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CYTOGENETIC STUDIES OF THE DRY BEANS, SPECIES OF THE GENERAL PHASEOLUS AND VIGNA (LEGUMINOSAE)

By

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ABSTRACT

CYTOGENETIC STUDIES OF THE DRY BEANS,
SPECIES OF THE GENERA PHASEOLUS AND VIGNA (LEGUMINOSAE).

BY

GARY R. BAUCHAN

Most of the species in the genera Phaseolus and Vigna are diploid (2n=2X=22) with only two exceptions, both of them being tetraploid species (2n=4X=44). The basic chromosome number is X=11. Most of these species have small chromosomes with a homogeneous karyotype, resulting in difficult karyotypic analysis. The discovery of polytene chromosomes may prove to be helpful in attempts to identify the individual chromosomes. Observations of meiosis in the pollen mother cells has shown normal pairing at metaphase I, however, early bivalent separation, which appear as univalents, have been observed. Chromatin bridges and quadrivalent associations in the hybrids indicate that structural modifications, particularly inversions and translocations, comprise the main evolutionary force for genome differentiation.

The cytology of Vigna radiata (L.) Wilczek, V. umbellata, (Thunb.) Ohwi & Ohashi, their hybrid, amphiploid, backcross progeny, and the plants of subsequent generations were investigated to determine the genome relationship among these plants for use in a breeding program with the goal of incorporating beneficial characteristics of V. umbellata into V. radiata. Light microscope investigations included:

root tip chromosome counts; the analysis of chromosome pairing during all stages of microsporogenesis; and pollen stainability to estimate plant fertility.

In this study the presence of secondary associations, multipolar meiosis, the grouping of bivalents during meiosis, and the high basic chromosome number has led to a re-evaluation of the ploidy levels in these plants. Genome formulae have been assigned in accordance with these findings under the assumption of genetic control of pairing with dosage effects.

DEDICATION

This dissertation is dedicated to my late mother, Barbara, and to my father, Roy G. Bauchan.

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ABSTRACT

CYTOGENETICS OF PHASEOLUS AND VIGNA

By

Gary R. Bauchan

Most of the species in the genera Phaseolus and Vigna are diploid (2n=2X=22) with only two exceptions, both of them being tetraploid species (2n=4X=44). The basic chromosome number is X=11. Most of these species have small chromosomes with a homogeneous karyotype, resulting in difficult karyotypic analysis. Polytene chromosomes may prove to be helpful in attempts to identify the individual chromosomes. Observations of meiosis in the pollen mother cells have shown normal pairing at metaphase I, however, early bivalent separation, which appear as univalents, have been observed. Chromatin bridges and quadrivalent associations in the hybrids indicate that structural modifications, particularly inversions and translocations, comprise the main evolutionary force for genome differentiation. Cytological studies on these plants have been conducted which seem to indicate that they may not be diploids but rather allotetraploids with a basic chromosome number of X=6. The observation of secondary associations, multipolar meiosis, the grouping of bivalents during meiosis, and the high basic chromosome number has led to this proposed re-evaluation.

Introduction

Cytogenetic studies in species of the genera Phaseolus and Vigna are handicapped because these species have: 1) very small chromosomes, 2) homogeneous karyotypes (i.e., similar chromosomes within and between species), and 3) the lack of knowledge concerning the driving forces of evolution (i.e., chromosome structure modifications or chromosome number changes). It is difficult to obtain meiotic stages because usually only one bud in an inflorescence is undergoing meiosis at any given time. Therefore, to obtain a bud at a stage suitable for analysis of the chromosome behavior is a major problem.

Most of the cytogenetic studies in these genera have been conducted in southeast Asia, probably due to their agricultural and economic importance in this region. Many of the Vigna species are endemic to India, perhaps this may be a catalyst for active cytogenetic research in that region. In recent years, reports originating in the laboratories in the western countries, including the United States, have increased.

Discussion

Karyotype

The basic chromosome number of X=11 has been reported for all of the species thus far examined of the genera Phaseolus and Vigna (Table 1). Most species are diploids with a somatic number of 2n=2X=22 (Figure 1). Dana (1964) was the first to report a spontaneously occurring allotetraploid, Vigna glabrescens with 2n=4X=44. A similar chromosome count has been reported by Frahm-Leliveld (1965b) for Vigna radiata var. sublobatus, however, this plant may be the same species that Dana

Table 1. Chromosome numbers of some economically important species in the genera Phaseolus and Vigna.

Phaseolus	Gametophyte	Sporophyte	References
P. acutifolius		22	Karpechenko, 1925; Marechal, 1969 & 1970; Shibata, 1962; Smartt, 1970; Sarbhoy, 1978; Sinha & Roy, 1979; Sarbhoy, 1980.
P. coccineus =P. multiflorus	11	22	Karpechenko, 1925; Senn, 1938; Nagl, 1962; Thomas, 1964; Lamprecht, 1966; Marechal, 1969 & 1970; Smartt, 1970; Cionini & Avanzi, 1972; Mok & Mok, 1976; Joseph & Bouwkamp, 1978; Sinha & Roy, 1979; Blackson & Tai, 1982.
		24	Kleinman, 1923.
P. lunatus	11	22	Karpechenko, 1925; Kawakami, 1930; Senn, 1938; Berger et al., 1958; Dhaliwal et al., 1962; Thomas, 1964; Marechal, 1969 & 1970; Smartt, 1970; Sinha & Roy 1979; Sarbhoy, 1980.
P. polystachys	11	22	Allard & Allard, 1940; Dhaliwal et al., 1962.
P. vulgaris	11	22	Karpechenko, 1925; Weinstein, 1926; Katayama, 1928; Kawakami, 1930; Malinowski, 1935; Senn, 1938; Takagi, 1938; Thomas, P., 1945; Berger et al., 1958; Shibata, 1962; Moh, 1962; Thomas, H., 1964; Marechal & Otoul, 1965; Yarnell, 1965; Lamprechet, 1966; Nagl, 1969; Marechal, 1969 & 1970; Smartt, 1970; Mok & Mok, 1976; Biswas & Bhattacharya, 1976; Bhattacharya, 1978; Sarbhoy, 1978; Sinha & Roy, 1979; Sarbhoy, 1980; Blackson & Tai, 1982.
P. vulgaris var. aborigineus = P. aborigineus	11	22	Burkart & Brucher, 1953; Blackson & Tai, 1982.

Table 1. (continued)

Vigna	Gametophyte	Sporophyte	References
V. aconitifolia = P. aconitifolius	11	22	Tschechow & Kartaschow, 1932; Marechal, 1969 & 1970; Biswas & Dana, 1976a; Sinha & Roy, 1979; Sarbhoy, 1980.
V. angularis	11	22	Marechal, 1970; Joseph & Bouwkamp, 1978; Sinha & Roy, 1979.
V. glabrescens = V. radiata glabra	22	44	Dana, 1964, 1965a, b, & 1966d; Krishnan & De, 1965, 1968, & 1970; Swindell et al., 1973; Sarbhoy, 1980.
V. mungo = P. mungo	11	22	Karpechenko, 1925; Simmonds, 1954; Sen & Chheda, 1958; Dana, 1966b; De & Krishnan, 1966a & b; Bir & Sidhu, 1967; Krishnan & De, 1968; Biswas & Dana, 1975a; Sinha & Roy, 1979; Sarbhoy, 1980.
		24	Rau, 1929
V. radiata = P. aureus = P. radiata	11	22	Karpechenko, 1925; Kawakami, 1930; Frahm-Leliveld, 1953 & 1957; Simmonds, 1954; Shibata, 1962; Krishnan & De, 1965; Dana, 1966a, b, & c; De & Krishnan, 1966b; Bir & Sidhu, 1967; Krishnan & De, 1968; Marechal, 1969 & 1970; Kaul, 1970; Biswas & Dana, 1975; Josepha & Bouwkamp, 1978; Sinha & Roy, 1979; Sarbhoy, 1980.
		24	Karpechenko, 1925; Rau, 1929.
V. radiata var. sublobatus = P. sublobatus		44	Frahm-Leliveld, 1965b.
V. umbellata = P. calcaratus = P. ricciardianus	11	22	Janaki-Ammal, 1945; Frahm-Leliveld, 1953 & 1957; Shibata, 1962; Dana, 1966c & d; Singh & Roy, 1970; Biswas & Dana, 1975a; Joseph & Bouwkamp, 1978; Sinha & Roy, 1979; Sarbhoy, 1980.
V. unguiculata = V. catjang = V. cylindrica = V. sesquipedalis = V. sinensis	11	22	Karpechenko, 1925; Tschechow & Kartaschowa, 1932; Senn, 1938; Saunders, 1960; Sen & Bhowal, 1960a & b; Shibata, 1962; Faris, 1964; Frahm-Liliveld, 1965; Kodama, 1967; Marechal, 1969 & 1970.
		24	Rau, 1929; Kawakami, 1930; Berger et al., 1958; Floresca et al., 1960; Miege, 1962.

