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Allelic Relationship and Location of Gene Controlling Short Stature in Thai Wild Rice 'SPR 82-83'

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ABSTRACT

The Thai wild rice, 'SPR 82-23', re-identified as *Oryza nivara* was reciprocally crossed with IR 8 to test the allelic relationship of 'SPR 82-23' with Dee-geo-woo-gen derivatives; and as a male parent was crossed with 12 primary trisomic stocks to locate its short stature gene.

F_1 and F_2 Analysis the F_1 and F_2 generations of the cross between 'SPR 82-23' and Dee-geo-woo-gen derivatives showed that the short stature genes of 'SPR 82-23' share the same sd_1 compound locus but the two parents differed in the ultrastructure of the locus. Differences between parents in the genetic materials adjacent to the sd_1 locus could give rise to *trans* and *cis* configurations which lead to the appearance of intermediate and tall F_2 phenotypes. In the case of IR36/'SPR 82-23', larger proportions of intermediate and tall F_2 plants were obtained. Different genetic backgrounds of the two parents could allow modifying genes (modifiers) in the positive direction (taller heights) to express themselves, although IR 36 and 'SPR 82-23' also share the sd_1 compound locus.

The chromosomal location of 'SPR82-23' was determined by trisomic segregation ratios. Due to low F_2 population size, conclusive evidence was not obtained on the exact location of the two semi-dwarfing genes. It is probable that the genes might be located in either chromosome 4, 1, 2, 3, 9 or 12.

Further studies are needed to confirm the gene system controlling the short stature of 'SPR 82-23'. Moreover, refined techniques and larger population sizes are needed to pinpoint the exact location of the semi-dwarfing genes in the wild rice.

INTRODUCTION

Semidwarfs have played an important role in rice improvement, both in direct releases as cultivars and as parents in breeding programs. Short and sturdy culms confer lodging resistance.

The concept of breeding tropical indica rice varieties for semidwarfness was first practiced in Taiwan. The semidwarf height of TN1 is controlled primarily by a single recessive gene, sd_1 (Aquino and Jennings, 1966).

At IRRI, the development of IR 8, a line derived from the cross between a tall Indonesian variety Peta and the semidwarf Dee-geo-woo-gen, marked the beginning of the development of high yielding varieties adapted to tropical conditions. This led to the release of IR 8 in 1966 (Chandler, 1966). TN1 and IR 8 heralded the so-called "green revolution" in the tropics. IR 8 also carried the semidwarfing gene (sd_1). Almost all of the high yielding varieties of IRRI, have derived from Dee-geo-woo-gen, (DEWG) and

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as well as several modifiers (Chang and Li, 1980). Only 'IR 5', which is not a true semidwarf, does not carry this sd_1 gene (IRRI, 1968).

Now many rice varieties of economic importance have the single sd_1 gene for semidwarf height, mostly coming from DGWG. The first semidwarf rice cultivar in California Calrose 76, which came from irradiation treatment of the tall Calrose, also possesses a single recessive gene for semidwarfism (Rutger *et al.*, 1976, 1985).

Carnahan *et al.*,(1981) reported that M-401 has a semidwarfing gene allelic to sd_1, indicating that this is a recurrent mutation from the tall cultivar Terso.

Some of the Thai rice varieties (RD1, RD2, and RD3 varieties), derived from the IRRI hybridization programs, have also the sd_1 gene (Pushpavesa, 1987).

The frequent use of the same semidwarfing gene may reduce genetic diversity and bring about genetic vulnerability (Chang and Li, 1980). New sources of semidwarfism are necessary to broaden the genetic base of the high-yielding varieties.

Staff of the National Rice Seed Storage Laboratory for Genetics Resources of Thailand at the Pathum Thani Rice Research Center, found that the wild rice G.S. 6048 (named 'SPR 82-23'), has several interesting characters, such as short plant height (about 80 cm.), good tillering, white awn, black seeds, and 120 day maturity. It was collected from Song Phi Nong District, Suphan Buri Province. It would be of interest for rice workers to know if the gene system controlling the short stature of the wild rice is different from that of DGWG or not.

This study is aimed to : i) test the allelic relationship of 'SPR 82-23' with DGWG derivatives (IR 8, IR 36), and ii) determine the location of the gene controlling short stature of 'SPR 82-23', using the primary trisomic stocks with the assumption that it differs from that of DGWG.

