Crop Breeding and Applied Biotechnology 7:103-110, 2007
Brazilian Society of Plant Breeding. Printed in Brazil

PLANT BREEDING PROGRAM

Establishment of the irrigated rice cultivar SCSBRS Tio Taka by recurrent selection

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Received 13 September 2005

Accepted 6 April 2006

ABSTRACT - *This study aimed to present and discuss the recurrent selection methodology used in the development of the rice cultivar SCSBRS Tio Taka. This cultivar, derived from the population CNA-IRAT 4, achieves high yields and has agronomic traits related to a better performance in the pre-germinated cultivation system used in Santa Catarina State. The molecular characterization of Tio Taka and CNA-IRAT 4 parents with 16 SSR markers showed that one of the parents, BG 90-2, was highly similar to SCSBRS Tio Taka. The most probable reason was that SCSBRS Tio Taka was extracted from CNA-IRAT 4 in the second recombination cycle, preventing the occurrence of a high level of recombination and favoring the phenotypic selection of plants derived from crosses with BG 90-2 as parent, once this line has one of the best plant architectures and yields.*

Key words: *Oryza sativa*, recurrent selection, population improvement, SSR markers, grain yield.

INTRODUCTION

The method of recurrent selection was developed in 1945 by Hull, with a view to gene recombination by continuous re-selection, generation after generation, intercrossing the selected families. The method had first been proposed for cross-pollinated species (Hallauer 1992) and is nowadays successfully used in self-pollinated species such as soybean (Uphoff et al. 1997), wheat (Wang et al. 1996), common bean (Sing et al. 1999) and rice (Rangel and Neves 1997, Rangel et al. 2000, Rangel et al. 2003).

In Brazil recurrent selection is being used as an alternative method that aims to improve populations of wide genetic base, for the extraction of lines with high grain yield and other agronomical traits superior to those of the currently used commercial cultivars. The implantation of this recurrent selection program further has the objective of broadening the genetic base of the irrigated rice cultivars (Rangel et al. 1996, Breseghello et al. 1999) to reduce the risks of epidemics of pests and diseases in the plantations.

Population improvement by means of recurrent selection in rice has been considered the most appropriate option of achieving genetic gains for quantitative traits such as grain yield (Rodriguez et al. 1998, Rangel et al. 1998, Rangel et al. 2002).

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The SSR (Simple Sequence Repeat) or microsatellite markers are very important in the genomic analysis, since they are co-dominant, abundant, distributed across the entire genome, multialleleic, depend on a small DNA quantity only, and are based on the PCR (Polymerase Chain Reaction). SSR markers are available in large number for rice (McCouch et al. 2002), so the markers with the greatest information potential as well as widely dispersed in the genome can be chosen.

The objective of this study was to present and discuss the recurrent selection methodology used in the development of cultivar SCSBRS Tio Taka and analyze its genetic similarity with the parents of the population using microsatellite molecular markers.

MATERIAL AND METHODS

Population CNA-IRAT 4

Cultivar SCSBRS Tio Taka was derived from the population CNA-IRAT 4 that was synthesized by Embrapa Arroz e Feijão and the Institut de Recherche en Agronomie Tropicale, (IRAT), in France, by the intercrossing of 10 lines of the subspecies *indica* (Table 1). Nine lines were used as male parents in crosses with a source line of male sterility genetic, derived from cultivar IR 36 by mutation. This mutant has a recessive allele (ms), which in homozygosis (msms) produces the sterility of the pollen grains. This allows field recombinations of the selected families, which spares manual crossings (Singh and Ikehashi 1981). Male-sterile plants are easily identified at flowering, by the white atrophied anthers and growth of a defective panicle, part of which gets entangled with the flag leaf sheath. The F_1 plants derived from the crossing of IR 36 male-sterile with the nine lines were backcrossed as male parents, with the nine lines, in order to ensure the cytoplasm diversity in the population and reduce the participation of the mutant. The F_2 seeds of the heterozygous BC_1 plants were mixed and intercrossed on the field three times, harvesting always the male-sterile plants, originating the population CNA-IRAT 4/0/3. The early and mean cycle plants of this population were extracted and originated, respectively, the populations CNA-IRAT 4P/0/3 and CNA-IRAT 4M/0/3. Population CNA-IRAT 4M/0/3 underwent recurrent selection in a first mass selection cycle and a second selection cycle with evaluation of $S_{0:2}$ families, according to the methodology proposed by Rangel and Neves (1997), which led to the establishment of the new population CNA-IRAT 4M/2/1.

