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Five primary trisomics from translocation heterozygote progenies in common bean, *Phaseolus vulgaris* L.*

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Summary. Twelve distinct phenotypic groups of plants were isolated from nondisjunction progenies of 11 translocation heterozygote stocks. All the plants in these phenotypic groups originated in the light weight seed class. Five of the 12 phenotypic groups of plants have been verified as primary trisomics. They are all phenotypically distinguishable from each other and from disomics. One of the five primary trisomic groups, puckered leaf, was directly recovered as a primary trisomic from the original translocation heterozygote progenies. Three of the five trisomics – weak stem, dark green leaf, and convex leaf – originated first as tertiary trisomics. The related primary trisomics were isolated later from progenies of selfed tertiary trisomics. The fifth group, chlorotic leaf, originated at a low frequency among the progenies of three other trisomics: puckered leaf, convex leaf, and dark green leaf. The chlorotic leaf did not set seed under field conditions. The remaining four groups – puckered leaf, dark green leaf, convex leaf, and weak stem – are fertile, though sensitive to high temperature conditions. The transmission rate of the extra chromosome on selfing ranges from 28% to 41%. Physical identification of the extra chromosome has not been achieved for any of the five trisomic groups. Two trisomic groups, dark green leaf and convex leaf, have produced tetrasomics at low frequency. The phenotypes of these two tetrasomics are similar to the corresponding trisomics but more exaggerated.

Key words: Tertiary trisomics – Tetrasomics – *Phaseolus vulgaris*

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Introduction

The discovery of the globe trisomic of *Datura stramonium* was the first report of a primary trisomic; thus began the pioneer investigations of trisomics by Blakeslee and Avery (1919). Some of the crops for which primary trisomics have been developed and are being used for genetic studies include maize (McClintock 1929), wheat (Sears 1939), tomato (Rick and Barton 1954), barley (Tsuchiya 1960), pepper (Pochard 1970), sorghum (Schertz 1974), and rice (Khush et al. 1984).

A primary trisomic is one having its extra chromosome as a normal chromosome (not translocated). Since the trisome modifies the genetic segregation ratios of genes located on that chromosome, primary trisomics provide an efficient means to associate genes with their linkage groups. The linkage map of common bean is still rudimentary and a series of primary trisomics is much needed to expand and verify the linkage groups already established (Lamprecht 1961).

Primary trisomics have appeared spontaneously among the progeny of normal diploids of many species, but are obtained more frequently from asynaptic and desynaptic mutants, from the progeny of polyploids (most notably triploids), and from the progeny of translocation heterozygotes (Khush 1973). Among the different sources of primary trisomics mentioned above, triploids are the best and most dependable source (Khush 1973). There have been no reports of the spontaneous occurrence of trisomics in disomic progenies nor any reports regarding the presence of asynaptic or desynaptic mutants in the common bean. There is only one report of trisomics in common bean, which were produced as a byproduct of research intending to transfer cold tolerance genes from *Phaseolus ritensis* Jones to *P. vulgaris* (Braak and Kooistra

Table 1. A translocation heterozygote on selfing produces eight types of trisomics, among which primary trisomics can be distinguished by the following procedures

Trisomic classes[a]		Selfing $(2n+1)$ or crossing $[(2n+1)\times 2n]$	Cytology[b] of PMC from S_1 or F_1 of the progeny	Pollen fertility analysis[c] of the trisomic plant progeny (including disomics and trisomics)
Chromosome background	Type of trisomic			
Homozygous normal	Tertiary[d]	Selfing Crossing	Predominantly a pentavalent formation at M I	All S_1 and F_1 fertile
Heterozygous translocation	Primary	Selfing Crossing	Predominantly a trivalent or a ring of 4+1 univalent	S_1 and F_1 segregate for 1 fertile:1 semisterile
Heterozygous translocation	Tertiary	Selfing Crossing	Predominantly a pentavalent formation or a ring of 4+1 univalent	S_1 and F_1 segregate for 1 fertile:1 semisterile
Homozygous translocation	Primary	Selfing	Predominantly a trivalent formation	All S_1 fertile
		Crossing	PMC show a tetravalent +1 univalent. A pentavalent may also appear at a very low frequency	All S_1 semisterile

