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Short Communication

Ploidy estimation in West Indian gherkin, horned melon and wild *Cucumis* spp. by flow cytometry

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The ploidy level of wild *Cucumis* spp. differs among species and accessions. Therefore, estimations of ploidy levels are important in the breeding of species and accessions of this plant. Flow cytometry is a powerful technique for estimating ploidy level. This study examined the applicability of flow cytometry analysis for estimating ploidy levels in wild *Cucumis* spp. West Indian gherkin and horned melon. The relative DNA content of these accessions was highly correlated with their ploidy levels. The results suggest that flow cytometry analysis can be used for estimating ploidy levels in these species.

Key words: West Indian gherkin, horned melon, *Cucumis* spp., ploidy level, flow cytometry.

INTRODUCTION

Plant breeding programs consistently require effective tools that can confer timesaving benefits and increased efficiency in improving cultivars; for example, generating doubled haploids is of great interest for the rapid production of pure lines (Griffing, 1975). Because the *Cucumis* plant is cross-pollinated, it has a long breeding cycle. Therefore, to produce pure lines in the shortest possible time, a collaborative study was set up to obtain irradiated pollen-induced parthenogenetic haploid embryos that were raised into complete haploid plants (Gursoz et al., 1991). The classic method of counting chromosomes is considered the most accurate way of determining ploidy level. However, other indirect methods can also be used satisfactorily. For example, flow cytometry (FCM) is a powerful technique for estimating plant nuclear DNA content and ploidy level because it permits sensitive measurements of the florescence intensity of large numbers of stained nuclei within seconds (Arumuganathan and Earle, 1991; Ushio et al., 2002). FCM analysis has been successfully used in melon (Ogawa et al., 2002) and cucumber (Kubaláková et al., 1996), but not yet in other *Cucumis* spp., for which microscopic counting of chromosomes in root-tip cells is conventionally done to determine the ploidy level (Dane and Tsuchiya, 1976). The genus *Cucumis* consists of about 30 species and is further classified into 2 subgenera representing different base chromosome numbers: *Cucumis* subgen. *Cucumis* (x = 7) and *Cucumis* subgen. *Melo* (x = 12) (Krkbride, 1993). The ploidy level of this genus differs among species or accessions, unlike that of melon and cucumber.

Variations in ploidy levels have been reported, especially in accessions of wild species of the subgenus *Melo*. Most accessions of wild species have been found to be 2n = 24 (*Cucumis africanus*, *Cucumis dipsaceus*, *Cucumis ficifolius*, *Cucumis hirsutus*, *Cucumis humifructus*, *Cucumis myriocarpus*, *Cucumis prophetarum*, *Cucumis pustulatus*, *Cucumis sagittatus*, *Cucumis sacleuxii* and *Cucumis zeyheri*). However, among these species, the accessions of 3 species have also been reported to be polyploid: *C. ficifolius*, *C. africanus*, *C. prophetarum*, *Cucumis heptadactylus* and *C. zeyheri*, 2n = 48; *C. pustulatus*, 2n = 48 or 72 (Dane and Tsuchiya, 1976; Krkbride, 1993). Therefore, ploidy level estimations are necessary in breeding of these species and accessions. In this study, we examined the applicability of the FCM analysis for estimating ploidy levels in West Indian gherkin, horned melon and wild

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Table 1. Accessions and ploidy level of *Cucumis* species used in this study.

Species	Accession No.	Ploidy level*
C. africanus	PI 275571	2x
	PI 299569	2x
C. anguria	PI 147065	2x
	PI 196477	2x
	PI 233646	2x
	PI 320052	2x
	PI 364475	2x
C. dipsaceus	PI 236468	2x
C. metuliferus	PI 292190	2x
C. myriocarpus	PI 203977	2x
	PI 282449	2x
	PI 282450	2x
	PI 299568	2x
	PI 374153	2x
C. sagittatus	PI 282441	2x
C. africanus	PI 299571	4x
	PI 273648	4x
C. ficifolius	PI 299570	4x
	PI 299572	4x
C. heptadactylus	PI 282446	4x
C. prophetarum	PI 193967	4x
C. subsericeus	PI 273650	4x
C. pustulatus	PI 343699	6x
	PI 343700	6x
	PI 343701	6x

* Ploidy level was according to Dane and Tsuchiya (1976).

Cucumis spp.

MATERIALS AND METHODS

Plant materials

Fresh leaf material of 10 wild *Cucumis* species, West Indian gherkin, and horned melon were collected, and a total of 25 accessions were used (Table 1). The seeds of these accessions were obtained from Germplasm Resources Laboratory (Agricultural Research Service, U.S. Department of Agriculture).

Furthermore, leaves of *Oryza sativa* L. cv. Nipponbare were used to internally calibrate the flow cytometer. The ploidy levels of each accession were taken from Dane and Tsuchiya (1976).

