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Dynamic QTL analysis of seed reserve utilization in sh_2 sweet corn germination stages

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ABSTRACT. In this study, dynamic quantitative trait loci (QTL) were mapped in a recombinant inbred line ($F_{2:4}$) population derived from a BF3109 x Q267 cross. This was done during the unconditional (4, 7, and 10 days) and conditional (0 to 4, 4 to 7, and 7 to 10 days) germination stages in sh_2 sweet corn. The values of seedling dry weight, weight of mobilized seed reserve (WMSR), seed reserve depletion percentage (SRDP), seed reserve utilization efficiency (SRUE), and their heritability differed across the investigated stages. The heritabilities of these traits were lower at 7D/4D and 10D/7D compared with those at the 4- (4D/0D), 7- and 10-day (D) developmental stages. WMSR and SRDP were significantly negatively correlated with SRUE at the early stage. The unconditional QTL mapping can explain the accumulation of genetic effects of seed reserve utilization from the starting time, whereas the conditional QTL mapping can reveal genetic expression in the time intervals. Fifteen and fourteen additive QTLs were identified

by the unconditional and conditional mapping, respectively. The number of additive QTLs and their effect values varied among the different stages. More additive QTLs were found at the later stage (7 to 10 days), based on the conditional mapping results. The identification of QTL mapping based on a combination of time-dependent measurements is important for a better understanding of the genetic bases of seed reserve utilization. It is also important for the improvement of relevant variety traits for subsequent sweet corn-breeding studies using marker-assisted selection.

Key words: Sweet corn; Quantitative trait loci; Seed reserve utilization

INTRODUCTION

Maize can be divided into normal, waxy, and sweet corn, depending on the starch composition of the endosperm in the seed. Sweet corn (*Zea mays* L. var. *saccharata* Bailey) is a special variety of cultivated maize with a high sugar content that is the result of a naturally occurring recessive mutation in the genes on chromosome. These genes control the conversion of sugar to starch inside the endosperm of the corn kernel (Sprague et al., 1943; Nelson and Rines, 1962; Carey et al., 1984). However, the utilization of wrinkled and fragile seeds can lower the rate of sweet corn germination. This is an important initial step for successful seedling establishment, which begins with the appearance of the radicle and ends after the seedling has exhausted the seed's energy reserves and starts to photosynthesize (Ichie et al., 2001).

The seedling-establishment processes, such as germination and heterotrophic growth, are complex traits that are influenced by both genetic and environmental factors (Hodgkin and Hegarty, 1978). As seeds absorb water, the mobilization of seed reserves from storage tissues, including starch, proteins, and lipids, is initiated and provides energy to stirring and seedling growth until the seedling becomes photoautotrophic (Pritchard et al., 2002). Heterotrophic seedling growth can be identified by the weight of the mobilized seed reserve and the conversion efficiency of utilized seed reserves to seedling tissue (Soltani et al., 2006; Mohammadi et al., 2011). Over the years, studies of the phenotypes of seed reserve utilization have been reported in soybean (Mohammadi et al., 2011), wheat (Soltani et al., 2006), rice (Cheng et al., 2013), and chickpea (Soltani et al., 2002), but no publications in this area exist on sweet corn. In the present study, unconditional and conditional quantitative trait loci (QTLs) for seed reserve utilization at different growth stages were determined. To this end, we used two methods of composite interval mapping (CIM) in WinQTL Cartographer (Wang et al., 2005) and the method of inclusive composite interval mapping (ICIM) in QTL IciMapping (Li et al., 2007, 2008). These results will contribute to the revelation of the genetic basis of seed germination. Furthermore, by marker-assisted selection, the identified QTLs could be employed to substantially improve seed germination in sweet corn.

MATERIAL AND METHODS

Plant materials

In this study, we used a mapping population of 190 recombinant inbred lines (RILs) ($F_{2:4}$) developed from BF3109 and Q267, sh_2, sweet corn inbred lines. BF3109 was utilized

as one of the parental lines developed from exotic germplasm, whereas Q267 was derived from a local variety. All seeds were dried at the Experimental Station of Anhui Science and Technology University.

