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Cytogenetic Analyses of Intragenomic Rice Hybrids Derived from *Oryza sativa* L. and *O. nivara* Sharma et Shastry

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Abstract: Several approaches of plant breeding demand constant reference to the chromosomal status of the breeding materials. The degree of variability, viability and stability desired in the plant breeding often depends on the efficiency of parental chromosomes pairing and subsequent recombination. In order to assess meiotic affinity between *O. sativa* (IR 64, Kalanamak and Manshara) and *O. nivara*, chromosome behaviours in pollen mother cells (PMCs) of parents and their hybrids (*O. sativa cv.* IR64/*O. nivara*, Kalanamak/*O. nivara* and Manshara/*O. nivara*) were analyzed at metaphase I and anaphase and telophase I. The frequency of abnormalities such as univalent (I) and quadrivalent (IV) were slightly increased in all the hybrids. Despite these minor abnormalities, on an average all the intragenomic hybrids showed normal meiosis with remarkably high degree of chromosome pairing. At metaphase I, all the hybrids had more than 11.8 bivalents and 22 chiasmata/PMC. Other meiotic irregularities were comparable with parental meiosis except for the presence of a few bridges and bridges + fragments. The bridge + fragments were only observed for *O. sativa* cv. Kalanamak/*O. nivara* hybrid in 3.08% of the PMCs. Bridges at anaphase and telophase I were recorded only in IR64/*O. nivara* hybrids in 7.07% PMCs. This cytogenetical study revealed that the chromosome pairing between cultivars of domesticated rice (*O. sativa*) and wild rice (*O. nivara*) is essentially normal.

Key words: Interspecific Hybrid · Meiotic Configuration · *O. nivara* · Wild Relatives · Metaphase I · Anaphase I · Telophase I

INTRODUCTION

Wide hybridization and chromosome manipulation are the important techniques to transfer useful genes from wild to cultivated rice, *Oryza sativa* L. Following these methods, several genetic and cytogenetic stocks such as monosomic alien addition lines (MAALs), introgression lines (ILs) and mapping population have been developed in rice [1]. Introgressive hybridization can often lead to rapid genomic changes, including chromosomal rearrangements, genome expansion, differential gene expression and gene silencing, some of which are mediated by transposable elements [2]. These genomic changes may lead to beneficial new phenotypes and selection for fertility and ecological traits may in turn alter genome structure [2]. Knowledge of the cytogenetic relationships between cultivated species and their wild relatives has still greater scope to transfer alien genes of interest. Several innovative approaches in breeding crops have been developed and one of them demands constant reference to the chromosomal status of the breeding materials. The chromosomal status and ploidy level of species in gene introgressive breeding programme ultimately depends on the meiotic behaviour at different stages of meiosis [3]. Moreover, cytogenetic basis of genome and evolutionary analyses are crucial for directing our efforts to search for beneficial gene/s in wild species of rice. Although landraces and wild species are in general agronomically inferior to cultivated rice, the transfer of favorable alleles for disease resistance, tolerance to abiotic stresses, agronomic traits such as yield heterosis, higher protein quantity and other quality related traits is still feasible [1, 4-6].

O. nivara is one of the most potent annual wild species and constitutes the primary gene pool of cultivated rice. Being the close relative of domesticated

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rice, it can also offer an ideal experimental system to geneticists and breeder for the characterization of adaptive traits [7, 8]. Economic genes such as grassy stunt virus resistance and source of cytoplasmic male sterility have already been transferred into cultivated *indica* background and several other potential genes source for blast resistance and drought avoidance have also been identified [1]. Nepal also harbors *O. nivara* species with wide arrays of ecotypic variation and could be the potential source for rice breeding.

Earlier cytogenetic study revealed that chromosome pairing between cultivated rice and *O. nivara* (IRGC 101508), originally collected from Uttar Pradesh of India, was normal [9]. They reported that 82.3-90% and 85.9-98.0% cells had 12 II at metaphase I and normal separation at anaphase and telophase I, respectively. Although they obtained irregularities, such as univalent to hexavalent, aneuploidy with nucleolar bodies at diakinesis and metaphase I and fragments, bridges, bridges + fragments, laggards, early separation, bridge + laggards and late disjunction at anaphase and telophase I in small number of cells, chromosome pairing was essentially normal. Such anomalies were not only comparable to their respective parents, but also similar to meiosis those found in a number of true bred and intervarietal rice hybrids [10]. The chromosome relationship between *O. sativa* and *O. nivara* of Nepal origin has not been investigated yet. Therefore, for the manipulation of cytogenetic knowledge as a keystone to the rice breeding and to establish the provision of adequate access to this species, the present study was carried out to elucidate the crossability and chromosome affinity at different stages of meiotic division in F_1 hybrids obtained from *O. sativa*/*O. nivara* of Nepal origin.

