Scientia Horticulturae 134 (2012) 121–126

Contents lists available at SciVerse ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Difference in the polysomaty degree during fruit development among plants with different ploidy levels produced by artificial chromosome doubling of a pepper (*Capsicum annuum*) cultivar 'Shishitou No. 562'

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ARTICLE INFO

Article history:
Received 23 March 2011
Received in revised form
12 September 2011
Accepted 14 November 2011

Keywords:
Capsicum
Endoreduplication
Flow cytometry
Fruit size
Polyploidy
Polysomaty

ABSTRACT

Polysomaty status of fruit pericarp was compared between a diploid cultivar of pepper (*Capsicum annuum* 'Shishitou No. 562') and its artificially induced triploid and tetraploid plants. In pepper fruits of different ploidy levels, fruit size as assessed by fruit length reached a plateau from 3 to 4 weeks after anthesis, and the maximum fruit length of triploid and tetraploid fruits was about 43% and 88% of the diploid counterpart, respectively. However, the maximum fruit diameter of both triploid and tetraploid plants was almost the same as that of diploid plant. Polysomaty status of fruit pericarp increased during fruit development and the maximum DNA content of the pericarp cells in the diploid, triploid and tetraploid fruits showed 64C, 96C and 128C, respectively. These results indicate that the same number of endoreduplication is programmed to occur in pepper fruits irrespective of the difference in the ploidy level of plants.

1. Introduction

The presence of cells of various ploidy levels in the one and the same organ during differentiation of plant tissues is called polysomaty (Smulders et al., 1994). Polysomaty has been found in tissues and organs of various species in angiosperms (D'Amato, 1984). Somatic polyploidization is mainly caused by endoreduplication, which undergoes several rounds of DNA replication without mitosis. DNA endoreduplication is regarded as an evolutionary strategy for achieving the high nuclear DNA content of cell (Nagl, 1976). Besides, endoreduplication usually induces larger cell, and consequently, the plants grow larger than their low ploidy counterparts (Kondorosi et al., 2000; Mizukami, 2001). Increase of tissue and organ size with increase of cell size by endoreduplication may be advantageous in accumulation of secondary metabolites.

The fruits of *Capsicum* cultivars have the wide phenotypic variation in shape, size and color (Bosland and Votava, 2000). The yellow, orange and red color of *Capsicum* fruit originate from carotenoid pigments produced during ripening. The red chilli and paprika carotenoids are used by the food industry as natural red colorants, also have immense nutritional value as provitamin A and antioxidants. Improvement of *Capsicum* for carotenoid and nutrient content is the goal of several breeding programs (Wall et al., 2001). Another goal of *Capsicum* breeding is the modification of fruit morphology, which consists of various components such as length, diameter, fresh weight and shape of fruit (Bosland and Votava, 2000). Although some loci of these factors are analyzed in QTLs (Rao et al., 2003), there remain some more factors affecting the morphology to be investigated.

As one of the factors affecting the size, polysomaty is reported in tomato (Bergervoet et al., 1996), which belongs to the same Solanaceae family as *Capsicum*. Polysomaty has also been recognized in fruit pericarp tissues in *Capsicum* (Ogawa et al., 2010b), in which all the 12 genotypes examined were classified into four groups according to the difference in the maximum DNA content, i.e., 32C, 64C, 128C and 256C. In this study, moreover, high correlation was observed between the maximum DNA content in cells of pericarp and pericarp thickness. The stability of maximum DNA content in each genotype was also confirmed throughout the cultivation in different seasons. These results suggested that the maximum DNA content and the number of endoreduplication cycle (or the degree of polysomaty) in pericarp tissue were genetically controlled in pepper. Therefore, it is interesting to know whether both maximum DNA content of pericarp cells and thickness of pericarp might be affected by polyploidization in each genotype.

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doi:10.1016/j.scienta.2011.11.015

In the previous study, we established the method for obtaining tetraploid pepper by colchicine treatment of the seeds to evaluate the possible merits of tetraploid pepper in fruit production and nutritional aspects (Ishikawa et al., 1997). By using this method, tetraploid lines of various types of pepper, for example, bell type 'Chigusa', hot pepper type 'Jalapeño', and small bell type 'Shishitou' were obtained. In the present paper, we report the successful production of triploid lines of 'Shishitou' by utilizing its tetraploid lines and compared the degree of polysomaty of fruit pericarps from initial stage to mature stage among the three different ploidy peppers (diploid, triploid and tetraploid) in one particular genotype.

2. Materials and methods

2.1. Plant materials

Capsicum annuum 'Shishitou No. 562', an inbred line established by Japan Horticultural Production and Research Institute was used in this study. Tetraploid Shishitou plant was induced from this inbred line by colchicine treatment (1.0%) of the seeds according to the method described by previous paper (Ishikawa et al., 1997) and grown in a greenhouse to harvest the seeds of tetraploids. Triploid seeds were produced by the cross between diploid and tetraploid plants. The triploid and tetraploid seeds thus obtained were sown with the diploid seeds in the tray and the seedlings were transplanted once to the pots. They were finally planted to the soil in the greenhouse with the temperature of maximum at 30 °C and minimum at 18 °C. Consequently, five diploid, two triploid and four tetraploid plants were used for the measurement of fruit size and polysomaty status.

