


QTL mapping for anthocyanin and proanthocyanidin content in red rice

T. Y. Xu  · J. Sun · H. L. Chang · H. L. Zheng · J. G. Wang · H. L. Liu · L. M. Yang · H. W. Zhao · D. T. Zou

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Abstract Anthocyanins and proanthocyanidins are the primary pigments of red rice and are also important functional nutrients for human health. To identify novel quantitative trait loci (QTLs) underlying anthocyanins and proanthocyanidins (ANC and PAC) in rice, a recombinant inbred line (RIL) derived from a cross of red rice ‘Hong Xiang 1’ (‘HX1’) and white rice ‘Song 98-131’ (‘S98-131’) was cultivated in six environments. A genetic map containing 126 markers covering 1833.4 cM with an average of 14.55 cM between markers was constructed. A total of 21 additive QTLs (A-QTLs) for ANC and PAC were identified from six environments using the IciMapping v3.3 software. Two new QTLs, *qANC3* and *qPAC12-4*, were detected in several environments, and explained significant phenotypic variance. Nine QTLs of ANC and PAC were detected with additive × environmental interaction effects (AE effects) by QTLNetwork 2.1 software, but no epistatic and epistatic × environmental interaction effects (AA and AAE effects) were detected. The information obtained in this study could be useful for fine mapping

and molecular marker-assisted selection of ANC and PAC in rice.

Keywords *Oryza sativa* · Anthocyanin · Proanthocyanidin · QTLs · Environmental interaction

Introduction

Rice is the most important food for more than 50% of the world’s population, and approximately 90% of the world’s rice is produced and consumed in Asia (Liu et al. 2016; Ghosh et al. 2014). Rice, as one of the most important cereals, can provide human beings with energy, vitamins, minerals and amino acids (Cheng et al. 2016). Rice has been cultivated for nearly 10,000 years (Molina et al. 2011). Red rice, rice varieties having a red pericarp, is a kind of colored rice and has gained attention for its nutritional and medicinal value (Furukawa et al. 2006). Anthocyanins (ANC) and proanthocyanidins (PAC) are the primary pigments in colored rice. These pigments accumulate in the seed coat, pericarp, and aleurone layers of the rice grain that gives the rice different colors (Han et al. 2009). Anthocyanins are also important functional nutrients having huge health benefits for humans (Acquaviva et al. 2003). ANC have been suggested to have a positive effect on obesity, diabetes and cardiovascular disease (Williamson and Clifford 2010; Miller and Shukitt 2012; Norberto et al. 2013;

T. Y. Xu, J. Sun and H. L. Chang have contributed equally to this work.

T. Y. Xu · J. Sun · H. L. Chang · H. L. Zheng · J. G. Wang · H. L. Liu · L. M. Yang · H. W. Zhao · D. T. Zou (✉)
College of Agriculture, Northeast Agricultural University,
Harbin 150030, People’s Republic of China
e-mail: zoudtneau@126.com

Tsuda 2012). The hydrolysate of ANC can inhibit the growth of tumor cells significantly, and its antioxidant and anti-inflammatory activity can also reduce the physiological activity of serum cholesterol and cancer (Koide et al. 1996; Acquaviva et al. 2003; Lazze et al. 2003; Russo et al. 2005). Proanthocyanidins have important applications not only in agriculture but also in health care products, cosmetics and as functional polymers (Shi and Du 2006). PAC have inhibitory effects on cancer-causing peroxide and protect against DNA damage and apoptosis (Bagchi et al. 1999; Agarwal et al. 2000). Consumption of proanthocyanidin-rich foods, such as red wine and cocoa (chocolate), seems to decrease blood pressure and insulin resistance and reverse endothelial dysfunction (Grassi et al. 2005; Heiss et al. 2007, 2005; Taubert et al. 2007; Hayashi et al. 2010).

The breeding of rice varieties with high ANC or PAC is an effective method to increase the daily intake of these antioxidants for people. Using identified genes/QTLs and molecular markers associated with ANC and PAC for marker-assisted selection (MAS) is a promising method for breeding high ANC or PAC containing rice varieties. The ANC and PAC content were considered to be the quantitative traits. Quantitative trait loci (QTLs) of ANC and PAC have been identified in pepper, eggplant, grape, mimulus and carrot (Chaim et al. 2003; Barchi et al. 2012; Huang et al. 2012; Yuan et al. 2013; Cavagnaro et al. 2014), but no QTLs were detected in rice. Though certain genes necessary for ANC and PAC have been reported in rice (Furukawa et al. 2006; Kim et al. 2007), more gene loci should be identified to explain the complex ANC and PAC traits.

