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Full Length Research Paper

Determination of chromosomal ploidy in *Agave* ssp.

Lingling Lv[1,2,3], Guangming Sun[1], Jianghui Xie[1], Xiaoping Zang[1], Yulin Hu[1] and Jun Duan[2]*

[1]South Subtropical Crops Research Institute, Chinese Academy of Tropical Agricultural Sciences. Zhanjiang 524091, China.
[2]South China Botanical Garden, Chinese Academy of Sciences. Guangzhou, 510650, China.
[3]Graduate School, Chinese Academy of Science, Beijing 100039, China.

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Chromosome observation is necessary to elucidate the structure, function and organization of *Agave* plants' genes and genomes. However, few researches about chromosome observation of *Agave* ssp. were done, not only because their chromosome numbers are large, but also because their ploidies are complicated. The root tips of 19 *Agave* ssp. germplasms were used as materials for determining their chromosomal ploidies. Through normal pre-treatment, fixation, digesting and Giemsa staining, the glass slides with expelled cells on them were obtained. Observed with a light microscope, the results showed that 10 germplasms are diploids, including 4 wild species and a local variety which are good parents for cross-breeding. The main cultivar in China *A.hybrid* cv NO 11648 is also a diploid. *A. cantala* Roxb used as parent for disease-resistant breeding is a triploid. *A. hybrid* cv nanya NO.1 and *A. hybrid* cv nanya NO.2 are tetraploids. The other germplasms belong to polyploids. Although three germplasms' ploidies were reported before, the other 16 germplasms' were first reported in this paper. These results will provide theoretical basis for cross-breeding.

Key words: Chromosomal Ploidy, *Agave* ssp., germplasm, polyploidy.

INTRODUCTION

Agave ssp. is the most important fiber crop in tropical and subtropical areas. It is also a dominant crop with potential export capacity in China. *Agave* ssp. was brought to China in 1970s and now over 60 *Agave* germplasms are conserved in South Subtropical Crops Research Institute, Chinese Academy of Tropical Agricultural Sciences. The germplasms have different morphologies and their chromosomal ploidies are very complicated. There are diploid, triploid, tetraploid and pentaploid in different germplasms or the same germplasm. The base chromosome number of *Agave* ssp. appears to be 30.

Moreno-Salazar et al. (2007) and Guadalupe et al. (2008) analyzed the karyotypes of several agave cultivars. Doughty (1936) reported the chromosomal ploidies of three varieties. However, little is known about the chromosome numbers of wild and local breeding varieties, especially in China. So it is necessary to determine their ploidies.

Due to the polyploids of the *Agave* germplasms, it is possible for a gametophyte to have different chromosome numbers which leads to different heredities. The fertility of cross-offspring is directly related to the chromosomal ploidies of parents. As the chromosomal ploidy of female parent is even, there are many seeds. In contrast, as the chromosomal ploidy of female parent is odd, there are no seeds (Institute of Bast Fiber Crops, Chinese Academy of Sciences, 1993).

Chromosome observation has been used in many plants to identify chromosomal ploidy (Nathewet et al., 2007; Kazuo and Maltide, 1993; Nankui and Paul, 2003; Li et al., 2008; Ramon and Manuel, 2004; Brutovska et al., 2000; Agnieszka et al., 2006; Kazuo et al., 2000). In this paper, 19 germplasms in *Agave* ssp. (Table 1; Guo, 2006) were analyzed to identify their chromosomal ploidies. Knowledge of the chromosomal ploidies of germplasms will provide theoretical basis for cross-breeding.

*Corresponding author. E-mail: lianzi9381@yahoo.com.cn. Tel: (+86)13922727215.

Table 1. Material and its origin and type.

