A COMPARATIVE STUDY OF INDICA AND JAPONICA RICES USING RFLP AND MORPHOLOGICAL CHARACTERS

A Thesis
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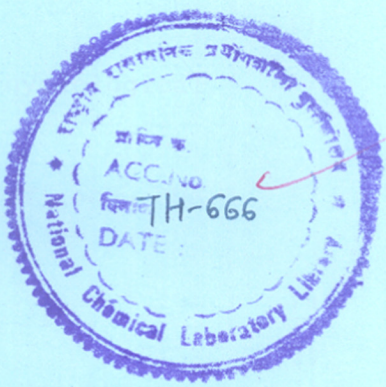
For the Degree of
Doctor of Philosophy
(IN Chemistry)



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C O N T E N T S **Page no.**

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DECLARATION

Certified that the work incorporated in the thesis "A comparative study of indica and japonica rices using RFLP and morphological characters", submitted by Mr. Prafulla K. Chitnis was carried out by the candidate under my supervision. Such material as has been obtained from other sources has been duly acknowledged in the thesis.

P K Ranjekar

11/10/92

(P.K.Ranjekar)

Research Guide

LIST OF ABBREVIATIONS

bp	:	base pairs
kbp	:	kilo base pairs
gm	:	grams
mg	:	milligram
ug	:	microgram
ml	:	millilitre
ul	:	microlitre
U	:	units of enzyme
Mol. wt.	:	molecular weight
nm	:	nanometer
Ci	:	Curie
mCi	:	milli Curie
uCi	:	micro Curie
cpm	:	counts per minute
A	:	Adenine
T	:	Thymine
G	:	Guanine
C	:	Cytosine
dCTP	:	deoxycytidine 5'- triphosphate
dNTP	:	deoxynucleotide 5'-triphosphate
Tris	:	Tris - hydroxymethyl amino methane
SDS	:	Sodium dodecyl sulphate

EDTA	:	Ethylene diamine tetra acetate
TBE	:	Tris-Borate-EDTA buffer
cv.	:	cultivar
SSC	:	Saline sodium citrate
kcal	:	kilo calories
mol	:	mole
mM	:	milli mole

LIST OF FIGURES

Figs. 5.4a

to 5.4f: Free energy distribution comparison between rice glutelin and rice prolamin (5.4a), zein (5.4d), hordein (5.4e) and gliadin (5.4f); wheat glutelin and gliadin (5.4c). Breslaur's free energy values are used with sliding blocks of 12 bases. The genes are aligned at the translation start site (position 0) and the length of DNA sequence analyzed is from -250 to +10 bp. Legend
5.4a : x rice glutelin and - rice prolamin
5.4b : x rice glutelin and - wheat glutelin
5.4c : + wheat gliadin and - wheat glutelin
5.4d : x rice glutelin and - zein
5.4e : x rice glutelin and - hordein
5.4f : x rice glutelin and - gliadin

LIST OF TABLES

SYNOPSIS

Rice is a self-pollinated crop and, therefore, has most of the problems associated with the self-pollinated system like lack of heterosis or sufficient vigour and lack of variability. These two areas have a direct impact on the breeding of this crop for important parameters like yield. Several attempts were made and are being made to identify sufficiently diverse parents in order to achieve the above mentioned objectives, one of this being the inter-racial or inter-subspecific crosses like indica x japonica or indica x javanica. The indica x japonica combination is better than the other as indica and japonica are more diverse.

A wealth of information is available regarding the variations between indica and japonica rices based on conventional characters. Taking these observations as the base, many programmes were initiated to derive suitable hybrids from an indica x japonica cross. These programmes were however, hindered due to some serious problems like high degree of F1 sterility and skewed segregations giving rise to the unavailability of desirable recombinants (1). To understand these problems, studies were carried out with the then available tools viz. morphological characters, biochemical markers and cytogenetical

approaches (2,3,4,5). These studies pointed out many a possibilities of which the major ones were genic causes and chromosomal structural alterations. (6,7,8,9). With a view of reassessing the genetic diversity amongst the indicas and japonicas, Glaszmann (4) made an attempt to study the genetic variation in rice using isozymes and reclassified Oryza sativa into six groups of which group I and VI corresponded to indica and japonica rices, respectively.

With the advent of RFLPs, Wang et al. (10) and Zhang et al. (11) studied RFLPs in Oryza sativa L. and concluded that indica rice was genetically more diverse than japonica and that the conventional indica-type could not clearly group together as a single unit whereas the japonica could group as a single unit. In addition to these findings, McCouch et al. (12) reported the skewing of some loci during segregation in an indica x javanica cross towards the preference for indica alleles. All these studies, even though did reveal some interesting facts about indica-javanica relationships, could not probe into the cause of occurrence of skewing and semi-sterility in the indica-javanica hybrids. A comparative analysis of molecular and morphological variation between indica and japonica subspecies of rice was, therefore, carried out with an aim of gaining an understanding about the genetic

divergence and separating mechanisms operating between these two subspecies.

Summary of work

In the study of indica-japonica comparison, a total of four aspects were considered and the details of these aspects are summarized as follows :

1. Assessment of genetic variability between indica and japonica rice cultivars by using RFLP and morphological characters

As a first step to assess the genetic heterogeneity between indica and japonica cultivars, I decided to study the morphological and molecular variability at the varietal level. For this purpose, I selected 5 cultivars representing each group viz. Ambemohar-157, Barmolbora, Basamti-370, Malkolam, Manharasali (indica) and Akawa, Habakati, Kerunmochi, Ritutonorin, Taichung-65 (japonica) as my sample population for the study. These were screened for morphological characters namely plant height, number of tillers, days to flowering and panicle length. For the RFLP analysis, four probes viz. pOSM1C-2, pOSM1D-9, pOSM5F-3, and mouse rDNA (18S subunit) and two enzymes EcoRI and BglI were used to construct a dendrogram using a computer program written by me.

The important findings of this study were as

follows :

1. The indica and japonica cultivars grouped into two groups based on morphological characters.
2. Of the 4 probe-enzyme combinations used, 3 could distinguish between indica and japonica whereas one could distinguish most of the cultivars from each other.
3. The dendrogram based on Nei's F values did group the indicas into one group and japonica into one group. But when the dendrogram based on genetic distance was constructed, it revealed an entirely different pattern.

Thus, though the indicas and japonicas can be distinguished based on their morphological characters, the pattern of molecular variation seems to be more complicated to enable clearcut differentiation of the two. Some of the sequences show their involvement in the differentiation process by revealing subspecies specific nature.

2. Segregation at the F2 level of an indica - japonica cross for the morphological traits

Another aspect of genetic diversity assessment is the study of the pattern of recombination and behaviour of hybrids of the relevant taxa. Recombination can be best studied at the F2 level (the F2 progeny shows maximum variation as compared to the

BC1 population) as it enables one to visualize the recombinational events by looking at the products of recombination that appear in the progeny. In order to study the above mentioned aspect, I selected two cross combinations viz. indica x japonica and indica x indica - japonica derivative for the study of the segregation of morphological traits. The parents selectd were as follows : Basmati 370 as an indica, Taichung 65 as a japonica and Adt 27 as an indica - japonica derivative. The F2 progeny when studied revealed the following important features

1. There was more skewing of quantitative traits as compared to the qualitative traits
2. The skewing was towards indica type
3. Bushy plant types with less to no productive tillering were observed
4. Grain type showed preference of japonica type.

The quantitative traits tend to show more of skewing than the qualitative traits suggesting the occurrence of small multiple changes than major changes in the genetic structure as the cause for unavailablity of proper intermediates between the indica and japonica subspecies. This probably maintained the subspecies integrity of indica and japonica acting a separating mechanism.

3. Analysis of RFLP markers segregating in the indica -

japonica cross at the F2 level

In the previous section, I have described the use of the F2 progeny for segregation studies using morphological characters. In this section the data on segregation of RFLP markers in the same population is presented.

Initially to determine the most polymorphic cross, I used 8 probes and 6 enzymes to assess the level of polymorphism exhibited by both the crosses viz. Bamsati-370 x Taichung-65 and Basmati 370 x Adt 27. Basmati 370 x Taichung 65 revealed polymorphism for 24 probe-enzyme combinations as compared to 11 in the Bamati-370 x Adt-27, and, therefore, the former cross was selected for the analysis. The cross was then analyzed for the segregation of 5 clones viz. pOSM1C-2, pOSM1D-9, pOSM5F-3, rice glutelin cDNA and mouse 18S rDNA using BglI as the enzyme. The results of this analysis are as follows :

1. All the probes revealed multiple loci wherein on an aggregate 14 loci are deciphered.
2. A minimum of 4 and a maximum of 7 plants did not carry the alleles which is present in both the parents.
3. rDNA and glutelin showed a differential organization in Basmati-370 and Taichung-65.
4. A few loci showed skewing in the progeny.

These results indicate the presence of chromosomal

alterations in the indica-japanica hybrids at a very finer level, so far undetected by conventional methods. Our finding supports the element of chromosomal changes involved in the differentiation of indica and japonica.

4. A computational analysis of 5' upstream DNA sequences of rice (*indica* and *japonica*) genes

I attempted to study the DNA sequence variation of 5' upstream regions of the published rice gene sequences with a view to study their phylogenetic implications, as such studies have not be carried out in rice. Secondly the regulatory domains provide a good system for study as they are slow as well as gradually evolving types and contribute to the existing information regarding evolutionary relationships. A total of 18 rice genes were used for the analysis of which 13 were derived from japonica and 5 from indica. These domains were assessed for the homology exhibited amongst them and the free energy distribution in the 5' regions of genes contributed to by the dinucleotides. (For this analysis the computer program was written by me). The results of this analysis are as follows :

1. The dendrograms constructed using homology percentages revealed that the indica and japonica types could not cluster into separate groups.

2. Free energy distribution of indica and japonica showed a variation, where japoncia had more free energy as compared to indica.
3. The frequencies of AA, AG, CA, CG, and TG showed significant differences between the two.

Actual DNA sequence comparisons reveal the complex pattern of variation between the two subspecies. This is further demonstrated by the presence of more low free energy contributing elements in indica than japonica, keeping the pattern of high free energy contibuting elements more or less constant in both. Secondly the free energy patterns tend to positively correlate with the gene expression as seen from the example of glutelin and prolamin.

Overall conclusions

Indica and Japonica subspecies are diverse as can be seen from their morphological differences. But this seems to be a fairly simplistic picture, for the pattern of molecular variation reveals complex results. Based on three parameters namely, DNA sequence variation, skewing of quantitative traits and detection of very small deletions, it appears that the japonica probably differentiated from the indicas by small multiple changes rather than major changes.

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CHAPTER 1

Compilation of conventional and modern approaches to evolve a better understanding of indica–japonica interrelationships

1.1 Introduction

Rice belongs to the genus Oryza and has two cultivated species viz. Oryza sativa and Oryza glaberrima which are grown in the Asian and African continents, respectively. The Asiatic cultivated rice belonging to the sativa complex is further classified into two major groups or ecotypes namely, indica and japonica and a third minor group viz. javanica. The two subspecies, indica and japonica, show specific habitat preferences such as tropical by indicas and temperate by japonicas and are restricted to specific geographical regions.

In an attempt to increase the hybrid vigour and thereby yield, it is necessary to cross diverse parents/genotypes. Indica and japonica are genetically diverse and are therefore, good candidates for the crossing programs. The two subspecies, however, offer a barrier in crossing amongst themselves as is seen from very low success rates in indica- japonica crosses. Another problem encountered is the high F1 sterility which causes skewed segregations resulting in the non-availability of suitable intermediates. Several morphological, cytological and biochemical studies have been carried out in order to understand the probable causes

for the two problems in indica and japonica breeding programs. Recently RFLP has been employed to assess the similarities and differences at DNA level between these two - subspecies and these studies have revealed that the two subspecies of indica and japonica differ significantly.

In the present review, the available data on indica and japonica have been considered including the most recent molecular data to gain a better understanding about the phylogenetic relationships and evolutionary status of indica and japonica subspecies of rice and to throw some light on the problems encountered in the breeding programs between these two subspecies.

1.2 **Geographical, Morphological and Physiological Aspects**

The first systematic study on intraspecific classification of rice has been carried out in 1928 by Kato (1). Based on morphological characters, hybrid sterility data and serological analysis, he has divided about 100 cultivated rice varieties representing different geographical regions, upland and lowland rices, glutinous and non- glutinous rices, normal and long glumed rices, scented and non-scented rices and

also rices with red and white pericarp into indica and japonica subspecies. The studies regarding the geographical distribution of the subspecies reveal that the japonicas include the varieties of northern China, Korea and Japan whereas the indicas include varieties from Burma, Malaya, Taiwan, South China, Java, India and Ceylon (2,3). Subsequent studies have indicated that almost all the cultivars grown in temperate and subtropical regions would fall under the japonica category. The distribution of the japonica is found in the Jeypore tract of Orissa (4,5), Nepal (6), northeast India (7), northern Thailand (8), Vietnam (9) and northern Laos (10). Varieties indigenous to Japan, Korea and northern China belong to the japonica subspecies, whereas those indigenous to India, Burma, Ceylon, Malaysia, Java, Taiwan, south China and other countries mostly belong to the indica sub-species (3).

Matsuo (11) and Oka (12, 13) have used the `phenol reaction' test, degree of endosperm disintegration in KOH solution, and a few other diagnostic tests in the classification of the varieties and have pointed out the existence of a continuous variation in these characters. Recently, Takahashi and Hamza have studied the colour reaction with phenol to differentiate ecotypes in Oryza sativa L. (14). Considering the

TABLE 1.1

CHARACTERISTICS OF *SATIVA* RACES (a,b)

	Character	A:japonica	B:javanica	C:indica
1.	Grain shape	Short	Large	Narrow
2.	Length of 2nd leaf blade	Short	Long	Long
3.	Angle between 2nd leaf and stem	Short	Small	Large
4.	Texture of plant parts	Hard	Hard	Soft
5.	Angle between flag leaf and stem	Medium	Large	Small
6.	Flag leaf	Short, narrow	Long, wide	Long, narrow
7.	Tiller number	Large	Small	Large
8.	Tiller habit	Erect	Erect	Spreading
9.	Leaf pubescence	None	Little	More
10.	Glume pubescence	Dense	Dense	Sparse
11.	Awns	Usually absent	Usually present	Usually absent
12.	Shattering	Difficult	Difficult	Easy
13.	Panicle length	Short	Long	Medium
14.	Panicle branching	Few	Many	Intermediate
15.	Panicle density	High	Moderate	Moderate
16.	Pancile weight	Heavy	Heavy	Light
17.	Plant height	Short	Taller	Tall

a) Matsuo, T. (1952) Bull. Nat. Inst. Agr. Sci. Japan. D3, 1 - 111.

b) Chandaratna, M.F. (1964) Genetics and Breeding of Rice 389 pp. Longmans Green, New York.

detailed studies on the variability of cultivated rices, it is seen that the morphological variations are more or less continuous (15) and it is not possible to place each and every variety into japonica and indica category. Japonica is characterized by narrow and deep green leaves, wide angle between the top-most leaf and the stem, broad and thick grain, prominently awned to awnless types, erect habit and better response to manuring. Indica is characterized by broad, light green leaves, narrow angle between the topmost leaf and the stem, grains generally slender and somewhat flat, usually awnless with rare occurrence of awned types, thin and short hairs, usually lodging habit and poor response to manuring (16). Compared to japonica, indica shows more rapid germination, higher tiller capacity, lower milling output and more drought and disease resistance. Japonicas, however, are more resistant to low temperature than indicas (17). More detailed studies have been carried out later on the indica and japonica subspecies, results of which are summarized in Table 1.1.

Recently, Ueno et al. (18) have studied rice cultivars belonging to five ecotypes (aus, aman, boro, bulu and tjereh) and two to groups of

Japanese rice (lowland and upland) with respect to physiological characters such as $KClO_3$ resistance, phenol reaction and morphological characters involving apiculus hair length. They state that the aman, boro and tjereh ecotypes should be classified as typical indica, and that the Japanese lowland rice cultivars are mainly typical japonica. Some of the aus, bulu and Japanese upland rice cultivars differ from typical indica and typical japonica and hence the respective terms aus type, bulu type and Japanese upland rice type have been proposed. Finally they have concluded that lowland rice cultivars could be clearly grouped into indica and japonica whereas upland cultivars could not be grouped.

In another recent morphological study, Sato (19) has studied variation in the spikelet shape of the indica and japonica rice cultivars in Asian origin and has classified the cultivars into 108 a-type, 273 b-type and 244 c-type and also as 98 temperate japonicas, 255 tropical japonicas and 272 indicas. The coefficient of correspondency (C.C.) for all of Asian cultivars is 0.582 which is too low to suggest that the two classifications viz. (i) based on spikelet type and (ii) based on the habitat, correspond to each other. The differences in grain shape, awning and the

pattern of hairiness of fertile glumes show subspecies differences.

The grouping of the Japanese varieties as a valid subspecies is now generally accepted with an emphasis on certain physiological differences such as the response to differences in mean temperature, duration of basic vegetative growth, and reaction of varieties to variation in day-length in which the subspecies show definite adaptations.

1.3 Karyomorphology, nucleolar organizer and DNA content

An additional line of approach regarding indica and japonica subspecies is the study of variations at the cytological level which include parameters like karyomorphology, nucleolar organizer and DNA content. These studies have been made in order to detect subtle changes amongst the subspecies at the cellular level. In this regard, the first significant report is from Selim (20) wherein he has reported that the japonica has a single nucleolus while the indica and bulu forms have two nucleoli each in their microspore mother cells. He has further attributed the sterility in intervarietal rice hybrids to the difference between the number of their nucleoli. In anoth-

er study, Sakai (21) has observed the behaviour of nucleolar chromosomes in several varieties of rice and has divided the varieties into "bi-nucleolar" and "quadrinucleolar" types corresponding to japonica and the indica types respectively. Later, Oka and Kao (22) have reported that about two thirds of the "continental" varieties and a half of the "tropical-insular" varieties are of quadri- nucleolar type and the mean number of nucleoli ranges from 2.2 to 3.6. They further state that rice might have two pairs of nucleolar chromosomes and the mean number of nucleoli indicates that the nucleolus forming power of one pair of chromosomes is relative to the other.

In order to obtain a clearer picture, Misra and Shastry (23) have studied the karyotype variation in Oryza sativa and have found considerable variation at the varietal and specific levels and have concluded that the bulu or javanica and japonica forms are more evolved and have more asymmetrical karyotypes than that of subspecies indica. This observation of asymmetry of karyotypes may favour the hypothesis that the indica rices have given rise to japonica forms by structural changes either of larger magnitude like reshuffling of satellites or of smaller magnitude like small deletions or translocations. Another interesting

finding has been reported by Shastry et al. (24) who have noticed for the first time the presence of supernumerary nucleoli in O. sativa at pachytene. In the light of the above information, a study of karyomorphology in different species of Oryza by Misra (25) reveals that the species and the varieties within a species show great amount of variation in their nucleolar chromosomes.

In order to analyze any probable variation in the total nuclear DNA content in the two subspecies, Nagato et al. (26) have found that the relative nuclear DNA content of indicas is 66.5 ± 10.8 whereas that of the japonicas is 60.0 ± 9.0. They further conclude that in Asian rice, mean DNA content has decreased in the course of speciation (domestication).

1.4 **Genetic Approaches**

Extensive studies using genetic approaches have been carried out to understand the sterility problem in indica - japonica hybrids and several divergent views including gene action, accumulation of mutations and some unknown phenomena have emerged as the causes of sterility. According to Henderson (27), single inversions, even if numerous, will not account

for the higher degree of sterility. The absence of evidence for translocations in the indica-japonica hybrids combined with the cytological proof that inversions are commom has led Henderson (27) to the opinion that sterility in inter varietal hybrids of cultivated rice may be a result of cryptic structural hybridity arising from included inversions. Cryptic structural hybridity cannot be detected at meiosis by the above criteria but can be inferred by more indirect criteria namely reduction in quadrivalent frequency in amphidiploids and block transfer of characters (28). Shastry and Misra (29) have clearly established that the sterility in intervarietal rice hybrids is due to cryptic structural changes in chromosomes rather than due to genic causes. For example, in some hybrids, as much as 31.04% of the chromosome length is involved in pairing abnormalities. The chromosomal differentiation between japonica and indica rices, as reflected in pairing at pachytene, strongly suggests that these two subspecies should be treated separately in linkage studies, as pointed out by Shastry and Misra (30). Oka (31), on the other hand, states that the intervarietal F1 sterility in Orzya sativa is characterized by modification of segregation ratios (restric-

tion in recombination of independent genes) and occurrence of partly sterile or weak segregants .

According to Samptah et al. (32), the occurrence of sterility in hybrids between varieties of the indica zone (monsoon lands of India, Burma, Indonesia, Thailand and South China) and those from Japan, North China and Northern half of Taiwan provides a clear-cut expression of racial differentiation.

1.5 **Isozyme variation**

Isozymes in general provide a very useful dimension for assessment of genetic variability and phylogenetic analysis as a single gene product can be traced in different taxa. Table 1.2 lists isozyme loci showing marked differences in allelic frequency between the indica and japonica types. Based on a survey of the esterase isozymes in an Asian collection of O. sativa, Nakagahara (33) has concluded that genetic diversity of Oryza sativa centres around Indochina and Burma. It is observed that the indica and japonica types classified on the basis of character association are clearly related to isozyme genotype comprising Pgi A, Pgi B, Cat A and Est - 2, further

Table 1.2

Isozyme loci differing in allelic frequency between indica and japonica

Locus	Type (no. of cvs.)	Frequencies of alleles				Ref.
		-4	+9	null		
Acp-1	Indica (197)	0.981	0.015	0.004		1
	Japonica (295)	0.041	0.959	0.000		
		4C	null			
Px-2	Indica (21)	0.952	0.048			2
	Japonica (20)	0.100	0.900			
		S	F	null		
Est-2	Indica (185)	0.686	0.157	0.157		3
	Japonica (332)	0.033	0.012	0.955		
		S	F	null		
Est-3	Indica (185)	0.078	0.859	0.063		4
	Japonica (332)	0.926	0.015	0.009		
		1	2			
Pgi-A	Indica (78)	0.756	0.244			4
	Japonica (75)	0.013	0.987			
		1	2	3	4	
Pgi-B	Indica (77)	0.351	0.623	0.013	0.013	4
	Japonica (75)	0.933	0.067			
		1	2			
Cat-A	Indica (61)	0.968	0.032			4
	Japonica (61)	0.082	0.918			

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referred to as 1, 2, 3 and 4, respectively. The genotypes (with reference to above mentioned four isozymes) of japonicas including so-called Javanica type are 21--, while indicas are either 11--, 12--, 22-- or 23-- (34). Later, in his pioneering effort, Glaszmann (35) has reclassified Oryza sativa L. by surveying 1688 traditional rices from Asia for 15 polymorphic loci coding for 8 enzymes. Multivariate analysis of the data has resulted in identification of six varietal groups with two major ones (groups I and VI), two minor ones (groups II and V), and two satellite ones (groups III and IV). Group I is found throughout tropical Asia, and it encompasses most Aman rices in Bangladesh, the Tjereh rices in Indonesia and the Hsien rices in China. Group VI is found mostly in temperate regions and in high elevation areas in the tropics; it encompasses most upland rices from Southeast Asia, the Bulu rices from Indonesia and the Keng rices from China. Groups II and V are found in the Indian sub-continent from Iran to Burma viz. Aus rices from Bangladesh (group II) and Basmati rices from Pakistan and India (group V). Groups III and IV are restricted to some deepwater rices in Bangladesh and Northeast India. Based on the analogy with other classifications, group I might be considered as the indica type

and group VI as the japonica type. Comments on gene diversity and intergroup differentiation by Glaszmann suggest that the most polymorphic groups are I and V, followed by II and VI, then V and lastly III. The diversity of groups I and V suggests possible further subgroupings with one subgroup consisting of I , II and III and other subgroup consisting of IV, V and VI. According to Glaszmann, Basmati 370 is the only variety of enzymatic group V, which has an indica type morphology.

During the comparison of IRRI and IBPGR classification systems, Glaszmann (35) has found that many varieties described as indica also belong to group VI, and most of them are upland rices. There is an agreement between the two classifications when the variation is restricted to tropical indica versus temperate japonica. However, there is no concordance when the variation is more complex like in the hilly areas of Southeast Asia or in the Indian subcontinent. Some varieties differ from the typical indica and japonica types and are present mostly in the Indian sub-continent. These kind of varieties represent more than a third of all the varieties and especially those along the Himalaya foothills. These varieties show a

differentiation analogous to that of indica - japonica differentiation but the extent of differentiation is much smaller. One may assume that this ensemble which differs considerably from the typical indica and japonica has arisen from alternative evolutionary processes.

1.6 **Evolutionary aspects**

An understanding of the evolutionary process leading to the formation of different rice races would help in the better utilization of the various types of rice and also would facilitate the synthesis of new varieties of cultivated rices. In order to understand the effect of evolutionary pressure at the chromosomal level, Shastry and Misra (29) have carried out a pachytene analysis of the genus Oryza and have suggested that the subspecies, japonica and indica, are differentiated by a series of small structural differences in their chromosome complements. Based on their data regarding the unravelling of a high degree of structural hybridity, they have referred to the sterility in the hybrid progeny, an important parameter in the process of indica - japonica differentiation, as being a result of recombination. The recombination leads to the formation of different density of segments

varying in their genetic composition. These segments in combination with the varying degrees of tolerance for deletions in different chromosomal regions determine the extent of sterility of indica - japonica hybrids. It is also envisaged that the major line of evidence of the relationship between differential nature of chromosomes, F1 sterility and occurrence of abnormal phenotypes in hybrid progenies indicates that small translocations have played a significant role in the differentiation of the varieties of these subspecies.

In another study Oka and Chang (36) have shown that the indica and japonica are monophyletic in origin and the distance between them increases as the indica and japonica approach their cultivated forms. A line of thought that has emerged from their studies is the involvement of duplicate genes in the differentiation of the indica and japonica subspecies. When genes are duplicated, a recessive mutation at one locus may be concealed by the non-mutated duplicate gene at the other locus, so that genic changes which are otherwise fatal may be retained in the population. Further, the differences in duplicate genes will result in the occurrence of restricted to free recombination

of other independent genes as certain genotypes are eliminated by gametic or zygotic selections. In view of these aspects, Oka and Chang (36) have suggested that the potentiality of wild rice to produce different cultivated types such as the indica and japonica is essentially due to the duplication of genetic materials. However, if many recessive mutations or deficiencies of duplicate genes are combined at random, they will give rise to various genotypes which are mutually partially isolated by different kinds of barriers rather than resulting in a few major groups such as the indica and japonica. Therefore, it cannot be accepted in totality that duplication of genes is the cause of the differentiation of indica and japonica. This concept finds support in Oka's (37) report wherein a situation analogous to the above has been found among the derivatives of a hybrid between an indica and a japonica variety. In such a case, the indica - japonica differentiation could be more clearly recognized by character association rather than by hybrid sterility relationship (hybrid sterility is considered here to be the result of the breeding of the mutually partially isolated genotypes). This suggests that the partial sterility of indica - japonica hybrids is rather an outcome of dif-

ferentiation which has been advanced by several factors. One such factor is the differential adaptability of the two types to their environments which acts as a forceful agent in promoting indica-japonica differentiation. This is supported by the fact that northern Thailand has rice varieties grown in the mountain region resembling japonicas and those in the low valleys resembling indicas.

