5. DISCUSSION

5.1. Morphology of trisomics

Although the utility of trisomic analysis in assigning genes and their respective linkage groups to specific chromosomes have long been realised following the classical discovery of different types of trisomics in *Datura* by Blakeslee and his associates (Blakeslee, 1924, 1930; Blakeslee and Belling, 1924a; Avery and Blakeslee, 1948), such an elegant technique could not profitably be employed in rice as the earlier investigators could not establish a complete set of 12 trisomics in rice (Nakamori, 1932; Ramanujam, 1938; Nishimura, 1961; Katayama, 1963; Jachuck, 1963; Sen, 1965; Jagdish, 1969). Only recently, however, a beginning was made in this direction after the identification of a complete set of 12 primaries in Taiwanese and Japanese rices (Hu, 1968; Iwata et al., 1970; Watanabe et al., 1975). This would help strengthening the basic and applied genetics of rice. Since the two sub-species, *japonica* and *indica* are known to have non-identical linkage groups, (Nagao and Takahashi, 1963; Misro et al., 1966), it is desirable to develop a complete set of trisomics independently for *indica* sub-species. Development of 12 primary trisomics in two different varieties of *indica* rices independently by Sur (1975) at International Rice
Research Institute, Manila, and Sen (1977) at Central Rice Research Institute, Cuttack marked the beginning of a new era of research on the use of trisomic in rice breeding and genetics.

The efficiency of establishing a trisomic series depends upon the source utilised. Of the different sources known to produce trisomics (Khush, 1973), autotriploids have been used effectively for isolation of trisomics in rice. Indian workers had earlier utilised a spontaneous autotriploid of a very late photosensitive variety, 'T-1242' and reported few trisomics, (Jachuck, 1963; Sen, 1965; Jagdish, 1969). Their failure to obtain a complete set of primaries might be due to the use of such a long duration variety which did not offer the opportunity for raising a large progeny from the seeds. Sen (1977), however, was successful in using a photoinsensitive high yielding variety 'Sona', the autotriploid of which gave enough seeds to raise a fairly large progeny from which he could get 11 primary trisomics. The 12th one was identified by him from another autotriploid of a high yielding semidwarf variety 'Ratnagiri 242'. In the present study the autotriploid 'Sona' was used as the source, the seed progeny of which gave all the 12 possible types of primary trisomics. This is, therefore, the first report of establishing a complete set of 12 trisomics in an uniform genetic background.
The 12 trisomics obtained here exhibited conspicuous variation with regard to a number of morphological characters and fertility. Of them 11 types are distinctly different from the disomic 'Sona'. The other one, designated as 'pseudonormal', though largely resembled the disomic can be distinguished from the latter for its having the longest ligule (2.7 cm compared to 1.5 cm in disomic). Similar type of trisomic designated as 'pseudonormal' was reported in japonica rice by Iwata et al. (1970) and Watanabe and Koga (1975) and in indica sub-species by Sur (1975) and Sen (1977).

The 11 types are morphologically different from each other and from the disomic as well. On the basis of differences in a number of morphological traits, such as, plant height, general vigour, colour, shape and disposition of leaves, awning, panicle length, length of anther and style, and the size of ovary. All these could together be helpful in the identification of trisomics from a very large population. Besides, for other quantitative characters, variation was observed among these trisomics. Excepting these quantitative characters, most of the major morphological traits did not show much variation. Further these trisomics, reported in the present study, were developed from the autotriploid of one single variety only, and consequently the variation observed were largely due to
addition of one extra chromosome each in their complements. Hence, it would be expected that all the trisomics have same degree of heterozygosity or homozygosity as that present in the autotriploid source and the observed morphological difference (Table 6-10) among the trisomics, therefore, might be largely due to the effect of the extra chromosome present in each of them.

In barley (Tsuchiya, 1967), Datura (Blakeslee, 1922), tomato (Rick and Barton, 1954), Sorghum (Schertz, 1966), Capsicum (Pochard, 1968) and Spinach (Janick et al., 1959), it is easy to distinguish the trisomics morphologically from each other and from their disomic counterpart. In some crops like maize (Rhoades, 1955) and Hyacinthus (Darlington & Mather, 1944) no morphological differences were found among the trisomics. In Solanum (Vogt and Rowe, 1968), though the trisomics were initially not distinguishable from each other and from their diploid counterpart, they were clearly distinguishable from their diploid sibs after a backcrossing within the family. From these observations they suggested that, if the trisomics are developed in a genetically uniform background, the effect of extra chromosome on the phenotypic characters of the trisomics would be more apparent and clear. The morphological differences among the 12 trisomics and from their diploid parent are distinct since these are developed from one uniform genetic background.
The morphological deviations (Table 10) of the trisomics, from the normal diploid, therefore, can easily be attributed to be due to the presence of an extra chromosome in each of them.

Though the addition of an extra chromosome resulted in altered morphology, coupled with reduced fertility, and vigour, some trisomics, especially Triplo-9 (stout) and Triplo-12 (robust) exhibit increased vigour. The 'stout' trisome is characterized by dark green erect and thick leaves with thick and stout culm. In another background recently developed stout trisomic had not only thick culm and tough erect and thick leaves but also had very large spikelets resembling to that of the spikelet of a tetraploid rice (Misra, unpublished). Such vigour exhibited by these few trisomics may be due to the gene content and the dosage effect of the extra chromosomes (Khush, 1973).