[a] Trisomic progeny appear with nearly equal frequencies in the four classes listed below
[b] Chiasma terminalization or failure of chiasma formation may reduce the pentavalent or trivalent associations to smaller configurations (Khush and Rick 1967)
[c] Pollen fertility of trisomic segregates is generally lower than normal disomics, depending upon extra chromosome and several other factors (Khush 1973). If pollen fertility analysis is to be employed for preliminary observations, translocation heterozygosity of the background complement with respect to pollen abortion, must be taken into account
[d] Tertiary trisomics regularly produce related primaries among their selfed progenies (Khush and Rick 1967) hence these are another excellent source of primary trisomics in addition to the translocation heterozygote progenies

1975). For the cross (*P. vulgaris* × *P. ritensis* amphidiploid) × *P. ritensis* 25,000 pollinations produced only one triploid plant. After making 1,600 pollinations of the cross *P. vulgaris* × triploid, they obtained a single trisomic plant (the reciprocal cross being completely unsuccessful). A small number of additional trisomics were obtained by selfing the triploid, which had been multiplied vegetatively. No further reports were made about these trisomic materials. It should also be noted that development of triploids from autotetraploids is not very promising because of the latter's great infertility (Monge and Moh 1963).

A project was started by Bassett and Hung (1982) in which reciprocal chromosome translocations were induced by gamma irradiation. Twelve translocation lines were established and genetically verified. Five of the 12 translocation stocks were also verified cytologically (Ashraf and Bassett 1986). Furthermore, cytological analysis of a half diallel set of crosses indicated that these five stocks involve eight different chromosomes [($n=11$ in *P. vulgaris*) Weinstein 1926; Mok and Mok 1976] in reciprocal translocations (Ashraf and Bassett 1986). This paper reports the discovery of five primary trisomics isolated primarily from nondisjunction progenies of 11 translocation heterozygote stocks.

Materials and methods

The small size of common bean chromosomes prevents pachytene analysis of PMCs. It is necessary to analyze patterns of chromosome associations at diplotene, diakinesis, and metaphase I (MI) to distinguish tertiary from primary trisomics. Whenever the trisomic phenotypes are favorably distinctive, the cytological evidence can be supplemented by genetic classification data from segregating progenies derived from selfs and test crosses with trisomics.

Procedure for isolating primary trisomics

Table 1 summarizes the procedures followed in isolating primary trisomics from tertiary trisomics. Since common bean cytology is laborious, pollen fertility analysis was employed at a preliminary stage to reduce cytological analysis to a minimum. A translocation heterozygote upon selfing produces eight types of trisomics falling into four phenotypic classes, provided that each chromosome (including its translocated segment) has some effect on the morphology of the trisomic plant that carries it as the extra chromosome. The two primary trisomic classes do not have a normal chromosome background. It is easier to identify the primary trisomic class with a homozygous translocation background because the primary trisomics of this class yield F_1 progeny (disomics as well as trisomics), all having semisterile pollen. The semisterile primary trisomic plants originating in the F_1 progeny of a primary trisomic (with homozygous translocation background) crossed with a normal disomic, if backcrossed with the normal disomics, can be converted to a homozygous normal primary trisomic. In BC_1F_1 trisomic progeny, only fertile trisomic plants are selected (Khush, IRRI, Philippines, pers. commun.). Fertile trisomic plants in BC_1F_1 progeny can then be verified cytologically. If a high percentage of PMCs show a trivalent association with all other chromosomes appearing as bivalents, the plants in question are primary trisomics with a homozygous normal chromosome background (Khush 1973). This procedure avoids a lot of cytological analysis during preliminary stages.

In case one is unable to recover any primary trisomics from the progeny of a translocation heterozygote, the best alternative is to grow a few selfed progenies of a tertiary trisomic and in each progeny look for any deviates from the tertiary trisomic phenotype. One of the unique features of the breeding behavior by which tertiary trisomics differ from other types of trisomics is the regular appearance of one or both of the two related primary trisomics in their progenies (Khush and Rick 1967).

Preparation of seed stocks

Eleven out of 12 heterozygous translocation stocks established by Bassett and Hung (1982) were first developed into homozygous translocation lines by test crosses of their progenies. These homozygous translocation stocks were then crossed with a standard normal bean breeding line and F_1 seed was harvested. The F_1 generation was grown to produce F_2 seed. For each translocation stock, seed from each F_1 plant was handled separately as follows: each seed was weighed and arranged in ascending order according to seed weight. Seed was then separated into two weight classes, light (the lower 30% of seed weight distribution) and heavy (the remaining 70%) on an individual plant basis.