FCM analysis

FCM analysis was performed with a PA flow cytometer (Partec, Münster, Germany). Samples were prepared according to Ushio et al. (2002). Leaf segments of 0.5 cm^2 were chopped with a razor blade in the presence of a nucleus isolation solution (high-resolution DNA kit type P solution A; Partec). The suspension was filtered through a 30-µm nylon mesh, and nuclei were stained with 4′,6-diamidino-2-phenylindole solution (high-resolution DNA kit type P solution B; Partec). In each sample, 5000 nuclei were analyzed, and the analysis was replicated 3 times. The gain of the instrument was calibrated so that the G_1 peak of 'Nipponbare' was set at channel 100. The relative DNA content of individual plants was calculated according to the following formula:

$$\text{Relative DNA content} = \frac{\text{Mean of the peak channel of the } G_1 \text{ of the sample}}{\text{Mean of the peak channel of the } G_1 \text{ of 'Nipponbare' nuclei}}$$

RESULTS AND DISCUSSION

The relative DNA contents of the 25 accessions analyzed were clearly classified into 3 groups: 0.7 to1.0, 1.6 to 2.0 and 2.4 to 2.8. These relative DNA contents were highly correlated with ploidy levels (R^2 =0.97)

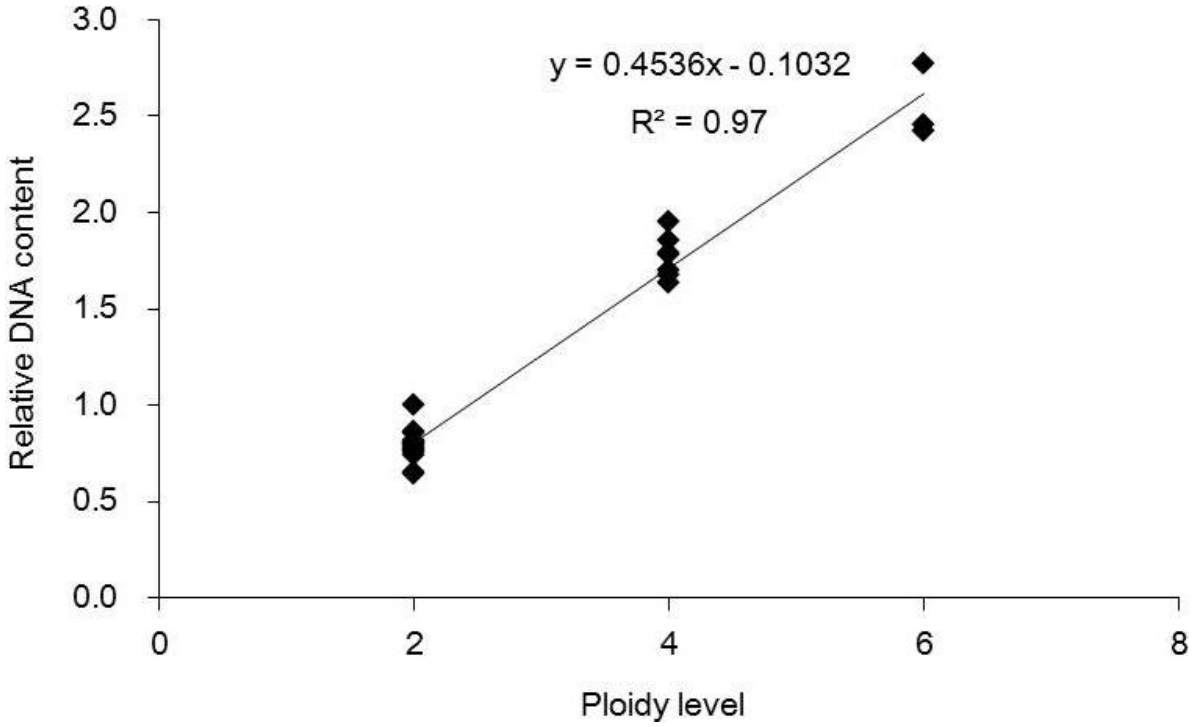

Figure 1. Relationship between the relative DNA content and ploidy level.

(Figure 1). The relative DNA contents of diploid, tetraploid and hexaploid accessions were 0.7 to 0.9, 1.6 to 2.0 and 2.4 to 2.8, respectively. Therefore, the results suggest that FCM analysis can be used for estimating the ploidy level of West Indian gherkin, horned melon and wild *Cucumis* spp. Furthermore, we used rice cultivar 'Nipponbare' as a calibration. 'Nipponbare' is one of the model plants, and widely used for genomic research (Ohmido et al., 2000). Therefore, these results could be used as comparison between other species. FCM analysis can be used as a simple and rapid method for estimating the ploidy level of a large number of accessions. Although, this technique has been used successfully in melon (Ogawa et al., 2002) and cucumber (Kubaláková et al., 1996), it has not been applied to West Indian gherkin, horned melon and wild *Cucumis* spp. The results of this study may have applications in the breeding of West Indian gherkin, horned melon and some *Cucumis* species, especially as an early and easy marker for ploidy manipulations (for example, polyploidization, haploidization and somatic hybridization).

Furthermore, FCM analysis might be suitable for detecting mixoploidy as well as for determining the ploidy level in progenies of conventional intra- and interspecific crosses of *Cucumis* spp.

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