LIFE SCIENCES

Khulna University Studies 2(1): 119-122

STUDY ON IDENTIFICATION OF PLOIDY LEVEL OF REGENERATED PLANTS OBTAINED FROM CULTURED WHEAT ANTHERS USING LENGTH OF GUARD CELLS

Md. Monirul Islam[a*], Mahtalat Ahmed[a], G.I. Ivanov[b] and P. Gain[a]
[a] *Discipline of Agrotechnology, Khulna University, Khulna 9208, Bangladesh*
[b] *Department of Biotechnology, Kuban State Agrarian University, Krasnodar, Russia*

Manuscript received: August 28, 2000; Accepted: March 13, 2001

Abstract: A fast and reliable method for ploidy determination of regenerated *Triticum aestivum* from anther culture is described in the present work. The anthers of 47 hybrid combinations of winter wheat were cultured in vitro and the length of the guard cells was measured in regenerated plants. Thus, plants with a guard cell length of 32-46 µm were haploid, whilst plants with a guard cell length of 54-74 µm were diploid. Plants with a guard cell of 46-54 µm required further chromosome investigation for the exact determination of ploidy level.

Key words: Guard cell; Ploidy level; Diploid; Haploid; Chromosome number

Introduction

Determination of ploidy in regenerated plants from wheat anther culture is one of the major problems of developing the technology for mass production of homozygous lines of wheat. Traditionally, ploidy level is determined using cytological method by counting the chromosome number in the cells of root tips (Waninge, 1965). But this method is costly, laborious, time consuming and tedious. The present experiment was designed on the basis of well established fact of the morphological and anatomical differences between haploid and diploid plants (Karpechenko, 1935) and using this fact, length of the guard cells were measured for the determination of ploidy level in regenerated plants obtained through cultured wheat anthers. The objective of the present work was to develop an express-method for easy identification of ploidy level of regenerated plants without counting the chromosome number.

Materials and Methods

The present experiment was conducted in 1994 at the laboratory of Cell Engineering and Biotechnology of Lukyanenko Agricultural Research Institute, Krasnodar, Russia. Eight

* Corresponding author. Tel.: 88-(041)-721791, 720171-3; Fax: 880-41-731244; E-mail: <sls@ku.khulnanet.net>
DOI: https://doi.org/10.53808/KUS.2000.2.1.119-122-Ls

hundred twenty regenerated green plants obtained from the wheat anthers of 47 hybrid combinations were used as experimental materials. For the identification of haploid and diploid (produced by spontaneous doubling of chromosomes) plants, length of the guard cells of green regenerated plants was measured. For this purpose a small piece of leaf was dissected out from the middle portion of the second leaf and observed under microscope. From each plant, length of 25 guard cells was measured by the scale of microscope and at the same time the chromosome number of the cells of root tips were counted from the same plants. Potato-II (Chuang *et.al*., 1978) liquid medium and MS medium (Murashige and Skoog, 1962), both supplemented with 2, 4 D- 0.75 mgl^{-1}; ∝ NAA- 0.1 mgl^{-1}; kinetin- 0.75mgl^{-1} were used for callus induction and plant regeneration subsequently.

Results and Discussion

Ninety haploid (n=21) and 82 diploid plants (2n=42) were identified as a result of length measurement of the guard cells and simultaneously counting the number of chromosomes in the cells of root tips.

Table 1. Determination of ploidy of regenerated plants by the measurement of length frequency of guard cells and simultaneously counting of the chromosome numbers.

Class size (µm)	Frequency of haploid plants (n=21)	Frequency of diploid plants (2n=42)
32	00	
34	00	
36	01	
38	04	
40	15	
42	24	
44	16	
46	12	00
48	12	01
50	04	02
52	02	01
54	00	04
56		04
58		12
60		22
62		13
64		07
66		09
68		04
70		02
72		01
74		00

Table 1 shows that the length of the guard cells in haploid plants varies from 36 µm to 50 µm and in diploid plants from 50 µm to 72 µm respectively. It was observed that some plants with length of the guard cells of 48 to 52 µm were identified both as haploid and diploid and these plants are termed as transgressive plant.