Figure 1. Root tip cell in Vigna radiata (2n=22). (2000x)

Figure 2. Metaphase I in V. umbellata showing secondary associations of two pairs of bivalents and co-orientation of the other chromosomes. (700X)

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

Figure 4. Anaphase I in the interspecific hybrid V. radiata x V. umbellata with 5/6 disjunction and 11 lagging chromosomes. (650X)

Figure 5. Metaphase I in the amphiploid showing 22 bivalents. (1000X)

Figure 6. Metaphase I in the backcross progeny (amphiploid x V. radiata) showing 11 bivalents and 11 univalents. (950X)

(1964) studied. Faris (1964) surveyed 192 cultivars and strains of Vigna unguiculata from 42 countries and found that all had 2n=2X=22. Previous reports listed this species as having 2n=2X=24 (Rau, 1929; Kawakami, 1930; Berger et al., 1958; Floresca et al., 1960; and Miege, 1962). hromosome counts of 2n=2X=24 have also been reported for Phaseolus coccineus (Kleinmann, 1923) and Vigna mungo (Rau, 1929), but chromosome counts reported prior to 1930 may be incorrect.

Chromosome size differences have been reported among different chromosomes of the same chromosome complement as well as the total chromatin length of the haploid set in different species. However, the small size of all the chromosomes may make these measurements less significant than in other species, with larger chromosomes.

Phaseolus vulgaris has the greatest total chromosome length of the haploid set, 42.26 µm, and P. lunatus the least, 35.2 µm. The greatest length in Vigna species is found in V. radiata, 37.4 µm, and the smallest in V. umbellata, 28.4 µm (Sarbhoy, 1980). Different cytological pretreatments or measurements at different stages of meiosis can lead to variations in chromosome length, thus the difference between the longest and the shortest complement may not be statistically significant.

The range of individual chromosome size ranges from 0.17 µm to 3.1 µm for Phaseolus and from 0.64 µm to 3.7 µm for Vigna (Sen and Bhowal, 1960; Joseph and Bouwkamp, 1977; Sinha and Roy, 1979; and Sarbhoy, 1980).

Mok and Mok (1976) observed two pairs of SAT-chromosomes in Phaseolus vulgaris and P. coccineus, while Bhattacharya (1978) and Sarbhoy (1977) observed only one pair in P. vulgaris. Sinha and Roy (1979) reported that Vigna radiata, and V. radiata var. sublobatus have one pair of chromosomes with a satellite. Sen and Bhowal (1960) have seen two pairs of SAT-chromosomes in Vigna catjang, V. sesquipedalis, and V. sinensis (V. unguiculata).

Sarbhoy (1977) suggested that Phaseolus vulgaris possesses a symmetrical karyotype and the other Phaseolus species have an asymmetrical karyotype. In Vigna species, V. radiata is the only species with a symmetrical karyotype. A symmetrical karyotype is one in which the chromosomes are all approximately the same size, with median or submedian centromeres. A change in the position of a centromere or a change in the relative length of a chromosome arm due to chromosome aberrations can convert a karyotype from a symmetrical to an asymmetrical karyotype (Stebbins, 1971). Chromosome aberrations such as inversions, translocations, duplications, and deletions have been detected in both Phaseolus and Vigna species (Dana, 1966a & d; De and Krishnan, 1966b; Honma, 1968; Biswas & Dana, 1975, 1976a; and Sarbhoy, 1977).

Since the small chromosome size makes karyotypic analysis of mitotic metaphase chromosomes very difficult, other techniques (i.e., pachytene analysis, Giemsa banding, and fluorescent banding techniques) have been used.

Pachytene chromosomes have been studied extensively in corn because the different sizes and shapes of the heteropycnotic knobs have diagnostic value in karyotype analysis. Krishnan and De (1965 and 1970) and De and Krishnan (1966a) used pachytene chromosomes to karyotype Vigna radiata, V. mungo and V. glabrescens. They observed "knobs" of varying lengths near the centromere. The difficulty in distinguishing between individual chromosomes at the pachytene stage, makes the results drawn from this technique inconclusive.

Giemsa and fluorescent banding techniques are rather new in cytogenetic studies. In most plant species it is difficult to obtain a good banding pattern with any degree of consistency. However, banding patterns have been used to produce a karyotype of Phaseolus vulgaris and P. coccineus. All the chromosomes showed bands around the centromere and terminal bands in one or both arms. The number of bands varied from two to five bands per chromosome (Schweizer, 1976; Bhattacharya, 1978; and Mok and Mok, 1976).

Nagl (1974) has written a review paper on the polytene chromosomes found in suspensor cells of Phaseolus. The suspensor consists of a basal cell plus several other cells which connect the embryo proper to the embryo sac. Polytene chromosomes have been found in P. coccineus and P. vulgaris. These polytene chromosomes are similar to those found in dipteran insects. The chromosomes show distinct banding patterns if the plants are subjected to cold temperatures, unfavorable light periods, inhibitions of RNA synthesis by actinomycin D, and exposure to high concentrations of calcium ions, thus making it possible to identify individual chromosomes and possibly the loci of individual

genes. Puffs, the site of RNA synthesis, also have been observed in the polytene chromosomes (Nagl, 1969). These chromosomes are about 30 times longer and 15 times larger in diameter than metaphase chromosomes found in root tips of the same plants.

Meiosis

Early reports indicated that meiosis in the pollen mother cells was normal in most Phaseolus (Weinstein, 1926; Dhaliwal et al., 1962; Sarbhoy, 1977; and Sinha and Roy, 1979b) and Vigna species (Sen and Chheda, 1958; Sen and Bhowal, 1960 a & b; Krishnana and De, 1965; Frahm-Levliveld, 1965a & b; De and Krishnan, 1966b; Dana, 1966a, b, & c; Biswas and Dana, 1976a; Sarbhoy, 1977; and Sinha and Roy, 1979). The 22 chromosomes form 11 bivalents at diakinesis and metaphase I. Segregation of the chromosomes was reported to be normal in subsequent stages, resulting in pollen grains with 11 chromosomes each.

Marechal (1971) observed the meiosis of Phaseolus coccineus, P. formosus, P. obvallatus, P. polyanthus, and P. vulgaris and noted the nuber of chiasmata that formed between the homologous chromosomes. He then made a series of crosses between these species and observed the number of chiasmata that were formed. From this study he concluded that P. formosus, P. obvallatus, and P. polyanthus are similar to P. coccineus but were distinct enough to reclassify them as subspecies of P. coccineus i.e., P. coccineus subsp. formosus, etc.

Irregular meiotic behavior has been occasionally reported. The presence of univalents has been observed in P. acutifolius, P. coccineus, P. lunatus, P. vulgaris, Vigna aconitifolia, V. radiata, and V. umbellata (Sarbhoy, 1977; Sinha and Roy, 1979; and Machado et al.,

1982). The univalents may be attributed to 1) failure of the chromosomes to pair at zygotene, 2) early separation of bivalents at metaphase I, or 3) inversion heterozygosity (Sarbhoy, 1977).

Lack of homology has been the most important factor to cause univalent formation during meiosis I in most plant species. However, lack of pairing also can be caused by genetic factors as reported in corn (Beadle, 1930), rice (Ramanujan and Parthsarthy, 1935), wheat (Feldman, 1966), pea (Gattschalk and Baquar, 1971), and other plants.

