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CYTOGENETIC ANALYSIS OF THE INTERSPECIFIC HYBRID,

VIGNA RADIATA X V. UMBELLATA

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Marcia Machado

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J R Baker

Major professor

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CYTOGENETIC ANALYSIS OF THE INTERSPECIFIC HYBRID, *VIGNA RADIATA* X *V. UMBELLATA*

by

Marcia Machado

A THESIS

Submitted to
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ABSTRACT

CYTOGENETIC ANALYSIS OF THE INTERSPECIFIC HYBRID,
VIGNA RADIATA X *V. UMBELLATA*

BY

Marcia Machado

Vigna radiata and *V. umbellata* and their interspecific F_1 were examined cytologically to confirm hybridity of the F_1, determine the causes of sterility in the hybrid, and study the nature of genome relationships between the two species. Meiosis in the parents was mostly normal. Split spindles showing 5/6 segregation and secondary association of 1-5 bivalents were observed at metaphase I. Meiosis in the interspecific hybrid was irregular. The mean pairing at metaphase I was 13.40 I + 3.95 II + .18 III + .01 IV. Spindle abnormalities were observed in which the bivalents were segregated from the univalents. The number of microspores formed from each microsporocyte was irregular with 42% dyads, 9% triads, and 38% tetrads. Pollen stainability in the interspecific hybrid was low (1.5%); however, the size of the small percentage of stainable pollen was noticeably larger than that of the parent pollen. These observations suggest that these two species have a lower basic chromosome number and may be polyploid in nature.

Guidance Committee:

This thesis is condensed into a format suited and intended for publication in Crop Science.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Vigna radiata (L.) Wilczek (mungbean) and *V. umbellata* (Thunb.) Ohwi and Ohashi (rice bean) are major food legume crops in Asia and Africa. Interspecific crosses, all with limited success, have been previously reported between these two species (1,3,10,18,28). In all cases, the F_1 was highly sterile.

Meiosis in both species has been reported to be normal with 11 bivalents (1,8,9,10,11,29) except for occasional precocious separation of 1-2 pairs of chromosomes in both species (1,22). Cytogenetic studies on *V. radiata* were reported by Bose (6), Sen and Ghosh (29,30), and Krishnan and De (24).

Barriers to genetic exchange between *V. radiata* and *V. umbellata* were expressed as failure of hybrid embryogenesis and sterility of the infrequent hybrid plants. With the broad objective of introducting new and valuable genetic variation into *V. radiata*, certain treatments were successfully used to overcome the crossability barriers (5,7); however, complete seed sterility of the hybrid prevented the exchange of genetic material between the two species.

The objectives of this cytological analysis were to further confirm hybridity of the interspecific F_1; study the cytological causes underlying sterility in this hybrid; and clarify the nature of genomic homology in these two species.

MATERIALS AND METHODS

The cultivars used as parents for the hybrid were 'Tainan #1', 'M4', and 'PHLV #18' for V. radiata and 'HK' for V. umbellata from the accession collection of the Asian Vegetable Research and Development Center, Tainan, Taiwan, R.O.C. Hybridization was accomplished as reported by Chen et al (7). The plants used for cytogenetic study (five each of both parents and the hybrid) were greenhouse-grown plants.

All cytological observations were made on pollen mother cells (PMC). The flower buds were fixed for at least 12 hours in Carnoy's fixative of ethanol, chloroform and glacial acetic acid (6:3:1). Anthers were squashed in propiono-carmine. Photomicrographs were taken for a permanent record.

To estimate fertility, dehisced anthers were tapped lightly on a drop of I_2-KI solution (21). Those that stained darkly were counted as viable. One flower was collected from each of five plants for both parents and the hybrid. Five hundred pollen grains were counted for each flower.