In the present study, a recombinant inbred line (RIL) population derived from a cross between rice cultivars, HX1 and S98-131, was used to determine ANC and PAC content in six environments. The objectives of this study were (1) to identify the stable QTLs controlling ANC and PAC in different environments and (2) to measure the additive and additive \times environment interaction effects of QTLs controlling ANC and PAC. The results contribute to the fine mapping of ANC and PAC QTLs in rice and provide a foundation for breeding high ANC and PAC content rice varieties.

Materials and methods

Plant materials and growth conditions

The mapping populations of 182 F₇, F₈, and F₉ recombinant inbred lines (RILs) that were advanced by single-seed-descent from the cross between two *japonica* varieties namely, Hongxiang No. 1 (HX1, developed by Jilin Academy of Agricultural Sciences, Changchun, China) and Song 98-131 (S98-131, developed by Heilongjiang Academy of Agricultural Sciences, Harbin, China). The unpolished rice of HX1 was red, and the ANC and PAC content was high. The unpolished rice of S98-131 was white, and the ANC and PAC content was low. Parents and RIL populations were planted in Harbin (N45°28', E128°08') in 2013, 2014 and 2015 (E1, E3, E6), Acheng (N44°29', E126°42') in 2013 and 2014 (E2, E4) and Suihua (N48°02', E124°13') in 2014 (E5). All of the test materials were grown in a randomized block design with three replications of single row plots with a 5 m row length. The space between each row was 30 cm, and each single plant per row was 10 cm apart. Field management followed standard agricultural practices. Only three middle plants per row were used for measuring the ANC and PAC content. After harvest, the seeds were dried naturally for 3 months to stabilize the physical and chemical characteristics.

Measurement of ANC and PAC

The ANC content was measured according to the methods used by (Pang et al. 2009). The unpolished rice samples from each parent and RIL was ground to a fine flour (100 mesh) using a grinding miller. Next, 0.1 g of ground sample was added to 2 mL of methanol-concentrated HCl (99/1 v/v) solution and incubated for 24 h at 4 °C. Next, the samples were centrifuged at 25,000 RPM for 10 min. An equal volume of water and chloroform was added to remove the chlorophyll, and the absorption of the aqueous phase was detected at 530 nm using an AAS-3500 atomic absorption spectrophotometer (Shanghai HP analysis instrument, China). The molar absorbance of cyanidin-3-glucoside was used to calculate the total ANC content, expressed as cyanidin glucoside equivalents in μg per g of dry matter.

The PAC content was measured according to the method described by (Przybylski et al. 1998). The

unpolished rice flour of 0.1 g (100 mesh) was dissolved in a 70% acetone solution containing 0.5% acetic acid and was slowly shaken on a shaking Table (20 r/min) at 25 °C for 15 h. After centrifuging at 3500 RPM for 15 min, the supernatant was mixed with *n*-hexane and was centrifuged again at 3500 RPM for 10 min. The supernatant was removed, and the remaining sample was dried at 40 °C for 4 h. A solution of methanol containing 1.0% (w/v) of vanillin and 10.0 M H₂SO₄ was added to the sample solution dissolved in methanol. The mixture was allowed to stand for 20 min at 40 °C, and the absorbance was measured at 500 nm. To determine the content of PAC, catechin was used as a standard, and PAC content was expressed as catechin equivalents.

Construction of a genetic linkage map and QTL analysis

Total DNA of the parents and of each RIL were isolated from fresh leaf tissues by the CTAB method (Doyle 1990). A total of 1000 SSR markers, covering the whole genome of rice, were used to detect polymorphisms. The PCR reaction conditions were set at 94 °C for 5 min, followed by 35 cycles of 30 s at 94 °C, 30 s at 55 °C, and 30 s at 72 °C followed by 5 min at 72 °C after the last cycle. The PCR products were separated by 6% (w/v) polyacrylamide gel electrophoresis and visualized by silver staining (Trigiano and Caetano 1998). The 126 polymorphic markers were integrated into the genetic linkage map

covering a total length of 1833.4 cM with an average distance of 14.55 cM between adjacent markers.