Early separation of bivalents at metaphase I has been observed in Phaseolus coccineus, P. lunatus, P. vulgaris, Vigna radiata, and V. umbellata (Sarbhoy, 1977; and Machado et al., 1982). Short chromosomes, such as those found in Phaseolus and Vigna, have a lower frequency of chiasma formation (Darlington, 1930 and 1937; and Kostoff, 1940), and thus have a tendency to separate early (Machado et al., 1982). These separated bivalents appear as univalents which orient themselves opposite each other in the equatorial region. These paired univalents behave normally and do not affect normal segregation (Sarbhoy, 1977).

Inversion heterozygosity is known to cause univalent formation in various species of Citrus (Raghuranshi, 1962a & b). Chromatin bridges with their accompanying fragments suggests the presence of inversion heterozygotes. Chromatin bridges have been observed in Phaseolus acutifolius, P. coccineus, P. vulgaris, Vigna mungo, and V. radiata (Sarbhoy, 1978 and Sinha and Roy, 1979).

Dana (1964) reported that the spontaneously occurring allotetraploid 2n=2X=44 shows regular meiosis with 22 bivalents at metaphase I. Dana (1964 and 1966d), Krishnan and De (1968 and 1970),

and Biswas and Dana (1976b) have used Vigna glabrescens in many of their crossing programs. When V. glabrescens was used as the female parent with Vigna umbellata as the male parent, Dana (1964) reported that the offspring was triploid with the majority of the metaphase plates possessing 11 bivalents and 11 univalents. Other meiotic abnormalities included failure of the chromosomes to line up on the metaphase plate, early movement of the bivalents at metaphase I, laggards at anaphase I, and unequal distribution of chromosomes during the second division. When Dana (1966d) studied the pachytene stage of this hybrid, he discovered inversion loops, duplications, deletions, and heteromorphic regions. Dana (1965a) crossed V. glabrescens with Vigna umbellata. Biswas and Dana (1976b) treated this hybrid with colchicine to produce a plant with 2n=66. These plants showed meiotic irregularities such as multivalents, laggards, and unequal segregation of chromosomes. In 72% of the cells observed, 33/33 segregation occurred and autosyndetic pairing was observed. Krishnan and De (1968) used Vigna mungo as the female parent and V. glabrescens as the male parent. The resulting hybrid had 33 chromosomes with the majority of the cells containing 12 bivalents and 9 univalents. Quadrivalents, trivalents, early separation of the bivalents, two to six spindles during anaphase II, and one to five micronuclei in the tetrads also were observed. Krishnan and De (1970) completed a pachytene analysis of the allotetraploid and produced a karyotype of 19 of the 22 pairs of chromosomes. They suggested that the chromosomes of V. glabrescens are similar to some of the chromosomes of Vigna radiata and V. mungo. These authors have called this spontaneous tetraploid an allotetraploid

with the different genomes being derived from V. radiata and V. mungo; however, the genomic relationships between these two species have not been well established.

A number of hybrids have been produced (Table 2), but not all F_1s produced viable seeds, due to the presence of isolating mechanisms. These isolating mechanisms include: 1) hybrid inviability or weakness, 2) developmental hybrid sterility, 3) segregational hybrid sterility, and 4) F_2 breakdown (Stebbins, 1971b). Through the use of embryo culture, some of these barriers have been overcome.

Only a few of the hybrids have been studied cytogenetically (Table 3). Some of the meiotic abnormalities that have been observed include: 1) failure of the chromosomes to align themselves on the metaphase plates, 2) multivalent formation, 3) early bivalent separation, 4) chromatin bridges with fragments, 5) laggards, 6) univalents, 7) multiple spindles, and 8) micronuclei formation. See Table 3 for specific information on each hybrid studied.

Some of these hybrids have been treated with colchicine to produce amphiploids. If the hybrids were infertile, the amphiploids proved to be more fertile as shown by the percentage of viable pollen (Table 3). Complete fertility was not restored due to abnormal meiosis. Multivalents, unequal separation of chromosomes, and micronuclei were observed in the amphiploids.

In one of the crosses, Phaseolus vulgaris x P. ritensis, the amphiploid was produced and then backcrossed to P. ritensis, yielding a triploid. This triploid was then backcrossed producing a trisomic of 2n=23. The trisomics were selfed and produced a small number of

Table 2. Intergeneric, interspecific, and intraspecific crosses that have been performed.

Phaseolus

Interspecific

P. acutifolius x P. coccineus - Al-Yasiri & Coyne, 1966.

P. acutifolius x P. vulgaris - Mok et al., 1978.

P. coccineus x P. acutifolius - Al-Yasiri & Coyne, 1966.

P. coccineus x P. lunatus - Honma & Heeckt, 1958; Wolfenbarger & Sleesman, 1961.

P. coccineus x P. vulgaris - Honma & Heeckt, 1952; Al-Yasiri & Coyne, 1966; Smartt & Hag, 1972; Imbrahim & Coyne, 1975.

*P. lunatus x P. polystachyus - Lorz, 1952; Dhaliwal et al., 1962; LeMarchand et al., 1976.

P. lunatus x P. ritensis - LeMarchand et al., 1976.

P. ritensis x P. lunatus - LeMarchand et al., 1976.

P. vulgaris x P. acutifolius - Honma, 1956; Al-Yasiri & Coyne, 1966; Mok et al., 1978.

*P. vulgaris x P. coccineus - Lamprecht, 1941; Tschermak-Seysenegg, 1942; Gates, 1951; Wall & York, 1957; Bemis & Kedar, 1961; Al-Yasiri & Coyne, 1966; Marechal, 1971.

P. vulgaris x P. dumosus - Jaarerslag, 1957.

*P. vulgaris x P. filiformis - Marechal & Baudoin, 1978.

P. vulgaris x P. lunatus - Honma & Heeckt, 1959; Al-Yasiri & Coyne, 1966; Mok et al., 1978.

P. vulgaris x P. ritensis - Braak & Kooistra, 1975.

Intraspecific

*P. vulgaris x P. vulgaris - Honma, 1958.

*Crosses that have been studied cytogenetically.

Table 2. (continued)

Vigna

Interspecific

V. angularis x V. mungo - Al Yasiri & Coyne, 1966.

V. angularis x V. umbellata - Al Yasiri & Coyne, 1966; Chen, 1980; Parrot, 1981.

V. glabrescens x V. mungo - Parrot, 1981.

V. glabrescens x V. radiata - Parrot, 1981.

V. glabrescens x V. umbellata - Dana, 1964 & 1965a; Biswas & Dana, 1976b; Parrot, 1981.

V. mungo x V. angularis - Chen, 1980.

*V. mungo x V. glabrescens - Krishnan & De, 1968.

V. mungo x V. radiata - Luyeye, 1975.

*V. mungo x V. umbellata - Al-Yasiri & Coyne, 1966; Biswas & Dana, 1975a.

V. radiata x V. angularis - Ahn & Hartman, 1978a; Chen, 1980.

*V. radiata x V. glabrescens - Dana, 1965b; Parrot, 1981.

*V. radiata x V. mungo - Sen & Ghosh, 1960; Bhag-Singh et al., 1964; Chavan et al., 1965; Dana, 1966b; De & Krishnan, 1966b; Luyeye, 1975; Ahn & Hartman, 1977; Chen, 1980.

V. radiata x V. umbellata - Dana, 1966c; Sawa, 1974; Evans, 1975; Ahn & Hartman, 1977; Chen, 1980; Parrot, 1981; Machado et al., 1982.

*V. radiata x V. angularis - Evans, 1975; Ahn & Hartman, 1978b; Chen, 1980.

V. umbellata x V. mungo - Evans, 1975.

*V. umbellata x V. radiata - Evans, 1975.

Intraspecific

V. unguiculata x V. unguiculata - Premseker & Raman, 1972.

Intergeneric

Phaseolus vulgaris x Vigna mungo - Strand, 1943; Al-Yasiri & Coyne, 1966.

Phaseolus vulgaris x Vigna umbellata - Al-Yasiri & Coyne, 1966.

Vigna aconitifolius x Phaseolus trilobus - Chavan et al., 1965; Biswas & Dana, 1976a.

Vigna angularis x Phaseolus acutifolius - Al-Yasiri & Coyne, 1966.

Vigna angularis x Phaseolus vulgaris - Al-Yasiri & Coyne, 1966.

Vigna mungo x Phaseolus vulgaris - Wolfenbarger & Cleesman, 1961; Al-Yasiri & Coyne, 1966.

