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Epistatic interaction has the reverse effects with its constitutive quantitative trait loci

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Epistasis is one of important genetic components for a quantitative trait in plant. Eshed and Zamir found negative epistatic interactions of quantitative trait loci in Tomato first. We detected that positive (negative) QTLs generated mostly negative (positive) epistatic interactions on heading date in rice, and then proposed the hypothese that QTL epistasis plays a role of homeostasis in one of our recent papers. In order to further provide additional evidence, the effects of QTLs and their epistatic effects on two quantitative traits of plant height (ph) and thousand kernel weight (tkw) were analyzed in this study. The same regularity was verified again. We detected that positive ph QTLs and negative tkw QTLs always generated reverse epistatic effects, respectively. Moreover, high-order epistatic effects were estimated on these two traits. The sum of all epistatic effects would partially neutralize the additive of constitutive QTL effects. This feature of epistsis would be the mechanism for bionts to maintain homeostasis while the obstacle for human to achieve the pyramiding breeding objectives. More evidences are still being collected to support our assumption.

Keywords Epistatic interaction, Quantitative trait locus, Reverse effect, Single segment substitution line

The interaction among non-allelic genes, known as epistasis, is a crucial genetic component of quantitative traits with practical significance in determining the achievement of expected objectives in pyramiding breeding[1,2]. However, researches on epistasis have significantly lagged behind due to the limitations in experimental materials and statistical methods, making it challenging to quantitatively estimate epistatic effects at different levels and types[3,4]. Based on the single segment substitution lines, we effectively quantified the epistatic effects of various orders and types on lots of important agronomic traits in rice[5–7]. However, epistatic laws and mechanisms remained still mysterious due to the poverty in genetic theory and cognition. Eshed and Zamir[8] observed that the combination effects between QTLs were always less than the additive of individual QTL effects, thus suggesting that the epistasis was accompanied by negative effects. Inspired by this, we carried out extensive researches on QTL epistasis to explore epistatic laws. Researches indicated that two positive QTLs always generated a negative epistatic effect, while two negative QTLs did a positive epistatic effect[5–7,9–11].

In one of our recent articles, QTL epistasis on heading date were analyzed by four single segment substitution lines in rice[12]. We found that QTLs of three positive effects and one negative effect generated 62.5% negative dual QTL epistatic effects and 57.7% positive triple QTL epistatic effects, namely forming the relationship "positive QTLs—negative first-order epistasis—positive second-order epistasis". Thus we suggested that the aggregation effect of QTLs was partially neutralized by the opposite epistatic effect sum, QTL epistasis playing a role of homeostasis on heading date in rice[12]. To provide additional evidences for this hypothesis, this paper analyzed QTL epistatic effects on two traits of plant height (ph, cm) and thousand kernel weight (tkw, g) based on five single segment substitution lines.

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Results

Additive effects (*a*) and dominant effects (*d*)

A genotypic effect includes additive, dominance and epistasis, which can be estimated based on SSSLs and their pyramiding materials. Additive effects (*a*) and dominant effects (*d*) were estimated by the differences of genotypic values between the homozygotes and the heterozygotes of SSSLs and the receptor HJX74, respectively (Table 1).

Except for *S2* without ph QTL, all SSSLs carried with significant ph QTLs and tkw QTLs. The additive effects and dominant effects of all QTLs increased ph, while decreased tkw. They existed different estimations between the two season environments, and hadn't significant tkw effects in the late season. These QTLs showed reverse effect directions between two measured traits, which was helpful to explore the regularity of QTL epistasis.

First-order epistatic effects

Based on SSSLs and their pyramiding materials, the epistatic effects between dual QTLs, known as first-order epistasis, were estimated by the residual effects between the pyramiding effects and the sum of two single QTL effects (Table 2). Here epistatic effects estimated included four components such as additive-additive (*aa*), additive-dominance (*ad*), dominance-additive (*da*) and dominance-dominance (*dd*) epistasis.

