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IDENTIFICATION OF QTLS TOLERANCE TO SALINITY IN RICE (*ORYZA SATIVA* L.)

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ABSTRACT

Our attempts in this study were to identify QTLs which controlling the salinity tolerance of rice by using F2/F3 population which derived from the crossed combination between the Chanhtrui (high salinity tolerance) and Khangdan18 (susceptible) rice cultivars. The results have shown that: salinity tolerance was controlled by multiple genes. fresh and dried shoot weights of F3 rice lines ranged from 0.13g to 0.40g and from 0.05g to 0.12g respectively; Natri ion^{+} concentration in dry roots of the susceptible lines was higher than those of tolerance lines; Kali ion+ concentration in dried shoots and roots of the susceptible lines showed lower than those of tolerance lines; Natri ion^{+}/Kali^{+} ion ratio in dried shoots and roots of tolerance lines were lower than those of susceptible lines. The molecular map was constructed to cover by 192 polymophic SSR markers which distributed on the 12 chromosomes with the total distance 1.797cM, and the average distance between two markers was 9.4cM. 10 QTLs were detected on the chromosomes 1, 3, 4, 6, 7 and 9; QTLs named as: *qSFW-1a-CK, qSFW-1b-CK, qRK-1-CK, qSN-1-CK, qSDW-3-CK, qSTR-4-CK, qSNK-6-CK, qSDW-7-CK, qSNK-9-CK, qRNK-9-CK;* flanking markers of QTL as: RM323-RM8144, RM449-RM8094, RM1287-RM8094, RM3412-RM220, RM2593-RM563, RM1359-RM127, RM141-RM253, RM5481-RM5720, RM242-RM460, RM105-RM287 respectively. Six QTLs (*qSFW-1b-CK, qRK-1-CK, qSN-1-CK, qSTR-4-CK, qSNK-6-CK, qSDW-7-CK*) had the nagetive AE (*Additive effect*) values which explained Khangdan18 variety contributed to increasing salinity tolerance. The *qSTR-4-CK* located on chromosome 4 is major QTL, because this QTL had the largest LOD (13.25) and phenotypic variation (39%).

INTRODUCTION

Salinity is one of main serious factors constraining the productivity of rice crop in many rice growing areas in the world (Islam *et al*., 2011). Currently, over 1.3 billion hectares of growing food crops, most areas are producing rice, have been severly affected by salt (Ammar *et al*., 2007; Islam *et al*., 2011). In Asia alone, 21.5 million hectare of arable lands are facing with salinity problem and estimated to cause the loss up to 50% fertile land by the 21st midcentury (Nazar *et al*., 2011; Huyen *et al*., 2013). Rice is a cash crop in Vietnam and providing daily food for nearly 90 million persons in this country. Also, Vietnam is a leading biggest rice exporter in the world, accounting for 50% of the world rice trade (Khanh *et al*., 2013). However, rice cultivating areas in Vietnam including two large rice producing areas, Red River Delta (RRD) and Mekong River Delta (MRD) are being seriously influenced by salt intrustion with estimated to be about 19.0% - 37.8% of MRD and about 1.5% - 11.2% of RRD. Vietnam is formidably dealing with salinity problem which is causing adverse influence on 1 million ha, equally with 3% of total Vietnam areas (Nguyen *et al*., 2006; Linh *et al*., 2012). On the other hand, the economic loss annually by salt intrusion is up to 45 million USD, which is equivalent to 1.5% of rice productivity per year in MRD (MARD, 2005). To overcome reduction of rice yield affected by salt in the country, one of the feasible method is to use the salinity tolerance of rice cultivars as the target crop. The work on mapping and identifying QTLs which are responsibe and controlled salinity tolerance play a key role to generate the rice lines with high salinity tolerance. Therefore, the objective of the current study was to identify and map the QTLs which controlling salinity tolerance of rice. The data will provide good information for the breeders to further generate salt tolerance rice cultvars and grow in the salt affected areas to enhance rice yield and ensure food security in the country.

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PLANT MATERIALS

Chanh Trui (CT) is a local rice cultivar with high salinity tolerance. While, Khang Dan 18 (KD) is popularly grown rice cultivar with low and supsebtible salinity tolerance (Nghia *et al*., 2012). The 250 individual plants in the F2 population (for DNA extraction) from the previous crossed combination CT x KD and selfing F3 lines (salinity tolerance evaluation) were used for QTL mapping in this study.