Evaluation of seed reserve utilization

The RILs and their parents were incubated in an incubator in the dark at 30° ± 1°C. First, the initial seed dry weight (ISDW; mg/seed) of all seeds was determined; three seed replicates were weighed (W_1), dried at 104°C for 24 h in an oven, and then reweighed (W_2). Fifty seeds per RIL replicate and their parents were weighed. Second, the seed water content (WC) was calculated [$(W_1 - W_2) / W_1$], and the ISDW in each replicate was then computed [W_1 x (1 - WC)].

Finally, four traits were investigated on days 4, 7, and 10 after the germination as three independent experiments, respectively. The weight of mobilized seed reserve (WMSR; mg/seed) was calculated as the dry weight of seed remnant subtracted from the ISDW. Seed reserve depletion proportion (SRDP; mg per mg) was calculated as the ratio of WMSR to ISDW. Seed reserve utilization efficiency (SRUE; mg per mg) was estimated by dividing the seedling dry weight (SDW; mg/seed) by the WMSR (Soltani et al., 2006). Three replicates were included in the design of the experiment.

Data analysis

The data from the experiment were analyzed using the Statistical Analysis System (SAS8.1) software, and the traits of the parents were compared by the Student *t*-test at the 5 and 1% levels of probability. The correlations of the traits were computed using PROC CORR by the SAS software.

QTL mapping

The genetic effect measured at time *t* is the result of genes expressed before (*t*-1) and the extra effects observed within the period [from (*t*-1) to *t*]. These two types of genetic effects are usually not independent (Yan et al., 1998). Unconditional QTL mapping was conducted based on the phenotypic value at time t[$y_{(t)}$]; where $y_{(t)}$ indicates the phenotypic values on days 4, 7, and 10 after germination. The conditional QTL mapping was performed based on the phenotypic mean at time t in relation to the phenotypic means measured at time t-1[$y_{(t|t-1)}$]; where $y_{(t|t-1)}$ denotes the phenotypic values in the intervals from 0 to 4, 4 to 7, and 7 to 10 days after germination. Additive QTLs were identified using the CIM method in WinQTL Cartographer (Wang et al., 2005), and the ICIM method in QTL IciMapping (Li et al., 2007, 2008) through the single-environment phenotypic values.

RESULTS

Phenotypic analysis

For the unconditional analysis, the seed reserve utilization phenotypes were established at three different time points (4, 7, and 10 days) (Table 1). The results from this analysis showed

that there were no significant differences in SDW between the two inbred lines at the 4-day (4D/0D), 7-day, 10-day, or 10D/7D developmental stages. However, at the 7D/4D stage, the SDW of BF3109 was higher than in Q267 (Table 1). The WMSR and SRDP of BF3109 were greater than those of Q267, at the 7-day, 10-day, 7D/4D, and 10D/7D developmental stages, whereas the opposite was observed at the 4-day (4D/0D) stage. In contrast, the Q267 had a higher SRUE compared to the BF3109 at the 7-day, 10-day, 7D/4D and 10D/7D developmental stages, but not at the 4-day (4D/0D) stage. We observed a continuous frequency distribution and transgressive segregation in these traits in the RIL population. The heritability of SDW, WMSR, SRDP, and SRUE varied at different developmental stages, ranging from 17.8 to 63.8%, 22.9 to 67.5%, 26.1 to 70.5%, and 28.6 to 58.1%, respectively. The heritability of those traits was relatively low at 7D/4D and 10D/7D compared to those at the 4-day (4D/0D), 7-day, and 10-day developmental stages.

Table 1. Phenotypic values of seed reserve utilization among parents and the recombinant inbred lines (RIL) population at different germination stages.