2.2. Measurement of fruit size

Flower buds 1 day before anthesis were tagged in all the diploid, triploid and tetraploid plants. Then 1, 2, 3, 4, 6, 8 and 10 weeks after anthesis, 5 fruits were arbitrarily taken from the diploid plants, and fruit sizes (fruit length and diameter) were measured. In triploid and tetraploid plants, fruit size was measured on 3–6, and 9 fruits arbitrarily taken from the plants, respectively.

Fruit sizes, i.e., fruit length and diameter of each ploidy plants were measured in millimeters as the length from the highest part of the shoulder to the bottom and that at the widest part of the fruit, respectively. All the fruits used for measuring the fruit sizes, were then subjected to flow cytometric analysis.

2.3. Flow cytometric analysis of pericarp tissue in pepper fruit

Polysomaty status of pericarp tissue in pepper fruit was analyzed by flow cytometry (PA-II, Partec, Münster, Germany) according to our previous report (Ogawa et al., 2010b). One piece (0.5 cm × 0.5 cm) of fresh pericarp tissue was taken from the middle height of the fruit and put on a plastic Petri dish, and chopped with a razor blade in 0.25 ml of solution A of plant high-resolution DNA kit type P (Partec, Münster, Germany). Then 1.0 ml of staining solution B consisting of 10 mM Tris, 50 mM sodium citrate, 2 mM $MgCl_2$, 1% (w/v) PVP, 0.1% (v/v) Triton X-100 and 2.0 mg l^{-1} 4′,6-diamidino-2-phenylindole (DAPI), pH 7.5 (Mishiba and Mii, 2000) was added to the crude suspension and filtered through a 30 μm nylon mesh. After staining by solution B, the suspension of nuclei was subjected to flow cytometric analysis for determining the relative nuclear DNA content on a semilogarithmic scale histogram. At least 5000 nuclei were counted for each sample. The percentage of nuclei with each ploidy level in each sample was expressed as the histogram. The height of all the ploidy level was measured, and the frequency of each ploidy level was calculated from the height of the peaks of the histograms. To determine the standard peak position of 2C nuclei, the 2C peak of young leaves was analyzed.

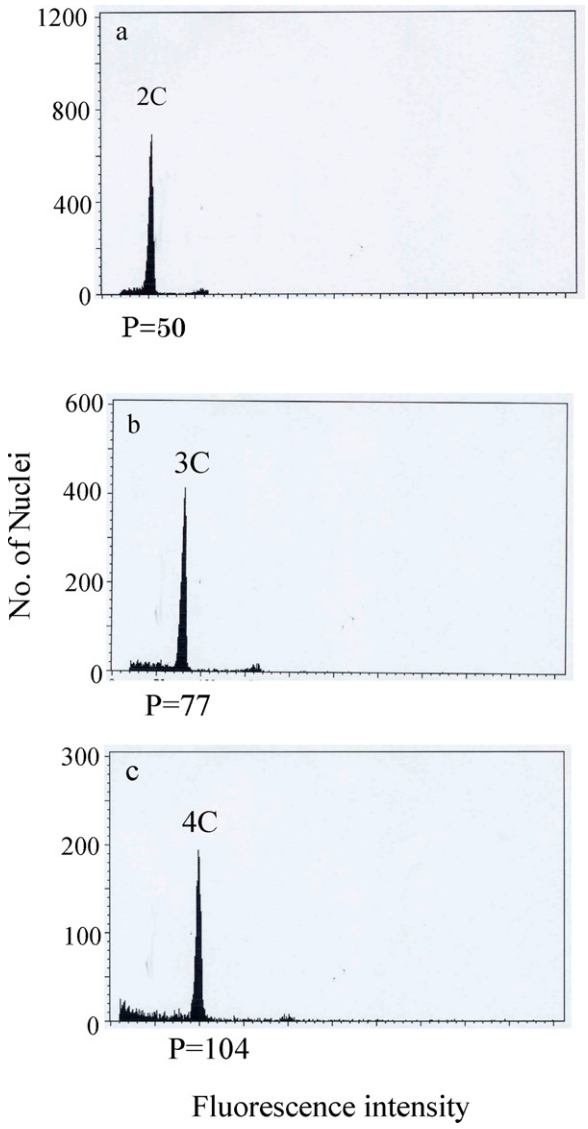

Fig. 1. Difference in nuclear DNA content in leaves between diploid pepper (*Capsicum annuum* L.) cultivar 'Shishitou No. 562' and its artificially induced triploid and tetraploid shown as histograms of flow cytometric analysis. (a) Diploid, (b) triploid and (c) tetraploid. P, peak position; i.e. channel with the most numerous numbers of the counts in a peak.

3. Results and discussion

3.1. The ploidy level of triploid plant

In order to examine the ploidy levels of triploid plants produced by the cross between diploid and tetraploid plants, the leaves of their plants were analyzed by flow cytometry. Diploid leaf samples were used as control to set peak position corresponding to 2C at channel 50 (Fig. 1a), and the fluorescence intensity (relative DNA content per nucleus) of putative triploid and tetraploid leaf samples were analyzed by comparing with the diploid leaf samples. Since putative triploid and tetraploid plants had the peak corresponding 3C and 4C, respectively (Fig. 1b and c), we identified these plants as triploid and tetraploid plants.