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Analysis of Cytoplasm Polymorphism on the TR2 Locus of Mitochondria Genome in Leaf Beet Line SK-5

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Abstract. One mitochondrial minisatellite locus was used to study cytoplasmic diversity in leaf beet lines SK-5 population from Chongqing, China. The analysis of one mitochondrial minisatellite locus (TR2) was conducted using variable number of tandem repeats (VNTRs) molecular markers method within 50 single plants of leaf beet line SK-5 material. Over the whole sample, TR2 show the polymorphic with two alleles. The variable numbers of tandem repeats on TR2 locus is 3 and 6 respectively among the 50 plants examined. TR2 of a six-copy array of 33-bp sequence was first discovered in 2 single plants among the population. 1 plants can not bolting and 1 plant bolting late can not form normal seeds among the 2 plants which VNTRs copy number is 6. Only 1 plants among the 48 plants with three-copy in TR2 locus fails to bolting normally and others bolting normally and form seeds.

Introduction

Sugar beet is one of the main sugar crops in the world, which belong to typical cross -pollinated cultivate plants [1]. Cytoplasmic male sterility (CMS) is a mainstay for the hybrid seed production in sugar beet because large-scale emasculation is not feasible, owing to the small perfect flowers and indeterminate flowering habit of beet plants [2, 3]. Male sterility lines selection is a time-consuming and costly laborious work during sugar beet hybrid seed production [4]. The production of all hybrid beet cultivars relies upon a single source of CMS at present, which was discovered by Owen [4]. As the gene pool of current sugar beet cultivars may be genetically narrow, new sterility germplasm resources is highly desirable for sugar-beet breeding [5]. Leaf beet has never been bred intensively, thereby leading us to assume that a lot of variation is still present in leaf beet cultivars or landraces [6]. Leaf beet can be considered as a potential, valuable source of desirable characters lacking in sugar beet breeding programs [7]. With this in mind, we have decided to estimate the extent of genetic diversity within leaf beet germplasm resources.

A variable number of tandem repeats (VNTRs) is abundant and ubiquitously present in eukaryotic genomes [8, 9]. These sequences hybridize to multiple loci and are hypervariable in nature, thus proving highly informative for genetic analysis [10]. Japanese researchers first report the VNTR loci found in the mitochondrial DNAs (mtDNAs) of sugar beet, which discover four unrelated tandem repeat loci (TR1, TR2,

TR3 and TR4) in the mitochondrial genomes of beets [10]. TR1 is composed of an array of 32-bp tandem repeats, the number of which varies from 2 to 13 among the seven beet genotypes examined. PCR assay shows TR3, TR4 and TR1 to be polymorphic in terms of the number of repeat units among the seven beet genotypes.TR2 is composed of a three-copy array of a 33-mer and no polymorphic [10].

To find new type cytoplasm germplasm within the leaf beet, we examined here cytoplasmic diversity among 50 plants in leaf beet lines SK-5 using polymorphisms in mitochondrial minisatellite loci TR2. Bolting and pollen fertility observation were conducted in the field. Leaf beet also named Swiss chard, belong to biennial herbaceous plant. Leaf beet native to the coast of the Mediterranean Sea, and is widely used in cooking [11]. We expect to provide the theoretical foundation of new type cytoplasm germplasm resources use, and rich new research materials and data for explaining male sterility mechanism at the same time.

Materials and Methods

Plant Materials

The leaf beet (Beta vulgaris) line used in this study is SK-5 from Chongqing, China. The plants of SWK5 were provided by Breeding Department of Sugar beet Research Institute of HIT.

DNA Isolation

Sample seeds were grown and DNA was extracted from leaves of single plant of SK-5 following a modified CTAB extraction protocol as described in Liu et al [12].

VNTRs Polymerase Chain Reaction

Oligonucleotides for polymerase chain reaction (PCR) primers were designed in accordance with the conserve sequence of mitochondrial minisatellite genomes TR2 loci of beets [10]. The primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd. Polymerase chain reaction (PCR) was conducted in a 20μL reaction mixture containing 16μL water, 2.0μL Taq PCR buffer (3mM Mg^{2+}), 0.5μL dNTP mix (10 mmol/L), 0.5μL primer (10 μmol/L), 0.5μL g DNA (100ng/μL) and 0.5μl Taq polymerase (TaKaRa, Dalian, China). DNA amplification was performed in a Thermal Cycler (TaKaRa, Japan). Amplifications were performed with an initial denaturing temperature of 94 ℃ for 3 min, followed by 30 cycles of 94 ℃ for 30 s, 45 ℃ for 1 min and 72 ℃ for 1 min, and ended with an extension step of 72 ℃ for 5 min. Then amplified products were stored in 4 ℃.

Agarose-gel Electrophoresis Procedure

Amplified PCR products electrophoresis were run on 1.2% agarose gel (including 0.05% ethidium bromide) under conditions of 1 ×TAE electrode buffer solution and 5 V/cm voltage,and photographed by gel imaging analyzer. Each gel consisted of 10 PCR products from 10 plant and one DNA marker lanes (DL2000) on the left of the gel serving as fragment-size standards.

