World Applied Sciences Journal 10 (2): 143-146, 2010
ISSN 1818-4952

Genetic Study on Colchicine Induction of Parthenogenesis in Maize

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Abstract: Silks of two maize crosses 5003×Qi310 and 478×5011 were treated with colchicine for cytogenetics and agronomic traits analysis of parthenogenetic offsprings at Shenyang Agricultural University (China) during 2007-10. Among the 1814 root-tips from 60 Pa_1 plants, diploid cells were the majority, accounting for 65.7%, secondly aneuploids for 29.5%, haploids and heteroploids for 4.8%. In the field experiment, the major agronomic traits of Pa_1 plants from the same cross had significant differences. The differences between the agronomic traits of Pa_1 and Pa_2 offsprings showed that the higher generations of parthenogenetic offsprings, the more degradation in agronomic traits after comparing Pa_1 plants and Pa_2 lines.

Key words: Agronomic trait · Maize · Cytogenetics · Parthenogesis · Genetic stability

INTRODUCTION

In plants, parthenogenesis means development of embryos from egg cells without being fertilized by pollen, the male part of the plant, which results in offspring that are genetically identical to the mother plant. Parthenogenetic haploid plants were reported in melon [1], mandarin [2], Poaceae [3], wheat [4] and maize [5-7].

Maize (*Zea mays* L.) is a typical diploid plant (2n = 20), haploid individuals (n = 10) occur naturally at a low rate [8]. Although the rate of haploids was still low and depended on the genotypes, 1-2% of maize haploids were obtained when the line Stock 6 was used as pollinator [9]. At present, maize haploids can be derived either through *in vitro* (tissue culture technique) or through *in vivo* (genetic induction). Since introduction *in vitro* can not provide a perfect condition of embryogeny in parent plant, maize haploid inducing *in vivo* is widely used in the maize improvement. As one of the basic methods inducing haploids *in vivo*, chemical induction were successfully used to obtain haploids in rice [10], wheat [4] and maize [9].

For the mechanism of haploid-inducing capacity in maize, several hypotheses have been put forward to explain the haploid formation through *in vivo*. Chalyk *et al.* [11] suggested that the abnormality in microsporogenesis and fertilization might be the reasons of haploid induction. Coe [12] also supported the irregularities during microsporogenesis and fertilization. There were also reports that chromosome elimination after fertilization might be the major mechanism in maize *in vivo* haploid induction [13, 14].

The purpose of this study was to determine the effectiveness of chemical induction in obtaining haploids from genotypes that are widely used in maize breeding in China. Since maize haploids can be produced by chemical induction *in vivo*, we treated non-pollinated silk of two F_1 of maize crosses using colchicine to determine its efficiency in obtaining haploids from genotypes and study them more extensively from agronomic traits and the cytological point of view.

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MATERIALS AND METHODS

Plant Materials: Maize crosses 5003×Qi310 and 478×5011 were obtained from Maize Science Institute, Liaoning Academy of Agricultural Sciences, Shenyang (110161), China.

Obtaining Maize Haploids (n = 10) and Their Selfing Progenies: Field experiments were conducted in experimental station in Maize Science Institute, Liaoning Academy of Agricultural Sciences, Shenyang, China. In 2007 spring, two F_1 of maize crosses 5003×Qi310 and 478×5011 were planted in the field. Tassels were removed from all F_1 plants before anthesis and the ear shoots were bagged to prevent contamination. After silks reached 3 to 5 cm in length, they were trimmed to 1 to 2 cm and injected 2 ml colchicine using a sentry needle. In the end of October 2007, Seeds of maize haploids (Pa_0 generation) from the treated ears of F_1 plants of two maize crosses were harvested, replanted and selfed in greenhouse in the same year for seeds of Pa_1 generation. In 2008 spring, seeds of Pa_1 generation of two maize crosses were planted and selfed again for seeds of Pa_2 generation in the field.