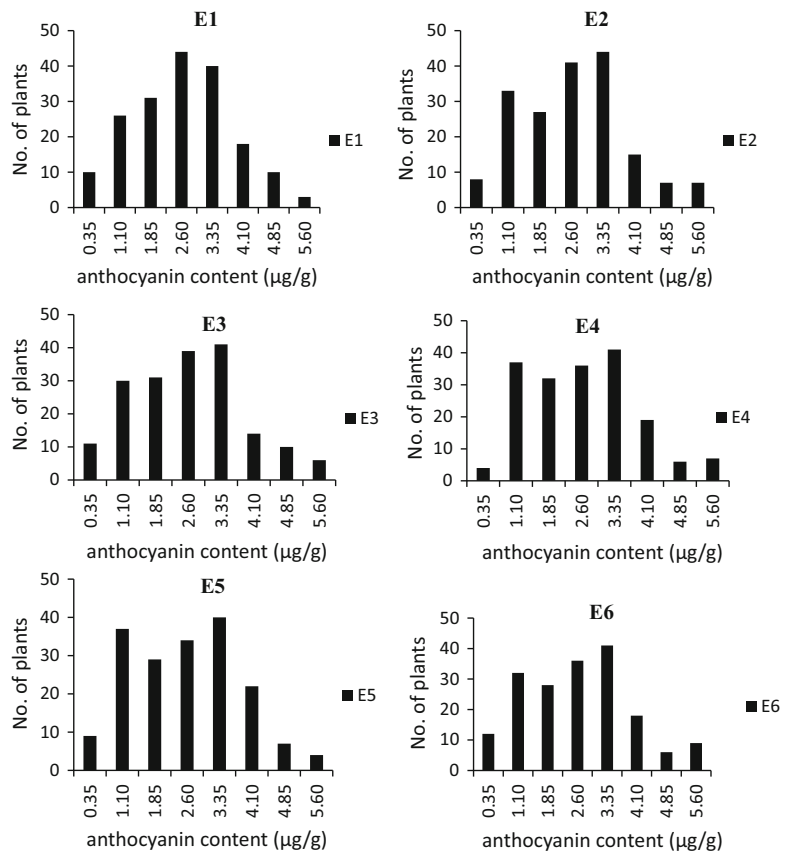
The identification of QTLs was performed using QTL IciMapping v3.3 (Li et al. 2007) software. For single environment analysis, additive effect QTLs (A-QTLs) were identified in the biparental populations (BIP) module using the Inclusive Composite Interval Mapping (ICIM) method. An LOD value corresponding to an experimental threshold of LOD > 2.5 was used to declare a QTL as significant. The estimate of the QTL position was the point of maximum LOD score in the region under consideration. QTLNetwork 2.1 (Yang et al. 2007), which is based on mixed-model-based composite interval mapping (MCIM), was used to conduct two-locus QTL analysis. This method allows identification of QTLs involved in additive effect (A-QTL), epistatic interactions (AA-QTL), additive × environmental interactions (AE-QTL), and epistatic × environmental interactions (AAE-QTL). A significance level of $P < 0.05$ was used to select associated markers and to declare a putative A-QTL or AA-QTL. This QTL nomenclature follows that of (McCouch et al. 1997). The ANC and PAC data and the correlation between ANC and PAC levels were analyzed by SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). Values of $P < 0.05$ were considered to be statistically significant, and values of $P < 0.01$ were considered to be highly significant.

Table 1 Phenotypic values for ANC and PAC content in parents and RILs in different environments