METHODS

The seedling stage of rice lines/cultivars were screened in the laboratory condition to evaluate the salinity tolerance by use the method of IRRI, 2002. Physiological and biochemical factors of rice lines/cultivars such as shoot fresh weight (SFW), shoot dried weight (SDW), Na^+ and K^+ concentrations content and ration in the shoots and roots dried materials were conducted following the method of Saeedipour (2011). Total DNA extraction of the parental lines and F2 was carried out as the method of Keb-Llanes (2002). PCR reaction with SSR primers by using recycle temperature:

After initial denaturation for 5 min at 94˚C each cycle comprised 94˚C for 1 min, 55˚C - 58˚C for 45 s, and 72˚C for 2 min at the end 35 cycles. The PCR products were kept at 4˚C util for use. For statistical analyses, excel ver. 2007, IRRISTAT ver. 5.0 were used. The Mapmaker/QTL ver 1.1, Mapmaker/EXP ver 3. softwares were applied to contruct the linkage map and identify the salintiny tolerance QTL/gen in studied rice lines (Lander, 1989; Lincoln, 1992). The names of QTLs were given as the reports of McCouch (1997) and Tabien (2002), for instance, qSDW-7-CK that implies QTL for shooot dried weight, locted on the chromosome 7 "q": means QTL, " CK " was abbreviated from the local names of rice cultivars "Chanh trui và Khang dan 18".

RESULTS AND DISCUSSION

Phenotyping

Evaluation of salinity tolerance of F3 lines

Two hundred fifty lines of F_3 were screened to evaluate salinity tolerance based on the STR-Standard Tolerance Rankin, (Yoshida culture, with adding EC_{NaCl} = *10dS/m, after 12 days salt treatment)*. The obtained results showed that the salinity tolerance of the tested rice cultivars were ranged from 1 to 9 score, of which, 2 rice lines showed salinity tolerance with score 1, and 4 rice lines were score 2; 32 lines were score 3; 51 lines were 4; 98 lines showed score 5; 17 lines were at score 6; 13 rice lines wre score 7; 25 lines exhibited score 8; 8 lines were score 9; among those rice cultivars, Chanh trui showed salinity tolerance at score 2, while, Khang dan 18 was 7, respectively (Unpublished data).

As shown in Figure 1, the diagram of salinity tolerance of parental and F_3 lines exhibited the standard distribution with the square figure. Hence, the results showed that salinity tolerance of the rice lines/cultivar should be quantitative traits and controlled by the complex genes

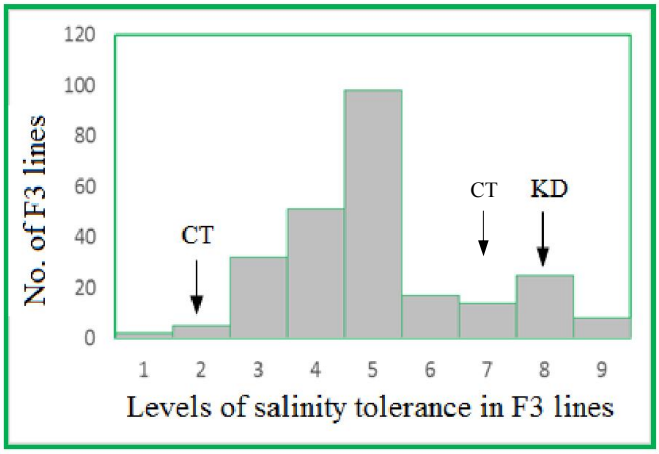

Figure 1. The diagram to evaluate salinity tolerance of the parental and F3 lines

Characteristics of physiology and biochemistry of F_3 lines

Weight of fresh shoots and dried shoots at seedling stage

To identify the fresh shoots and dried shoots of the F_3 lines, the seedling at 3 weeks growth (planted in the Yoshida culture, with adding NaCl at 100 mM) were used. The results showed that the weight of fresh shoots of the rice lines were from 0.13g to 0.40g, with average weight was 0.26g. While, the weight of dried shoots were ranged from 0.06g to 0.12g, and average weight was 0.08g (Figure 2).