MATERIALS AND METHODS

The study was conducted at two sites. The first site was in the greenhouse of Bangkhen Rice Experiment Station, Bangkhen, Bangkok, Thailand from November 1985 to April 1986, and from June 1986 to September 1986. The second site was the nursery area and field facility of the International Rice Research Institute, Los Baños, Philippines, from March 1987 to June 1987 and May 1987 to September 1987.

Materials

1. One Thai Wild rice ('SPR 82-23'), two semidwarf varieties (IR 8, IR 36) and primary trisomic stocks were used in this study.

The semidwarf wild rice 'SPR 82-23' (G.S. 6048) was taken from the National Rice Seed Storage Laboratory for Genetics Resources, Pathum Thani Rice Research Center, Rice Research Institute, Thailand.

2. The improved semidwarfs IR 8 (IRRI Acc. 10320) and IR 36 (IRRI Acc. 30416) were obtained from IRRI, Los Baños, Philippines.

3. The twelve primary trisomic stocks used in this study were obtained from the seed supplied by Dr. G.S.Khush of Plant Breeding Department, International Rice Research Institute.

Methods

'SPR 82-23' was reciprocally crossed with IR 8, using the clip method (Coffman and Herrera, 1980). F_1 progenies of the 'SPR 82-23'/IR 8 cross and its reciprocal were grown in the greenhouse to produce F_2 seeds. Parents and F_2 progenies of 'SPR 82-23'/IR 8 and its reciprocal were grown in a field at IRRI at a spacing of 25 × 20 cm, with 1 plant/hill.

Crosses with the 12 primary trisomic lines were made, using the wild rice as the male parent. Cytological examination on the F_1 plants of the

12 primary trisomic lines/'SPR 82-23' were undertaken to identify trisomics (2n + 1) and normal (2n) plants. F_1 progenies of triplo 1-4 which were highly sterile were backcrossed to the wild rice parent. The trisomic F_1 plants of triplo 5-12 which were highly fertile were allowed to self-pollinate to obtain F_2 seeds. F_2 progenies of 12 primary trisomic lines/'SPR 82-23' (2n + 1 plants and 2n plants) were grown in the greenhouse together with IR 36 at a spacing of 25 × 25 cm. between rows and between plants with 1 plant/hill.

Trisomics were identified as early as they could be distinguished morphologically from disomics. Trisomic characteristics described by Khush *et al.* (1984) were used to identify the trisomics. Triplo 4, 5, 7 and 8 were identified at the tillering stage. Triplo 3, 6 and 10 were identified after heading, while triplo 11 was identified cytologically by counting the chromosomes because of its pseudonormal morphology.

RESULTS AND DISCUSSION

The wild rice 'SPR 82-23' was re-identified as *Oryza nivara.*, using the taxonomic key to *Oryza* species (Chang, 1976). The chromosome number was 2n = 24.

Analysis of IR8/'SPR 82-23' and its reciprocal crosses

Plant height data taken from IR 8, 'SPR 82-23' and the F_1 plants of the reciprocal crosses were 88, 84, 95 and 106 cm, respectively (Table 1). The F_1 plants were taller than both parents probably due to heterosis. The distribution of F_2 plants in plant height is shown in Figure 1 and Figure 2 for the reciprocal crosses. The F_2 height distribution ranged from 38-153 cm. with a mean and mode of 92 and 105.5 cm, respectively, in IR 8/'SPR 82-23'; and 48-157 cm. with a mean of 98 cm. and a mode of 96 cm. in 'SPR 82-23'/IR 8.

Because of the continuous distribution in the F_2 population of 'SPR 82-23'/IR 8, it is not feasible to divide the F_2 population into 2 height groups.

The only plausible interpretation is that the Dee-geo-woo-gen derivatives have a pair of sd_1 alleles, which constitutes a compound locus that contains pseudo-alleles that are all functionally related to plant height. This phenomenon

Table 1 Mean, variance and percent coefficient of variation (c.v.) for plant height of parents, $F_{1's}$ and $F_{2's}$ populations.

