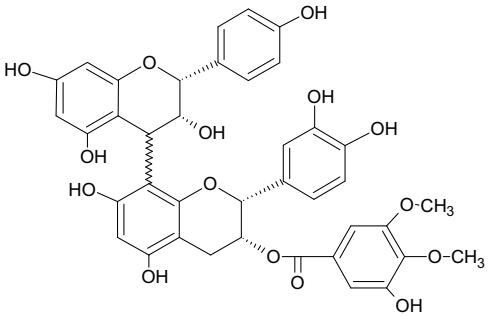
c: weak path towards hydroxycinnamic acids with a percentage of < 30% of ([total phenolics] – [simple phenolics])

D: strong path towards flavonols with a percentage of ≥ 50% of ([total phenolics] – [simple phenolics] – [hydroxycinnamic acids])

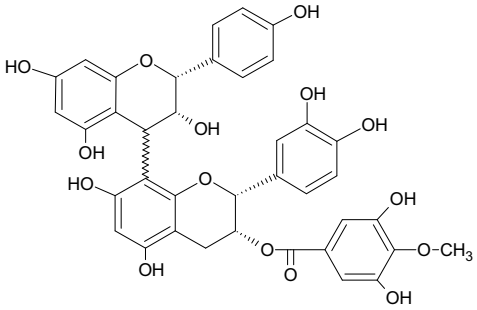
Propelargonidins



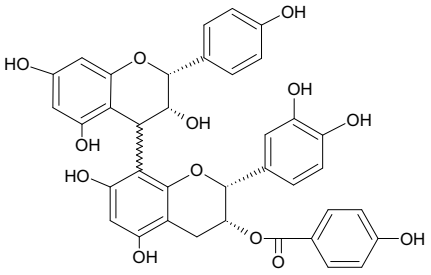
Epiafzelechin-(4-6)-epicatechin (Ölschläger *et al.* 2008)



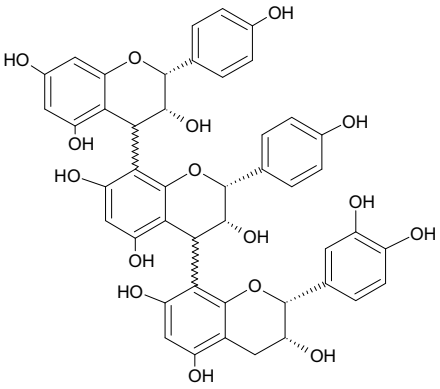
Epiafzelechin-(4-8)-epicatechin 3-*O*-(3,4-dimethyl)gallate (Ölschläger *et al.* 2008)



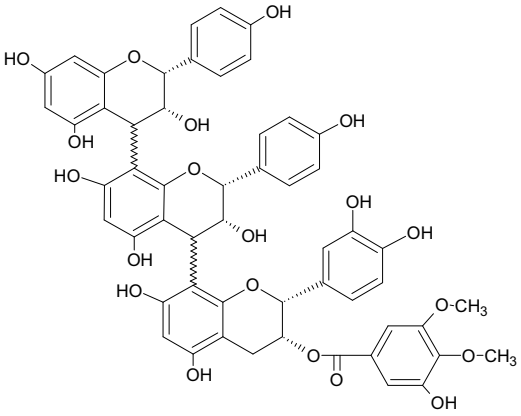
Epiafzelechin-(4-8)-epicatechin 3-*O*-(4-methyl-gallate) (Ölschläger *et al.* 2008)



Epiafzelechin –(4-8)-epicatechin-[(-3-*O*-[*p*-OH-benzoate] (Ölschläger *et al.* 2008)



Epiafzelechin-(4-8)-epiafzelechin-(4-8)-epicatechin (Ölschläger *et al.* 2008)



Epiafzelechin-(4-8)-epiafzelechin-(4-8)-epicatechin-3-*O*-(3,4-dimethylgallate) (Ölschläger *et al.* 2008)

d: strong path towards flavan 3-ols with a percentage of ≥ 50% of ([total phenolics] – [simple phenolics] – [hydroxycinnamic acids]), complementary to a weak flavonol pathway.

RESULTS AND DISCUSSION

Phenolic profiles of parental plants used for crossing experiments

The analyses of single grains of the parental plants show total phenol contents between 100 and 400 mg/100 g DW (**Fig. 2**). The phenolic profiles concerning the classes of phenolic compounds differ qualitatively (**Fig. 3**) and demonstrate the variability of the profiles which is a prerequisite for breeding purposes. These lines exhibit a predominating accumulation of either rutin (flavonol) or flavan 3-

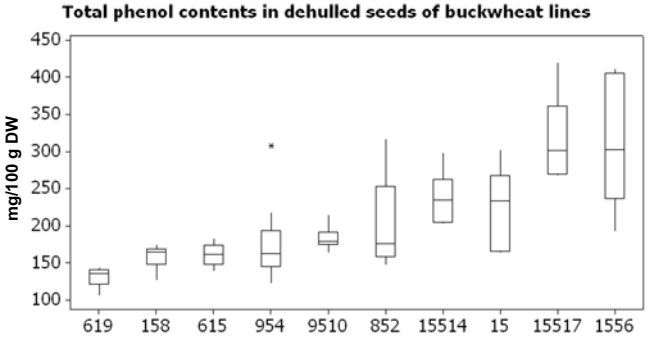


Fig. 2 Box plots of total phenol contents in dehulled grains of *F. esculentum* x *F. homotropicum* hybrids used as parental lines in this study.