In a different kind of study of indica - japonica differentiation, Sampath and Seetharaman (32) have attempted to look at this event from the viewpoint of the progenitors of indica and japonica. The study of the interrelationships between the proposed progenitors and their derivatives reveals a wealth of information about the differentiation of the derivatives from the progenitors and the following three conclusions have been drawn (i) Wide distribution of forms of O. perennis is the putative ancestral, cosmopolitan and widely variable wild species. (ii) Occurrence of natural crossing to a considerable extent between the progenitor and their derivatives in the regions of initial race formation. and (iii) The consequent prevalence of variable spontanea types in the region of indica - japonica differentiation suggests that intro-

gressive hybridization between the progenitor and its derivatives has played an important role in the origin of subspecies in the cultivated rices of Asia.

Stebbins (38) in his studies on evolution of karyotypes has recognized 12 classes of which class 1a is most symmetrical and is encountered in primitive taxa whereas the class 4c is most asymmetrical and is encountered in most advanced taxa. Based on the above classification Misra and Shastry (23) have found that the karyotypes of japonica fall in the class 2c, whereas the karyotypes of all the indicas fall under class 2b. The karyotypes of two javanicas are divergent - one falling under 2b and the other under 2c. Hence it is obvious that the japonica types investigated have more asymmetric karyotypes in comparison to the indica types which are presumably more primitive. In their continued effort of studying the causes of indica - japonica differentiation at the chromosomal level Misra and Shastry (23) have observed that the japonicas exhibit less chromatin length indicating the probable operation of chromosomal diminution in subspecific differentiation.

Recently, Sato (39) has studied the evolutionary dynamics of the indica-japonica differentiation from the viewpoint of population genetics.

Indica and japonica are distinguished by genes and characters associated with each other nonrandomly. Since in the indica/japonica hybrid progenies, the same direction of gene and character association is found as amongst the parent cultivars, a trend towards restriction of recombination among several independent loci can be inferred. Accordingly, intermediate types between indica and japonica are relatively infrequent, even if natural hybridization occurs frequently between them. Hence it is concluded that indica and japonicas are isolated by restrictive recombination in hybrids. In an attempt to study the genetic diversity and inter-varietal relationships in rice (Oryza sativa L.) in Africa, Pham (40) has concluded that the indica-japonica distinction as revealed by isozyme studies corresponds to a distinction based on reproduction barriers like hybrid sterility and abnormal transmission of genetic information in F2 progeny. The evolutionary trends described so far clearly indicate that the japonicas are more evolved than the indicas and that they have a monophyletic origin. Secondly, the environmental factors along with the genetic factors seem to have influenced the differentiation of the indica and japonica subspecies and the

pattern of their differentiation is certainly complex.

1.7 **RFLP Approach**

With the advent of molecular tools like RFLP, it has become possible to track a DNA fragment like a Mendelian gene. Secondly the molecular markers are virtually limitless in number as against the limited number of morphological and biochemical markers. The RFLP markers are devoid of any environmental effects and hence can be used to screen any plant material from any geographical region with the guarantee of obtaining the same results. These DNA markers offer the possibility to screen any plant part and yet give a complete picture about the genotype. Lastly the technology involving RFLP markers is speedy, reliable and very efficient and it has opened up new vistas in the study of phylogeny amongst different taxa as it is exciting to observe the differences detected in the same sequence in different taxa.

Using the RFLP technique, Wang *et al.* (41) have studied variations in *Oryza sativa* L. by surveying 70 varieties polymorphic for 10 RFLP markers. Based on genetic distance calculations, the ratio of the genetic variation between versus within rice varieties has been estimated to be approximately 12:1. From

the RFLP based dendrogram, conventional indica - type varieties (tropical rice) cannot be clearly grouped together as a single unit whereas all the japonica type (temperate rice) group into one cluster. Secondly, several indica varieties cluster together with japonica type varieties indicating that these indica varieties may have been originally misclassified. This possibility is also supported by isozyme data in which these same indica varieties clustered with the japonica group (35). Analysis with additional probes and varieties may shed more light on this issue. Recently, Zhang et al. (42) have studied the genetic diversity and differentiation in indica and japonica groups of cultivated rice (Oryza sativa) by assaying RFLPs amongst 12 indicas and 14 japonicas with three enzymes and 49 probes derived from the RFLP map constructed by S.D.Tanksley. The results demonstrate that the indica rice is genetically more diverse than the japonica rice. Significant differentiation between the two rice groups are detected by 33 probes representing 11 of the 12 rice chromosomes. It is further observed that the process leading to differentiation has probably involved a combination of molecular events including base substitutions

and insertions/deletions.

RFLP provides a new molecular tool with extremely high resolving power and high reliability to study the distribution of loci in a segregating progeny. In order to contruct a RFLP map, McCouch et al. (43), have studied the indica x javanica cross for the segregation of RFLP markers wherein they have found skewed segregations occurring in favour of indica alleles. Further inferences have yet to be drawn from the above data as more studies in this context are necessary. Cordesse et al. (44) have cloned a rice rDNA probe and have analyzed 105 accessions including 58 cultivated and 47 wild rices. They have found a general tendency in the japonicas to have a smaller spacer than the indicas and that the classification as japonica or indica based on rDNA pattern agrees well with the classification based on isozyme pattern.

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CHAPTER 2

Use of a few low copy repeat sequences from a rice landrace to differentiate, fingerprint and decipher subspecific sequences in five indica and japonica cultivars

CHAPTER II

Summary

Use of three low copy repeat DNA clones derived from a landrace cv. Malkolam is described in differentiating and fingerprinting five indica and japonica cultivars. Two repeat clones viz. pOSM1C-2 and pOSM5F-3 reveal the ability to differentiate between the indica and japonica cultivars. The pOSM1C-2 clone enables to identify japonica specific fragments in the range of 3.4 kb to 1.3 kb. The third clone, pOSM1D-9 can differentiate eight out of ten cultivars from each other based on its hybridization pattern or DNA fingerprints. It shows one major band common to indicas except Manharsali and one to the japonicas while the other bands vary in the range of low (5.7 kb) to high (19.3 kb) molecular weight.

2.1 Introduction

Indica and japonica, the two subspecies of cultivated rice Oryza sativa, have been extensively studied using morphological, biochemical and cytological aspects. In early fifties and sixties, attempts have been made to introgress a few good characters of japonica into indica and vice versa using conventional methods. However, the success in this work has been rather limited due to lack of availability of sufficient molecular markers. With the advent of RFLP, it has been possible in recent years to develop DNA fingerprints and species or subspecies specific probes. For example, Dallas (1) has used the human minisatellite probe to fingerprint indica and javanica cultivars. Later, Wang et al. (2) have studied RFLPs in Oryza sativa L. by surveying 70 varieties polymorphic for 10 RFLP markers. From the RFLP based dendrogram, conventional indica - type varieties (tropical rice) can not be clearly grouped together as a single unit whereas all japonica type varieties (temperate rice) did cluster into a single group. Kochko et al. (3) have used a tandem 352 bp repeat from rice to distinguish between the indica and japonica subtypes of O. sativa. Recently Zhang et al. (4) have studied the genetic diversity and differentiation in indica and japonica groups and have demonstrated that indica rice

is genetically more diverse than japonica.

From the above studies, it is clear that no specific attempts have been made in identifying indica or japonica specific sequences or development of their DNA fingerprint profiles. Such an information will definitely aid in obtaining hybrid varieties having the good of both indica and japonica subspecies. In the present work, I describe the use of repeat DNA sequences derived from a landrace cv. Malkolam for the differentiation between a few indica and japonica cultivars. The DNA sequences being from a conserved gene pool have enabled me to detect japonica specific sequences and helped to differentiate and fingerprint five indica and japonica cultivars.

2.2 Materials and Methods

2.2.1 Plant Material

Table 2.1 gives the details of the cultivars used for the analysis. These cultivars were obtained from Dr. A. Krishnamurthy, Cuttack., Prof. S.D.Kalke, ARS Vadgaon(Maval), and Prof. Verghese, ARS Palghar, and were maintained at the College of Agriculture, Pune where facilities were kindly made available by Prof. B.S.Shah and Prof. D.R.Bapat, Mahatma Phule Agriculture University, Rahuri. The phenotypic observations were

List of cultivars

S.No.	Name of the cultivar	Source from where obtained	Classification
1.	Ambemohr - 157	ARS Vadgaon(Mawal), Pune	Indica
2.	Barmolbora	CRRI, Cuttack	Indica
3.	Basmati - 370	CRRI, Cuttack	Indica
4.	Malkolam	ARS Palghar, Thane	Indica
5.	Manharsali	CRRI, Cuttack	Indica
6.	IR 64	CRRI, Cuttack	Indica
7.	Akawa	CRRI, Cuttack	Japonica
8.	Habakati	CRRI, Cuttack	Japonica
9.	Kerunmochi	CRRI, Cuttack	Japonica
10.	Ritutonorin	CRRI, Cuttack	Japonica
11.	Taichung 65	CRRI, Cuttack	Japonica
12.	Adt 27	CRRI, Cuttack	Indica - Japonica derivative
13.	Mahsuri	ARS Vadgaon(Mawal), Pune	Indica - Japonica derivative

recorded at ARS Vadgaon (Maval) for two seasons.

2.2.2 Plant DNA extractions

2 - 3 week old tissue (leaves) was harvested, immediately frozen in liquid nitrogen and stored at -70^{o}C. The plant DNA extractions were done according to Shure et al. (5).

2.2.3 Restriction enzyme digestions, Agarose gel electrophoresis and Southern blotting

10 - 30 ug plant DNA was digested overnight at 37^{o}C with two restriction enzymes EcoRI and BglI (5U/ug) (Bangalore Genei Pvt. Ltd., India) as per the manufacturer's instructions. The DNA was then electrophoresed in 1.0% agarose gel in 1X TBE buffer. The gels were then blotted onto Genescreen Plus as per the manufacturer's instructions.

2.2.4 DNA Probes used and Southern hybridizations

The probes namely pOSM5F-3, pOSM1D-9 and pOSM1C-2 were derived from a partial PstI genomic library of cv. Malkolam. A 18s subunit of mouse ribosomal DNA was kindly supplied by Dr. P. Roy, Hindustan Lever Research Centre, Bombay, India.

Plasmid DNA extraction was done according to Dretzen et al. (6) and the DNA was subjected to PstI

digestion followed by elution of insert. About 40ng of the insert DNA was used for labelling. Random primer labelling kits were supplied by Amersham and Bhabha Atomic Research Centre and the DNA labelling was carried out according to their instructions.

Southern hybridizations were performed according to Sambrook et al. (7) using labelled DNA (approx. 1 x 10^8cpm) at 55°C with 5x SSC and 0.1% SDS. The filters were washed with 0.1x SSC and 0.1% SDS at 55°C and autoradiography was done using Amersham Hyperfilms and Kodak X-Omat films.

2.2.5 Data Analysis

Each DNA band on the autoradiogram was considered as an independent unit for the analysis. The Nei's F value and genetic distance were calculated according to Nei (8). The dendrograms were constructed using the UPGMA (9) method on a computer with the help of a computer software developed by me in collaboration with Mr. Dinesh Lagu.

2.3 Results

2.3.1 Morphological characters

The morphological data is presented in two sets viz. (i) a comparison of indica and japonica cultivars and (ii) a comparison between indica, japoni-

Fig. 2.1 : Indica - Japonica comparison of morphological characters (i) panicle length, (ii) plant height, (iii) days to flowering and (iv) number of tillers. Fertilization 100 : 50 : 50 (N:P:K) Row spacing 15 cms and plant spacing 15 cms.

INDICA JAPONICA COMPARISON BASED ON MORPHOLOGICAL CHARACTERS

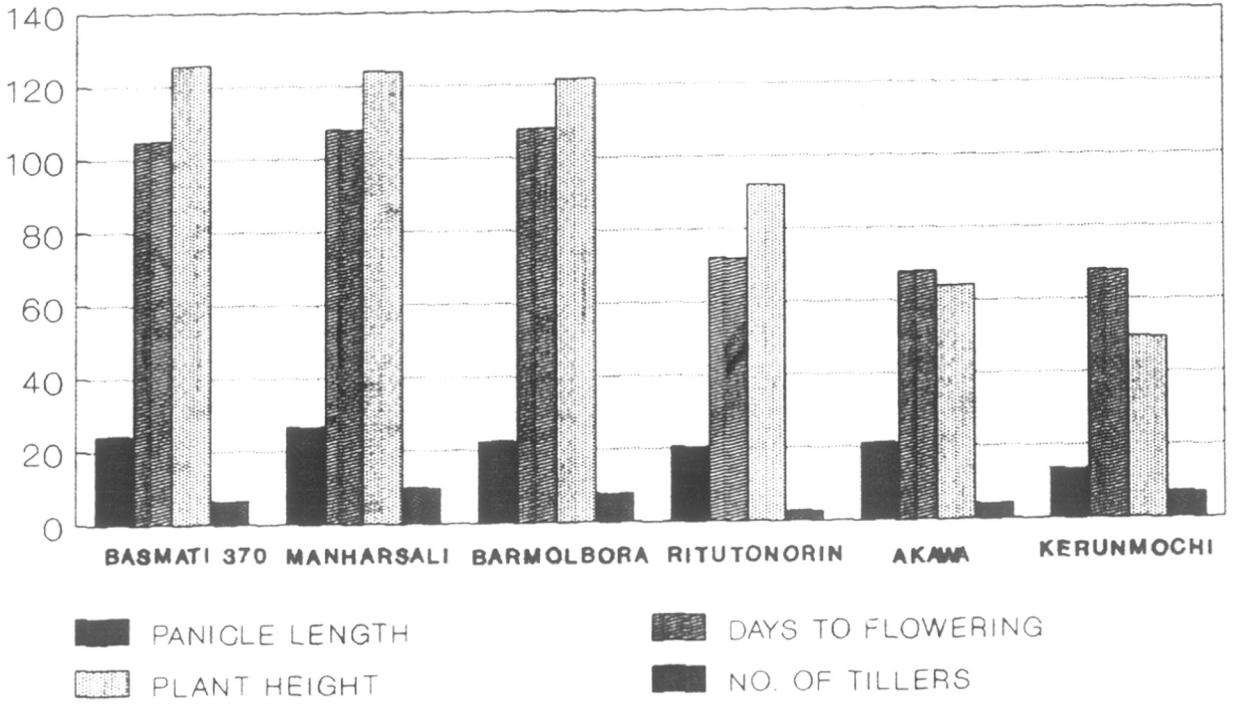


FIG 2.1

ca and indica-japonica derived cultivars. These two sets of data will give some insight about the possible relationships i.e. indica - indica, indica - japonica and japonica - japonica at the morphological level under tropical conditions.

(i) A comparative data for indica and japonica cultivars was collected for plant height, number of tillers, panicle length and days to flowering and is depicted in Table 2.2 and Fig. 2.1. From these data, it can be deduced that Basmati 370, Manharsali and Barmolbora form a group whereas Ritutonorin, Akawa and Kerunmochi form another group. The panicle length is longest in Manharsali and shortest in Kerunmochi. In indicas, the panicle length ranges from 22 to 26 cms whereas in japonicas it ranges from 13 to 21 cms. Referring to days to flowering, indicas range from 105 to 108 days whereas japonicas range from 68 to 72 days only. In terms of plant height, indicas are the tall ones ranging from 121 to 125 cms and japonicas are the dwarf ones ranging from 49 to 92 cms. The tiller number is higher in indicas ranging from 6 to 10 and lower in case of japonicas ranging from 3 to 7.

(ii) Table 2.3 and Fig. 2.2 present a comparative data of three morphological characters viz. plant height, number of tillers and panicle length of IR64, Malkolam,

Table 2.2

Screening for morphological characters of indica and japonica cultivars

S.No.	Name of cultivar	Plant height	No. of tillers	Panicle length	Days to flowering
1.	Basmati 370	125.7	6.4	24.2	105
2.	Manharsali	124.1	9.8	26.6	108
3.	Barmolbora	121.6	7.7	22.4	108
4.	Ritutonorin	92	2.7	20.3	72
5.	Akawa	64.1	4.1	21.1	68
6.	Kerunmochi	49.6	7.0	13.4	68

Table 2.3

Screening for morphological characters of indica, japonica and indica - japonica derivatives

S.No.	Name of cultivar	Plant height	No. of tillers	Panicle length
1.	Malkolam	78.0	15.0	17.3
2.	IR 64	60.4	39.1	18.3
3.	Basmati 370	111.8	49.9	24.2
4.	Adt 27	92.6	19.0	21.3
5.	Mahsuri	101.5	35.0	25.7
6.	Taichung 65	96.1	19.4	22.2

Fig. 2.2 : A comparison of indica, japonica and indica - japonica cultivars based on (i) plant height, (ii) number of tillers and (iii) panicle length. Fertilization 100 : 75 : 75 (N:P:K). Row spacing 45 cms and plant spacing 30 cms.

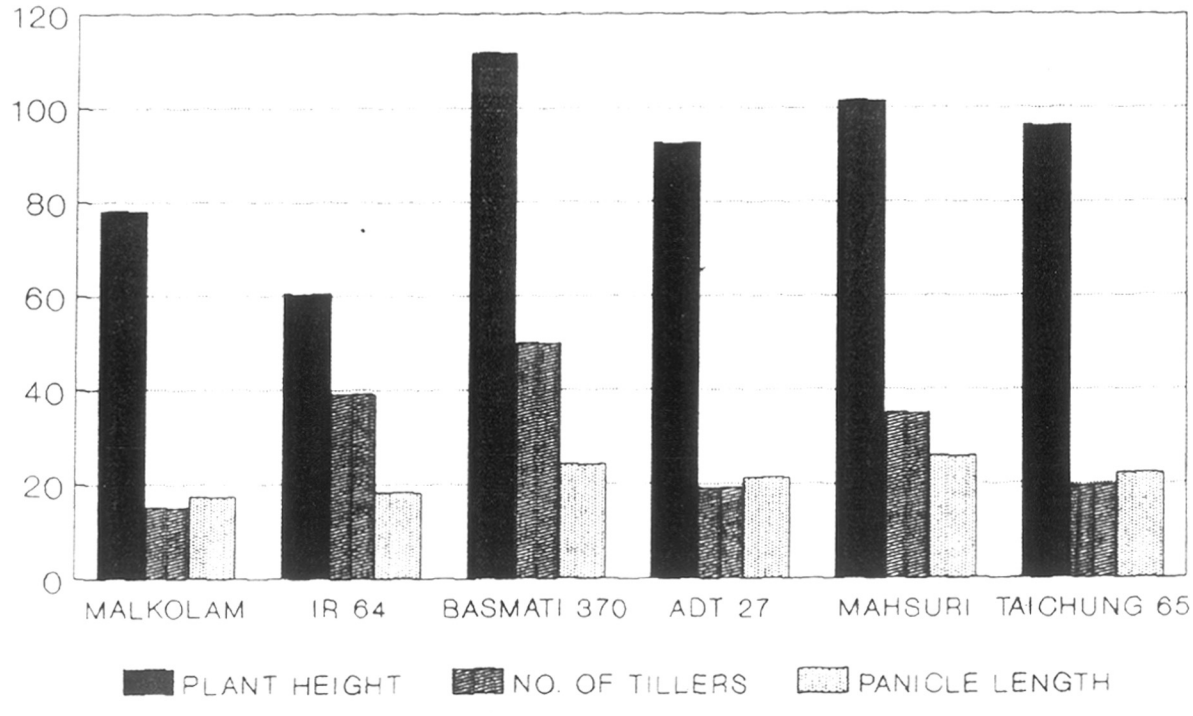

A COMPARISON OF INDICA, JAPONICA AND INDICA-JAPONICA CULTIVARS
120
100
80
60
40
20
0
MALKOLAM
IR 64
BASMATI 370
ADT 27
MAHSURI
TAICHUNG 65
PLANT HEIGHT
NO. OF TILLERS
PANICLE LENGTH

FIG 2.2

Basmati 370 (indica), Adt 27 , Mahsuri (indica - japonica derivative) and Taichung 65 (japonica). There is a lot of variation in these six cultivars in terms of plant height. The indica group comprising IR64, Malkolam and Basmati 370 exhibits a plant height ranging from 60 cms to 111 cms (The dwarfness in IR64 is due to the presence of DGWG gene or the Dee-Gee- Woo-Gen gene). On the other hand, the japonica Taichung 65 and the other two indica - japonica derivates viz. Adt 27 and Mahsuri (both derivatives of the Mayang Ebos 802 x Taichung 65) have plant height ranging from 92 to 101 cms. The number of tillers is maximum in case of Basmati 370 and minimum in case of Malkolam (Malkolam being an early cultivar, it has a low tiller number). But in comparison to Taichung 65 (19), Adt 27 (19) and Mahsuri (35), the indica types have a higher tiller number (The fertilization and the plant spacing have played a role in giving such high number of tillers in the latter data as compared to the former data.) The panicle length seems to be more in Basmati 370 and Mahsuri, slightly smaller in Adt 27 and Taichung 65 and lowest in IR 64 and Malkolam.

The overall morphological data thus indicate that there is more variation within indica as compared to japonica and that the japonicas and their derivatives do not appear to perform well under tropical

conditions.

2.3.2 RFLP analysis

In all 8 DNA probes and two restriction enzymes were used in the analysis of 5 indicas viz. Ambemohr -157, Barmolbora, Basmati 370, Malkolam, Manharsali and 5 japonicas viz. Akawa, Habakati, Kerunmochi, Ritutonorin, Taichung 65. About 10 probe - enzyme combinations were tried of which four were found to be polymorphic. Out of the four probes, one was a dispersed repeat, two were moderate repeats and one tandem repeat. Since repeated DNA sequences have a higher turn over rate as compared to the single - copy sequences, they exhibit more sequence diversity as compared to the other sequences. Hence generally the species specific or genome specific sequences are repeats. In our analysis, repeats were used in order to track the variations in the non-coding regions. The results with each probe - enzyme combination will be dealt with separately in the following paragraphs :

(i) pOSM5F-3/BglI

In the autoradiogram (Fig. 2.3) a clear-cut difference is observed between the indica and japonica cultivars. In indicas, there is a variation in terms of bands of different molecular weights. In japonica, one band is present at 6.2 kb which is intense in

Fig. 2.3 : Autoradiogram depicting hybridization of pOSM5F- 3 insert with EcoRI digests of DNAs of indica and japonica cultivars. lane a - Ambemohr 157, lane b - Barmolbora, lane c - Basmati 370, lane d - Malkolam, lane e - Manharsali, lane f - lambda/Hind III molecular weight marker, lane g - Akawa, lane h - Habakati, lane i - Kerunmochi, lane j - Ritutonorin and lane k - Taichung 65.

Fig. 2.4 : Autoradiogram showing hybridization of pOSM1C-2 insert with EcoRI digests of DNAs of indica and japonica cultivars. lane order same as Fig. 2.3.

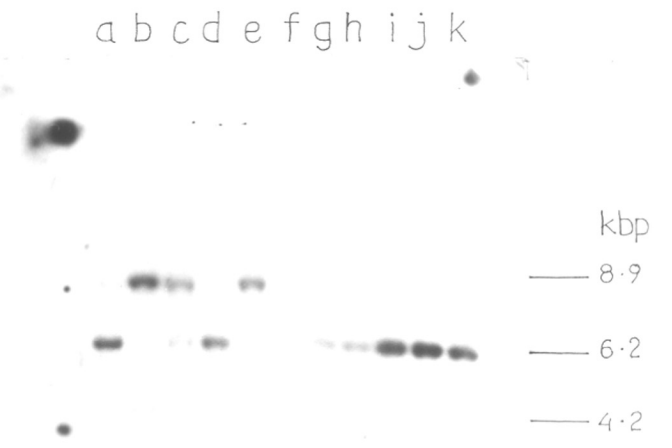

a b c d e f g h i j k
kbp
8·9
6·2
4·2

Fig. 2·3

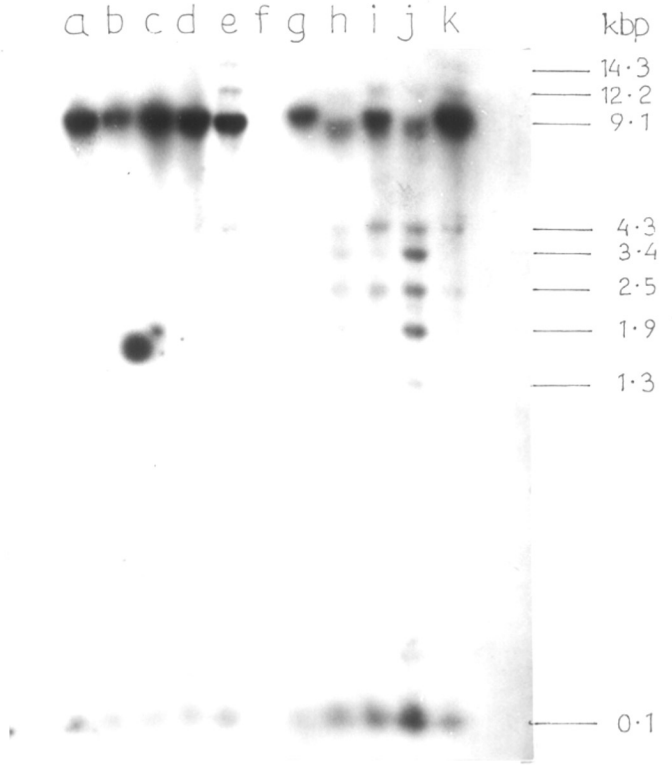

a b c d e f g h i j k
kbp
14·3
12·2
9·1
4·3
3·4
2·5
1·9
1·3
0·1

Fig. 2·4

Kerunmochi, Ritutonorin and Taichung 65 and faint in Akawa and Habakati indicating copy number variation.