Excepting these two, all other trisomics had reduced vigour by which they could be distinguished from the disomic sibs in terms of reduced height, reduced leaf size and reduction in other plant parts. Reduced vigour also reflected in the pollen and spikelet fertility. Most of the trisomics had considerable reduction in pollen and spikelet fertility ranging from complete to nearly 50 per cent fertility. From the present investigation and from earlier reports (Sur, 1975 and Sen, 1977) it is evident that the
trisomics of rice in general, have reduced vigour and vitality. These morphological and physiological deviations have been attributed to the genetic imbalance caused by the addition of genes by the extra chromosome (Bridges, 1922). However, there is some evidence in tomato which may well be applicable to other crops that the differences between the trisomics are not related to qualitative genes with major effects. On the contrary the differences are more likely to be due to imbalance of the genomes, especially with regard to gene determining, quantitative characters brought about by the extra chromosome as a whole (Sampson et al., 1961). The morphological and physiological differences in rice trisomics are, therefore, more likely due to the effect of extra chromosome each of which imparts certain definite effect on cellular and developmental rhythm of the individual trisomics.

Although trisomics were identified on morphological basis by Hu (1968) and Watanabe et al. (1970), it was Sur (1975) who was first to associate each trisomic phenotype with a cytologically identifiable extra chromosome. He designated the trisomics as Triplo-1 to Triplo-12, Triplo-1 having chromosome-1 in triplicate, Triplo-2 having chromosome-2 as extra chromosome and so on. However, his identification needed further confirmation. Sen (1977), likewise, designated the trisomics according to the extra
chromosome present in the complement from Triplo-1 to Triplo-12. Similar designation was used in the present study and associating the extra chromosome with each of them, the trisomics were designated from Triplo-1 to Triplo-12.

Besides, the trisomic are also named on the basis of one of the major conspicuous character that distinguishes a particular trisomic from rest of the trisomics and disomic (Blakeslee et al., 1924; Khush and Rick, 1967; Rhoades and McClintock, 1935; Venkateswarlu and Reddi, 1968; Gill et al., 1970). Datura trisomics were given names generally signifying some morphological changes in fruit shape such as Globe, Poinsettia, Cocklebur, Ilex, Echinus, Rolled, Reduced, Buckling, Glossy, Microcarpic, Elongate and Spinach. In barley (Tsuchiya, 1960) the primaries were, likewise, named as slender, bush, pale, robust, pseudonormal, purple and semi-erect signifying one of the few striking characters. Likewise, names signifying one of the few changed characters have been proposed for rice in the present study and as far as possible wherever any earlier names were existing, they were retained in the present study. Thus the names pale, dwarf, awned, sterile, twisted, bushy, rolled, recurved, stout, boat, pseudonormal and robust are given to the 12 trisomics of which some are similar to that reported earlier by Sur (1975) and Sen (1977).
Such of these names are retained here in the present study as these names were appropriately given by them and proposing any new names further create confusions.

However, the observed differences in morphological characters, flowering duration and fertility (pollen, spikelet fertility) helped in precise identification of the trisomics. Once their extra chromosomes are precisely and correctly identified, the trisomics can profitably be utilized for genetical investigations.

5.2. Cytology of trisomics

The present study of the meiotic consequences of extra chromosomes at diakinesis and Metaphase-I revealed that the longer chromosomes tend to form trivalents more frequently than the short chromosomes. The extra chromosome usually pairs with its two homologous counterparts in the complement forming trivalents of which the frying pan types are most frequent. However, the trisomics with shorter chromosomes form univalents at diakinesis and Metaphase-I because the number of chiasmata are less which terminalise completely at these stages. Ring trivalents were rarely observed at Metaphase-I indicating the occurrence of non-homologous pairing of chromosomes. In the present study the trisomics with extra submedian (4, 6 and 9) and sub-telocentric (5) chromosomes did not form ring trivalents.
These five chromosomes are more assymetrical than others. The next frequent classes are the Y and chain trivalents. In pachytene Y configurations are most frequent. The triangle type configurations are most infrequent which indicates that the trisomics were all having single intact chromosomes.

The frequency and type of trivalent formation have direct correlation with the size of the chromosome which is in agreement with the earlier findings of Belling (1925), Darlington (1932), Rick and Barton (1954), Iwata et al. (1970), Sur (1975) and Sen (1977). This phenomenon of trivalent formation inferred that the longer chromosomes would have a greater physical chance to synapse with a larger number of chiasmata than the trisomics with shorter chromosomes.

At Anaphase-I regardless of the trivalent configurations, the disjunction of chromosomes are in 2-1 to either pole and also due to the belated separation of the chromosomes the trivalent forms a laggard with a bivalent and univalent moving towards the poles. Persistent laggards of 1-2 univalent are observed at the time of orientation of chromosomes on the spindle of the first meiotic division. From the above phenomenon it is apparent that two of the gametes or spores will be normal haploid, whereas others will have n+1 type of chromosomes and will be able to
transmit the trisomic condition provided the gametes are viable. The above cytological consequences had an impact on the gametophyte which upsets the balance of genes (Bridges, 1922) and the gametophyte generation would be abnormal. Abnormal disjunction of chromosomes to poles result in lowered viability of the trisomic gamete in male side.

