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CYTOGENETICAL STUDIES ON A TISSUE CULTURE SELECTED SALT TOLERANT IR-6 RICE *(ORYZA SATIVA L.)* MUTANT

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This study was undertaken on the anthers of callus born IR-6 variant, *in vitro* selected for salt tolerance. Cytological study of meiosis is reported. In total 300 cells were observed. Out of these, 57% of the cells were aneuploids 23% PMCs with 10 (II) and 33% with 11 (II). The remaining 43% of the PMCs (pollen mother cells) observed contained the normal number of chromosomes. No other association (trivalent, quadrivalent) was found in this material. Micronuclei and laggards were not observed. It is hypothesised that 2, 4-D (2, 4-dicholorophenoxy acetic acid) and Kinetin (6-furfuryl amino purine) interaction enhances the DNA replication, and the DNA polymerases cannot complete the job of proof reading satisfactorily. SOS repair system which is an inducible repair system to cope with the very fast changes in DNA, operates and the mutation is enhanced more in the presence of NaCl. This gives rise to the cells with different ploidy-hyperploidy, hypoploidy, polyploidy and mixoploidy.

Key words: Tissue culture, Chromosome, *Oryza saliva*.

Introduction

To understand the nature of change in our salt tolerant variant of IR-6, cytogenetical studies were suggested. Plant tissue culture systems generate epigenetic changes and/or mutations. If the trait is not based on alteration of the genetic material the change is termed as epigenetic and does not transmit through meiosis. These changes are brought about by the mechanisms which normally operate during cellular differentiation and are more frequent and less persistent than mutations (Maliga, Meins,) [1,2]. Mutations are recovered in many crop plants including rice (Oono) [3]. In fact plant tissue cultures seldom keep to a rigid geometric series of chromosome doubling and cells with numbers intermediary in the series start to emerge. Shamina [4] found about 13% of mitosis with a triploid number in Haplopappus after only 4 months of culture, while Sidorenko and Kunakh [5] examining the same strain several years later found a range of ploidies including 3n, 5n, 7n, 9n and so on. A similar wide range of ploidy was found by Torrey [6] in long established Pisum cultures. The auxin 2, 4-D has been implicated in accelerating the rate of change and was established to be more effective than NAA (Naphthelene acetic acid) for this purpose [4]. It had already been known to cause chromosomal mutations [7]. However, the concentration of 2, 4-D is also a very important factor in this regard [9].

Unfortunately, rice chromosomes are very small and difficult to prepare [10]. However, meiotic chromosomes are easier to identify than somatic chromosomes because of their size and numerous other distinguishing features and due to their paired nature, the number of chromosomes is half of the somatic number. This renders the task of identification easier. Chromosomal deficiencies are best identified through detection of a deficiency loop in the paired condition at pachytene. This cannot be done with somatic chromosomes [11]. These observations led us to analyse pollen mother cells in meiosis.

Materials and Methods

Plant material was regenerated from (1-0.5%) NaCl selected IR-6 seed born callus. The callus was induced, multiplied and screened at 2 mg/1 2, 4-D and 1 mg/1 Kinetin. A plantlet was transferred to a pot and kept at the NARC Glass house. Another plant was grown on basal medium devoid of any growth regulator and NaCl. No callus formation took place and this plant was used as control for chromosomal comparison. For cytological studies young panicles were collected at 8-9 a.m and fixed for 24 hrs in 1:3 acetic alcohol solution. For the cytological preparation the method described by Sato [12] was used.

Results and Discussions

In total 300 cells were observed. The results are shown in the Table 1 and Figs. 1,2,3.

Table 1. Cytological Observations of 300 PMCs of Salt Selected Rice. Chromosome Association at M1.

Plant	Chromosome numbers			
	12(II)	11(II)	10(II)	Others
Mutant	130	100	70	0
	(43)	(33)	(23)	
IR-6 (control)	100	0	0	0
	(100)	(0)	(0)	(0)

Parenthesis denotes percentage.

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Chromosomal pairing at early metaphase I of meiosis revealed in these preparations was found complete, but aneuploids were found in about 57% of cells observed. 23% PMCs had only 10 bivalents, and 33% had 11. Out of total cells observed about 43% were found with normal chromosome complements (12 bivalents). All the cells observed in IR-6 control contained 12 bivalents. Moreover, no other association (trivalent, quadrivalent) was found in this material. Micronuclei and laggards were not observed at quartel and anaphase stage.