Parents and Generations	Known Genotypes	No. of Plants	Plant Height (cm)				
			Mean	Range	s^2	$\bar{X} \pm S_{\bar{X}}$	C.V. (%)
'SPR 82-23'		30	84	64-99	59.43	84±1.41	9.23
IR8	sd_1	30	88	74-98	23.83	88±0.9	5.5
F_1 (IR8/'SPR 82-23')		59	95	66-114	152.91	95±1.6	13.0
F_1 ('SPR 82-23'/IR8)		21	106	94-119	35.76	106±1.3	5.7
F_2 (IR8/'SPR 82-23')		125	92	38-153	486.10	92±1.9	24.0
F_2 ('SPR 82-23'/IR8)		210	98	48-157	501.68	98±1.6	22.7
IR36	sd_1 + negative modifiers	30	88	83-97	11.87	88±0.63	3.9
TP6		18	71	54-83	50.93	54±1.68	10.05
F_2 (IR36/'SPR 82-23')		525	116	60-162	555.14	116±1.03	20.32

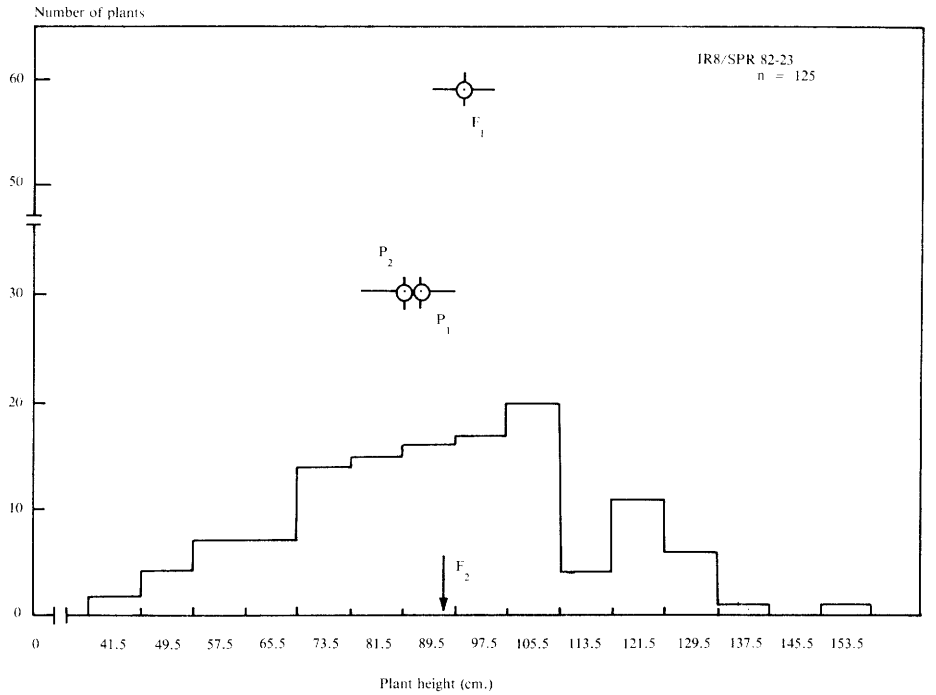

Figure 1 Distribution and means of parents (P_1, P_2), F_1 and F_2 plants by plant height classes in the cross IR8 (P_1) and 'SPR 82-23' (P_2). Solid horizontal lines show the range of the parents and F_1 plants about the means (dotted circles) while the arrow shows the mean of the F_2 population.