VNTRs Sequencing

PCR products were either cloned into the pBluescript vector (Stratagene, La Jolla, CA, USA) prior to sequencing or directly sequenced by Sangon Biotech (Shanghai) Co., Ltd.

The numbers of tandem repeat loci (TR2) in the mitochondrial genomes of every plant were determined according with sequencing analysis.

Bolting and Pollen Fertility Observation

Plants were cultivated in the field after vernalization under low tempreture in spring. Individual plants were carefully evaluated for bolting, anther dehiscence, pollen production and seed during the flowering period.

Result and Discussion

Cytoplasm Polymorphism on the TR2 Locus of Mitochondrial within Leaf Beet Line SK-5 Population

VNTRS polymorphism on the TR2 locus of cytoplasm mitochondrial was tested among 50 individual plants within the leaf beet line SK-5 by PCR products electrophoresis. During agarose gel electrophoresis**,** 3-copy CK sample identified by sequencing, DNA Marker and individual plants PCR product were drip into the gel lane as shown in Fig. 1. The total nucleotides length of TR2 PCR products for 3- copy VNTRs plant is 362bp, and 461pb for 6copy VNTR plant. Two individual plants difference in banding pattern was observed after the PCR products from TR2 region of 50 individual plants were separated by electrophoresis. The PCR products of two plants were bigger than the size of three-copy VNTR plants (Fig. 1). Nucleotide sequencing revealed that six-copy array of a 33-bp sequence was discovered in 2 single plants among the population, and the nucleotides length is 461bp. The numbers of tandem repeats among 50 individual plants within the leaf beet line SK5 were list in Table 1.

The number of repeats in mitochondrial minisatellite TR2 locus was 3(in 48 cases) and 6(in 2 cases). We found cytoplasmic diversity on the TR2 locus of mitochondria within individual plants of leaf beet line SK-5. The results showed that the Numbers of Tandem Repeats in mtDNA TR2 locus were three-copy array of tandem repeats in 48 single plants among the 50 plants of leaf beet line SK-5, which consistent with sugar beet. Six-copy VNTRs in mtDNA TR2 locus were found in 2 single plants. So far, polymorphism of mitochondrial TR2 locus has never been found in beet [10]. Six-copy VNTRs in mtDNA TR2 locus was first found in leaf beet in this paper, and a new cytoplasmic individual plant is exist. Experimental results indicate that diversity of the cytoplasm more widely in leaf beet group than sugar beet.

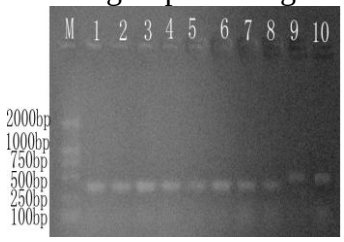

Figure 1. PCR product electrophoretogram for VNTRs polymorphism on mitochondrion TR2 locus. M denotes DL2000 DNA marker, Number 1 denotes three-copy CK, Number 2 to 8 denote three-copy VNTRs single plant, Number 9 to 10 denote six-copy VNTRs single plant

Bolting and Fertility Trait

No obvious difference was found in growth habit and plant type among the 50 plants. The result of fertility trait was show in Tab.1. Only one plant among the 48 plants with three-copy in TR2 loci failed to bolting normally and others bolting normally and form

seeds. One plant could not bolt among the 2 plants which VNTRs copy numbers were 6, and one plant bolting late could not form normal seeds. Normal seed can not be form in the six-copy plant, and the reasons of this phenomenon need further explanation and research.

Table 1. Copy number of VNTRs on TR2 locus and fertility trait

Sequence numbers of single plant	VNTRs copies	Fertility trait	Total number
1-15	3	Bolting and seed setting	15
16	3	Not bolting	1
17-18	3	Bolting and seed setting	2
19	3	Bolting and seed setting	1
20-21	3	Bolting and seed setting	2
22	6	Not bolting	1
23-46	3	Bolting and seed setting	24
47	6	Bolting late and no seed	1
48-50	3	Bolting and seed setting	3

Conclusions

Molecular identification of cytoplasm polymorphism within leaf beet line SK-5 was conducted in this paper. Polymorphisms in mtDNA TR2 locus were analyzed using variable number of tandem repeats (VNTRs) method. The result showed that the Numbers of Tandem Repeats in mtDNA TR2 locus were three-copy array of tandem repeats in 48 single plants among the 50 plants of SK-5, which consistent with sugar beet. Six-copy VNTRs in mtDNA TR2 locus were first found in 2 single plants among the population. One plant could not bolt among the two plants which VNTRs copy numbers were six. One plant bolting late could not form normal seeds among the two plants which VNTRs copy number were six. Only one plant among the 48 plants with three-copy in TR2 loci failed to bolting normally and others bolting normally and form seeds. The results show diversity within the leaf beet population. The discovery of the special polymorphism of cytoplasm provides a new germplasm resources materials for further explore the mechanism of f plant fertility research.

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