The chromosome analysis of Hibiscus spp. is difficult, rare and tedious due to numerous number chromosomes and genome sizes are small. The aim of this research is to know chromosome number, chromosome morphological distributions, ribosomal DNA position of different chromosomes, individual chromosomes length and a total genome length of *H. sabdariffa*. The root tips were collected and treated with α-bromonaphthalene solution for 1–2 h at room temperature. The root tips were washed with double-distilled water and transferred into aceto-ethanol (1:3, v/v) solution to fix the roots overnight. After rinsing with distilled water, 70% ethanol was used to preserve the roots at −20°C until further use. For slide preparation, the root tips were washed with distilled water to remove the ethanol solution and incubated with enzyme mixture consisting of 0.3% pectolyase (Duchefa, Haarlem, The Netherlands), 0.3% cellulase (Duchefa) and 0.3% cytohelicase (Sigma, St. Louis, MO, USA) at 37°C for 40 min. Digested root tips were transferred to a clean slide and squashed under a microscope. Finally, 17 µL acetic acid (70%) slide−1 was added and the slides were air-dried. The number of chromosomes and detailed karyotype of *H. sabdariffa* was analyzed by fluorescence in situ hybridization (FISH) technique with using 18S rDNA and 5S rDNA probes. *H. sabdariffa* is tetraploid and the chromosome number of somatic cell is 2n = 4x = 72. According to FISH analysis, ten loci of 18S rDNA signals (red) and four loci 5S rDNA signals (green) were detected in chromosome set 1, 3, 4, 6, 13, 15, 17, 18 and chromosome set 3, 9, 10, 15 respectively. The length of metaphase-2 chromosome range from 3.42 to 6.64 µm, with a total length/genome is 94.64 µm. The chromosome composed of 8 sets (32) metacentrics and 10 sets (40) submetacentrics. The karyotype formula is K (2n) = 4x = 72 = 32m + 40sm. This result will greatly contribute to breeding and cytogenetic study of *Hibiscus*. (This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry (IPET) through Agri-Bio industry Technology Development Program Program (or Project), funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (Grant Number. PET311821-4).)