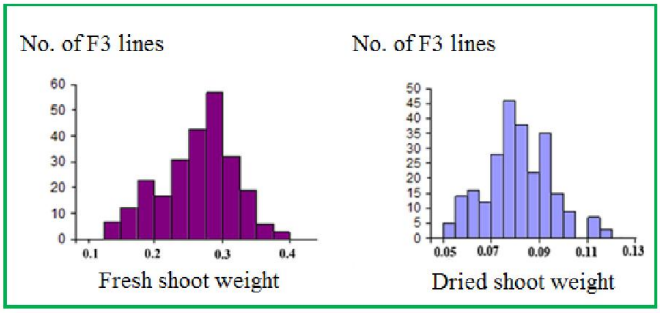

Figure 2. Distribution of the fresh and dried shoots weight of F3 rice lines grown in the Yoshida culture, adding NaCl, with concentration 100mM

Concentration of Na^+, K^+ content in the dried shoots at seedling stage

After 12 days of salt treatment by using NaCl with 100mM, the fresh shoots were well soaked by tap water, and dried at 70ºC, concentration of Na^+, K^+ content was also recorded as following the method of (Ghomi *et al*., 2013). The results showed that the concentration content of Na+ of the dried shoots of F3 was ranged from 0.70 to 1.76 meq/g dried weight, the average arrangement was 1.03 mep/g of dried weight. Chanh trui and Khang dan 18 cultivars revealed 0.84 and 1.52 meq/g of dried weight. Concentration of K+ content in dried weight of F3 lines was shown from 0.53 to 1.62 meq/g of dried weight), the average was 1.09 meq/g of the dried weight. While, Chanh trui and Khang dan 18 cultivars were 0.63 and 2.67 meq/g of dried weight, respectively (Figure 3)

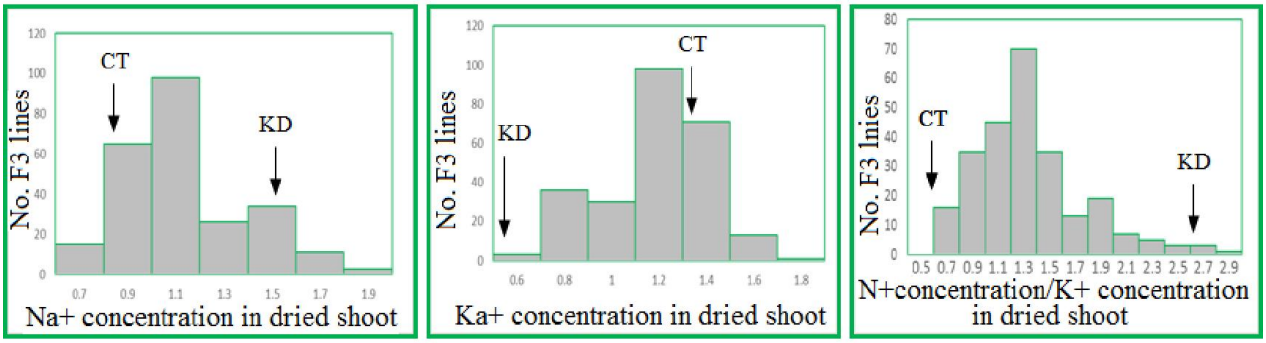

Figure 3. Concentration of Na^+, K^+ and ration between concentration of Na^+/K^+ in the dried shoot of the F_3 rice lines grown in Yoshida culture, adding NaCl (100mM)

