LOCATION IRRI

CALL # SB208.2004.T5 .N48.

**AUTHOR** 

Nguyen, Thi Ngoc Hue.

TITLE

Homoeologous chromosome pairing and alien introgression

analysis in wide-cross derivatives of Oryza through

flourescence in situ hybridization.

IMPRINT

Los Baños, Laguna, 2004.

DESCRIPT

xiv, 157 leaves : col. ill. ; 28 cm.

NOTE

Thesis (Ph. D.) -- University of the Philippines at Los Baños,

2004.

**NOTE** 

Includes bibliographical references (p. [137]-150).

**KEYWORDS (NAL)** 

Rice; Chromosome pairing; Introgression; In situ

hybridization; Hybrids; Plant genetics; Plant

breeding.

# HOMOEOLOGOUS CHROMOSOME PAIRING AND ALIEN INTROGRESSION ANALYSES IN WIDE-CROSS DERIVATIVES OF *ORYZA* THROUGH FLUORESCENCE *IN SITU* HYBRIDIZATION

#### **NGUYEN THI NGOC HUE**

SUBMITTED TO THE FACULTY OF GRADUATE SCHOOL UNIVERSITY OF THE PHILIPPINES LOS BANOS IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY (Genetics)

**APRIL 2004** 

## **TABLE OF CONTENTS**

	<u>Page</u>
TITLE PAGE	i
APPROVAL PAGE	ii
BIOGRAPHICAL SKETCH	iii
ACKNOWLEDMENT	iv
TABLE OF CONTENTS	V
LIST OF TABLES	viii
LIST OF FIGURES	
LIST OF APPENDICES	xii
ABSTRACT	xiii
INTRODUCTION	1
REVIEW OF LITERATURE	
Key Traits of Wild Species	5
Production of Interspecific Hybrids and Alien Introgression Lines	9
Homoeologous Chromosome Pairing in Wide-Cross Hybrids	10
Molecular Characterization of Introgression	. 12
Isozyme Markers	12
DNA Markers	13
In Situ Hybridization	16
Genomic In Situ Hybridization (GISH)	17
Fluorescence <i>In Situ</i> Hybridization (FISH) and Physical Mapping	24
MATERIALS AND METHODS	32
Experiment 1. Developing FISH Protocol for Characterization of Pachytene Chromosome of Rice	32
Plant Material	32
Chromosome Preparation	32

		<u>Page</u>
	Fluorescence In Situ Hybridization (FISH)	33
	Experiment 2. Analysis of Homoeologous Chromosome Pairing using Genomic <i>In Situ</i> Hybridization and Centromere Specific Probes	37
	Plant Material	37
	Meiotic Chromosome Preparation	37
	Chromosome Association	38
	Characterization of Chromosome Pairing through GISH	38
	FISH using Centromere Specific Probe	38
	Experiment 3. GISH Analysis of Wide-Cross Derivaties	39
	O. minuta x O. ridleyi Hybrid	39
	Backcross Progenies and Monosomic Alien Addition Lines (MAALs)	39
	GISH Analysis of Alien Introgression Lines	40
RES	ULTS	41
	FISH Protocol for Pachytene Chromosomes of Rice	41
	Pollen Mother Cell (PMC) Collection	41
	Chromosome Preparation	43
	Modification in FISH Protocol	44
	Chromosomal Analysis of Interspecific Hybrids of Oryza	51
	O. sativa (AA) x O. officinalis (CC)	51
	O. sativa (AA) x O. brachyantha (FF)	57
	O. sativa (AA) x O. granulata (GG)	60
	O. sativa (AA) x O. minuta (BBCC)	60
	O. sativa (AA) x O. ridleyi (HHJJ)	62
	Characterization of Parental Genomes and Homoeologous Pairing through FISH in Interspecific Hybrids of <i>Oryza</i>	64
	O. sativa (AA) x O. officinalis (CC).	64
	O. sativa (AA) x O. brachyantha (FF)	70

	<u>Page</u>
O. sativa (AA) x O. granulata (GG).	76
O. sativa cv NPT (AA) x O. minuta (BBCC)	85
O. sativa (AA) x O. ridleyi (HHJJ)	94
FISH using Centromere Specific Probes	104
Characterization of Parental Genomes in Hybrids of O. minuta x O. ridleyi through GISH.	107
FISH Analysis of Backcross Progenies and MAALS Derived from O. sativa x O. ridleyi.	111
Backcross Progenies	111
Characterization of MAALs from O. ridleyi.	114
FISH Analysis of Advanced Introgression Lines Derived from O. sativa x O. australiensis	117
DISCUSSION	122
Modified FISH Protocol for Meiotic Chromosomes of Rice	123
Homoeologous Chromosome Pairing in Wide-Crosses of Rice	124
Characterization of Wide-Cross Derivatives through <i>In Situ</i> Hybridization	127
SUMMARY AND CONCLUSION	133
LITERATURE CITED	137
APPENDICES	151