(ii) **pOSM1C-2/BglI**

The autoradiogram in Fig. 2.4 reveals that the probe pOSM1C-2 has the ability to differentiate between the indica and japonica cultivars. Two bands viz. 9.1 kb and 0.112 kb are common to all the cultivars. The 14.3 kb band is present only in Manharsali and Taichung 65 whereas the 12.2 kb band is present as a faint but clear band in one indica (Manharsali) and four japonicas (Habakati, Kerunmochi, Ritutonorin, Taichung 65). The 4.3 kb band is present more prominently in all the japonicas excepting Akawa whereas it is present as a faint band in all the indicas. Habakati and Ritutonorin exhibit the bands 3.4 kb and 1.9 kb which are absent in all the other cultivars. All the japonicas excepting Akawa show the presence of the 2.5 kb band as a strong band when compared to the faint band seen in the indicas. The 1.3 kb band seems to be present only in Ritutonorin.

(iii) **pOSM1D-9/BglI**

In the autoradiogram depicted in Fig. 2.5, indicas are characteristic in having one band at 11.8 kb with the exception of Manharsali where it is absent. In japonica, one strong band at 9.7 kb is present in

Fig. 2.5 : Hybridization pattern revealed by probing EcoRI digests of indica and japonica cultivars with pOSM1D-9 insert. lane order same as Fig. 2.3.

Fig. 2.6 : Autoradiogram depicting a hybridization pattern revealed when mouse rDNA (18s subunit) insert was hybridized to EcoRI digests of DNAs of indica and japonica cultivars

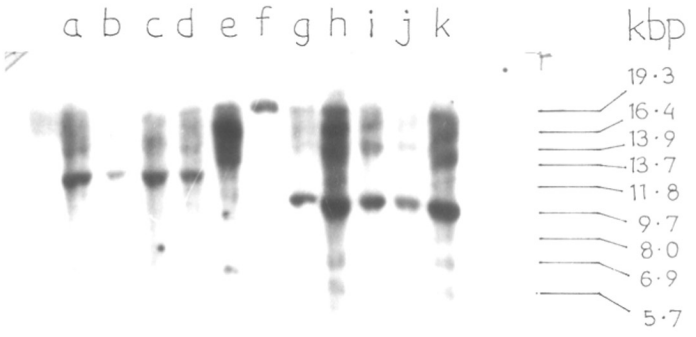

a b c d e f g h i j k
kbp
19·3
16·4
13·9
13·7
11·8
9·7
8·0
6·9
5·7

Fig. 2·5

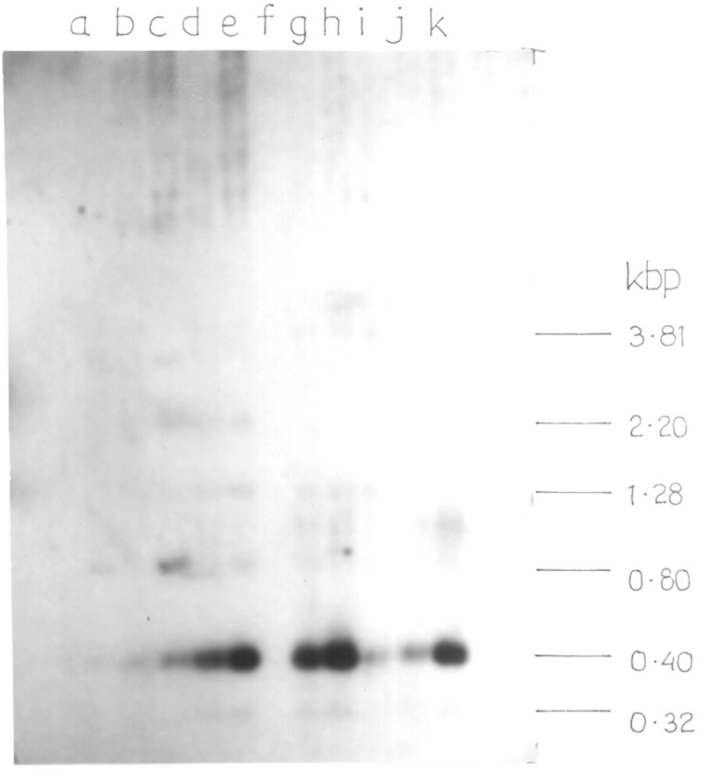

a b c d e f g h i j k
kbp
3·81
2·20
1·28
0·80
0·40
0·32

Fig. 2·6

all the cultivars. Manharsali exhibits two intense bands viz. 16.4 kb and 13.7 kb with background on the lane and its pattern is totally different from the other cultivars. Barmolbora, on the other hand, shows only one clear band at 11.8 kb. Ambemohr- 157 has two distinct bands at 13.9 kb and 11.8 kb and two very faint bands at 6.9 kb and 5.7 kb. Basmati 370 and Malkolam both show three bands each viz. 16.4 kb, 13.9 kb and 11.8 kb. Considering the japonicas, Habakati and Taichung 65 have a very similar pattern in having six bands in common viz. 19.3 kb, 16.4 kb, 13.7 kb, 9.7 kb, 6.9 kb and 5.7 kb. However, a faint band at 8.02 kb is seen only in Taichung 65. Out of the other three, the dense band at 9.7 kb being common to all, a possible distinction can be made based on the faint bands. Akawa displays three faint bands of 19.3 kb, 16.4 kb and 13.9 kb whereas Kerunmochi displays only two bands of 16.4 kb and 13.9 kb and Ritutonorin only one at 13.9 kb.

(iv) **mouse r DNA (18s subunit)/BglI**

Figure 2.6 exhibits the pattern of variation of 18s rDNA sequences in indica and japonica cultivars. A prominent band at 0.407 kb can be seen in all the cultivars excepting Ambemohr 157 and Barmolbora. The band exhibits less intense hybridization in Basmati

370, Kerunmochi and Ritutonorin as compared to Malkolam, Manharsali, Akawa, Habakati and Taichung 65 indicating copy number variation. A few faint bands can be seen at higher and lower molecular weights. However, these cannot be utilized in deriving significant conclusions. All the same, these faint bands are seen to vary in the indicas as compared to the japonicas.

2.3.3 Dendrograms based on Nei's F and Genetic distance

Using the Nei's F value and genetic distance (Nei, 1987), the molecular data was processed to obtain a graphical representation i.e. a dendrogram. The Nei's F value is a measure of divergence whereas the genetic distance is a measure of divergence derived by processing the similarity index.

In Fig. 2.7, a dendrogram representing indica - japonica relationships based on Nei's F value is depicted. It is clear from the dendrogram that the indicas and japonicas cluster separately. Within the indica cluster, two sub-clusters are formed, one with Ambemohr - 157 and Barmolbora and the other with Basmati 370, Malkolam and Manharsali. In the japonica cluster again two sub-clusters are formed viz. Akawa and Habakati forming one group as against Kerunmochi, Ritutonorin and Taichung 65 forming the other.

Figure 2.8 depicts a dendrogram based on

Fig. 2.7 : Dendrogram of indica and japonica cultivars based on Nei' F values

FIG 2.7

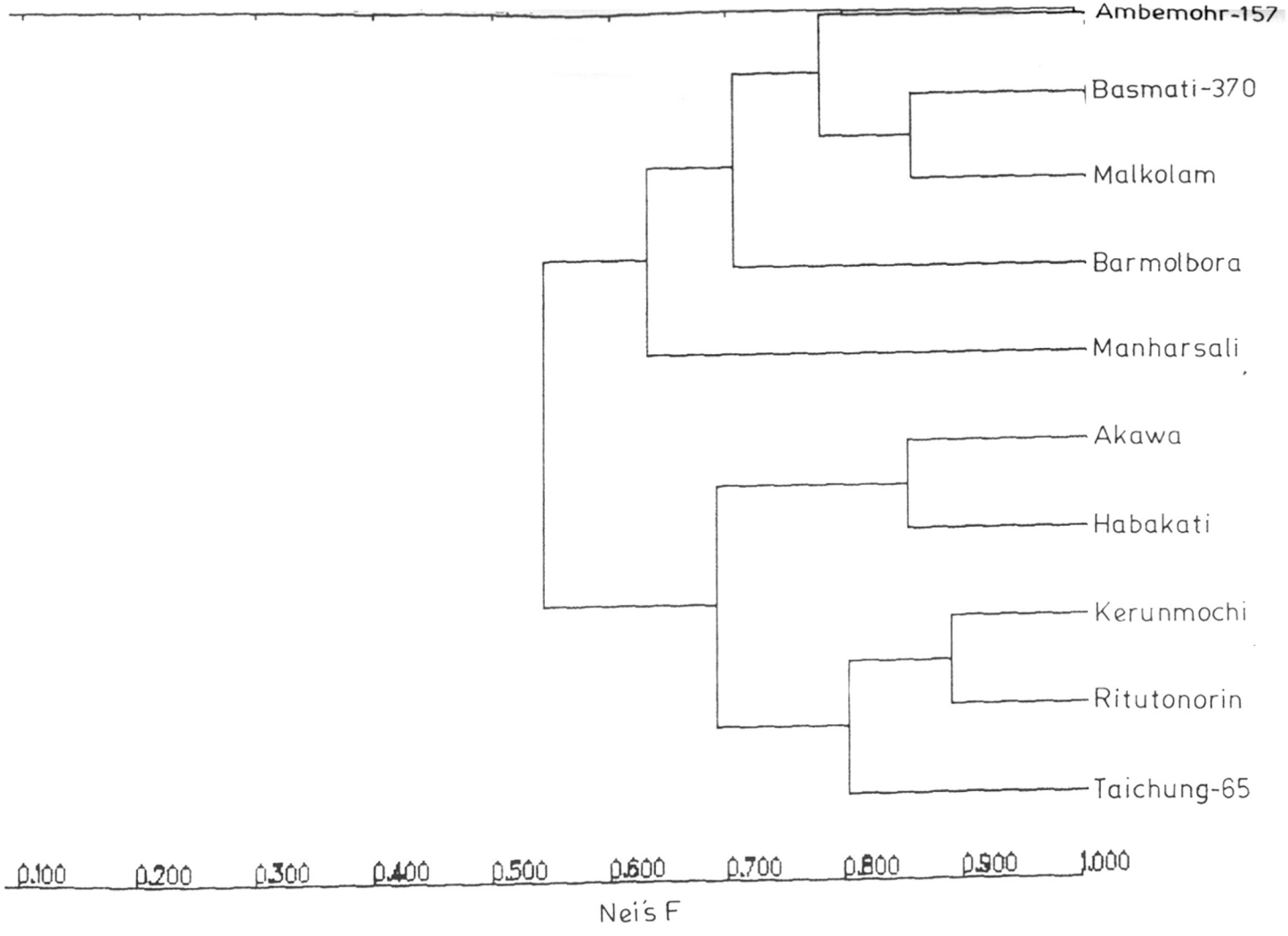
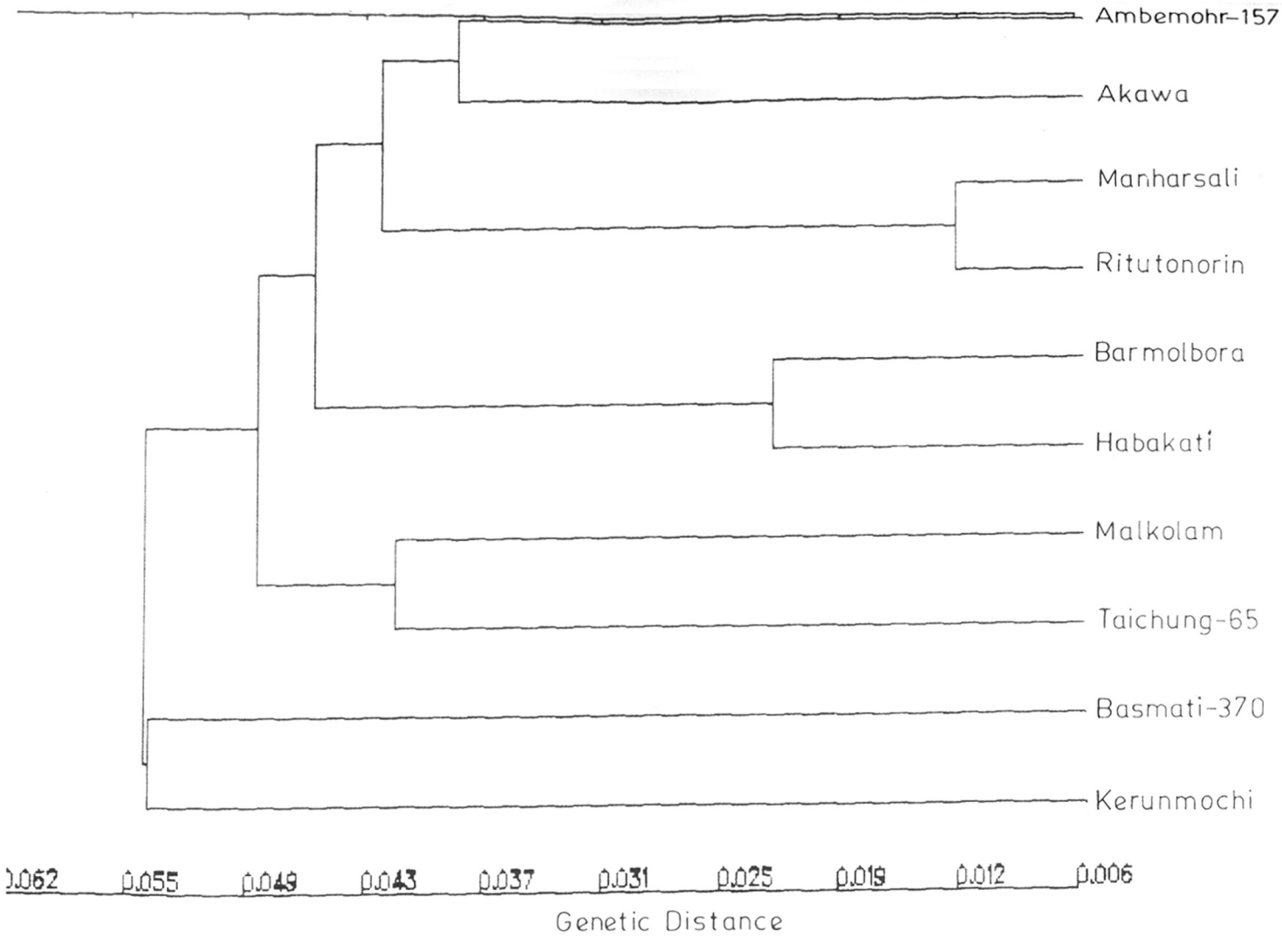

Ambemohr-157
Basmati-370
Malkolam
Barmolbora
Manharsali
Akawa
Habakati
Kerunmochi
Ritutonorin
Taichung-65
0.100
0.200
0.300
0.400
0.500
0.600
0.700
0.800
0.900
1.000
Nei's F

Fig. 2.8 : Dendrogram of indica and japonica cultivars based on genetic distance

FIG 2.8


Ambemohr-157
Akawa
Manharsali
Rituronorin
Barmolbora
Habakati
Malkolam
Taichung-65
Basmati-370
Kerunmochi
0.062
0.055
0.049
0.043
0.037
0.031
0.025
0.019
0.012
0.006
Genetic Distance

genetic distance between 5 indica and 5 japonica cultivars. When Nei's F value is iterated to obtain the genetic distance, significant differences are observed between the two dendrograms. Unlike the other dendrogram, in this dendrogram these characteristics are seen namely (i) no definite relationship between the indica and japonica cultivars, (ii) no definite character based clustering and (iii) a mix-up of indica and japonica cultivars . Several pairs of indica and japonica like Manharsali - Ritutonorin, Barmolbora - Habakati and Malkolam - Taichung 65 can be observed in Fig. 2.8. Grossly, three clusters can be visualized viz. (Ambemohr 157, Manharsali, Ritutonorin, Akawa, Kerunmochi), (Barmolbora, Habakati, Basmati 370) and (Malkolam , Taichung 65).

2.4 Discussion

In the previous limited studies on indica and japonica, the DNA probes have been mainly derived from two sources namely IR 36 and Nipponbare while IR 36 is a synthetic indica variety with a number of genotypes ranging from wild to cultivated indica subspecies (2,4) Nipponbare is the japonica subspecies (10). In the present work, the DNA probes have been derived from Malkolam which probably is the second source of probes from indica subspecies.

One of the important findings in the present work is the detection of japonica specific sequences deciphered by pOSM1C-2, a dispersed repeat. The size of the probe is 1.55 kbp and it hybridizes with the bands of much higher molecular weight, indicating that this repeat containing fragments along with some other sequences are subspecies specific. The japonicas are believed to have evolved from the indicas based on several observations (11,12,13,14,15,16). Considering this fact and looking at the pattern revealed by the probe in the present study, it can be seen that the DNA probes derived from an indica library are able to reveal japonica specific sequences. Since the number of bands is increased in the japonicas as compared to the indicas, a duplication of the sequence can be inferred and the subsequent divergence of these sequences in the course of evolution has resulted in the specificity towards the japonica subspecies. The above explanation is supported by the fact that between the indica cultivars there seems to be less variation compared to between the japonicas considering the bands that are revealed.

The next significant observation is that pOSM1D-9 exhibits an entirely different and complex pattern with both indica and japonica wherein a maximum of nine bands with a minimum of four bands are re-

vealed. The probe can differentiate eight of the ten cultivars from each other based on its pattern of hybridization. Secondly, there is one major band common to indicas and one to the japonicas whereas the other bands vary from lower molecular weight to higher molecular weight. These patterns can be termed as DNA fingerprints of the genotypes based on DNA analysis. Especially the DNA profile of Manharsali which is totally different from the others indicates its diverse nature. Thirdly, making a comment about its evolutionary implications, this sequence seems to be highly variable in comparison to the earlier ones.

Another salient feature of the present study is that the pattern revealed by pOSM5F-3 is a fairly simpler pattern wherein the variation is seen only amongst the indica cultivars as against the japonica cultivars. As the probe deciphers bands of higher molecular weights in the indicas as compared to the japonicas, it is likely that there is a gain or modification of restriction sites during the evolution. This probe too has a size of about 800 bp and yet it hybridizes to high molecular weight fragments and thereby indicates that in conjunction with other sequences it forms an unit which shows this variation between the indicas and the japonicas.

Lastly the mouse ribosomal RNA genes have

revealed that there is more intense hybridization in low molecular weight region (0.407 kb) with very faint hybridization in the high molecular weight region (3.81 kb to 0.805 kb). These results indicate that there are more BglI sites in the rRNA genes which give rise to smaller fragments with very few large fragments.

In summary, two of the four probes viz. pOSM1C-2 and pOSM5F-3 have the ability to differentiate between the indica and the japonica cultivars whereas pOSM1D-9 has even more resolution wherein within indica and within japonica variations can also be tracked by it. Three probes viz. pOSM1C-2, pOSM5F-3 and pOSM1D-9 are new probes derived from a landrace cv. Malkolam, and have probably enabled the differentiation between indica and japonica because of their being from a conserved gene pool.

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CHAPTER 3

Genetic analysis of qualitative and quantitative traits at the F_2 level of an indica – japonica cross : a possible evidence for disturbed genetic coherence

CHAPTER III

Summary

The genetic analysis of two crosses viz. Basmati 370 x Adt 27 and Basmati 370 x Taichung 65 is described using both qualitative and quantitative characters. The F1 sterility of Basmati 370 x Adt 27 is 16% whereas that of Basmati 370 x Taichung 65 varies from 18% to 38%. Out of the two crosses, the Basmati 370 x Taichung 65 exhibits deviations from the normally expected phenotype and hence this cross is analyzed in greater detail. The plant type variation observed indicates the presence of parental types as well as intermediates. But in addition to parental and recombinant types, occurrence of a few dwarf types exhibiting almost no to less tillering is observed. The panicle morphology varies from one parent to the other with intermediates while the spikelet morphology reveals the occurrence of more of japonica type grains. Of the three qualitative traits analyzed, aroma segregates as 3 : 1 (scented : non-scented) in both the crosses while apiculus colour and awn segregate as 3 : 1 (coloured : colourless) and 15:1(awned : awnless), respectively in Basmati 370 x Adt 27. The plant height, one of the quantitative traits analyzed, is seen to be more towards the indica parent in the proge-

ny whereas the days to flowering is more towards the japonica parents. The number of tillers is again tending towards indica parent which has the higher number of the two.

Considering the above data, the cross Basmati 370 x Taichung 65 can be used as a good representative of an indica x japonica cross for RFLP analysis as it reveals most of the features plus a few abnormalities.

3.1 **Introduction**

One of the major aspects of genetic divergence between the indica and japonica subspecies is the occurrence of high F1 hybrid sterility (1). The two subspecies of indica and japonica offer a barrier in crossing amongst themselves as is seen from very low success rates in indica x japonica crossing programmes (2). The segregation studies show a marked skewing in the F2 population of japonica or indica alleles, indicating the presence of recombination barriers in these two subspecies. Several studies todate have explained the basis of the hybrid sterility as being controlled by mainly genic and chromosomal causes (3).

Joshua et al., (4) have attempted to explain the mode of evolution of the japonica rices from the indica and to elucidate the mechanism of intersubspecific hybrid sterility, on the basis of the radiobiological response. From the occurrence of albina and tigrina mutants in the progeny of untreated hybrids and deviations from the expected spectra in the irradiated hybrid progeny, they have suggested that the linkage groups of these subspecies are different. The mutation rate and the spectra indicate that both the subspecies are diploids and the duplication of genes has resulted from translocations but not as a result of doubling of the chromosomes. Recently, Siddiq (5) has stated that

the study of stable mutant affecting key diagnostic characteristics has revealed that constellation of such characteristics may be affected either individually or in clusters.

In the present study, I have carried out an analysis of segregation of qualitative and quantitative characters in an indica x japonica cross in order to assess whether the genetic material is appropriate for the RFLP analysis. The qualitative and quantitative characters studied are aroma, awn, apiculus colour and plant height, number of tillers, days to flowering, respectively. The results of this study has enabled me to confirm that the Basmati 370 x Taichung 65 cross represents a typical indica - japonica cross as it exhibits most of the peculiar features observed.

3.2 **Materials and methods**

3.2.1 Plant material

The seeds of Taichung-65 (japonica), Adt 27 (indica-japonica) and Basmati 370 (indica) were obtained from the Central Rice Research Institute, Cuttack .

3.2.2 **Crossing**

The crossing was performed using conventional

method viz. emasculation (manually with forceps) (6) followed by pollination (pollen applied onto stigma with a brush). The resultant F1 seed was sown at the Agriculture School, Roha as a summer paddy in order to save time. The F1's were confirmed by analyzing the morphological characters like plant height, tiller number, anthocyanin pigmentation and panicle length. The F2 progeny was grown at CRRI, Cuttack for further work (Normal fertilization of NPK, 100:50:50 per hectare was used) under the guidance of Dr. R. N. Misra, Head, Department of Genetics and Plant Breeding, Central Rice Research Institute, Cuttack.

3.2.3 Screening of sterility, aroma & other ancillary characters

a) Sterility - For sterility, the filled spikelets were considered fertile as against unfilled spikelets as sterile (Randomly 10 panciles were selected for observations).

b) Aroma - Leaf pieces were incubated in 1.5% KOH at 50°C for 10 min and then checked for the presence of aroma (7). This work was carried out in collaboration with Dr. R.N. Misra, Dr. M. Nagaraju and Dr. Nirajana Murthy from CRRI, Cuttack.

* - Selection of progeny - the pooled seeds of a

single plant were sown and plants were selected randomly to be planted in a randomized block design - 10 x 15 ft^2 spacing - 15 x 30cm.

c) Ancillary characters Plant height, number of tillers and days to flowering were measured according to the standard evaluation system for rice.

3.3 Results

In a morphological analysis of a cross, characters which are sensitive and which are affected considerably are generally screened. In the present analysis, data regarding crossing, F1 data and screening of F2 progeny for plant height, number of tillers, days of flowering, aroma, awn and apiculus colour are considered.

3.3.1 Field data regarding crossing

The details of crossing give a rough picture about the success rate which is indicative of the compatibility between the two parents involved in the crossing. Table 3.1 shows the data regarding the crossing programme carried out to obtain indica x japonica (Basmati-370 x Taichung-65) and indica x indica -japonica derivative (Basmati-370 x Adt-27) crosses. In Basmati-370 x Taichung-65 cross, only 8 seeds were obtained from crossing 153 spikelets whereas

Table 3.1

Field data regarding the inter-subspecies crosses performed at ARS Vadgaon (Maval), Pune

S.No.	Name of the cross	No. of crosses performed	No. of crossed spikelets	No. of seed obtained
1.	Basmati 370 x Adt 27	12	237	14
2.	Basmati 370 x Taichung 65	7	153	8

14 seeds were obtained from crossing 237 spikelets in the Basamti-370 x Adt-27 cross. The percentage success is thus quite low in both the cases.

3.3.2 Panicle types of parents (indica, japonica and indica- japonica types)

The reproductive organs form a very important system to study the effects of hybridization. In the present study, Fig. 3.1 - shows the morphology of panicles from seven different varieties viz. Gu Meaung Luang, Adt-27, Taichung-65, Mahsuri, Malkolam, IR64 and Basmati-370. One can see the unbranched and compact panicle of Taichung-65, a japonica as compared to the branched and less compact pancile of Basmati-370, Malkolam, IR64 and Gu Meaung Luang, which are all indicas and have branched and dense panicles. Mahsuri and Adt-27, both indica-japonica derivatives, show branched, compact and fairly dense panciles. The panicle type of Mahsuri and Adt 27 is different though they are derivatives of the same cross Mayang Ebos 802 (indica) x Taichung 65 (japonica).

3.3.3 Phenotypes of hybrids

The overall phenotype of an hybrid indicates the level of heterosis and the recombination. In Fig. 3.2a and 3.2b the F1 phenotype of Basmati-370 x Adt 27

Fig 3.1 : Spikelet morphology of hybrids and parents of Basmati 370 x Taichung 65 cross.

Fig. 3·1

Fig 3.2a : F1 hybrid morphology of Basmati 370 x Adt 27 cross

Fig 3.2b : F1 hybrid morphology of Basmati 370 x Taichung 65 cross

Fig. 3·2 a

Fig. 3·2 b

and Basmati-370 x Taichung 65 cross is seen. The Basmati-370 x Taichung-65 F1 is around 95cm tall as compared to Basmati- 370 x Adt-27 F1 which is exceeding approx 100cms. The distinct anthocyanin pigmentation can be seen at the base of culms in the F1 of the Basmati-370 x Atd-27 cross.

3.3.4 Confirmation of hybrids based on spikelet morphology

In rice, spikelet morphology is a very stable character and is often used to identify genotypes, especially the hybrids. In case of indica and japonica, the spikelet morphology is considerably different and hence has been used in addition to other characters to confirm the hybrid nature.