Table 1. Crosses that originated the parents of population CNA-IRAT 4

Cultivars/lines	Parents
BG 90-2	IR 262/Remadja
CNA 7	T 141/IR 665-1-1-75-3
CNA 3815	CICA 4/BG 90-2//SML 1517
CNA 3848	IR 36/CICA 7//5461
CNA 3887	BG 90-2/Tetep//4440
Colombia 1	Napal/Takao Iku 18
Eloni	IR 454/SML Kapuri//SML 66410
Nanicão	Traditional Brazilian variety
UPR 103-80-1-2	IR 24/Cauvery
IR 36 (msms)	IR1561-228-1-2/IR1737//CR94-13

Establishment of cultivar SCSBRS Tio Taka

The sequence of cultivar establishment of SCSBRS TioTaka is shown in Figure 1, with the following stages:

S_0 generation: selection of male-fertile plants in population CNA-IRAT 4M/2/1, on the experimental field of the Fazenda Palmital, Goianira, state of Goias, in the crop year 1991/92.

$S_{0:1}$ generation: multiplication of $S_{0:1}$ families on the experimental field of Formoso do Araguaia, state of Tocantins, in the between harvests of 1992, originating $S_{0:2}$ families.

$S_{0:2}$ generation: evaluation of 164 $S_{0:2}$ families in the 1992/93 cropping season, in trials with 10 x 10 and 8 x 8 triple lattice design at two localities in Rio Grande do Sul and one in Santa Catarina state. Considering mainly the mean grain yield at the three sites family number 75 was selected.

Generation $S_{0:3}$: beginning of the individual plant selection within family 75 using the pedigree method on the experimental field of the Fazenda Palmital, in the 1993/94 cropping season.

Generation $S_{3:4}$: re-selection of individual plants and elimination of the male sterility gene, on the experimental field of the Fazenda Palmital, in the 1994/95 cropping season.

Generation $S_{4:5}$: advance of line CNA-IRAT 4M/2/1-75-B-B-2-2 in generation $S_{4:5}$ to generation $S_{4:6}$, (CNA-IRAT 4M/2/1-75-B-B-2-2-B) in the between harvests of 1995 on the experimental field of Formoso do Araguaia, TO, for seed multiplication for the realization of the first phenotypic evaluations.