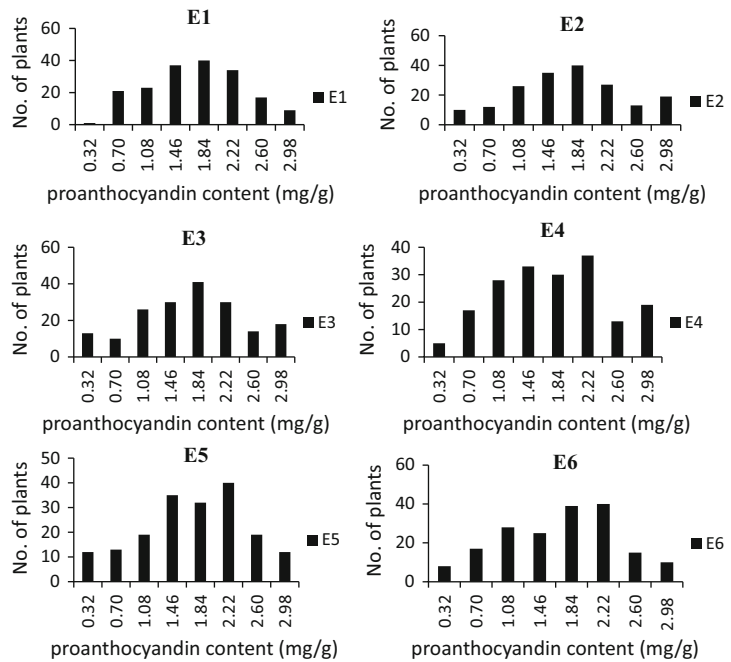
Traits	Env	Parents		RIL			
		'HX1'	'S98-131'	Mean ± SD	Range	Skewness	Kurtosis
ANC (µg/g)	E1	6.25	0.35**	2.57 ± 1.18	0.91–5.55	0.57	– 0.66
	E2	6.35	0.41**	2.61 ± 1.17	0.57–5.14	0.41	– 0.87
	E3	6.67	0.79**	2.54 ± 1.21	0.94–5.28	0.58	– 0.79
	E4	6.83	0.67**	2.49 ± 1.19	0.91–5.34	0.64	– 0.67
	E5	6.23	0.87**	2.46 ± 1.17	0.87–5.15	0.68	– 0.48
	E6	6.19	0.43**	2.50 ± 1.13	0.91–5.36	0.65	– 0.52
PAC (mg/g)	E1	2.27	0.32**	1.81 ± 0.55	0.91–3.36	0.47	– 0.33
	E2	2.18	0.28**	1.63 ± 0.49	0.81–3.40	0.80	0.72
	E3	2.38	0.17**	1.77 ± 0.56	0.71–3.26	0.66	– 0.19
	E4	2.03	0.36**	1.81 ± 0.58	0.88–3.17	0.48	– 0.57
	E5	2.78	0.46**	1.76 ± 0.59	0.90–3.17	0.51	– 0.54
	E6	2.41	0.37**	1.74 ± 0.63	0.93–3.21	0.56	– 0.50

*** mean significant difference at $P < 0.05$ and $P < 0.01$, respectively, according to the Student's *t* test

Fig. 1 Distributions of the ANC and PAC in RIL population at E1, E2, E3, E4, E5 and E6



Distributions of the ANC in RIL population at E1, E2, E3, E4, E5 and E6



Distributions of the PAC in RIL population at E1, E2, E3, E4, E5 and E6

Table 2 Correlation of ANC and PAC content in different environments

Traits	Experiment	PAC
ANC	E1	0.271**
	E2	0.159*
	E3	0.348**
	E4	0.268**
	E5	0.163*
	E6	0.247**

*and **mean significance at $P < 0.05$ and $P < 0.01$, respectively

Results

Phenotypic variation and correlation among traits

Table 1 shows the statistical analysis for ANC and PAC content of the parental lines and RIL populations from the six environments. ANC and PAC levels were significantly higher in ‘HX1’ compared to ‘S98-131’ for all six environments. For ANC and PAC content, the frequency of six environments in the RIL were normally distributed, and the absolute values of skewness and kurtosis for most environments were less than one (Fig. 1), which indicates that the data for ANC and PAC were suitable for QTL analysis.

The correlation analyses showed that ANC content was significantly and positively correlated with PAC content in the six environments, and the correlation coefficients were 0.271, 0.159, 0.348, 0.268, 0.163 and 0.247 for E1, E2, E3, E4, E5, and E6, respectively (Table 2).

A-QTLs for ANC and PAC content

A total of 21 A-QTLs for ANC and PAC content was detected using the ICIM method and QTL IciMapping v3.3 software (Table 3; Fig. 2). These QTLs were detected with LOD scores ranging from 2.5 to 4.7 and were distributed on chromosomes 1, 2, 3, 7, 8, 10 and 12. The explanation for the phenotypic variation by a single QTL varied from 3.8 to 34.8%.

Of the 21 A-QTLs, seven were detected for ANC content in all six different environments (Table 3). Two, three, three, two, two and one QTLs were detected in E1, E2, E3, E4, E5 and E6, respectively.

The phenotypic variances of QTLs ranged from 7.1 to 34.8%. *qANC7*, *qANC1-1* and *qANC3* were detected in more than one environment. *qANC3* was detected in E1, E3, E4, E5 and E6 and explained 17.6–34.8% of the phenotypic variance. The positive allele (an allele increasing the trait value) of *qANC3* in five environments was carried by ‘HX1’. 14 A-QTLs were detected for PAC content in six different environments (Table 3). One, four, five, one, five and two QTLs were detected in E1, E2, E3, E4, E5 and E6, respectively. The phenotypic variances of the QTLs ranged from 3.8 to 23.3%. *qPAC12-4*, detected in E2, E3, E5 and E6 explained 14.0–23.3% of the phenotypic variance and was a major effect QTL for PAC content. The positive allele of *qPAC12-4* in four environments was carried by ‘HX1’.

qANC2-2 and *qPAC2-3* were in the SSR interval of RM530–RM166, and *qANC10* and *qPAC10* were in the SSR interval of RM24992–RM474. The phenotypic variances of the four QTLs were 10.4, 13.1, 10.1 and 11.2%, suggesting that common loci control the ANC and PAC content of rice.