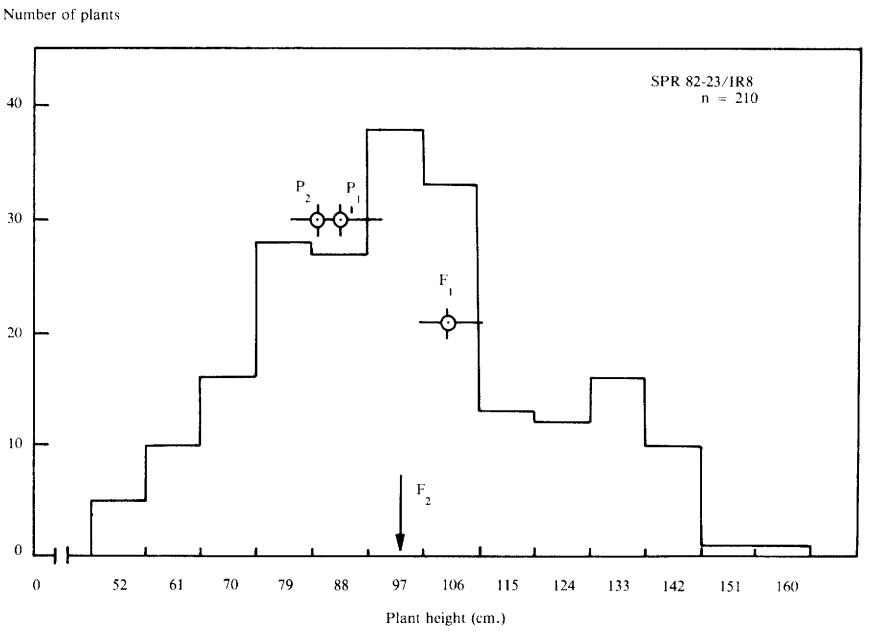

Figure 2 Distribution and means of parents (P_1, P_2), F_1 and F_2 plants by plant height classes in the cross 'SPR 82-23' (P_2) and IR8 (P_1). Solid horizontal lines show the range of the parents and F_1 plants about the means (dotted circles) while the arrow shows the mean of the F_2 population.

is termed the clustering of functionally similar genes or a genetic unit having fine ultrastructure (Grant, 1975). The logical interpretation is that on both sides of the sd_1 gene, there are small segments of genetic material which also affect plant height. If IR 8 and 'SPR 82-23' differ in the additional genetic material on both sides of the sd_1 gene, it is possible to have new recombinants involving such adjacent segments in *cis* and *trans* forms. This type of pseudoalleles could produce the following classes of recombinants:

Ho: IR8 $\frac{+_1\ sd_1\ +_2}{+_1\ sd_1\ +_2}$ × 'SPR 82-23' $\frac{m_1\ sd_1\ m_2}{+_1\ sd_1\ +_2}$

F_1 $\frac{m_1\ sd_1\ m_2}{+_1\ sd_1\ +_2}$

possible gametes

1) $m_1\ sd_1\ m_2$
2) $+_1\ sd_1\ +_2$
3) $m_1\ sd_1\ +_2$ *(trans)*
4) $+_1\ sd_1\ m_2$ *(cis)*

F_2 genotypes and phenotypes

	$m_1\ sd_1\ m_2$	$+_1\ sd_1\ +_2$	$m_1\ sd_1\ +_2$	$+_1\ sd_1\ m_2$
$m_1\ sd_1\ m_2$	$\frac{m_1\ sd_1\ m_2}{m_1\ sd_1\ m_2}$ (dwarf)	$\frac{m_1\ sd_1\ m_2}{+_1\ sd_1\ +_2}$ (semidwarf) as 'SPR 82-23'	$\frac{m_1\ sd_1\ m_2}{m_1\ sd_1\ +_2}$ (semidwarf)	$\frac{m_1\ sd_1\ m_2}{+_1\ sd_1\ m_2}$ (semidwarf)
$+_1\ sd_1\ +_2$	$\frac{m_1\ sd_1\ m_2}{+_1\ sd_1\ +_2}$ (semidwarf) as 'SPR 82-23'	$\frac{+_1\ sd_1\ +_2}{+_1\ sd_1\ +_2}$ (semidwarf) as IR8	$\frac{+_1\ sd_1\ +_2}{m_1\ sd_1\ +_2}$ (intermediate)	$\frac{+_1\ sd_1\ +_2}{+_1\ sd_1\ m_2}$ (intermediate)
$m_1\ sd_1\ +_2$	$\frac{m_1\ sd_1\ +_2}{m_1\ sd_1\ m_2}$ (semidwarf)	$\frac{m_1\ sd_1\ +_2}{+_1\ sd_1\ +_2}$ (intermediate)	$\frac{m_1\ sd_1\ +_2}{m_1\ sd_1\ +_2}$ (semidwarf)	$\frac{m_1\ sd_1\ +_2}{+_1\ sd_1\ m_2}$ (tall) *trans*-arrangement
$+_1\ sd_1\ m_2$	$\frac{+_1\ sd_1\ m_2}{m_1\ sd_1\ m_2}$ (semidwarf)	$\frac{+_1\ sd_1\ m_2}{+_1\ sd_1\ +_2}$ (intermediate)	$\frac{+_1\ sd_1\ m_2}{m_1\ sd_1\ +_2}$ (tall) trans-arrangement	$\frac{+_1\ sd_1\ m_2}{+_1\ sd_1\ m_2}$ (semidwarf)