5.3. Identification of chromosomes

Associating the extra chromosome with trisomic phenotype is fairly a difficult task since somatic metaphase chromosomes of rice are too small to permit a clear identification of individual chromosomes in the complement (Nandi, 1936; Sakai, 1938). The primary and secondary constrictions also do not differentiate clearly. Due to these difficulties reliable karyotypic data that could be useful for identification of extra chromosomes at mitotic metaphase are not yet available for rice. These difficulties imposed restriction in proper identification of extra chromosomes in trisomics developed by Kurata et al. (1981) who identified only 5 out of 12 chromosomes from mitotic prophase clearly. They pointed out the limitations of the use of somatic metaphases as there was differential contraction of the chromosome arms due to pretreatment. Therefore, root tip mitosis would be of little help in precise identification of individual chromosomes in rice unless more refined techniques are available.
The improved leaf-tip squashing technique employed in the present study offered help in initial counting of chromosomes of the trisomic individuals. Since, the size of the chromosomes in a complement at leaf-tip mitosis varied from 3.2 to 0.7 \( \mu \), it was possible to identify the longest and the shortest chromosomes with reasonable accuracy when present as extra chromosome in trisomics. Besides, leaf-tip squash offered scope for identification of primaries at seedling stage.

It is very often fruitful to employ pachytene chromosome analysis for correct identification of extra chromosomes in trisomics as had been done in Maize (Rhoades and McClintock, 1955), tomato (Rick and Barton, 1954), Sorghum (Venkateswarlu and Reddi, 1968) and Solanum (Wagenvoort and Ramanna, 1979). In rice, Sur (1975) and Sen (1977) were able to identify the extra chromosomes of trisomics at pachytene. The problems encountered by them were as follows:

1) Irrespective of the length, the extra chromosome in the complement never remains as univalent at pachytene.

2) In cases where greater length of extra chromosome remains as univalent, the chromomeric details of the extra chromosome are not clear.
3) Wherever the extra chromosome paired with its homologous bivalent the pairing is both between nonhomologous and homologous regions of the constituent chromosomes.

The above difficulties, however, could be avoided by confining the analysis to 3-5 good PMCs at mid pachytene where all the bivalents and the extra chromosome (in trivalent configuration) are clearly analysable. The individual bivalents were matched with their counterparts in the standard pachytene karyotype of the disomic control thereby eliminating the eleven bivalents not involved in trivalent formation in trisomics, so that the particular bivalent involved in trivalent formation could be inferred. The constituent chromosomes of the trivalents were then traced to find out the nature of pairing. This method was followed for each trisomic and the extra chromosome was placed at right position in the karyogram. For perfect identification of extra chromosome emphasis was placed on PMCs in which the other chromosomes were available for verification.

The three homologous chromosomes in each trisomic usually form a trivalent association. Generally two of the three homologous pair at any given point and the third one can pair only after the exchange of pairing partners. But in the present study close pairing was observed for a considerable part of their length. In addition to this,
nonhomologous association (McClintock, 1933, Vogt and Rowe, 1968) of the three chromosomes, were also observed which complicate the identification. The pairing path of extra chromosome involved in a trivalent formation was traced for homologous and non-homologous bivalents from which some were clearly identifiable and some were not. The trisomies for 12th chromosome sometimes can be wrongly identified as 9 or 10.

Some peculiar configuration that deserves consideration are the triradial configuration at pachytene which normally would be mistaken as due to the pairing of the iso-chromosome of the secondary trisomics as has been observed in Solanum (Wagen Voort and Ramanna, 1979). But by careful tracing the constituent chromosomes, it revealed the peculiar pairing of the nonhomologous regions of two homologous chromosomes which are due to stretching and adjustment of chromosome arms. Most of these triradial configurations obtained at pachytene resembles the so called T-configuration recorded by McClintock (1933) in Maize, Khush (1973) in tomato and Sur (1975) and Sen (1977) in rice.

Sur (1975) stated that nonhomologous pairing of unequal arms is due to considerable adjustment of arm length through differential condensation.
adjustment of arm length to effect non-homologous pairing have also been observed in Sorghum (Venkateswarlu and Reddi, 1968). Moreover, the length differences of chromosome 8, 9, 11 and 12 were not that conspicuous and the similar chromomeric pattern of some of these bivalents do not permit clear identification of the extra chromosome. Hence, to overcome this confusion Giemsa or Fluorescence technique would be helpful to differentiate the constituent chromosome involved in trivalent formation. Till then the present identification of extra chromosome remains tentative.

5.4. Transmission of Trisomics

In the present study transmission of extra chromosomes in each of the trisomics was studied. The transmission of extra chromosome through pollen was estimated from the appearance of parental trisomics in the progeny of the crosses involving the disomic with each of the trisomics. Of the 8 trisomics studied, parental trisomics appeared only in the progenies of the crosses involving Triplo-6 and Triplo-11 as the pollen parents. The frequency of transmission in these two trisomics were low (4.0 and 3.1 per cent respectively). There was no pollen transmission in Triplo-1, Triplo-3, Triplo-5, Triplo-8, Triplo-9, Triplo-10 and Triplo-12. Complete pollen sterility in Triplo-4 and small number of progenies in Triplo-2 crosses
did not permit the estimation of male transmission in these two trisomics. However, the study of fairly adequate progenies from 2n x 2n + 1 crosses for the above 8 trisomics clearly revealed that the extra chromosomes in rice trisomics are not transmitted or very rarely transmitted through male sides.