Germination stages (days, D)	Traits[a]	Parents[b]		RIL population[c]						
		BF3109	Q267	Mean	Max	Min	SD	Skewness	Kurtosis	Heritability (%)
0	ISDW	28.12 ± 0.61	26.79 ± 1.01	24.99	34.89	17.84	2.94	0.35	0.93	86.6
4 (4D/0D)	SDW	2.81 ± 0.14	2.54 ± 0.37	3.73	5.96	1.41	0.88	-0.06	-0.04	63.8
	WMSR	2.50 ± 0.49	3.33 ± 0.26	4.33	8.25	1.74	1.21	0.48	0.28	67.5
	SRDP	0.10 ± 0.02	0.15 ± 0.02*	0.20	0.38	0.10	0.05	0.49	0.29	70.5
	SRUE	0.80 ± 0.17	0.75 ± 0.09	0.82	0.99	0.33	0.11	-1.57	3.78	49.6
7	SDW	4.55 ± 1.02	3.71 ± 0.81	5.74	9.77	2.61	1.29	0.68	1.03	57.1
	WMSR	10.52 ± 0.27**	4.88 ± 0.60	8.85	15.95	4.40	1.90	0.93	1.90	53.1
	SRDP	0.35 ± 0.10**	0.20 ± 0.02	0.41	0.61	0.23	0.06	0.01	0.24	50.2
	SRUE	0.54 ± 0.035	0.86 ± 0.02**	0.65	0.80	0.48	0.06	-0.16	0.04	56.0
10	SDW	5.93 ± 0.87	5.43 ± 1.49	6.93	11.13	2.53	1.32	0.12	0.57	58.0
	WMSR	11.44 ± 0.48**	8.43 ± 1.15	10.79	16.28	5.09	1.84	0.13	0.40	56.7
	SRDP	0.48 ± 0.04**	0.37 ± 0.04	0.50	0.62	0.29	0.05	-0.58	0.91	44.5
	SRUE	0.52 ± 0.03	0.60 ± 0.13	0.64	0.94	0.32	0.07	-0.58	5.63	58.1
7D/4D	SDW	2.46 ± 0.14*	1.62 ± 0.83	2.44	6.61	0.11	1.21	0.70	0.58	32.9
	WMSR	6.02 ± 1.60**	1.82 ± 0.29	4.74	12.97	0.74	1.82	0.95	2.69	34.6
	SRDP	0.25 ± 0.06**	0.04 ± 0.02	0.21	0.45	0.05	0.07	0.25	0.47	34.8
	SRUE	0.42 ± 0.10	0.57 ± 0.24	0.45	0.74	0.06	0.13	-0.30	0.12	38.4
10D/7D	SDW	1.82 ± 0.40	1.72 ± 1.00	1.75	4.37	0.08	1.00	0.57	-0.32	17.8
	WMSR	4.51 ± 2.00	3.75 ± 1.10	2.69	7.06	0.06	1.43	0.54	0.40	22.9
	SRDP	0.17 ± 0.08	0.16 ± 0.04	0.11	0.25	0.01	0.05	0.36	-0.01	26.1
	SRUE	0.41 ± 0.05	0.51 ± 0.29	0.58	2.13	0.02	0.28	2.02	11.32	28.6

[a]ISDW = initial seed dry weight, mg/seed; SDW = seedling dry weight, mg/seed; WMSR = weight of mobilized seed reserve, mg/seed; SRDP = seed reserve depletion percentage, mg per mg; SRUE = seed reserve utilization efficiency, mg per mg; [b]Means ± SD (standard deviation); * and **indicate significance at the levels of 5 and 1%, respectively, according to the Student *t*-test; [c]RIL sample size N = 190, replicates r = 3.

Correlations of the four traits

At all developmental stages, there were significant positive correlations between ISDW and SDW, under both the unconditional and conditional environments (Tables 2 and 3). The correlations between ISDW and WMSR were significantly positive at the later 7-day, 10-day, 7D/4D, and 10D/7D developmental phases, but they were not correlated with SRDP or SRUE. The SDW for 4 days (4D/0D) was significantly and positively correlated with the WMSR and SRDP of 4 days (4D/0D), and the SDW for 7 days, 10 days, 7D/4D, and 10D/7D was strongly and positively correlated with WMSR, SRDP, and SRUE. The WMSR was markedly

and positively correlated with the SRDP during all developmental stages, except 7D/4D and 10D/7D. Furthermore, the WMSR and SRDP were significantly and negatively correlated with SRUE at the early 4-day (4D/0D) stage, whereas opposite results were observed at the 7D/4D developmental period.

Table 2. Correlation coefficients between seed reserve utilization traits at different germination stages under the unconditional setup.