Expected phenotypic ratio: 1 dwarf : 9 semidwarf : 4 intermediate : 2 tall

Observed phenotypic ratio:

IR8/'SPR 82-23' : 6 : 76 : 35 : 8

'SPR 82-23'/IR8: 5 : 119 : 46 : 40

Chi-square value and probability level

IR8/'SPR 82-23' : X^2 = 5,0516** P = 0.025 - 0.01

'SPR 82-23'/IR8 : X^2 = 13.0434** P = 0.05 - 0.025

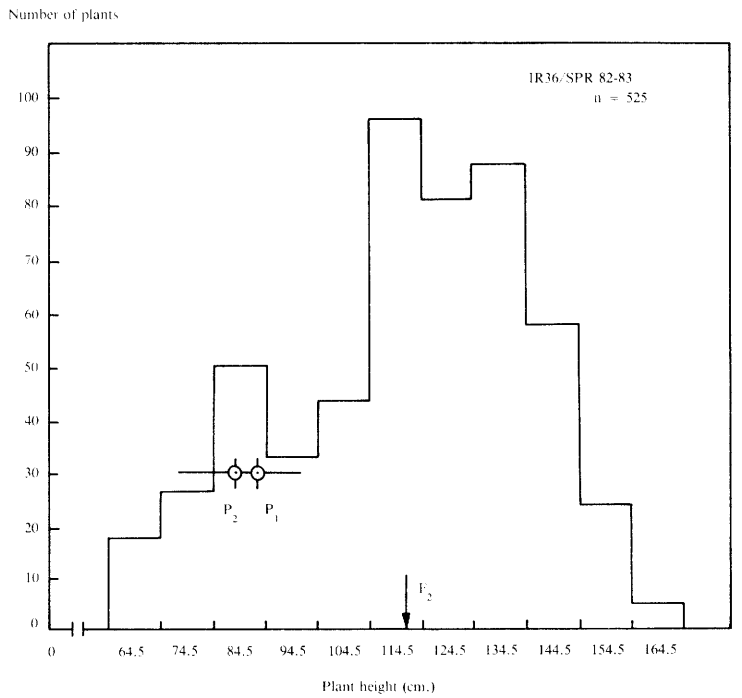

Figure 3 **Distribution and means of parents (P_1 , P_2) and F_2 plants by plant height classes in the cross triplo 6 (2n) of IR36 (P_1) and 'SPR 82-23' (P_2). Solid horizontal lines show the range of the parents about the means (dotted circles) while the arrow shows the mean of the F_2 population.**

Differences in the plant height of the F_1 and F_2 segregation patterns between the reciprocal crosses of IR 8/'SPR 82-23' indicated that there are cytoplasmic-nuclear interaction effects. Previously, in crosses involving *O. nivara* and *O. sativa* cultivars also involving sd_1 gene, maternal effects were observed in the percentage of pollen sterility (Dolores *et al.*, 1979).

Analysis of IR 36/'SPR 82-23 (2n progeny of Triplo 6/'SPR 82-23') Crosses

The F_2 population of IR 36/'SPR 82-23' was derived from the diploid (2n) $F_{1's}$ of the cross, triplo 6/'SPR 82-23', since the extra chromosome from triplo 6 was not transmitted to the diploid (2n) plants. The primary trisomics were derived from the progenies of IR 36 by backcrossing (Glaszmann, personal communication).

The mean plant height of IR 36 and 'SPR 82-23' were 88 and 84 cm, respectively. The female parent used in the cross was 71 cm. The average plant height of the F_2 population was 116 cm (Table 1). The F_2 distribution showed two peaks (Figure 3) ranging from 60-162 cm having a c.v. of 20.3%. The mode of the F_2 distribution was at 114.5 cm (Figure 3). About 82.5% of the F_2 population were taller than IR 36, and 12.2% were shorter than 'SPR 82-23'.