Scientific name of germplasm	Germplasm origin	Germplasm type
A. attenuata var.	Central America	Wild species
A. attenuata	Central America	Wild species
A. angustifolia Haw	Mexico	Wild species
A. angustifolia Haw.var.marginata Frel.	Mexico	Wild species
A. sisalana Perrine var.Yuexi	China	Cultivar
A. hybrid cv No.487	Eastern Africa	Cultivar
A. americana.L	Central America	Wild species
A. fourcroydes Lem.	Mexico	Wild species
A. hybrid cv nanya NO.1	China	Cultivar
A. hybrid cv nanya NO.2	China	Cultivar
A. hybrid cv Yuexi No.114	China	Cultivar
A. hybrid cv Yuexi No.75	China	Cultivar
A. hybrid cv Dongfang hong No.16	China	Cultivar
A. cantala Roxb.	Eastern India	Wild species
A. hybrid cv Yuexi No.117	China	Cultivar
A. hybrid cv Dongfang hong No.292	China	Cultivar
A. Potalorum Var.a.h cv Dongfang hong No.109	China	Cultivar
A. potatorum Zucc.var.verschaffeltii Bgr.	Mexico	Cultivar
A. hybrid cv No.11648	Eastern Africa	Cultivar

MATERIALS AND METHODS

Plant materials

Chromosome observation was carried out in cells of the root tips. These plants (10 to 20 cm height) were cultured in water in a greenhouse at the South Subtropical Crops Research Institute, zhanjiang, guangdong, China. The culture water was replaced every 3 days.

Chromosome preparation and staining

Pre-treatment and fixation

Both pre-treatment and fixation were carried out using the respective methods reported by Iwatsubo and Naruhashi (1991). The root tips were collected from young plants in the morning (around 9:00 a.m.), pre-treated with 0.002 mol/L 8-hydroxyquinoline solution for one to two hours at room temperature. After short-rinsed in distilled water, the roots were fixed in a 3:1 methanol and acetic acid mixture solution for 24 h at room temperature. Finally, they were preserved in a 70% ethanol solution at -20°C.

Digesting and Giemsa staining

The fixed root tips were short-rinsed in distilled water and subsequently kept in distilled water for 10 min at room temperature. Then root tips were cut to around 1 mm long and digested using the enzyme mixture of 3% cellulase (BBI) and 0.1% pectinase (Worthington Biotechmical Corporation) solution at room temperature for 12 min, then short-rinsed in distilled water. After that, the root tips were fixed again for over 20 min so that the chromosomes became hardened and easily been identified. After the root tips were expelled onto a clean glass slide, they were stained with 3% Giemsa solution for 15-18 min.

Chromosome observation

Chromosomes stained with Giemsa solution were viewed and counted using a light microscope (BX51, Olympus Optical Co.Ltd.) at 40× and 100× magnifications. Well-spread chromosomes at the metaphase stage were selected and photographed using a digital camera (A80; Canon Co.Ltd.). For each variety, the chromosomes of 30 cells were observed.

RESULTS

The chromosomal ploidy of germplasm

Among these germplasms, 10 germplasms are diploids (Figure 1A), including 4 wild germplasms and a local variety which are good parents for cross-breeding. The main cultivar in China *A. hybrid* cv No 11648 is also a diploid. '*A. cantala* Roxb.' is a triploid (Figure 1B). Tetraploids include '*A. hybrid* cv nanya NO.1' and '*A. hybrid* cv nanya NO.2' (Figure 1C). The other 6 varieties belong to polyploids (Table 2).

The form of polyploid

In this paper, 6 varieties are polyploids including 5 forms. The first form includes diploid and pentaploid (*A. fourcroydes* Lem.); the proportion of diploid is 70%. The second form includes diploid, triploid, tetraploid and pentaploid (*A. sisalana* Perrine var.Yuexi); the proportion of diploid is 57%, followed by triploid 13%, tetraploid 15% and pentaploid 15%. The third form includes triploid and