In other plant species where trisomics have been studied, male-transmission of trisomics are rare or extremely low. Buchholz and Blakeslee (1922, 1932) observed the pollen behaviour of trisomics of Datura and suggested that no germination of pollen, poor germination, germination but slow growth of pollen-tube, and burst of pollen tubes are the main causes of poor pollen transmission of n+1 gametes. The low transmission of n+1 gametes through pollen in rice trisomics observed here suggests that gametophytic selection against n+1 gametes in addition to the above factors is more severe in male side resulting in low rate of transmission through the pollen in comparison with egg.

The frequency of transmission through female was estimated from the appearance of parental trisomics in their selfed progenies. Of the 10 trisomics studied (Triplo-1 and Triplo-4 are not included because of poor seed set and high sterility respectively) the average transmission rate for all the trisomics was 26.16 per cent.
The variation range of transmission through females was 4.50 per cent (for chromosome-2) to 50 per cent (for chromosome-12). Sur (1975) studied the transmission rates of different primaries and found the average transmission rate through females to be 32.1 per cent. The average female transmission studied here is 26.16 per cent but after deleting the exceptionally low transmission value of 4.5 per cent for Triplo-2, the average transmission frequency for all the trisomics was 29.68 per cent which is more or less close to that reported by Sur (1975) for the trisomics studied by him. However, the transmission rate for different trisomics obtained by Sur (1975) were entirely different from the values obtained in the present study. Special mention may be made here regarding Triplo-12, the trisomic for smallest chromosome, where a transmission frequency of 50.00 per cent obtained in the present study contrary to that of 22.2 per cent for Triplo-12 reported by Sur (loc.cit.). Here again this discrepancy may be due to the difference in the genetic background of the materials.

From the present finding as well as that reported by Sur (1975), it is clearly established that transmission of \( n+1 \) gamete through female is high compared to transmission through male. Similarly, all the earlier workers reported very low or no transmission of \( n+1 \) gametes through male sides and comparatively much higher frequency of female
transmission in the trisomics of other crops (Blakeslee and Belling, 1924; McClintock and Hill, 1931; Einset, 1943; Kamanoi and Jenkins, 1962; Khush et al., 1967). Khush (1973) provided many other examples where transmission of extra chromosome in trisomics occurred with a far greater frequency through females than through male gametes.

Theoretically, the primary trisomic could produce \( n \) and \( n+1 \) gametes in equal proportion in female and male sides, and often selfing the trisomic, the progeny would be \( 2n \), \( 2n+1 \) and \( 2n+2 \) individuals in a ratio of 1:2:1. However, from the data available so far on trisomics of different plant species (Hermsen, 1970; Khush, 1973) including rice such expectations are never realised and the percentage of \( 2n+1 \) individuals, as evident from the appearance of parental trisomics in the progeny of trisomics upon selfing, is much lower than the theoretical limit of 50 per cent. Only Triplo-12 was exceptional where female transmission was 50 per cent which was the theoretical limit. In other trisomics the parental types appeared in the frequency of 4.50 to 39.00 per cent. Hence, it is clearly established that the female transmission data on rice trisomics are mostly in agreement with that of other crops.

The reduction (from theoretical limit of 50 per cent) in transmission frequency has been attributed due to a variety of factors such as (i) the elimination of extra
chromosome during meiosis, (ii) megaspore replacement, (iii) reduced viability of n+1 gametes and (iv) malformation of embryo, endosperm and seed coat, as suggested by McClintock and Hill (1931) for maize and Sreeramulu et al. (1978) for Lycopersicum. One or more such factors are possibly operating in case of rice. Growing conditions (Rajahathi, 1975) and extension of germination time (Blakeslee, 1928; Goodspeed and Avery, 1939; Khush, 1973) are also known to exert influence in transmission rate and in the final proportion of aneuploid gametes among the offsprings of trisomics. It is also expected that the reduction in germination percentage of selfed seeds of trisomics which range from 12.8 to 80.0 per cent in different trisomics. With the overall average of 49.3 per cent germinability might be contributing for the low frequency of transmission among the selfed progenies of rice trisomics. The rare appearance of unrelated types of trisomics observed in very low frequency might be due to the presence of extra chromosome in a trisomic which frequently interfere with meiotic chromosome behaviour resulting the production of unrelated types. Moreover, the production of unrelated types may be due to the phenomenon of "univalent shift" as reported by Person (1956) in monosomics of wheat.

But the above considerations were not propitious in the case of trisomics for twelveth chromosome, which yielded
more trisomies on selfing. Hence, it is probable that under such circumstances very little selection pressure might be operating against the transmission of trisomic gametes, as reported by Sreeramulu et al. (1978) for *Lycopersicon*. Moreover, it is well proved that shorter the extra chromosome the greater would be the tendency for transmission to the offsprings (Chen and Grant, 1968b). The maximum frequency of trisomic transmission may be attributed due to the greater chance of transmission of univalents at the gametic level.