While both IR 8 and IR 36 have the sd_1 gene for short stature, the F_2 segregation pattern of the crosses with 'SPR 82-23' differed significantly. In the cross with IR 8, most of the F_2 plants were of the dwarf to semidwarf phenotypes, while in the cross with IR 36 more taller phenotypes were recovered. The segregation of a quantitative character observed in the progeny of a hybrid does not necessarily expose for analysis all of the multiple genes involved in the development of that character. The segregation reveals only those genes that are represented by different

Table 2 Trisomic analysis for 8 triplo plants in the F_2 or backcross generations of primary trisomics of rice.

	F_2 or BC	Total	2n		X^2 F_2 = 8 : 1 BC = 2 : 1	2n + 1		Total		X^2 F_2 = 12.5 : 1 BC = 3.5 : 1
			Tall	Dwarf		Tall	Dwarf	Tall	Dwarf	
TP3/'SPR 82-23'	BC	2	1	—	—	1	—	2	—	—
TP4/'SPR 82-23'	BC	21	15	1	5.2812*	3	2	18	3	0.7654
TP5/'SPR 82-23'	F_2	411	369	41	0.5125	—	1	369	42	4.7370*
TP6/'SPR 82-23'	F_2	990	886	93	2.5746	10	1	896	94	6.2902*
TP7/'SPR 82-23'	F_2	498	411	71	6.3924**	15	1	426	72	36.0925**
TP8/'SPR 82-23'	F_2	474	360	71	12.5476**	34	9	394	80	61.9808**
TP10/'SPR 82-23'	F_2	163	127	25	4.3824*	9	2	136	27	19.9274**
TP11/'SPR 82-23'	F_2	56	31	16	25.0241**	7	2	38	18	49.9565**

alleles in the two parents (Grant, 1964).

The recovery of more taller phenotypes in the F_2 populations could be attributed to the differences in the genetic background of IR 36 and 'SPR 82-23'. IR 36 is a progeny of multiple crosses, having the semidwarfs CP-231/SLO-17, DGWG derivatives, and *O. nivara* in the parentage. It is probable, therefore, that 'SPR 82-23' has modifiers having both positive and additive effects and/or the interaction of the background genes with the major genes could result in short and tall recombinants.

Genetic studies involving wild rices are still limited, thus further studies are needed to confirm the present findings and elucidate the gene mechanism controlling the short stature in wild rices. Crosses with tall phenotypes could clarify the gene action of the wild rice 'SPR 82-23'.

Chromosomal location of dwarfing genes in the Thai wild rice 'SPR 82-23' was determined through primary trisomic tests. The F_1 plants with $2n+1$ chromosome number were selfed and backcrossed to the parent for F_2 study. Of the 184 F_1 progenies studied, eight segregated in the trisomic of triplos 3, 4, 5, 6, 7, 8, 10 and triplo 11.

In analyzing the F_2 progenies, 90 cm was used to separate between intermediate-tall-to-tall and dwarf classes in this particular study because the mean plant height of both 'SPR 82-23' and 12 primary trisomics was nearly 90 cm.

Among the backcross progenies of triplo 3/'SPR 82-23' only 2 plants out of 8 were left and X^2-test was not possible. It is difficult to determine the cause of seedling death whether it was due to seedling weakness or to trisomic weakness. Chu and Oka (1980) found seedling weakness in F_1 hybrids between strains of *O. breviligulata* (wild) and *O. glaberrima* (cultivated rice).

Chi-square analysis of four F_2 populations involving triplo 7, triplo 8, triplo 10 and triplo 11 showed highly significant X^2-values (Table 2), indicating that the short stature gene of 'SPR 82-23' is not present on these chromosomes.

In the crosses of TP5/'SPR 82-23', and TP6/'SPR 82-23' significant X^2-values were observed in the populations but non-significant in diploid plants. The result would still indicate the absence of semidwarfing gene in chromosome 5 and 6.

In the cross of TP4/'SPR 82-23', X^2-test value (5.2812*) was significant in diploid population but non-significant in the total population (0.7654). Since, the population is relatively small, no conclusion can be drawn.

The present findings suggest the possibility that the 'SPR 82-23' semidwarfing genes are located on either chromosomes 1, 2, 3, 9 or 12. Unfortunately, the F_1 of these triplo plants could not be obtained, which could be attributed to the low percentage transmission of the extra chromosome (33.3%) (Khush 1973).

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