Germination stages (days)		0	4				7				10			
	Traits	ISDW	SDW	WMSR	SRDP	SRUE	SDW	WMSR	SRDP	SRUE	SDW	WMSR	SRDP	SRUE
0	ISDW	1												
4	SDW	0.312**	1											
	WMSR	-0.187	0.806**	1										
	SRDP	-0.188	0.668**	0.913**	1									
	SRUE	0.073	0.131	-0.284**	-0.295**	1								
7	SDW	0.601**	0.317**	0.265**	0.079	0.119	1							
	WMSR	0.606**	0.307**	0.282**	0.096	0.072	0.929**	1						
	SRDP	0.041	0.245**	0.297**	0.328**	0.045	0.731**	0.796**	1					
	SRUE	0.113	0.058	-0.013	-0.059	0.139	0.328**	-0.026	-0.075	1				
10	SDW	0.683**	0.392**	0.269**	0.047	0.160	0.569**	0.546**	0.227**	0.174	1			
	WMSR	0.736**	0.415**	0.276**	0.033	0.150	0.565**	0.568**	0.226**	0.097	0.883**	1		
	SRDP	0.062	0.322**	0.231**	0.235**	0.137	0.222**	0.238**	0.296**	-0.022	0.540**	0.668**	1	
	SRUE	0.086	0.110	0.097	0.080	0.082	0.127	0.062	0.019	0.213**	0.466**	0.035	-0.038	1

ISDW = initial seed dry weight, mg/seed; SDW = seedling dry weight, mg/seed; WMSR = weight of mobilized seed reserve, mg/seed; SRDP = seed reserve depletion percentage, mg per mg; SRUE = seed reserve utilization efficiency, mg per mg; **indicates significance at the level of 1%.

Table 3. Correlation coefficients between seed reserve utilization traits at different germination stages under the conditional setup.

Germination stages (days, D)		0	4D/0D				7D/4D				10D/7D			
	Traits	ISDW	SDW	WMSR	SRDP	SRUE	SDW	WMSR	SRDP	SRUE	SDW	WMSR	SRDP	SRUE
0	ISDW	1												
4D/0D	SDW	0.312**	1											
	WMSR	-0.187	0.806**	1										
	SRDP	-0.188	0.668**	0.913**	1									
	SRUE	0.073	0.131	-0.284**	-0.295**	1								
7D/4D	SDW	0.379**	-0.256**	-0.225**	-0.336**	0.072	1							
	WMSR	0.488**	-0.153	-0.323**	-0.471**	0.279**	0.751**	1						
	SRDP	0.181	-0.306**	-0.469**	-0.503**	0.314**	0.623**	0.849**	1					
	SRUE	0.051	-0.268**	-0.121	-0.146	-0.082	0.519**	0.224**	0.228**	1				
10D/7D	SDW	0.259**	0.092	0.022	-0.092	-0.012	-0.039	-0.078	-0.214**	-0.019	1			
	WMSR	0.249**	0.123	0.037	-0.076	0.011	-0.114	-0.158	-0.292**	-0.026	0.692**	1		
	SRDP	0.029	0.022	-0.082	-0.125	-0.034	-0.243**	-0.239**	-0.358**	-0.148	0.617**	0.804**	1	
	SRUE	-0.089	-0.098	-0.121	-0.102	0.045	-0.088	-0.107	-0.083	-0.068	0.247**	0.157	0.127	1

ISDW = initial seed dry weight, mg/seed; SDW = seedling dry weight, mg/seed; WMSR = weight of mobilized seed reserve, mg/seed; SRDP = seed reserve depletion percentage, mg per mg; SRUE = seed reserve utilization efficiency, mg per mg; **indicates significance at the level of 1%.

Unconditional QTLs

Three additive QTLs (*qISDW1*, *qISDW5.1*, and *qISDW5.2*) for ISDW were detected simultaneously by both the CIM and ICIM approaches (Table 4). In the marker intervals bnlg1614-bnlg421, umc1692-umc2116, and umc2116-umc1171 on chromosomes 1 and 5, *qISDW1*, *qISDW5.1*, and *qISDW5.2* were mapped. The phenotypic variance that could be

explained by each QTL ranged from 5.73 to 11.92%. The positive alleles *qISDW1* from Q267 and *qISDW5.1* and *qISDW5.2* from BF3109 contributed to the increase of ISDW.

Table 4. Putative additive QTLs for seed reserve utilization traits under unconditional environments by CIM and ICIM.