Although, Einset (1943) reported a positive correlation between length of extra chromosome and transmission rate, it does not hold true for rice as revealed from the present study and also from the reports by Chen and Grant (1968b) in *Lotus* and Rick and Barton, (1954) in *Lycopersicon*. It is therefore, suggested that the presence of an extra chromosome would cause genic imbalance and that this imbalance may result in abortion of some of the n+1 gametes and 2n+1 zygotes at different stages in ontogeny. The longer the extra chromosome, the greater would be the imbalance and hence there would be less chance for n+1 gametes and 2n+1 zygotes to be viable. Therefore, it may be inferred that the univalents might be taking part in the transmission.
Einset (1943) from his study on the transmission rate of extra chromosome in maize reported a correlation between chromosome length and transmission rate. Trisomics for longer chromosomes in maize yielded a higher frequency of trisomics in progenies than did the ones for shorter chromosome. In *Datura* the chromosome length, however, had been shown to have no relation to the transmission rates. While the smallest chromosome in maize showed 32.7 per cent transmission, the third smallest chromosome of *Datura* in the complement had only 3.0 per cent transmission (Blakeslee and Avery, 1938). Likewise Tsuchiya (1967) found no correlation with chromosome length, germination per centage and transmission rate in trisomics of barley. In the present study the correlation coefficient between chromosome length and transmission rate through females was -0.6044 which was negative and not significant indicating that, though transmission rate in smaller chromosome is higher than that of longer chromosomes the relationship is not consistent.

In trisomics of *Mathiola* higher percentage of germination is associated with higher frequency of transmission (Frost, 1919). In the present study Triplo-7 had the highest germination percentage (80 per cent) associated with high frequency of female transmission (38.67 per cent). But in Triplo-12, where the germination was only 12.8 per cent, 50.0 per cent transmission was recorded indicating that
almost all the n+1 gametes were viable in egg side which is a rare phenomenon in trisomics of rice.

Khush (1973) discussed the cytological reasons for the low transmission rate in female and rare or no transmission through male gametes. The reasons for the reduction in the number of 2n+1 individual to less than the expected 50 per cent has been attributed by him to be due to several causes of which the following are important.

i) Elimination of the extra chromosome during meiosis due to lagging or misdivision,

ii) Megaspore replacement

iii) Reduced viability of n+1 spore

iv) Sub-normal development of 2n+1 zygotes

v) Poor and delayed germination of 2n+1 seeds

vi) Reduced vigour of 2n+1 seedlings

vii) The effect of genetic background

The essential features in meiosis of trisomics is the presence of the extra chromosome as either univalent or trivalent. Since the extra chromosome has two more homologs present in the complement, it would be expected that the three homologs would compete for pairing during early stages of meiosis. It was observed in the present study as well as the available reports comparing the behaviour of the extra chromosomes of maize and tomato trisomics that pairing occurs always between two chromosomes
at any given region along the chromosome length. Hence, the pairing affinity of the constituent chromosomes forming a trivalent in a primary trisomic depends upon the magnitude of pairing site and the number of chiasmata formation. Einset (1943) observed in the trisomic stocks of maize that long chromosomes form relatively more trivalents because of the available long regions for pairing and greater opportunity for more chiasma formation. Since shorter chromosomes have less opportunity for chiasma formation and have shorter pairing sites, trivalent formation in them is relatively low. Darlington and Mather (1932), Rick and Barton (1954); Tsuchiya (1960); and Sreeramulu et al. (1977) also had similar observations in the materials studied by them.

Einset (1943) reported higher average transmission frequency for longer chromosomes and comparatively lower frequency for trisomics with short chromosomes. In the present study there was no positive correlation between chromosome length and transmission frequency (\( r = -0.6044 \)) indicating that, unlike maize trisomics, rice trisomics with longer chromosomes do not have high transmission rate. Also as discussed above that larger chromosome have relatively more chance of forming trivalents, is not corroborated from the present findings. The relative chromosome length and trivalent frequency of the trisomic studied here showed a
negative correlation \( (r = -0.3280) \). Frequent non-homologous pairing other than the magnitude of pairing site might be contributing for the above non-significant negative relationship.

Cytological analysis of percentage of trivalents in different trisomics was undertaken here to estimate the expected transmission of \( n+1 \) gametes indirectly. The trisomics with the longer and shorter extra chromosomes show much difference in trivalent frequency and consequently the expected transmission rate estimated on trivalent percentage basis show much difference. If the trivalent percentage in a trisomic was assumed to be \( x \), then the expected \( n+1 \) gamete would be \( x/2 \), since on disjunction of the trivalents, one univalent goes to one pole, two members will go to the other poles and therefore, the percentage of \( n+1 \) gametes would be equal to half the trivalent frequency. If the actual transmission rate of the trisomics to the progeny be \( Y \), then the difference, \( \frac{x}{2} - Y \) would account for the abortion of \( n+1 \) gametes through female. If the fertility is relatively high as observed in case of the trisomics studied here, and the fertility difference between the trisomics and the disomics is equal to \( \frac{1}{u} \), then the total number of gametes aborted in female would be equal to \( \frac{1}{u} - (\frac{x}{2} - Y) \). This above relationship was found to hold good for some of the trisomics with longer chromosomes. Therefore, the reduction in transmission
to less than the expected 50 per cent might be due to the abortion of n+1 gametes.

