Identification of QTLs related to rice seedling traits under K deficiency stress in Iranian inbred lines population

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Abstract

A mapping population of 96 inbred line derived by cross Neda (NAD) and Ahlamitaroum (ATM), was used to detect quantitative trait loci (QTLs) for fresh biomass (FB), root length (RL), shoot length (SL), root number (RN), leaf width (LW), root fresh weight (RFW), root dry weight (RDW), and K content (KC) under K deficiency condition in rice. Two parents and 96 inbred lines were phenotyped for the traits by growing them in K deficiency nutrient solution. Under K deficiency, 16 QTLs were able to explain a great deal of phenotypic variation in features. qSL-7a, qRL-6, qRL-10b, qRL-12b, qRN-6b, qRN-12b, qLL-6, qLL-7, qLW-6b, qLW-10b, qLW-12a, qLW-12b, qLW-12c, qKC-6b, qKC-10b, and qKC-12c were on chromosomes 6, 7, 10, and 12. Their LOD were 4.732, 5.826, 5.01, 5.067, 11.346, 5.867, 5.973, 5.85, 7.077, 8.055, 5.577, 6.439, 8.012, 14.057, 6.432, and 858/6, respectively. In normal conditions, 5 QTLs with a large effect were identified. qKCN-6c, qKCN-7a, qKCN-10, qKCN-12b, and qKUN-6 were located on chromosomes 6, 7, 10, 12, and 6, respectively. Owing to the high percentage of explanation, the major QTLs can be a suitable candidate for marker assistance programs in recombinant Iranian rice lines after validation.

Keywords: Gene mapping, major QTL, marker assisted breeding, potassium deficiency, seedling

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Introduction

Rice (*Oryza sativa* L.) is a grain and one of the most important foods in the world with a major role in human nutrition, especially in developing countries ((Dien et al., 2019); (Thiyagarajan et al., 2005)). Rice needs sunlight, water, and various nutrients to grow. From these various elements, 16 elements are essential in rice nutrition ((Islam et al., 2020)). Potassium is known as an essential macronutrient in plant growth and plays an important role in various biochemical and metabolic processes such as cell elongation, enzymes activation, turgor regulation, and osmotic adjustment ((Adams and Shin, 2014); (Islam et al., 2020); (Ye et al., 2020); (Fontana et al., 2020)). In addition, potassium enhances the absorption of nutrients such as N and P (Ye et al., 2019). Therefore, K deficiency limits the growth

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and development of the plant, ultimately limiting rice yields ((Safdar et al., 2020); (Fang et al., 2015)). This is why produced new tolerant cultivars to K deficiency finds significance. A host of research have found significant differences between rice genotypes in morphological, physiological, and biochemical traits in K deficiency conditions ((Liu and Liu, 2002); (Tai et al., 2004); (Yang et al., 2004)).

Nowadays, genomic technologies and molecular markers are valuable methods for identifying genomic regions for tolerance to abiotic stresses (Anis et al., 2018). Much effort has been made to reveal the genetic basis of complex traits such as yield, growth, and stress resistance in crops using quantitative trait locus (QTL) analyses (Zhang et al., 2020). QTL mapping determines the relationship between genotype and phenotype and identifies possible genes and alleles that are linked with specific traits (Patishtan et al., 2018). But the genetic structure of potassium deficiency in rice has been little studied. Three QTLs were identified on 3 chromosomes for three traits under low K and two QTLs were identified on 2 chromosomes for two traits under normal K by Islam et al. (2020).

Thus, this study aimed at identifying QTLs associated with K deficiency tolerance by using Iranian RIL population derived from rice varieties Ahlamitaroum × Neda.

Materials and Methods

Evaluations of K deficiency tolerances at the seedlings stage were performed in a greenhouse in the College of Agriculture Science and Natural Resource of Gonbad Kavous University. Two kinds of cultivars (*Oryza sativa* L.) Neda (NAD) and Ahlemitarom (ATM) were used as parents since NAD is sensitive to K deficiency while ATM is tolerant. From a cross between NAD and ATM, 94 lines F_8 generation were derived and used in this study. Completely randomized design (CRD) consisting of 96 lines (10 plants per accession) with 3 replicates were applied for both control and stress conditions.