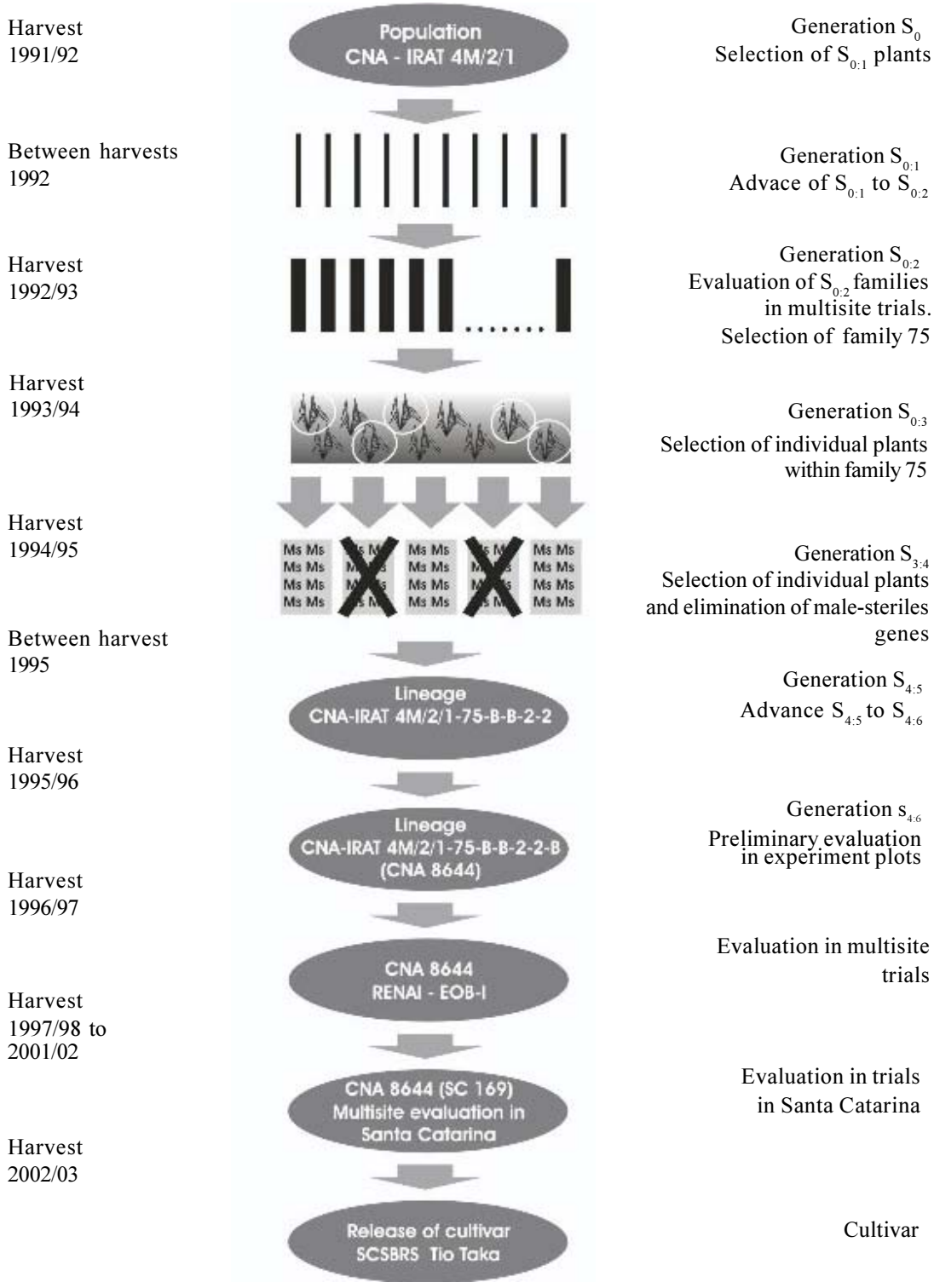

Figure 1. Scheme used in the establishment of cultivar SCSBRS Tio Taka

Generation $S_{4:6}$: in the 1995/96 cropping season, line CNA-IRAT 4M/2/1-75-B-B-2-2-B was designated CNA 8644 and preliminarily evaluated in experimental plots on the Experimental Field of the Fazenda Palmital, GO, for yield, disease resistance and industrial and culinary grain quality.

In 1996/97, line CNA 8644 was made available for other Brazilian research institutions linked up in a network, to be evaluated in the Trial of Observation of Irrigated rice (EOB-I) of the Technical rice commissions (Embrapa 1994), where it presented superior performance in Santa Catarina and was selected to pass on to the subsequent experiments.

Evaluation in Santa Catarina

In the 1997/98 cropping season, CNA 8644 (designated SC 169 in Santa Catarina) was evaluated preliminarily with 43 other lines, where it stood out for its high yield, blast resistance and tolerance to iron-toxicity and was forwarded to advanced experiments. In 1998/99 it was evaluated in the advanced yield trial, together with 15 other lines. These trials had the objective of evaluating all agronomic traits of economic interest, particularly lodging resistance, which is the main trait a variety must own to be suitable for the predominant pre-germinated cultivation system in Santa Catarina. The pre-germinated seeds of lines were planted into a layer of water, 15 to 20 cm deep, and this condition of irrigation was maintained until maturation with the objective of increasing the selection pressure for lodging resistance. The experiment was carried out at three sites of the State, where CNA 8644 stood out once more for its superior performance.