## **LIST OF TABLES**

<u>Table</u>		<u>Page</u>
1	Chromosome number, genomic constitution and useful traits of <i>Oryza</i> species	6
2	Frequency of ring and rod bivalents at diplotene/early diakinesis in rice and different wild species of <i>Oryza</i>	52
3	Frequency of univalents, bivalents and multivalents in F <sub>1</sub> hybrids of <i>O. sativa</i> and five wild species of <i>Oryza</i>	53
4	Chromosomes associations in $F_1$ hybrids of $O$ . sativa $x$ $O$ . officinalis, $O$ . sativa $x$ brachyantha and $O$ . sativa $x$ $O$ . granulata	58
5	Restitution cells with different configurations at DK/MI in some $F_1$ hybrids of rice and wild species of $Oryza$ .	59
6	Chromosome association in $F_1$ hybrids of $O$ . sativa $x$ $O$ . minuta and $O$ . sativa $x$ $O$ . ridleyi	63
7	Autosyndetic and allosyndetic pairing in F <sub>1</sub> hybrid between <i>O. sativa</i> and <i>O. officinalis</i> detected through FISH	66
8	Chromosome configurations classified through FISH in F <sub>1</sub> hybrid between <i>O. sativa</i> and <i>O. Officinalis</i>	67
9	Autosyndetic and allosyndetic pairing in F <sub>1</sub> hybrid between O. sativa and O. brachyantha detected through FISH	71
10	Chromosome configurations classified through FISH in F <sub>1</sub> hybrid between <i>O. sativa</i> and <i>O. brachyantha</i>	72
11	Autosyndetic and allosyndetic pairing in F <sub>1</sub> hybrid between O. sativa and O. granulata detected through FISH	77
12	Chromosome configurations classified through FISH in F <sub>1</sub> hybrid of <i>O. sativa</i> and <i>O. granulata</i>	78
13	Chromosome configurations at pachytene in F <sub>1</sub> hybrid of O. sativa and O. granulata	79

<u>Table</u>		<u>Page</u>
14	Comparison of allosyndetic and autosyndetic pairings in $F_1$ hybrids of O. sativa x O. officinalis, O. sativa x O. brachyantha, and O. sativa x O. granulata	84
15	Autosyndetic and allosyndetic pairing in F <sub>1</sub> hybrid between O. sativa and O. minuta detected through FISH	87
16	Meiotic chromosomes with different configurations in $F_1$ hybrid of $O$ . sativa and $O$ . minuta	88
17	Autosyndetic and allosyndetic pairing in F <sub>1</sub> hybrid between O. sativa cv IR56 and O. ridleyi detected through FISH	95
18	Chromosome configurations classified through FISH in F <sub>1</sub> hybrid of <i>O. sativa</i> cv. IR 24 and <i>O. ridleyi</i>	96
19	Chromosome configurations classified through FISH in F <sub>1</sub> hybrid of IR56 and <i>O. ridleyi</i>	98
20	Comparison of chromosome associations in different hybrids analyzed through FISH and classical cytogenetic techniques	102
21	Detection of introgression through FISH from <i>O. australiensis</i> in three advanced lines derived from <i>O. sativa x O. australiensis</i>	118

#### **LIST OF FIGURES**

<u>Figure</u>		<u>Page</u>
1	Pachytene cells of <i>O. sativa</i> cv IR56 prepared from pollen mother cell (PMC) at different collection times	42
2	Electrophoresis and dot blot analyses after labeling total genomic DNA of <i>O. minuta</i> by Nick translation	45
3	FISH on pachytene chromosomes of IR 56. The total genomic DNA of IR 56 was labeled with biotin 14-dATP and used as probe	47
4	FISH on pachytene chromosomes of two wild species, <i>O. granulata</i> and <i>O. autraliensis</i> . Total genomic DNA of respective wild species was labeled with biotin 14-dATP and used as a probe	48
5	Pachytene chromosomes of two tetraploid wild species after FISH. Total genomic DNA of wild species was labeled with biotin 14-dATP and used as probe	49
6	Diakinesis cells from wild species after FISH. Total genomic DNA of wild species was used as probe	50
7	Meiotic cells (pachytene, diakinesis) of O. sativa cv IR 56	55
8	Meiotic behavior of $F_1$ hybrids involving $\textit{O. sativa}$ and 3 diploid wild species	56
9	Meiotic behavior of O. sativa x O. ridleyi hybrid	61
10	FISH using genomic DNA of <i>O. officinalis</i> as a probe on meiotic chromosomes of <i>O. sativa</i> (NPT) x <i>O. officinalis</i> acc. 101399	68
11	FISH on meiotic chromosomes of IR74 x <i>O. officinalis</i> acc. 101399. Total genomic DNA of <i>O. officinalis</i> was labeled with biotin 14-dATP and used as probe	69
12	FISH using total genomic DNA of <i>O. brachyantha</i> as probe on meiotic and mitotic chromosomes of <i>O. sativa</i> cv IR56 x <i>O. brachyantha</i>	73