When the extra chromosome is present as univalent at diakinesis and metaphase-I, it is observed to either lag or misdivide during anaphase. If it is not included in any of the telophase-I nuclei, the spores produced thereby will have 'n' chromosomes and consequently there will be significant decrease of n+1 spores. If the reduced transmission of the extra chromosome is due to univalent formation and its elimination due to lagging or misdivision, there would be a positive correlation between the univalent frequency and transmission rate. From his critical study Einset (1943) established a positive correlation between these two phenomena. In the present study a large number of PMCs were examined in each of the 12 trisomics at diakinesis and metaphase-I of meiosis. The percentage of univalents found to have a positive correlation with the relative chromosome length, though the correlation is not significant (r = +0.3346). Since pollen fertility in the trisomics were high and germination percentage of trisomic for smaller chromosomes were comparatively lower than the disomic, the variable reduction of the transmission frequency of the rice trisomics studied here might possibly be due to univalent formation, subsequent lagging, misdivision and elimination of the extra chromosome.
5.5. Genetic analysis

In the present study, available data on the frequencies of female and male transmission of different trisomics, made it possible to calculate the expected gametic frequencies and the zygotic expectations at $F_2$ for different trisomics for both duplex and simplex genotypes (both the triplex AAA and nulliplex aaa would not show any segregation). Based on these, different expected trisomic ratios were calculated for different trisomic crosses for each character studied. Then the observed trisomic ratios were tested for its fitness with the expected trisomic ratios and those critical crosses which fitted into the expected ratios, the gene for that character was assigned to the extra chromosome of that particular trisomic.

In barley (Tsuchiya, 1960, 1967) where considerable achievements have been made on localisation of genes using trisomics, the different populations of 2n, 2n+1 were separately considered and genetic ratios for a trisomic condition of a particular gene fitted accordingly. However, as pointed out by Hermsen (1970) and Khush (1973) it is practically not possible to separate out disomic and trisomic fractions in large populations at $F_2$. Therefore, the entire $F_2$ population was considered here for the calculation of modified trisomic ratio.
Though trisomic-F\textsubscript{2} analysis is different from disomic-F\textsubscript{2} analysis, it is fairly easy to locate a gene on specific chromosome for the character under study which is determined by a single gene. When a character is controlled by more than one gene, complication arises during F\textsubscript{2} analysis unless ratios are calculated on different assumptions and are substituted for each locus and the phenotypic ratios are calculated considering one gene at a time on one chromosome. In the present study, two characters, clustered spikelets and gold hull were analysed in the above manner, since the genes responsible for the two traits are more than one. The results obtained in the present study are discussed separately for each character as follows:

5.5.1. Genes for clustered spikelets

F\textsubscript{2} populations derived from disomic x marker F\textsubscript{1} and 8 of 11 trisomic F\textsubscript{1}s fitted into a 45 (clustered) : 19 (non-clustered) ratio which indicated that the clustered character is controlled by three dominant genes which Cl\textsubscript{1} may be the basic gene for clustered spikelets and Cl\textsubscript{2} and Cl\textsubscript{3} are two independent non-allelic genes, any one or both of which together with Cl\textsubscript{1} express the clustered phenotype (while calculating the genetic ratios, the designation of these three genes Cl\textsubscript{1}, Cl\textsubscript{2}, Cl\textsubscript{3} were Cl, A and B for the sake of convenience).
This character was earlier reported to be controlled by one dominant gene (Parthasarathy, 1935; Rajappan Nair, 1958; Jodon, 1957; Kadam and D'Cruz, 1960; Seetharaman, 1964). Thakur and Roy (1975) reported two dominant complementary genes controlling this character. Kadam and Pant (1968) reported a ratio of 45 (clustered) : 19 (non-clustered) similar to that obtained in the present study. Three of the eight trisomic F2 ratios showed significant departure from the expected 45 : 19. This indicated that these three genes might be present on the extra chromosomes of these three trisomics. Theoretically expected trisomic ratios which were calculated taking into consideration the actual transmission rate both at female and male sides and for both random chromosome and chromatid segregation of simplex and duplex genotypes were tested against these three critical trisomic crosses.

The F2 ratio of the cross involving Triplo-5 and the marker fitted to the expected trisomic ratio of 29.44 (clustered) : 13.80 (non-clustered) ratio which could be obtained for a simplex condition with chromosome segregation. This expected trisomic ratio was estimated with the assumption that when the gene $C_{12}$ or $C_{13}$ was considered to be the basic gene for clustering which in presence of either one or both of the other two genes gives clustered phenotype.
Taking \( C_1 \) as basic in presence of \( C_{12} \) or \( C_{13} \) the observed phenotypic ratio under both chromosome and chromatid segregation did not give a good fit with the expected ratio. Therefore, the gene \( C_1 \) is not present in Triplo-5. From this it was inferred that the basic gene \( C_{12} \) or \( C_{13} \) might have come from chromosome-5 of male parent. Alternatively, if the dominant allele \( C_{12} \) or \( C_{13} \) could have come from female, it could not have given a simplex genotype in \( F_1 \).

In case of Triplo-7 x marker cross, the \( F_2 \) segregation fitted into 174.13 (clustered) : 62.04 (non-clustered) ratio that is expected from a duplex genotype with random chromosome segregation where \( C_{12} \) or \( C_{13} \) is assumed to be the basic gene. The observed \( F_2 \) ratio of this cross did not fit to any of the expected trisomic ratios where \( C_1 \) was assumed to be the basic gene. This excluded the possibility of the presence of \( C_1 \) locus on extra chromosome of Triplo-7. Therefore, one of the other two dominant genes, either \( C_{12} \) or \( C_{13} \) might be present on chromosome-7 and in this case the dominant allele must have come from the female side.