First field generation, summer 1984

The two seed weight classes from each F_1 plant were planted separately in adjacent plots. The F_2 progeny plots were screened for plants that were phenotypically distinguishable from each other and from normal plants. Putative trisomics (off-type plants) were not cross-pollinated with normal disomics in the field because of several difficulties under Florida field conditions. The selected off-type plants were classified into phenotypic groups in each translocation stock and seed (selfed) from every tagged (off-type) plant was harvested separately.

First greenhouse generation, fall 1984

S_1 seed from four phenotypic classes (putative trisomics), designated weak stem, puckered leaf, dark green leaf and convex leaf, was planted in the greenhouse. Hereafter, the off-type (putative trisomic) plants will simply be called trisomics, anticipating supporting evidence in the Results and discussion section, to avoid awkward circumlocutions. Immature flower buds were collected from the trisomic S_1 segregates that were identical to the seed parent (puckered leaf and weak stem only) between noon and 2 p.m. every other day, put in Carnoy's fixative solution for 24 h, and then changed to 70% ethanol for preservation. Microscope slides were prepared using Cheng and Bassett's technique (1981) with slight modification, i.e., the staining time of anthers with iron propionic carmine was reduced from 1 h to 20 min. The convex leaf and dark green leaf classes were allowed to self-pollinate, and some of the plants picked at random from both of these classes were also crossed with pollen from $2n$ plants. All the trisomic plants in puckered leaf and weak stem were searched for male-fertile segregates and, wherever found, were crossed with pollen from $2n$ plants. If male-fertile plants with the trisomic phenotype were not present in the progeny, semisterile trisomic plants were selected for crossing with $2n$ pollen.

Second greenhouse generation, spring 1985

F_1 seed from crosses (trisomic × disomic) with puckered leaf and weak stem was grown in the second greenhouse generation and all the F_1 progenies of male-fertile trisomic parents (including trisomic and disomic segregates) were tested for pollen fertility. The progenies having all semisterile plants were selected and trisomic segregates were backcrossed with pollen from a normal disomic plant. On the other hand, the F_1 progenies derived by crossing the semisterile trisomic plants with pollen from $2n$ plants in the first greenhouse generation were searched for male-fertile trisomic plants, which were tagged and allowed to self-pollinate.

Second field generation, summer 1985

F_2 seed from selfed male-fertile trisomics, BC_1F_1 seed from the all-semisterile trisomic groups, and S_2 and F_1 seed from dark green leaf and convex leaf trisomics from the first greenhouse generation were all planted in the second field generation. Male-fertile trisomic plants were identified in BC_1F_1 progenies and immature flower buds were collected from them for cytological analysis. Similarly, flower buds were also collected from F_2 trisomic segregates of weak stem trisomic. Selections were made among S_2 progenies of dark green leaf and convex leaf that were segregating for only one type of trisomic (off-type class). Another phenotypic class, chlorotic leaf, originating among most of the trisomic progenies at a very low frequency was analyzed cytologically. Plant photographs were made, and notes on pod and seed characteristics were recorded wherever possible. Other descriptive notes for all five groups of trisomics were recorded in the field along with data on ovule abortion rates and the frequency of transmission of the extra chromosome.

Results and discussion

Preparation of seed stocks

F_2 seed from crosses of the homozygous translocation stocks with a normal bean line was divided into light and heavy classes on an individual plant basis. On average, the individual seed weight ranged from 46 to 287 mg in the light seed weight class and from 107 to 670 mg in the heavy seed weight class. The wide range in each class (note the overlap) was probably due to: (a) individual plant differences due to environment; (b) genetic differences between different translocation heterozygote stocks in the rate of ovule abortion. In the translocation stocks used in this study, ovule abortion rates ranged from 19% to 60% (Bassett and Hung 1982; Ashraf and Bassett 1986). In lines with higher ovule abortion, the number of seeds per plant is reduced and individual seed weight increased. As a result, the lower and upper limits of the seed weight distribution both change, i.e., the whole distribution for each plant is shifted. The same type of compensation has been observed in translocation heterozygotes of soybean (Palmer 1976). For this reason, any seed weight that is chosen as the dividing line between heavy and light seeds to increase the efficiency of searching for trisomics within the progenies of different translocation heterozygotes is somewhat arbitrary. Out of 6,600 seeds, 2,100 were separated into the lower seed weight class.