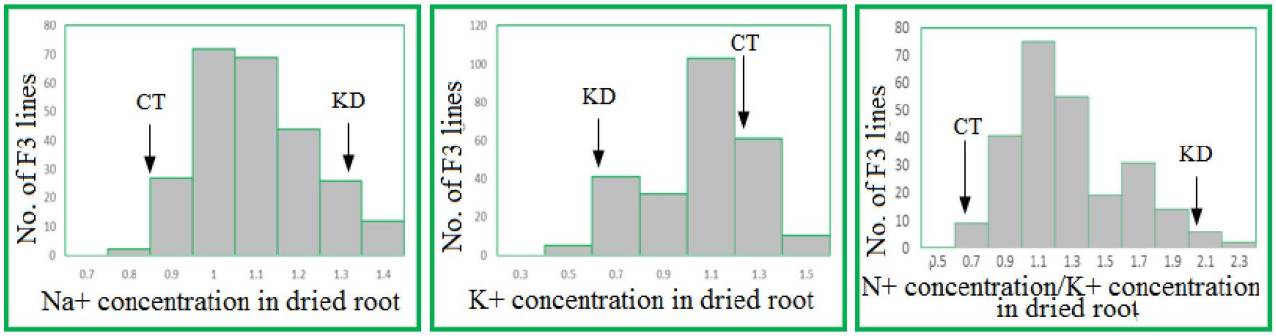

Figure 4. The difference of concentration of Na^+, K^+ and the ratio between concentration of Na^+/K^+ content in the dried roots of the F_3 grown in the Yoshida with NaCl (100mM)

As the recorded the concentration of Na^+, K^+ in the dried shoot weight of F_3 lines, it demonstrated that concentration of Na^+ in the dried shoot weight of the salinity subseptible cultivars showed higher to compare with the salintity tolerance cultivars. While, the concentration of K^+ content in the dried shoot weight of supseptible culivars was lower than the salinity tolerance cultivars. On the other word, the ratio between Na^+/K^+ of the lines/culivars with salinity tolerance disclosed lower to compare with the subseptible cultivars.

Concentration of Na^+ và K^+ content in dried roots at the seedling stage

After 12 days treatment with NaCl (100mM), roots of rice seedling were well cleaned by tap water, and dried at 70°C, concentration of Na^+, K^+ content in roots was determined as following the method of Ghomi *et al*. (2013). The results showed that the concentration of Na+ in the dried roots of F3 lines was ranged from 0.79 to 1.36 meq/g of dried weight, the average was 1.06 mep/g of the dried weight. For Chanh trui and Khang dan 18 cultivars, the concentration of Na+ in the dried roots were 0.85 and 1.32 meq/g of the dried weight. The concentration of K^+ content of the dried roots of the F3 lines were 0.41 to 1.5 meq/g of its dried weight. While, Chanh trui and Khang dan 18 were 1.24 and 0.64 meq/g of the dried weight. The ratio of Na^+/K^+ was ranged from 0.70 to 2.27, and 1.15 on average, Chanh trui and Khang dan 18 cultivars were reached 0.69 and 2.06, respectively (Figure 4). As the results of the concentration of Na^+, K^+ consisting in the dried roots of the F3 lines, it demonstrated that concentration of Na^+ in the dried root of the salinity supseptible rice cultivars showed higher to compare with the salinity tolerance of rice cultivars; Contrarily, concentration of K^+ in the dried root of subseptible rice cultivar exhibited lower to compare with the salinity tolerance of rice cultivars; the ratio of Na^+/K^+ of rice salinity tolerance showed lower than the salinity subseptible rice cultivars.

Genotyping

Constructing the linkage map by using the molecular markers

In the current study, 192 polymophic markers between Chanh trui and Khang dan 18 were used to identify DNA of Khang dan 18, which was encoded as A, and Chanh trui was B; the individual plants of F_2 had genetic similarity with Khang dan 18 were A, and for Chanh trui cultivar was B, respectively, hetergegous (the mediate between Khang dan 18 and Chanh trui) was determined as H, non-data was recorded as "–". DNA data of the parental plants, individual plants of F2, were used to contruct the linkage map based on the molecular markers. All data were analysed by Mapmaker/exp software. The SSR markers were distributed on the 12 chromosome with the total distance 1.797 cM, the average distance between the markers was 9.4 cM (Figure 5). The arrangement of SSR markers located on each chromosome in this study were correlative the linkage map of rice which was previous reported by Bonilla *et al.* (2002); and rice genome programe (RGP).

Identification of salinity tolerance QTL/gen

Genotyping data (DNA identification) and phenotying data (salinity tolerance level such as the fresh shoot weight; dried shoot weight, concentration content of Na^+, K^+. etc.) were used to analyse and identify QTL/gen by applying the MAPMAKER/QTL ver 1.1.

