Variation of Chromosome Number in Plants and Regenerated Plantlets of Bupleurum falcatum L.

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Variation of Chromosome Number in Plants and Regenerated Plantlets of *Bupleurum falcatum* L.

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Variation of chromosome number in plants and regenerated plantlets of *B. falcatum* was studied using three cultivars, originated from Kumamoto and Fukuoka, Japan and Jeongseon, Korea. Within and among cultivars, there was a variablity in chromosome number. The basic chromosome number from plants was shown as 2n=26, 2n=26 and 2n=20 for the cultivar from Kumamoto, Jeongseon, and Fukuoka, respectively. Various kinds of aneuploidy were observed from the plants as 2n=18, 22, 23, 24, 25, 48. Chromosome number of regenerated plantlets by anther culture was 2n=13, which was originared from a 2n=26 plant.

INTRODUCTION

B. falcatum has variation of chromosome number in different geographical origins as well as in the same cultivar with 2n=20, 26, 32 of three basic types (Amano *et al.*, 1989; Chung *et al.*, 1995; Ohta *et al.*, 1986; Ohta, 1991). Furthermore, variation of chromosome number was observed in the regenerated plants from tissue culture (Amano *et al.*, 1989; Kohda *et al.*, 1990). Therefore, it is necessary to observe chromosome numbers for roots from seeds of various cultivars and for plantlets regenerated through anther culture to confirm the haploidy.

The objective of this study is to obtain more information on the chromosome number variation for different cultivars and regenerated plantlets through anther culture.

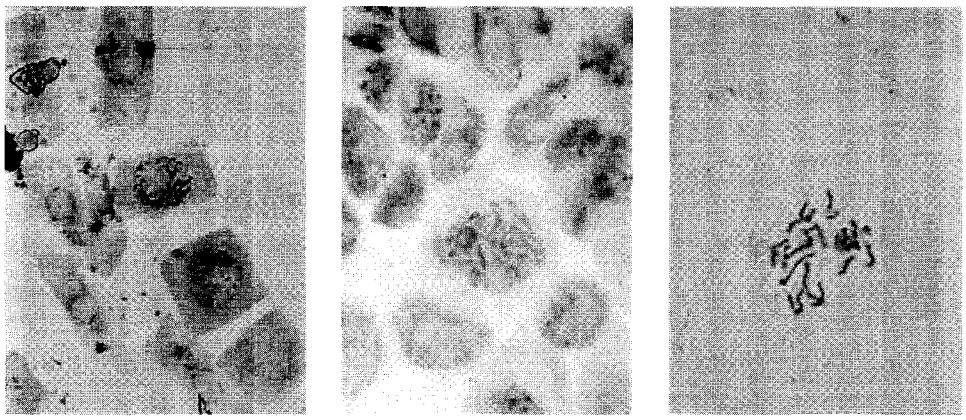
MARERIALS AND METHODS

Three cultivars, originated from Jeongseon, Korea and Fukuoka and Kumamoto, Japan, were used for somatic chromosome observation. Seeds were germinated for 1 month in a jiffy pot at 20°C and the root tips were used for chromosome observation. Root tips were pretreated with a solution of 0.1% colchicine for 2 hours at 20°C. After fixation in ethanol/acetic acid (3:1%) for 30 min. at 5°C and macerated with 1N HCl for 5 ~7 min. at 60°C, they were stained in an 1% aceto-orcein solution.

For chromosome observation of regenerated plantlets, root tips, obtained from regenerated plantlets through anther culture, were treated with 2M 8-hydrooxyquinline for 4~5 hours at 20°C, fixed in 45% glacial acetic acid for 5 to 10 min. at 5°C, macerated with a mixture solution of 1N HCl and 45% glacial acetic acid (2:1) for 10 seconds at 60°C and stained with 1% aceto-orcein.

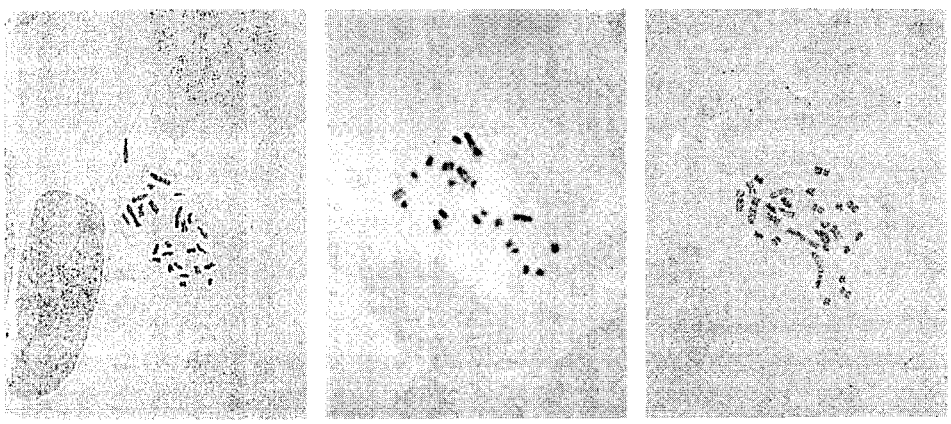
RESULTS

In *B. falcatum*, the chromosome formed dark stained chromatin threads and spread over the nucleus uniformly at interphase (Fig. 1, A). At prophase, all chromosomes were concentrated homogeneously along with their long axis, thus early and late concentrated segments were not distinguishable (Fig. 1, B and C). At metaphase, all chromosomes were observed very clearly (Fig. 2).