In Triplo-12 x marker cross, the observed \( F_2 \) ratio was fitted to the expected trisomic ratio of 30 (clustered) : 18 (non-clustered). This is expected from a simplex genotype with random chromosome segregation when \( C_1 \) is assumed to be the basic gene. Observed \( F_2 \) ratios of other critical crosses did not fit with any of the estimated trisomic ratios for
this cross. This indicated that the gene \( C_{1} \) might be present on chromosome-12 of this Triplo-12, and is in dominant form in the male parent. Since one of the other two genes was in duplex condition in Triplo-7, it was likely that chromosome-7 in female side might be dominant homozygous for \( C_{2}C_{2} \) or \( C_{3}C_{3} \). In Triplo-5 cross, the fitness to simplex ratio gave indication of the presence of one of the two alternative dominant gene \( C_{2} \) or \( C_{3} \) in chromosome-5 of the male parent.

The genotypic constitution of three triplos might be as follows:

Triplo-5. \( C_{2} \) or \( C_{3} \)
Triplo-7. \( C_{3} \) or \( C_{2} \)
Triplo-12. \( C_{1} \)

Hence the complete genotypic constitution for the female parent, Triplo-5, 7 and 12 and along with the marker 'AC 1224' might be as follows:

1. (\( C_{1} \) as basic gene and \( C_{2} \) other dominant gene)

(a) Triplo-5.

\[
\begin{align*}
& \Phi \quad C_{1}C_{1}, C_{2}C_{2}C_{1}C_{3}C_{1}C_{3} \\
& \times C_{1}C_{1}, C_{2}C_{2}C_{3}C_{3}C_{1}C_{3}
\end{align*}
\]

(b) Triplo-7.

\[
\begin{align*}
& \Phi \quad C_{1}C_{1}, C_{2}C_{2}C_{1}C_{3}C_{1}C_{3}C_{1}C_{3}C_{1}C_{3} \\
& \times C_{1}C_{1}, C_{2}C_{2}C_{3}C_{3}C_{1}C_{3}C_{1}C_{3}
\end{align*}
\]

(c) Triplo-12.

\[
\begin{align*}
& \Phi \quad C_{1}C_{1}C_{1}, C_{2}C_{2}C_{1}C_{3}C_{1}C_{3}C_{1}C_{3}C_{1}C_{3}C_{1}C_{3} \\
& \times C_{1}C_{1}C_{1}, C_{2}C_{2}C_{1}C_{3}C_{1}C_{3}C_{1}C_{3}
\end{align*}
\]
2. (Cl$_1$ as basic gene and Cl$_3$ other dominant gene)
   
   (a) Triplo-5.
   
   $* \text{Cl}_1 \text{Cl}_1 \text{Cl}_2 \text{Cl}_2 \text{Cl}_3 \text{Cl}_3 x \text{Cl}_1 \text{Cl}_1 \text{Cl}_2 \text{Cl}_2 \text{Cl}_3 \text{Cl}_3*$
   
   (b) Triplo-7.
   
   $* \text{Cl}_1 \text{Cl}_1 \text{Cl}_2 \text{Cl}_2 \text{Cl}_3 \text{Cl}_3 x \text{Cl}_1 \text{Cl}_1 \text{Cl}_2 \text{Cl}_2 \text{Cl}_3 \text{Cl}_3*$
   
   (c) Triplo-12.
   
   $* \text{Cl}_1 \text{Cl}_1 \text{Cl}_1 \text{Cl}_2 \text{Cl}_2 \text{Cl}_3 \text{Cl}_3 x \text{Cl}_1 \text{Cl}_1 \text{Cl}_2 \text{Cl}_2 \text{Cl}_3 \text{Cl}_3*$

According to Jodon (1963), the gene Cl which is designated by him as basic gene for clustering is present in linkage group-I while Misro et al. (1966) based on overall informations obtained by Indian workers showed the presence of gene Cl in two linkage groups i.e. I and XI. Kadam and Pant (1968) did not give the respective linkage groups of 3 genes for clustering obtained by them. With the help of the trisomics in japonica rice, Iwata and Omura (1976) identified the gene Cl on chromosome number 6 in B type of trisomic.

Their methodology was, however, not appropriate since they have tried to fit the entire F$_2$ population (both disomic and trisomic fractions) into disomic and trisomic ratios and not tried the ratios appropriate for the entire population as has been done in barley by Tsuchiya (1960).
In the present study since the entire F₂ population (both disomic and trisomic fractions) was fitted in appropriate expected trisomic ratios and, therefore, the results are more appropriate and authentic than that of Iwata and Omura (1975, 1976). However, it is evident from the present study that the basic gene 01 belonging to the linkage group I is present on chromosome number 12 and the other two genes are located on chromosome number 7 and 5.

5.5.2. Gene for gold hull character

The inheritance pattern of the gene for gold hull character have been studied by Misro et al. (1966) who reported it to be controlled by a single recessive gene. This gene belonged to linkage group VI (Misro et al., 1966).

All the F₁s were normal and 8 out of 11 trisomic and the disomic crosses gave a F₂ ratio of 55 (straw hull) : 9 (gold hull) indicating that the gold hull is a recessive character and 3 genes are responsible for this character out of which, one gene (gh₁) along with two more non-allelic dominant genes are responsible for gold hull character. The presence of 3 genes for the gold hull character is further substantiated indirectly by the significant departure in three trisomic crosses indicating that these three genes are independently present on these three chromosomes. Since the F₁s of all the trisomic crosses were straw hull,
it is probable that the male parent might be having the genotype $gh_1gh_1$, $GH_2GH_2$, $GH_3GH_3$. From F$_2$ segregation Triplo-12 showed duplex mode of segregation and Triplo-2 and Triplo-8 showed simplex mode of segregation for gold hull character (while calculating the genetic ratios the designation of these three genes $gh_1$, $GH_2$, $GH_3$ were $gh$, A and B for the sake of convenience).