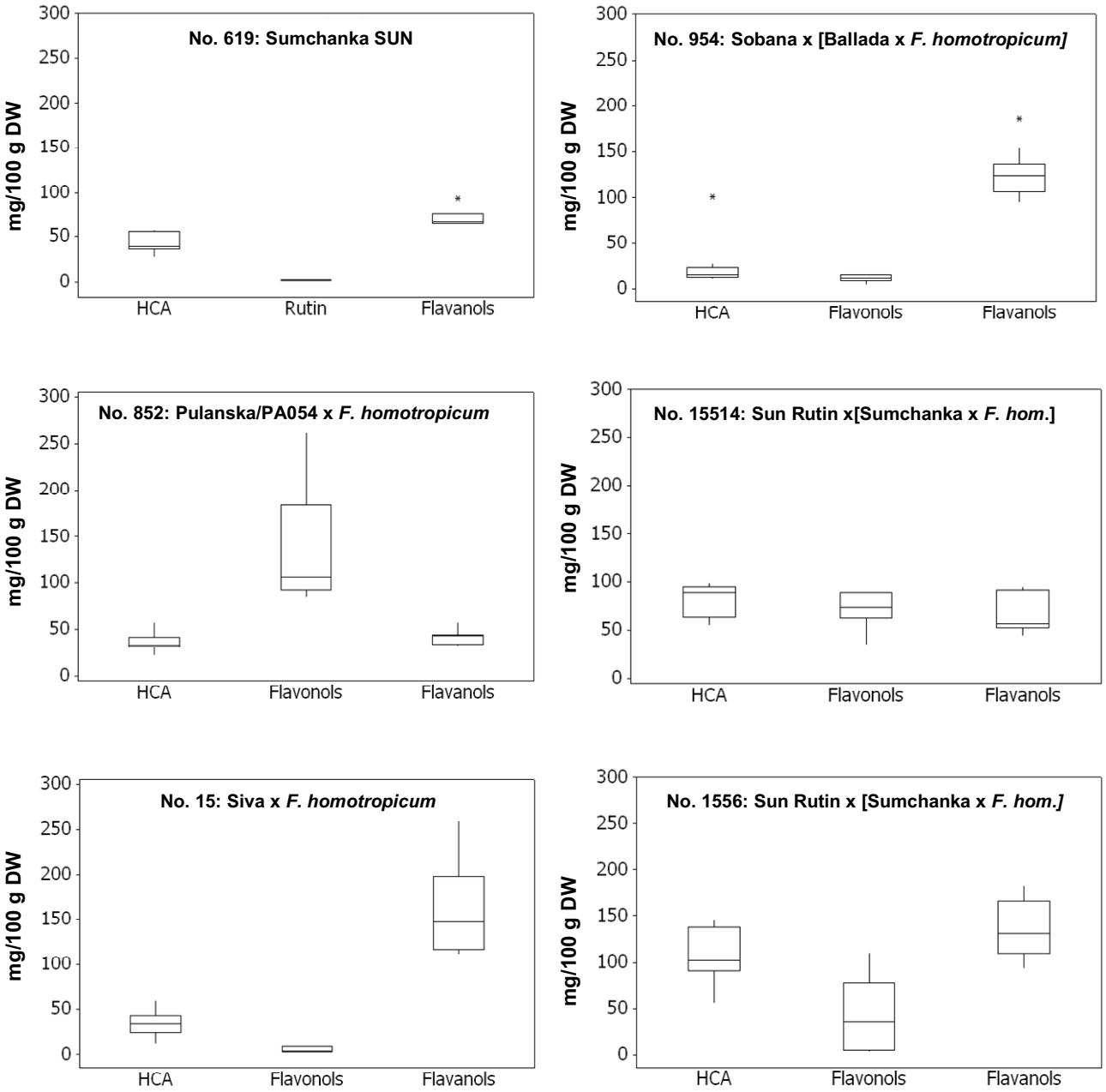


Fig. 3 Phenolic class profiles including hydroxycinnamic acids (HCA), flavonols (mainly rutin) and flavanols of selected parental lines.

ols or hydroxycinnamic acids. Furthermore, a balanced accumulation of all the three phenolic classes also occurs.

Breeding buckwheat for specific flavonoid profiles

In order to study if the phenolic profile is inherited and how it segregates the parental plants as well as the progenies were classified according to their metabolic channelling into distinct groups of phenotypes. The biosynthetic pathways leading to the main phenolic classes of buckwheat grain are shown in a simplified scheme in **Fig. 4**. The benzoic acids are thought to be derived from the shikimate pathway which is also the origin of the aromatic amino acid phenylalanine. The latter is the starter molecule for the biosynthesis of phenylpopanoids represented by hydoxycinnamic acids, and of flavonoids. A number of enzymes are directing intermediate metabolites towards the pools storing the end-products which can be detected by the analyst and are marked with boxes in **Fig. 4**. Flavones are not found in our studies and would be derived from the flavanone naringenin and/or eriodictyol by the activity of a flavone synthase. To our knowledge, the enzymes catalyzing the different steps of these pathways in buckwheat have not been characterized so far. The scheme (**Fig. 4**) is therefore based on the general knowledge on flavonoid biosynthesis in higher plants.