Dormancy of seeds was broken at 50 ℃. After 3 days, the seeds germinated at 35 ℃. The germinated seeds were cultivated in the *Styrofoam* plates with a nylon net bottom. This system floated on distilled water for 3 days. Then, the *Styrofoam* plates were relocated on Yoshida culture solution (Yoshida et al., 1971)for 11 days. Its macro elements nutrients consisted of 50 mg/L Si, 40 mg/L N, K, Mg, and Ca, and 10 mg/L P. The micro elements nutrients were made with 2.0 mg/L Fe, 0.5 mg/L Mn, 0.2 mg/L B, 0.05 mg/L Mo, 0.01 mg/L Zn, and 0.01 mg/L Cu. The solution culture was changed weekly and the pH was regulated to 5.5 with 1-N NaOH/HCl twice a week. One week after culturing, two K conditions were applied: normal condition (40 mg L^{-1} K^{+}) and low potassium stress (4 mg L^{-1} K^{+}). Screening was conducted in a controlled condition with 16 h photoperiod, irradiance of 1500 µmolm^{-2} s^{-1}, day/night temperature of 29/21 ℃, and minimum relative humidity of 70%.

After plants were harvested, length of shoot (SL) and length of root (RL), fresh weight of root (FWR), fresh weight of shoot (FWS), and root number (RN) were measured. The concentration of K in shoot samples under stress conditions were determined by atomic absorption spectrometry (AAS, Series2, Thermo Electron Corporation) with wet digestion method (GB/T 14609–2008). Forty (40) SSR primer pairs, 16 ISSR markers (76 alleles), 2 IRAP markers (7 alleles), and 1 iPBS marker (3 alleles) were appropriately distributed on 12 rice chromosomes chosen according to (Chen et al., 1997), (Temnykh et al., 2000), and (McCouch et al., 2002). The SSR marker Saltol was used on chromosome 1. Also, markers ISSR, iPBS, and IRAP were used to check the rate of polymorphism from previous articles.

PCR was applied in volume of 10 ml with 2 ng of DNA, 39.2 µmol dm^{-3} of primers, 117.6 mmol dm^{-3} of each dNTP, 156.8 mmol dm^{-3} $MgCl_2$, 19.6 unit of Taq polymerase, and 0.098 cm^3 of 10 × PCR buffer. PCR amplification was carried out a thermal cycler (BIORAD, America) in Genetic Laboratory of Gonbad Kavous University, Iran. PCR products were separated on 6% (m/v) polyacrylamide gels (38:2 acrylamide:bis acrylamide) and detected by fast silver staining as described by An et al. (2009). Using Mapmanager QtbX17, 12 linkage groups were constructed with a minimum LOD score of 2. Map distances were presented in Centi Morgan

