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## Full Length Research Paper

# Determination of chromosomal ploidy in *Agave* ssp.

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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.

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**Table 1.** Material and its origin and type.

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

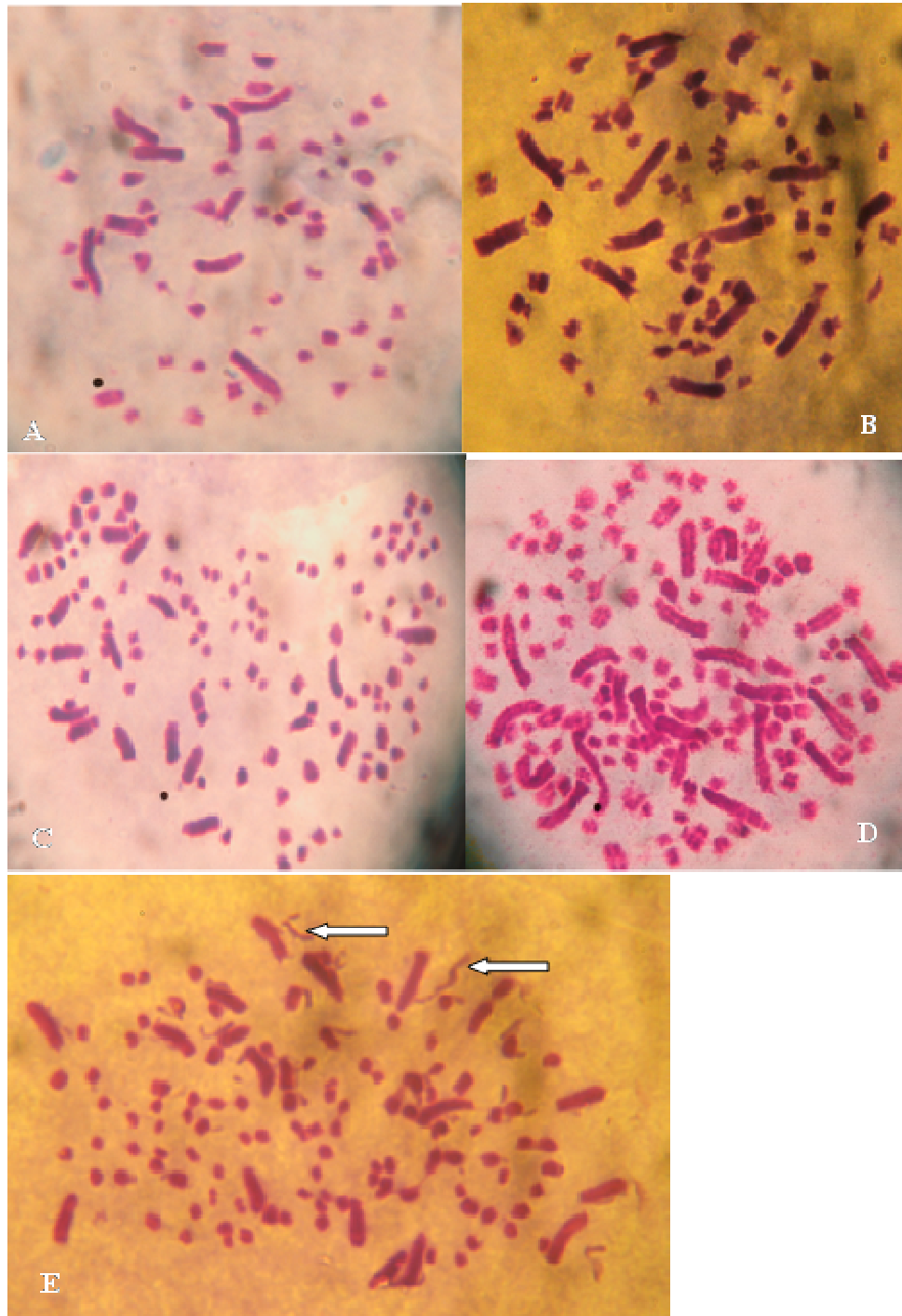
## RESULTS

### 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).  $\times 4300$

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

- Agnieszka M, Hitoshi M, Keiichi O (2006). The origin of Darwin hybrid tulips analyzed by flow cytometry, karyotype analyses and genomic *in situ* hybridization. *Euphytica*, 151: 279-290.
- Brandham PE (1969). Inversion Heterozygosity and Sub-chromatid Exchange in *Agave stricta*. *Chromosoma (Berl.)*, 26: 270-286 .