AE-QTLs for ANC and PAC content

A total of nine QTLs were detected for ANC and PAC content by the combined analysis of phenotypic values using the MCIM method and QTLNetwork 2.1 software (Table 4; Fig. 3). All nine loci were detected with AE effects for the six environments but no AA and AAE effects were detected. Except for the locus RM1379–RM1347 on chromosome 2, other loci were detected by the ICIM method using QTL IciMapping v3.3 software including the two major effect QTLs, *qANC3* and *qPAC12-4* (Table 3).

For A-QTL, the effects of the QTLs ranged from -0.1476 to 0.2205 , and the explanation for the phenotypic variation by a single QTL varied from 0.25 to 2.01%. For AE-QTL, the effects of the QTLs ranged from -0.441 to 0.1947 , and the explanation for the phenotypic variation by a single QTL varied from 0.06 to 0.38%.

Discussion

Anthocyanins and proanthocyanidins are important bioactive substances in plants and constitute a major group of natural pigments in flowers, fruits and

Table 3 A-QTL for ANC and PAC

Traits	Env	QTL	Chr ^a	Marker interval	CI ^b	Position (cM)	LOD	A ^c	R ² (%) ^d	
ANC	E1	<i>qANC1-1</i>	1	RM580–RM562	105.6–118.3	112.3	2.7	0.35	8.8	
		<i>qANC3</i>	3	RM411–RM448	90.0–101.3	99.0	3.1	0.57	22.5	
	E2	<i>qANC2-1</i>	2	RM13844–RM1367	0–16.3	13.7	2.7	-0.36	9.3	
		<i>qANC2-2</i>	2	RM530–RM166	168.0–180.7	168.2	3.0	0.27	10.4	
		<i>qANC10</i>	10	RM24992–RM474	0–19.3	13.7	2.6	0.40	11.2	
		<i>qANC1-2</i>	1	RM582–RM580	97.8–103.6	101.0	3.5	0.40	11.9	
	E3	<i>qANC3</i>	3	RM411–RM448	87.3–107.5	99.0	4.7	0.69	34.8	
		<i>qANC7</i>	7	RM1364–RM501	18.8–24.6	22.4	3.0	0.36	9.6	
	E4	<i>qANC3</i>	3	RM411–RM448	85.2–115.1	99.0	2.6	0.51	17.6	
		<i>qANC7</i>	7	RM1364–RM501	18.0–22.6	19.7	2.5	0.28	7.1	
	E5	<i>qANC1-1</i>	1	RM580–RM562	107.6–120.9	113.5	2.5	0.32	7.9	
		<i>qANC3</i>	3	RM411–RM448	85.3–110.2	98.1	3.3	0.55	23.1	
	E6	<i>qANC3</i>	3	RM411–RM448	92.7–112.1	99.0	4.0	0.43	17.9	
	PAC	E1	<i>qPAC7</i>	7	RM445–RM432	44.9–57.2	45.8	2.5	0.11	3.8
		E2	<i>qPAC2-1</i>	2	RM1367–RM1379	18.2–24.7	21.2	2.8	0.16	11.4
			<i>qPAC2-2</i>	2	RM1379–RM1347	23.4–33.7	31.1	3.6	0.18	13.7
			<i>qPAC8-1</i>	8	RM281–RM23065	181.3–192.2	189.7	3.5	0.15	10.9
			<i>qPAC12-4</i>	12	RM1302–RM1310	28.7–51.6	39.4	3.0	0.42	23.3
E3		<i>qPAC1-1</i>	1	RM11273–RM1282	0–10.1	3.3	2.5	0.14	7.0	
		<i>qPAC1-2</i>	1	RM294–RM5	152.8–171.1	171.0	3.0	0.13	5.7	
		<i>qPAC2-3</i>	2	RM530–RM166	167.8–180.3	168.4	2.5	-0.20	13.1	
		<i>qPAC12-1</i>	12	RM1261–RM309	99.4–110.8	107.6	3.7	0.21	14.0	
		<i>qPAC12-4</i>	12	RM1302–RM1310	30.0–54.1	39.4	2.5	0.32	17.0	
E4		<i>qPAC7</i>	7	RM445–RM432	44.9–59.4	45.8	2.6	0.12	3.8	
E5		<i>qPAC8-3</i>	8	RM339–RM342	100.6–110.7	101.1	2.7	-0.14	4.9	
		<i>qPAC10</i>	10	RM24992–RM474	0–20.6	6.6	3.1	0.19	10.1	
		<i>qPAC12-4</i>	12	RM1302–RM1310	26.7–49.7	39.4	2.7	0.22	14.0	
		<i>qPAC12-2</i>	12	RM1337–RM511	78.7–86.3	86.3	2.8	0.13	4.7	
		<i>qPAC12-3</i>	12	RM1300–RM1246	149.2–157.1	154.2	2.5	0.17	8.5	
E6		<i>qPAC8-2</i>	8	RM80–RM1345	143.6–150.4	149.7	3.0	0.09	7.3	
		<i>qPAC12-4</i>	12	RM1302–RM1310	26.7–50.2	39.4	2.8	0.32	15.3	