All of nine pairs of SSSL combinations were detected with significant epistatic effects. Of 36 epistatic components, 20 and 21 estimations reached the significant level of $p < 0.05$ or 0.01 on ph and tkw in both seasons, respectively. One striking feature was that all significant epistatic components were negative on ph but positive on tkw. Additionally, despite the same effect directions, the effect magnitudes appeared differences between two season environments. Especially, no epistatic effects was detected significantly on tgw in the late season.

Second-order epistatic effects

The epistatic effects among three QTLs, known as second-order epistases, were estimated by the residual effects between the pyramiding effects and the sum of single QTL effects and the all dual QTL epistasis (Table 3). Here epistatic effects estimated included eight components such as additive–additive–additive (*aaa*), additive–additive–dominance (*aad*), additive–dominance–additive (a*da*), dominance–additive–additive (*daa*), additive–dominance–dominance (*add*), dominance–additive–dominance (*dad*), dominance–dominance–additive (*dda*), and dominance–dominance–dominance (*ddd*) epistases.

All of six SSSL combinations were detected with significant epistatic effects. Of 48 epistatic components, 35 and 18 estimations reached the significant level of $p < 0.05$ or 0.01 on ph and tkw in the both seasons, respectively. Oppositely with first-order epistasis, all significant second-order epistatic components were positive on ph but negative on tkw. They had the same effect directions but the different effect magnitudes in the two season environments.

Third-order epistatic effects

The epistatic effects among four QTLs, known as third-order epistases, were estimated by the residual effects between the pyramiding effects and the sum of single QTL effects and the all low-order epistases (Table 4). Here epistatic effects estimated included sixteen components such as additive–additive–additive–additive (*aaaa*), additive–additive–additive–dominance (*aaad*) and additive–additive–dominance–additive (*aada*) epistases etc., respectively.

Of sixteen epistatic effects, 13 and 11 estimations reached the significant level of $p < 0.05$ or 0.01 on ph and tkw in both seasons, respectively. All significant epistatic components were negative on ph but positive on tkw. They had the same effect directions but the different effect magnitudes appeared in the two season environments.

SSSL	Effect	Early season		Late season	
		ph/cm	tkw/g	ph/cm	tkw/g
S1	*a*	5.74*	−8.75**	8.73**	0.24
	d	6.19*	−5.06**	13.93**	0.93
S2	*a*	3.47	−4.75*	2.48	0.05
	d	1.00	−1.10	0.31	0.78
S3	*a*	3.82	−5.8**	6.32**	0.73
	d	5.33*	−5.97**	5.28*	0.44
S4	*a*	5.74*	−4.25*	0.93	0.44
	d	5.70*	−3.36	2.06	0.48
S5	*a*	2.81	−5.11**	5.36*	−0.54
	d	2.29	−4.73*	4.90	−0.66

Table 1. Additive effects (*a*) and dominant effects (*d*) of SSSLs on plant height (ph, cm) and thousand kernel weight (tkw, g). "−" indicated that the allele from the donor shorten the measurement traits. "*" and "**" represented the significance at the probability levels 0.05 and 0.01, respectively.