Figures 3.3a and 3.3b show the comparison of spikelet morphology of the parents with their F1s. In Fig. 3.3a, the L/B ratio of the F1 is seen to be intermediate to that of Basmati-370 and Taichung-65. Secondly the awn also is seen in the F1, a character derived from the female parent. In Fig. 3.3b, the F1 shows the presence of coloured apiculus and stigma and absence of awn. The anthocyanin is derived from the male parent and is a good phenotypic marker. The L/B ratio of the F1 spikelet tends towards Adt- 27 but does show some elongation, a character derived from Basmati-

Fig 3.3a : Spikelet morphology of hybrids and parents of Basmati 370 x Taichung 65 cross

Fig 3.3b : Spikelet morphology of hybrid and parents of Basmati 370 x Adt 27 cross

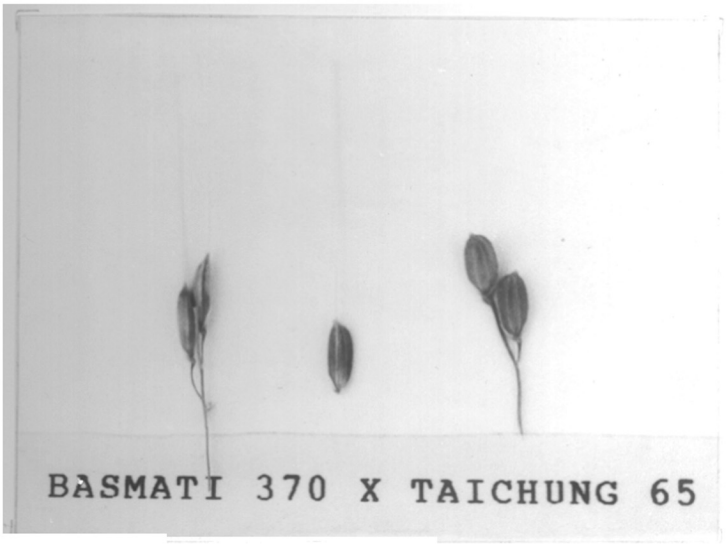

Fig. 3·3a

Fig. 3·3b

370.

3.3.5 Phenotypic data of parents and hybrids

The overall phenotypic data regarding the confirmation of F1's of the crosses at a single location is presented in Table 3.2. The Basmati-370 is the tall parent whereas Taichung-65 is dwarf and Adt-27 is intermediate of both. The tiller number is more in the case of Basamti-370 and least in the case of Taichung-65. Comparing the panicle lengths, not much differences are found between Basmati 370 and Taichung 65. Looking at the Basmati-370 x Adt-27 cross, it shows a tall phenotype with extensive tillering (more than both the parents) and panicle length comparable to that of Adt-27. In the Basmati-370 x Taichung-65 cross, observations for two plants were recorded. The plant height in both the F1's is tending towards Basmati-370, whereas the tiller number is more than Taichung-65 in both the cases. The panicle length in both the plants is similar to that of Basmati-370.

In order to compare both the above crosses, another cross Basmati-370 and IR64 (both indicas) was done along with the two crosses. The F1 plant type of this indica cross was tall, low tillering and with panicle length longer than both the parents. These observations regarding the plant type, indicate the

Table 3.2

Morphological characters screened for the parents and F1s at ARS Roha, Raigarh

S.No.	Name of parent/cross	Plant height (cms)	No. of tillers	Panicle length (cms)
1.	Basmati 370	108	35	25
2.	Adt 27	98	22	28
3.	Taichung 65	80	10	22
4.	Basmati 370 x Adt 27	115	55	27
5.	Basmati 370 x Taichung 65	102	28	25
6.	Basmati 370 x Taichung 65	102	40	25
7.	IR 64	54	28	22.5
8.	Basmati 370 x IR 64	100	16	29

diverse nature of Basmati-370 - Adt-27 and Basmati-370 -Taichung-65 as compared to Basmati- 370 - IR64.

3.3.6 F1 sterility data of hybrids

The F1 sterility is an important parameter in the study of combining ability and the degree of genetic divergence of the parents involved in the crossing program. Table 3.3 shows the F1 sterility data collected for both the cross combinations for a sample of 10 panicles selected randomly. From this table, it can be seen that Basmati-370 shows an unusally high percentage of sterility. The probable cause of this can be pinned to the summer paddy trial wherein there is low humidity and higher temperatures. In comparison Taichung-65 and Adt-27 exhibit only 21% and 10% F1 sterility, respectively. The F1's of Basmati-370 x Adt-27 show 16% sterility whereas in the case of Basmati-370 x Taichung-65 sterility varies from 18% to 38%.

3.4 Generation of F2 progeny and its characterization

3.4.1 Plant type variations

F2 progeny of the three crosses was obtained at Central Rice Research Institute, Cuttack (Fig. 3.4) and it was characterized for qualitative as well as quantitative traits. It is important to study the plant type as it plays a significant role in the deci-

Table 3.3 F1 sterility data of parents and hybrids

Parent/ Cross	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	Mean
Basmati 370	82.6	42.0	70.2	78.3	16.0	41.9	25.3	61.1	69.8	47.1	53.4
Basmati 370 x Taichung 65 #1	12.6	13.5	45.4	10.4	4.7	9.6	24.5	20.9	19.7	20.0	18.1
Basmati 370 x Taichung 65 #2	35.0	27.5	36.9	30.9	31.3	28.5	26.5	17.6	30.5	44.4	30.9
Basmati 370 x Taichung 65 #3	48.5	25.0	35.4	50.5	36.6	25.0	61.3	41.2	37.5	24.1	38.5
Taichung 65	12.6	13.7	5.0	15.7	84.5	5.0	36.8	8.9	8.5	19.1	21.0
Basmati 370 x Adt 27	25.1	19.1	18.6	14.1	11.7	14.9	23.2	13.6	12.2	12.1	16.5
Adt 27	2.6	12.6	10.1	9.0	20.0	3.3	4.1	14.7	17.6	10.3	10.4

Fig 3.4 : F2 progenies of Basmati 370 x Taichung 65 and Basmati 370 x Adt 27 crosses at Central Rice Research Institute, Cuttack.

Fig . 3·4

phering of the relatedness of the parents. Moreover, the plant type also plays an important role in the determination of yield of the plant. Indica - japonica hybrids are known to exhibit weak and sterile plant types due to several genic interactions. Figure 3.5a shows a plant which is tall with extensive tillering, a typical Basmati-370 like phenotype whereas Fig. 3.5b shows a dwarf plant type with moderate tillering, a combination of dwarfness from japonica and a slight increase in tillering derived from indica. Figure 3.6a shows a plant type which is tall with less tillering, again a combination of tallness from indica and low tillering from japonica. Figure 3.6b exhibits a bushy habit with only one productive tiller and is a "novel phenotype" totally different from both the parents.

Figure 3.7a depicts a dwarf plant type with moderate tillering, a combination of japonica type height and indica type tillering whereas Fig. 3.7b shows a dwarf bushy type phenotype with no productive tillering, again a novel phenotype appearing in the progeny. In addition to these plant types, a striata phenotype (Fig. 3.8) has appeared in the progeny (both the parents did not exhibit such types earlier). In another trial conducted at Agriculture College, Pune with the same cross, albino plants were found.

Fig 3.5a : Phenotype of a tall with extensive tillering plant type of a segregant of a Basmati 370 x Taichung 65 cross.

Fig 3.5b : Phenotype of a dwarf plant type with less tillering of a segregant of a Basmati 370 x Taichung 65 cross

Fig. 3·5a

Fig. 3·5 b

Fig 3.6a : Phenotype of a tall with less tillering plant type of a segregant of a Basmati 370 x Taichung 65 cross

Fig 3.6b : Phenotype depicting a bushy habit with only one productive tiller of a segregant of a Basmati 370 x Taichung 65 cross

Fig. 3·6 a

Fig. 3·6 b

Fig 3.7a : Dwarf bushy plant type with moderate tillering of a F2 segregant of a Basmati 370 x Taichung 65 cross

Fig 3.7b : Dwarf bushy plant type with no productive tillers of a segregant of a Basmati 370 x Taichung 65 cross

Fig. 3·7a

Fig. 3·7b

Fig 3.8 : Striped leaf of a F2 segregant of a Basmati 370 x Taichung 65 cross.

Fig. 3·8

The above data thus shows the presence of odd plant types indicative of the divergence of indica and japonica subspecies.

3.4.2 Panicle type variation

Rice varieties are known to have typical panicle types and are fairly stable in terms of variations. Secondly, this parameter directly affects the yield of the plant and is important from a breeder's point of view. The data of the panicle type variation for the cross Basmati 370 x Taichung 65 is presented here as the other cross viz. Basmati 370 x Adt 27 did not exhibit the presence of odd types other than the parental and recombinant types. In Fig. 3.9a a low branched, compact pancile with Taichung-65 like short bold (SB) grains is seen whereas Fig. 3.9b shows a highly branched panicle with SB type or japonica like grains. One of the segregants exhibits a low branched, less dense and sparsely seeded pancile (Fig. 3.10a) compared to the one depicted in Fig. 3.10b wherein a branched dense panicle with L/B ratio of grains intermediate to that of Basmati-370 and Taichung-65 is seen. However, typical parental types are also observed such as a japonica type less branched and a compact panicle with SB grains (Fig. 3.11a) and a Basmati-370 like more branched and less compact panicle with MS

Fig 3.9a : A panicle resembling Basmati 370 and having japonica type grains of a segregant of a Basmati 370 x Taichung 65 cross

Fig 3.9b : A branched panicle with japonica type grains of a segregant of a Basmati 370 x Taichung 65 cross

Fig. 3·9 a

Fig. 3·9 b

Fig 3.10a : A sparsely seeded panicle of a F2 segregant of a Basmati 370 x Taichung 65 cross

Fig 3.10b : A dense panicle of a F2 segregant of a Basmati 370 x Taichung 65 cross

Fig. 3·10a

Fig. 3·10b

Fig 3.11a : A typical Taichung 65 like panicle of a F2 segregant of a Basmati 370 x Taichung 65 cross

Fig 3.11b : A typical Basmati 370 like panicle of a F2 segregant of a Basmati 370 x Taichung 65 cross

Fig. 3·11 a

Fig. 3·11 b

Fig 3.12 : A panicle type intermediate to that of Basmati 370 and Taichung 65 observed in a F2 segregant of a Basmati 370 x Taichung 65 cross

Fig 3.13 : Variation observed in the spikelet type in a Basmati 370 x Taichung 65 cross

Fig. 3·12

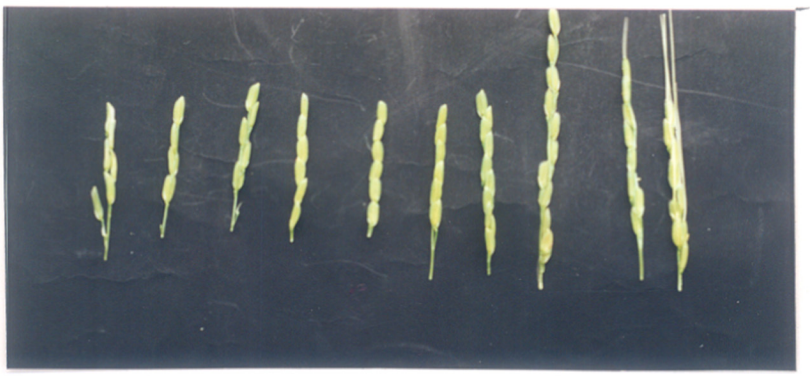

Fig. 3·13

grains (Fig. 3.11b). The occurrence of intermediate type of panicle in comparison to the above two is also observed (Fig. 3.12).

From these data, it can be concluded that the panicle type variation shows a good recombination in the cross Basmati 370 x Taichung 65. The field data about the morphology of the segregants of the Basmati-370 x Taichung-65 and Basmati-370 x Adt-27 crosses indicates that both progenies show variation in plant type, panicle type and grain type indicating a broad range of variation, a typical feature of wide crosses.

3.4.3 Spikelet morphology of indica x japonica cross

Figure 3.13 depicts the spikelets variation in the F2 progeny of Basmati-370 x Taichung-65 wherein awned and awnless plants are seen. Considering the L/B ratio, there is a range of variation from left to right showing Basmati-370 like grain type to Taichung-65 like grain type and intermediates. On the extreme right of Fig. 3.13 japonica like grains with awns can be seen whereas on the extreme left (Fig. 3.13) indica like grains without awns are seen, both being recombinants.

3.4.4 Qualitative characters

In addition to the plant type, panicle type and spikelet variation, a few qualitative and quantita-

Table 3.4

Screening of the F2 progenies at CRRI,Cuttack

S.No.	Character	Name of the cross	Segregation	X^2 value	Probability	Ratio
1.	Aroma	Basmati 370 x Adt 27	141 : 40	0.8121	0.30 - 0.50	3 : 1
		Basmati 370 x Taichung 65	114 : 38	0.0000	>0.99	3 : 1
2.	Awn	Basmati 370 x Adt 27	161 : 12	0.1394	0.70 - 0.90	15 : 1
3.	Apiculus colour	Basmati 370 x Adt 27	132 : 42	0.0689	>0.80	3 : 1

tive characters were also screened for both the progenies to determine the extent of skewing exhibited by both the crosses.

These characters govern economically important traits such as aroma. In case of rice, aroma is of prime importance from the quality aspect whereas the apiculus colour and awn indirectly affect the yield.

Aroma

Table 3.4 shows the segregation of aroma in Bamsati- 370 x Adt-27 and Basmati-370 x Taichung-65 crosses. Both the crosses show a distinct 3:1 ratio for scented: non-scented, thereby indicating a monogenic dominant type of inheritance.

Awn

The F1s of both the crosses viz. Basmati 370 x Taichung 65 and Basmati 370 x Adt 27 differed in terms of the awn trait. The Basmati 370 x Adt 27 cross showed a lack of awn, implying the need to study the segregation of this trait in the F2 progeny. Accordingly upon analysing the trait it is found that the Basamti-370 x Adt-27 cross displays a 15:1 segregation of awnless : awned (Table 3.4) suggesting a duplicate gene inheritance.

Apiculus colour

This trait is particularly observed in the Basmati-370 x Adt-27 cross. The coloured apiculus : colourless apiculus segregated as 3:1, indicating the presence of a monogenic dominant gene governing this trait.

The segregation of the above 3 characters does not show any skewing and is normal in both the crosses.

3.4.5 Quantitative traits

Three quantitative traits viz. plant height, number of tillers and days to flowering which directly influence the yield of a plant were screened in 3 progenies. In the present analysis, two crosses viz. Basmati-370 x Adt-27 and Basmati-370 x Taichung-65 are screened for the above mentioned traits and the data is presented below.

BASMATI-370 x ADT-27

Plant height

In rice, plant architecture breeding has been done since the 1960's to obtain an ideal plant type wherein the maximum biomass is in terms of the yield. With the advent of dwarf varieties, a significant increase in yield was observed. Hence it is necessary to study this parameter in all the rice crosses. In Fig. 3.14, the plant height in the progeny ranges from

Fig.3.14 : Frequency distribution of plant height (in cms) in a Basamti-370 x Adt-27 cross. Classes A(90-95), B(96-100), C(101- 105), D(106-110), E(111-115), F(116-120), G(121-125), H(126-130), I(131-135), I(136-130), K(141-145), L(146-150). Basamti-370 : 108 cms & Adt-27 : 98 cms.

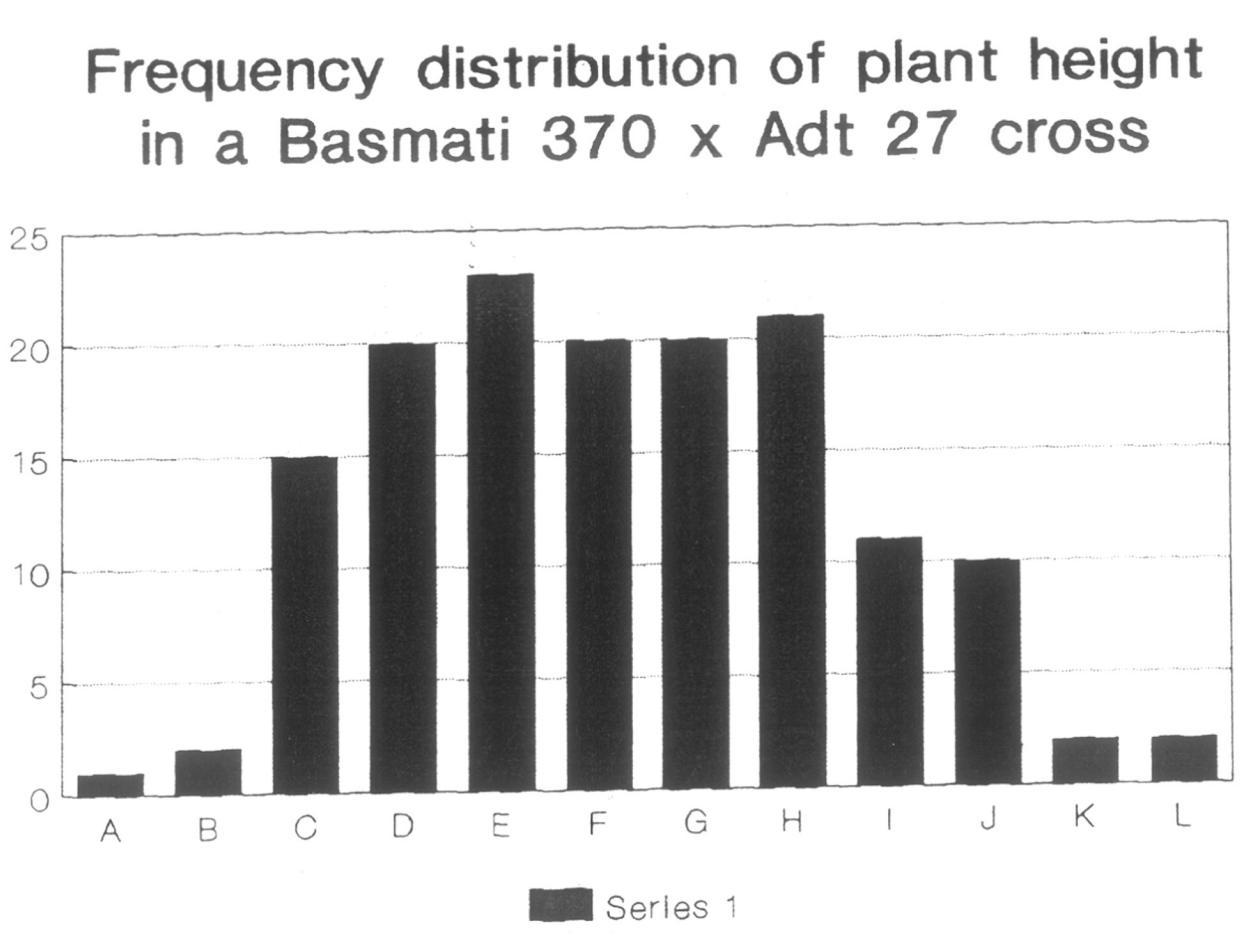
Frequency distribution of plant height
in a Basmati 370 x Adt 27 cross
25
20
15
10
5
0
A
B
C
D
E
F
G
H
I
J
K
L
Series 1

Fig. 3.14

90cms to 150cms. The entire-range was divided into 12 classes of 5cms each. The maximum number of plants falls in the class 111-115cms and the overall skewing towards indica type is observed.

Number of tillers

The number of tillers is one of the parameters which directly contributes towards the yield of the plant. The data regarding this parameter is extremely important and hence is always considered in all the crossing programs.

Figure 3.15 shows the frequency distribution of number of tillers in the segregants of the F2 progeny of Basmati-370 x Adt-27 cross. The number of tillers ranges from 6 to 60 per plant and the maximum number of plants fall in 3 classes, (16-20), (21-25) and (26-30). indicating the indica type dominance in the progeny.

Days to flowering

The number of days required for the plant to flower indirectly affects the yield of the plant. In an attempt to observe the behaviour of this trait in the cross, the date of flowering rather than days to flowering was considered (data not shown). The flowering of the progeny begins on 25/4 and has extended upto 10/6, a period of six weeks. I have divided this period

Fig. 3.15 : Frequency distribution of number of the tillers in a Basmati-370 x Adt-27 cross classes A(0-5), B(6-10), C(11-15), D(16-20), E(21-28), F(26-30), G(31-35), H(36-40), I(41-45), J(46-50), K(51-55), L(55-60). Basmati-370 : 35 and Adt-27 : 22

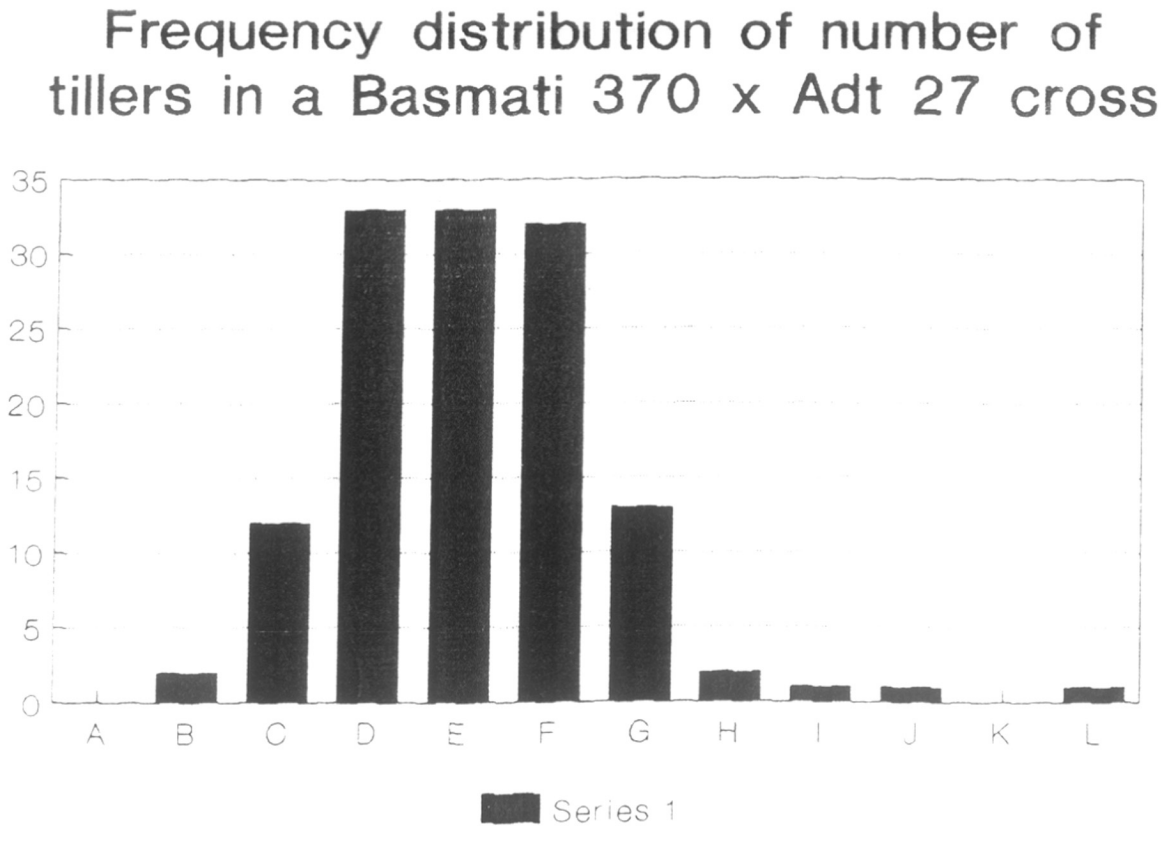
Frequency distribution of number of tillers in a Basmati 370 x Adt 27 cross
35
30
25
20
15
10
5
0
A
B
C
D
E
F
G
H
I
J
K
L
Series 1

Fig. 3.15

of six weeks into classes of a period of 5 days each in order to study the frequency distribution of this trait in the progeny. The maximum number of plants is observed to be distributed in the 2nd, 3rd and 4th classes indicating the tendency towards earliness, a japonica character.

BASAMTI-370 x TAICHUNG-65

Plant height

Figure 3.16 shows the frequency distribution of plant height which ranges from 76cms to 155cms. There seems to be an overall skewing towards the Basmati-370 type plant height and the maximum number of individuals fall under the class 111-115 cms. This cross exhibits a more distributed variation as compared to the Basmati 370 x Adt 27 cross.

Number of tillers

In Fig. 3.17, the frequency distribution of number of tillers is seen where the number of tillers range from (0-5) to (41-45). The majority of the individuals fall in three classes viz. (11-15), (16-20) and (21-25), the maximum being in the (16-20) class. This shows again the tendency of skewing towards the indica type character of high tillering.

Days to flowering

Fig. 3.16 : Frequency distribution of plant height (in cms) in a Basmati-370 x Taichung-65 cross. Classes (same as Fig 3.14) Basmati-370 : 108 cms and Taichung-65 : 80 cms.