RESULTS

Cytology. The two parental species and their interspecific F_1 had 2n=22 chromosomes. Mostly regular meiosis was observed in both parents. At diakinesis, 2 pairs of chromosomes were associated with the nucleolus. Eleven bivalents were regularly seen at this stage (Fig. 1), as well as at metaphase I (MI) (Fig. 2). Precocious separation of 1 or 2 bivalents (II) at MI was observed. Occasionally, a split metaphase plate was observed with 5 chromosomes in 1 part and 6 in another (Figs. 3,4). Normal distribution of chromosomes occurred at anaphase I (AI) (Fig. 5).

Pairing or "secondary association" of bivalents was observed at MI in both V. radiata and V. umbellata (Figs. 6,7,8). The number of obvious secondary associations per metaphase plate ranged from 1 to 5. In cells showing less than 5 pairs, most of the remaining chromosomes were co-oriented in a manner suggestive of a relationship between them (Fig. 9).

Meiosis in the interspecific F_1 was highly irregular. At diakinesis, generally, no pairing was found (Fig. 10), although occasionally a loose juxtapositioning of chromosomes was evident (Fig. 11). A total of 82 PMCs were carefully analyzed at MI. No normal appearing, tightly paired bivalents were observed. In all PMCs, at least 2 loosely paired bivalents were observed with a maximum of 9 such pairs in one PMC (Fig. 12). The mean pairing configuration at MI was 13.40 I + 3.95 II + .18 III + .01 IV (Table 1).

Table 1. Chromosome configurations in meiosis of the interspecific hybrid, *Vigna radiata* x *V. umbellata*.

Metaphase I Configurations				Cells	
Univalents	Bivalents	Trivalents	Quadrivalents	No.	%
4	9			1	1.2
6	8			3	3.7
8	7			2	2.4
10	6			9	11.0
12	5			16	19.5
14	4			14	17.1
16	3			12	14.6
18	2			11	13.4
20	1			5	6.1
11	4	1		2	2.4
13	3	1		1	1.2
15	2	1		1	1.2
6	5	2		1	1.2
10	3	2		1	1.2
12	2	2		1	1.2
8	1	4		1	1.2
7	4	1	1	1	1.2
			Total	82	

Figure 1. Diakinesis in V. radiata showing 11 bivalents (1268x).

Figure 2. Metaphase I in V. radiata showing 11 bivalents (1268x).

Figure 3. Metaphase I in V. umbellata showing split plate with 5/6 segregation of bivalents (750x).

Figure 4. Metaphase I in V. radiata showing split plate with 5/6 segregation of bivalents (1375x).