The high number of enzymes involved in the biosynthesis of phenolics indicates an obvious quantitative genetic basis behind the formation of a single endproduct like rutin, for instance. The accumulation of an individual compound is determined by the activity and specificity of the biosynthetic enzymes and also by the availability of the substrates for each enzymatic step and a competition for metabolites may occur. However, four main junctions leading to the different storing pools may be postulated (**Fig. 5**). First of all, metabolic precursors derived from primary metabolism must enter the secondary metabolism, in the case of phenolic compounds, via the shikimate pathway. At this level, a first branching is possible which may lead to the formation of benzoic acid derivatives (**Fig. 5B**). Alternatively, metabolites from the shikimate pathway may be channelled via phenylalanine towards the phenylpropanoids and flavonoids (**Fig. 5A**). The phenylpropanoids or, in a narrow sense, the hydroxycinnamic acids are precursors of flavonoids. However, they also can be stored as esters or glycosides (**Fig. 5C**). The flavonoid path of buckwheat is branching at the

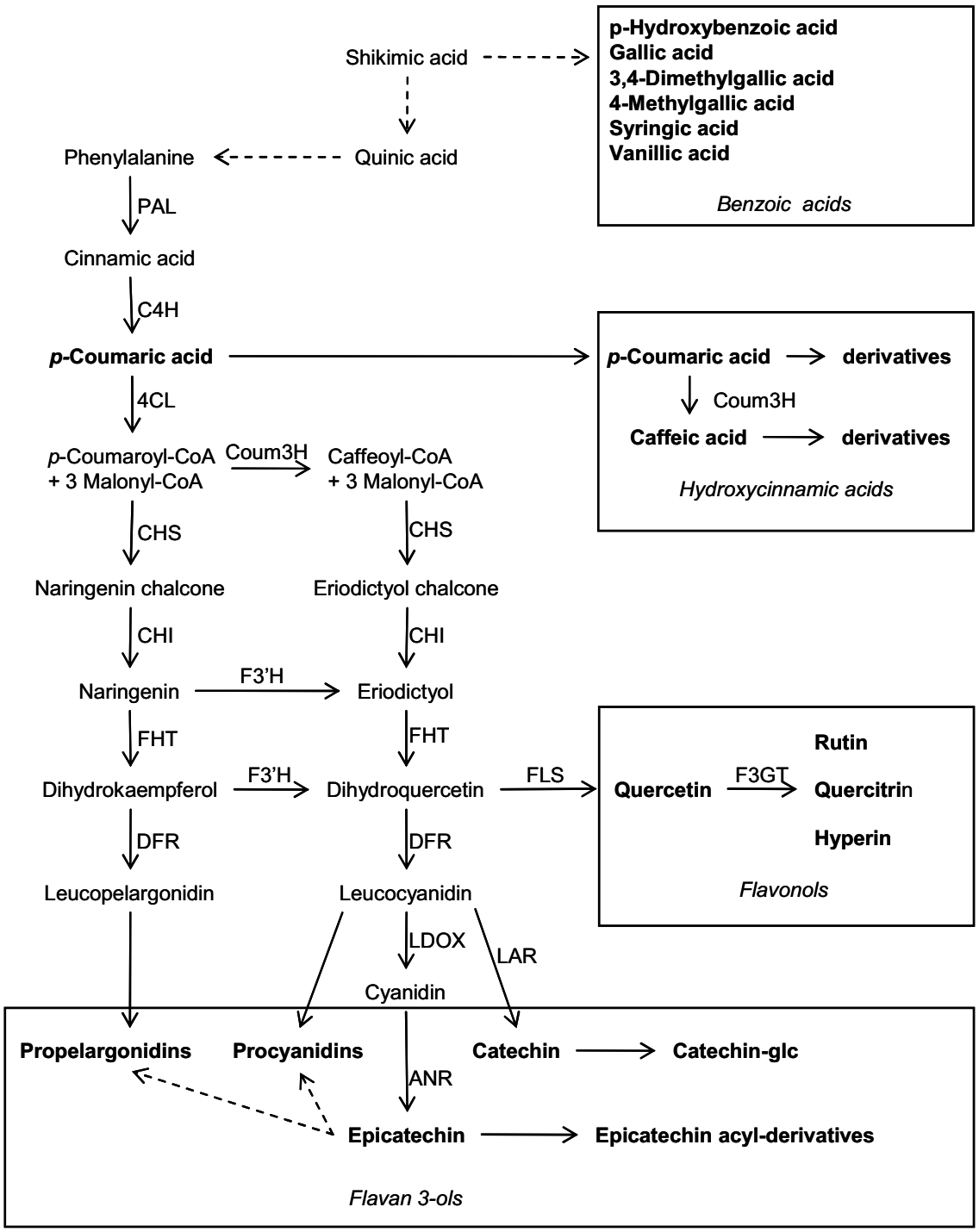


Fig. 4 General biosynthetic scheme of phenols, phenylpropanoids and flavonoids in buckwheat grain. Abbreviations for enzymes: ANR, anthocyanidin reductase; C4H, Cinnamate 4-hydroxylase; CHS, chalcone synthase; CHI, chalcone-flavanone isomerase; 4Cl, 4-OH cinnamate-ligase; Coum3H, coumaroyl 3-hydroxylase; DFR, dihydroflavonol reductase; F3GT, flavonoid 3-glycosyltransferase; F3'H, flavonoid 3'-hydroxylase; FHT, flavanone 3-hydroxylase; FLS, flavonol synthase; LAR, leucoanthocyanidin reductase; LDOX, leucoanthocyanidin dioxygenase; PAL, phenylalananine ammonia lyase.

level of dihydroflavonols being precursors for flavonols (**Fig. 5D**) and for flavan 3-ols.