*Vigna radiata x Macroptilium lathyroides - Biswas & Dana, 1975b.

Vigna radiata x Phaseolus trilobata - Dana, 1966a.

*Cross that have been studied cytogenetically.

Table 3. Observations of meiosis and pollen fertility in the hybrids.

Crosses Studied:	1	2	3	4	5	6	7	8	9	10	11
P. aconitifolia x *P. trilobus*			X				X		6%	90%	$1_{IV}+9_{II}$
P. lunatus x *P. polystachyus*			X	X	X	X	X	X	44-74%		$1_{IV}+8_{II}+2_{I}$
P. lunatus x *P. ritensis*			X			X			64-86%		$1_{IV}+8_{II}+2_{I}$
P. vulgaris x *P. coccineus*		X							15-20%		22_{II}
P. vulgaris x *P. filiformis*			X			X			1.2%		$1_{IV}+5_{II}+9_{I}$
P. vulgaris x *P. ritensis*			X			X					$1_{IV}+3_{II}+12_{I}$
P. vulgaris x *P. vulgaris*		X									22_{II}
V. glabrescens x *V. umbellata*	X	X			X		X		8%		$11_{II}+11_{I}$
V. mungo x *V. glabrescens*	X	X					X	X	4%		$1_{IV}+1_{III}+8_{II}+10_{I}$
V. mungo x *V. umbellata*	X	X	X	X	X	X	X		0.7%		$1_{IV}+6_{II}+6_{I}$
V. radiata x *V. angularis*						X	X	X			$2_{II}+18_{I}$
V. radiata x *V. mungo*	X	X	X	X	X	X	X	X	21%	63-77%	$3_{IV}+4_{II}+2_{I}$
V. radiata x *V. umbellata*		X	X	X	X	X	X	X	0.5-2%	69%	$1_{IV}+6_{II}+6_{I}$
V. umbellata x *V. angularis*		X							49-93%		22_{II}
V. radiata x *P. trilobus*		X	X	X	X	X		X	31%	75-90%	$1_{IV}+1_{III}+6_{II}+3_{I}$
V. radiata x *M. lathyroides*		X	X	X		X	X	X	24%		$1_{IV}+5_{II}+8_{I}$

Observations:

1. Failure of the chromosomes to line up at metaphase.
2. Early bivalent separtation.
3. Multivalents.
4. Chromatin bridge.
5. Laggards.
6. Univalents.
7. Multiple spindles.
8. Micronuclei.
9. Pollen fertility of the F_1.
10. Pollen fertility of the amphiploid (if produced).
11. Maximum meiotic configurations.

semi-sterile trisomics, plus fertile normal diploid plants (Braak and Kooistra, 1975). A similar situation occurred when the amphiploid of Vigna radiata x V. umbellata was backcrossed to V. radiata. The resulting plants, when selfing occurred, produced a small number of plants that were trisomic (Bauchan and Tai, 1982).

Mok and Mok (1977) treated shoot tips with 0.1% colchicine for two days, six hours each day. Along with the expected autotetraploids, they discovered two monosomics, 2n=21. The use of aneuploids, such as 2n+1=23 or 2n-1=21, can prove to be useful in genetic studies and/or in a breeding program.

Shoot tips of different varieties of Phaseolus vulgaris (Biswas and Bhattacharya, 1976), Vigna mungo (Sen and Chheda, 1958), and V. unguiculata (Sen and Bhowal, 1960b) were treated with 0.25% colchicine for two days, six hours each day, to produce autotetraploids. Numerous examples of meiotic abnormalities were observed: multivalents, early separation of bivalents, laggards, nondisjunction, three or more spindles at anaphase II, unequal segregation of the chromosomes, formation of micronuclei, and polyspory.

Kaul (1970) treated Vigna radiata with 0.01, 0.025, and 0.05%, 2,2 dichloropropionic acid (DPA). The treated plants showed 50% pollen sterility at 0.01% DPA and 100% pollen sterility at 0.025% and 0.05% DPA. Thirty-eight percent of the cells showed a normal meiosis with 11 bivalents. Multivalents, early separation of bivalents, bivalent associations (both side-to-side and end-to-end), laggards, unequal segregation of chromosomes into 10/12 groupings, chromatin bridges, and polyspory were observed.

Goswami (1980) exposed Vigna mungo seeds to a 35Kr dosage of x-rays. He studied the meiosis of these plants and observed the lack of chromosome pairing at diakinesis and metaphase I. Chromatin bridges, lack of cytokinesis at the end of telophase I, and the formation of micronuclei and microcells at the quartet stage were some of the irregularities that were observed. Ojomo and Chheda (1971) exposed Vigna unguiculata seeds to x-rays; they observed chromatin bridges, sticky chromosomes, fragments and/or laggards in these plants.

Conclusions

1.) Except for one spontaneous allotetraploid, all studied species of Phaseolus and Vigna are diploids with 2n=2X=22, with the basic chromosome number being X=11.

2.) There is no evidence to support the idea that changes in chromosome number contribute to genome differentiation.

3.) Most of these species have small chromosomes with homogeneous karyotypes. Therefore, karyotyping may not be as useful for cytotaxonomic studies as in other species.

4.) Meiosis is normal with the formation of 11 bivalents at metaphase I for the diploid species, however, early bivalent separation, which appear as univalents, do occur. The allotetraploid species also have normal meiosis with 22 bivalents forming at metaphase I.

5.) Chromatin bridges and quadrivalent associations in the hybrids, although found at a low frequency, have led authors to believe that structural modifications, particularly inversions and translocations, comprise the main evolutionary force for genome differentiation.

APPENDIX

Appendix

Cytological studies in the genera Phaseolus and Vigna have been conducted at Michigan State University. These studies have yielded different results and interpretations than those reviewed above. The presence of one to five bivalents in association with each other per metaphase plate have been observed in Vigna angularis, V. mungo, V. radiata, V. umbellata, Phaseolus coccineus, and P. vulgaris var. aborigineus (Blackson and Tai, 1982; Hussein, personal communication; and Machado et al., 1982) (Figure 2). In cells with less than five bivalents in association, most of the remaining chromosomes were co-oriented in a manner suggestive of an interrelationship.

According to Rieger et al. (1976), this attraction of bivalents, known as secondary association, occurs if they are related by genetic and evolutionary factors. There is general agreement that the occurrence of secondary associations indicates homology between the chromosomes involved (Lawrence, 1931; Meurman, 1933; and Therman, 1951).

Along with the secondary associations, split spindles showing five/six segregation of bivalents at metaphase I and multipolar meiosis have been observed for all of the species described (Figure 3). Multipolar meiosis is a spontaneous or induced spindle apparatus abnormality resulting in genome separation during meiosis (Li & Tu, 1974; Thompson, 1962; Tai, 1970). Tai (1970) proposed that multipolar meiosis occurs via genome specific spindle organizer, cell organelles which govern chromosome migration and cytokinesis, which provide an

evolutionary mechanism for haploidization in higher plant polyploids. The spontaneous appearance of multipolar meiosis in the Phaseolus and Vigna species mentioned suggests that these species may be comprised of two genomes.

In the Phaseolus and Vigna species studied; the existence of secondary associations, multipolar meiosis, the grouping of bivalents into groups of five and six, and the high basic chromosome number (X=11) suggests that these species may be allotetraploids functioning at the diploid level. These species would consist of two genomes, one containing six and the other five chromosomes. Six would most likely be the basic chromosome number and five the derivative number arising due to a loss of a pair of chromosomes from the other genome.

Additional evidence to support this theory has been collected by studying the cytology of the hybrid between Vigna radiata and V. umbellata, (Bauchan and Tai, 1982; and Machado et al. 1982).

Machado et al. (1982) observed up to nine bivalents in the hybrid, suggesting allosyndetic pairing, while the presence of multivalents, suggested autosyndetic pairing. This ambivalence suggests that there must be more than one genome in each parent and the presence of some homology between the genomes of V. radiata and V. umbellata (Figure 4).