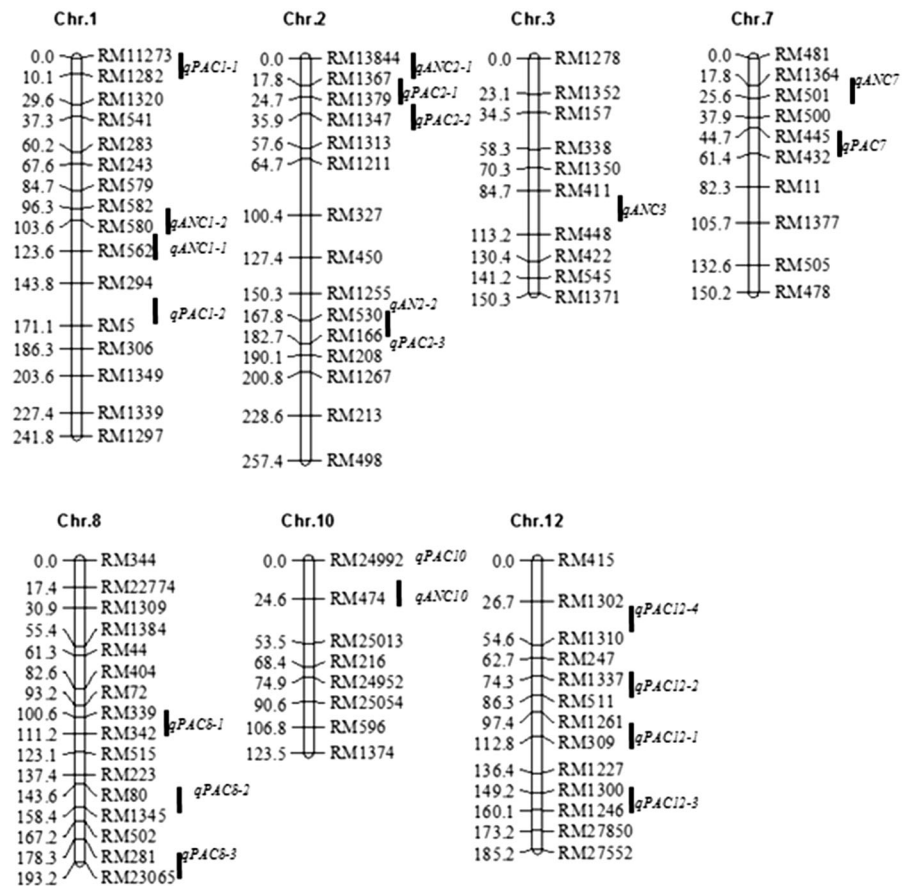
^aChromosome on which the QTL was located^bConfidence interval^cThe additive effect^dPhenotypic variation explained by each QTL

vegetables (Koponen et al. 2007; Finocchiaro et al. 2007). In this study, two parents of a RIL population, HX1, having red-colored rice, and S98-131, with white-colored grains, were crossed and studied. The ANC and PAC content were significantly higher in HX1 than in S98-131, indicating that the red rice has more anthocyanins and proanthocyanidins compared to common rice. In HX1, the PAC content was higher

than the ANC content, suggesting that red rice has more proanthocyanidins compared to anthocyanins. This result was consistent with the research of (Oki et al. 2002) and (Finocchiaro et al. 2007, 2010).