The single grain analyses show that the parental plants are not homogeneous with respect to the channelling characters of their seeds (**Figs. 6-8**). However, with respect to the strength of the phenylpropanoid/flavonoid pathway in general, which is described as characters 'A' or 'a' for strong and weak, respectively, it can be seen in **Fig. 6** that the phenotype 'A' is dominating over 'a' . If the two parental plants used for crossing are characterised as 'a', this phenotype could also be found in the majority of the progenies (**Fig. 6A**) whereas the use of one 'A'-type parent produces mostly 'A'-type offspring (**Fig. 6B**). A similar observation can be made regarding the hydroxycinnamic acid channel (**Fig. 7**) described as 'C' or 'c' for a strong or a weak pathway, respectively. This character is also inherited and the phenotype 'C' is dominating over 'c'. The inheritability of the flavonol pathway (D/d) remains unclear. It seems that the flavan 3-ol channel ('d') is prevailing (**Fig. 8**) in the progenies.

The frequency distribution of rutin and flavan 3-ol contents in the progeny encoded as D or d for a strong and a weak flavonol path, respectively, both in combination with a strong and a weak general phenylpropanoid/flavonoid path are demonstrated in **Fig. 9**. Highest rutin concentrations (**Fig. 9A**) are found if a strong phenylpropanoid/flavonoid path (A) is combined with a strong flavonol path (D). The combination of these metabolic channels results in a

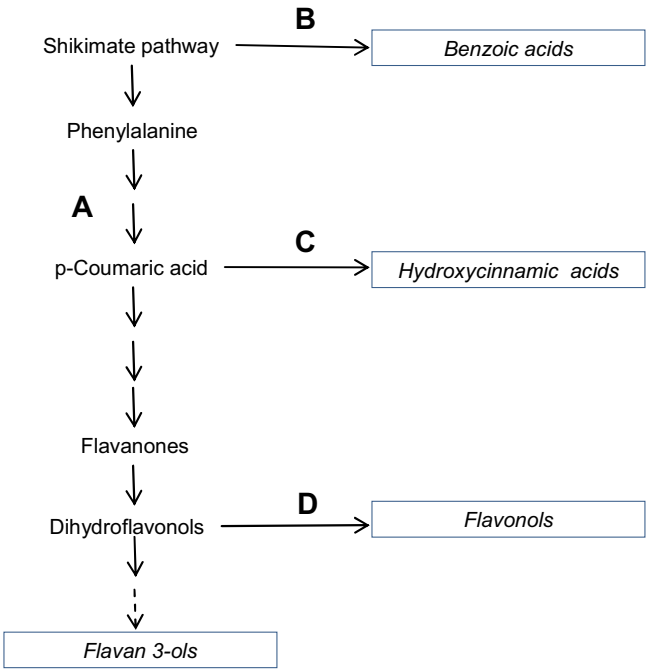


Fig. 5 Junctions within the biosynthesis of buckwheat phenolics leading to different storage pools. The postulated regulatory steps are indicated with A (marking the channelling towards phenylpropanoids and flavonoids), B (channelling towards benzoic acids), C (channelling towards hydroxycinnamic acids), D (channelling towards flavonols and/or flavan 3-ols)

number of grains with rutin concentrations exceeding the total flavan 3-ol amount occurring in the group of 'dA' phenotypes (**Fig. 9D**). However, it is obvious that the maximum frequencies for flavan 3-ols for all phenotype groups are between 60 and 120 mg/g DW and the grains scarcely show that low values as for rutin in 'da' phenotypes. This may indicate that there is no simple competition for metabolites at the junction between flavonols and flavan 3-ols. Moerover, another kind of regulation is obvious. A regulated competition between biosynthesis of anthocyanidins and proanthocyanidins in buckwheat stems was assumed by Matsui *et al.* (2008). Differential activation of distinct phenylpropanoid and flavonoid pathways may happen by the competition of pathways for a common substrate or by differential transcription of structural genes in distinct branches of the corresponding pathway. Numerous regulators controlling the transcription of structural genes have been identified in *Arabidopsis*, maize and petunia (Koes *et al*. 2005). On the transcript level, Takos *et al.* (2005) found out the biosynthesis of proanthocyanidins in apple fruits being separately regulated from other flavonoid biosynthesis genes.

As compared to rutin concentrations of common buckwheat grain and flour described in the literature ranging from 0.2 to 58.4 mg/100 g (Kitabayashi *et al.* 1995; Oohmnah and Mazza 1996; Watanabe *et al.* 1997; Dietrych-Szostak and Oleszek 1999; Quian *et al.* 1999; Steadman *et al.* 2001; Holasova *et al.* 2002; Briggs *et al.* 2004; Park *et al.* 2004; Jiang *et al.* 2005; Brunori and Végvári 2007). Some of the interspecific hybrids exhibit more than 400 mg/100 g (**Fig. 9, Table 2**).

Flavanol and proanthocyanidin concentrations in grains of the hybrids do not substantially exceed the values estimated in *F. esculentum* by Quettier-Deleu *et al.* (2000). They found about 220 mg/100 g of total flavanols and 159 mg/100 g proanthocyanidins and among these the procyanidin B2 with 52 mg/100 g, B2-gallate with 0.5 mg/100 g and traces of procyanidin B5. The monomeric flavanols epicatechin and epicatechin-gallate exhibited concentrations of 4.0 and 0.9 mg/100 g, respectively.