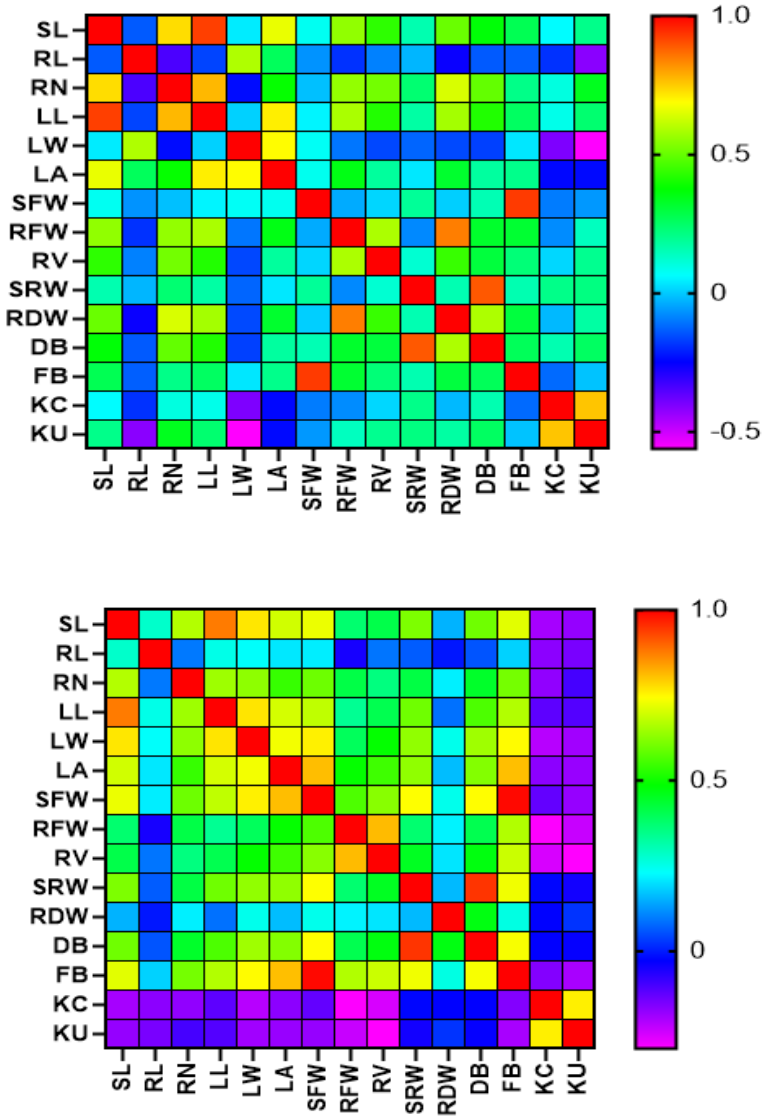

Fig. I : Correlation coefficients among traits studied in 96 Iranian inbred lines in normal condition (up) and K deficiency condition (down).

(cM) derived using the Kosambi function (Kosambi, 1944) of the program.

Results

Thirty-six (36) QTLs were determined in normal condition, which covered 12 rice chromosomes (Table 1 and Fig. I). In normal conditions, a QTL was identified in chromosome 12 for fresh biomass weight. This QTL explaining 9.7% of phenotypic variation. A QTL was identified on chromosome 12. qSLN-12 explaining 10% of phenotypic variance. NAD alleles have reduced this trait. A QTL was detected for root number on chromosome 3 and close to ISSR16-3 marker. Three QTLs were detected for leaf width on chromosomes 2, 7, and 12, qLWN-2, qLWN-7, and qLWN-12 close to ISSR1-1, ISSR20-2, and ISSR14-2 markers, respectively. Their additive effects were 0.024, 0.328, and -0.42, respectively. Also, 17 QTLs were identified for K content (KC) on chromosomes 1, 5, 6, 7, 8, 9, 10, and 12 in normal condition.

Among the QTLs, qKCN-6c, qKCN-7a, qKCN-10, and qKCN-12b were major effect QTLs, explaining 41.9%, 28.8%, 22.1%, and 24.6% of phenotypic variance, respectively. Also, 13 QTLs were identified for K uptake (KU) on chromosomes 2, 5, 6, 7, 8, 9, 10, and 12, qKUN-6 explained 24.3% of

the phenotypic changes of the trait with LOD of 5.805 and an additive effect of -2.656.

A total of 36 and 91 QTLs were identified in K deficiency condition, and distributed across all 12 chromosomes (Table 2 and Fig. II). In K deficiency conditions, six QTLs for shoot length (SL) were detected on chromosomes 6, 7, 9, and 12. qSL-7a explained 20.3% of phenotype changes of the trait. qSDW-10a and qSDW-10b were detected on chromosomes 10. These QTLs explained 9.5 and 9.6% of phenotypic changed of the trait. The alleles of the parent ATM reduced this trait. qRDW-6 was detected on chromosome 6 for root dry weight (RDW) in stress conditions. This QTL was close to the ISSR9-1. A QTL was detected on chromosome 6 for dry biomass (DBW). qDBW-6 had positive additive effect of 0.005 gr.

A QTL was detected on chromosome 10 fresh biomass weight (FBW). This QTL had an LOD of 2.105 and an additive effect of 0.11 gr. Fourteen (14) QTLs were identified on chromosomes 2, 4, 6, 7, 8, 9, 10, and 12 for root length (RL). qRL-6, qRL-10b, and qRL-12b were close to ISSR9-1, ISSR14-2, and ISSR15-1 markers, respectively. These QTLs justified 24.4%, 21.4%, and 21.6% of phenotypic variance, respectively. Thirteen (13) QTLs were identified on chromosomes 1, 2, 5, 6, 7, 8, 9, 11, and 12 for root number (RN). qRN-6b and qRN12b were major effect QTLs and justified 42% and 24.5% of phenotypic variance, respectively. In stress conditions, six QTLs were identified on chromosomes 1, 6, 7, 9, and 12 for leaf length (LL). qLL-6 and qLL-7 explained 24.9% and 24.5% of phenotypic variance, respectively. Their additive effects were 2.313 and 24.495 cm, respectively.