Methods	Treatments (days)	Traits[a]	Chr.[b]	QTLs	Marker interval	LOD	r^2 (%)[c]	Add[d]
CIM	0	ISDW	1	*qISDW1*	bnlg1614-bnlg421	2.91	5.73	-0.81
			5	*qISDW5.1*	umc1692-umc2116	2.54	9.22	0.94
	4	WMSR	4	*qWMSR4.2*	umc1294-umc2062	2.90	8.17	-0.26
		SRDP	4	*qSRDP4.2*	umc2188-umc1101	3.72	11.40	-0.03
		SRUE	6	*qSRUE6*	umc1979-umc1796	2.58	9.21	-0.04
	7	WMSR	2	*qWMSR2*	umc2129-umc1536	3.06	8.58	0.02
		SRDP	1	*qSRDP1.2*	umc1403-umc1292	2.99	7.68	-0.52
	10	SDW	4	*qSDW4*	umc2062-bnlg1169	3.95	7.68	-0.43
		WMSR	4	*qWMSR4.1*	umc1767-umc1784	2.75	8.50	-0.35
		SRDP	4	*qSRDP4.1*	umc2009-umc1808	3.83	11.14	-0.04
		SRUE	4	*qSRUE4*	umc1656-umc2266	2.65	7.44	-0.02
ICIM	0	ISDW	1	*qISDW1*	bnlg1614-bnlg421	2.90	9.62	-1.02
			5	*qISDW5.2*	umc2116-umc1171	2.62	11.92	1.02
	4	SDW	2	*qSDW2*	umc1261-umc1555	3.02	10.85	-0.31
		WMSR	4	*qWMSR4.2*	umc1294-umc2062	2.61	9.39	-0.38
		SRDP	4	*qSRDP4.2*	umc2188-umc1101	3.35	9.33	-0.02
	7	WMSR	1	*qWMSR1*	umc1222-umc2025	2.79	10.41	-0.70
	10	SDW	1	*qSDW1*	bnlg1556-bnlg400	2.62	11.32	-0.53
		SRDP	4	*qSRDP4.1*	umc2009-umc1808	3.73	9.83	-0.02

[a]ISDW = initial seed dry weight, mg/seed; SDW = seedling dry weight, mg/seed; WMSR = weight of mobilized seed reserve, mg/seed; SRDP = seed reserve depletion percentage, mg per mg; SRUE = seed reserve utilization efficiency, mg per mg; [b]chromosome on which the QTL was located; [c]variation explained by each putative QTL; [d]additive effect is the effect of substituting a BF3109 allele for an Q267 allele; a positive value indicates that BF3109 has the positive allele and a negative value indicates the opposite.

Three additive QTLs (*qSDW1*, *qSDW2*, and *qSDW4*) were identified for SDW by the CIM and ICIM approaches (Table 4), but the same QTLs were not identified by both methods. The SDW at 10 days were controlled by *qSDW4* and *qSDW1* on chromosomes 4 and 1 in marker intervals umc2062-bnlg1169 and bnlg1556-bnlg400, respectively. Each QTL could explain phenotypic variance and ranged from 7.68 to 11.32%. The three QTLs from Q267 were the positive alleles that accounted for the increase of SDW.

Via CIM and ICIM, four additive QTLs (*qWMSR1*, *qWMSR2*, *qWMSR4.1*, and *qWMSR4.2*) were determined responsible for controlling the WMSR (Table 4). Among these, QTL *qWMSR4.2* at 4 days was detected, by both the CIM and ICIM approaches, on chromosome 4 in the marker interval umc1294-umc2062. The WMSR at 7 days was controlled by *qWMSR1* and *qWMSR2* on chromosomes 1 and 2 in the marker intervals umc1222-umc2025 and umc2129-umc1536, respectively. Finally, *qWMSR4.1*, on chromosome 4 in the marker interval umc1767-umc1784, was responsible for the WMSR at 10 days. Each QTL accounted for phenotypic variance ranging from 8.17 to 10.41%; of these, four QTLs from Q267 were positive alleles that contributed to the increase in WMSR.

Three additive QTLs (*qSRDP1.2*, *qSRDP4.1*, and *qSRDP4.2*) were identified for SRDP, according to the CIM and ICIM methods (Table 4). Among these, QTLs *qSRDP4.1* and *qSRDP4.2* were identified by both methods. The SRDP at 4 and 10 days were controlled by *qSRDP4.2* and *qSRDP4.1* in the marker intervals umc2188-umc1101 and umc2009-umc1808,

respectively. Each QTL accounted for phenotypic variance ranging from 7.68 to 11.40%.

Each additive QTL for SRUE at the 4-day (*qSRUE6*) and 10-day (*qSRUE4*) stages were identified by CIM (Table 4), while no QTL for the 7-day stage was recognized. The QTLs *qSRUE6* and *qSRUE4* were detected on chromosomes 6 and 4 in the marker intervals umc1979-umc1796 and umc1656-umc2266, accounting for 7.44 and 9.21% of the phenotypic variance, respectively.