SSSL combination	Epistasis	Early season		Late season	
		ph/cm	tkw/g	ph/cm	tkw/g
S1/S2	*aa*	− 0.89	5.71*	2.27	0.17
	ad	− 5.01	6.80**	− 12.31**	0.45
	da	− 7.76*	6.44*	− 12.07**	1.24
	dd	− 1.03	6.16*	− 14.89**	0
S1/S3	*aa*	− 8.51*	9.12**	− 10.42**	1.23
	ad	− 2.64	11.66**	− 11.17**	1.35
	da	− 0.42	11.07**	− 11.96**	1.11
	dd	− 7.62*	7.05**	− 14.73**	1.23
S1/S4	*aa*	− 8.81*	9.92**	− 1.42	1.60
	ad	− 5.99	5.21*	− 6.44*	1.09
	da	− 7.20*	6.71**	− 12.33**	1.84
	dd	− 3.21	3.19	− 16.37**	1.27
S1/S5	*aa*	4.36	7.43**	3.43	0.54
	ad	− 5.36	4.53	− 2.87	− 0.95
	da	3.46	5.27*	1.75	− 2.20
	dd	− 4.84	2.55	− 8.99*	− 1.79
S2/S3	*aa*	− 8.56*	4.90	− 5.94	0.12
	ad	− 10.57**	5.49*	− 3.54	0.29
	da	− 3.18	2.94	− 0.35	0.46
	dd	− 1.19	2.01	− 0.55	0.55
S2/S4	*aa*	− 8.86*	3.94	− 3.03	0.18
	ad	− 1.02	3.05	− 5.24	0.73
	da	− 5.26	− 0.07	− 0.31	1.01
	dd	− 2.65	3.19	1.03	0.05
S2/S5	*aa*	− 4.33	3.79	–	− 0.22
	ad	− 2.95	2.28	–	1.24
	da	− 3.46	6.21**	− 11.03**	0.90
	dd	− 0.09	1.76	− 15.14**	− 0.07
S3/S4	*aa*	0.88	7.01**	− 2.92	0.66
	ad	− 1.53	4.89	− 5.13	0.38
	da	− 6.61	5.47*	2.80	0.63
	dd	− 11.97**	1.74	− 13.34**	0.92
S3/S5	*aa*	− 13.08**	10.58**	− 9.58*	2.99
	ad	1.88	10.80**	− 5.70	2.30
	da	0.29	7.23**	− 4.39	0.05
	dd	− 4.43	8.18*	− 11.46**	− 0.21

Table 2. The epistatic effects between dual SSSLs on plant height (ph, cm) and thousand kernel weight (tkw, g). *aa, ad, da* and *dd* indicated the additive–additive, additive–dominance, dominance–additive and dominance–dominance epistases, respectively. "–" indicated that the allele from the donor decreased the measurement traits. "*" and "**" represented the significance at the probability levels 0.05 and 0.01, respectively.

Discussions

The order and type of epistasis

Since genotype is composed of genes, genotypic effect can be divided into gene effects. Gene effects generally include additive, dominance and epistasis. Thus epistasis is one of important genetic components for a complex quantitative trait[1,2]. Epistasis was defined as the effect of one gene modified by another gene or several other genes (biological epistasis)[1–4], and epistatic effect is estimated as the deviation from additivity in a linear statistical model (statistical epistasis)[8]. According to the genetic model $G = a + d + e$, the additive (a), dominance (d) and epistasis (e) can be estimated on the genotypic effect G value[8,13,14]. This paper estimated effectively a and d by using the data from the homozygous and heterozygous of SSSLs, respectively. While e was done based the effects of pyramiding materials, a and d. When one locus is considered, there are a or d only. While involving in multiple loci, there are a, d and e. Epistasis between dual loci is known as first-order epistasis, including 2^2 types of components, *aa, ad, da* and *dd*. Epistasis among three and four loci are known as second-order and third-order epistasis, which includes 2^3 and 2^4 types of epistatic components, respectively. This paper estimated effectively various orders and types of epistases. High-order epistasis is even more common in the multiple gene system