Fourteen (14) QTLs were identified on chromosomes 2, 6, 7, 8, 9, 10, and 12 for leaf width (LW). qLW-6, qLW-10b, qLW-12a, qLW-12b, and qLW-12c were major effect QTLs and explained 28.8%, 32.1%, 23.5%, 26.6% and 31.9% of phenotypic variance, respectively. qLA-12 was detected on chromosome 12 for leaf area (LA). This QTL was close to ISSR15-3 marker and explained 17.8% of phenotypic variance.

In K deficiency conditions, a QTL was identified on chromosome 10, explaining 14.8% of phenotypic variance. qRFW-6 was detected on chromosome 6 for root fresh weight (RFW). The additive effect was 0.019 gr, explaining 12.4% of phenotypic variance. Four QTLs were identified on chromosomes 1, 6, and 12 for root volume (RV). These QTLs explained 9.2% to 19.8% of phenotypic variance.

In K deficiency conditions, nine QTLs were identified on chromosomes 1, 2, 3, 6, 10, and 12 for K content (KC). These QTLs explained 9.5% to 19.8% of the variance of the adjective phenotype. Seventeen (17) QTLs were detected for K uptake (KU) on chromosomes 1, 2, 3, 5, 6, 8, 9, 10, and 12. Among the QTLs, qKU-6b, qKU-10b, and qKU-12c were major effect QTLs. These QTLs justified 49%, 26.5%, and 28% of phenotypic variance and were close to the ISSR9-1, ISSR13-4, and ISSR15-1 markers.

Among the QTLs, several similar QTL were detected under normal and stress conditions. At the 120 cM position of chromosome 12, similar QTLs were identified for KC in both conditions. Also, three similar QTLs were detected in both normal and stress conditions on chromosomes 10 and 12 (two cases) at 22, 44, and 120 cM positions, respectively.

Discussion

Potassium is an essential element in most plants that plays an important role in the activity of enzymes, turgor provision, and water homeostasis (Hartley et al., 2019). In this study, genetic linkage map was formed with a length of 1419 cM and an interval space between two locus of 13.07 cM. Fang et al. (2015) used doubled haploid population derived from the cross between a *japonica* cultivar CJ06 and an *indica* accession TN1. They identified 30 QTLs for different traits including height of shoot, length of root, dry weight of shoot, dry weight of root, and total dry weight under the normal (40 mg L^{-1} K^{+}) and low potassium stress culture (4 mg L^{-1} K^{+}). Wu et al. (1998) used doubled haploid population derived from a cross between *indica* rice cultivar, IR64, and a *japonica* variety Azucena in a hydroponic experiment. In their study, several QTL for plant height, tiller number, shoot dry weight, root dry weight, K concentration in plant, K uptake, and K use efficiency were detected.

Table 1
Putative QTLs for normal condition in seedling stage the F8 population derived from Ahlemi Tarom (AHT; a K tolerance variety) and Neda (NAD; a K-susceptible variety). Only QTLs with LOD higher than 3 are shown.

Traits	QTL	Chr.	Flanking markers	LOD	Location (cM)	Additive effect	R2	Direction of ph
Content K	qKCN-5a	5	ISSR2-1-RM39	3.087	40	-0.556	13.8	AHT
	qKCN-5b	5	RM39-RM194	3.415	50	-0.455	15.1	AHT
	qKCN-5c	5	ISSR10-2-ISSR4-3	3.25	92	-0.341	14.4	AHT
	qKCN-6c	6	RM597-ISSR9-1	11.325	86	-0.817	41.9	AHT
	qKCN-7a	7	ISSR20-2-ISSR12-1	7.087	16	-6.387	28.8	AHT
	qKCN-7b	7	ISSR4-6-RM500	3.557	46	-0.353	15.7	AHT
	qKCN-7c	7	ISSR5-4-ISSR4-7	3.607	114	-0.632	15.9	AHT
	qKCN-8a	8	ISSR4-6-ISSR13-3	3.204	20	-0.627	14.2	AHT
	qKCN-8b	8	RM331- ISSR2-5	3.276	66	0.543	14.5	NAD
	qKCN-9b	9	RM205-ISSR8-7	3.058	98	-0.43	13.6	AHT
	qKCN-10	10	ISSR14-2-ISSR13-4	5.21	22	-0.632	22.1	AHT
	qKCN-12b	12	RM83-ISSR15-1	5.886	120	0.916	24.6	NAD
K uptake	qKUN-5b	5	ISSR4-3-ISSR9-4	3.282	94	-1.595	14.6	AHT
	qKUN-6	6	ISSR2-3-ISSR4-5	5.805	18	-2.656	24.3	AHT
	qKUN-7a	7	ISSR20-2-ISSR12-1	3.461	16	-22.514	15.3	AHT
	qKUN-7c	7	ISSR8-6-RM248	3.503	90	-2.52	15.5	AHT
	qKUN-7d	7	RM248-ISSR5-4	3.287	98	-2.004	14.6	AHT
	qKUN-10	10	ISSR14-2-ISSR13-4	3.967	22	-2.708	17.3	AHT
	qKUN-12b	12	ISSR15-1-IRAP17-3	3.212	120	3.376	14.3	NAD

