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Some Morphological Characters and the Reproductive Method of Diploid and Tetraploid Varieties in Rhodes Grass, *Chloris gayana* Kunth

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Introduction

Rhodes grass, *Chloris gayana* Kunth, is a ley grass introduced into cultivation in South Africa[4]. It is now becoming one of the promising grasses for green fooder and hay in Japan[11]. As its breending is now under way in our country, many vague points have been yet left to be clarified.

In spite of the fact that Rhodes grass contains diploid (2n=20) and tetraploid (2n=40), the morphological differences between them, such as the characteristics available for the discrimination of the ploidy of the plants, have been yet scarcely known, though such physiological difference as the diploid is a neutral day plant, and tetraploid a short day plant, has been pointed out up to now[12].

Furthermore, whether the reproductive method of this grass is allogamy or apogamy, is the fundamental problem to be solved[11].

This experiment was designed to get the informations about the morphological difference between a diploid variety and a tetraploid one, and those about their reproductive methods.

Materials and Methods

Four varieties, Pioneer and Katambora as the diploid, Masaba and Mbarara as the tetraploid, respectively, which were got from a seed company in this city, were used. These varieties were sown in pots laid in a green house April 23, 1979 and 30 plants per variety were trans-planted to the laboratory field. Among the plants, 20 plants per variety which grew vigourously, were selected and used for the following experiments.

1. The root tips got from the tillers of the respective plants, which were cultured in running water, were used for the observation of the chromosome. The root tips were ketpt in 4°C water for 4 hours, then fixed in Farmer's fluid, and reserved in 70% alcohol solution. They were macerated by means of being kept in 1 N HCl solution at 60°C, 15 min., washed with cool water and then stained with Shiff's fluid for 30 minuts, again washed with water and stained with acetic-Orsein. These double stained materials were smeared on slide glass and observed.

2. The mature leaf got from the respective plant was used for the observation of the stoma. The size of 10 stomata per leaf was measured by means of the SUMP-method, and the average of the data was used as the value of the respective plant. The number of the stoma in a visual field (about 6 mm^2) under a microscope of 150 magnification, was counted, and the average of the data counted in 10 visual field, which were selected at random, was used as the value of the respective

plant.

3. In order to investigate the size and fertility of pollens, the panicle just before heading was collected from the respective plant and fixed with Farmer's fluid. The average of the data obtained by measuring 50 pollens got from 5 floret, was used as the value of the respective plant.

Pollen fertility was decided on the basis of the staining with acetic-carmin fluid. The pollens not to be stained with carmin fluid were judged sterile ones. The number of fertile and sterile pollens in a visual field (about 6 mm^2) under a microscope of 150 magnification was counted. The average pollen fertility calculated from the data ascertained by counting in 25 visual fields (5 visual field $\times$ 5 floret) was used as the value of the respective plant.

4. In order to get the informations about the reproductive method, the following pollinations were carried out, and the rates of the setting and the germination of the seeds were investigated.

In order to carry out different kinds of pollination, 4 plants, namely 2 different plants from each variety of the same ploidy, which were grown in a pot, respectively, were laid together in close positions. 10 sets composed of 4 plants mentioned above, were used for the pollination in diploid and tetraploid, respectively.

Self-pollination: the panicle just before flowering was bagged with a glassine bag.

Open-pollination: the panicles were left unbagged.

Intra-variety cross-pollination: 2 panicles just before flowering of different plants of a variety, were inserted together in a bag.

Inter-variety cross-pollination: 2 panicles just before flowering of different plants of different varieties were inserted together in a bag.

The panicles were harvested about 20 days after flowering. 100 spikelets from a panicle were selected at random for the investigation of the setting of seeds. The setting of seeds was examined with naked eye, operating the floret with forceps in a laboratory dish. The setting of seeds was judged at every spiklet. The ripening seeds were seeded on filter paper held water in a laboratory dish, which was laid in a incubater kept at 30°C, and their germination was investigated 10 days after seeding. The investigations of setting and germination of seed were repeated twice per panicle.