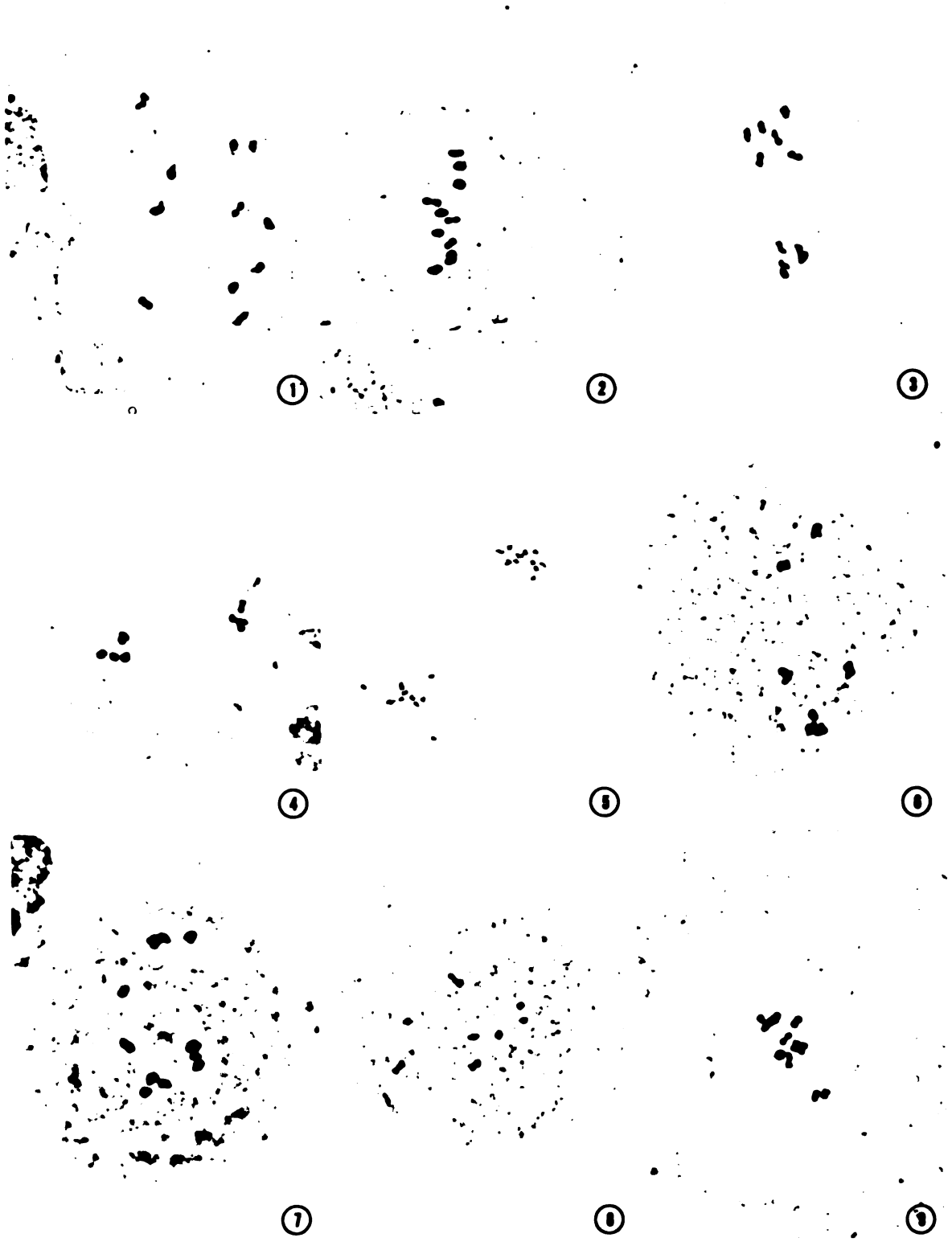
Figure 5. Anaphase I in V. umbellata (950x).

Figure 6. Secondary association in V. radiata showing 4 pairs of bivalents and 1 group of 3 bivalents (1575x).

Figure 7. Secondary association in V. radiata (1900x).

Figure 8. Secondary association in V. radiata showing 3 pairs of bivalents (1150x).

Figure 9. Secondary association in V. umbellata showing 2 pairs of bivalents and co-orientation of other chromosomes (1650x).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The degree of pairing in the interspecific hybrid varied from actual (but not intimate) contact to wide separation with a thin strand of chromatin joining or nearly joining the 2 chromosomes. Both side-by-side and end-to-end associations were seen (Figs. 13,14,15). Within each PMC, in addition to the obviously associated or paired chromosomes, some of the remaining chromosomes were juxtaposed in a way that suggested an association (Fig. 14). Very few obvious multivalent configurations were seen; however, groups of 3 or 4 chromosomes were commonly observed in possible associations.

In the majority of PMCs at MI, chromosomes were distributed on the plate with no consistent orientation of pairs. Occasionally, spindle abnormalities were seen which appeared to represent split or multiple spindles (Fig. 15). In some of these, bivalents were segregated from univalents (Fig. 16). Only one occurrence of equal distribution of dyads at AI was observed. Lagging chromosomes (from 1 to 11) were observed in 73% of the PMCs (Fig. 17). In these cells, the univalents which were situated on the plate were oriented axially and dividing precociously (Fig. 18). The distribution of chromosomes at AI appeared to be random. Chromatin bridges between the 2 nuclei were occasionally observed at telophase I.

Second division was also irregular. Out of 68 PMCs observed at prophase II, 40 cells (59%) contained the normal 2 nuclei while 28 (41%) appeared to contain more than 2.