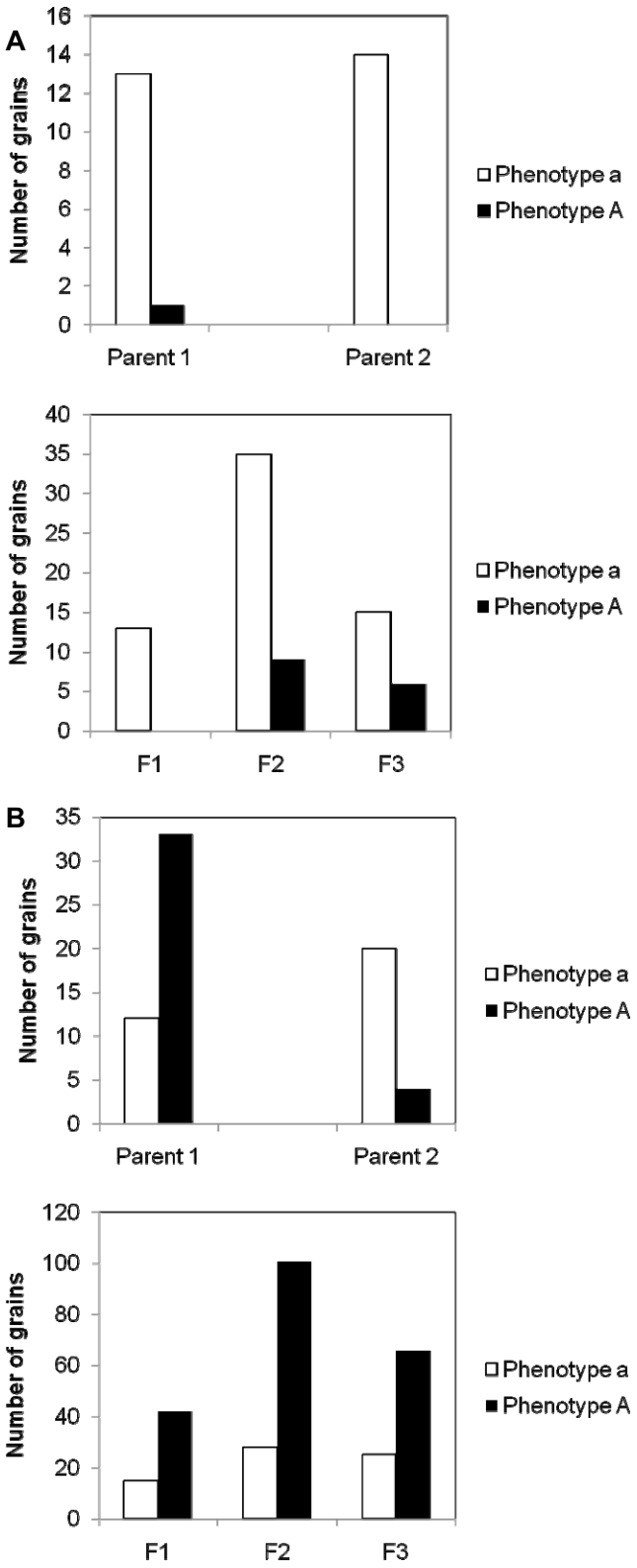


Fig. 6 Channelling characters of the grains of parental plants and of their F1, F2, F3 progenies with respect to the strength of the phenylpropanoid/flavonoid pathway with characters 'A' or 'a' for strong and weak, respectively. (**A**) Crossing of parent plants both exhibiting phenotype 'a'. (**B**) Crossing of parent plants exhibiting 'A'- and 'a'-phenotypes. The numbers of single grains for each phenotype are given.

CONCLUSION

The evaluation of our metabolomic studies revealed that grains with a high total phenolic content do not necessarily contain a high amount of rutin. On the other hand, grains with low total phenolics may exhibit a relatively high percentage of rutin as compared to flavanols and other phe-