Results

1. Chromosome number

The ploidy of 20 plants per variety was ascertained on the basis of the observation of the chromosomes in the root tips. All plants in Pioneer and Katambora were diploid ($2n=20$), and those in Masaba and Mbarara were tetraploid ($2n=40$), as reported by other investigators[3,4,10].

2. Heading and flowering

First heading dates of the respective varieties under natural day-length, were as follows: Pioneer, July 17; Katambora, July 25; Masaba and Mbarara, October 25.

Diploid varieties, Pioneer and Katambora, began to flower about 5 days after heading, but tetraploid ones, Masaba and Mbarara, did not flower at all after heading. The variations of heading dates among plants in the respective varieties were observed.

As the heading of tetraploid varieties under natural day-length condition was too late to be used for the pollination, the short day treatment for them was carried out from August 24. They did not show even the boot stage at the time when they were grown under 10 hours day-length for 16 days, thereafter they were grown under 11 hours day-length. By 21 days after the beginning of

the latter treatment, almost the all plants, excepting 3 plants in Masaba and 2 ones in Mbarara, headed.

3. Morphological Character

For the purpose of using for the discrimination of ploidy of growing plant, some morphological characters were investigated.

(1) The size and number of the stoma

The size and number of the stoma in the face and back of the leaf were measured with the SUMP method. As the differences of these characters between ploids were more remarkable in the back of the leaf than in the face, though they showed the same tendency in both sides, the data in the back of the leaf are shown in Table 1. The differences of means of the length, width and the number of the stoma in the back of leaves between the two diploid varieties, and those between the two tetraploid ones were not significant, but the differences among diploid varieties and tetraploid ones were significant. Namely, the size of the stoma of tetraploid varieties was larger than that of diploid ones, and the number of the stoma of tetraploid varieties was smaller than that of diploid.

Table 1. Length, width and number of stoma in the back of leaves and the difference of the mean values among varieties (μ)

1-1. Length

Variety	Mean	Difference		
		Katambora	Masaba	Mbarara
Pioneer (2x)	17.47	0.61	4.16**	4.40**
Katambora (2x)	18.08		3.55**	3.79**
Masaba (4x)	21.63			0.24
Mbarara (4x)	21.87			

1-2. Width

Variety	Mean	Difference		
		Katambora	Masaba	Mbarara
Pioneer (2x)	15.29	0.77	3.06**	2.76**
Katambora (2x)	14.52		3.83**	3.53**
Masaba (4x)	18.35			0.30
Mbarara (4x)	18.05			

1-3. Number

Variety	Mean	Difference		
		Katambora	Masaba	Mbarara
Pioneer (2x)	46.10	1.82	18.33**	17.55**
Katambora (2x)	44.19		16.50**	15.73**
Masaba (4x)	27.69			0.77
Mbarara (4x)	28.46			

**, Significant at 1% level

The differences of the mean values of length, width and the number of the stoma between the face of a leaf and the back in the respective varieties are shown in Table 2. In any varieties of diploid and tetraploid, the length of the stoma in the back of the leaf was shorter than that in the face, and the number of a stoma in the back of the leaf was larger than that in the face.

Table 2. Differences of the mean values of the length, width and the number of stoma between in the face and in the back of leaves (B–F)*

Variety	Difference		
	Length	Width	Number
Pioneer (2x)	−3.47**	−1.86**	36.47**
Katambora (2x)	−2.04**	−0.60	32.34**
Masaba (4x)	−2.80**	+0.26	19.22**
Mbarara (4x)	−1.66**	−0.39	17.43**

* (B–F), Subtracted mean value in face from those in back

** Significant at 1% level

(2) Size and fertility of the pollen grains

Mean values of the diameter of the pollen grains of the respective varieties and the differences of the mean values among varieties are shown in Table 3. The differences of mean values among tetraploid Masaba and diploid varieties, Pioneer and Katambora, were significant at 1% level, but concerning tetraploid Mbarara, only the difference between this variety and diploid variety Katambora was significant at 1% level. Namely, the differences of size of pollen grains among diploid varieties and tetraploid ones were not so larger as those in stomata.