Observations of meiosis in the amphiploid by Bauchan and Tai (1982) showed normal bivalent pairing (Figure 5). The absence of multivalents in both the parental species and the amphiploid may be the result of genetic control of pairing with dosage effects; i.e., a single dose allows homoeologous pairing, whereas a double dose promotes

homologous pairing (Riley and Chapman, 1958; Feldman, 1966; Driscoll, 1972; and Starks and Tai, 1974). These observations further support the theory that the parental species are allotetraploids.

Split metaphase plates resulting in the 12/10 segregation of bivalents were also observed in the amphiploid. This adds evidence to the hypothesis that these species consist of two genomes, one containing six chromosomes and the other five.

The meiosis of the backcross progeny (amphiploid x V. radiata) was very irregular (Bauchan and Tai, 1982). A majority of the cells observed contained 11 bivalents and 11 univalents (Figure 5).

Although the evidence thus far seems to suggest that the species contained in the genera Phaseolus and Vigna are allotetraploids with the basic number of X=6, only seven species have been studied critically. Much more research needs to be done on the cytology of these species and their hybrids in order to learn their relationships.

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SECTION II

Cytology and Fertility of *Vigna radiata* and *V. umbellata*

ABSTRACT

CYTOLOGY AND FERTILITY OF VIGNA RADIATA AND V. UMBELLATA

By

Gary R. Bauchan

The cytology of Vigna radiata (L.) Wilczek, V. umbellata (Thunb.) Ohwi & Ohashi, their hybrid, amphiploid, backcross progeny, and the plants of subsequent generations was investigated to determine the genome relationship among these plants for use in a breeding program with the ultimate goal of incorporating beneficial characteristics of V. umbellata into V. radiata. Light microscope investigations included: root tip chromosome counts; the analysis of chromosome pairing during all stages of microsporogenesis; and pollen stainability to estimate plant fertility.

In this study the presence of secondary associations, multipolar meiosis, the grouping of bivalents during meiosis, and the high basic chromosome number has led to a re-evaluation of ploidy levels in these plants. Genome formulae have been assigned in accordance with these findings under the assumption of genetic control of pairing with dosage effects.

Introduction

The food legumes are an important component for increasing the protein portion of primarily vegetarian diets. As a group, the food legumes include species adapted to a broad range of conditions. However, since most of the cultivated food legumes are self pollinated, the selection pressures imposed upon them have created individual species with relatively narrow gene bases. The Leguminosae as a whole, is endowed with a richness of genetic diversity. Increased genetic recomination within this extensive gene pool would significantly increase the genetic base of the agricultural legumes. One method of accomplishing this exchange is through the use of wide hybridization, to transfer beneficial genes from the wild species to the cultivated species (Adams, personal communication).

Vigna radiata (L.) Wilczek (mungbean) is an important food legume crop in Asia and Africa. In the 1972-73 Asian Vegetable Research and Development Center (AVRDC) Annual Report (1974) it is stated that mungbeans are an excellent source of protein, but its yield potential is low and it is susceptible to many insect and disease pests. V. umbellata (Thunb.) Ohwi & Ohashi (rice bean) is a species closely related to V. radiata and is reported to be highly resistant to bean fly (Melanagromyze phaseoli), Cercospora leaf spot, powdery mildew and root diseases (AVRDC, 1975). A goal of this project is to incorporate these characteristics of V. umbellata into V. radiata.

Stebbins (1950) and Grant (1975) discussed in detail the pre-zygotic and post-zygotic isolating mechanisms that prevent the achievement of a cross between two species which would allow the

transfer of desirable traits. There are methods for overcoming these barriers, one of which is to produce the F_1 hybrid, double the chromosome number to produce an amphiploid, and backcross the amphiploid to the cultivated parent with the eventual goal of producing trisomics (2n+1). These trisomics can then be used for genetic studies to assign genes to particular chromosomes. They also can be used for genetic recombination through crossing-over of homologous, homoeologous, or secondarily associated chromosomes; or by the breakage of the chromosomes using mutagens to facilitate translocation of a piece or pieces of chromosomes from one species into another (Kimber and Sears, 1980).

Limited success has been reported in achieving the interspecific cross Vigna radiata x V. umbellata (Dana, 1966; Evans, 1975; AVRDC, 1975; Ahn and Hartman, 1977; and Chen et al., 1977). In all cases the F_1 hybrid was highly sterile. Meiotic studies of this cross (Dana, 1966; Ahn and Hartman, 1977; and Machado et al., 1982) showed many cytological irregularities with incomplete bivalent pairing.

The objectives of the present study were: 1) to determine the cytological relationships between V. radiata and V. umbellata, their hybrids, amphiploids, backcross progeny and the plants of the subsequent generations; 2) to determine the relationship between cytology and fertility in these plants.

Materials and Methods

All original plant material was obtained from the AVRDC. The cultivars used in the breeding program were V. radiata PI377276 and AVRDC accession number 2013; and V. umbellata AVRDC accession number 4065. Hybridizations and recovery of the hybrids via embryo culture were accomplished as reported by Chen (1980) and Parrot (1981). Among the sterile hybrids grown at the AVRDC, one plant was found to have some fertility. This plant was sent to our laboratory at Michigan State Unviersity for cytological analysis. Preliminary studies of this plant indicated that chromosomal doubling of the original F_1 hybrid had occurred. The F_1 was successfully backcrossed to V. radiata by the use of embryo culture. These backcrossed plants grew slowly but flowered profusely and produced a few self-pollinated seeds which upon germination produced mature plants morphologically similar to V. radiata. The petigree is diagramed in Figure 1.

All plants were greenhouse grown. Four plants of the amphiploid, three BC1 plants and four S1 plants were studied cytologically.

Germinated seedlings and roots from stem cuttings were used to provide root tip material for chromosome counts. Roots were harvested and placed into Carnoy's fixative of ethanol, chloroform and glacial acetic acid (6:3:1, v/v/v) for 24 hours. The roots were hydrolyzed for 15 minutes in 1N HCl at 60°C, placed in Feulgen's stain for 30 minutes, and squashed in propiono-carmine.

Meiotic observations were made on pollen mother cells (PMC's). The flower buds were fixed in Carnoy's fixative for 24 hours, transferred to 70% ethanol and stored in a refrigerator. PMC's were