The goodness of fit of 55 (straw hull) : 9 (gold hull) indicates that the (gold hull) character can express when two recessive genes $gh_1gh_1$ along with two dominant non-allelic genes $GH_2GH_2$, $GH_3GH_3$ either in homozygous or heterozygous condition should be present. Since the cross involving Triplo-12 showed a duplex mode of segregation, it implies that one recessive gene must have come from male and two dominant genes must have come from female trisomic parent for chromosome-12. The other two chromosomes, 2 and 8 might be having two other non-allelic dominant genes either in homozygous or heterozygous conditions. Alternatively, it might be possible that chromosome-2 and 8 have two dominant alleles $GH_2GH_2$ and $GH_3GH_3$, respectively. The F$_1$ trisomics in that case would not give simplex ratio but both will show duplex inheritance. Therefore, chromosome-2 and 8 in male might be having both dominant alleles $GH_2$ and $GH_3$ and the Triplo-12 have corresponding recessive alleles for these chromosomes. Since, the male expressed the gold hull character, the basic gene $gh_1$ might be present in recessive
homozygous form in addition to two other dominant genes $GH_2$, $GH_3$ in chromosome-12. On the other side chromosome-12 in female would have a dominant allele $GH_2$. Hence, the basic gene for gold hull character $gh_1$ is found to be present in chromosome-12 and other two $GH_2$ and $GH_3$ are present in chromosome-2 and 8 or vice versa. Iwata and Omura (1971a) assigned the gene $gh$ in chromosome-2. In the present study, the gene for gold hull belonging to linkage group VI is found to be present in chromosome-12 and the other complementary genes on chromosome-2 and 8.

5.5.3. Genes for long glume and brittle culm character

The character long glume designated as $g$ belongs to linkage group IV and is reported to be controlled by a single recessive gene $g$ (Jodon, 1957; Hsieh, 1960; Thakur and Roy, 1975). However, Kadam and D'Cruz (1960) reported a digenic mode of inheritance for this gene. The character brittle culm designated as $bc$ which has not yet been assigned to any indica linkage group for indica rices and is found to be controlled by a single recessive gene (Hsieh, 1960; Sastry, 1977) and is reported to be present in linkage group XI of japonica rice (Nagao and Takahashi, 1963).

From the present study 10 out of 11 crosses of trisomic x marker gave a $F_2$ segregation ratio of 3 (normal): 1 (long glume) indicating a monogenic inheritance with
normal glume as dominant. The crosses for brittle culm character also showed a monogenic recessive nature of the gene $bc$ and the $F_2$ population of 9 out of 10 trisomic crosses fitted to a $3:1$ ratio. The critical crosses involving Triplo-9 for long glume character and Triplo-3 for brittle culm character showed a striking departure from $3:1$ ratio indicated the presence of these two genes on chromosome-9 and 3 respectively and their goodness of fit to the duplex mode of segregation implies the presence of gene $g$ on chromosome-9 $bc$ on chromosome-3.

The modified trisomic ratios gave a good fit to both duplex genotypes with both random chromosome and chromatid segregation. Random chromatid segregation is taken into consideration the gene under study is to be situated farthest away from the centromere allowing maximum number of chiasma formation and terminalisation leading to the occurrence of univalents in low frequency which is not observed in the present study. But univalent frequency was more or less equal in both the trisomics which indicated that the chromosome segregation was more likely than chromatid segregation. Further comparing both the chi-square values it could be seen that chromosome segregation had a better fit than chromatid segregation. Therefore, chromosome segregation have been considered in these cases.
From the present genetic analyses the relationship between seven types of trisomics and the linkage groups have been tentatively classified with supporting reasons and the genes belonging to their respective linkage groups are identified which are given in Table 52 below:

Table 52. Relationships among types of trisomics, linkage groups and kind of extra chromosome.

<table>
<thead>
<tr>
<th>Type of trisomic</th>
<th>Linkage group</th>
<th>Genes involved</th>
<th>Kind of extra chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triplo-12</td>
<td>I</td>
<td>$G_1$</td>
<td>12</td>
</tr>
<tr>
<td>Triplo-5</td>
<td>I</td>
<td>$G_2/G_3$</td>
<td>5</td>
</tr>
<tr>
<td>Triplo-7</td>
<td>I</td>
<td>$G_3/G_2$</td>
<td>7</td>
</tr>
<tr>
<td>Triplo-12</td>
<td>VI</td>
<td>$gh_1$</td>
<td>12</td>
</tr>
<tr>
<td>Triplo-2</td>
<td>VI</td>
<td>$GH_2$</td>
<td>2</td>
</tr>
<tr>
<td>Triplo-8</td>
<td>VI</td>
<td>$GH_3$</td>
<td>8</td>
</tr>
<tr>
<td>Triplo-9</td>
<td>IV</td>
<td>$g$</td>
<td>9</td>
</tr>
<tr>
<td>Triplo-3</td>
<td>XI</td>
<td>$bc$</td>
<td>3</td>
</tr>
</tbody>
</table>

Further studies are needed to identify more number of genes in each of the linkage groups so that a clear picture of gene - chromosome relationship would be obtained which would be of great use for manipulation of genes and chromosomes in plant breeding.