Many researchers have reported similar results. Bajaj and Bidani [13] studied 796 dividing cells in embryo derived Basmati rice callus. They reported diploids, polyploids and aneuploids in these calli. An almost similar pattern was observed when mature and immature endosperm was used [14-15] reported 10 tetraploids (3.1%) and 7 sterile diploid (2.2%) in plantlets regenerated from somatic cell cultures using IR-36 and IR-54 cultivars. Ling [16] regenerated a plant from somatic cell culture of IR-54. Chromosome distribution during the haplophase proved to by very irregular. The number of bivalents at diakinesis varied from 6 to 12 with only 5.7% of the cells showing 12 (II), 89% of PMCs examined proved to be aneuploids. Ogura *et al.* [17] reported one triploid, 10 tetraploids and one aneuploid among 126 protoplast derived plants. Zhang and Chu [18] concluded from their experiments that aneuploidy was one of the causes of trait variation in somaclonal variants. Chu *et al.* [19] studied 1715 anther regenerated plants. Out of these 10.7% were aneuploids. The most common among aneuploids being trisomics (5.4–6.7%) but tetrasomic, monosomic, nullisomics and double trisomics were also found. Anther cultures of 46 japonica enteries during 1980–82 produced 864 clumps of plantlets, of which 1.9–8.0% were polyploids, 43.3–72.0% diploid and 20–48.4% haploid. Mixed clumps with plantlets of different ploidy were also noted in the 1982 experiment in 2.5% cases [20].

2, 4-D causes an increase in the amount of several *in vitro* translation products, including the release of some specific mRNA and specific proteins [21,22]. Cytokinins do not prevent auxin mediated mRNA although they prevent auxin induced cell elongation [23]. Within 60 mins of 6-Benzyl amino purine (a cytokinin) application observable messenger RNA changes can be observed in pumpkin cotyledon tissue culture [24]. These mRNAs are also specific. Hyberidization of pBR 322 cloned DNA repeats with nuclear DNA from cultured rice cells showed that most of the sequences underwent amplification in cultured cells up to 75 fold [25]. Nair and Rana [26] reported that the chiasma number per cell increased when rice varieties were treated with alkalinity and salinity and the tolerant varieties were more stable towards chiasma number.

These investigations lead to the hypothesis that the epigenetic changes are caused by 2, 4-D mediated specific mRNAs and proteins which cause activation of some specific genes or cause the alteration of gene products. 2, 4-D releases some specific mRNAs. Kinetin releases some other specific mRNAs and somehow they antagonize each other (in case of cell elongation for example). These changes may be caused by their interactions. Since 2, 4-D causes DNA amplification, DNA synthesis is enhanced. This in turn causes some errors in the replication. Due to very fast replication and very high rate of mutations (2, 4-D is a carcinogen), SOS repair system is evoked and this is an error prone system. This causes mutation in the DNA.

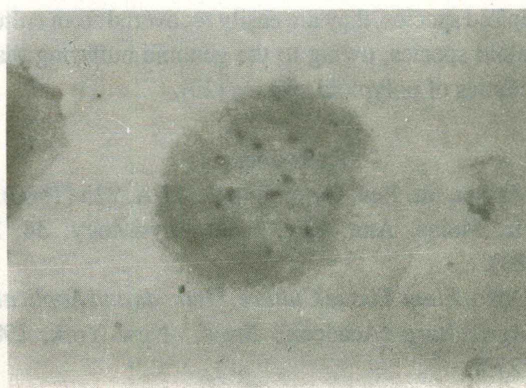

Fig. 1. Metaphase stage showing twelve bivalents (2n=24).

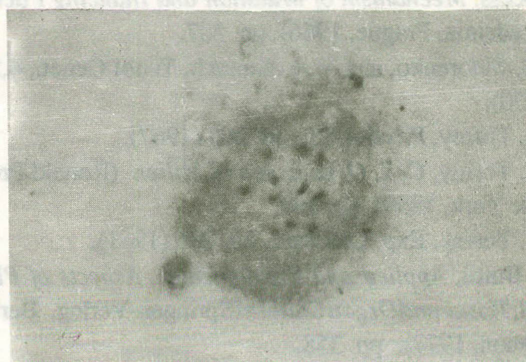

Fig. 2. Mataphase nucleus showing eleven bivalents (2n=22).

Fig. 3.Metaphase nucleus showing ten bivalents (2n=20).