MATERIALS AND METHODS

Oryza nivara Sharma et Shastry (originally collected from Nepalgunj, Nepal) was crossed with three *indica* rice varieties viz. Kalanamak, Manshara and IR64 as male and female parents, respectively. Both hand pollination and approach methods were adopted for interspecific pollination. Up on pollination panicles were covered with crossing bags. Cross seeds were harvested after 25 day of pollination. Crossability between wild and cultivated rice was calculated as follows: crossability = number of true F_1 hybrids obtained/total number of spikelet pollinated X 100. The recovered F_1 hybrids and their parents were pregerminated at 33°C for 3 day and planted in plastic pot containing well fertilized soils. Then the seedlings were grown in the glasshouse of Institute of Agriculture and Animal Science (IAAS), Nepal until harvest.

Meiotic analyses from F_1 hybrids were carried out at different stages of meiosis using traditional cytogenetic techniques. Young spikelets at suitable stage were fixed in acetic alcohol (1:3 v/v) containing traces of ferric chloride as described previously [11]. The materials were kept in the fixative for 2 4 hours at low temperature (14-25°C) and then transferred to 70 % ethanol until used for smearing. Fixed anthers were removed from the spikelets and smeared in one to two drops of 1% acetocarmine. Slight warming and gentle tapping were provided to promote the excellent spreading of the chromosomes. Data were recorded based on the analysis of 10 randomly selected immature spikelets, including five plants of each for each of the stages studied. At metaphase I and anaphase and telophase I, data for meiotic behavior were recorded only from PMCs with well-spread and distinct configuration after screening a large number of dividing cells. Microscopic photographs were taken from temporary slides with the aid of microscopic camera. Meiotic chromosomes were observed at 1000X magnification using Olympus Microscope (CX-41). Meiotic mean configurations and chiasma frequency were determined based on the meiotic observations at metaphase I as described previously [12].

RESULTS AND DISCUSSIONS

The crossability between *O. sativa* and *O. nivara* was varied from 35-44.75 (%). Among hybrids, the highest crossability was observed in *Manshara/O. nivara* (44.75%) and least in IR 64/*O.nivara* (35%). Based on crossability, Manshara showed a close affinity with *O. nivara* (Table 1). All the hybrid plants obtained from *O. sativa*/ *O. nivara* were intermediate between two parents in some of respects, but there was a preponderance of wild traits. Dominance of wild traits were apparent in leaf and basal leaf sheath coloration, seed characters (apiculus and seed coat colour and awning pattern), panicle characters (type, exertion and shattering), shoot characters (culm number, internode colour, branching of culm and growth habit) and reproductive organ such as stigma color and anther size. (Table 2, Fig. 1 a, b and c). All the hybrid plants were vigorous and fully fertile (Fig. 1b).

Among three cross combinations, landraces of Nepal showed higher crossability than the improved varieties (IR64). Degree of cross affinity was varied with

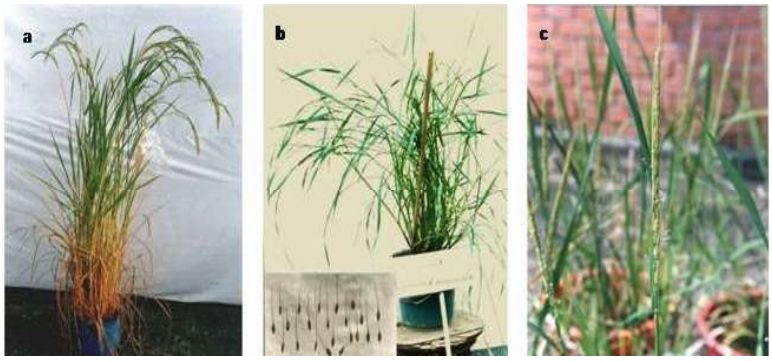

Fig. 1: The morphological feature of parents and hybrid. a: female parent (cultivated landrace, Manshara), b: F_1 hybrid and c: male parent (*O. Nivara*, common wildrice)