From 1999/00 to 2001/02 CNA 8644 was evaluated in regional trials of Santa Catarina that have the main objective of defining new lines that would be released as new irrigated rice cultivars for the State. These trials were conducted in the pre-germinated cropping system at six sites (in Itajaí, Joinvile, Massaranduba, Pouso Redondo, Tubarão and Turvo), for three cropping seasons. The treatments were sown on 60 m^2 plots at each site, distributed in random blocks, without replication per year. Data of grain yield (in kg ha^{-1}), lodging, plant height, and number of days from planting to harvest were collected as well as data of tolerance to iron toxicity and blast (*Pyricularia grisea)* incidence, according to the Guideline of rice research methods (Embrapa 1976). The data of whole-grain yield were obtained in the EPAGRI (Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina) Laboratory of Grain Quality and the amylose content and gelatinization temperature, according to criteria of the International Center of Tropical Agriculture (Jennings et al. 1981), by the Laboratory of Grain Quality of Embrapa Arroz e Feijão.

In the joint analysis of the trials, the effects of local and years were considered to be random and the effects os treatments were fixed since data of only four lines were taken into consideration, aside from control cultivar Epagri 108, which participated as common treatment in all trials.

The null hypothesis of the treatment effects was evaluated by the *F* test obtained by:

$F[\alpha:(t-1),n]=Q4/[Q5+(Q6-Q7)]$, where

α: level of significance,

t-1:degrees of freedom for treatments,

n: degrees of freedom related to combination $Q5+(Q6-Q7)$, estimated by

$n \equiv \sum_i Qi^2 / \sum_i (Qi^2 / gli)$, according to Satterthwaite (1946), for $i = 4, 5$ and 6; *gli* stands for the degrees of freedom associated to *Qi*.

Q4, Q5, Q6 and *Q7*: mean squares of the sources of treatment variation (lines and cultivar control), treatments x years, treatments x local and treatments x local x years interactions (considered as error in the analysis of variance), respectively.

Molecular analysis by SSR markers

DNA extraction

Seeds of cultivar SCSBRS Tio Taka and of the 10 parents, members of the population CNA-IRAT 4 (Table 1) were germinated in a paper tube and the DNA was extracted from the seedlings according to the protocol described by Doyle and Doyle (1987). The DNA concentration was estimated by electrophoresis in 0.8% agarose gel by comparison with the DNA-standard of the lambda phage, adjusted to 3 ng mL^{-1}.

PCR reaction

The genetic analysis was carried out with markers described in previous publications (Chen et al. 1997, Temnykh et al. 2000, Brondani et al. 2001). Sixteen

markers were chosen, based on their information content and representativeness in the 12 rice chromosomes. The amplification reactions were run in a final volume of 13 mL containing: 0.3 mM of each primer (Forward and Reverse), one unit of the enzyme Taq DNA polymerase, 0.2 mM of each dNTP, 1 mM TRIS-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 1.3mL DMSO (50%) and 7.5 ng total DNA. The PCR amplification reactions were performed in a PT-100 (MJ Research) thermocycler as follows: one pre-cycle of 96 °C for 2 minutes; followed by 30 cycles at 94 °C for 1 minute, 56 °C for 1 minute and 72 °C for 1 minute; and a final step at 72 °C for 7 minutes. The amplification was checked by horizontal electrophoresis in 3% agarose gel containing TBE 1 X (0.09M Tris Borato and 2mM EDTA) buffer and 0.2 mg mL^{-1} ethidium bromide. Allele polymorphism was detected in 6% polyacrylamide. The amplified products were visualized in polyacrylamide gels stained with silver nitrate, according to Bassam et al. (1991).

Statistical analysis

The allele frequencies were determined using software Fstat (Goudet 2001). The dendrogram was constructed based on the genetic distance matrix obtained from the genetic distance coefficients of Rogers modified by Wright (1978). The accessions were clustered by the criterion of cluster UPGMA, using software NTSYS (Rolhf 1989). The stability of the clusters, visualized in the dendrograms was tested by the boostrapping procedure with 10.000 randomizations using Software Bood version 3.04, available on request (Coelho, acoelho@icb.ufg.br). The private alleles were estimated using software GDA (Lewis and Zaykin 2000).