- Guadalupe P, Martinez J, Mendez I (2008). Karyotype studies in cultivars of *Agave tequilana* Weber. *Caryologia*, 61(2): 144-153.
- Guo CM (2006). Genetic diversity analysis by AFLP and identification of disease resistance for agave germplasm, Dissertation for Master's Degree. Hainan: South China University of Tropical Agriculture.
- Iwatsubo Y, Naruhashi N (1991). Karyotypes of *Fragaria nubicola* and *F.daltoliana* (*Rosaceae*). *Cytologia*, 56: 453-457.
- Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences (1993). *Cultivation Sciences of Bast Fiber Crops in China* [M]. Beijing: Agricultural Publication in China: p. 490.
- Kazuo NW, Matilde O (1993). An Alternative Pretreatment Method for Mitotic Chromosomes Observation in Potatoes. *Am. Potato J.* 70: 543-548.
- Kazuo O, Tanguy J, Hiroshi T (2000). The Karyotype Analysis of Somatic Chromosomes in *Amblystella trichopoda* (Amborellaceae). *J. Plant Res*, 113: 281-283.
- Li J, Chen SM, Chen FD (2008). Karyotype and meiotic analyses of six species in the subtribe Chrysantheminae. *Euphytica*, 164: 293-301.
- Li M, Zhao JC, Wang LB, Fan ST, Li QS (2004). Chromosome Observation of Two Species of Bryophytes from Hebei Province, China. *J. Hebei Normal University, (Natural Science Edition)* 28(3): 296-297.
- Lin XH (2005). Chromosome Location and Molecular Marker of Resistant Gene to Powdery Mildew from *Elytrigia Intermedium*. Dissertation for Doctoral Degree, Shandong: Shandong Agricultural University.
- Liu ZS, Chen RX (2000). Observation of Chromosomal G-type for Ramie. *Guizhou Agric. Sci.* 28(3): 21-22.
- Doughty LR (1936). Chromosome Behaviour in Relation to Genetic of *Agave*. I. Seven Species of Fibre *Agave*. *J. Genet.* 33(2): 198-205.
- Moreno-Salazar SF, Esqueda M, Martinez J, Palomino G (2007). Nuclear genome size and karyotype of *Agave angustifolia* and *A-rhodacantha* from Sonora, Mexico. *Revista Fitotecnia Mexicana*, 30(1): 13-23.
- Nankui T, Paul WB (2003). Observations on interspecific compatibility and meiotic chromosome behavior of *Capsicum buforum* and *C. lanceolatum*. *Genet. Res. Crop Evol.* 50: 193-199.
- Nathewet P, Tomohiro Y, Kazumi S, Shin T, Nobuyuki O (2007). Chromosome observation method at metaphase and pro-metaphase stages in diploid and octoploid strawberries. *Scientia Hort.* 114: 133-137.
- Palomino G, Dolezel J, Mendez I, Rubluo A (2003). Nuclear genome size analysis of *Agave tequilana* Weber. *Caryologia*, 56(1): 37-46.
- Ramon RC, Manuel UA (2004). Karyotype analysis of *Eucinostomus argenteus*, *E. gula*, *E. harengulus*, and *Eugerres plumieri* (Teleostei, Gerreidae) from Florida and Puerto Rico. *Environ. Biol. Fishes.* 67: 269-276.
- Brutovska R, Kusnirikova P, Bogyiova E, Cellarova E (2000). Karyotype analysis of *Hypericum perforatum* L. *Biol. Plant* 43(1):133-136.
- Tian XH, Xiao YP, Liu QH, Zhu BC, Wang ZH, Yuan SQ (1994). Observations on chromosomes of fourteen moss species from the Qinling Range, China. *Acta Phytotaxonomica Sinica*, 32(3): 240-245.
- Xue CX, Cui MW, Zhao J, Liu MJ (2007). Observation on chromosomes of European blackberry. *J. Agric. University HeBei.* 30(6): 50-53.
- Zhang AJ (2005). The Karyotype analysis of tea plant chromosomes and the preliminarily mapping of TCS gene in chromosome. Dissertation for Master's Degree. Anhui: Anhui Agricultural University.
- Zhao ZJ (2006). Tissue Culture and Karyotype Analysis of *Catha Edulis*. Dissertation for Master's Degree, Nanjing: Nanjing Agricultural University.