SSSL combination	Epistasis	Early season		Late season	
		ph/cm	tkw/g	ph/cm	tkw/g
S1/S2/S3	*aaa*	9.81*	−5.45	11.52**	1.04
	aad	12.88**	−6.18	−5.57	1.30
	ada	2.52	−11.25**	12.93**	−1.91
	daa	5.70	−5.24	16.25**	0.43
	add	7.16	−10.04**	9.06*	−0.48
	dad	12.43*	−9.65**	7.94	−1.26
	dda	−0.01	−11.17**	19.80**	−0.44
	ddd	2.10	−1.48	16.56**	0.85
S1/S2/S4	*aaa*	15.51**	−3.10	3.64	0.13
	aad	10.38*	−5.50	6.29	1.08
	ada	4.78	−10.91**	2.47	−4.91*
	add	16.28**	−10.59**	16.10**	−2.88
	dad	7.45	−6.46	14.93**	0.36
	dda	8.36	−0.52	18.45**	0.61
	daa	−1.36	−8.66*	18.85**	0.01
	ddd	−3.19	−11.11**	20.61**	−0.63
S1/S2/S5	*aaa*	−7.40	−5.46	–	−0.56
	aad	10.60*	−2.03	–	0.68
	ada	0.50	−12.11**	1.84	−0.14
	daa	13.74**	−3.55	–	1.71
	add	−2.82	−6.54	12.71**	2.31
	dad	−1.46	−1.99	–	0.90
	dda	6.22	−6.53	5.54	2.83
	ddd	6.18	−2.61	9.99*	−4.22*
S1/S3/S4	*aaa*	14.68**	−13.64**	2.03	−2.83
	aad	−2.10	−7.94*	9.23*	0.86
	ada	0.16	−12.44**	−3.09	−3.23
	daa	10.44*	−8.52*	14.94**	1.99
	add	−4.39	−5.38	12.17**	0.13
	dad	1.16	−7.75*	14.92**	1.55
	dda	6.48	−1.92	11.55**	1.07
	ddd	5.31	−3.83	29.94**	1.07
S2/S3/S4	*aaa*	8.40	−3.64	13.06**	0.03
	aad	1.97	−0.58	7.98	1.38
	ada	19.93**	−6.12	−1.15	1.71
	daa	8.40	−5.45	−0.59	1.96
	add	11.86*	−0.96	13.40**	0.15
	dad	12.14*	−5.98	4.78	2.04
	dda	3.25	1.84	−2.14	0.10
	ddd	0.48	−2.06	4.86	1.73
S2/S3/S5	*aaa*	16.59**	−3.23	17.62**	−3.88
	aad	−4.73	−10.02**	–	−2.62
	ada	5.28	−3.77	–	−3.11
	daa	3.89	−11.93**	15.46**	−4.45*
	add	15.28**	−5.76	–	0.27
	dad	8.78	−9.76**	12.53**	−2.09
	dda	8.57	−0.64	5.34	0.58
	ddd	4.80	−4.30	19.75**	1.23

Table 3. The epistatic effects among three SSSLs on plant height (ph, cm) and thousand kernel weight (tkw, g). *aaa*, *aad*, a*da, daa, add, dad, dda* and *ddd* indicated the additive–additive–additive, additive–additive–dominance, additive–dominance–additive, dominance–additive–additive, additive–dominance–dominance, dominance–additive–dominance, dominance–dominance–additive, and dominance–dominance–dominance epistases, respectively. "–" indicated that the allele from the donor decreased the measurement traits. "*" and "**" represented the significance at the probability levels 0.05 and 0.01, respectively.

SSSL combination	Epistasis	Early season		Late season	
		ph/cm	tkw/g	ph/cm	tkw/g
S1/S2/S3/S4	*aaaa*	–	3.93	− 21.76**	5.15*
	aaad	2.18	11.10**	− 18.22**	4.08*
	aada	–	2.46	0.75	0.11
	adaa	− 12.97*	18.52**	− 8.17	1.94
	daaa	− 4.06	− 2.17	− 34.82**	1.81
	aadd	0.21	1.59	4.94	1.16
	adad	1.21	14.52**	− 27.98**	4.68*
	adda	− 10.87*	8.66**	− 8.62	5.43**
	daad	− 11.75*	− 0.50	− 22.29**	0.03
	dada	− 2.84	5.85**	− 9.25*	0.45
	ddaa	− 9.26*	3.92	− 32.24**	5.98**
	addd	–	11.95**	− 5.21	1.52
	dadd	− 8.50	6.61**	− 25.24**	3.15
	ddad	− 10.57*	15.02**	− 32.99**	1.33
	ddda	3.65	–	− 18.93**	1.74
	dddd	− 13.21*	5.35**	− 32.21**	1.39

Table 4. The epistatic effects among four SSSLs on plant height (ph, cm) and thousand kernel weight (tkw, g). *aaaa*, *aaad* and *aada* etc. indicated the additive–additive–additive–additive, additive–additive–additive–dominance and additive–additive–dominance–additive epistases etc., respectively. "–" indicated that the allele from the donor decreased the measurement traits. "*" and "**" represented the significance at the probability levels 0.05 and 0.01, respectively.

and more important to keep homeostasis of organism[2,4,12]. Various types of epistatic components play different roles in biological evolution and breeding practice[12].