RESULTS AND DISCUSSION

Despite the relative recent beginning only 12 years ago, the Brazilian population improvement program of irrigated rice by recurrent selection began to be fruitful with the release of the high-yielding cultivar SCSBRS TioTaka for cultivation in Santa Catarina. This is the first rice cultivar derived from a program of this kind and released for cultivation in Brazil. It is expected that this cultivar is formed by a gene combination of the different parents that represent the base population, and that it is therefore genetically more divergent from the currently used commercial cultivars of irrigated rice.

Evaluation in Santa Catarina

Table 2 shows the data of mean grain yield and the number of days from planting to harvest (Cycle) of lines evaluated in the regional trials conducted at six sites in Santa Catarina state between 1999/00 and 2001/02. There were highly significant ($P < 0.01$) differences by the F test between the means of lines evaluated for the trait grain yield (Table 2). Cultivar SCSBRS Tio Taka was the most productive with 8.561 kg ha^{-1} in the mean of the 18 environments, differing significantly from control Epagri 108 and the evaluated lines by the test of Dunnett (Dunnett 1955, 1964) at the level of 5% probability. The superiority of the cultivar in terms of grain yield is an expression of the genetic potential of population CNA-IRAT 4 to provide high-yielding genotypes throughout the recurrent selection cycles.

The cycle from planting to harvest lasts 141 days, which is considered long (Table 2). Since the lowlands allow only one harvest per year, long-cycle cultivars with less than 150 days are preferred in the state, as a possibility of boosting the yield and profit of the farmers.

Table 2. Data of mean grain yield and cycle of the cultivars/lines evaluated in the regional trials performed at six localities in Santa Catarina state in the cropping seasons 1999/00 to 2001/02

Lines	**Grain yield** (kg ha^{-1})	**Cycle** (days)
SCSBRS Tio Taka	8.561 a[1]	141
SC 170	8.194 b	137
Epagri 108	8.044 b	137
SC 167	7.628 c	148
SC 168	7.083 c	141
CV %	7.49	

[1] Means followed by letters different from b differ from the control mean at the level of 5% probability by the test of Dunnett

Cultivar SCSBRS Tio Taka presented high resistance to lodging, a trait considered essential for a cultivar that is designed for the pre-germinated cultivation system, used in nearly all of the 130 thousand hectares of irrigated rice in Santa Catarina.

The cultivar presented moderate susceptibility to iron toxicity and greater blast resistance compared with the control Epagri 108. The disease is one of the chief problems of the rice crop in Brazil, where it is present in all plant growth stages and causes considerable losses in the productivity of plantations and raises the production costs by fungicide applications for the control. The release of resistant cultivars such as SCSBRS Tio Taka is of great importance for the rice farmers of Santa Catarina for reducing yield losses and costs, besides lessening the environmental impact of plantations.

In the evaluations of the industrial grain traits SCSBRS Tio Taka presented a very good whole grain yield of 63%, and high amylose contents and gelatinization temperature. It was also adequate for the parboiling process and presented a glassy appearance of the polished as well as the parboiled grain. In the cooking tests the grains were loose, soft and of normal taste. The grain quality of the cultivar is therefore satisfactory for the process of white as well as parboiled grains.

Genetic diversity of SCSBRS Tio Taka and population CNA IRAT 4 parents

The analysis with microsatellite markers (SSR) of cultivar SCSBRS Tio Taka and its parents detected variation in the number of alleles from 2 (MRG4879) to 8 (OG61), with a mean of 4.9 alleles per locus (Table 3). The gene diversity (He) varied from 0.17 (MRG4879) to 0.89 (OG61), with a mean of 0.64 (Table 3). There was a direct relation between the higher He values with the lower frequency of the allele identified in SCSBRS Tio Taka among the parents, except for marker OG44 (He= 0.57, an allele identified only in the parent Nanicão). Another SCSBRS Tio Taka allele, also found in the parent Nanicão only, was obtained by marker OG17. Interestingly, allele SCSBRS Tio Taka for marker OG61 was not identified in any of the 10 parents, that is, it had been derived from a pollen source outside the original population. This result is of great relevance, in the sense that in populations of recurrent selection, whose recombination are given by the presence of the male sterility gene, the maintenance of a great distance and installation of plant barriers must be painstakingly observed to avoid the undesirable gene flow coming from foreign rice plants.