Table 2. Transmission of the extra chromosome in four trisomics of common bean (first greenhouse planting, fall, 1984)

Translocation stock	Seed parent phenotype	No. seeds planted	No. trisomics scored	% transmission on selfing
II-70	Puckered leaf	100	26	26
I-97	Weak stem	19	6	32
I-52	Dark green leaf	125	39	31[a]
I-52	Convex leaf	50	17	34[a]

[a] The transmission rate of dark green leaf and convex leaf is based on the progeny test of these classes in the second field generation. For details, see text

First field generation

All the light and heavy seeds from each F_1 plant were planted separately in adjacent plots. It was found that 96 phenotypically distinct plants (off-type) originated among the progeny and all appeared in the light weight seed class. These results were similar to those reported in barley (Ramage and Day 1960). The off-type plants were allowed to self-pollinate. Out of 96 plants, 60 set pods and seeds. These plants were divided into 17 phenotypic classes distinguishable from each other and from normal plants (Ashraf 1985). Seed was recovered from only 12 of these phenotypic classes.

First greenhouse generation

The seed from four phenotypic classes was planted in the first greenhouse generation (Table 2). Immature flower buds were collected from each putative trisomic separately for puckered leaf and weak stem, but within each trisomic class the buds from the plants were composited. For each of these phenotypic classes, slide preparations of PMCs were searched until a total of 25 PMCs were found that fell into one of the following categories: 11 bivalents and one univalent at diplotene or diakinesis (Fig. 1 A, B), 23 chromosomes at early anaphase 1 (Fig. 1 C), or 11 vs 12 chromosomes at late anaphase 1 (Fig. 1 D). During this search a very low frequency of certain anaphase 1 cells was also observed with 11 chromosomes at each pole and with one laggard still in the middle (Fig. 2 A). When the normal segregates arising among the progenies of these trisomics were analysed meiotically, these never showed any extra chromosome in the late prophase 1, metaphase 1, or anaphase 1. We realize that due to the small size of bean chromosomes, which range from 1 to 3 microns at diplotene (Cheng and Bassett 1981), it is fairly difficult to distinguish between univalents and bivalents at late prophase 1 or metaphase 1. However, the presence of 23 univalents (Fig. 1 C) at early anaphase 1 or 11 vs 12 univalent chromosomes at late anaphase 1 (Fig. 1 D) is conclusive evidence that these phenotypic classes are trisomics. The extra chromosome was always associated only with the altered phenotype during the course of this study. Thus it is concluded that the puckered leaf and weak stem phenotypic classes (Table 2) are trisomics. In puckered leaf and weak stem, the trisomic segregates were easily differentiated from normal plants at the seedling stage. In the other two classes, dark green leaf and convex leaf, it was not possible to distinguish (in the greenhouse) between trisomic and normal segregates in the seedling stage. Cytological verification of these two trisomics was obtained in the second field generation where the trisomic phenotypes were once again distinctive under full sunlight unfiltered by glass.

The method of isolation of primary trisomics from teritary trisomics differs slightly from one trisomic to the other (with one exception). Thus they are treated under separate headings, and the research results of the second greenhouse and field generations have been incorporated therein.

Puckered leaf trisomic

Out of 100 S_1 seeds of puckered leaf planted in the first greenhouse generation, 26 plants were identified as phenotypically identical to the seed parent (Table 2). Five of these 26 plants were identified as male-fertile on the basis of pollen abortion rates. In these five male-fertile trisomics, the pollen abortion rates ranged from 20 to 36%. This gave an indication that these male-fertile plants could be either tertiary or primary trisomics, both either on a homozygous normal or homozygous translocation background.

The five S_1 male-fertile trisomic segregates mentioned above were crossed with pollen from $2n$ plants and their progenies grown. In the second greenhouse generation, one of these five male-fertile plant progenies produced an all semisterile F_1. The 23 plants produced segregated into 12 trisomics and 11 normal disomics. Pollen abortion in the trisomics ranged from 50% to 65%, whereas in the semisterile disomics it was 40% to 45%. This gave an indication that the plant progeny might be primary or tertiary with translocation heterozygote background. These 12 trisomic plants