Table 1: Seed set and crossability between *O. sativa* L. and common wild rice species (*O. nivara*)

Cross combination Female/ Male	No. of spikelets pollinated	Seed set %	No. of F_1 plants obtained	Crossability %
Kalanamak/*O. nivara*	120	40.00	48	40.00
Manshara/*O. nivara*	181	45.86	81(3)*	44.75
IR 64/*O.nivara*	140	35.00	49	35.00

*Figure in parenthesis indicates number of non germinated seeds

Table 2: Phenotypic characters of parents and their F_1 hybrids and manifestation of dominant traits

Characters	Male parent, *O. nivara* (4)	Female parent, Manshara (1)	Female parent, Kalanamk (2)	Female parent, IR 64 (3)	F_1 (1/4, A)	F_1 (2/4, B)	F_1 (3/4, C)	Domina-nce of (A)	Domina-nce of (B)	Domina-nce of (C)
Apiculus color	dark brown	brown weak red	white	light golden	dark brown	dark brown	dark brown	4	4	4
200 grain wt.	4.9gm	3.46gm	3.1gm	3.91gm	4.5gm	4.4gm	4.9gm	4	4	4
Awn density	long and fully	-	small	minute partly	long andfully	long andfully	long andfully	4	4	4
Awn length	4.0cm	-	-	0.5mm	3.78cm	5.09cm	3.8cm	4	4	4
Branching Culm	present (2-3)	present (1-2)	intermediate	-	present (2-3)	present (2-3)	present (2-3)	4	4	4
Heading days	80	96	109	104	74	101	100	4	I	3
Height	57cm	112.14cm	132.72cm	112.28cm	76.43cm	82.6cm	123cm	4	I	3
Internode color	purple lines	pale green	pale green with green dot	light green	purple lines	purple lines	purple line	4	4	4
Leaf color	dark green with light purple tip	green	green	green	dark green with light purple tip	dark green with purple tip	dark green with purple tip	4	4	4
Panicle exerted	just exerted	moderately exerted	well exerted	well exerted	just exerted	just exerted	just exerted	4	I	4
Panicle length	15.3cm	19cm	22.73cm	23.84cm	15.65cm	19.05cm	16.95	4	I	4
Panicle shattering	high	-	-	-	high	high	high	4	4	4
Panicle type	open	intermediate	compact	intermediate	open	open	intermediate	4	I	I
Seed coat color	dark brown	dark brown	black	yellow	dark reddish	dark brown	dark brown	4	4	4
Stigma color	dark purple	white	white	white	dark purple	dark purple	dark purple	4	4	4
BLSC*	purple	green	green	straw green	purple	purple	purple	4	4	4
Culm number	>30	25	24	22	>30	>30	>30	4	4	4

*BLSC = basal leaf sheath coloration

the genetic makeup of female parents (Table 1) suggesting that different cultivars have different cross affinity with their ancestral wild species. Similar such variations in crossability have been reported in literatures [13, 14, 15]. Therefore, this slight discrepancy in crossability obtained in this study might be attributed to differences in geographical race of wild species. Regarding the dominance of wild traits, similar observations were reported in several intragenomic and intergenomic hybrids [13, 10].

Table 3: Meiotic configurations of parents and their hybrids, *O. sativa/O. nivara*, at metaphase I

		Meiotic mean configuration					
			II				
Parents and hybrids	No. of cells observed	I	Total	Rod	Ring	IV	Chiasmata/ PMC
Oryza nivara	69	0.06(0-2)	11.94(10-12)	0.77(0-4)	11.17(8-12)	0.01(0-1)	23.17±1.04(20-24)
Kalanamak	121	0.03(0-2)	11.96(9-12)	0.50(0-3)	11.46(9-12)	--	23.42±0.99(18-24)
Manshara	82	0.02(0-2)	11.99(11-12)	0.84(0-3)	11.15(9-12)	--	23.13±0.99(21-24)
IR64	139	0.01(0-2)	11.97(10-12)	0.96(0-9)	11.01(3-12)	0.01(0-1)	23.03±1.79(15-24)
Manshara /*O. nivara*	156	0.37(0-10)	11.69(7-12)	0.82(0-6)	10.90(6-12)	0.01(0-1)	22.81±1.73(13-24)
Kalanamak/ *O. nivara*	91	0.22(0-4)	11.84(10-12)	0.93(0-5)	10.91(7-12)	0.02(0-1)	22.83±1.47(19-24)
IR64/*O. nivara*	106	0.23(0-4)	11.8(10-12)	0.88(0-6)	10.92(6-12)	0.05(0-1)	22.90±1.26(18-24)