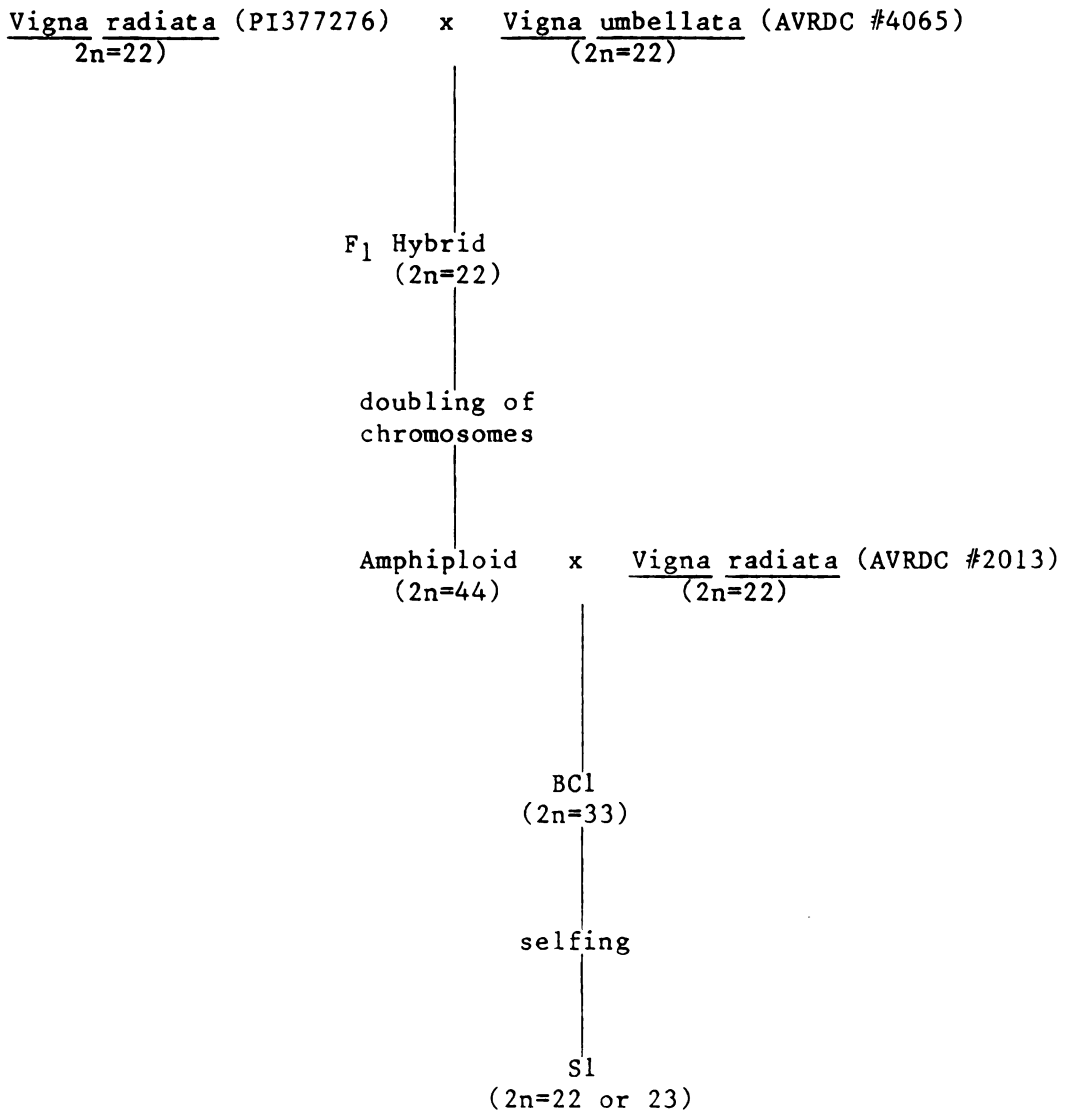


Figure 1. Diagram of a cross between Vigna radiata and V. umbellata.

squashed in propiono-carmine. All cytological interpretations were based upon observations from temporary slides.

These plants were extremely difficult to work with owing to their small chromosomes (1-4 μm in length) (Sen and Bhowal, 1960; Joseph and Bauwkamp, 1978; Sarbhoy, 1977; and Sinha and Roy, 1979), homogeneous karyotype, and the fact that only one bud per inflorescence is undergoing meiosis at any one time (Bauchan and Tai, 1982).

To estimate fertility, pollen grains from mature anthers were stained with cotton blue. Only those grains of normal shape staining blue were counted as stainable and hence assumed viable.

All observations were made using a Zeiss Photoscope II phase-contrast microscope with a Planapo 63X, N.A.=1.4 objective. Photomicrographs were taken with an attached Linhof 4X5 box camera using Kodak Contrast Process Ortho film.

Results

Amphiploid of V. radiata x V. umbellata

Root tip chromosomes of this plant were 2n=44 (Figure 2). Meiosis was mostly regular with 184 of the 248 metaphase I cells observed containing 22 bivalents (Figure 3). Early separation of one or two bivalents occurred in 19% and 7% of the cells, respectively. Occasionally (4%), a split metaphase plate was discovered with one half containing 12 bivalents grouped together and the other half containing 10 bivalents grouped together (Table 1). Normal distribution of the chromosomes occurred at all of the remaining stages of meiosis. However, only 44% of the 3500 pollen grains counted proved to be

Figure 2. Root tip cell in the Amphiploid (2n=44). (1700X)

Figure 3. Metaphase I in the Amphiploid showing 22 bivalents. (675X)

Figure 4. Root tip cell in BC1 (2n-33). (1350X)

Figure 5. Metaphase I in BC1 showing 11 bivalents and 11 univalents. Arrows indicate an association of an univalent to a bivalent. (950X)

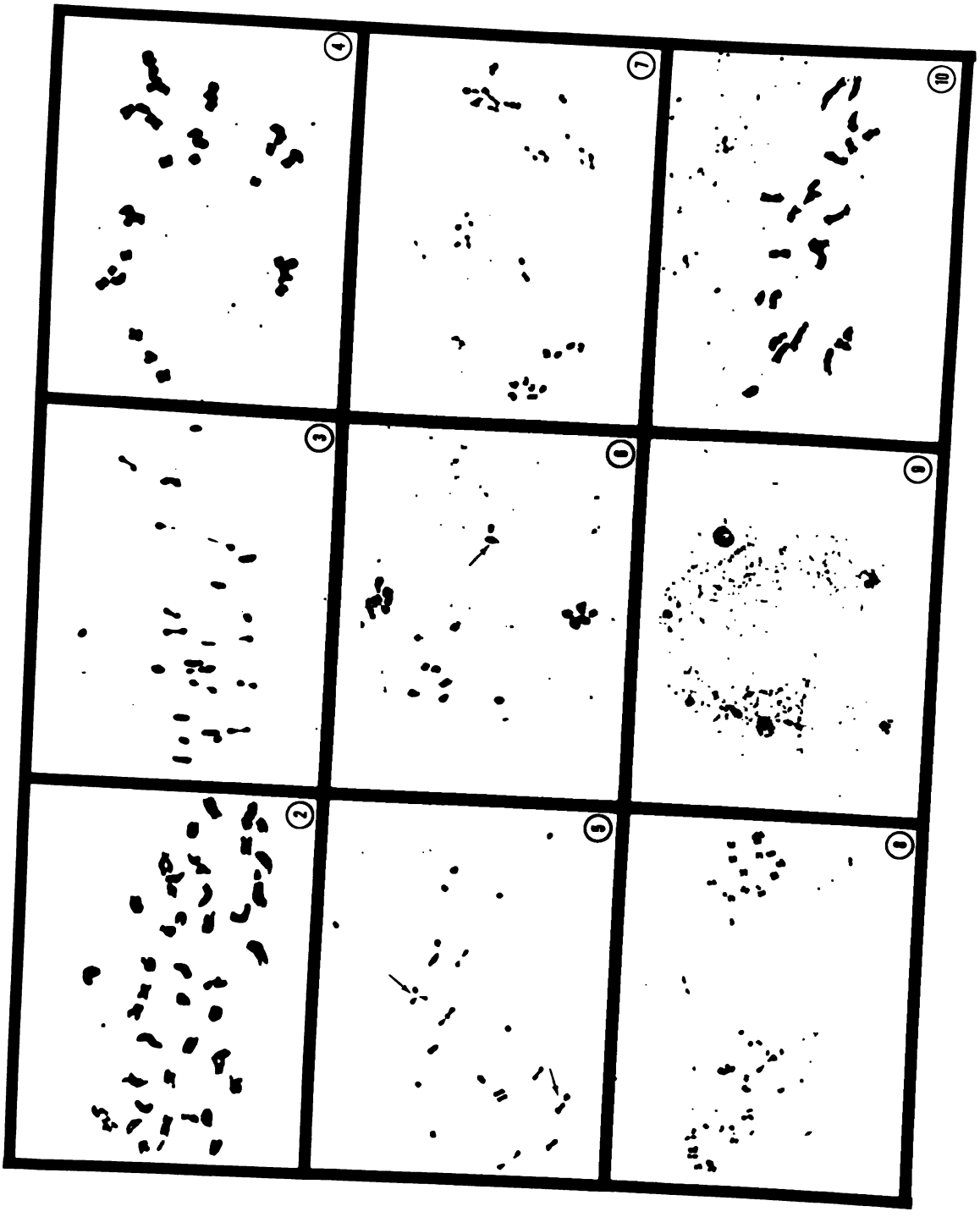
Figure 6. Metaphase I in BC1 showing a split plate with 5/6 segregation of bivalents scattered throughout the cell. Arrow indicates an overlap. (1300X)

Figure 7. Anaphase I in BC1 showing 11/11 segregation of chromosomes and 11 lagging chromosomes. (850X)

Figure 8. Metaphase II in BC1 showing 11 chromosomes per cell with several fragments. (850X)

Figure 9. Quartet stage in BC1 showing four "normal" cells plus two microcells. (475X)

Figure 10. Root tip cell in S1 (2n=23). (1350X)



stainable (Table 3).

Table 1. Metaphase I chromosome associations in the amphiploid (2n=44).