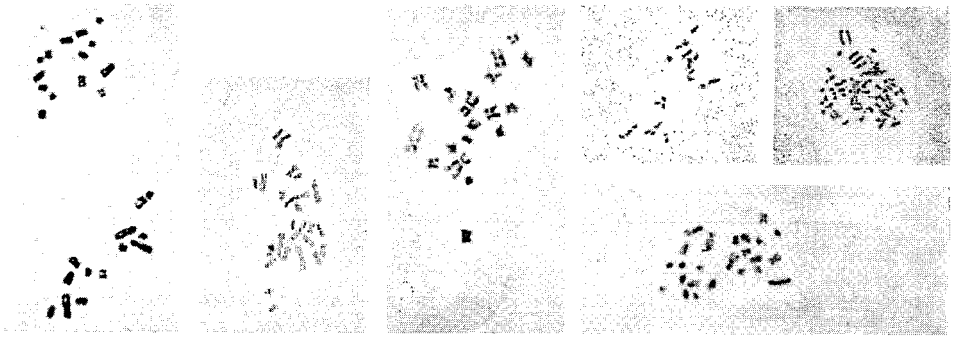
Interphase(A) Early prophase(B) Late prophase(C)

Fig. 1. Somatic chromosomes at interphase and prophase in *B. falcatum*.



Jeongseon (2n=26) Fukuoka (2n=20) Kumamoto (2n=26)

Fig. 2. Somatic chromosomes of *B. falcatum* of three cultivars from Jeongseon, Fukuoka and Kumamoto.



Jeongseon (2n=25, 23) Fukuoka (2n=18) Kumamoto (2n=48, 24, 22)

Fig. 3. Variation of somatic chromosomes of *B. falcatum* from seeds of three cultivars from Jeongseon, Fukuoka and Kumamoto.

2n=13

Fig. 4. Chromosome in a root tip cell of a regenerated plantlet.

Fig. 2 shows somatic chromosomes of three different cultivars. Chromosome number was determined to be 2n=20 (the cultivar from Fukuoka), and 2n=26 (the cultivars from Kumamoto and Jeongseon).

Variation of somatic chromosomes from seeds within a cultivar is represented in Fig. 3. Aneuploids of somatic chromosome were found as follows; 2n=23, 25 for the cultivar from Jeongseon, 2n=18 for that from Fukuoka, and 2n=22, 24, 48 for that from Kumamoto (Fig. 3 and Table 1). Fig. 4 shows that the haploid plantlets originated from anther culture of *B. falcatum* had a half of the diploid chromosome number (n=13) of their originated plant (2n=26).

DISCUSSION

In this study, somatic chromosome numbers of 2n=20, 26 (the cultivars from Fukuoka and Kumamoto) were observed. This number confirms previous reports of Ohta *et al.* (1986) and Ohta (1991). The chromosome number of the cultivar from Jeongseon was determined for the first time as 2n=26. Most of the chromosome numbers was the same

Table 1. Chromosome numbers of the plants from seeds regenerated plantlets by anther culture of *B. falcatum*.

Cultivars from	Plants from seeds		Plantlets from anther culture	
	Chromosome number	Number of plants observed	Chromosome number	Number of plantlets observed
Kumamoto	48	1	13	5
	26	22		
	24	1		
	22	1		
Jeongseon	26	17	13	4
	25	1		
	23	1		
Fukuoka	20	6		
	18	1		

as the basic type with a few variation in this study. Ohta (1991) reported that *B. falcatum* showed polymorphic variation in external morphology such as plant size, shapes of stem and basal leaves, size of florets, and had various chromosome numbers, *i.e.* 2n=19, 20, 21, 22–23, 24, 25, 27, 27–28, 27–29, 29–31, 30, 30–31, 32–34, 33, 34, 37, 40.

In regenerated plants by tissue culture, the variation in chromosome number differed among different cultivars; large frequency of chromosome number variation for the cultivars from Hiraodai (93.9%) and that from Kirishima (94.1%) and no variation for that from Mishima (Amano *et al.*, 1989). Kohda *et al.* (1990) reported that propagations of the shoot primordia of *B. falcatum* were highly stable in the chromosome number as less than 1% in frequency of chromosome number variation. In the present study, all regenerated plantlets by anther culture were stable in chromosome number as n=13, though a few materials were counted for regenerated plantlets.

B. falcatum is a cross pollinating species and genetically heterozygous and was found to have a lot of intraspecific variation in external morphology as well as internal cytology. Thus, for classification and identification of cultivars and production of high yield and quality cultivars, homozygous plants through tissues culture are needed.

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