Several genes necessary for anthocyanins and proanthocyanidins have been identified in rice seeds. Kim analyzed the expression levels of homologous genes from the anthocyanin biosynthetic pathway of

Fig. 2 Distribution of the QTL on linkage map for ANC and PAC



Arabidopsis (Kim et al. 2007). Two genes, *DFR* and *ANS*, located on chromosome 1, had relatively high expression levels in the seeds from the black rice, Heugjinju. Some transcription factors associated with production of anthocyanin pigments were up-regulated or down-regulated in Heugjinju, but their functions in regulating anthocyanin biosynthesis need to be studied more deeply (Kim et al. 2010, 2011). Furukawa reported that the *Rc* and *Rd* genes are involved in proanthocyanidin synthesis in rice pericarp (Furukawa et al. 2006). The *Rc* gene encodes a bHLH protein and is located on 42.6–47.7 cM of chromosome 7; the *Rd* gene encodes a DFR protein and is located on 103.7–106.2 cM of chromosome 1. *Rc*, as a positive regulator of proanthocyanidin in rice, was reported by (Sweeney et al. 2006) around the same time. Dong reported four QTLs associated with the degree of red coloration (DRC) on rice chromosomes 1, 7, 9 and 11, and loci on chromosome 1 and 7 had the same location with previously characterized *Rc* and *Rd*

genes (Dong et al. 2008). Therefore, the PAC content in rice was considered to be controlled by these genes on chromosome 1 and 7. In this study, 21 A-QTLs for ANC and PAC content were detected using the ICIM method. Among these QTLs, the major effect QTLs were *qANC3* for ANC content and *qPAC12-4* for PAC content, and each were detected in multiple environments. The two QTLs were located on different chromosomes from known genes, indicating that *qANC3* and *qPAC12-4* were new loci affecting ANC and PAC content. The accurate location of these loci on the chromosomes needs to be determined by QTL fine mapping.

qANC1-1 and *qANC1-2* for ANC were located on 105.6–118.3 cM and 97.8–103.6 cM of chromosome 1, respectively. The two adjacent QTLs were most possibly the same QTL, but further fine mapping need to perform to verify it. *qPAC2-1* and *qPAC2-2* were also the possibility to be the same QTL. *qANC1-1* and *qANC1-2* locus had the same location on chromosome

Table 4 QTLs for ANC and PAC on additive × environment (AE) interaction effects

Traits	Chr ^a	QTL	Marker interval	A ^b	AE1 ^c	AE2	AE3	AE4	AE5	AE6	r ² (A)(%) ^d	r ² (AE)(%) ^e
ANC	1	<i>qANC1-1</i>	RM580–RM562	0.19	0.11	-0.16	0.11	0.07	0.08	-0.20	1.53	0.14
	2		RM1379–RM1347	-0.15	-0.08	0.18	-0.05	-0.07	-0.07	0.09	0.94	0.09
	3	<i>qANC3</i>	RM411–RM448	0.22	0.17	-0.50	0.26	0.14	0.17	-0.24	2.01	0.25
	7	<i>qANC7</i>	RM1364–RM501	0.13	-0.04	0.27	0.10	0.03	-0.26	0.06	1.37	0.11
	10	<i>qANC10</i>	RM24992–RM474	-0.01	0.19	0.06	0.03	-0.10	0.10	0.07	0.42	0.06
PAC	2	<i>qPAC2-2</i>	RM1379–RM1347	0.20	0.11	-0.08	0.06	0.06	0.07	-0.06	1.31	0.24
	8	<i>qPAC8-1</i>	RM281–RM23065	-0.03	0.11	0.05	0.03	0.04	-0.02	0.07	0.25	0.11
	12	<i>qPAC12-1</i>	RM1261–RM309	0.01	-0.10	0.03	0.13	-0.12	0.04	0.01	0.83	0.13
	12	<i>qPAC12-4</i>	RM1302–RM1310	0.02	0.12	-0.44	0.08	0.11	0.05	-0.13	1.41	0.38