Figure 10-18. Meiosis in the interspecific hybrid, *V. radiata* x *V. umbellata*.

Figure 10. Diakinesis with no pairing (1250x).

Figure 11. Diakinesis with loosely paired chromosomes (1250x).

Figure 12. Metaphase I with 9 bivalents and 4 univalents (arrow indicates overlapping bivalents) (1450x).

Figure 13. Metaphase I with 6 bivalents and 10 bivalents (1116x).

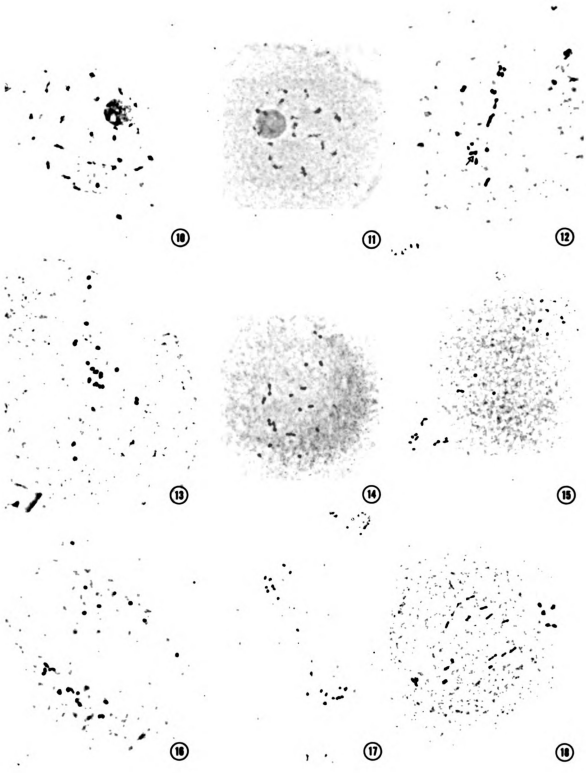
Figure 14. Metaphase I with 6 bivalents and 10 univalents. Positions of univalents suggest associations (1200x).

Figure 15. Metaphase I showing split spindle (1025x).

Figure 16. Metaphase I showing split spindle and segregation of bivalents and univalents (1025x).

Figure 17. Anaphase I with 9/12 disjunction and 1 lagging chromosome (1050x).

Figure 18. Anaphase I with 5/6 disjunction and 11 lagging chromosomes dividing precociously (975x).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Three groups of chromosomes were seen in 36% of the PMCs observed (44 cells) at MII, while 4, 5, or 6 groups were seen at AII and TII. The distribution of chromosomes in the various stages of the second division varied widely and did not show any consistent pattern.

In Vigna, cytokinesis does not occur after first meiosis. It is a single process of quadri-partitioning after second meiosis. Examination of 223 sporads revealed that the number of microspores formed from each microsporocyte was abnormal with 42% dyads, 9% triads, and 38% tetrads (Figs. 19,20). The remaining sporads contained 6, 7, or 8 microspores (Fig. 21). Micronuclei were commonly observed in many PMCs.

Fertility. Pollen stainability was high in the parents with 98% for V. radiata and 94% for V. umbellata. Pollen size in the parents was uniform (Figs. 22,23). In the F_1, pollen stainability was only 1.5%. The pollen size was extremely variable; however, the stainable pollen was much larger than the parent pollen (Fig. 24).

Figure 19. Sporad stage of the interspecific hybrid, V. radiata x V. umbellata showing dyad (3125x).

