

氏名 (本籍・生年月日)	上野広樹 (長野県・1986年5月17日)
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学位論文題目	Structural and functional analysis of sex chromosomes in papaya ( <i>Carica papaya</i> ) (パパイヤ性染色体のゲノム構造および機能の解析)
論文審査委員	主査 (准教授) 松村英生 (教授) 海老沼宏安 (教授) 林田信明 (准教授) 堀江智明 (准教授) 菅野明 (東北大学)

## 論文内容の要旨

Sexual reproduction in higher plants is highly divergent. The majority of higher plants are hermaphrodite species, and approximately 6% of flowering plants are dioecious. Of these, 48 species have been identified to have sex chromosomes, which are responsible for their sex determinations. According to the previous studies, those dimorphic sex chromosomes have derived from ancestral autosomes.

Papaya (*Carica papaya*), which belongs to the Caricaceae family, is known to be a species harboring sex chromosomes. Particularly, papaya is a trioecious plant, and its sex types are determined by the pairing of three sex chromosomes, XX, XY and XY<sup>h</sup> for females, males and hermaphrodites. Phylogenetic analysis presumed that hermaphrodite of papaya has derived from a natural mutation from male plants. Draft genome sequence was accessible in papaya, and partial sequences of X and Y<sup>h</sup> chromosomes are also available as BAC (Bacterial Artificial Chromosome) clone sequences. On the other hand, Y chromosome sequence information is limitedly analyzed. In spite of these studies, any genes for papaya sex determination have not been found in these sex chromosomes. Therefore, the present study aimed to identify candidate genes for sex determination in papaya by comparing structure and function of genes in the three sex chromosomes.

For identifying candidate genes for female/hermaphrodite or male determination, genome-wide gene expression was analyzed using High-throughput SuperSAGE in flower buds in all three sex types of papaya. Of the expressed genes in these tissues, expressed genes mapped in the sex chromosome sequences were focused. This analysis allowed to

identifying 47 tags (genes) in the sex chromosomes, showing sex type dependent expression. Among these genes, Cp2671, uniquely mapped on Y<sup>h</sup> chromosome, encoded a MADS-box protein, which is a homologue of *Short vegetative phase* of *Arabidopsis thaliana*. Since allele of this gene was absent in the X chromosome, it was suggested that this MADS-box gene was responsible for determination between female and other sex types.

For elucidating details of genome structures and functions of papaya sex chromosomes, reference sequences of X and Y<sup>h</sup> chromosome were constructed by own genome sequence analysis and assembly. Whole genomes of female and hermaphrodite plants were sequenced by next generation sequencer, and obtained sequence reads were re-assembled with publicly available BAC clone sequences of sex chromosomes. By comparison of predicted genes and genome sequences between the X and Y<sup>h</sup> chromosomes, rearrangements process could be predicted during sex chromosome revolution. Transcriptome analysis of 99 unique genes on the X chromosomes, using RNA-seq revealed that no significant difference in expression level was found in the leaves between female and hermaphrodite plants, regardless of difference of their gene dosage, suggesting the presences of their compensation mechanism.

For identifying genes for male/hermaphrodite determination, genome structure of Y and Y<sup>h</sup> chromosomes were compared by reference mapping the male genome sequence reads to the Y<sup>h</sup> reference sequence. In total 15,493 polymorphisms were found between the Y and Y<sup>h</sup> chromosome, together with three highly diverged regions. Of these polymorphisms between the Y<sup>h</sup> and Y chromosomes, specific insertion of a putative transposon in the *SVP-like* gene was identified in the Y<sup>h</sup> chromosome. Sequence of its cDNA demonstrated that truncation of MADS-box domain was found in the transcripts from the Y<sup>h</sup> chromosome, suggesting its concern to male/hermaphrodite determination.

These results in the present genomic studies must be greatly helpful for elucidating sex chromosome evolution in papaya. Particularly, the *SVP-like* gene was a candidate for sex determination (female-hermaphrodite, male-hermaphrodite). Further functional studies for this gene should shed light on sex determination mechanisms in papaya.