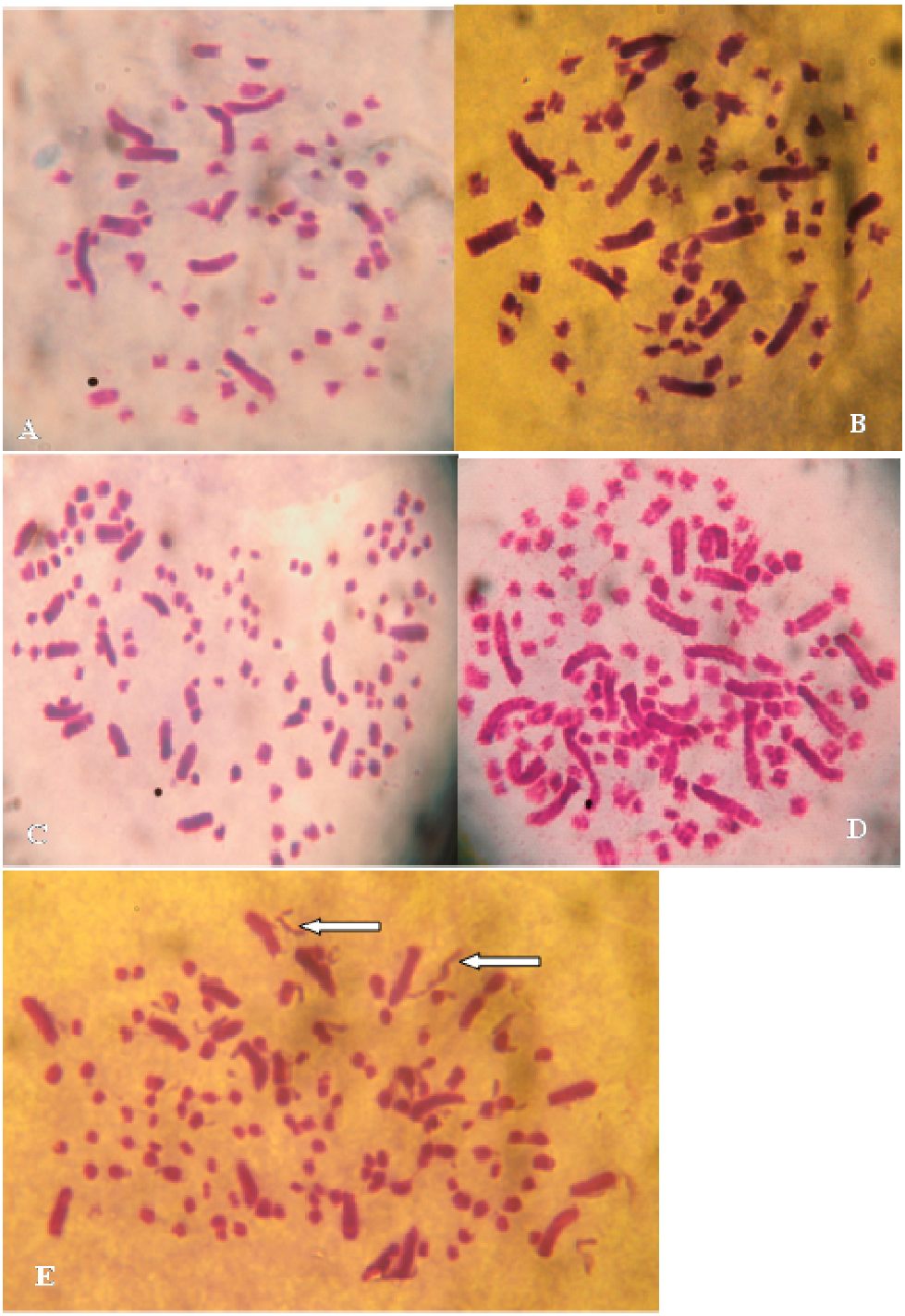

Figure 1. Chromosomes in root tips' cells of agave plants stained with Giemsa. **A.** Metaphase chromosomes in the root tips' cells of *A. angustifolia* Haw (2n = 60). **B.** Metaphase chromosomes in the root tips' cells of *A. cantala* Roxb. (3n = 89). **C.** Metaphase chromosomes in the root tips' cells of *A. hybrid* cv nanya NO.1 (4n = 120). **D.** Metaphase chromosomes in the root tips' cells of *A. sisalana* Perrine var.Yuexi (5n = 137). **E.** Metaphase chromosomes in the root tips' cells of *A. americana*.L (4n = 120, including 14 small chromosomes). ×4300

tetraploid (*A. hybrid* cv Yuexi No.114); the proportion of triploid is 50%. The fourth form includes tetraploid and pentaploid (*A.hybrid* cv Dongfang hong No.16); the proportion of tetraploid is 73%. The last form includes diploid, triploid and tetraploid, 2 varieties belong to this form: *A. americana*.L and *A. hybrid* cv Yuexi No.75. The

Table 2. The chromosomal number and ploidies of 19 agave germplasms.