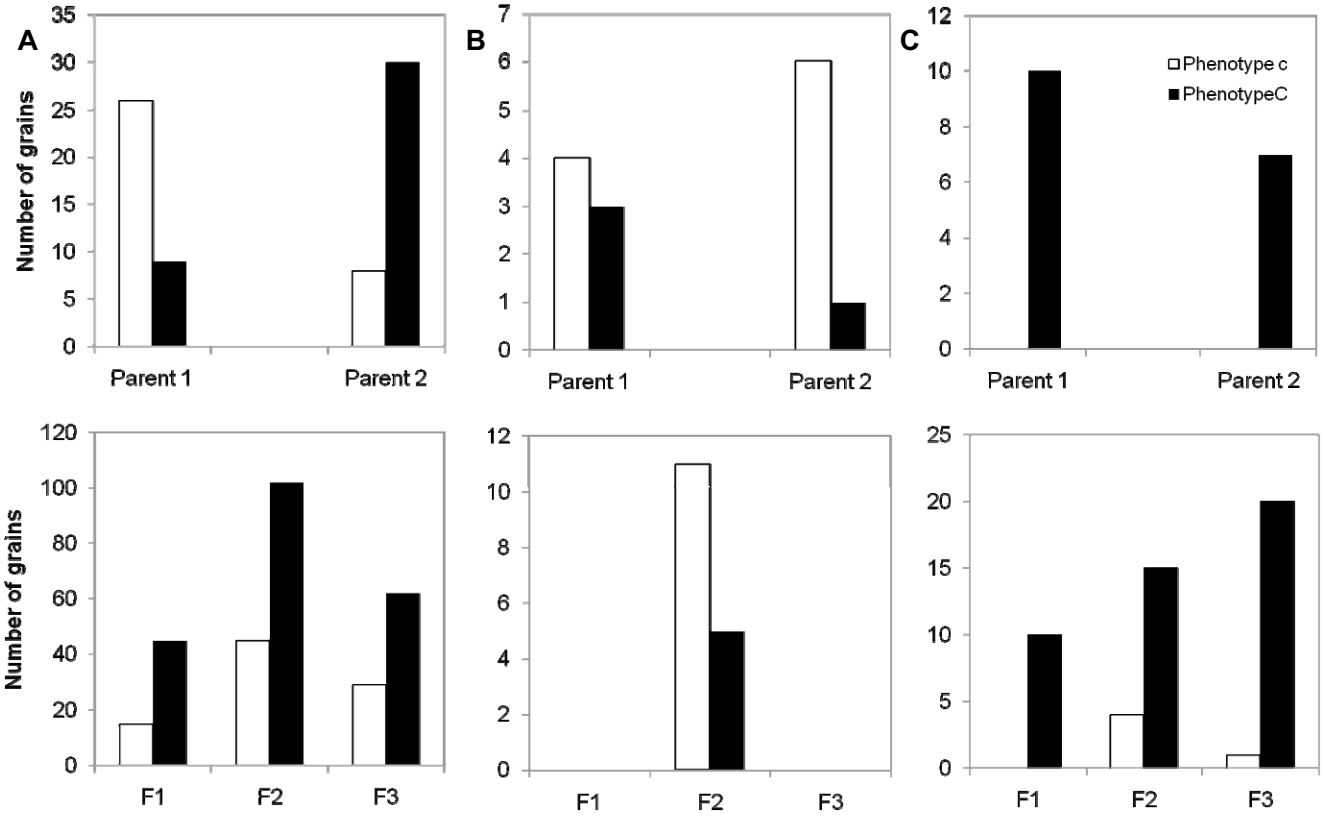


Fig. 7 Channelling characters of the grains of parental plants and of their F1, F2, F3 progenies with respect to the strength of the hydroxy-cinnamic acid pathway with characters 'C' or 'c' for strong and weak, respectively. The number of single grains for each phenotype is given. (**A**) Crossing of parent plants exhibiting phenotypes 'c' and 'C'. (**B**) Crossing of parent plants exhibiting mostly 'c'-phenotypes. (**C**) Crossing of parent plants exhibiting both 'C'-phenotypes.