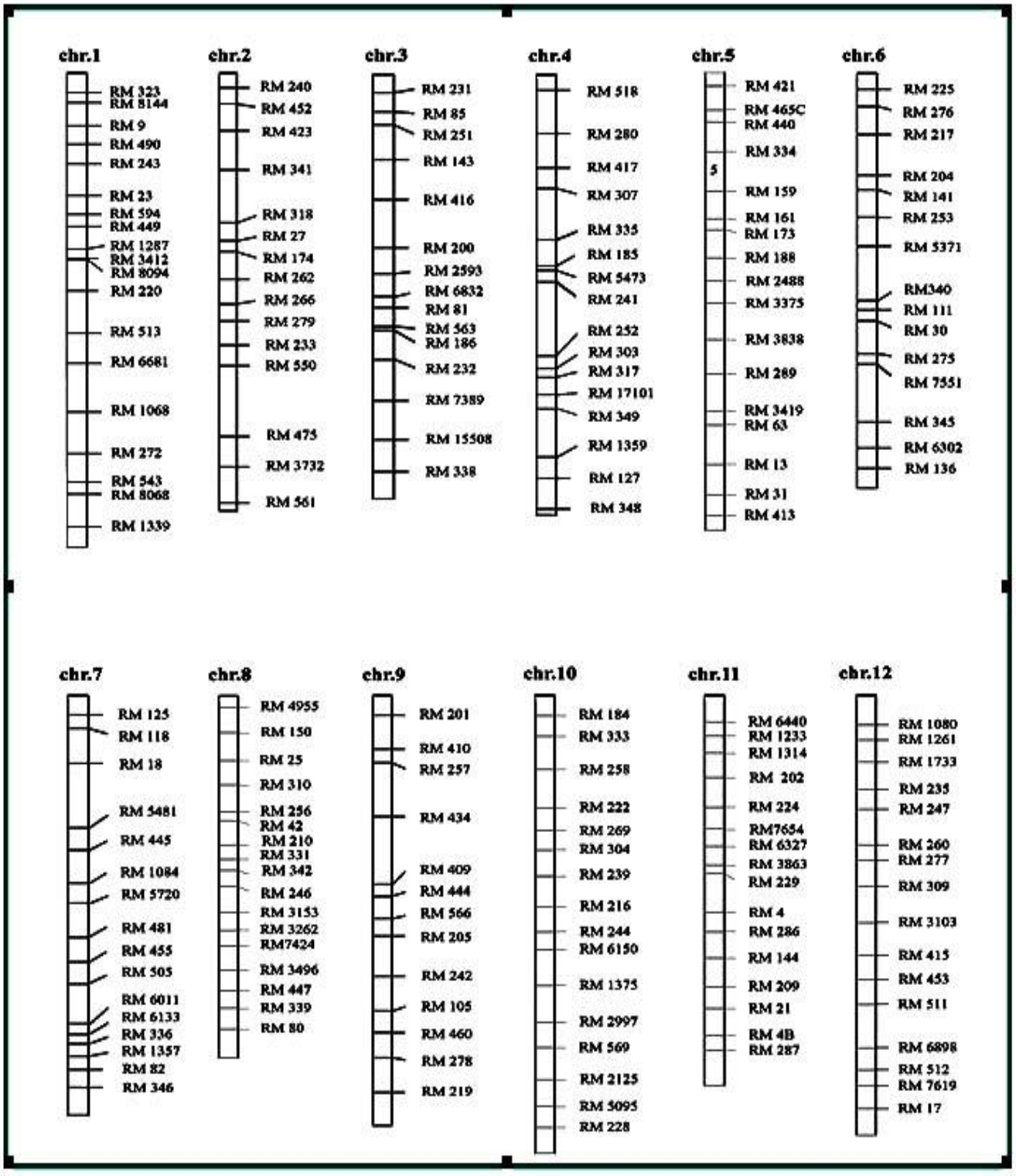

Figure 5. Linkage map of the molecular markers distributed on the 12 chromosomes was constructed from the crossed combination between Chanh trui and Khang dan 18 cultivars

Table 1: The distance and number of molecular markers distribute on 12 chromosomes in the linkage map