Chromosome Associations			Cells	
I	II		No. of Cells	Percent
0	22		184	74
2	21		48	19
4	20		16	7
		Total	248	100

Backcross Progeny (BC1), Amphiploid x V. radiata

Mitotic chromosome counts of the backcross progeny showed that BC1 has 33 chromosomes (Figure 4). A summary of the meiotic behavior is shown in Table 2. Meiosis was highly irregular. 221 PMC's were observed at metaphase I and most (99%) of the cells contained 11 bivalents and 11 univalents (Figure 5). Periodically one to three bivalents were found to have a univalent loosely associated (Figure 5). This association varied from close proximity to wide separation with only a thin strand joining the chromatin. Side-to-side, side-to-end, and end-to-end associations were also noted. A few of the cells (12%) showed a split spindle with one part containing six bivalents and the other containing five bivalents with the 11 univalents scattered throughout the cell (Figure 6). Early separation of a bivalent was observed in 2% of the cells.

Table 2. Meiotic chromosome behavior of BC1 (Amphiploid x V. radiata backcross).

Metaphase I				Anaphase I			Metaphase II			Anaphase II			Quartets		
Chromosome Associations		No. of		Laggards per	No. of		Fragments per	No. of		Laggards per	No. of			No. of	
I	II	Cells	Percent	Cell	Cells	Percent	Cell	Cells	Percent	Cell	Cells	Percent		Cells	Percent
11	11	219	99	11	61	100	11	2	22	11	5	71	Normal	26	31
13	10	2	1	Total	61	100	14	1	11	6	1	14	Micronucleated*	10	12
	Total	221	100				15	3	33	5	1	14	Microcells**	48	57
							16	1	11	Total	7	100	Total	84	100
							18	1	11						
							20	1	11						
							Total	9	100						

*The number of micronuclei per quartet varies from 1-6 with the most common being 1.

**Contains more than four cells per quartet. The number varies from 5-11, the most common being 5 or 6.

Anaphase I displayed the normal bipolar distribution of chromosomes with 11 univalents remaining at the metaphase plate in all of the observed cells (Figure 7). These univalents invariably underwent precocious centromere division, but generally migrated to the poles and were included in the telophase I nuclei.

The second division of meiosis was difficult to obtain in this material probably due to the very short length of these stages. Of the cells observed at metaphase II, 11 chromosomes plus several fragments could be found in each cell (Figure 8). The distribution of chromosomes during anaphase II varied widely and did not show any consistent pattern.

The quartet stage reflected the irregular behavior noted in the previous stages. Of the 84 quartets observed, 69% contained micronuclei or consisted of more than four cells; 31% of the quartets appeared normal at this stage. Many (57%) of the quartets consisted of four "normal" sized cells and one to several smaller cells (Figure 9). Of the 1800 mature pollen grains counted only 15% were stainable (Table 3).

Table 3. Pollen stainability of the Amphiploid and BC1.

Plant	No. Stainable Pollen	Counted	Percent Stainable Pollen
Amphiploid	1554	3500	44
BC1	274	1800	15

Progeny from Selfing of BC1

Only mitotic studies were made on these plants. From the four plants studied, 79 root tip cells were counted with 70% containing 23 chromosomes (Figure 10), and 30% containing 22 (Table 4).

Table 4. Chromosome number counts from self progeny of BC1.

Chromosome Number	No. of Cells	Percent
22	24	30
23	55	70
Total	79	100

Discussion

Cytological studies of the parents, V. radiata and V. umbellata, were reported by Machado et al. (1982), who stated that microsporogenesis was mostly normal. At metaphase I, 11 bivalents could be found, with one to five of the bivalents found in association with each other. According to Rieger et al. (1976), this attraction of bivalents, known as secondary association, occurs if they are related by genetic and evolutionary factors. There is general agreement that the occurrence of secondary association indicates homology between the chromosomes involved (Lawrence, 1931; Meurman, 1933; and Therman, 1951). Hussein (personal communication) found secondary associations in other closely related species such as V. angularis and V. mungo.

Similar findings were also reported in Phaseolus coccineus, P. vulgaris, and P. vulgaris var. aborigineus (Blackson and Tai, 1982).

Along with the secondary associations, split spindles showing five/six segregation of bivalents at metaphase I and multipolar meiosis have been described for both parental species (Machado et al., 1982). Multipolar meiosis is a spontaneous or induced spindle apparatus abnormality resulting in genome separation during meiosis (Li and Tu, 1947; Thompson, 1962; and Tai, 1970). Tai (1970) proposed that multipolar meiosis occurs via genome specific spindle organizers, cell organelles which govern chromosome migration and cytokinesis, which provide an evolutionary mechansim for haploidization in higher plant polyploids. The spontaneous appearance of multipolar meiosis in Vigna species may be the first indication that these plants may contain more than a single genome.

The existence of a high basic chromosome number, especially uneven ones such as 11 found in Vigna, suggests that the diploid genome is possibly comprised of two genomes differing by structural modifications (Stebbins, 1950 and Grant, 1963). The basic chromosome number may be either five or six with one of the two being an aneuploid number (either a gain or a loss of a chromosome). The existence of secondary associations, multipolar meiosis, the grouping of bivalents into groups of five and six, plus the high basic chromosome number suggests that the parental species used in this breeding program are perhaps segmented allotetraploids consisting of two genomes of five and six, respectively. Thus the following genome formula $V_rV_rV'_rV'_r$ for V. radiata and $V_uV_uV'_uV'_u$ for V. umbellata (V=6 chromosomes and

V'=5 chromosomes) are suggested (Figure 11).

The subscripts r and u are used to designate the origin of the genomes from V. radiata and V. umbellata. Since only 11 bivalents were observed in the parental species V and V' are not homologous. The presence of secondary associations, suggests some interrelationships between these genomes.

The question exists, which is the basic number and which is the derivative number, five or six. The basic number could be six with five being the derivative number, five arising due to the loss of a pair of chromosomes from the other genome. This loss would not have severe genetic consequences.

Machado et al. (1982) reported the meiotic irregularity of the F_1 hybrid between V. radiata and V. umbellata. A maxium of nine loosely paired bivalents was noted as well as a few trivalents and quadrivalents at metaphase I. Multipolar meiosis was also observed with the bivalents segregating from the univalents. During the latter stages of meiosis, fragmentation of the univalents occured forming microcells at the quartet stage. In the parents, the homologous chromosomes and spindle organizers allow a normal meiosis; in this interspecific hybrid, non-homology of the chromosomes and spindle organizers lead to fractionation of the chromosome complement.

Due to the presence of up to nine bivalents in the hybrid, allosyndetic pairing, pairing of the chromosomes from different gametes, is suggested. It is believed, therefore, that there must be some relationship between the genomes of V. radiata and V. umbellata. However, the rare occurrence of multivalents also suggests some

