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Chromosome Duplication of *Brachiaria decumbens* Grass Using Colchicine

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ABSTRACT

Brachiaria decumbens is one of important tropical forage grasses. This grass has exceptional adaptation to acidic soils, vigorous growth, ease of establishment, and good forage value throughout the year, but it is susceptible to spittlebugs pest. Breeding and improvement of forage grasses can be approached through cytological and genetic investigations. These research can contribute fundamental information that can be applied to the breeding of grasses. This research was aimed to optimize chromosome duplication of tetraploid *B. decumbens* (2n=4x=36) grass using colchicine. Explant of immature inflorescences were isolated and cultured in MS medium supplemented with 4 mg/L 2,4D and 0.2 mg/k kinetin for 4 weeks. The embryogenic calli were then transferred to regeneration medium MS supplemented with 4mg/l kinetin. Basal segment of the planlets then were immersed with a range of colchicine concentration (0.01%, 0.05%, and 0.1%) for 24 and 48 hours. After that, the buds were again placed in an MS medium without colchicine for regrowth. The chromosome number were confirmed by cytological analysis of root tips. The treatment of 0.1% colchicine for 48 hours can induce ploidy increase up to 15%. There was an increase in the rate of ploidy related to the increase in colchicine concentration.

Keywords: *Brachiaria decumbens*, grass, chromosome, duplication, colchicine

INTRODUCTION

Livestock productivity is attributed to high quantity and quality of feed for the livestock. Traditionally, livestock have been a key component of farming systems in developing countries. Natural range pastures constitute the highest source of forage for ruminant livestock. One of the important grasses in tropics is *Brachiaria*. *Brachiaria* originated from Africa and then were introduced into the humid tropical regions of Latin America, Southeast Asia, and Northern Australia. *Brachiaria decumbens* Stapf cv. Basilisk grass is tolerant to drought and have rapid growth and establishment, but they are susceptible to fungal diseases and spittlebug (Valle & Savidan, 1996; Miles et al., 2004). There is an interest in development of this grass to increase biomass production, nutritional quality and resistance to pest and diseases.

Breeding and improvement of forage grasses can be approached through cytological and genetic investigations. These research can contribute fundamental information that can be applied to the improvement of grasses. Cytological and genetic studies contribute fundamental information on chromosome numbers, the nature of polyploidy, and the existence of aneuploids and chromosome series that is useful in breeding. A knowledge of chromosome numbers can be

applied in plants crossing. In this study we conducting experiment on application of colchicine in *B. decumbens* Stapf cv. Basilisk (apomictic tetraploid) in order to know the effect of colchicine in polyploidization of this grass.

MATERIALS AND METHODS

Explant of immature inflorescences were isolated and cultured in MS medium supplemented with 4 mg/L 2,4D and 0.2 mg/L kinetin for 4 weeks. The embryogenic calli were then transfer to regeneration medium MS supplemented with 4mg/L kinetin. Basal segment of the planlets then were immersed with a range of colchicine concentration (0.01%, 0.05%, and 0.1%) for 24 and 48 hours. After that, the buds were again placed in an MS medium without colchicine for regrowth. The chromosome number were confirmed by cytological analysis of root tips.

The number of chromosomes was determined by evaluation of meristematic cells obtained from root tips pre-treated with water cooled for 24 h, and fixed in Carnoy (ethyl alcohol:acetic acid, 3:1). The slides were prepared by the squash technique and stained with Giemsa 3% for 5 minutes.

RESULTS AND DISCUSSION

Table 1. Percentage of survival and of hexaploid plants of *B. decumbens* in treatments with colchicine for inducing chromosome duplication

Treatment	Time (h)	Survival (%)	Hexaploids (%)
0.01% colchicine	24	25	5
0.05% colchicine	24	30	7
0.1% colchicine	24	30	8
0.01% colchicine	48	17	12
0.05% colchicine	48	20	14
0.1% colchicine	48	19	15

Effectiveness of polyploidization in this study was relatively high. The treatment of 0.1% colchicine for 48 hours can induce ploidy increase up to 15%. There was an increase in the rate of ploidy related to the increase in colchicine concentration.

The survival percentage of plants ranged from 17% (colchicine 0.01% for 48h) to 30% (colchicine 0.05 and 0.1 % for 24h). The percentage of hexaploids obtained among the surviving plants was 7 % to 15%. Of these, the treatment that provided the best results used colchicine 0.1% for 24h, and allowed 15% regeneration of plants with a duplicated chromosome number. In previous studies in induction of chromosomal duplication of *Brachiaria* using colchicine, the time of exposure to colchicine used was 48 h (Simioni and Valle, 2009) and 2 and 3 h (Ishigaki *et al*., 2009, Timbó *et al*., 2014). Percentage of polyploidization using different materials showed variation of polyploid plants obtained. Simioni and Valle (2009) used meristem culture and only 3.9% of the regenerated and assessed plants resulted in tetraploids. Higher number of successful plant was obtained

using seedlings material, namely 31.3% (Ishigaki *et al*., 2009) and 12.5% (Timbó *et al*., 2014).

CONCLUSIONS

Colchicine treatment can induce increase of ploidy number in *Brachiaria*. The treatment of 0.1% colchicine for 48 hours can induce ploidy increase up to 15%. There was an increase in the rate of ploidy related to the increase in colchicine concentration.

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