No	Chro	Number of marker	Total distance (cM)	No	Chro	Number of marker	Total distance (cM)
1	1	19	187,7	7	7	16	138,3
2	2	15	162,6	8	8	17	126,4
3	3	15	132,5	9	9	13	133,5
4	4	16	164,0	10	10	16	163,0
5	5	18	167,0	11	11	16	134,0
6	6	15	132,6	12	12	16	156,0

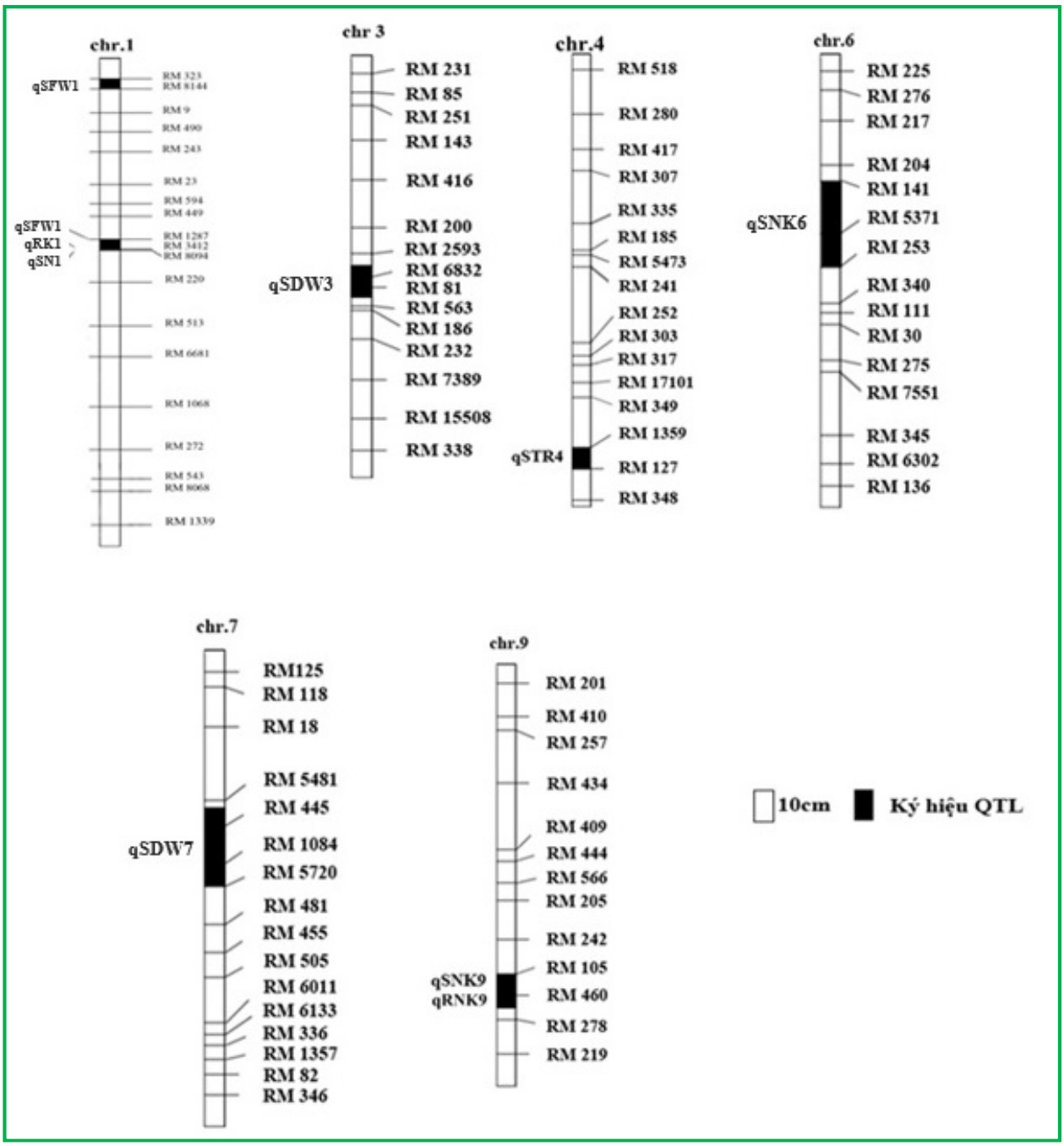

Figure 6. Location map of QTL/gen related the salinity tolerance was identified based on the crossed combination of Chanh trui and Khang dan 18 cultivars