Numbers in parenthesis denote for range

Table 4: Chromosome behavior at anaphase I and telophase I in parents and their F_1s

		Percent cells observed						
Parents and hybrids	No. of cells observed	Normal	US	B	B + F	B + L	L	LD
Oryza nivara[1]	117	93.16	1.71	0.85	-	-	2.56	0.85
Kalanamak	203	92.12	1.48	1.48	-	-	2.96	1.97
Manshara[2]	101	96.04	-	-	-	-	1.98	-
IR64	241	94.76	1.0	-	-	-	1.83	2.41
Manshara/*O. nivara*	146	91.10	2.74	2.05	-	1.37	1.37	1.37
Kalanamak/ *O. nivara*	130	90.77	0.77	2.31	3.08	2.31	0.77	-
IR64/*O. nivara*	198	70.71	7.58	7.07	-	-	5.56	9.09

[1]and[2] Denote early division of chromosomes observed at 0.85 and 1.98 % PMCs, respectively, US=Unequal Segregation, B= Bridges, F= Fragments, L= Laggard and LD= Late Disjunction

All the parental PMC's had a consistent chromosome number of 2n=24 with normal meiosis. At metaphase-I, all the PMCs had 12 II with predominant of ring bivalent (Table 3). An average of higher than 11.94 bivalent per cell was scored in all the parents and it was highest in *O. sativa* cv. Manshara (11.99). Among parents the frequency of the univalents (I)/PMC ranged from 0.01-0.06. No quadrivalents were found in Manshara and Kalanamak, but their occurrence was scored at low frequency (0.01/PMC) in *O. nivara* and IR 64. Chiasma frequency/cell was varied from 23.03±0.99-23.42±0.99. At anaphase and telophase I, percentage of cells with normal behaviour varied from 92.12-96.04 (Table 4). Laggards were observed in *O. nivara*, Kalanamak, Manshara and IR 64. All types of anomalies were not recorded within a single parent; however, more than two irregularities were commonly observed (Table 3 and 4). Moreover, small percent of cells had also unequal segregation and late disjunction except Manshara.

Meiotic behaviour was regular in all the interspecifc hybrids at metaphase I (Table 4). Chromosome configurations of the interspecific hybrids were not differed appreciably from those of the parental species. The total number of bivalent ranged from 11.69-11.84/cell with preponderance of ring bivalents (Fig. 2a). Chiasma frequencies were also comparable and varied from 22.81±1.73 to 22.91±1.26. Except IR 64/ *O. nivara,* more than 90% of the cell had normal chromosome separation at anaphase and telophase I (Table 4, Fig. 2c, d and e). Most of the cell in IR 64/*O. nivara* had higher frequency of unequal segregation, bridges, laggards and late disjunction. Other aberrations were comparable to their respective parents except bridges and fragments in Kalanamak/*O.nivara*. In this hybrid 3.08% cells showed single bridge + fragment (Table 4).

Based on Meiosis of intervarietal and interspecifc hybrids, Shastry [16] suggested that A genome species are differentiated by chromosome structural changes to various degrees. However in this study, the chromosome pairing in all F_1's hybrids at metaphase I was essentially normal. The type and frequency of meiotic

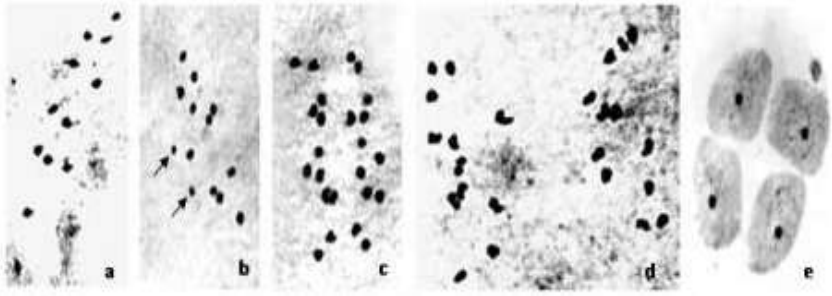

Fig. 2a-e: Representative photomicrograph of chromosome behavior during meiosis at metaphase l, anaphase and telophase l in the interspecific rice hy brids, a: Showing 12 rings ll, Kalanamak/ *O. Nivara*, b: 11 ll+2 l (arrows), IR 64/ *O. Nivara*, c: Normal separation of chromosome at anaphase l, Manshara/ *O. Nivara*, d: early telophase l, normal disjuction, Kalanamak/ *O. Nivara* and e: Four normal daughter cells at late telophase, IR 64/ *O.nivara*