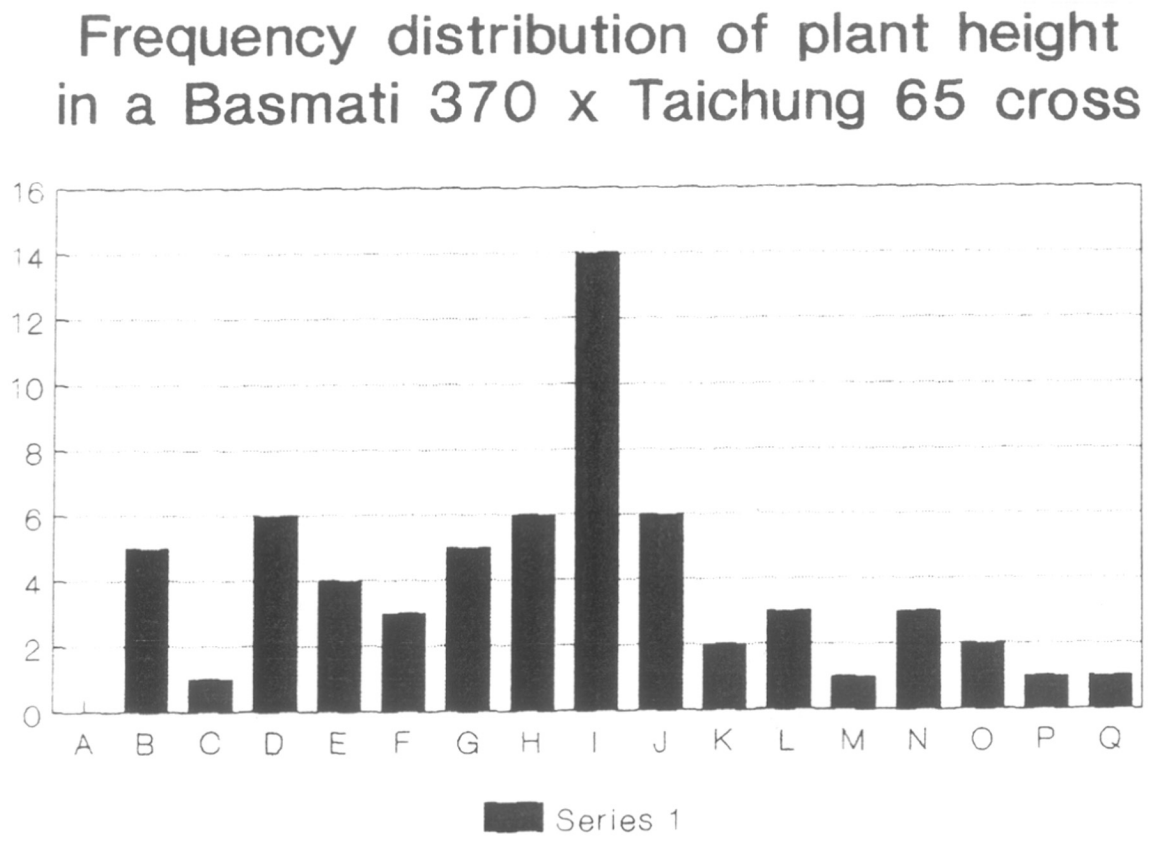
Frequency distribution of plant height
in a Basmati 370 x Taichung 65 cross
16
14
12
10
8
6
4
2
0
A B C D E F G H I J K L M N O P Q
Series 1

Fig. 3.16

Fig. 3.17 : Frequency distribution of number of tillers in a Basmati-370 x Taichung-65 cross (same as Fig. 3.16) Basmati-370 : 35 and Taichung-65 : 10

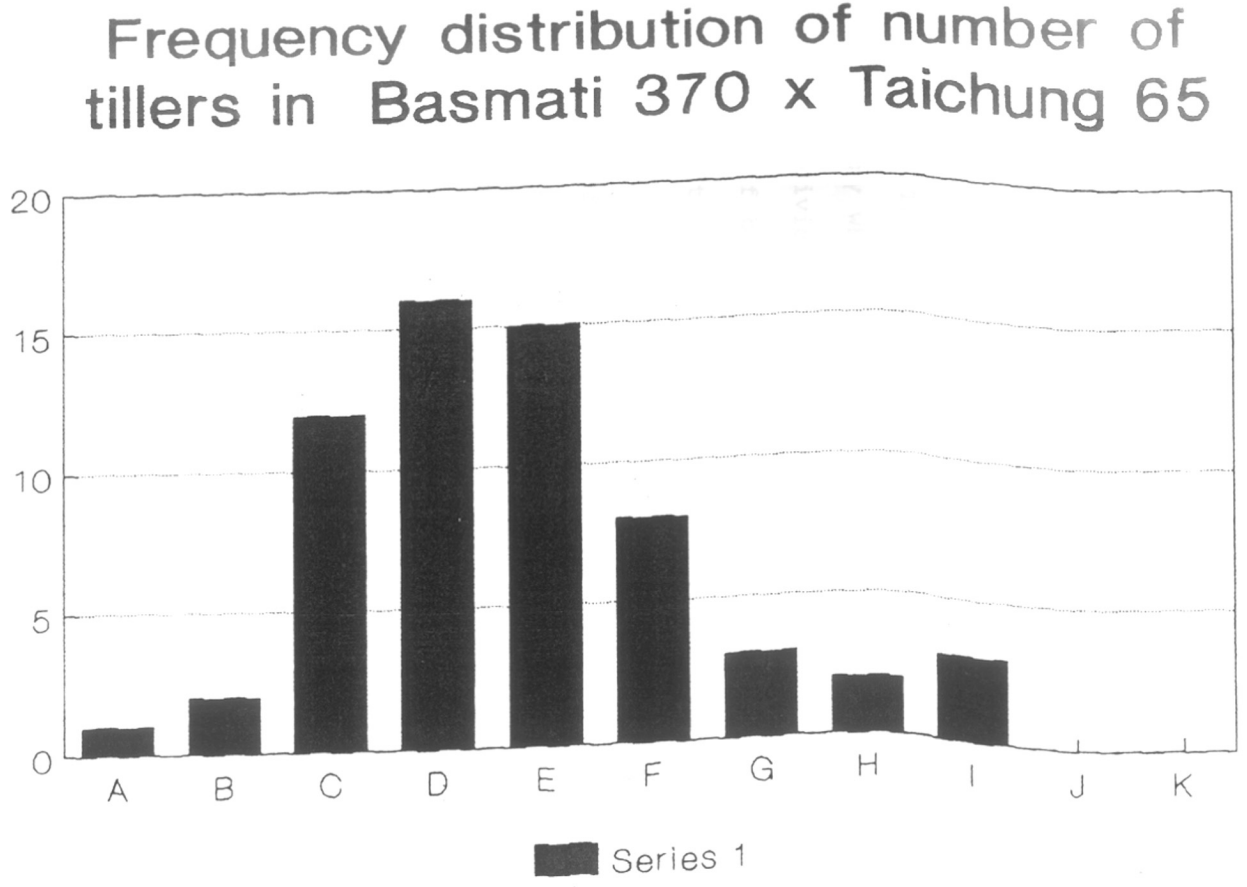
Frequency distribution of number of tillers in Basmati 370 x Taichung 65
20
15
10
5
0
A
B
C
D
E
F
G
H
I
J
K
Series 1

Fig. 3.17

As observed in the earlier cross here also the flowering period begins from 25/4 and extends upto 10/6, a period of six weeks (data not shown). I have divided this period of six weeks into classes of a period of 5 days each in order to study the frequency distribution of this trait in the progeny. The majority of the individuals fall in the 1st, 3rd, and 4th classes indicating a tendency towards earliness . Another fact is regarding the significant number of individuals being distributed into three classes viz. 3rd, 4th and 6th. This shows a distribution/segregation in the progeny which may call for a screening of large number of individuals in this progeny in order to verify such kind of behaviour. It is rather difficult to make any comment about the skewing in this population.

Now considering the two populations, one can conclude that there is no skewing with respect to the qualitative characters/traits and there is significant skewing with respect to the quantitative traits wherein indica type domination can be seen.

3.5 Discussion

3.5.1 F1 sterility

In the present study, we have detected 53% sterility in Basmati 370 which can be attirubted to environmental variation (8,9,2). The F1 sterility

varying from 18% to 38% in the Basmati-370 x Taichung-65 and 16% in Basmati-370 x Adt-27 is in concurrence with earlier observations of spikelet sterility (10,11). Shastry (12) has observed that reciprocals in general do not differ in sterility in a limited number of cross combinations wherein the difference between the cross and its reciprocal ranges from 20 - 30%. However in a MO.2 x Taikoku 6 cross, the sterility of the reciprocal hybrids was found to be 28 and 98%. The role of cytoplasm, although limited in evidence, is positive at least in some cases. This view has been confirmed by the differences in sterility which persist even in the F2 generation. Oka (11) has found that in most cases, reciprocal crosses showed no significant differences in the percentage of good pollen in F1 hybrids.

3.5.2 Plant morphology

According to Shastry (12) in several F2-F4 populations, albino, xantha and "non-flowering" plant are observed which he feels may have resulted due to disharmonious recombinations in hybrids. Venkataswamy (13) reports "narrows leaf", "sterile lemma" and dwarf sterile "types in some progenies. In the present study too, dwarf bushy plants with very few to no flowering and a striata mutant are observed in the Basmati-370 x

Taichung-65 cross. These plants were weak and narrow leaved. This observation is in concurrence with the previous ones and indicates the presence of anamolies in the cross. According to Oka (11), the occurrence of sterile or weak plants in F2 and in later generations can be largely explained by double-recessive combinations of duplicate genes and may be regarded as a tendency of hybrids to break down. Further, he states that these phenomena will be freqently encountered in hybrids between distantly related sativa varieties and may be used as an index of phylogenetic relationships in the a manner as F1 sterility. However, an important difference between F1 sterility and the present phenomenon is that the former occurs in heterozygotes and causes genetic selection while the latter occurs in homozygotes and results in zygotic elimination.

3.5.3 Spikelet morphology

The spikelet morphology shows the dominance of japonica type "short bold" grains in the Basmati-370 x Taichung-65 progeny. In the Basmati-370 x Adt-27, there is a tendency of more Adt-27 like grains (data not shown). This indicates the skewing of spikelet morphology determining traits in the populations.

3.5.4 Panicle type

Nayar (14) states that the panicle type of an

indica is of medium length, intermediate branching type having moderate density and light in weight as against the japonica having a short, less branching and having high density with heavy weight. In the present study, most of these traits are seen to manifest in the indica and the japonica parents. In the segregating progeny, variants ranging from indica to japonica types can be seen but no significant skewing in panicle types can be observed. .pa

3.5.5 Qualitative traits

Aroma

From many investigations in the past, aroma in rice is known to be genetically controlled and is found to be either monogenic recessive or dominant, digenic, trigeneic and polygenic in inheritance (7,15,16,17,18,19,20,21,22,23,24,25).

In the present study, we report a 3:1, aroma : non- aroma type segregation indicating a monogenic dominant mode of inheritance.

But as it is evident from the above examples, there seems to be no consensus on the nature of inheritance of aroma in rice. However, Tsuzuki and Shinokawa (1990) suggest that the genes responsible for this character many differ with aromatic varieties.

Awn

In the case of Basmati-370 x Adt-27, the inheritance for awn shows a 15:1 (awnless : awned) mode of inheritance which is indicative of duplicate gene loci. Oka (11) states that both the haplontic and diplontic sterilities, as well as the occurrence of weak F2 plants found in intervarietal hybrids of sativa, can be interpreted on the hypothesis of duplicate genes. The presence of duplicate genes controlling empty-glume length, pigmentation of certain organs and other character has already been known on the basis of 15:1 F2 ratios (Oka 11) implying that the germplasm of sativa might be duplicated to some extent. Duplication of gene loci results either from doubling of chromosomes or from translocations. Although a large number of cytological workers are inclined to assume the presence of cryptic structural differences, the evidence is not sufficient because if there are many minute structural differences, there should also be differeneces large enough to produce visible disturbance of meiosis.

Apiculus colour

The apiculus colour segregates in 3:1 (coloured : colourless) manner and shows a monogenic dominant behaviour. According to Qi Zu-ben et al. (26), the genetic pattern of wide compatibility is governed

by nuclear genes in the cross of indica with japonica and is probably dominanted by the major effect gene along with the minor effect gene. The major gene, which is a dominant single gene, has a remarkable influence on wide-compatibility and is closely linked with the marker gene of apiculus color. The minor gene possesses only slight effect on compatibility and bears no relationship with the marker gene.

When the progeny for Basmati-370 x Adt-27 is observed, one can notice the "probable effect" of the "wide compatibility" gene.

3.5.6 Quantitative traits

Looking at the frequency distribution of plant height in both the crosses, a distinct preference for tallness can be seen indicating a skewing towards indica type. The number of tillers seems to be more in the F2 segregants in both the populations and again this demonstrates skewing towards indica type.

When the days to flowering are considered in the Basmati-370 x Adt-27 cross, a tendency for preference to earliness is seen whereas the distribution is completely distorted in the Basmati-370 x Taichung-65 cross.

The overall comment about qualitative and quantiative characters is that more skewing is observed in the case of quantitative traits.

According to Siddiq (5), disturbed genetic coherence rather than chromosomal differences may be responsible for both the semi-sterility and skewed segregation ratios observeed in the indica-japonica hybrids progenies. In my work, it is found that there is a preference in the quantitative traits with a non-preference in quanlitative traits which hints at a disturbed genetic coherence. Thus it appears that the differentiation of these two racial groups does not involve a systematic or macromutation, but has probably proceeded through a series of independent mutations affecting grain and plant characteristics brought together in a cluster, probably under the influence of disruptive selection. If many of the mutant loci have an antimorphic effect in relation to the orignal allele, sterility can be the result in hybrids combinations.

As per Oka (11), we have assumed that in the course of evolutionary change toward cultivated forms, the differentiation in certain characters might proceed ahead of that of sterility relationship. If duplicate genes controlling intervarietal sterility are linked with other gene complexes determining adaptability to different environments, it will be possible that plants selected for different ecological requirements are gradually differentiated into partly inter-sterile

groups. As the indica-japonica differentiation seems to have advanced in parallel with that of wild plants to cultivated forms, its initiating factor might be a differential response to a certain new environmental condition arising in a cultivated habitat.

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CHAPTER 4

Use of RFLP approach to study an indica-japonica cross : evidence for occurrence of small deletions leading to abnormal segregations

CHAPTER IV

SUMMARY

Indica-Japonica diversity is assessed by studying the RFLP marker behaviour in the hybrid progenies of two crosses namely indica x indica - japonica derivative (Basmati-370 x Adt-27) and indica x japonica (Basmati-370 x Taichung-65). The RFLP analysis reveals (i) at least one and maximum of seven plants with the absence of a band present in both the parents and (ii) peculiar "allele-like" behavior of the loci deciphered by pOSM1C-2. The findings indicate the deciphering of multiple loci by these probes and hint the possibility of small deletions which may be the result of included inversions.

4.1 Introduction

An important aspect of indica-japonica interrelationship is to study their hybrids and progeny. If the two subspecies are diverse, it will reflect in their hybrids and progeny in terms of their genetic make-up. In the case of indica x japonica hybrids, F1 sterility is observed from 0 to 90 % and the segregation is skewed, where the skewing is either towards the indica or japonica parent (1,2,3,4). Further work on cytogenetics of the F1 hybrids on these lines has revealed that there are recombinational abnormalities where approximately 30% of the chromosomes are not involved in pairing (5,6,7). From the subsequent work in this direction, it appears that there are two probable reasons for the sterility exhibited by the F1s of indica x japonica crosses viz. (i) genic and (ii) chromosomal (structural) (8,9,10). However, there are no adequate evidences for and against both these reasons.

With the recent advent of RFLPs, a powerful tool for genetic analysis, it is possible to throw some light on some fine changes which are undetectable by cytological methods. In the present work, I have made an attempt to use RFLP approach to look into the problem of the cause of sterility and other features exhibited by indica x japonica hybrids. This is proba-

bly one of the first attempts to correlate RFLP with the cytogenetics to solve some of the long pending problems in indica-japonica relationship/genetics.

4.2 MATERIALS & METHODS

4.2.1 Plant materials

The seeds of Taichung-65, Adt-27 and Basmati-370 were obtained from Central Rice Research Institute, (CRRI), Cuttack, India. The crosses were performed using conventional methods to obtain F1 and F2 progenies and were confirmed by normally used methods involving morphological data.

4.2.2. Plant DNA extractions, agarose gel electrophoresis and Southern blotting

The 3-4 week old leaf tissue, used for DNA extractions, was harvested and immediately frozen in liquid nitrogen and was stored at - 70°C. The DNA extractions were done according to Shure et al. (11). For restriction enzyme digestion, 10ug DNA was used with enzyme concentration of 5U/ug of DNA. The restriction enzymes used were obtained from Bangalore Genei Pvt. Ltd., Boehringer Mannheim and Bethesda Research Laboratories. The DNA digestions were carried out overnight as per the respective instructions of the

manufacturer. The DNAs were resolved onto 1% agarose gels in 1x Tris-Borate-EDTA buffer and the gels were blotted using Genescreen Plus membrane, as per the manufacturer's instructions.

4.2.3 Southern hybridizations and autoradiography

Insert DNA of the following probes were used in Southern hybridization experiments

(i) Rice glutelin c-DNA obtained from Dr. S. Muthukrishnan, Kansas State University, U.S.A.

(ii) Mouse rDNA 18S subunit obtained from Hindustan Lever Research Centre, Bombay.

(iii) pOSM1C-2, pOSM1D-9 and pOSM5F-3 were from the parital PstI library of cv. Malkolam constructed by Dr. (Ms.) V.V. Pethe from our laboratory.

Plasmid DNA isolation was carried out according to Birnboim et al.(12) and Dretzen et al. (13). 40ng of insert DNA was labelled using alpha-P-32-dCTP (3000 Ci/mM) and random primer labelling kit from BARC, Bombay. The autoradiography was done using Amersham Hyperfilms and Kodak X-Omat films.

4.3 RESULTS

Inter-species or intra-species interrelationships have been studied considering specific genes or sequences amongst inbred lines. But another aspect of

these relationships is the recombination barriers offered by the species to maintain the species integrity. In the case of indica-japonica interrelationship analysed so far, investigations have been made at the molecular level using the approach of utilizing inbred lines (14,15). In the present study, we have made an attempt to observe the molecular marker segregation in order to understand or probe the probable cause(s) of the F1 sterility and skewed segregations due to intraspecific barriers in recombination.

In RFLP aided analysis , it is observed that there is a preference to use either single copy or low copy clones. This is due to the ease of interpretation of the patterns revealed by these probes, especially in segregating progenies. The repeats have been seen to be used only in the case of genetic diversity or fingerprinting. In our study, we have decided to use repeats as the probes because these sequences tend to differ much more as compared to the single- low copy sequences. Secondly, repeats provide an opportunity to assess the genetic make up for more than one locus simultaneously. We used probes from the following two classes of DNA sequences viz.

(a) Repeated DNA : pOSM1C-2 .. dispersed repeat
: rDNA .. tandem repeat
: pOSM1D-9 .. low copy

: pOSM5F-3 .. low copy

(b) Low copy DNA : cDNA clone of rice glutelin

(5 - 10 copies)

4.3.1 Parental analysis

In our RFLP analysis of F2 segregations, since parental DNA was available in abundance, parental analysis was carried out first to identify polymorphic probe-enzyme combinations. As the F2 DNAs were a limiting factor, these already tried and proved combinations were then used to assess the F2 level segregation.

Table 4.1 shows the number of polymorphic probe- enzyme combinations observed with each probe and six enzymes viz. Bgl I, Cla I, Dra I, Hae III, Hinf I and Pvu II. The results indicate that Basmati-370 x Taichung-65 cross exhibits more polymorphism where 24 probe - enzyme combinations are polymorphic as against only 11 in the case of the Basmati 370 x Adt 27 cross. Secondly, based on these results we selected Bgl I as the enzyme as most of the clones showed polymorphism in terms of more polymorphic probe-enzyme combinations with it.

4.3.2 F2 analysis

Out of the two crosses, the Basmati 370 x Taichung 65 showed more of skewing and F1 sterility. We

TABLE 4.1

Sreening of the two crosses with different probe/enzyme combinations

S.No.	Name of probe used	Basmati 370 x Adt 27	Basmati 370 x Taichung 65
		No. of polymorphic probe/ enzyme combinations	No. of polymorphic probe/ enzyme combinations
1.	Glutelin cDNA (rice)	3	3
2.	pOSM1D-9	2	3
3.	pOSM1A-4	1	3
4.	pOSM1F-2	1	2
5.	pOSM1C-2	3	6
6.	pOSM5F-3	0	3
7.	pOSM4B-6	1	3
8.	pOSM1A-3	0	1
Total polymorphic probe/enzyme combinations		11	24

judiciously selected the progeny of the Basmati-370 x Taichung-65 cross which had only 18% F1 sterility to enable us to observe most of the genotypes occurring at the F2 level. Secondly, at the molecular level the screening of the F2 plants had to be done using a limited yet statistically significant number of plants. The field data was collected using 100 odd plants and out of these a stratified random sampling was done to select 50 plants, which represented well the ratios of qualitative characters. To determine the minimum number of progeny required to be screened, we made use of the tabulated data provided by Mather (16). In a RFLP marker screening, one expects either a 1 : 2 : 1 or a 3 : 1 segregation. In order to obtain at least one plant of the double recessive class in the ratio 1 : 2 : 1 at a probability of 0.99, a minimum of 16 plants are required whereas in the ratio 3:1 at a probability of 0.99, a minimum of 9 plants are required. Hence we decided to use 20 plants which satisfies both the conditions. The DNAs of these 20 plants were digested with Bgl I, blotted and screened with the probes. The segregation of each probe is mentioned separately in the following paragraphs.

pOSM1C-2

In Fig 4.1, the parents reveal 4 bands each

Fig. 4.1 : Southern hybridization of pOSM1C-2 insert (1.55kb) with 20 F2 DNAs of Basmati-370 Taichung-65 cross. Lanes 1-20 : F2 DNAs; lane 21 (P2) : Taichung-65; lane 22 (P1): Basmati-370.

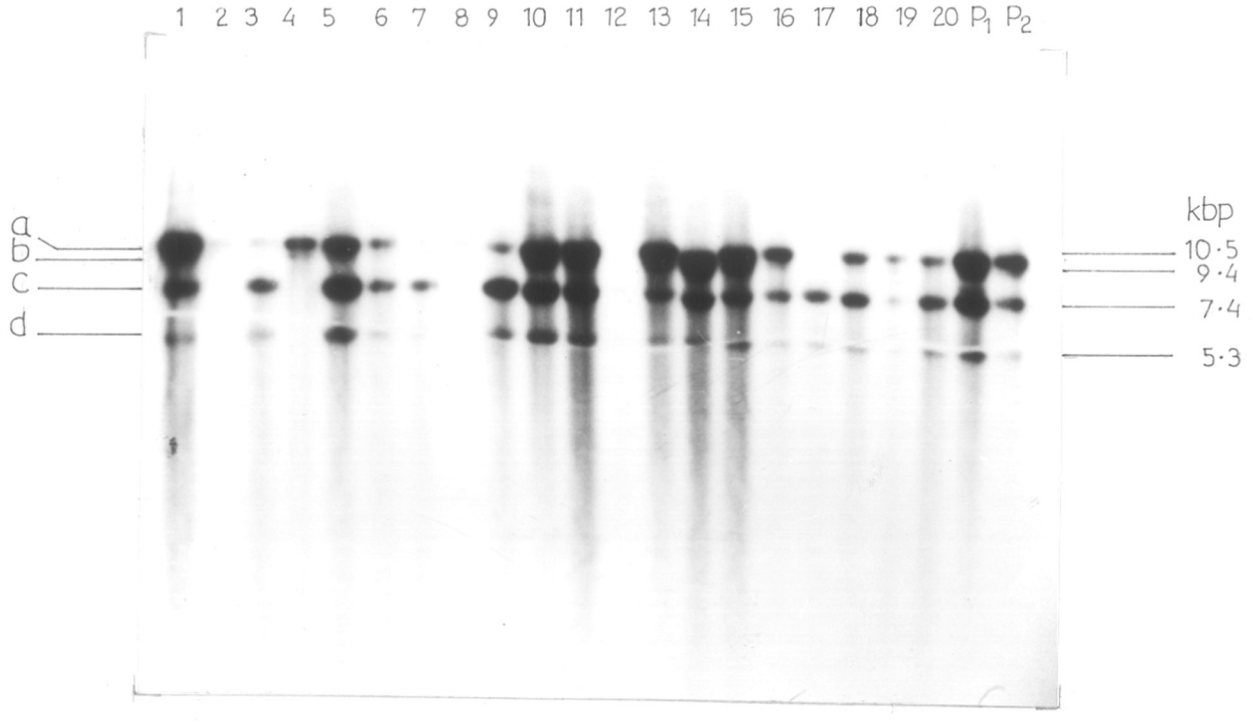
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 P1 P2
a
b
c
d
kbp
10·5
9·4
7·4
5·3

Fig. 4·1

Fig. 4.2 : Southern hybridization of mouse rDNA (18s subunit) insert (1.9 kb) with 20 F2. As of Basmati-370 x Taichung-65 cross lane 1 (P1) : Basmati-370; lane 2 (P2) : Taichung-65; lanes 3-22 : F2 DNAs.

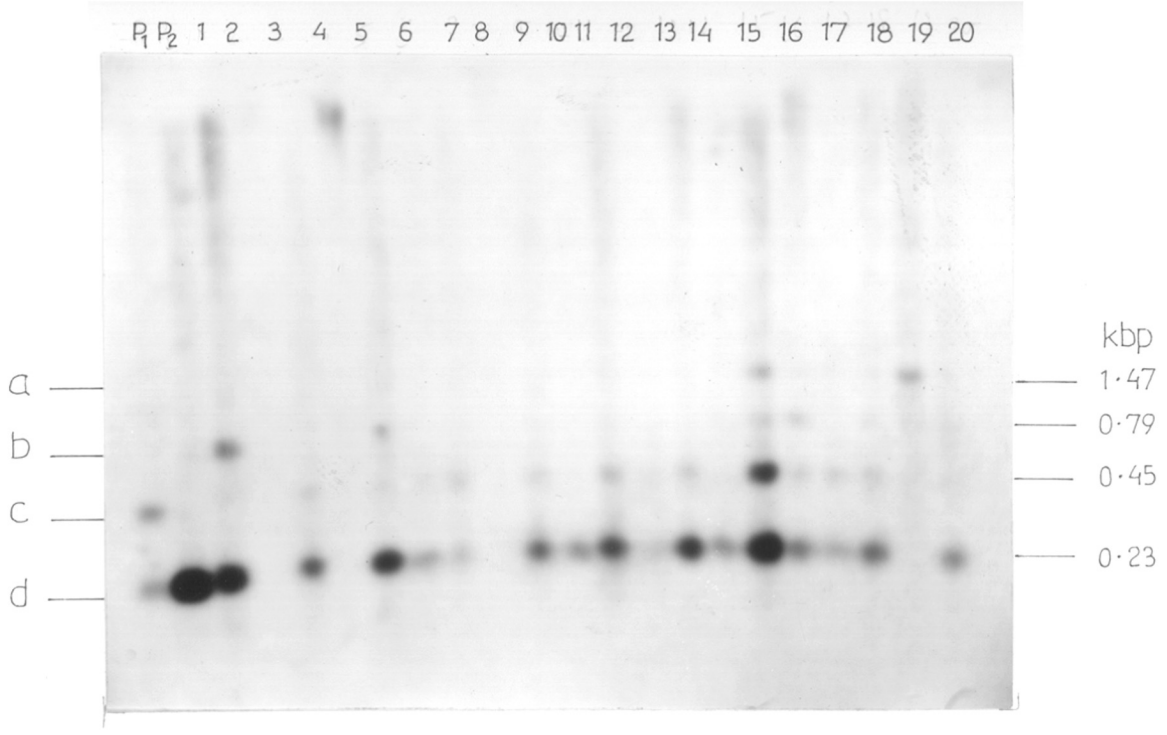
P1 P2 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
a
b
c
d
kbp
1·47
0·79
0·45
0·23

Fig. 4·2

with no polymorphism for three bands except for the second band (9.4 kb). The lanes marked with an arrow exhibit the absence of fragments which are present in both the parents. Secondly in the two lanes viz. 2 and 4, only the upper band (10.5 kb) is present whereas in lanes 7 and 17, only the two lower bands are present. These results indicate that the 10.5 kb band and the 7.4 kb and 5.5 kb bands are mutually exclusive. Third ly, the 10.5 kb and 9.5 kb bands and the 7.4 kb bands and 5.3 kb bands segregate independent of each other suggesting the presence of multiple loci.

rDNA

Figure 4.2 indicates that the parents have a clear cut difference in the organization of this locus. The Basmati-370 has two bands (0.45 kb and 0.23 kb) whereas the Taichung-65 has only one band (0.23 kb). In the F2 progeny, some plants viz. 1, 15, 16 and 19 reveal the presence of bands of different molecular weights (0.79 kb and 1.47 kb) when compared to the parents. The origin of these bands has to be elucidated as these are not present in the parents. The rest of the plants show normal segregation for the (0.45 kb) band in the ratio 11 : 6 (P = 0.30 - 0.50 for 3 : 1). These results suggest the differential organization of the rDNA in Basmati-370 and Taichung-65 as well

as some recombinational abnormalities existing in the event of recombining the nucleolar organizer regions from these two plants.

pOSM1D-9

In Fig 4.3b, the autoradiogram exhibits a difference in the banding pattern in the parents. The Basmati-370 DNA reveals faint two bands (8.56 kb and 6.18 kb) whereas Taichung 65 has distinct three bands (8.58 kb, 6.18 kb and 4.45 kb) with a more intense middle band. These three bands are labelled as 'a','b'and 'c', respectively. As the bands 'a' and 'b' are monomorphic, only the 'c' band segregates as 11 : 5 (presence : absence; P = 0.50 - 0.70 for 3 : 1), indicating the dominance of a japonica allele. Secondly, the 'b' band in the Taichung 65 cultivar can be traced by the band intensity as a japonica allele. This band is absent in lane 19 even though it is present in both the parents. It is present in 6 out of 15 plants. Lastly, the 'a' band too, though present in both the parents, is absent in 2 plants. This absence of bands 'b' and 'a' in a few F2 plants indicates an abnormality hinting a deletion of that allele in the plants.

pOSM5F-3

Fig. 4.4a : Southern hybridization of rice glutelin cDNA insert (1.2 kb) with 20 F2 DNAs of a Basmati-370 and Taichung-65 cross lane 1 (P1): Basmati-370; lane 2 (P2): Taichung-65; lanes 3-22 : F2 DNAs (lane 4 not considered in the analysis due to excess DNA).