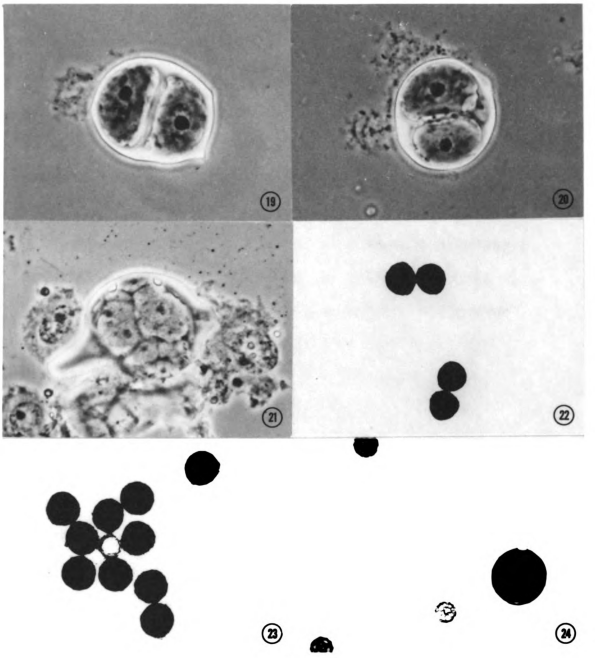
Figure 20. Tetrad of the interspecific hybrid, V. radiata x V. umbellata showing 2 large and 2 small cells (3125x).

Figure 21. Sporad stage of the interspecific hybrid, V. radiata x V. umbellata showing many cells (2500x).

Figure 22. Pollen of V. radiata (288x).

Figure 23. Pollen of V. umbellata (288x).

Figure 24. Pollen of the interspecific hybrid, V. radiata x V. umbellata (288x).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DISCUSSION

The hybridity of the interspecific F_1 from the cross of V. radiata x V. umbellata was previously confirmed on the basis of morphological characteristics (7). It is further substantiated by the meiotic irregularities observed in this study.

Whether the "loose" associations observed at metaphase I represented asynapsis or desynapsis of bivalents which had paired at zygotene and pachytene was uncertain. Examination of PMCs at diakinesis showed essentially no pairing. This does not preclude the possibility that the separation of paired chromosomes occurred prior to diakinesis. Short chromosomes, such as those of Vigna, have a lower chiasma frequency than long chromosomes (13,14,15,23). Given the low chiasma frequency in chromosomes of the interspecific hybrid, together with partial homology, it is conceivable that the bivalents would separate early.

The secondary pairing of bivalents exhibited by the parents of this cross complicated interpretation of the pairing behavior of the hybrid. Since the purpose of making the cross was to recombine characters of the parents, pairing (or the lack thereof) is of prime significance in assessing potential success. A maximum of 9 loosely paired bivalents

was found at metaphase I; however, the pattern of pairing was similar to the secondary associations seen in the parents. Since individual chromosomes have not yet been identified, it was not possible to determine if the observed pairing was auto- or allosyndetic.

The very low incidence of obvious multivalent association in the hybrid was surprising considering the secondary associations observed in the parents and the fact that some homology would be expected between the two species. However, it has been found that plants, even autotetraploids, with small chromosomes tend to have few multivalents because of low chiasma frequency (23). In addition, chiasma frequency may be under the control of additive genes or polygenes (19, 20). A genic influence can not be excluded as an explanation for low multivalent frequency in this Vigna interspecific hybrid. The occurrence of a few multivalents does, however, indicate possible allosyndetic pairing and partial homology between the two species.

The early division of univalents observed at anaphase I is typical behavior for univalents (16). In the present Vigna interspecific hybrid, the univalents lying on the equatorial plate at anaphase I were arranged axially and the chromatids were observed moving toward the poles. Therefore, it appears likely that they would have been included in the daughter nuclei. The scattered univalents lying far from the plate were more apt to be lost in the cytoplasm. These excluded chromosomes could

account for the micronuclei seen in the tetrads of this hybrid.

Since 41% of the prophase II cells examined contained more than the normal 2 nuclei, the polyspory observed at the sporad stage was not surprising. However, the high frequency of dyads (42%) was an unexpected occurrence. A similarly high proportion of dyads to tetrads has been reported (2) in the cross between V. radiata and V. angularis. Apparently in many cells, normal nuclear envelope formation and cytokinesis were hindered as a result of multipolar division and non-congregating chromosomes. This often led to the formation of only two microspores. This phenomenon could also explain the remarkably large size of stainable pollen. If a restitution nucleus were formed as a result of inability to segregate the chromosomes into individual nuclei, a polyploid nucleus might be formed which would exhibit normal meiosis and give rise to a viable gamete.