Table 3. Diameter of pollen grain and the differences of the mean values among varieties (μ)

Variety	Mean	Difference		
		Katambora	Masaba	Mbarara
Pioneer (2x)	28.26	0.72	2.59**	1.27
Katambora (2x)	27.54		3.31**	1.99**
Masaba (4x)	30.85			1.32
Mbarara (4x)	29.53			

*, Significant at 1% level

The fertilities of the pollen grains of the respective varieties are shown in Table 4. All plants investigated showed very high pollen fertility and the differences of this character among the diploid varieties and the tetraploid ones were not observed.

Table 4. Pollen fertility (%)

Variety	Mean	Range
Pioneer (2x)	91.84	55.80– 99.30
Katambora (2x)	97.24	95.60–100.00
Masaba (4x)	97.51	92.60– 99.60
Mbarara (4x)	96.53	91.40– 98.90

(3) Colour of stigma

The colour of the stigmas of all plants in diploid varieties, Pioneer and Katambora was bright purple, whereas the colour of the stigmas in tetraploid varieties, Masaba and Mbarara, was dark purple. The colour variations among the plants in the same ploidy varieties were not observed.

4. Reproduction

(1) Ripening of seeds

In order to get informations concering the reproductive methods, the rates of the setting of seeds after different pollination method were investigated. The results obtained are shown in Table 5, in which the data of the two varieties are included in diploid and tetraploid, respectively, since the number of the panicles of different plants, which flowered at the same time and were available to the pollination, was not satisfactory.

Table 5. Percentage of the spiklet in which floret set seed after different pollination method and the differences among the mean values

5-1. diploid variety

Pollination	Mean	Range	Difference		
			Open	Cross A	Cross B
Self	4.28	0.0–19.5	32.86**	16.86**	10.32**
Open	37.14	4.5–79.0		16.00**	22.54**
Cross A	21.14	0.0–60.5			6.54
Cross B	16.60	0.0–59.0			

5-2. tetraploid variety

Pollination	Mean	Range	Difference		
			Open	Cross A	Cross B
Self	1.06	0.0–13.5	28.59**	9.90*	14.92*
Open	29.65	0.0–77.0		18.69**	13.67
Cross A	10.96	0.0–47.5			5.02
Cross B	15.98	0.0–49.0			

*: significant at 5% level
**: significant at 1% level
self: self-pollination
open: open-pollination
cross A: intra-variety cross-pollination
cross B: inter-variety cross-pollination

In diploid variety, the mean percentage of the spikelet in which floret set seeds after self-pollination was the lowest of the four values after different pollination and that after the open pollination was the highest. It seems to show some artificial effects of the bagging that the value after open pollination was larger than those after cross A (pollination between different plants in a variety) and cross B (pollination between plants from different varieties). Though the difference between the value after cross A and that after cross B, was not significant, all the other differences among the values after different pollinations were significant.

In tetraploid variety, the percentages of the spikelet in which flolets set seeds after different pollinations, showed almost the same tendency as in the diploid varieties. Namely, the percentage of the spikelet in which floret set seeds after self pollination was the lowest of four ones after different pollinations and that after open pollination was the highest.

Generally, in both diploid and tetraploid varieties, the percentages of setting of seeds after cross pollinations were higher than those after self pollinations.