^aChromosome on which the QTL was located

^bAdditive effect

^cAdditive × environment interaction effects in the six environments

^dPhenotypic variation explained by additive effects

^ePhenotypic variation explained by additive × environment interaction effects

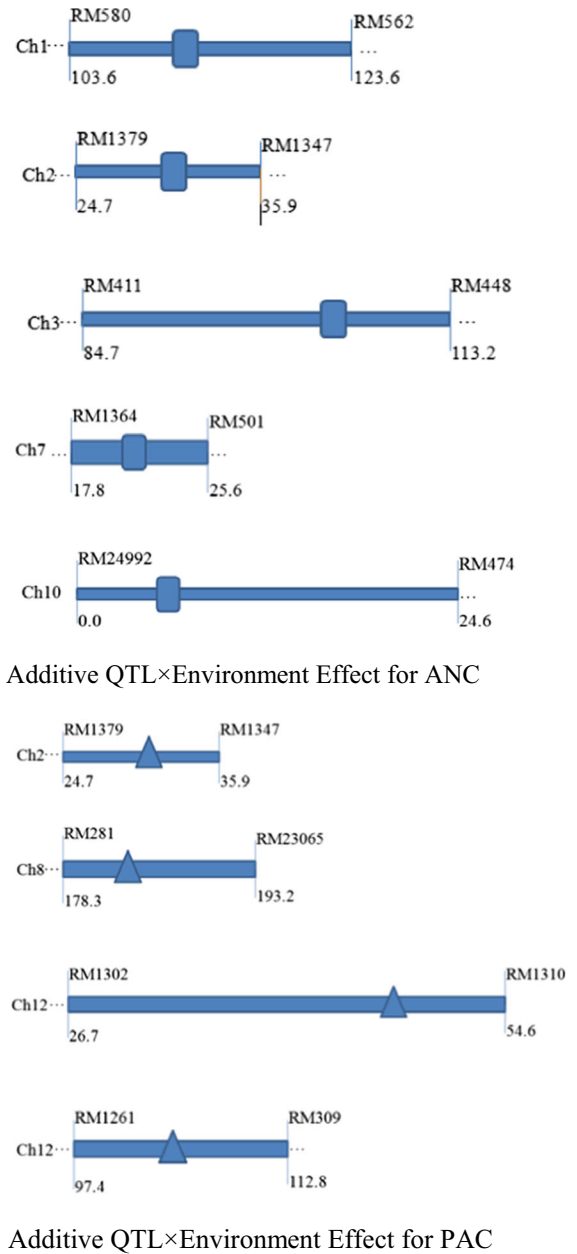


Fig. 3 The Additive QTL × Environment Effect for ANC and PAC

1 with *Rd* gene controlling the PAC, and it was coincident with the positively correlated result of ANC and PAC. It is pleiotropism of *Rd* gene or close linkage of the two loci, which needs further verification. *qPAC7* detected in E1 and E4 environments had the same location on chromosome 7 with *Rc* gene controlling the PAC. *qPAC7* maybe the *Rc* gene

detected in this study, but it has the very low LOD value and PVE. The key roles of *Rc* and *Rd* genes involve in PAC synthesis in red rice had been reported (Furukawa et al. 2006; Sweeney et al. 2006), but in this study, major effect QTL in *Rc* gene locus were not detected. QTL mapping using different genetic populations or in different environments usually got various QTL loci. For example, thirteen of the 19 (68%) QTLs for rice grain appearance traits detected in Ha/Ne population did not appear in the Ha/IR population, including some large effect QTLs (Hosseini, et al. 2012). In addition, the measure method of PAC were obviously different from (Furukawa et al. 2006) and (Sweeney et al. 2006), so some new QTL loci for PAC were detected but the major effect *Rc* gene locus was not discovered.

qANC2-2 and *qPAC2-3* on chromosome 2 and *qANC10* and *qPAC10* on chromosome 10 were detected within the same marker intervals, indicating a similar genetic background. The significantly positive correlation between ANC and PAC content also supports this conclusion. Several studies have demonstrated that anthocyanin and proanthocyanidin are produced in the early and middle steps of the flavonoid biosynthetic pathway and have a number of intersections (Winkel-Shirley 2001; Furukawa et al. 2006). The two pairs of QTLs detected in this study may play a common role to regulate the ANC and PAC content in rice.

Epistatic effects have been demonstrated to be prevalent for the inheritance of quantitative traits (Yu et al. 1997). When a QTL is involved in digenic epistatic interaction, its effects are mostly dependent on the genotypes at other QTLs (Hosseini et al. 2012). However, AA-QTLs were not detected in this study, suggesting that the interaction between QTLs affecting ANC or PAC content is feeble. QTL \times environment interactions have been discussed in different plant breeding programs (Messmer et al. 2009; Ha et al. 2012; Bonneau et al. 2013). In the present study, eight of the total 21 A-QTLs were detected with AE effects by the combined analysis of phenotypic values in six environments using the MCIM method of QTLNetwork 2.1 software. From a breeding standpoint, a QTL with only additive effects that are independent of the environment are particularly appealing for MAS (Cao et al. 2001; Hosseini et al. 2012). However, QTLs dependent on the environment could also be useful in a specific environment, but be

less important in other environments (Lark et al. 1995). Even so, the contribution rate of the explained phenotypic variation by A and AE-effects should also be considered. All of the AE-QTLs detected in this study have a tiny contribution rate of AE-effect compared to the A-effect; therefore, the AE effect of QTLs for ANC and PAC content could be negligible when using MAS.

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