In this hybrid, aberrant meiotic behavior of the chromosomes would rarely allow development of normal, fertile gametes. The lack of pairing of chromosomes at metaphase I was responsible for irregularities in later stages. The presence of many univalents and lack of co-orientation of bivalents led to unequal distribution of dyads at anaphase. This was reflected in all subsequent stages.

Multipolar divisions evident at anaphase I and in the second division would also produce gametes with incomplete chromosome complements. Non-homology between genome specific spindle

organizers was considered to be responsible for multipolar meiosis in Agropyron Cristatum (32). According to this model, when the spindle organizers are homologous, they either fuse or one degenerates and a single spindle is formed. However, in a species hybrid, non-homologous spindle organizers may both persist and cause multipolar division. A similar system may be operating in this interspecific hybrid.

Although meiotic irregularities could account for sterility in our hybrid, it is very difficult, as Stebbins (31) pointed out, to distinguish between genic and chromosomal sterility. Genic imbalance and chromosome anomalies may be acting jointly to produce sterility in this interspecific hybrid.

The most interesting feature of cytogenetic behavior in this study was the strong tendency for pairing of bivalents. This feature, previously overlooked by investigators in this genus, significantly affects the interpretation of other cytological observations. This type of pairing, termed secondary association (12, 25, 27), is exemplified by nonrandom distribution of bivalents during meiosis. There is general agreement that this attraction of bivalents indicates homology between the chromosomes involved (15,25,26,33). High basic chromosome numbers, especially uneven ones such as 11 found in Vigna, are likely derived from originally lower numbers and are secondarily balanced (14,25,26).

The discovery of secondary associations between chromosomes belonging to the basic genome of Vigna suggests that this genome was possibly derived from an ancestral lower set during the course of evolution. Split spindles showing 5/6 segregation in the parents and multipolar division at metaphase I in the hybrid further contribute to the hypothesis that the genome of Vigna is comprised of two genomes. One genome would contain a basic number of 5 and the second genome 6. Each genome may have a specific relationship to a special spindle or spindle region as was found in some polyploids (4,32). In the parents, the homologous chromosomes and spindle organizers allow a normal meiosis; whereas, in the interspecific hybrid, non-homology of chromosomes and spindle organizers lead to fractionation of the chromosome complement. Examination of the few reports available (17,24) on the karyotypes of Vigna do not preclude the possibility that the diploid set actually is comprised of two genomes differing by structural modifications.

Although assessing the significance of chromosome pairing and drawing conclusions about polyploidy and genome associations need be done with caution, secondary association strongly suggests that these two species are actually polyploids functioning as diploids. Further, the presence of multivalents implies that there is partial homology between the two species. This information may prove to be useful in future comparative studies in the genus Vigna.

REFERENCES

1. Ahn, C. S. 1976. Interspecific hybridization among four species of the genus Vigna Savi. Ph.D. Thesis, Univ. of Hawaii, Honolulu.

2. ____, and R. W. Hartmann. 1978. Interspecific hybridization between mung bean (Vigna radiata (L.) Wilczek) and adzuki bean (V. angularis (Willd.) Ohwi and Ohashi). J. Amer. Soc. Hort. Sci. 103:3-6.

3. Anonymous. 1975. AVRDC Annual Report for 1974. The Asian Vegetable Research and Development Center, Tainan, Taiwan.

4. Avivi, L. and M. Feldman. 1973. Pattern and control of non-random arrangement of chromosomes in the somatic nucleus of common wheat. Genetics 74:12-13.

5. Baker, L. R., N. C. Chen, and H. G. Park. 1975. Effect of an immunosuppressant on an interspecific cross of the genus Vigna. HortScience 10:313.

6. Bose, R. D. 1939. Studies in Indian Pulses IV. Mung or green gram (Phaseolus aureus Roxb.). Indian J. Agric. Sci. 2:607-624.