Table 2. The identified QTLs involving in salinity tolerance

QTL	SSR flanking marker	Chro	AE[a]	LOD	Var.exp. (%)[b]
qSFW-1a-CK	RM323-RM8144	1	0,422	4,43	13,24
qSFW-1b-CK	RM449-RM8094	1	-0,423	2,56	25,13
qRK-1-CK	RM1287-RM8094	1	-0,448	3,65	5,52
qSN-1-CK	RM3412-RM220	1	-0,279	3,49	10,67
qSDW-3-CK	RM2593-RM563	3	3,198	2,16	14,56
qSTR-4-CK	RM1359-RM127	4	-1,384	13,25	39,0
qSNK-6-CK	RM141-RM253	6	-0,284	3,38	8,00
qSDW-7-CK	RM5481-RM5720	7	-0,138	2,67	17,88
qSNK-9-CK	RM242-RM460	9	0,246	2,52	23,5
qRNK-9-CK	RM105-RM287	9	0,142	10,47	9,33

[a] *:Genetic effect increased by the interaction between 2 allens of Chanh trui and Khang dan 18. AE with negative value implied the allen was improved the salinity tolerance of Khang dan 18*

[b] *:Influence of QTL on the salinity tolerance.*

As the results obtained, 10 locations were identified with the LOD (Log likelihood) was higher than 2. It suggested that 10 QTLs were involved in the salinity tolerance in the crossed plants between Chanh trui and Khang dan 18. The factors such as LOD value, percentage of influence, location of the chromosome and flanking markers (markers on the both sides of QTL) as shown in the Figure 6 and Table 2. The identified QTLs were named as *qSFW-1a-CK, qSFW-1b-CK, qRK-1-CK, qSN-1-CK, qSDW-3-CK, qSTR-4-CK, qSNK-6-CK, qSDW-7-CK, qSNK-9-CK, qRNK-9-CK*. Among those, 6 QTLs included *qSFW-1b-CK, qRK-1-CK, qSN-1-CK, qSTR-4-CK, qSNK-6-CK, qSDW-7-CK* showed nagetive AE (*Additive effect*) values which explained Khangdan18 cultivar contributed to increasing salinity tolerance. It suggested further that Khang dan 18 was subseptible with salinity but obtaining alen activation of Chanh trui, showed to increase salinity tolerance level in the population. There were 4 QTLs with positive value included *qSFW-1a-CK, qSDW-3-CK, qSNK-9-CK, qRNK-9-CK* showed similar salinity tolerance to Chanh trui. The *qSTR-4-CK* located on chromosome 4 is major QTL, because this QTL had the largest LOD (13.25) and phenotypic variation (39%). It implied that this QTL has played an important role in controlling salinity tolerance in the studied population of rice.

Conclusions

Salinity tolerance in the F_3 population was controlled by complex QTL genes with the standard frequency distribution of the square diagram. The physiological and biochemical characteristics of F3 showed that: Concentration of Na^+ containing in the dried shoot and dried roots of the salinity subseptible cultivars revealed higher than those salinity tolerance rice cultivars. While, concentration of K^+ content in the dried shoots and dried roots of the subseptible cultivars showed lower content to compare with the salinity tolerance rice cultivars. The ratio of Na^+/K^+ of the rice salinity tolerance demonstrated lower than the subseptible salinity rice cultivars. Moreover, the linkage map was contructed based on 192 polymophic SSR markers which were distributed on the 12 chromosomes of rice with the total distance (1.797 cM) and average distance between the markers was 9.4 cM. Ten QTLs involving in salinity tolerance, namely *qSFW-1a-CK, qSFW-1b-CK, qRK-1-CK, qSN-1-CK, qSDW-3-CK, qSTR-4-CK, qSNK-6-CK, qSDW-7-CK, qSNK-9-CK, qRNK-9-CK,* which located on the chromosome 1, 3, 4, 6, 7 and 9 were identified. Among them, The *qSTR-4-CK* located on chromosome 4 is major QTL, because this QTL had the largest LOD (13.25) and phenotypic variation (39%), respectively.

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