-igure		<u>Page</u>
13	FISH on meiotic cells of <i>O. sativa</i> x <i>O. brachyantha</i> hybrid. Total genomic DNA of <i>O. sativa</i> was used as probe	74
14	FISH on meiotic chromosomes of $F_1$ hybrid of $O$ . sativa $x$ $O$ . granulata. Total genomic DNA of $O$ . granulata was used as probe	80
15	FISH on meiotic chromosomes in hybrid of <i>O. sativa</i> x <i>O. minuta</i> . The DNA of <i>minuta</i> was used as probe	90
16	FISH on meiotic chromosomes of <i>O. sativa</i> cv IR 24 x <i>O. ridleyi</i> F₁ hybrid. <i>O. ridleyi</i> DNA was used as probe	99
17	FISH on meiotic chromosomes of <i>O. sativa</i> cv IR 56 x <i>O. ridleyi</i> F₁ hybrid. <i>O. ridleyi</i> DNA was labeled with biotin 14-dATP and used as probe	100
18	FISH of centromere specific probe to <i>O. sativa</i> meiotic and mitotic chromosomes. Centromere specific sequence (RCS2) labeled with biotin 14 dATP and used as probe	105
19	Meiotic chromosomes in $F_1$ hybrid of $O$ . minuta $\times$ $O$ . ridleyi (WHD IS 806-2) after FISH	109
20	Characterization of parental genomes of WHDIS 806-3 through FISH	110
21	Characterization of parental chromosomes of backcross progenies derived from a cross between <i>O. sativa</i> cv IR56 <i>x O. ridleyi</i> . Total genomic DNA of <i>O. ridleyi</i> labeled with bio 14 dATP and used as probe	112
22	Meiotic chromosomes after FISH in MAALs derived from the cross of <i>O. sativa</i> and <i>O. ridleyi</i> . Genomic DNA from <i>ridleyi</i> labeled with biotin-dATP was used as probe	115
23	Meiotic chromosomes after FISH in introgression lines derived from the cross of O. sativa x O. australiensis	119

### **LIST OF APPENDICES**

<u>Appendix</u>		<u>Page</u>
1	Solutions used in chromosome preparations	151
2	Solution used in dot blot hybridization	152
3	Chemicals and Solutions used in in situ Hybridization	154
4	Composition of solutions used in DNA extraction	157

#### **ABSTRACT**

NGUYEN THI NGOC HUE, University of The Philippines, Los Baños, April 2004, Homoeologous Chromosome Pairing and Alien Introgression Analyses in Wide-Cross Derivatives of *Oryza* through Fluorescence *In Situ* Hybridization.

Major Professors: Dr. A. A. Barrion and Dr. D. S. Brar.

Homoeologous chromosome pairing among different genomes of Oryza was studied through classical and molecular cytogenetic techniques using genomic in situ hybridization (GISH) and fluorescence in situ hybridization (FISH). FISH protocols were modified for analysis of pachytene and other meiotic chromosomes. Meiotic chromosome analysis showed limited homoeology among AA genome of rice and FF, GG and HHJJ genomes of wild species. FISH was used successfully to characterize parental genomes and homoeologous pairing in hybrids involving different genomes: AA x CC, AA x FF, AA x GG and AA x HHJJ. Both allosyndetic and autosyndetic pairing was detected in the hybrids, which was not possible through classical cytological techniques. Chromosome segments introgressed from wild species into the rice genome were also identified through GISH. Autosyndetic pairing within sativa genome is indicative of duplication of chromosome segments, probably rice is secondary polyploid. Both O. minuta and O. ridleyi showed extensive pairing within BC and HJ genomes, respectively. FISH using centromere specific probe (pRCS2) showed hybridization signals on each of the 12 chromosomes at pachytene as well as on 24 somatic chromosomes of rice, however, no centromeric signal could be detected in five wild species (O. officinalis, O. brachyantha, O. granulata, O. minuta and O. ridleyi).

The parental chromosomes, extra alien chromosome in MAALs and introgressed segments in backcross progenies derived from *O. sativa* x *O. ridleyi* were clearly identified using GISH. Restructured chromosome(s) of both *sativa* and *ridleyi* were also detected. The introgressed segments of *O. australiensis* were located on rice chromosomes in the advanced breeding lines derived from *O. sativa* x *O. australiensis*.