Scientific name of germplasm	The number of chromosome	Chromosomal ploidy of germplasm
A. attenuata var.	45 - 62	2n
A. attenuata	45 - 60	2n
A. angustifolia Haw	45 - 62	2n
A. angustifolia Haw.var.marginata Frel.	45 - 60	2n
A. sisalana Perrine var.Yuexi	54 - 65; 77 - 99; 118 - 128; 137 - 151	2n, 3n, 4n, 5n
A. hybrid cv No.487	52 - 62	2n
A. americana.L	60; 81 - 104; 106 - 120	2n, 3n, 4n
A. fourcroydes Lem.	53 - 73; 144 - 158	2n, 5n
A. hybrid cv nanya NO.1	110 - 125	4n
A. hybrid cv nanya NO.2	109 - 132	4n
A. hybrid cv Yuexi No.114	79 - 104; 107 - 126	3n, 4n
A. hybrid cv Yuexi No.75	61 - 72; 76 - 104; 111 - 125	2n, 3n, 4n
A. hybrid cv Dongfang hong No.16	107 - 128; 135 - 143	4n, 5n
A. cantala Roxb.	77 - 97	3n
A. hybrid cv Yuexi No.117	48 - 64	2n
A. hybrid cv Dongfang hong No.292	50 - 64	2n
A. Potalorum Var.a.h cv Dongfang hong No.109	52 - 60	2n
A. potatorum Zucc.var.verschaffeltii Bgr.	45 - 60	2n
A. hybrid cv No.11648	46 - 60	2n

proportion of each ploidy of *A. americana*.L is 1, 50 and 49%, followed by the *A. hybrid* cv Yuexi No.75 14, 65 and 21%.

DISCUSSION

If the enzymes activities are low, more time is needed to digest the root tips, otherwise, the cytoplasm will not be digested and the chromosome cannot be seen clearly. On the other hand, if the enzymes activities are high, less time is needed, or the chromosomes can also be digested or look like floccules. In this research, the activity of cellulase is 113 U/mg, the activity of pectinase is over 30 U/mg, so the optical time of digesting is around 12 min, which is much shorter than the time (2 to 5 h) reported previously (Li et al., 2004; Xue et al., 2007; Zhang, 2005; Zhao, 2006; Lin, 2005).

Among the 19 germplasms, the two varieties '*A. americana*.L' and '*A. hybrid* cv Yuexi No.114' have large, middle and small chromosomes (Figure 1E); the other 17 agave germplasms have just large and middle chromosomes. Brandham (1969) found that there were 46 small chromosomes in *Agave stricta* SALM (2n = 60). The small chromosomes were reported in *Thuidium phitibertii* (Tian et al., 1994), Ramie (Liu and Chen, 2000) and fibre agave (Doughty, 1936) too. The function of small chromosomes is not clear.

Many varieties in *Agave* ssp. are noneuploid. When the chromosome number of a variety is between x - 15 and x + 14 (x = 2n, 3n, 4n, 5n), its ploidy is x. For example, the chromosome number of *A. attenuata* var. is between 2n - 15 and 2n + 2, so it is a diploid. Though *A. angustifolia* Haw is diploid, its chromosome number ranges from 45 to 62. It is probably because the cells' mitotic division is irregular (Doughty, 1936). Palomino et. al., (2003) observed a loss of certain DNA sequences after polyploidization.

Three germplasms' ploidies were reported by Doughty (1936) before. The ploidies of *A. cantala* Roxb. and *A.angustifolia* Haw in this paper are the same as before. However, '*A. fourcroydes* Lem.' was reported as a pentaploid (Figure 1D). In this paper, its ploidy is diploid and pentaploid. Maybe the ploidy varied through the long-term growth.

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