Conditional QTLs

The conditional QTLs at 4D/0D should be equal to the QTLs at 4 days under the unconditional environment (Tables 4 and 5). Four conditional QTLs were detected at the 4D/0D stage using the CIM and ICIM approaches (Table 5). A QTL for each of SDW, WMSR, SRDP, and SRUE was identified that explained the phenotypic variance ranging from 8.17 to 11.40%, and the positive alleles of these QTLs were contributed by Q267.

Table 5. Putative additive QTLs for seed reserve utilization traits under conditional conditions by CIM and ICIM.

Methods	Treatments (days, D)	Traits[a]	Chr.[b]	QTLs	Marker interval	LOD	r^2 (%)[c]	Add[d]
CIM	4D/0D	WMSR	4	*qWMSR4.2*	umc1294-umc2062	2.90	8.17	-0.26
		SRDP	4	*qSRDP4.2*	umc2188-umc1101	3.72	11.40	-0.03
		SRUE	5	*qSRUE5*	umc1221-umc1178	2.58	10.21	-0.04
	7D/4D	SDW	9	*qSDW9.1*	umc1571-umc1170	2.53	6.81	-0.32
			9	*qSDW9.2*	umc1657-bnlg1191	2.63	7.61	0.35
		SRDP	1	*qSRDP1.1*	umc2047-umc1035	3.24	8.16	0.02
		SRUE	10	*qSRUE10*	umc1785-umc1053	3.12	8.24	-0.04
	10D/7D	SDW	6	*qSDW6*	umc1979-umc1796	3.25	14.65	0.44
		SRDP	1	*qSRDP1.2*	umc1403-umc1292	4.20	9.19	0.02
			8	*qSRDP8*	umc1778-umc1034	3.75	9.64	0.02
		SRUE	3	*qSRUE3.2*	umc2024-umc1223	4.04	8.95	0.11
ICIM	4D/0D	SDW	2	*qSDW2*	umc1261-umc1555	3.02	10.85	-0.29
		WMSR	4	*qWMSR4.2*	umc1294-umc2062	2.61	8.39	-0.32
		SRDP	4	*qSRDP4.2*	umc2188-umc1101	3.72	11.40	-0.03
	10D/7D	SRDP	1	*qSRDP1.2*	umc1403-umc1292	2.94	9.54	0.02
		SRUE	1	*qSRUE1*	umc2025-umc2241	2.73	7.12	-0.07
			3	*qSRUE3.1*	umc1010-bnlg1144	3.84	11.76	0.11

[a]ISDW = initial seed dry weight, mg/seed; SDW = seedling dry weight, mg/seed; WMSR = weight of mobilized seed reserve, mg/seed; SRDP = seed reserve depletion percentage, mg per mg; SRUE = seed reserve utilization efficiency, mg per mg; [b]chromosome on which the QTL was located; [c]variation explained by each putative QTL; [d]additive effect is the effect of substituting a BF3109 allele for an Q267 allele; a positive value indicates that BF3109 has the positive allele and a negative value indicates the opposite.

Four conditional QTLs were determined by CIM at the 7D/4D stage (Table 5), while no QTL for ICIM was established. Two QTLs (*qSDW9.1* and *qSDW9.2*) for SDW were identified on chromosome 9 in the marker intervals umc1571-umc1170 and umc1657-bnlg1191, respectively. One QTL for each of SRDP (*qSRDP1.1*) and SRUE (*qSRUE10*) was detected on chromosomes 1 and 10 in the marker intervals umc2047-umc1035 and umc1785-umc1053. Each QTL explained phenotypic variance ranging from 6.81 to 8.24%.

Six conditional QTLs were revealed at the 10D/7D stage by the CIM and ICIM approaches (Table 5). One QTL, *qSDW6*, for SDW was identified on chromosome 6 in the marker interval umc1979-umc1796. Two QTLs (*qSRDP1.2* and *qSRDP8*) for SRDP were detected on chromosomes 1 and 8 in the marker intervals umc1403-umc1292 and umc1778-

umc1034. Three QTLs, *qSRUE1*, *qSRUE3.1*, and *qSRUE3.2*, for SRUE were detected on chromosomes 1 and 3 in the marker intervals umc2025-umc2241, umc1010-bnlg1144, and umc2024-umc1223, respectively. The positive allele of only one QTL (*qSRUE1*) from Q267 and five QTLs (*qSDW6*, *qSRDP1.2*, *qSRDP8*, *qSRUE3.1I*, and *qSRUE3.2*) from BF3109 contributed to the increase in seed reserve utilization.