Miyamato et al. (2012) used three *japonica* cultivars of rice (*Oryza sativa* L.), Koshihikari, Nipponbare, and Sasanishiki, studied three *indica* cultivars, namely IR36, IR64, and Kasalath and found some QTLs for potassium concentration in shoot and sodium concentration in shoot on chromosomes 3 and 6.

Veldboom et al. (1994) showed that correlated traits often have QTLs that map to the same chromosomal region. In the present study, the QTLs associated with LW, KC, and KU in the region of ISSR20-2-ISSR12-1 chromosome 7 overlapped in normal conditions. In K deficiency conditions, a large number of traits overlapped in different regions of the genome and traits SL, LL, LA, RFW, and RDW were detected on chromosome 6 at position 88. The QTLs of qSL-9a and qRN-9a, qLL-9a, qLW-9, and qKU-9 were on chromosome 9 and between markers ISSR20-5-ISSR14-1. Also, SL, RL, RN, LL, LW, RV, KU, and KC were detected on chromosome 12 and between markers ISSR15-1 and IRAP17-3. Although under K deficiency many QTLs were detected in similar regions, correlations were low. In the present study, under K deficiency conditions, SL, LL, LA, RFW, RDW, RN, RDW, and RFW were on chromosome 6, in the position of 88 cM, and showed a positive and significant correlation. Also, KU and KC were detected on chromosomes 1 and 12, which had a high correlation (0.758^{**}). RN and RV were detected on chromosomes 12 and 6 and in positions 120 and 32, respectively, with a positive and significant correlation. qFBW-10 and qSFW-10 were detected on chromosome 10 and between the markers RM216 and ISSR14-3, with the highest correlation (0.928^{**}).

According to the results in normal and K deficiency conditions, it seems that there is a pleiotropy effect of genes controlling the traits. Therefore, co-located chromosomal regions responsible for different traits may have an opportunity to introgress them together as a unit into rice varieties through MAS/MAB and to develop resilient tolerant cultivars (Gimhani et al., 2016).

Conclusion

In this study, genetic locations with more than 20% genes were identified for some traits, including qSL-7a, qRL-6, qRL-10b, qRL-12b, qRN-6b, qRN-12b, qLL-6, qLL-7, qLW-6b, qLW-10b, qLW-12a, qLW-12b, qLW-12c, qKC-6b, qKC-10b, and qKC-12c under K deficiency conditions and qKCN-6c, qKCN-7a, qKCN-10, qKCN-12b, and qKUN-6 were located on chromosomes 6, 7, 10, 12, and 6, at positions 86, 16, 22, 120, and 18 cM,

respectively in normal condition. Important linked markers to major QTLs, explaining high percentage of traits variation, could be a suitable candidate for MAS breeding programs.

Table
Putative QTLs for K tolerance in seedling stage the F8 population derived from Ahlemi Tarom (AHT; a K tolerance variety) and Neda (NAD; a K-susceptible variety). Only QTLs with LOD higher than 3 are shown.