(2) Germination of seeds

The germination percentages of the seeds set after the different pollination are shown in Table 6. The germination percentages of the seeds set after self pollination and that after open pollination in diploid varieties were almost same in their values, and the difference between the two was not significant. And the values after self and cross-pollinations in the tetraploid varieties and the

Table 6. Germination percentages of the seed set after different pollinating methods and the differences among the mean values

6–1. diploid variety

Pollination	Mean	Range	Difference		
			Open	Cross A	Cross B
Self	28.91	0.0–100.0	1.77	6.34	15.58
Open	27.14	0.0– 62.5		8.11	17.35*
Cross A	35.25	0.0–100.0			9.24
Cross B	44.49	0.0– 92.9			

6–2. tetraploid variety

Pollination	Mean	Range	Difference		
			Open	Cross A	Cross B
Self	53.70	0.0–100.0	0.42	25.43	6.29
Open	53.28	0.0–100.0		25.01*	5.87
Cross A	28.27	0.0– 57.2			19.14
Cross B	47.14	10.4– 77.6			

*: significant at 5% level
self: self-pollination
open: open-pollination
cross A: intra-variety cross-pollination
cross B: inter-variety cross-pollination

difference between the two showed the same tendency as in the diploid ones.

Though the difference between the values after open pollination and cross B in diploid variety, and the difference between the values after open pollination and cross B in tetraploid were significant, concerning these differences a certain tendency due to pollination method was not noticeable.

Discussion

Tarumoto and Mochizuki[15,16] reported that the heading of diploid varieties of Rhodes grass was sensitive to temperature and that of tetraploid was sensitive to day-length, and induced due to 12 hours day-length treatment.

The heading of the varieties used for this experiment also showed the same tendencies as mentioned above.

Nakagawa and Sato[12] observed tetravalent chromosomes in Masaba and Mbarara, and they estimated that these varieties are autotetraploid. The morphological difference of the stoma between diploid and tetraploid, shown in this experiment, seems to support the fact that these tetraploid are autotetraploid.

These morphological difference between diploid and tetraploid may be used as a simple method for the discrimination of ploidy of growing plants.

And the difference of colouration of the stigma between two ploidies also may be used as a indicator of the ploidy, excepting for the particular strains without antocyanin colouration reported by Bogdan[5].

Bogdan[1] reported that the dehiscence of the floret of this grass needed rising of the temperature of a floret due to the sun-light. In this experiment it was observed that the diploid varieties flowered on fine days and not flowering on cloudy days, and the tetraploid varieties which headed in October

under natural day-length, did not dehiscence their florets at all. The results reported by Hayasaki et al[8] that the panicles headed after the middle of October did not set seed, seem to show undehiscence of the floret due to lower temperature.

Namai, et al[14] reported that some plants without fertile pollens set seeds under the bagging. In this experiment such plants were not observed at all. Brown and Emery[6,7] inferred that diploid varieties of Rhodes grass are sexual and that tetraploid are apomictic. Hutton[9] reported that although Rhodes grass appears to be xenogamous, all the critical evidence points to an apomictic form of reproduction. But Bogdan[2,3,4] reported that in the progeny test both diploid and tetraploid behaved as sexual, xenogam plants. Jones and Pritchard[10] reported that cytological study of two diploids and five tetraploid Rhodes grass confirmed a normal sexual method of reproduction for both ploidy levels. Nakajima and Mochizuki[13] inferred that both diploid and tetraproid varieties are cross pollinative. The results in this pollination experiment also shows that both diploid and tetraploid varieties are probably prevailing of cross-fertilization, but there are some plants which set high percentage of seeds due to self-pollination.

Concerning the germination of seeds, a certain tendency according to pollination method were not observed. It seems that from the point of view of the effect of seed dormancy on the germination, further investigations must be carried out.

Summary

1) The chromosome number in Rhodes grass varieties were acertained: Pioneer (2n=20), Katambora (2n=20), Masaba (2n=40) and Mbarara (2n=40).

2) The size of stoma in the back of the leaf in tetraploid varieties was larger than that in diploid, and the number of stoma in tetraploid smaller than that in diploid.

These morphological difference between diploid and tetraploid may be used as the simplified method for the discrimination of ploidy of growing plants.

3) The rate of pollen fertility was generally high in both diploid and tetraploid varieties.

4) The results gained from open and controlled pollinations are considered to show that both diploid and tetraploid varieties in Rhodes grass are probably prevailing of cross pollination.

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