Fig. 4.3b : Southern hybridization of pOSM1D-9 insert (1.2 kb) with 20 F2 DNAs of a Basmati-370 x Taichung-65 cross lane 1 (P1) : Basmati-370; lane 2 (P2) Taichung-65; lanes 3-22; F2 DNAs.

Fig. 4.4c : Southern hybridization of pOSM5F-3 insert (0.8 kb) with 20 F2 DNAs of a Basmati-370 x Taichung-65 cross lane 1 (P1) : Basmati-370; lane 2 (P2): Taichung-65 ; lanes 3-22: F2 DNAs.

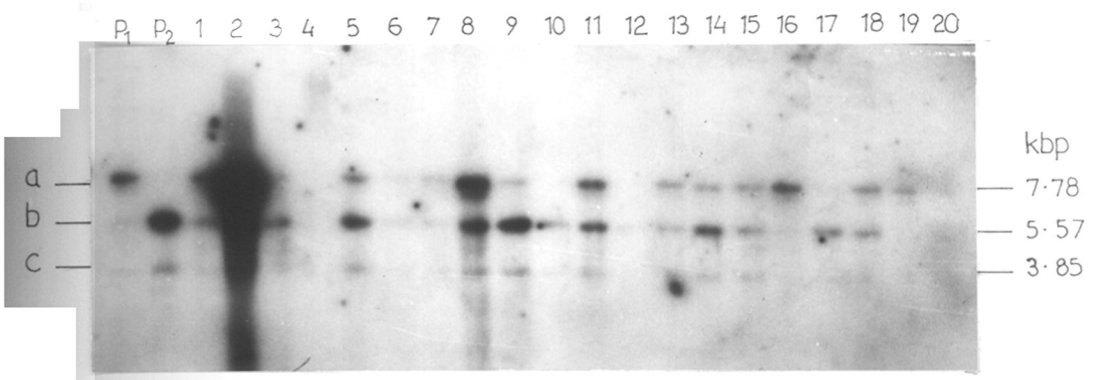

Fig. 4·3 a

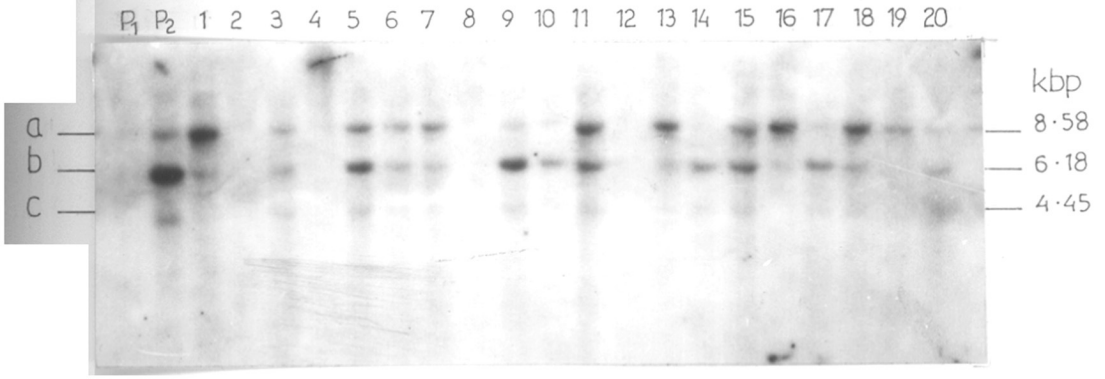

Fig. 4·3 b

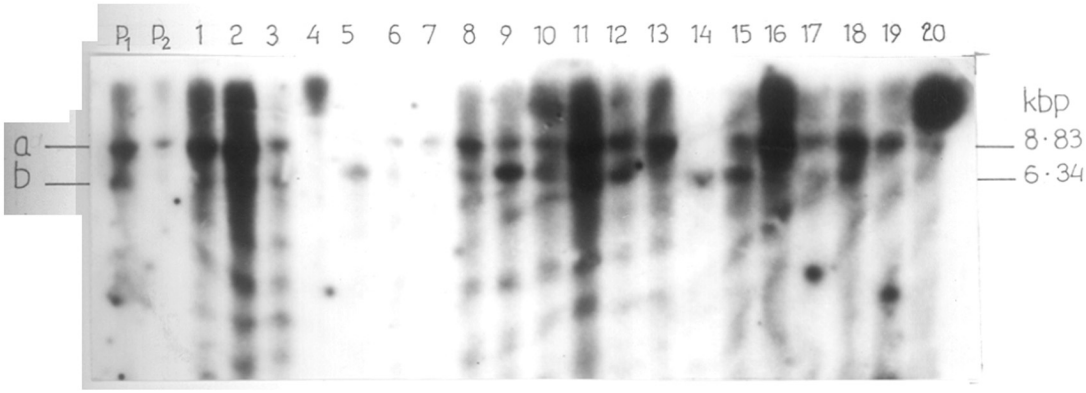

Fig. 4·3 c

For this locus, a difference between both the parents is seen (Fig 4.3c). Out of the total bands that are lighted out the lower band (6.34 kb) is absent in the Taichung-65 DNA which is segregating in the progeny. Lanes 5 & 14 reveal the absence of band 'a' (8.85 kb) which is present in both the parents suggesting some abnormality. The other segregants seem to be normal where the lower band segregates (6.34 kb) as 15 : 4 (P = 0.50 - 0.70 for 3 : 1). Based on these results, it appears that this locus is similar to pOSM1C-2 and pOSM1D-9 loci in getting involved in abnormal segregation.

Rice glutelin cDNA clone

In Fig 4.3a,the Basmati-370 DNA reveals three bands (7.78 kb, 5.57 kb and 3.85 kb) and the Taichung-65 DNA two bands (5.57 kb and 3.85 kb) of which the lower two are shared by both. The upper one from Basmati 370 segregates in the progeny. Some of the bands from Basmati-370 and Taichung-65 are mutually exclusive and hence are allelic according to the definition of RFLP alleles. These alleles of the locus segregate in a codominant manner. Secondly, the pattern also reflects the differential organization of glutelin genes in both these cultivars. The segregants show a 8 : 7 : 1 mode of inheritance which is

different from the expected 4 : 8 : 4 if the overall pattern in considered (P = 0.01< for 1 : 2 : 1). But apart from this, there are some abnormalities observed in lanes 6,7,13,16,18 & 19 where only some bands are seen, which are alleles derived form either parents. This indicates a possibility of a deletion occurring in these plants for one of the lower two bands. If the three bands are assumed to segregate independently, then the 7.78 kb and 5.57 kb segregate as 14 : 2 (P = 0.20 - 0.30 for 3 : 1) whereas 3.85 kb segregates as 9 : 7 (P = 0.05 - 0.10 for 3 : 1), suggesting differential genetic behavior.

4.4 Discussion

Rice is a self-pollinated crop and, therefore, has most of the problems associated with the self-pollinated system like lack of heterosis or sufficient vigour and lack of variability. These two areas have a direct impact on the breeding of this crop for important parameters like yield. Several attempts were made and are being made to identify sufficiently diverse parents in order to achieve the above mentioned objectives, one of this being the inter-racial or inter-subspecific crosses like indica x japonica or indica x javanica. The indica x japonica combination is better than the other as indica and japonica are more

diverse.

A wealth of information is available regarding the variations between indica and japonica rices based on conventional characters. Taking these observations as the base, many programmes were initiated to derive suitable hybrids from an indica x japonica cross. These programmes were however, hindered due to some serious problems like high degree of F1 sterility and skewed segregations giving rise to the unavailability of desirable recombinants. To understand these observations, studies were carried out with the then available tools viz. morphological characters, biochemical markers and cytogenetical approaches. These studies pointed out many a possibilities of which the major ones being genetic causes and chromosomal structural alteration. Sufficient proof could not be obtained for or against the structural alterations as there was no tool available which could give a very fine resolution.

In the present study, RFLP has been used as an attempt to gather some data regarding this aspect. A random population of 20 F2 plants derived from a Basmati 370 and Taichung-65 cross was assessed for segregations with 5 probes which revealed 17 loci on an aggregate. Two important observations emerge from these data : (i) At least one and maximum of seven plants are found to show a questionable pattern. On further

analysis one can find that these plants display the absence of a band which is present in both the parents. This calls for a serious thought. (ii) There is a peculiar "allele-like behavior" of the 'a', 'b' and the 'd', 'c' loci deciphered by the pOSM1C-2 probe. These loci are not allelic as they are not always mutually exclusive, a test for allelic complementation. Considering the above mentioned facts, it leads us to an obvious conclusion about the possibility of a deletion. But according to Henderson (1964), these deletions can occur as a follow up of a crossing over taking place between two heterozyotes for inversions. He also states that in rice hybrids may differ in a large number of inversions without this being detected. Further, he states that single inversions, even if numerous, will not account for the high degree of sterility that is characteristic of indica - japonica hybrids and hints at the possibility of an included inversion which can be illustrated as follows:

(I) A BCDE F
(II) AE DC BF
(III) AEC DBF

In this scheme, it is assumed that the original chromosome identified as (I) has the gene sequence ABCDEF and then an inversion occurs involving the segment B through E to form chromosome (II). It is then assumed

that a second inversion develops later in chromosome (II) involving the segment D through C forming chromosome (III) with the gene sequence AECDBF. The reinversion forms what is termed an included inversion because the inverted segment is entirely within the region of the first change.

The meiotic behavior in the hybrid which contains original chromosome I and reinverted chromosome (III) will differ in several important aspects. For one thing, the typical inversion will not occur at anaphase and consequently, there will be no anaphase cells with bridges and fragments. In short chromosomes, it is probable that "non-homologous pairing" will occur in the displaced B and E segments. It means that the presence of inversions in the hybrid cannot be detected by the usual cytological methods. On the other hand, the regions with CD in the two chromosomes will be brought into homologous association through pairing, despite the fact that this region is involved in the inversions:

(I) ABCDEF
(III) AECDBF

Chaisma formation in the CD regions will form deficient and duplicated chromatids, resulting in sterility.

The RFLP data obtained here does show the possibilities of occurrence of minor deletions and

suggests the presence of included inversions occurring in rice.

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CHAPTER 5

Computer analysis of DNA homology and Free Energy of regulatory domains in *Oryza sativa* L.: demonstration of intraspecific DNA polymorphisms

CHAPTER V

ABSTRACT

To assess intersubspecific variations at DNA level, 5' upstream sequences of 13 japonica and 7 indica genes are analyzed for DNA homology and transition free energy. The dendrograms based on percent homologies between indica and japonica 5' upstream regions do not show any separate grouping of the two suggesting that the regulatory domains of these two subspecies may not be directly involved in their differentiation process. The japonicas exhibit a higher free energy profile than the indicas in the 5' upstream region effected by the frequency distribution differences of AT, CA, CG, GC, TA and TG.

An attempt is next made to correlate the 5' upstream transition free energy and the level of gene expression using seed storage proteins as a model. Based on the proportion and free energy data of rice glutelin and other few seed storage proteins, a positive correlation is noticed between free energy of the DNA duplex and gene expression.

5.1 Introduction

The DNA sequence forms the crux of the genetic code and hence is highly significant in phylogenetic studies. To date extensive studies have been carried out using direct DNA sequences including comparisons of 5S RNAs, ribosomal DNA intergenic spacers, and repeat DNA sequences (1,2,3). Such studies have yielded a wealth of information regarding the consensus sequences amongst different genes. The DNA base composition has been considered to explain the basis of gene expression and provide an aid in the process of genetic engineering (4,5,6,7).

As early as in 1984, Nussinov (4) has shown a common pattern in the helical regions in the promoter of E.coli and has suggested that conformational features are important for the specific reaction with RNA polymerases. His later studies have shown that divergences from standard DNA conformations may serve as signals for RNA polymerase binding (5). Margalit et al. (6) have examined the relationship between the double - helix stability and promoter activity in the prokaryots by free energy computation based on reported values of dinucleotide free energies for strand separation. The high free energy peak and the mutation data strongly supports the conjecture that the instability

or melting properties of the -10 region plays an important role in promoter function. In his recent work, Nussinov (7) has analyzed sequence signals in eukaryotic upstream regions and has found two DNA sequence elements occurring frequently upstream of eukaryotic polymerase - II transcribed genes viz. TATAAA at -40 and GGCCAATCT at -80 with respect to transcription initiation site. Analysis of nucleotide tracts has indicated that these sequences are preferentially flanked by their complementary nucleotides with a pyrimidine - purine junction i.e. TTA_n, CCG_n, C_nGG, T_nAA flanking translational initiation and termination sites for various eukaryotic taxonomic groups and have conserved mechanisms of initiation and termination.

In rice, Nagato et al. (8) has observed that the japonicas possess a lower DNA amount as compared to the indicas and therefore according to one school of thought the japonicas have evolved from the indicas. Several studies on the mechanism of indica - japonica differentiation have pointed out two main causes viz. (i) genic and (ii) chromosomal structural alterations. As there are genic causes, it is likely that the regulatory domains may also reflect the effects of the events that have taken place during the indica - japonica differentiation. The regulatory domains are useful in understanding phylogenetic relationships as

they provide a good system for study due to their slow and gradual evolving nature. In the present work, I describe the intraspecific variation exhibited by the regulatory domains in terms of DNA homology and free energy profiles in Oryza sativa.

5.2 Materials and methods

5.2.1 Rice gene sequence data

Table 5.1 shows the list of rice genes which were used in the analysis. The sequence information was obtained from Prof. A.S.Kolaskar, Head, Bio-informatics Centre, Dept. of Zoology, University of Poona, Pune.

5.2.2. Computational analysis

(i) DNA homology analysis

The DNA homology analysis was carried out using the DNASIS software on an IBM PC/AT. The dendrogram was constructed using a computer program written by myself and Mr. Dinesh Lagu in Turbo C and operatable on an IBM PC/AT.

(ii) Free energy and dinucleotide frequency calculations

Breslauer et al. (9) have demonstrated that DNA duplex structures can be considered thermodynami-

Table 5.1

List of rice genes

No.	Name of the gene	Acronym	Indica/Japonica
1.	Abscisic Acid gene	RAB	I
2.	Waxy gene	WXY	J
3.	Phytochrome gene	PHY	I
4.	Alcohol dehydrogenase gene	ADH	J
5.	Phenyl Ammonia Lyase gene	PAML	J
6.	Oryzacystatin 1 gene	CYST1	J
7.	Oryzacystatin 2 gene	CYST2	J
8.	Amylase gene	AMY	J
9.	Constitutive gene	CONSI	I
10.	Light induced Shoot specific gene	LSSG	I
11.	Nitrate reductase gene	NIA1	I
12.	Light harvesting Core Protein 1 gene	LHCP1	J
13.	Light harvesting Core Protein 2 gene	LHCP2	J
14.	Ribulose bisphosphate carboxylase gene	RBPC1	I
15.	Prepro glutelin gene.	PPGLU	J
16.	Shoot Specific gene.	SSSG	I
17.	Coumarate gene.	COUM	J
18.	Proliferating cell nuclear antigen gene.	PCNA	J
19.	Prolamin gene.	-	J
20.	Glutelin gene.	GLU	J

cally to be the sum of their nearest-neighbor pairwise interactions and can be used to calculate the stability and to predict the melting behavior of any DNA double helix from the primary sequence. The free energy and the mono- and di- nucleotide frequencies calculations were done using a computer program written by myself and Vivek R.Varma in Quick Basic. The algorithm used was based on the method of Breslaur et al. (9).

5.3 Results

The computational analysis of the rice (indica and japonica) genes was done in two parts viz. DNA maximum homology analysis and the free energy profiles of their 5' upstream regions.

5.3.1 DNA homology of 5' upstream regions of rice genes derived from indica and japonica varieties

A general survey of available rice genes was first done and a few genes representing indica and japonica rices were selected. As these genes were coding for different proteins, it was apparent that the downstream sequences to 'ATG' would be different in terms of their nucleotide composition. Then it was thought that the 5' upstream regions should be compared as these would have some homology being from the same system. Accordingly the 5' upstream motifs were ana-

lyzed for maximum homology for two different lenghts of DNA viz.

(i) -225 bp

(ii) -400 bp

Figure 5.1 depicts the dendrogram which was constructed by using the homology percentages derived by comparing -225 bp of different rice genes. As seen from the dendrogram, the indica genes and the japonica genes do not tend to cluster together and that there are two major clusters comprising RAB1, RBPC1, LHCP1, CYST2, LHCP2, NIA1, PAML, AMY, LSSG and WXY, CONSI, PHY. The cluster I has further two sub-clusters comprising RAB, RBPC, LHCP1, CYST2, LHCP2, NIA1 and PAML, AMY, LSSG. The maximum homology between the genes varies from 0.45 to 0.54.

Figure 5.2 represents a dendrogram which was constructed by using homology percentages derived by comparing -400 bp. Here again indicas and japonicas do not show any grouping and two major clusters are seen viz. I - LHCP1, LHCP2, AMY3, RAB1, LSSG, PAML, PCNA and II - CONSI, PHY, WXY, GLU, PPGLU, COUM, SSSG. The homology again varies from 0.45 to 0.55 for most of the gene except for GLUG and PPGLU wherein it is 1.0.

5.3.2 Free energy distribution and dinucleotide frequencies of indica and japonica genes

Figs. 5.1 & 5.2 : A computer generated dendrogram based on homology percentages generated on DNASIS software using the UPGMA method is depicted. The genes are aligned at the translation initiation site and comparisons are made at two lengths viz. -225 bp (Fig. 5.1) and -400 bp (Fib. 5.2). The abbreviations of the genes are same as referred to in Table 5.1. 'I' and 'J' are the suffixes for indica and japonica, respectively.

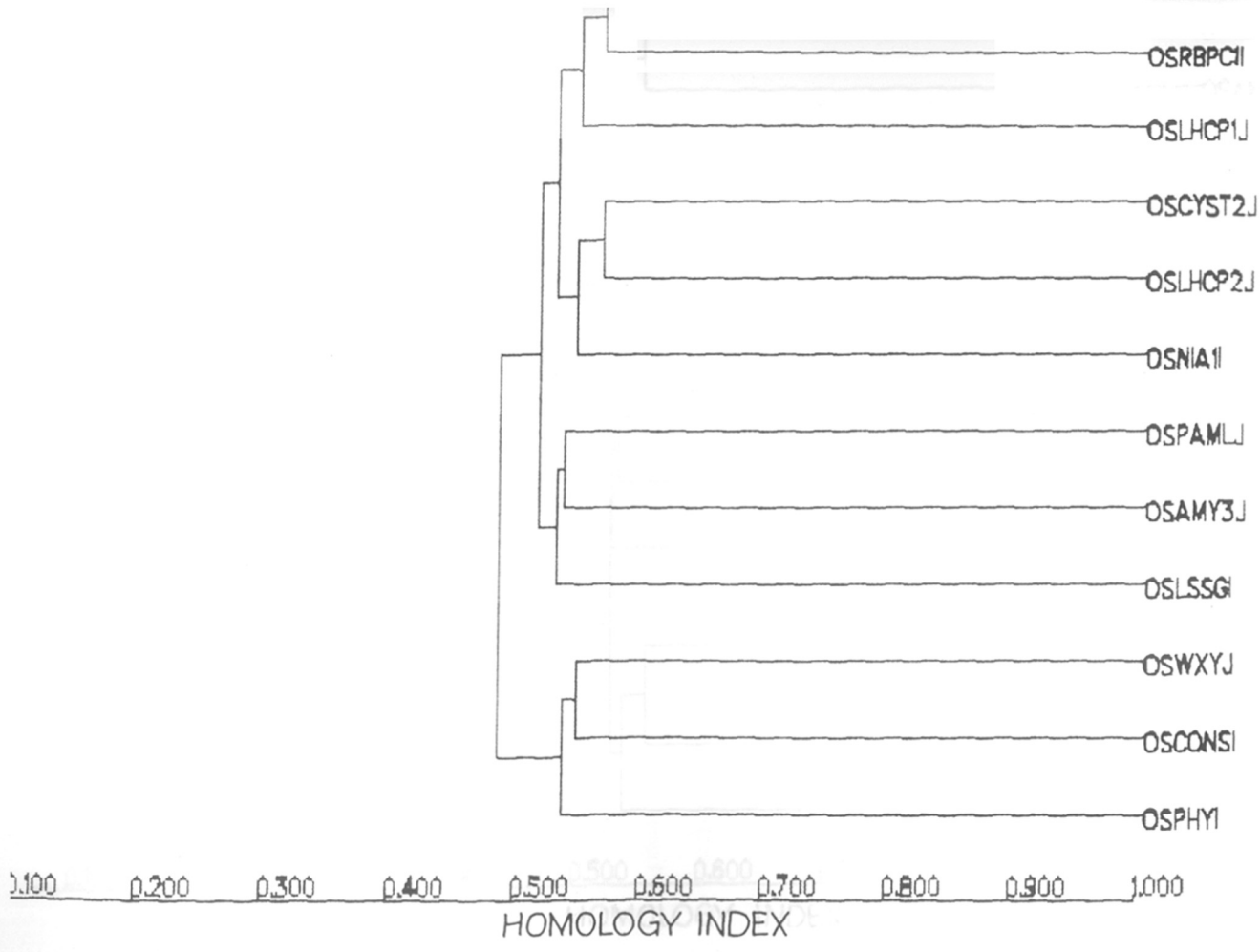
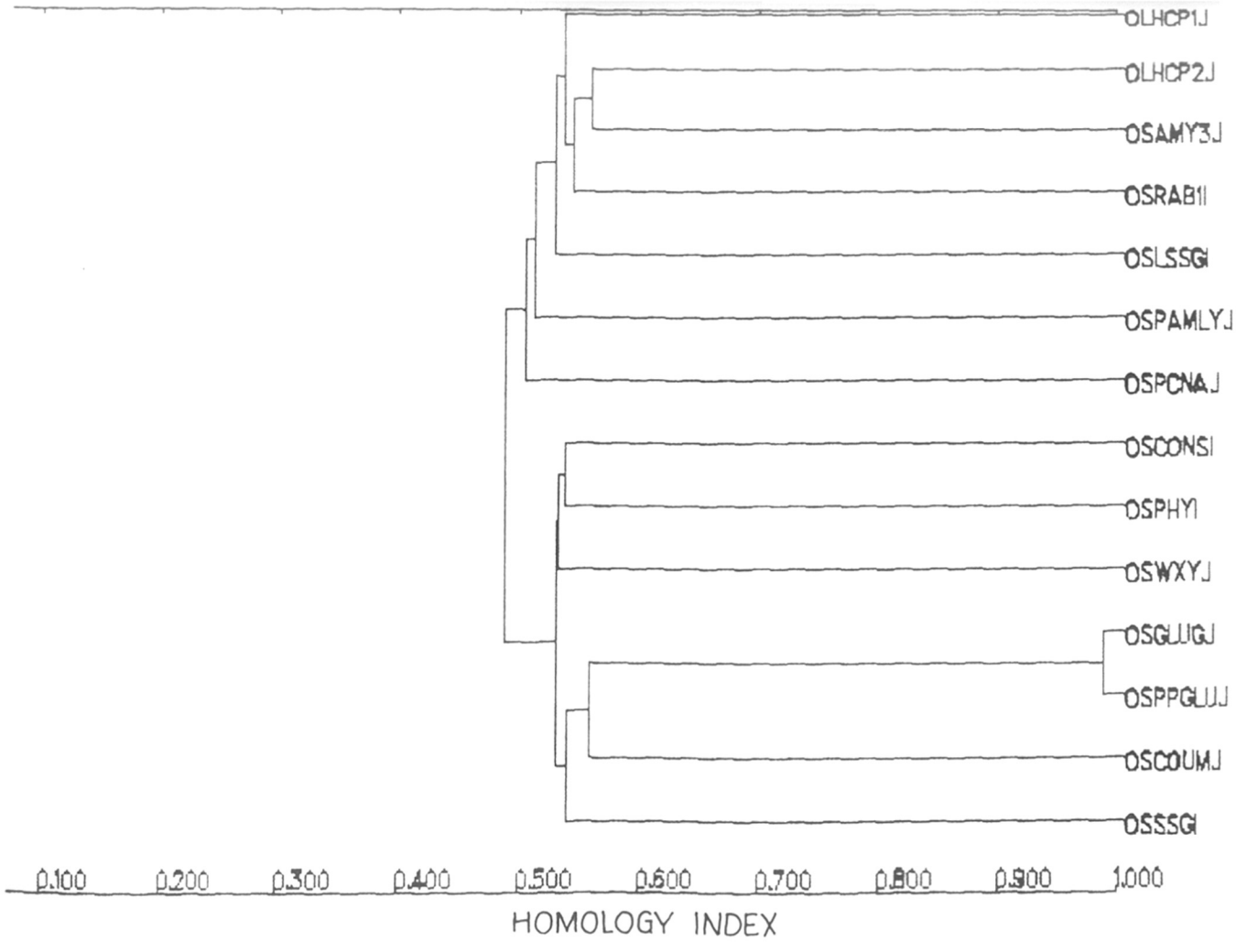

OSRBPC1I
OSLHCP1J
OSCYST2J
OSLHCP2J
OSNIA1I
OSPAML1
OSAMY3J
OSLSSGI
OSWXYJ
OSCONSI
OSPHY1
0.100
0.200
0.300
0.400
0.500
0.600
0.700
0.800
0.900
1.000
HOMOLOGY INDEX

FIG 5.2

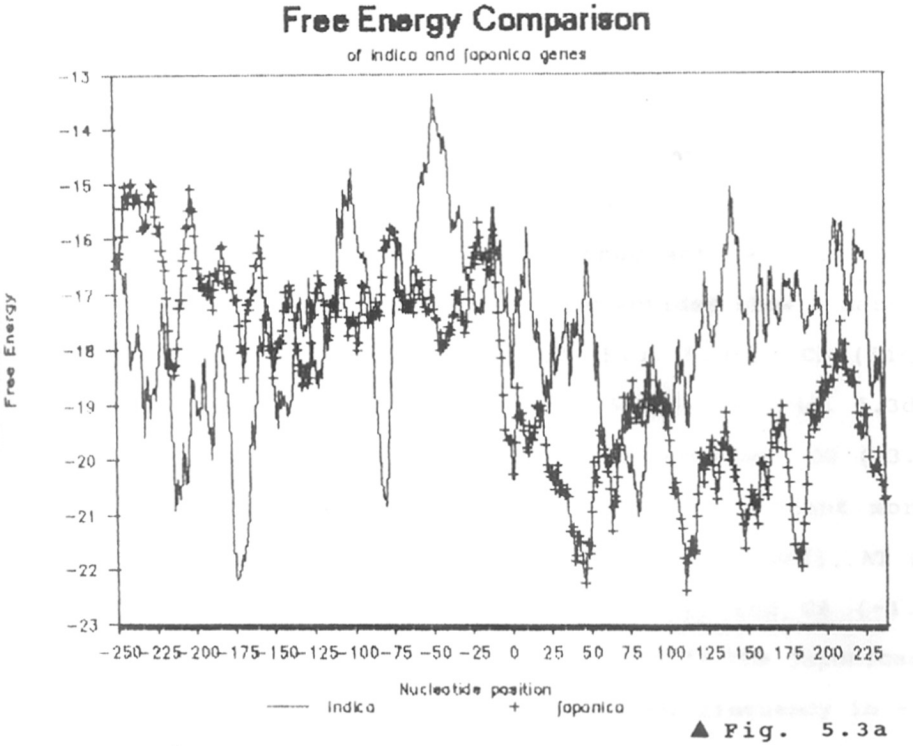

OLHCP1J
OLHCP2J
OSAMY3J
OSRAB1I
OSLSSGI
OSPAMLYJ
OSPCNAJ
OSCONSI
OSPHYI
OSWXYJ
OSGLUGJ
OSPPGLUJ
OSCOUMJ
OSSSGI
0.100
0.200
0.300
0.400
0.500
0.600
0.700
0.800
0.900
1.000
HOMOLOGY INDEX

To assess if there is a functional homology between indica and japonica genes, duplex stability which depends upon the free energies of the dinucleotides present in the duplex of the upstream regions of these genes was determined. The free energy analysis was done using indica and japonica genes for their free energy distribution patterns and their mono- and dinucleotide patterns.