The estimations of epistasis

How to estimate epistasis? People went through a long process of exploration[1,2]. The additive-dominant genetic model has been around for more than half a century when epistasis was ignored[3,4]. Since traditional method to analysis of quantitative traits didn't distinguish the effect of individual gene[15], it could only estimate epistasis mixed from multi-gene system[16]. QTL mapping methods based on bi-parental populations couldn't provide precise estimation of epistatic effects since the interference of genetic background[17]. Using near-isogenic lines or single segment substitution lines, some epistatic components between dual QTLs were estimated[17,18]. However, previous studies few estimated simultaneously various epistatic components[8]. Author ever constructed several secondary F_2 populations derived from crossing of two SSSLs, each of which pyramided dual QTLs to allow simultaneously analysis of four epistatic components[5,19]. Subsequently, a half-diallel hybridization design was proposed to improve secondary F_2 mapping population above[6,7,9–11]. A double QTL polymerization line was developed first, and then a half diallel crossing population from four parents (receptor, two SSSLs and their DSSL) was constituted to generate nine genotypes[6,7]. This method eased to get target genotypes, lowered the cost of molecular marker analysis, and could be constructed repeatedly[9–11]. Analyzing the genetic effects of the nine genotypes enabled to simultaneously estimate various epistatic components[6,7,9–11]. A mark advantage of this method is easy to extend to analyze epistasis among multiple QTLs. Using the extend half diallel crossing populations, we analyzed various epistatic components among three QTLs in a last paper[12], and then among four QTLs in this paper.

The characteristics of epistasis

In our past series of studies, a several common features of epistasis were detected[5–7,9–11]. Epistasis was very prevalent, almost all QTL combinations appeared epistatic interactions[5–7]. One QTL always interacted with multiple QTLs[9–11]. In this paper, we detected that the first-order, second-order and third-order epistases were mostly with high frequency. Eshed and Zamir[8] published the article of "Less-Than-Additive Epistatic Interactions of Quantitative Trait Loci in Tomato", where dominance-dominance epistasis were detected to mostly be negative values. In fact, this is caused by positive QTL values. We found that, negative epistases were derived mainly from interactions between positive QTLs, while positive epistases from negative QTLs[6,7,9–12]. Further researches indicated that there was the regularity "positive QTLs–negative first-order epistasis–positive second-order epistasis, while negative QTLs–positive first-order epistasis–negative second-order epistasis"[12]. Hereby, we proposed the hypothesis that QTL epistasis plays a role homeostasis[12]. In this paper, the above regularity of epistasis was confirmed again on two target traits of ph and tkw. Since all QTL effects were positive on ph, then the first-order epistases were negative while the second-order positive. Furthermore, the third-order epistases were mostly negative. Inversely, all QTL effects were negative on tkw, then the first-order, second-order and third-order epistases were successively positive, negative and positive. Epistasis may be brought about by modification of gene function due to alterations in the signal-transducing pathway[2,12]. Epistatic genes are more deleterious

in combination than separately, which are often accompanied by inverse epistatic interactions as homeostatic (that is, canalizing) mechanisms[2,12]. Further researches will provide additional evidences for this hypothesis.

Materials and methods

Materials

Huajingxian 74 (HJX74) and its five single-segment substitution lines (SSSLs) were applied in this trial. These experimental materials were described in our previous studies[11,12]. HJX74 is an elite indica variety with many excellent properties, which was cultured in our laboratory in South China[20]. Each SSSL possessed only a single substituted segment from a donor with the HJX74 genetic background, which was distributed in the related molecular marker regions on the corresponding chromosomes of given lengths[21] (Fig. 1). With these markers, the foreground selections of the donors and the background selections of HJX74 were performed in order to ensure that the single fragment was unique.