Observation of Chromosome Number: For the study of somatic chromosomes, the root tips of P_0 generation were collected and immediately pretreated in water at 4°C over 24 h, fixed in the mixture of 3 ethanol:1 acetic acid for 2~3 h at room temperature, dissociated for 20 min in 1N HCl at 25°C, washed 1~2, dyed 24 h in 2.0% acetic acid-orcein solution, squashed, examined under microscope (Olympus BX41-32H02-FLB3) and photographed.

Investigation of Agronomic Traits: In 2009 spring, seeds of Pa_1 and Pa_2 generations were planted in the field for the investigation of agronomic traits. Pa_1 seeds were space-planted about eight cm apart for facilitating note-taking of individual plants. For the Pa_2 RILs generation, about 20 seeds from each line were planted in a 1.5 m row with 20 cm apart between rows. Standard practices for fertilization and weed control common to the region were used for feld management. Agronomic traits including plant height, ear height, ear length, grain weight per spike and 100-grain weight were recorded.

RESULTS

The Effect of Colchicine on Induction of Maize Haploids: For crosses 5003×Qi310 and 478×5011, 200 spikes of each cross were treated with colchicine solution, 99 and 113 spikes were harvested later, respectively. The percentage of obtaining haploids were 0.21 and 0.35, respectively (Table 1).

Mitosis behavior of Pa_1 plants: Total of 1814 root-tips from 60 Pa_1 plants were squashed for microscopic examination (Table 2). Cytogenetic analysis revealed a high frequency of mixed ploids occurring in Pa_1 plants induced by colchicine.The majority of the observed cells within the same root-tip was diploid cells with chromosome number 2n=20, accounting for 65.7% (Fig.1A). Haploids (n=10) accounted for 4.2%, triploids (3n=30) and tetraploids (4n=40) were 4.2% and 0.6%, respectively. The aneuploids accounted for 29.5% (Fig.1B-C). Most of the aneuploids had 11~19 chromosomes. The chromosome numbers of 1~9, 21~29, 31~39 were rare observed.

Table 1: The effect of colchicine on induction of maize haploids

			Seeds	
Cross	No. of treated spike	No. of spikes with seeds	Total	Haploid, %
5003×Qi310	200	99	158	0.21
478×5011	200	113	255	0.35
Total	400	212	413	--
Average	--	--	--	--

Table 2: Somatic cell chromosomes in mitosis metaphase of Pa_1 plants

			Ploid			Aneuploid			
Cross	No. of observed Pa_1 plants	No. of observed cells	10%	20%	30~40%	10%	11-19%	21-29%	31-39%
5003/Qi310	30	1159	55	761	6	24	293	17	3
			4.7	65.7	0.5	2.1	25.3	1.5	0.3
478/5011	30	655	21	430	5	22	158	15	4
			3.2	65.6	0.8	3.4	24.1	2.3	0.6
Total	60	1814	76	1191	11	46	451	32	7
			4.2	65.7	0.6	2.5	24.8	1.8	0.4

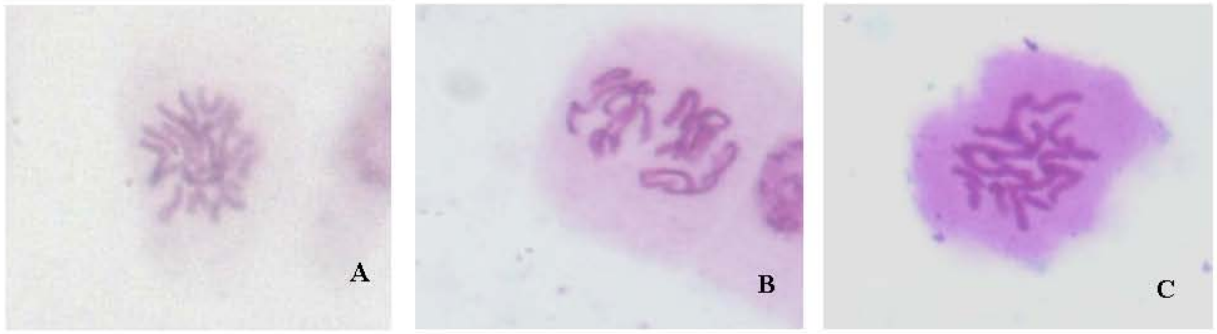

Fig. 1: The somatic cell chromosomes in mitosis metaphase of Pa_1 Plants (×120) A normal diploid cell 2n=20; B~C aneuploid cells