Traits	QTL	Chr.	Flanking markers	LOD	Location (cM)	Additive effect	R2	Direction of ph.
Shoot length	qSL-6	6	RM597-ISSR9-1	4.615	88	3.255	19.9	AHT
	qSL-7a	7	ISSR20-2-ISSR12-2	4.732	16	35.172	20.3	AHT
Root length	qRL-7c	7	ISSR5-4-ISSR4-7	3.17	112	-2.599	14.1	NAD
	qRL-8	8	ISSR4-6-ISSR13-3	4.001	18	-2.962	17.5	NAD
	qRL-9	9	RM205-ISSR8-7	3.561	100	-1.915	15.7	NAD
	qRL-10eb	10	ISSR14-2-ISSR13-4	5.01	24	-3.34	21.4	NAD
	qRL-10c	10	RM294A-RM591	3.379	90	-2.708	15	NAD
	qRL-12a	12	ISSR14-4-ISSR15-3	3.081	38	2.257	13.7	AHT
	qRL-12b	12	RM83-ISSR15-1	5.067	120	3.56	21.6	AHT
Root number	qRN-2	2	ISSR20-7-RM301	4.097	84	-1.235	17.8	NAD
	qRN-5	5	ISSR2-1-RM39	3.07	40	1.471	13.7	AHT
	qRN-6b	6	RM597-ISSR9-1	11.346	88	1.913	42	AHT
	qRN-9a	9	ISSR20-5-ISSR14-1	3.718	26	-1.596	16.3	NAD
	qRN-12b	12	RM83-ISSR15-1	5.867	120	-2.425	24.5	NAD
Leaf length	qLL-6	6	RM597-ISSR9-1	5.973	88	2.313	24.9	AHT
	qLL-7	7	ISSR20-2-ISSR12-1	5.85	16	24.495	24.5	AHT
	qLL-9b	9	RM205-ISSR8-7	3.314	100	1.512	14.7	AHT
	qLL-12	12	RM83-ISSR15-1	3.169	120	-2.886	14.1	NAD
Leaf width	qLW-6b	6	RM597-ISSR9-1	7.077	86	-0.669	28.8	NAD
	qLW-7	7	ISSR20-2-ISSR12-1	3.375	16	-4.543	14.9	NAD
	qLW-8a	8	ISSR4-6-ISSR13-3	3.205	12	-0.627	14.2	NAD
	qLW-10b	10	ISSR14-2-ISSR13-4	8.055	22	-0.752	32.1	NAD
	qLW-12a	12	ISSR14-4-ISSR15-3	5.577	38	0.702	23.5	AHT
	qLW-12b	12	ISSR15-3-RM12	6.439	44	0.669	26.6	AHT
	qLW-12c	12	RM83-ISSR15-1	8.012	120	1.03	31.9	AHT
Leaf area	qLA-12	12	ISSR14-4-ISSR15-3	4.081	42	6.638	17.8	AHT
Shoot fresh weight	qSFW-10	10	RM216-ISSR14-3	3.336	66	0.13	14.8	AHT
Root volume	qRV-6b	6	RM597-ISSR9-1	4.61	88	0.012	19.8	AHT
K content	qKC-2a	2	ISSR1-1-iPBS2078-2	4.041	34	0.572	17.6	NAD
	qKC-2b	2	iPBS2078-2-RM300	3.182	52	0.493	14.2	NAD
	qKC-3a	3	ISSR8-5-ISSR9-5	3.174	8	0.525	14.1	NAD
	qKC-6	6	RM597-ISSR9-1	4.471	88	0.496	19.3	NAD
	qKC-10	10	ISSR13-4-IRAP17-2	4.602	40	-0.856	19.8	AHT
K uptake	qKU-1	1	RM594-RM10720	3.309	98	-1.524	14.7	AHT
	qKU-2a	2	ISSR1-1-iBS2078-2	4.095	34	2.674	17.8	NAD
	qKU-5	5	ISSR2-1-RM39	3.557	38	2.957	15.7	NAD
	qKU-6b	6	RM597-ISSR9-1	14.057	88	3.675	49	NAD
	qKU-8a	8	ISSR4-6-ISSR13-3	3.297	14	3.034	14.6	NAD
	qKU-9	9	ISSR20-5-ISSR14-1	4.133	26	-2.976	18	AHT
	qKU-10a	10	ISSR14-2-ISSR13-4	3.214	22	2.395	14.3	AHT
	qKU-10b	10	ISSR13-4-IRAP17-2	6.432	38	-4.822	26.5	AHT
	qKU-12c	12	RM83-ISSR15-1	6.858	120	-4.608	28	AHT

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