Out of 18 rice genes used for comparison, 13 belonged to japonica and five were from indica. From Fig. 5.3a, which shows the free energy distribution patterns for indica and japonica a distinct difference between the free energies present upstream and downstream to the ATG can be seen where the upstream region exhibits higher free energy level than the downstream region. Comparing the indica and japonica free energy profiles in the 5' upstream region, it is clearly evident that the japonicas have a higher free energy as compared to the indicas. The downstream regions show a lower free energy for japonicas in comparison to the indicas. In order to verify the significance of this variation, 20 random rice DNA sequences were analyzed for their free energy distribution and considered as control (Fig. 5.3b). Comparing Figs. 5.3a & 5.3b, it can be seen that in the former the free energy varies from -11 kcal/mol to -17 kcal/mol as compared to the

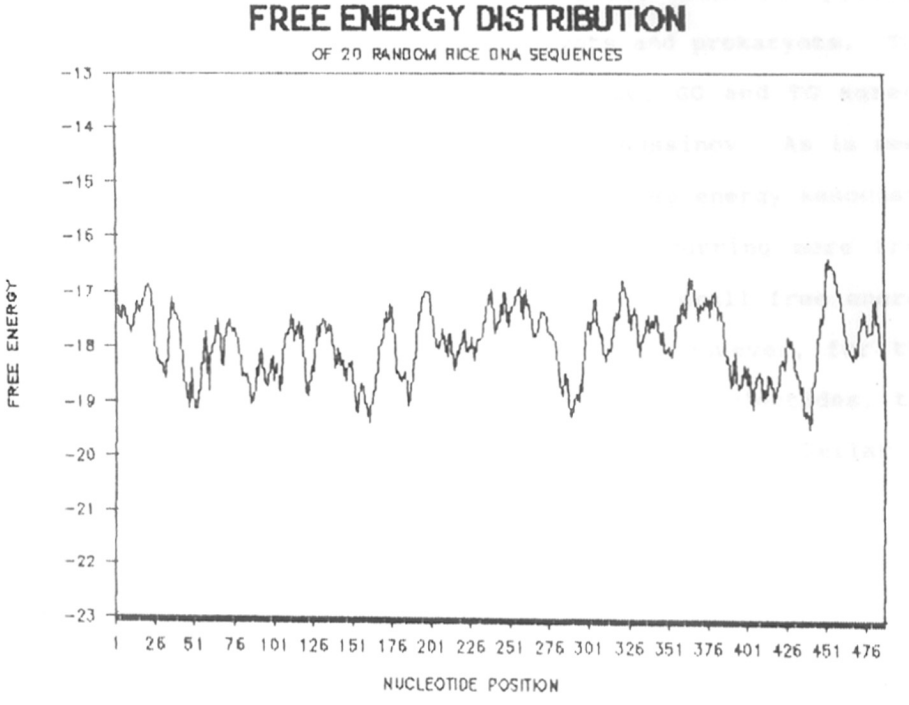
Fig. 5.3a : Distribution of free energies of 13 japonica and 5 indica genes around the translation start site. The graph is centered at the translation start position (position 0) and extends for 250 nucleotides on either side of position 0. Breslaur's free energy values are used with sliding blocks of 12 bases. Legend : + japonica and - indica.

Fig. 5.3b : Distribution of free energy of 20 randomly derived 500 bp long rice DNA sequences using Breslaur's free energy values with sliding blocks of 12 bases.



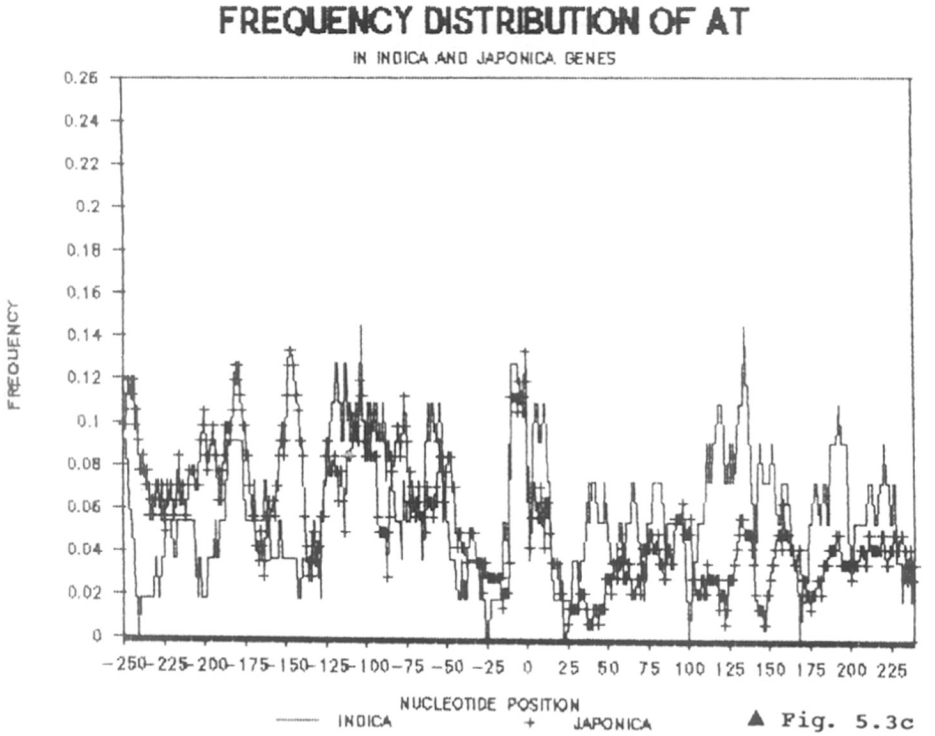
▲ Fig. 5.3a

▼ Fig. 5.3b



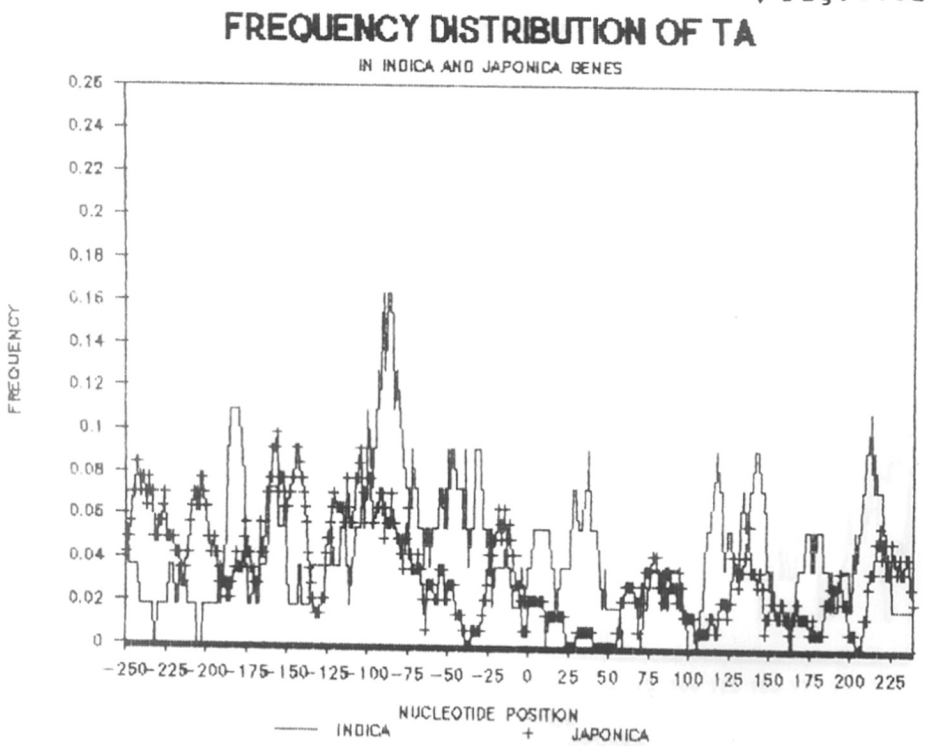
latter where it varies from -16 kcal/mol to -19 kcal/mol. To further probe the cause of such variation, it was felt necessary to look into the dinucleotide frequency distribution pattern in these regions. Looking at the possible 16 dinucleotide frequency distributions the following dinucleotides show a marked degree of difference viz. AT (Fig. 5.3c), CA (Fig. 5.3g), CG (Fig. 5.3f), GC (Fig. 5.3e), TA (Fig. 5.3d) and TG (data not shown). The dinucleotides CG (-3.1 kcal/mol) and GC (-3.6 kcal/mol) are present more frequently in the indicas and AG (-1.6 kcal/mol), AT (-0.9 kcal/mol) , TA (-1.5 kcal/mol) and CA (-1.3 kcal/mol) are present more frequently in the japonicas, except TA which is present at a higher frequency in -75 to -100 and -175 to -200 regions in indicas. According to Nussinov (7) there is a definite order of preference of dinucleotides in eukaryots and prokaryots. The more occurrence of AT, CA, CG, GC, GG and TG agrees well with the predictions made by Nussinov. As is seen in our analysis, most of the low free energy associated dinucleotide combinations are occurring more frequently in the indicas, making the overall free energy less as compared to the japonicas. However, for the other high free energy contributing dinucleotides, the frequencies of occurrence are more or less similar in the indica and japonica genes.

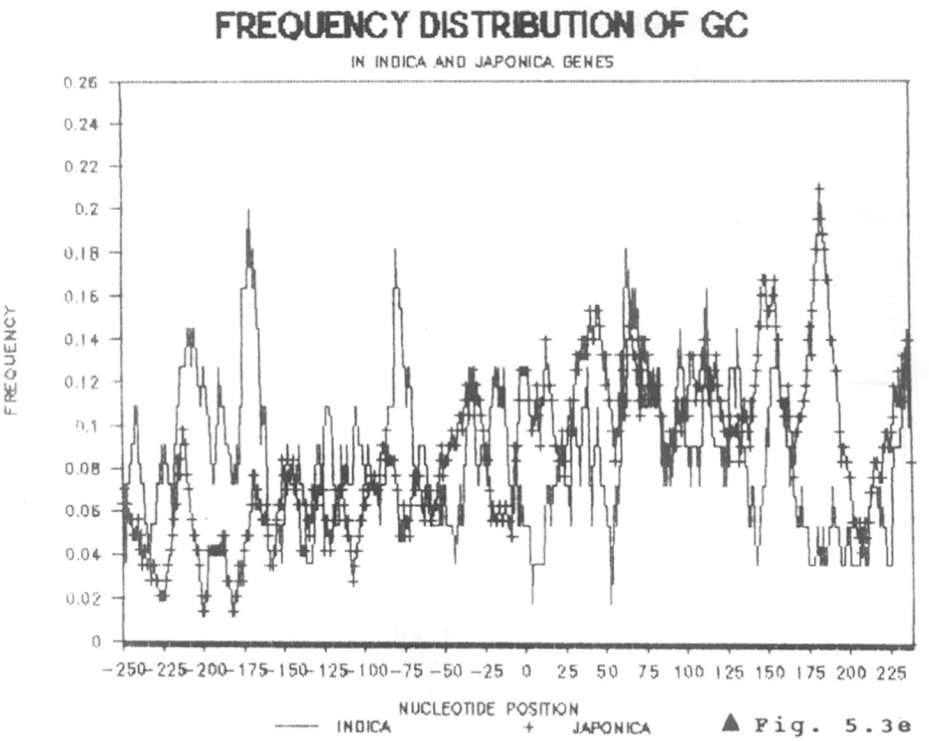
Figs. 5.3c to 5.3g: Distribution of dinucleotides around the translation initiation site. The sequences in the database are aligned at the translational start position (position 0). For each position, we calculate the frequency of each of the 16 possible dinucleotide combinations. The graphs illustrate the dinucleotide frequencies in overlapping blocks of length 12 bp. The frequencies are normalized by the number of entries in each block. Every point represents a mid-point of a block of 12 nucleotides. Legend : + japonica and - indica.



▲ Fig. 5.3c

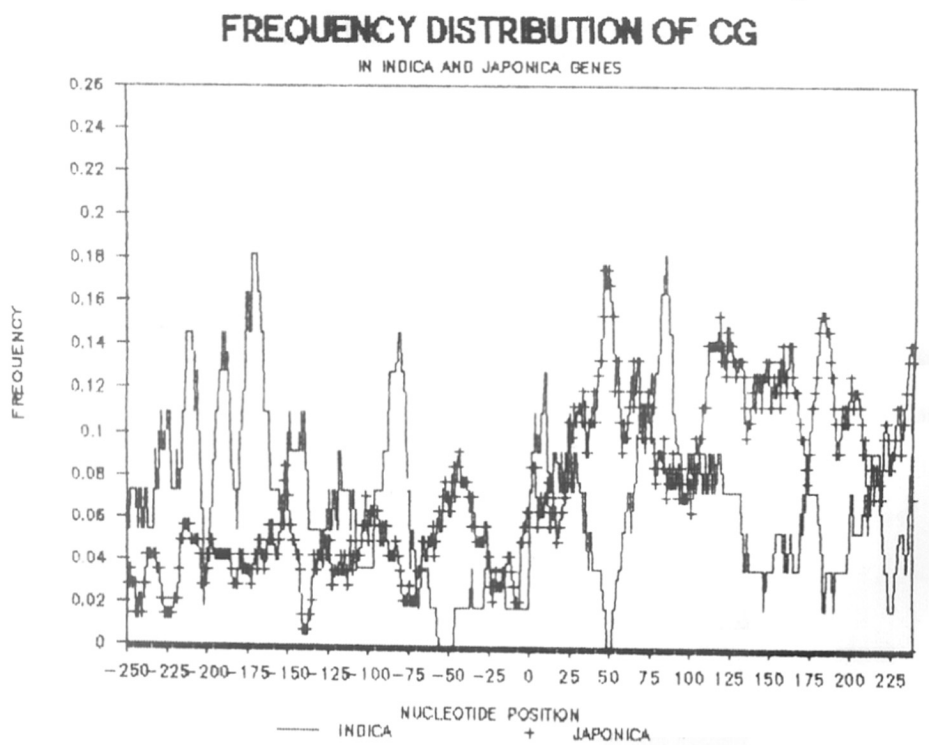
▼ Fig. 5.3d





▲ Fig. 5.3e

▼ Fig. 5.3f



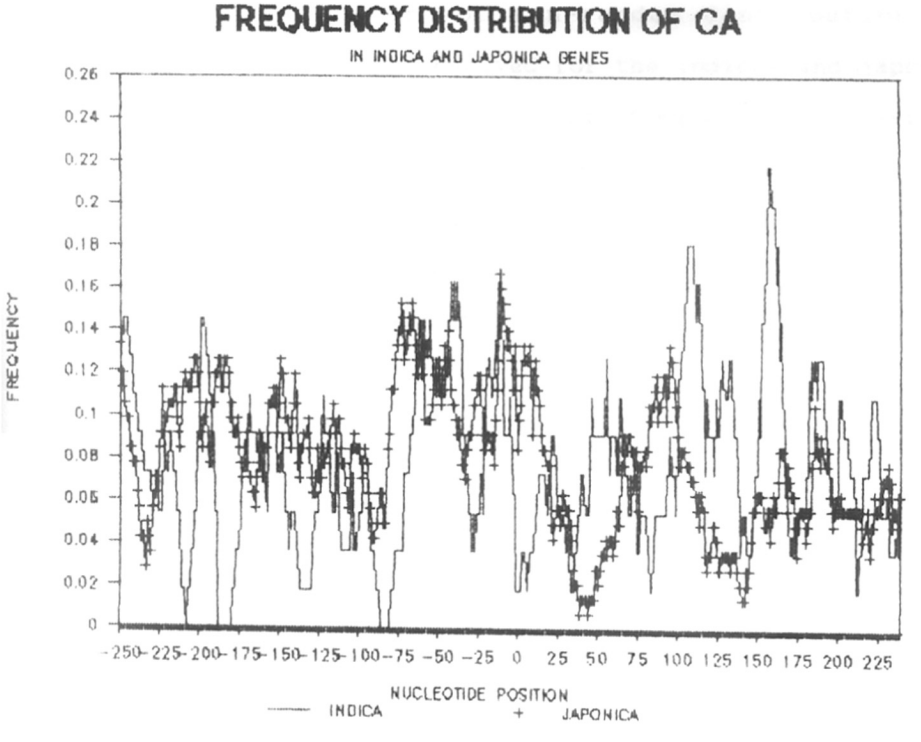

FREQUENCY DISTRIBUTION OF CA
IN INDICA AND JAPONICA GENES
FREQUENCY
0.26
0.24
0.22
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
-250 -225 -200 -175 -150 -125 -100 -75 -50 -25 0 25 50 75 100 125 150 175 200 225
NUCLEOTIDE POSITION
INDICA
JAPONICA

Fig.5.3g

5.3.3. Mononucleotide frequency for indica and japonica derived genes

With an aim of deciphering a possible consensus sequence, if present , mononucleotide frequency distribution was assessed in indica and japonica genes.

A pattern of non-random distribution of mononucleotides was evolved for the indicas and japonicas based on the mononucleotide frequencies at respective positions upto -50 bp and using the 50/75 rule (10), The indica consensus sequence is **TCNCTA-CAANTCNTCNCANN(A/T)NAGCTGNN(A/C)GCNNAN(A/G)(A/C)(C/T)NNACNA(A/C)NAA(A/C)NA**

whereas for the japonicas it is

TNNNNNTNNNNTCNCANNAGCNNCNNNNNNNTNNNNAGNNCNANGAANANA.

Comparing the two consensus sequences, it can be seen that there is more specificity in case of indicas as compared to the japonicas.

5.3.4 Comparative analysis of rice glutelin, prolamin and other like genes

In order to study the relationship between the free energy and the function of the gene, a case study was done using seed storage proteins. The seed storage protein genes are well characterized in many plant systems and hence provide an useful database for such studies.

Figure 5.4a shows the comparison of free energy profiles of rice glutelin and prolamin. On an average, it can be seen that rice glutelin has higher free energy levels as compared to that of rice prolamin. When the free energy profile of rice glutelin is compared with the other seed proteins which are the major seed proteins for the respective species, some interesting observations emerge. Rice glutelin when compared to hordein (Fig. 5.4e), wheat gliadin (Fig. 5.4f), zein (Fig. 5.4d) and wheat glutelin (Fig. 5.4b) exhibits a higher free energy profile. When wheat glutelin is assessed against wheat gliadin (Fig. 5.4c), it shows up a higher free energy profile than wheat gliadin. The dinucleotide frequency distribution in rice glutelin and prolamin shows more occurrence of AA (-1.9 kcal/mol), TA (-1.5 kcal/mol), TT (-1.9 kcal/mol) and AT (- 0.9 kcal/mol) in the glutelin as compared to prolamin (data not shown).

5.4 Discussion

The dendrograms drawn using the percent homologies between indica and japonica do not show any separate grouping of indica and japonica derived sequences. Instead a complex pattern is evolved from these dendrograms which suggests that the regulatory domains of these particular genes are not totally

Figs. 5.4a to 5.4f: Free energy distribution comparison between rice glutelin and rice prolamin (5.4a), zein (5.4d), hordein (5.4e) and gliadin (5.4f); wheat glutelin and gliadin (5.4c). Breslaur's free energy values are used with sliding blocks of 12 bases. The genes are aligned at the translation start site (position 0) and the length of DNA sequence analyzed is from -250 to +10 bp. Legend

5.4a : x rice glutelin and - rice prolamin

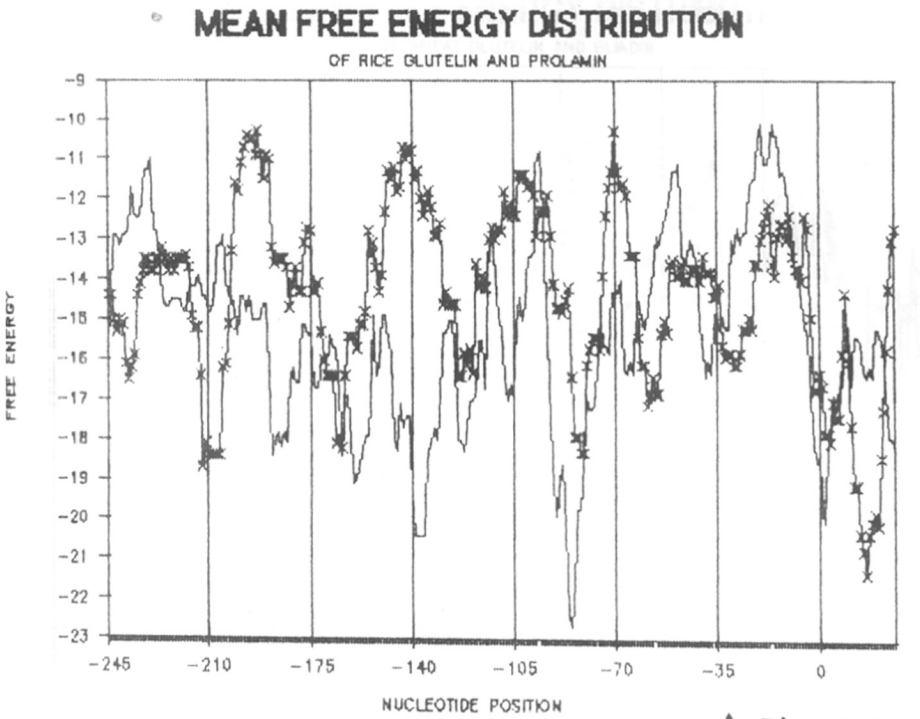
5.4b : x rice glutelin and - wheat glutelin