The parent BG 90-2 shared the largest number of

Table 3. Number of chromosomes (Chr.), number of identified alleles, expected heterozygosis (He), allele size in cultivar SCSBRS Tio Taka, allele frequency and parents with the allele of the cultivar

SSR	Chr.	Number of alleles	He	Allele size in Tio Taka	Allele frequency in the parents	Parents with the allele of Tio Taka
RM11	7	5	0.59	148	0.60	BG 90-2, CNA 3815, CNA 3848, CNA 3887, Eloni, Nanicão
RM204	6	7	0.87	116	0.20	BG 90-2, UPR
RM207	2	7	0.78	142	0.40	CNA 3848, Nanicão, UPR, IR 36
RM223	8	4	0.47	164	0.70	BG 90-2, CNA7, CNA3815, CNA3848, CNA3887, Eloni, UPR
RM229	11	4	0.64	134	0.20	BG 90-2, CNA3815
RM247	12	5	0.68	148	0.50	BG 90-2, CNA3815, CNA3848, CNA3887, UPR
RM304	10	3	0.33	182	0.80	BG 90-2, CNA3815, CNA3848, CNA3887, Colombia 1, Eloni, UPR, IR 36
OG61	5	8	0.89	108	-	-
OG106	9	5	0.74	218	0.35	BG 90-2, Nanicão, UPR, IR 36
MRG4961	11	3	0.66	140	0.40	BG 90-2, CNA 7, CNA 3815, UPR
OG17	2	7	0.88	126	0.10	Nanicão
OG44	3	4	0.57	158	0.10	Nanicão
RM224	11	6	0.81	158	0.30	BG 90-2, UPR, IR36
MRG4879	4	2	0.17	108	0.90	BG 90-2, CNA3815, CNA 3848, CNA 3887, Colombia1, Eloni, Nanicão, UPR, IR36
RM248	7	4	0.57	94	0.10	CNA 7
OG7	11	4	0.64	154	0.20	BG 90-2, CNA 3815
Mean	-	4.9	0.64	-	0.31	-

common alleles with SCSBRS Tio Taka (11 common alleles) and the parents Colômbia 1 (2 alleles) and CNA 7 (3 alleles) the fewest. According to these results, the parent closest to cultivar SCSBRS Tio Taka was BG 90-2, and the most distant were the parents Colômbia 1 and CNA 7 . There are two likely explanations for this result: The first is that SCSBRS Tio Taka was extracted from the population in the second cycle of recurrent selection, that is, after only two cycles of recombination, from the initial population. The second reason is related to the method of recurrent selection itself. As the most productive families were used in the recombination of this population, one would expect that they would be derived mostly from the most productive parents that contributed with the highest number of alleles to the following selection cycle. The genotypes derived from the crossings that included the contribution of alleles from the high-yielding parent line BG 90-2, may have therefore stood out amongst the others during the selection, and amongst these, the genotype that originated SCSBRS Tio Taka.

ACKNOWLEDGEMENTS

The authors wish to thank the FAO (Food and Agriculture Organization of the United Nations) for the financial support for the realization of the genetic analyses by microsatellite markers.

Obtenção da cultivar de arroz irrigado SCSBRS Tio Taka por seleção recorrente

RESUMO - *O presente trabalho teve como objetivo apresentar e discutir a metodologia de seleção recorrente utilizada no desenvolvimento da cultivar SCSBRS Tio Taka e a sua análise genética através de marcadores SSR. Esta cultivar, que é oriunda da população CNA-IRAT 4, além da alta produtividade de grãos apresentou também, outras características agronômicas adequadas ao cultivo no sistema pré-germinado de Santa Catarina. A caracterização da SCSBRS Tio Taka e os genitores da população CNA IRAT-4 com 16 marcadores SSR mostrou que um dos genitores, BG 90-2, foi altamente similar à SCSBRS Tio Taka. Provavelmente os motivos foram que houve apenas duas gerações de recombinação quando da extração da família que originou a SCSBRS Tio Taka, e que as famílias que continham a maior contribuição do genitor BG 90-2, que possui alta produtividade, tenham sido favorecidas na seleção.*

Palavras-chave: *Oryza sativa*, seleção recorrente, melhoramento populacional, marcadores SSR, produtividade de grãos.

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