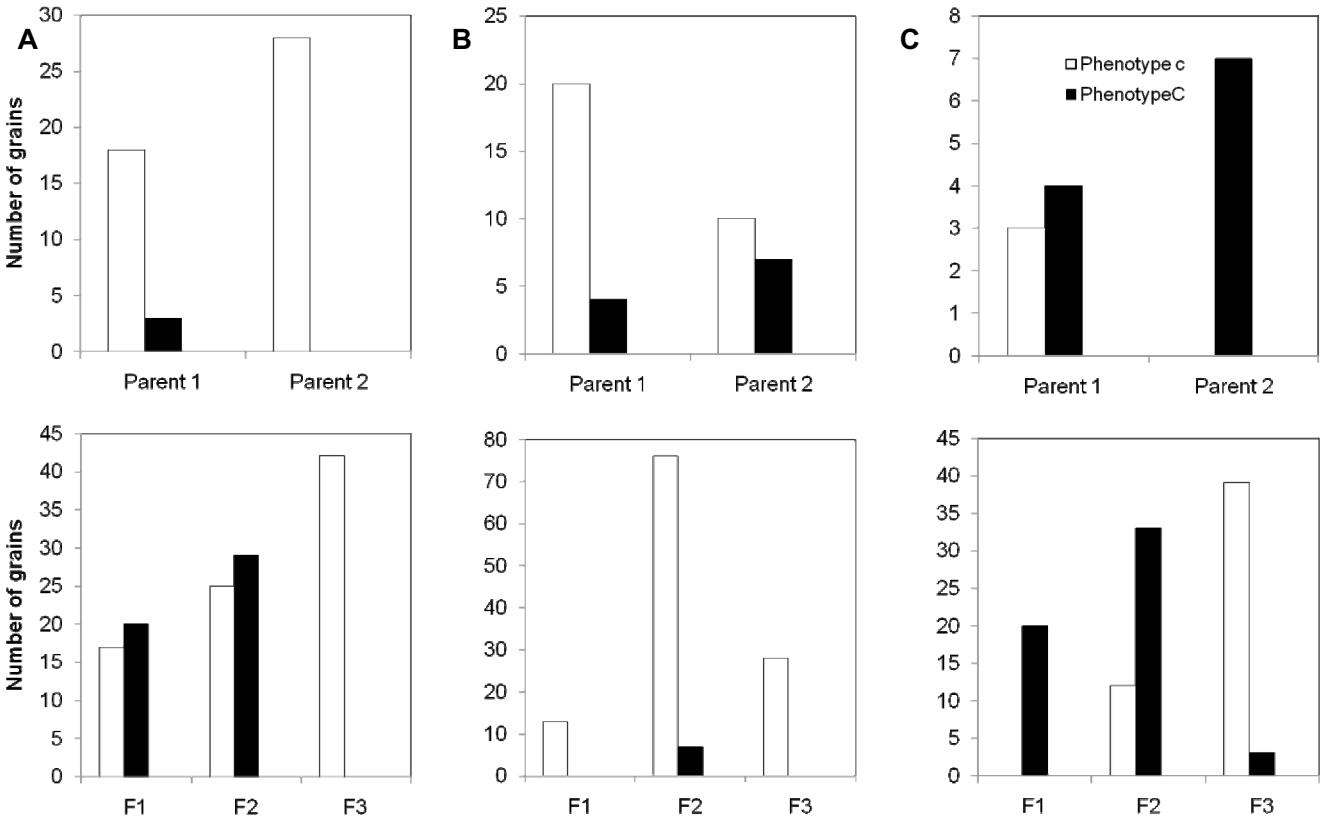


Fig. 8 Channelling characters of the grains of parental plants and of their F1, F2, F3 progenies with respect to the strength of the flavonol pathway with characters 'D' or 'd' for strong and weak, respectively. The number of single grains for each phenotype is given. (**A**) Crossing of parent plants exhibiting mostly phenotype 'd'. (**B**) Crossing of parent plants both exhibiting 'd'-phenotypes. (**C**) Crossing of parent plants exhibiting mostly 'D'-phenotypes.

Table 2 Concentrations of phenolic compounds in the grains of selected hybrids *F. esculentum* x *F. homotropicum* indicating the variability according to the metabolic phenotypes.

Phenotype	Rutin	Total flavanols	Simple phenolics	*p*-Coumaric acids	Caffeic acids	Catechin	Epicatechin	Epicatechin gallate	Epicatechin-dimethylgallate
acdE	6.0	75.7	13.1	46.2	28.5	0.5	3.1	0.0	2.8
acdE	13.4	100.7	13.6	36.0	31.2	1.8	21.2	0.5	17.0
acdE	16.3	29.6	16.2	12.5	18.3	0.1	1.9	0.0	0.1
acdE	35.4	98.2	9.9	29.3	6.4	6.6	18.4	0.6	13.1
aCdE	2.3	139.5	13.8	33.8	5.9	0.7	23.3	0.6	15.5
aCdE	5.4	113.8	25.7	24.6	15.6	6.0	18.5	0.8	11.1
aCdE	5.6	122.8	18.5	16.5	22.9	3.1	13.7	0.7	16.7
aCdE	10.4	74.3	18.8	6.4	27.6	1.8	9.2	0.3	10.0
acDe	83.5	15.2	18.5	7.7	47.8	0.5	1.7	0.1	4.9
aCDe	87.9	71.9	18.0	8.8	8.6	0.6	18.3	0.6	11.0
aCDe	91.9	60.3	12.3	4.3	21.9	0.1	9.8	0.4	12.3
AcdE	8.2	148.9	15.7	31.6	18.0	10.2	27.6	1.7	17.1
ACdE	3.4	195.9	22.7	22.5	8.6	0.2	17.1	1.4	20.8
ACdE	8.4	250.8	33.6	16.5	15.7	4.2	40.9	2.1	80.5
ACdE	90.3	119.6	17.9	29.0	2.0	4.7	34.5	0.8	10.8
ACdE	100.0	262.8	37.5	42.6	15.6	3.9	30.9	4.1	79.1
ACdE	104.3	130.2	60.0	19.4	42.0	0.9	29.1	0.4	23.7
AcDe	114.3	32.3	33.0	51.3	57.6	1.8	4.9	0.0	2.3
AcDe	174.1	114.4	12.4	12.4	9.4	6.2	19.0	1.3	27.9
ACDe	247.6	202.0	31.6	58.2	53.4	1.9	32.0	1.3	35.0
ACDe	299.0	41.9	23.0	22.5	40.3	8.2	6.5	0.5	8.4
ACDe	306.3	62.9	36.9	29.6	41.5	13.2	6.2	0.6	12.8
ACDe	320.0	116.9	14.9	18.4	13.1	2.2	11.1	0.9	23.1
ACDe	332.8	41.3	26.2	19.5	71.0	1.2	3.9	0.4	10.8
ACDe	422.0	116.4	34.2	23.5	31.4	2.7	18.87	0.6	21.4
acdE	0.5	0.4	0.4	0.1	1.3	0.5	0.5	1.0	64.3
acdE	4.0	0.3	1.2	0.9	8.2	2.5	4.1	4.5	34.0
acdE	0.6	0.1	0.1	0.1	0.2	0.0	0.0	0.3	25.9
acdE	2.0	1.1	0.1	1.6	6.6	0.4	3.5	3.2	40.5
aCdE	2.5	2.7	0.7	5.5	11.4	1.0	7.3	6.8	61.1
aCdE	4.1	1.6	0.1	2.5	6.4	0.4	4.3	3.1	54.3
aCdE	1.9	0.8	0.1	1.3	7.8	0.4	2.3	4.0	69.5
aCdE	1.7	1.0	0.2	1.0	5.1	0.1	1.7	3.1	38.4
acDe	0.1	0.2	0.0	0.1	1.1	0.1	0.1	0.7	5.2
aCDe	1.3	1.4	0.5	3.3	7.3	0.2	4.1	4.6	18.3
aCDe	1.7	0.8	0.1	1.3	7.2	0.4	2.3	3.8	19.6
AcdE	3.3	2.5	0.4	3.0	11.5	1.8	5.8	5.1	58.2
ACdE	2.5	3.3	1.1	6.3	15.8	1.5	5.2	11.6	108.7
ACdE	3.8	1.5	7.9	2.8	22.6	5.9	5.6	12.7	59.7
ACdE	3.6	0.7	2.5	3.1	7.9	0.7	5.9	4.5	39.1
ACdE	7.2	5.9	6.3	3.1	23.7	4.0	5.6	12.4	76.0
ACdE	6.6	1.3	2.3	3.4	11.6	0.2	7.9	6.7	35.4
AcDe	0.6	0.7	0.3	0.5	1.5	0.1	0.7	0.9	17.7
AcDe	2.9	1.1	2.4	2.9	13.6	0.8	3.5	9.0	23.3
ACDe	4.3	1.6	4.0	4.2	19.6	1.3	7.1	10.2	79.1
ACDe	0.8	0.6	0.1	0.4	4.0	0.3	0.4	3.0	8.2
ACDe	1.3	0.7	0.1	0.7	6.2	0.4	1.3	3.5	15.5
ACDe	2.1	0.3	1.7	2.3	11.3	0.5	3.0	7.6	50.4
ACDe	0.3	0.8	0.1	0.8	3.5	0.3	0.8	2.8	15.0
ACDe	3.9	0.3	1.7	3.4	14.4	0.9	5.7	8.8	33.2