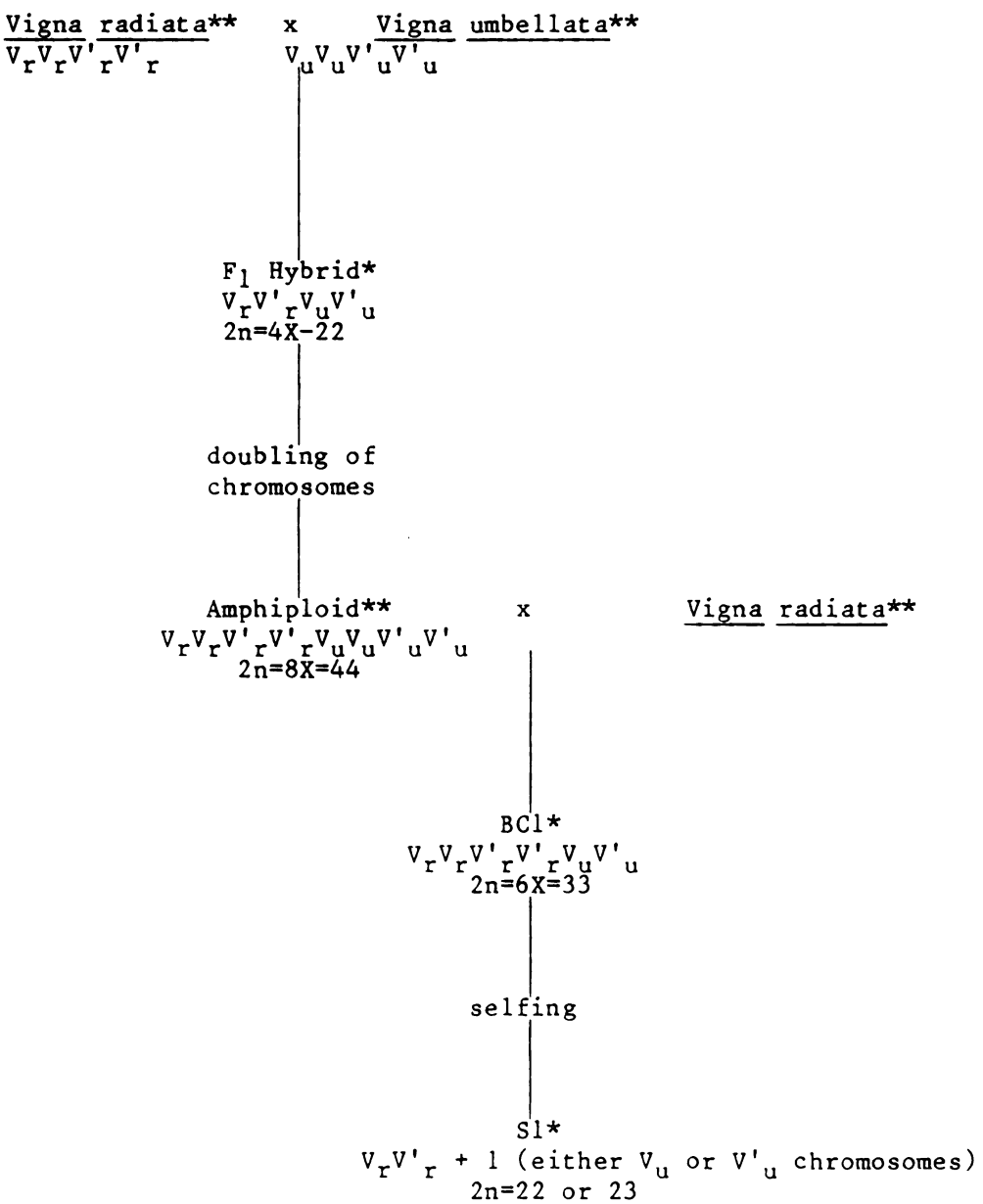


Figure 11. Summary of the proposed genome formulae.

autosyndetic pairing in addition to allosyndetic pairing. It is possible that there is some homology among all four genomes in the two parental species. Thus the genome formula $V_rV'_rV_uV'_u$ can be assigned to these hybrids.

Earlier workers have suggested that V. radiata and V. umbellata are diploids with the genome formula of AA and BB or AA and A'A', respectively. The F_1 hybrid then would be AB or AA'. If the genome formula for the hybrid was AB then only univalents could occur at metaphase I in the hybrid. The maximum chromosome association that could occur would be 11 bivalents as a result of allosyndetic pairing between the A and A' genomes. However, the occurrence of multivalents indicated autosyndetic pairing, thus substantiating the hypothesis that there are more than one homo- or homoeologous genome occuring in each parent.

Pollen viability of the F_1 hybrid was very low (1.5%) and the plants were sterile, probably due to the irregular meiosis. However, Stebbins (1958) pointed out that although meiotic irregularities exist, genetic causes may also account for sterility. Since the purpose of making the cross was to recombine characteristics of the parents, fertility (or lack thereof) is of prime concern.

The doubling of the chromosomes of the F_1 hybrid restored partial fertility, as shown in the normal bivalent pairing (22 II) at metaphase I. The absence of multivalents in both the parental species and the amphiploid further substantiates the theory that the parental species are allotetraploids.

Two factors may explain the absence of multivalents in these plants; low chiasma frequency of the chromosomes in Vigna because of their small size (Kostoff, 1940), and the genetic control of pairing. Chromosome pairing is governed by a gene or genes with the type of pairing resulting from a dosage effect; i.e. a single dose allows homoeologous pairing whereas a double dose promotes homologous pairing (Riley and Chapman, 1958; Feldman, 1966; Driscoll, 1972; and Starks and Tai, 1974).

Loosely paired bivalents were observed in both parental species suggesting the presence of a double dose of the gene(s) controlling pairing. In the F_1 hybrid, a single dose from each parent is present, thus allowing auto- and allosyndetic homoeologous pairing to occur as determined by the presence of bivalents and multivalents, respectively. The dosage of the gene(s) controlling pairing was doubled in the amphiploid, therefore no multivalents occurred and secondary associations were absent.

Split spindles occurred in 4% of the cells observed in the amphiploid, resulting in the 12/10 segregation of bivalents at metaphase I. This reflects the grouping of chromosomes of the same genome; groups of six and five in the parental species and groups of 12 and 10 after doubling in the amphiploid. This suggests that the parental species consist of two genomes, one containing six and the other five chromosomes.

Early separation of bivalents appearing as univalents at metaphase I may be caused by the low frequency of chiasma found in these small chromosomes. Succeeding stages of meiosis show that these aligned

univalents behave normally and do not affect the segregation of chromosomes. These observations suggest that the partially homologous V_r, V'_r, V_u, and V'_u genomes paired autosyndetically as bivalents and were doubled in their genomic constitution. Therefore, the genomic formula for the amphiploid is $V_rV_rV'_rV'_rV_uV_uV'_uV'_u$ (Figure 11).

The partial sterility of this amphiploid as seen in the reduced viability of the mature pollen (44%) may be caused by various kinds of physiological imbalances in spite of nearly regular meiotic behavior (Stebbins, 1971). This plant does have some of the desired characteristics such as disease and insect resistance (AVRDC, 1975). Due to its poor pollen production and sterility, this amphiploid has limited utilization in agriculture. Therefore, the backcross of the amphiploid to V. radiata was performed.

The backcross progeny (BC1) proved to be very weak plants with low fertility. Meiosis was very irregular, with a majority of the cells containing 11 bivalents and 11 univalents. The absence of multivalents at metaphase I would suggest homology between only two of the genomes. Because of the double dosage of the gene(s) controlling pairing from the V. radiata genomes, no secondary associations between the V. umbellata genomes could occur. The dosage effect also prevents the pairing of V. umbellata with V. radiata chromosomes. Therefore, it can be assumed that the univalents are of V. umbellata origin. However, the observation of loose associations suggests partial homology between all of the genomes present. This partial homology could be the result of genetically controlled homoeologous pairing. The genome formula

may be expressed as $V_rV_rV'_rV'_rV_uV'_u$ (Figure 11).

Multipolar meiosis occurred in Bc1 as in the others. At metaphase I, split spindles were observed, the bivalents segregating into groups of five and six, thus further substantiating the earlier hypothesis that the two genomes in the parental species contained six and five chromosomes, respectively. THe remaining 11 univalents were scattered throughout the cell. During anaphase I these univalents remained at the metaphase plate and eventually became fragmented due to precocious centromere division. These fragments will either be lost in the cytoplasm or become incorporated into the telophase nuclei.

During the second meiotic division these fragments become scattered and either formed micronuclei in a quartet of four cells or microcells during the final stages of meiosis. A high percentage of abnormal quartets were observed in these plants. Even the "normal" looking quartets may contain a portion of these fragments, accounting for the low percentage of viable pollen.

Despite chromosome abnormalities and possible genic imbalances, a few seed from BC1 were obtained through natural selfing. Upon germination of the seeds and analysis of the root tip cells, it was determined that these plants are trisomics (2n+1) with 2n=22 or 23. Due to the inability to distinguish between the different chromosomes of the original parents, the origin of the single additional chromosome was not discernable. It can be assumed that during the process of selfing, the progeny received its entire genome from V. radiata with the V. umbellata chromosomes being eliminated by multipolar meiosis.

Possibly only a fragment or a whole chromosome remains from the V_u or V'_u genome, depending quite possibly upon the degree of homology between the V. umbellata genomes and the genomes of V. radiata. THis is the optimum situation for incorporating beneficial genes into the cultivated variety. In addition, the morphological characteristics of the plants resemble the V. radiata parent. Thus, the genome formula could be $V_rV'_r$ + one V_u or V'_u chromosome (Figure 11).

Seed that was produced by the BC1 plants was sent to the AVRDC with these purposes in mind: 1) screening of the plants for beneficial characteristics, 2) mutigenesis to induce translocations, and 3) potential use in crop improvement.

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