In the present experiment, in 649 regenerated plants only length of the guard cells were measured, which ranges from 34 to 76 µm and were distributed in haploid and diploid according to Table 1. Chromosome number was counted in those plants whose length of the guard cells varies from 46 to 54 µm for the exact determination of the ploidy level (Table 2).

Table 2. Frequency distribution of regenerated plants in haploid and diploid according to the length of guard cells.

Class size (µm)	Frequency of haploid plants	Frequency of diploid plants	Frequency of transgresive plants
32	00		
34	02		
36	02		
38	07		
40	13		
42	14		
44	23		
46	19	00	19
48	18	17	35
50	16	32	48
52	06	29	35
54	02	27	29
56		34	
58		47	
60		54	
62		56	
64		47	
66		42	
68		51	
70		32	
72		19	
74		22	
76		18	
78		00	
Total	122	527	166

It is apparent from the table 2 that the regenerated plants with length of guard cells of 32-46 µm were found as haploid, 46-54 µm as mixture of haploid and diploid and 54-74 µm as diploid.

Chase (1964) and Zaitsev (1968) individually worked on maize and stated that haploid and diploid plants of maize could be determined using length of guard cells. Similar kind of work in wheat was also reported by Daophen *et al.* (1991). Du-LP *et al.* (1996) informed that regenerated plants of spring wheat with a guard cell length of 61 µm or less were found as haploid, whilst plants with a guard cell length of 64 µm or more were mostly diploid. And plants with guard cells of 61-64 µm required further chromosome investigation to determine ploidy levels. This result has little dissimilarities but not contrary to the present investigation. This dissimilarity may be due to use of different amount of samples. In the present experiment length of the guard cell was measured in 821 plants that belonged to 47 hybrid combinations, while Du-LP *et al.* (1996) used much smaller amount of plants belonging only to 16 wheat varieties.

Conclusion

The present research work has shown that regenerated wheat plant through in vitro anther culture with the guard cells length of 32-46 µm are haploid and 54-74 µm are diploid. Plantlets with guard cells of 46-54 µm required further chromosome investigation for exact determination of ploidy level, because regenerated plants belonging to this class were identified both as haploid and diploid.

References

Chase, S.S., 1964. Monoploids and diploids of maize: a comparison of genotypic equivalents. *American Journal of Botany*, 51: 924-933.

Chuang, C.C., Ouyang, T.W., Chia, H., Chou, S.M. and Ching, C.K., 1978. A set of potato media for wheat anther culture. Proceedings of Symposium on Plant Tissue Culture, Science Press, Peking, 51-56.

Daophen, X., Chunkhon, L. and Dhanpen, L., 1991. Length of the guard cells as indicator of ploidy of wheat plants, obtained through anther culture. TAA Bulletin, Tashkent, 3: 53-57.

Du-LP., Xu, H.J., Zhao, L.L., Zhang, C.X. and Ren, X., 1996. Identification of the ploidy of wheat pollen plants using the leangth of guard cells. *Ningxia Journal of Agriculture and Forestry Science and Technology*, 5: 10-12.

Karpechenko, P.O., 1935. Experimental methods for obtaining polyploids and haploids. In: *Fundamentals of Plant Breeding*. Moscow, pp. 197-434.

Murashige, T. and Skoog, F., 1962. A revised medium for rapid growth and bioassay tobacco tissue culture, *Plant Physiology*, 15: 473-497.

Waninge, J., 1965. A modified method of counting chromosomes in root tip cells of wheat. *Euphytica*, 14: 249-250.

Zaitsev, M.E., 1968. Determination of hoploid plants in maize by using anatomical elements. In: *Apomixis and Cytoembryology of Plants*. Saratov, pp. 142-149.