Some pyramiding materials of SSSLs (including homozygotes and heterozygotes) were configured to analyze epistasis among QTLs. The crossing between a SSSL and HJX74 would generate the heterozygote of SSSL. From the F_2 populations derived from the F_1 crossing combinations between two SSSLs, the homozygotes and the heterozygotes of dual segments could be obtained by marker assisted selection. Similarly, some pyramiding materials of triple- or tetra- segments could also be selected from the F_2 generation of three or four SSSL combinations[19].

Methods

Field trials. Phenotypic experiments were described in our previous studies[11,12]. The field trial site was the experimental farm of South China Agricultural University, Guangzhou (at ~ 113° east longitude and ~ 23°north latitude) and the field times were the early season (from March to July) and the late season (from July to November) in 2016, respectively. All experimental materials, including HJX74, homozygotes and heterozygotes of SSSLs, dual-, triple- and tetra-segment polymers, were grown in two season environments. In each experiment, the germinated seeds were sown in a seedling bed and seedlings were transplanted to a paddy field 20 days later, with one plant per hill spaced at 16.7 cm × 16.7 cm. A completely random design with three replications was adopted in the field trails, in which each plot was consisted of four rows with ten plants each row. The management of the field experiments was in accordance with local standard practices.

Traits measured

Plant height (ph, cm) per hill, the distance between the base and the top of the main panicle of the plant, was measured on 10 central plants in each plot when the plants approached maturity. After maturity, the seeds of single plant were harvested and put into a net bag to dry. The thousand kernel weight (tkw, g) was measured in each plot. The values of ph averages and tkw each plot were as inputting data for statistical analysis.

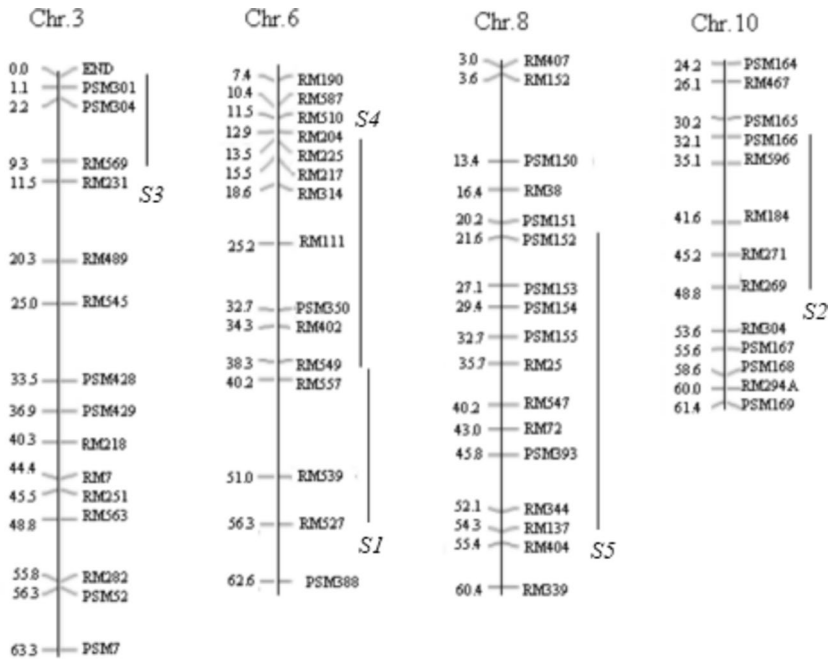

Figure 1. The approximate lengths and locations of substitution segments on the chromosomes for the five single segment substitution lines. Chr. and *S* were the abbreviation of chromosome and single segment substitution line, followed by serial numbers, respectively. The rectangular frames and the bold vertical lines represented chromosomes and substitution segments from donors of single segment substitution lines, respectively. The genetic distances (cM) between markers and the marker names were listed on either side of chromosomes, respectively.