5.4c : + wheat gliadin and - wheat glutelin

5.4d : x rice glutelin and - zein

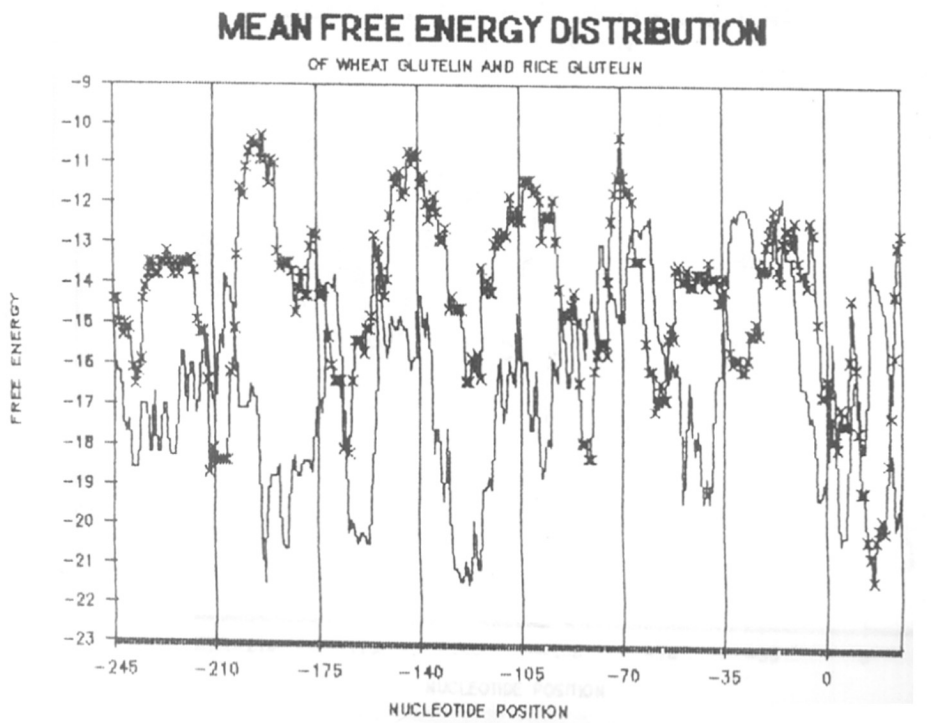
5.4e : x rice glutelin and - hordein

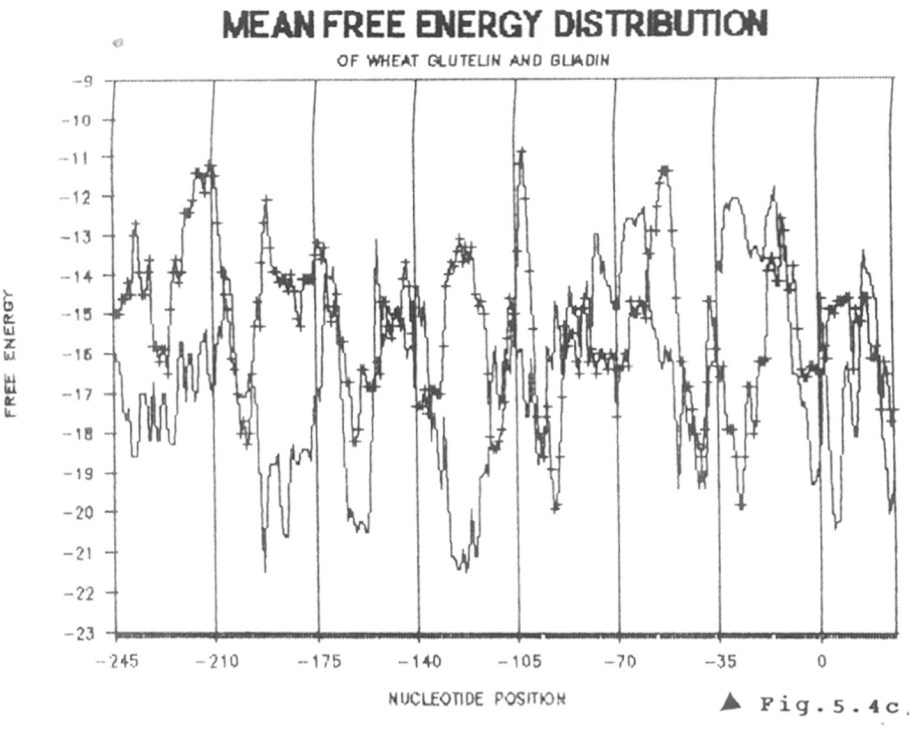
5.4f : x rice glutelin and - gliadin



▲ Fig. 5.4a

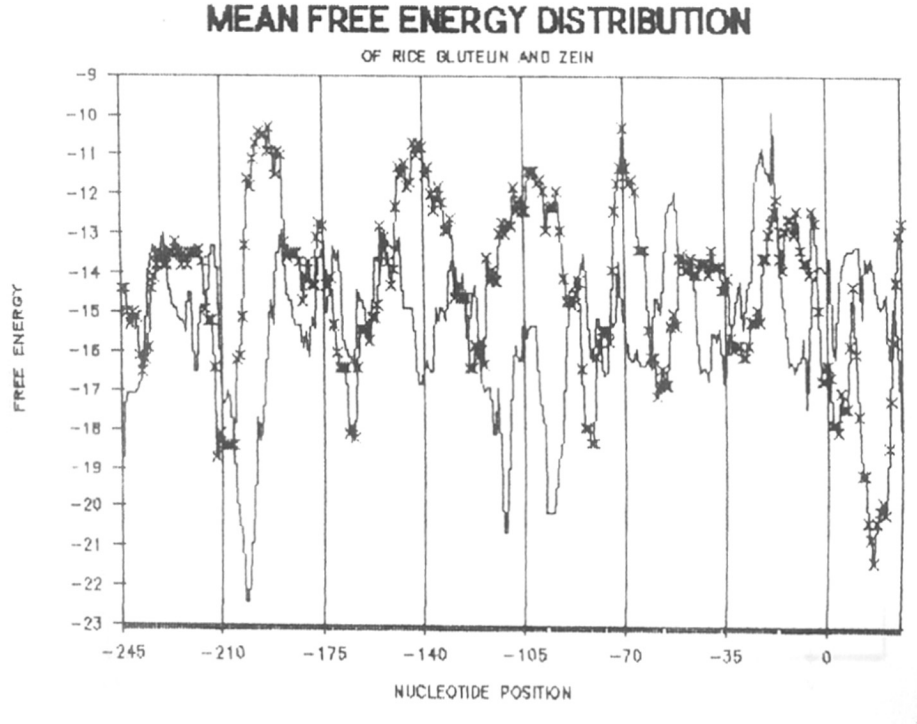
▼ Fig. 5.4b

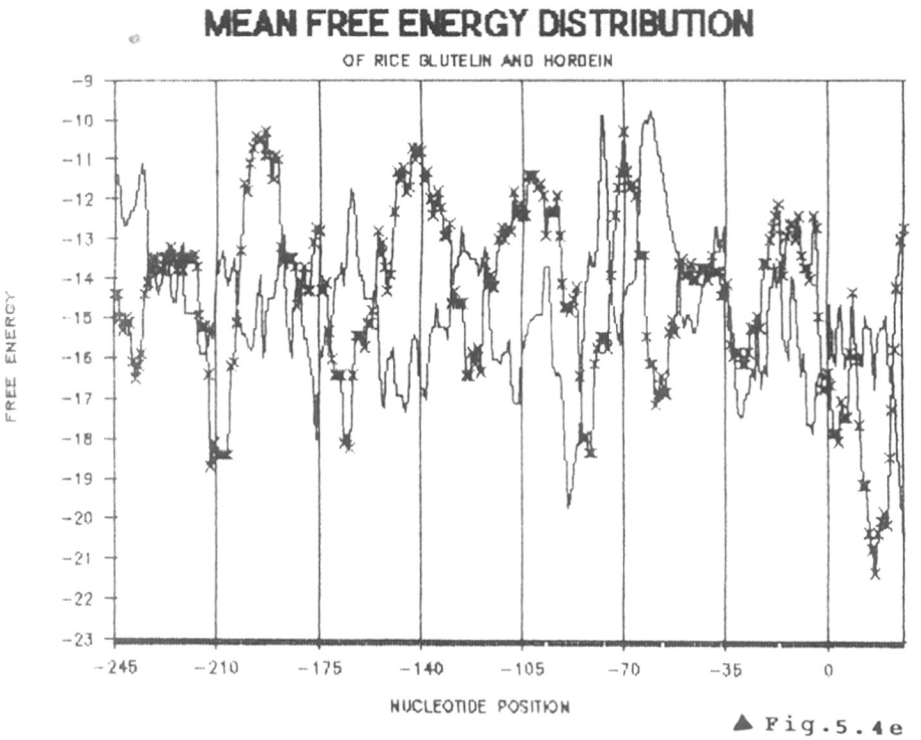




▲ Fig.5.4c.

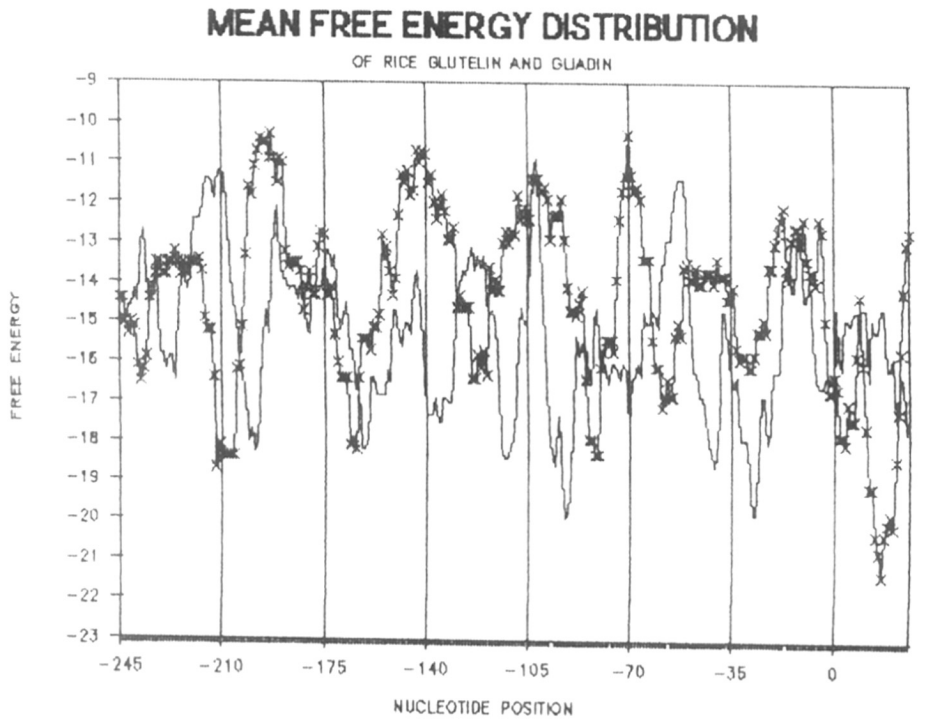
▼ Fig.5.4d





▲ Fig.5.4e

▼ Fig.5.4f



divergent and may not be directly involved in the process of indica - japonica differentiation. The pattern of homology as seen from the dendrograms is indicative of the fact that there is some order in their clustering which is complex and has to be deciphered. Another feature is the difference between the dendrograms of -225 bp and - 400 bp which implies that the regulatory region has separate motifs having distinct relationships and undergoes modifications irrespective of the neighbouring elements.

Next an attempt was made to assess for any other parameters reflecting the homogeneity or heterogeneity between the two subspecies. In another study conducted by us, our observation shows monocots exhibit a higher free energy profile than dicots (manuscript under preparation). Considering the evolutionary status of indicas versus japonicas and monocots versus dicots, the japonicas and the monocots are believed to be more evolved than their counterparts. This finding leads us to speculate that there is probably a tendency towards acquisition of higher free energy profiles during evolution from lower to higher types.

The above observation led us to think about the role of free energy distribution in the upstream regions of the genes. In order to assess the role of free energy in gene expression, two genes viz. glutelin

and prolamin were selected. Here the latter two account for 80% and 5% of the total seed proteins respectively and thus indicate a drastic difference in their expression (11).

A comparative study of free energy profiles reveals that the free energy of the duplex tends to positively correlate with the gene expression because the rice glutelin showed higher free energy as compared to the rice prolamin and all other genes to which it was compared. Secondly in wheat, gliadin (a prolamin comprising approximately 60% of the total seed protein) shows more high free energy compared to the glutelin (11).

In the light of the observation regarding the positive correlation of free energy and gene expression, a speculative comment can be made about the possible correlation between the temperate habitat of the japonicas and the high free energy profiles exhibited by the japonicas as against the tropical habitat of the indicas and the low free energy profiles exhibited by the indicas. It appears that these regulatory domains are in coherence with the ecological niches occupied by the indica and japonica subspecies of rice.

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GENERAL DISCUSSION

GENERAL DISCUSSION

Rice, the most widely cultivated cereal of the world, is a self pollinated crop and, therefore, exhibits a lack of heterosis or hybrid vigour during breeding for improved varieties. By involving sufficiently diverse parents in the breeding programs such as derived from indica and japonica subspecies, hybrid vigour and thereby yield can be enhanced. Compilation of conventional and modern approaches to evolve a better understanding of indica - japonica interrelationships reveals that the two subspecies exhibit complex genetic relations and raises questions regarding the causes for abnormalities observed in their breeding behaviour. A study is also warranted to understand the process of differentiation between indica and japonica subspecies to enable the development of genetically stable hybrids to be released as varieties. Based on my analysis, comprising conventional as well as modern approaches, I have made an attempt to get more information with respect to the breeding anamolies and the process of differentiation of indica and japonica subspecies of rice.

The first and foremost area of my interest was the genetic variability and interrelationships exhibited by the two subspecies. Inspite of the studies carried out using morphological, biochemical and RFLP markers, subspecies specific markers have not been found yet, excepting the r-DNA spacer specific to japonica. In my studies, I have, therefore, made an attempt to study a sample population of five indicas and five japonicas by both morphological and molecular approaches with a view to add to the existing information. Apart from confirming the earlier observations, I have detected with the help of a clone pOSM1C-2 **the presence of subspecies specific sequences.** These were probably not detected in the earlier RFLP studies, as the source of probes was a synthetic variety IR36 and not a landrace like Malkolam used in my analysis. **These subspecies specific sequences are japonica specific and hence can be used in indica - japonica breeding programs as markers to track the japonica alleles and detect the degree of introgression in the resultant progeny.** Secondly, these sequences also can be put to use in determining the true nature of unclassified or wrongly classified rice varieties. Another clone, pOSM1D-9, has been able to fingerprint eight of ten cultivars used in the present analysis. This implies the possible use of this sequence in the varietal

identification, gene introgression studies and genetic purity assessment of seed samples.

The second subject of my interest was the anamolies such as F1 sterility and abnormal segregation observed during the breeding of indica and japonica subspecies of rice. Till the mid 60's, several morphological, biochemical and cytological studies have been made without offering a definite explanation to the abnormalities. However, a few propositions were made, one of which interested me the most was the involvement of chromosomal structural hybridity. Analysis to probe the changes taking place in relation to the chromosomes could not be carried out further due to lack of tools having a higher resolution. Armed with a tool like RFLP, having extremely high resolving power, I have made an attempt to look into this area again. Initially, I took utmost care in selecting the material for my analysis. Out of two crosses made by me viz. Basmati 370 x Taichung 65 and Basmati 370 x Adt 27, I selected the Basmati 370 x Taichung 65 cross for the RFLP analysis ,based on morphological characters. My studies based on qualitative as well as quantitative characters have revealed most of the peculiar observations recorded in connection with the indica - japonica hybrids. In one of the crosses viz. Basmati 370 x Adt 27, I have Adt 27 with a coloured apiculus as a parent

which is known to be linked to the wide compatibility gene. This cross has revealed less anamolies than Basmati 370 x Taichung 65 and also has exhibited a good combination of both parents. It has also revealed the presence of a good degree of heterosis by exceeding the number of tillers of both the parents in the F1 hybrid. This data suggests the possible advantage of using an indica - japonica derivative instead of a pure japonica in a breeding program. Proceeding further with the RFLP analysis of the Basmati 370 x Taichung 65, **I have detected some anamolies in the segregation of low copy repeated DNA clones** viz. pOSM1C-2, pOSM1D-9, pOSM5F-3 together with a glutelin cDNA clone and a mouse 18S rDNA clone. Here, I have explained the disappearance of some bands in the progeny, as being deletions. Drawing an inference from the prediction made by Henderson (1964) based on extensive cytological analysis about the occurrence of included inversions leading to the deletions undetectable by cytological methods, I have been able to actually prove the existence of such a phenomena in indica - japonica hybrids. This perhaps is the first experimental evidence which shows the occurrence of chromosomal deletions in an indica - japonica cross.

It is tempting to postulate that these subtle small but multiple changes in chromosomes may be play-

ing an important role in the differentiation of japonica from indica. Another aspect of the occurrence of the small deletions is that they may seem to evolve a cumulative genotype. This cumulative genotype when considered for its phenotype it shows an intermingling of characters of parents and may also tend to be like either of the parents for some characters only and can be aptly termed as an example of disturbed genetic coherence. Based on the above observations, **it can be inferred that these two phenomena of occurrence of deletions and intermingled phenotypes showing extremes for particular characters may have contributed significantly in the differentiation of japonica from the indica subspecies.**

Later, I have tried to carry out the analysis of DNA sequences of a few genes of indica and japonica subspecies because to the best of my knowledge such attempts have not been made at the intraspecific level. I selected the regulatory domains for assessment of the DNA homology between genes derived from indica and japonica subspecies. These regulatory domains are known to be involved in the process of evolution and hence can serve as a good system. The homology studies evolved a dendrogram which could be closely compared with the dendrogram obtained based on actual RFLP data of the genetic variability analysis of indica and

japonica landraces performed by me, raising further questions and creating a need to study this observation in more details. Secondly, I studied another parameter viz. the free energy of the duplex contributed to by different dinucleotide combinations. This analysis helps to understand the functional implications involved about the genes. I have observed that the free energy exhibited by the japonicas is higher than that of the indicas in the -250 bp 5' upstream regions. In another analysis in monocots and dicots, I have found that monocots are associated with a higher free energy than that of dicots. Corroborating both the observations together it appears that there is a tendency towards gaining of higher free energy in the process of evolution. This also means that these regions can be "melted" with a lower energy for the japonicas as compared to the indicas. This may lead one to speculate that the habitats occupied by these two subspecies have some connection to this occurrence of differences in free energy profiles. To explain further, japonicas inhabiting temperate regions seem to require less energy to express genes as compared to the indicas inhabiting tropical regions. Can this requirement of needing low energy for expressing genes of japonicas be interpreted in terms of their efficiency ? Another major observation regarding seed storage protein genes

from rice, wheat, barley and maize has led me to conclude that there appears to be a positive correlation between the free energy of the regulatory domains and the degree of expression. It is evident from the observations that glutelin from rice which is expressed most exhibits a higher free energy than others and a hierarchy seems to emerge in terms of free energy and degree of expression. This observation can be put to use while doing gene analysis and manipulation and can serve as an important parameter for genetic engineers. It can also help in the designing of suitable primers based on free energy for the PCR analysis. Lastly, these studies point out towards the need to do more keen analysis of the DNA sequences to understand them better.

CURRICULUM VITAE

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Date of birth : 07-03-1965

Place of birth : Dharwar(Karnataka)

Marital Status : Unmarried

Educational Qualifications:

Degree	University	Year	Class	Subjects
B.Sc.	Poona, India	1985	I (78.15%)	Botany(Principal) Physics and Chemistry (Subsidiary)
M.Sc.	Poona, India	1987	I (76.60%)	Botany
Ph.D.	Poona, India	1987- *(work in progress)*		Biochemistry

Awards/Prizes/Fellowships :

1. "The M.S. Balakrishnan Shield for General Proficiency in SEM I and II" for the year 1986 at M.Sc.

2. "The Late Milind Gandhi Scholarship" for standing first at the M.Sc. Examination held in the year 1986.

3. Recipient of the Junior Research Fellowship of the Council of Scientific and Industrial Research (CSIR), New Delhi, India (1987-89).

4. Recipient of the Senior Research Fellowship of the Council of Scientific and Industrial Research (CSIR), New Delhi, India (1989-92).

Attendance of Symposia/Workshop:

1. Was one of the instruction faculty at the workshop on "Analysis of a cloned Plant DNA fragment and Agrobacterium mediated transfer in plants" held at the Division of Biochemical Sciences, National Chemical Laboratory, Pune, India, from 28th March to 17th April, 1989.

2. Attended the Rockefeller Foundation sponsored "RFLP Training Course" held at the Department of Plant Breeding and Biometrics, Cornell University, Ithaca, NY, USA,from 23rd July to 31st July,1989.

3. Attended on invitation the 4th Annual Rockefeller Sponsored International Program on Rice Biotechnology Meeting from 9th May to 12th May,1990 and the International Rice Genetics Symposium from 14th May to 18th May, 1990 held at the International Rice Research Institute, Manila, Philippines.

4. Attended the International Symposium on "Rice Research : New Frontiers" held at the Directorate of Rice Research, Ranjendranagar, Hyderabad from November 14-18,1990 on the occasion of the Silver Jubilee celeberations of AICRIP.

5. Participated on invitation in the "Annual Rice Workshop of Maharashtra State " held at Regional Rice Research Station, Karjat.(Dist. Raigarh) Maharashtra from February 6 - 8, 1991.

6. Participated on invitation in the "International Conference on Genetic Engineering and Biotechnology" organized by the Nepalese Biotechnology Association at Kathmandu, Nepal from April 15-19, 1991.

7. Attended the Symposium on `Molecular Biology of Microorganisms' sponsored by the Board of Research in Nuclear Sciences, Department of Atomic Energy, Govt. of India, held at Pune, January 28-30,1992. (I was also involved in the organization of the symposium).

Member of Learned Societies:

1. Member of the Society of Biological Chemists, INDIA.

2. Life Member of the Association of Rice Research Workers,Cuttack, India.

3. Member of the Rice Genetics Co-operative, International Rice Research Institute, Manila, Philippines.

4. Member of the International Society of Plant Molecular

Biologists.

5. Member of the Society of Plant Biochemistry and Biotechnology, New Delhi, India.

6. Life member of the Marathi Vidnyan Parishad, Pune, India

Research Experience :

1. Worked on induction of polyploidy with the use of colchicine in Guizotia abyssinica and Cajanus cajan.

2. One year research experience in crop genetics while working on `Interspecific hybridization in Pennisetum species. The work involved a crossing programme, embryo rescue, cytological analysis and induction of polyploidy.

3. Currently involved in the study of genetic variability and genetic mapping in rice using RFLP approach at the National Chemical Laboratory (NCL) under the guidance of Dr.P.K.Ranjekar. The work involves the use of molecular markers to study the variability exhibited among the indica cultivars grown in Maharashtra , study of DNA polymorphisms in indica and japonica subspecies of rice and the addition of new molecular markers to the existing map by using mapping populations not exploited for RFLP mapping.

Publications:

1. "Restriction Fragment Length Polymorphism : A recent approach in plant breeding"
Vaijayanti Pethe, Meena Lagu, P.K.Chitnis, Vidya Gupta and P.K.Ranjekar. Indian Journal of Biochemistry and Biophysics (1989), Vol. 26, pp. 285-288.

2. P.K. Ranjekar, V.S. Gupta, M.D. Lagu, P.K. Chitnis and V.V. Pethe, (1991)
"Applications of RFLP technology in rice breeding"
Page 1-6, Appeared in the Proceedings of the International Symposium on "Rice Genetics & Tissue Culture" held at Hyderabad during March 7-9.

3. P.K. Chitnis, V.R. Varma, V.S. Gupta and P.K. Ranjekar (1992)
"Interspecific DNA polymorphisms in Oryza sativa as revealed by DNA homology and free energy of regulatory domains" (Communicated to Nucl. Acids. Res.).

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"Use of RFLP approach and morphological characters to study indica-japonica cross : Evidence for occurrence of small deletions leading to abnormal segregations" (Communicated

to Theor. Appl. Genet).

5. P.K. Chitnis, M.D. Lagu, V.S. Gupta and P.K. Ranjekar (1992)
"Use of a few low copy repeat sequences from a rice landrace to differentiate, fingerprint and decipher subspecific sequences in five indica and japonica cultivars" (Communicated to Genome).

6. P.K. Ranjekar, V.V. Pethe, P.K. Chitnis, M.D. Lagu and V.S. Gupta (1992)
"Use of RFLP in detection of diversity in rice cultivars for directional breeding" (Communicated to Oryza).

Articles in books:

1. P.K. Ranjekar, V.V. Pethe, P.K. Chitnis, M.D. Lagu and V.S. Gupta (1992)
"Potential of plant repeated DNA sequences as species-specific markers, DNA fingerprint markers and transposable elements" In : Prof. U. Sinha commemoration volume.

Invited Talks :

1. Delivered an invited talk on ` Restriction Fragment Length Polymorphisms in Plant genetics' at the College of Agriculture, Pune to the faculty and students on 24th February,1990.

2. Delivered an invited talk on `Use of RFLP techniques in Plant breeding' at the Central Rice Research Institute, Cuttack, Orissa on 14th March, 1990.

3. Delivered an invited talk on behalf of Dr. P.K.Ranjekar on `Technique of RFLP in Germplasm characterization' during the symposium on "Techniques of in vitro conservation and cryo-preservation of plant germplasm" July 26 - Aug 10, 1991 sponsored by the Dept. of Biotechnology, Govt. of India, held at the National Facility for Plant Tissue Culture Repository, NBPGR, Pusa Campus, New Delhi, India.

4. Delivered an invited talk on the `Use of RFLP technology in the isolation of a gene by Reverse Genetics' to the students the final year M.Sc.Botany (Cytogenetics & Plant Breeding), Department of Botany, University of Poona, Pune 411007, on 8th February, 1992.

5. Delivered an invited talk on the `Use of RFLP technology in plant breeding' at the Vasantdada Sugar Institute (formerly Deccan Sugar Institute), Manjri (Bk.), Pune on May 15, 1992.

Presentations at National and International Symposia:

1. Presented a paper entitled " Genetic Variability and Mapping in rice using RFLP approach" P.K.Chitnis,V.V.Pethe,M.D.Lagu,V.S.Gupta and P.K.Ranjekar at the Rockefeller Foundation's 4th Annual Meeting of the International Program on Rice Biotechnology held at the International Rice Research Institute, Manila, Philippines from 9th May to 12th May,1990.

2. Presented a poster entitled "Genetic Varibality amongst the elite Maharashtra germplasm of rice using RFLP approach" V.V.Pethe, P.K.Chitnis, M.D.Lagu, V.S.Gupta and P.K.Ranjekar at the International Meeting "Rice Research : New Frontiers" held at the Directorate of Rice Research, Rajendranagar, Hyderabad in November, 1990.

3. Abstract entitled "Use of RFLP Technology in Plant Breeding" P.K.Chitnis and P.K.Ranjekar at the International Meeting sponsored by the Indo-American Seeds Corporation at Bangalore in November, 1990.

4. Lecture presentation entitled " Use of RFLP and allied techniques in rice genetics and breeding research " at the Annual Rice Workshop of Maharashtra State held at the Regional Rice Research Station, Karjat (Dist. Raigarh) Maharashtra from February 6 - 8 , 1991.

5. Presented a plenary lecture on behalf of Dr. P.K.Ranjekar entitled " Use of RFLP in selective rice breeding " V.V.Pethe, P.K.Chitnis, M.D.Lagu, V.S.Gupta and P.K.Ranjekar at the "International Conference on Genetic Engineering and Biotechnology " held at Kathmandu, Nepal from April 15-19,1991.

6. Poster presentation entitled " Rice Biotechnology Program at the National Chemical Laboratory, Pune 411 008 " at the " International Conference on Genetic Engineering and Biotechnology " held at Kathmandu, Nepal from April 15-19,1991.

7. Abstract entitled " Genome organization and RFLP mapping in rice" V.V.Pethe, P.K.Chitnis, M.D.Lagu, V.S.Gupta and P.K.Ranjekar at the "Rockefeller's Fifth International Meeting on Rice Biotechnology" held from Oct 2-5, 1991 at Tuscon, Arizona, U.S.A.