Table 3: Effect of colchicine treatment on agronomic traits of Pa_1 and Pa_2 generations

Parent and cross	Mumber of sample	plant height cm		ear height cm		ear length cm		grain weight per spike g		100-grain weight g	
		Mean	St	Mean	St	Mean	St	Mean	St	Mean	St
5003	13	230.6	30.51	83.9	13.00	18.4	3.70	153.4	50.30	33.8	4.20
Qi310	13	187.5	23.43	67.7	16.08	16.4	4.71	134.9	38.80	29.1	4.90
5003/Qi310	Pa_1 21	223.3	30.51	102.4	13.10	17.3	2.63	151.1	59.59	28.4	3.93
	Pa_2 21	195.2	18.30	76.9	8.29	15.0	1.75	110.4	23.89	25.9	4.30
478	20	212.7	21.10	95.3	15.00	18.1	3.07	130.5	51.80	31.5	4.30
5011	20	178.4	16.89	78.3	13.06	16.1	4.36	142.9	27.53	27.6	15.28
478/5011	Pa_1 11	228.6	23.46	96.6	16.90	17.8	3.12	121.8	50.63	30.3	3.68
	Pa_2 11	195.2	16.27	83.3	7.28	15.5	2.28	95.7	28.89	27.0	6.10

Effect of colchicine treatment on agronomic traits of Pa_1 and Pa_2 generations: Agronomic traits in progenies of Pa_1 and Pa_2 generations of crosses 5003/Qi310 and 478/5011 were listed in Table 3. Most of the Pa_1 plants grew normally in the field, except few deformed plants, plants without ears or tassels and plants with disproportionality of ears to tassels. Among the 32 Pa_1 plants, there were significant differences between different types of agronomic traits within the same cross (Table 3). All the agronomic traits of Pa_2 were little bit lower than Pa_1, suggested that the parthenogenetic offsprings gradually stable and degradating after selfing.

DISCUSSIONS

The methods of haploid induction in maize have been developed 60 years. Genetic induction can produce high percentages of maize haploids when certain gene stocks or unique genes were used as inducers, such as Stock 6 [9] and *igl* (interminate gametophyte) gene [15]. Certain chemicals have induced the haploid formation, such as maleic hydrazide (MH), 2,4-D, NAA-Na, GA3, IAA, colchicine [16, 17]. The mechanism for haploid induction is not still fully understood until now. Röber *et al.* [14] reported that chromosome elimination may be involved in the haploid production. Chaganti [18] found that various types of chromosome in haploid maize showed homology associations in metanaphase I. In the present study, chromosome disorder was found in the root-tip cells from Pa_1 plants. Different root-tip cells in the same plant carried different ploid chromosomes or different numbers of chromosomes. These results provide further evidences that Pa_1 progenies from maize hapoids induced with colchicine showed differences in major agronomic traits. Lashermes and Beckert [19] conducted analysis of agronomic traits and ELIASA and lead to the similar conclusion. Although the unclear mechanism of haploid-inducing capacity in maize, haploid plants are a perfect tool for the rapid production of homozygous lines and at the same time they can be used for an efficient improvement of quantitative traits important for breeding.

ACKNOWLEDGEMENTS

This project was supported by China Postdoctoral Science Foundation (grant no. 20080430187), Liaoning

Agricultural Science and Technology Research (grant no. 2008208001), Shenyang Agricultural Science and Technology Research (grant no. 1081191-3-00) and the project of Liaoning Provincial Department of Education (2009.1-2011.12).

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