Our plant was either regenerated from more than one callus cells (Chimeric) with different number of cells or the cells in the plantlet were mutated by 2, 4-D or by its conjugates [27]. Presence of NaCl may have enhanced the effect of 2, 4-D (as already discussed). It is speculated that some of the mutations caused during this process changed the chromosome origin of DNA replication site. The chromosomes with mutated origin of replication site could not replicate which resulted into 11 (II) and 10 (II) in PMCs. Moreover, tissue culture plants behave like intact ones. In this system endomitosis and resulting polyploidy do not prevent the induction and continuation of morphogenetic process [28]. While aneuploids are sporadically obtained from tissue cultures of truely diploid species, they are easily recovered from cultures of polyploid species, owing to the genome buffering that is characteristics of polyploid species [29].

References

1. P. Maliga, Int. Rev. Cytol. Suppl., **11** A, 225 (1980).
2. F. Jr. Meins, Ann. Rev. Plant Physiology, **34**, 327 (1983).
3. K. Oono, *Plant Tissue Culture, Methods and Application in Agriculture* (Academic Press, New York, 1981), pp. 273.
4. Z.B. Shamina, Proc. Symposium, The Mutational Process. *Mechanism of Mutation and Inducing Factors* (Academia, Prague, 1966), pp. 337.
5. P.G. Sidorenko, and V.A. Kunakh, Tsitol Genet., **4**,235 (1970).
6. J.G. Torrey, Physiol. Pl., **20**, 265 (1967).
7. J.G. Torrey, *Cell, Organism and Milien* (Ronald Press, New York, 1959), pp. 189.
8. J.G. Torrey, Exp. Cell Res., **23**, 281 (1961).
9. M. Buitti, *Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture* (Springer–Verlog, Berlon, London, 1977), pp. 358.
10. H.K. Wu and M.C. Chang, *Rice Genetics* (IRRI, Los Banos, Philippines, 1985), pp. 135.
11. G.S. Khush and R.J. Singh, *Rice Genetics* (IRRI, Los Banos, Philippines, 1985), pp. 239.
12. S. Sato, *Rice Genetics* (IRRI, Los Banos, Philippines, 1985), pp. 161.
13. Y.P.S. Bajaj and M. Bidani. Phytomorphology, **30**, 290 (1980).
14. Y.P.S. Bajaj, S.S. Saini and M. Bidani, Thero, Appl. Genet., **58**, 17 (1980).
15. D.H. Ling, W.Y. Chen, M.F. Chen and Z.R. Ma, Acta Genet. Sin., **14** (4), 249 (1987).
16. D.H. Ling, Cereal Res. Commun., **15**, 5 (1987).
17. H. Ogura, J. Kyozuka, Y. Hayashi, T. Koba and K. Shimamoto, Theor. Appl. Genet., **75**, 670 (1987).
18. L. Zhang and Q. Chu, Sci. Agr. Sin., **4**, 14 (1984).
19. Z.G. Chu, Z.H. Zhang and Y.H. Goo, Theor. Appl. Genet., **71** (3), 506 (1985).
20. Z.G. Chen, Z.M. Zhang, X.T. Lang and Z.D. Lui, Acta. Genet. Sin., **12** (4), 268 (1985).
21. L.L. Zurfluh and T.M. Guilfoyle, Plant Physiol., **69**, 332 (1982 a).
22. L.L. Zurfluh and T.M. Guilfoyle, Plant. Physiol., **69**, 338 (1982 b).
23. L.N. Vanderhoef and C.A. Stahl, Proc. Natl. Acad. Sci. USA, **72**, 1822 (1975).
24. C.M. Chen, J. Ertl, M.S. Yang and C.L. Chang, Plant Sci., **52**, 169 (1987).
25. K.L. Zheng, S. Castiglione, M.G. Biasini, A. Biroli, C. Morandi and F. Sala, Theor. Appl. Genet., **74**, 65 (1987).
26. Y.G.M. Nair and R.S. Rana, Tropical Ecology, **28**, 8 (1987).
27. G.H. Davidson, R.H. Hamilton, R.B. Vallego and R.O. Mumma, Plant Physiol., **70**, 357 (1982).
28. F. DAmato, *Crop Genetic Resources for Today and Tomorrow* (Cambridge Univ. Press, 1975), pp. 333.
29. F. DAmato, *Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture* (Springer – Verlog, Berlon, London, 1977), pp. 343.