7. Chen, N. C., J. F. Parrot, T. Jacobs, L. R. Baker, and P. S. Carlson. 1977. Interspecific hybridization of food legumes by unconventional methods of plant breeding. In: Proceedings First Int'l. Mungbean Symposium. Univ. Philippines, Los Banos. p. 1-9.

8. Dana, S. 1965. Phaseolus aureus Roxb. x tetraploid Phaseolus species cross. Revista de Biol. 5:109-114.

9. ____. 1966a. Cross between Phaseolus aureus Roxb. and P. mungo L. Genetica 37:259-274.

10. ____. 1966b. Cross between Phaseolus aureus Roxb. and P. ricciardianus Ten. Genet. Iberica 18:141-156.

11. Dana, S. 1966c. Chromosome differentiation in tetraploid Phaseolus species and P. ricciardianus Ten. Nucleus 9:97-101.

12. Darlington, C. D. 1928. Studies in Prunus, I, II. J. Genet. 19:213-256.

13. ____. 1930. Chromosome studies in Fritillaria, III. Cytologia 2:37-55.

14. ____. 1932. Recent Advances in Cytology. Ed. 1, London.

15. ____. 1937. Recent Advances in Cytology. Ed. 2, London.

16. ____. 1957. Messages and movements in the cell. Conference on Chromosomes, Wageningen. Willink/Zwolle.

17. De, D. N. and R. Krishnan. 1966. Cytological studies of the hybrid, Phaseolus aureus x Phaseolus mungo. Genetica 37:588-600.

18. Evans, A. M. 1975. Species hybridization in the genus Vigna. IITA Trop. Grain Legume Bull., Ibadan, Nigeria. p. 1-5.

19. Gale, M. D. and H. Rees. 1970. Gene controlling chiasma frequency in Hordeum. Heredity 25:393-410.

20. Jauhar, P. 1978. Primary trisomy in tall fescue. J. of Heredity 69:217-223.

21. Johansen, D. A. 1940. Plant Microtechnique. McGraw-Hill Book Co., Inc., New York.

22. Kaul, C. L. 1970. Investigation into causes of sterility. IV. Gametocide-induced male-sterile Phaseolus aureus Roxb.

23. Kostoff, D. 1940. Fertility and chromosome length. J. Heredity 31:33-34.

24. Krishnan, R. and D. N. De. 1965. Studies on pachytene and somatic chromosomes of Phaseolus aureus Roxb. Nucleus 8:7-16.

25. Lawrence, W. J. C. 1931. The secondary association of chromosomes. Cytologia 2:352-384.

26. Meurman, O. 1933. Chromosome morphology, somatic doubling and secondary association in Acer Platanoides L. Hereditas 18:145-173.

27. Nandi, H. K. 1936. The chromosome morphology, secondary association and origin of cultivated rice. J. Genet. 33: 315-335.

28. Sawa, M. 1973. On the interspecific hybridization between the adzuki bean, Phaseolus angularis (Willd) W. F. Wight and the green gram, Phaseolus radiatus L. I. Crossing between a cultivar of the green gram and a semi-wild relative of the adzuki bean, in endemic name "Bakaso". Jap. J. Breed. 23:61-66.

29. Sen, N. K. and A. K. Ghosh. 1960a. Interspecific hybridization between Phaseolus aureus Roxb. (green gram) and Phaseolus mungo L. (black gram). Bul. Bot. Soc. Bengal 14:1-4.

30. ____. 1960b. Studies on the tetraploids of six varieties of green gram. Proc. Natl. Inst. Sci. (India) 26:Sec. B. 291-299.

31. Stebbins, G. L. 1958. Hybrid inviability, weakness, and sterility of interspecific hybrids. Adv. in Genetics 9: 147-215.

32. Tai, W. 1970. Multipolar meiosis in diploid crested wheatgrass, Agropyron Cristatum. Amer. J. Bot. 57: 1160-1169.

33. Therman, E. 1951. Somatic and secondary pairing in Ornithogalum. Heredity 5:253-269.