nolic classes. In the latter case, a strong potency for rutin biosynthesis may not be represented by a high rutin accumulation. In order to describe the phenolic profiles and the metabolic potency of buckwheat lines and progenies we focused on the metabolic junctions (**Fig. 5**) which reflect the metabolic channelling of the different phenotypes. Two main regulatory steps of the phenylpropanoid/flavonoid pathway are postulated. They seem to be responsible for different flavonoid phenotypes, particularly for rutin- and flavan 3-ol-phenotypes. Metabolomic studies by using HPLC/DAD and HPLC/CRD methods allow the description of the flavonoid phenotypes for selection of valuable parents and hybrids for different purposes. However, the breeding goal "flavonoid"-phenotype still has to be combined with valuable agronomic characters. It is also noteworthy that those flavonoids being of interest for human nutrition may exhibit diverse physiological functions in the plant itself (Lattanzio *et al*. 2008). In particular, their significance in plant resistance (Treutter 2006) may be another interesting focal point for plant breeding.

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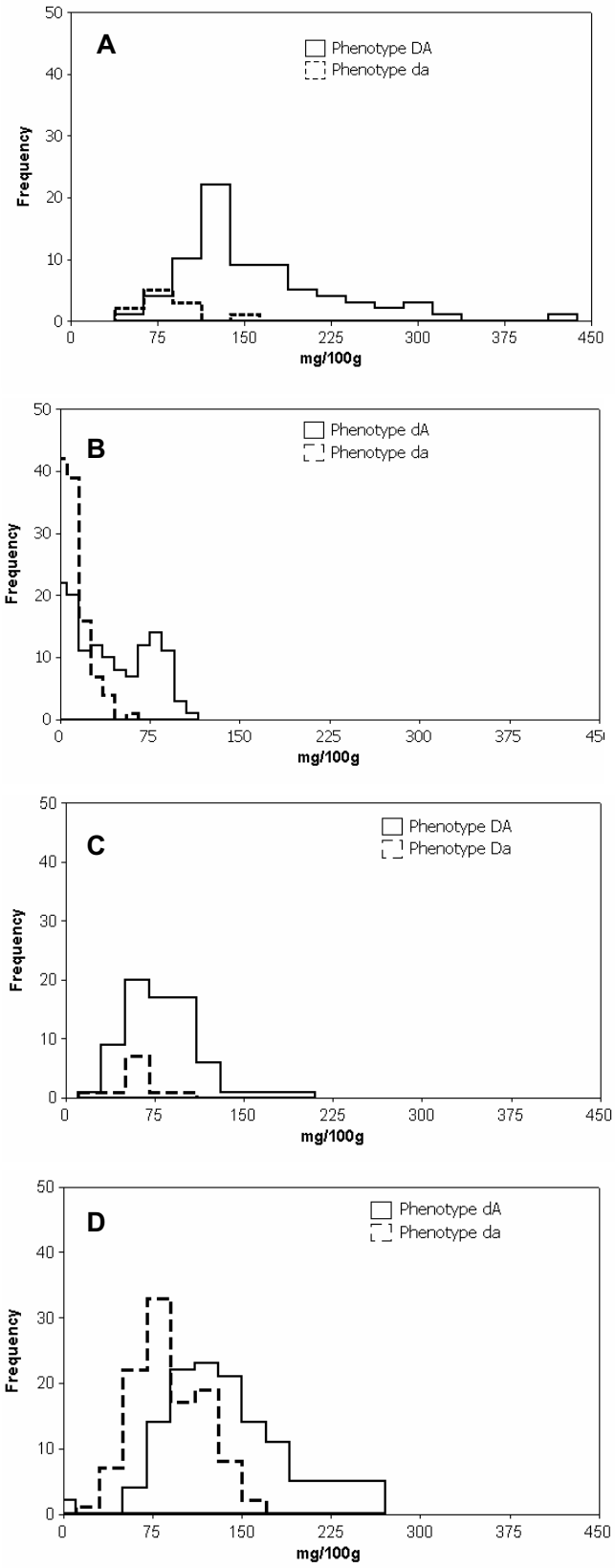

Fig. 9 Histogram of rutin (A, B) and flavan 3-ol (C, D) concentrations in the grains of 'DA'- versus 'Da'-phenotypes (A, C) and 'dA' versus 'da'- phenotypes (B, D).

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