aberrations observed in this study are similar to those found in interracial hybrids in *O. sativa* [10, 17, 18] and in *O. nivara*/*O. sativa* [9]. Hybrid among *sativa*, *sativa* var. *fatuwa, sativa* var. *formosana* and *perennis* var. *balunga* were possessed no cytogenetic differentiation and fertility of hybrids was as normal and regular as in intervarietal hybrids [10]. However, RFLP analysis indicated that some nuclear genome significantly differentiated in Asian rice varieties of *O. sativa* [19].

Univalents and quadrivalents were frequently observed not only in interspecific hybrids, but also commonly reported for interracial and intervarietal hybrids and even in true parental forms [10, 17]. Although the frequency of univalents were quite high (0-10) in Manshara/*O. nivara*, number of quadrivalents and univalents were similar to those reported earlier (Table 3). Similar observations were also reported in cross involving *O. sativa* and *O. nivara* [9]. High frequency abnormalities such as frequent formation of 0-3 quadrivalents, univalents and chromosomal elimination have been reported in *O. sativa*/*O. rufipogon* hybrids [21, 21]. The present study also revealed that no reduction in chiasma frequencies among hybrids and supports the observations in various interspecific rice hybrids by earlier workers [10, 12].

At Anaphase and telophase I all types of anomalies were not recorded within single parent and hybrid, however, more than two abnormalities were commonly observed (Table 4). Similar results were reported by many investigators in intragenomic cross hybrids [10, 21] and in intervarietal and interracial rice hybrids [17,22,33]. Dolores *et al*. [9] observed bridges + fragments in five of the 11 crosses involving *O. sativa* and *O. nivara*. However, in this study 3.08% PMCs showed bridges + fragments only in Kalanamak/*O. nivara* out of three hybrids (Table 4). Frequent abnormalities such as bridges, late disjunction and early division of chromosome were also reported in literatures [9]. The discovery of chromatin bridges without fragments at anaphases and telophase I in all the parental and hybrids form is not so noteworthy, because it could be found in homozygous varieties of rice [10]. However, few bridges and fragments observed in this study in Kalanamak/*O. nivara* is quite interesting and indicated that some form of structural differentiation was occurred in parental chromosomes.

Occurrence of structural changes at later stages such as quadrivalent formation at diakinesis and metaphase I and bridges and fragments at anaphase I can be taken as an indicator of translocation and inversion, respectively. However, the low frequency of such anomalies observed in this study is not enough to generalize the structural differentiations. Bridges + fragments also observed in 11 of the interracial hybrids out of 33 studied in <10% cells and concluded that bridges + fragments are mostly occurred by inversion [10]. Several other investigators reported occurrence of anaphase bridges only in interspecific crosses without mentioning their frequencies (17, 19). High frequency of formation of bridges without fragments at anaphase is mostly attributed to delay terminalization of chiasmata, sticky bivalent formation, or breakage and random reunion of the chromatids at earlier stages regardless of homology as reported by Walter [24]. The quite high frequency of laggard formation in this study is undoubtedly a result of univalents formation at metaphase I. Beside these aberrations, unequal separation and early and late disjunction observed in this study also confirmed the previous results [9]. These commonly occurring anomalies might be attributed to precocious separation of bivalent at metaphase I and or anaphase I. Such common anomalies can be frequently observed due

to environmental effect and leading to reduce number of chiasma formation and finally result into univalents at metaphase I and subsequent irregularities at later stages. Similar varied nature of data regarding the meiotic behaviour of the *O. sativa*/*O. glaberrima* has been reported in rice [10]. Therefore, the minor variation found in the meiotic behaviour among these workers might be due to their use of different strains of *O. nivara* and cv. of *O. sativa*. The results obtained herein also clearly indicate that the failure of bivalent formation at the later stages in hybrids is not always a proof of lack of homology. Such failure of synapsis can possibly be brought by many external and internal factors [18]. The present data indicate that the strain of *O. nivara* most likely has the same genome composition as in the *O. sativa* cultivars.

In summary, chromosome pairing between selected rice cultivars and Nepalese ecotype of *O. nivara* is essentially normal although these species are morphologically highly differentiated.

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