Analysis of variance. To estimate experimental error, analysis of variance was conducted on data of single season environment according to statistical model $y_{ij} = \mu + G_i + e_{ij}$, where y, μ, G *and* e were plot average, population mean, genotypic effect and error on phenotypic values of target traits, respectively. Analysis of variance was performed by using the function aov() of R language (http://cran.r-project.org).

QTL analysis

According to the genetic model of additive(a)–dominance(d)–epistasis(e), a genotypic effect (G) can be expressed by $G = a + d + e$. For instance, the effect of genotype *AABBCc* can be divided into $G_{AABBCc} = a_A + a_B + d_C + e_{AB} + e_{AC} + e_{BC} + e_{ABC}$. Using the homozygote and heterozygote of SSSLs and HJX74, a and d can be estimated by $\hat{a}_{AA} = G_{AA} - G_{HJX74}$ and $\hat{a}_{Aa} = G_{Aa} - G_{HJX74}$, respectively. While based on the pyramiding materials, some of e are estimated by,

$$\hat{e}_{aa} = G_{AABB} - \hat{a}_{AA} - \hat{a}_{BB}$$

$$\hat{e}_{ad} = G_{AABb} - \hat{a}_{AA} - \hat{d}_{Bb}$$

$$\hat{e}_{aaa} = G_{AABBCC} - \hat{a}_{AA} - \hat{a}_{BB} - \hat{a}_{CC} - \hat{e}_{AABB} - \hat{e}_{AACC} - \hat{e}_{BBCC}$$

$$\hat{e}_{aad} = G_{AABBCc} - \hat{a}_{AA} - \hat{a}_{BB} - \hat{d}_{Cc} - \hat{e}_{AABB} - \hat{e}_{AACC} - \hat{e}_{BBCc}$$

$$\hat{e}_{aaaa} = G_{AABBCCDD} - \hat{a}_{AA} - \hat{a}_{BB} - \hat{a}_{CC} - \hat{a}_{DD} - \hat{e}_{AABB} - \hat{e}_{AACC} - \hat{e}_{AADD} - \hat{e}_{BBCC} - \hat{e}_{BBDD} - \hat{e}_{CCDD} - \hat{e}_{AABBCC} - \hat{e}_{AABBDD} - \hat{e}_{AACCDD} - \hat{e}_{BBCCDD}$$

$$\hat{e}_{aaad} = G_{AABBCCDd} - \hat{a}_{AA} - \hat{a}_{BB} - \hat{a}_{CC} - \hat{d}_{Dd} - \hat{e}_{AABB} - \hat{e}_{AACC} - \hat{e}_{AADd} - \hat{e}_{BBCC} - \hat{e}_{BBDd} - \hat{e}_{CCDd} - \hat{e}_{AABBCC} - \hat{e}_{AABBDd} - \hat{e}_{AACCDd} - \hat{e}_{BBCCDd}$$

where,

$$\hat{e}_{aa} = \hat{e}_{AABB}\ or\ \hat{e}_{AACC}\ or\ \hat{e}_{BBCC}$$

$$\hat{e}_{ad} = \hat{e}_{AABb}\ or\ \hat{e}_{AACc}\ or\ \hat{e}_{BBCc}$$

$$\hat{e}_{aaa} = \hat{e}_{AABBCC}\ or\ \hat{e}_{AABBDD}\ or\ \hat{e}_{AACCDD}\ or\ \hat{e}_{BBCCDD}$$

$$\hat{e}_{aad} = \hat{e}_{AABBCc}\ or\ \hat{e}_{AABBDd}\ or\ \hat{e}_{AABbCC}\ or\ \hat{e}_{AABbCC}\ or\ \hat{e}_{AABbDD}\ or\ \hat{e}_{BbCCDD}\ or\ \hat{e}_{AaBBCC}\ or\ \hat{e}_{AaBBDD}\ or\ \hat{e}_{AaCCDD}$$

The other epistatic components can also be estimated by the same manner. All QTL effects were performed by using the function lm() of R language (http://cran.r-project.org).

Data availability

Data is provided within the manuscript.

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Competing interests

The authors declare no competing interests.

Additional information

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