DISCUSSION

Seed germination is a key period that substantially influences plant post-germination growth and yield. Seed reserve utilization is a complex trait, which involves the mobilization of storage material under the action of enzymes and the establishment of seedlings (Pritchard et al., 2002). Until now, some scholars have extensively studied the mobilization of starch, protein, and lipid during germination (Penfield et al., 2004; Aoki et al., 2006; Leonova et al., 2010). During the developmental phases, the dry weight of the growing seedling is always lower than that of the mobilized reserves, owing to respiration (Soltani et al., 2006). Several studies have revealed that seedling dry weight reduction is caused by a reduction in WMSR or SRDP, not in SRUE, under drought and salinity stress conditions (Soltani et al., 2002, 2006). Our results are in agreement with observations made in wheat (Soltani et al., 2001), establishing that seed reserve utilization exhibited significant differences among lines. We found that BF3109 had higher WMSR and SRDP, whereas Q267 displayed higher SRUE during the middle (from 4 to 7 days) and late (from 7 to 10 days) germination stages. In the RIL population, the mobilization of seed reserves takes place mainly during the middle stage (from 4 to 7 days), whereas higher conversion efficiency of the utilized seed reserve to germination and seedling growth occurs during the early stage (from 0 to 4 days). The significant genetic differences and heritability observed in WMSR, SRDP, and SRUE indicated that the genetic variation can be used to improve the seed reserve utilization in breeding programs.

Seed reserve utilization is a quantitative trait controlled by genetic and environmental factors. Molecular evidence has proven useful for the improvement of seed reserve mobilization (Fait et al., 2006; Bethke et al., 2007). Therefore, it is necessary to map QTLs of seed reserve utilization by marker-assisted selection. The unconditional QTL mapping can explain the accumulation of gene effects from the start to the end of the time period, whereas the conditional mapping can detect the QTLs acting at a specific growth periods (Yan et al., 1998). In this study, the dynamic unconditional QTLs for seed reserve utilization were investigated at different stages (4, 7, and 10 days), and the conditional QTLs were identified at the specific periods (from 0 to 4, 4 to 7, and 7 to 10 days). There were 15 and 14 QTLs determined with unconditional and conditional mapping, respectively. In addition, we found that four of the 15 QTLs were detected by both the unconditional and the conditional mapping, including *qISDW1*, *qWMSR4.2*, *qSRDP4.1*, and *qSRDP4.2*. The conditional QTL mapping showed that four, four, and seven QTLs, respectively, were identified from 0 to 4 days, 4 to 7 days, and 7 to 10 days of the germination period. It was evident that more QTLs were expressed at the later stage than at the early and middle stages, suggesting that it may be more important to control the genetic basis of seed reserve utilization at the later stages.

The use of different mapping methods at the different germination stages greatly accelerated the detection of QTLs. For comparison with the additive QTLs identified by CIM, we also performed a QTL analysis using ICIM. As a result, 19 QTLs were detected, of which 12 additive QTLs were established based on the findings of the CIM and ICIM, respectively,

and five additive QTLs were identified by both methods. We found that the expression of the additive QTLs for seed reserve utilization differed among the different germination stages. Although the various seed reserve utilization traits were correlated, their loci were co-localized in the genomic region bnlg1614-bnlg421 on chromosome 1 for *qISDW1*; the umc1403-umc1292 region for *qSRDP1.2*; the umc2009-umc1808 region on chromosome 4 for *qSRDP4.1*; and the umc1294-umc2062 and umc2188-umc1101 regions on chromosome 4 for *qWMSR4.2* and *qSRDP4.2*. These results illustrate that the genes controlling the different seed reserve utilization traits may be distinct and differentially induced during the development.

CONCLUSIONS

In this study, several QTLs for seed reserve utilization under different developmental stages were identified. The next step will be to perform fine-mapping of the identified QTLs, for the application of near isogenic lines. The QTLs detected here may be used to develop new varieties with a high level of seed reserve utilization, using the marker-assisted selection method.

Conflicts of interest

The authors declare no conflict of interest.

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