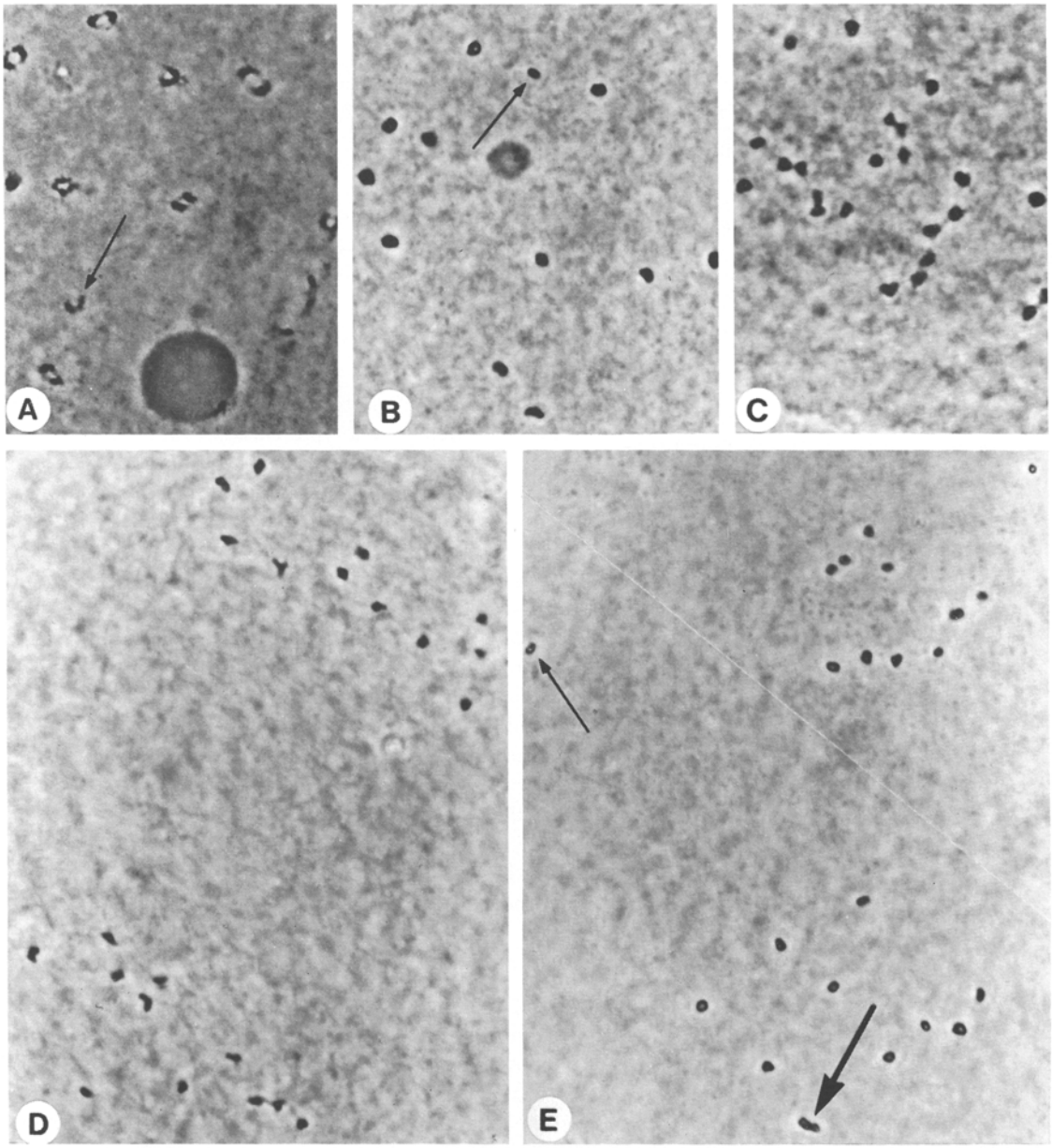

Fig. 1 A–E. Meiotic diplotene to anaphase I as observed in the trisomics of common bean (× 1,000). **A** a PMC at diplotene showing 11 bivalents and 1 univalent (*arrow*). One bivalent is still associated with the nucleolus; **B** diakinesis showing 11 bivalents and 1 univalent (*arrow*); **C** early anaphase showing 23 chromosomes; **D** anaphase I showing 11 chromosomes at one pole and 12 at the other pole (top); **E** anaphase I showing 11 chromosomes at each pole. The *thicker arrow* points to 2 chromosomes overlapping each other. The *thinner arrow* points to a univalent away from the poles, appearing as a micronucleus. The large size of the PMC made it necessary to take two photographs to cover the whole PMC, and these were later cut and joined

from the semisterile progeny were selected for backcrossing with pollen from $2n$ plants (Table 3). The BC_1F_1 seed produced from these plants was grown in the second field generation. The progeny numbered 283 plants in total with 97 trisomic segregates. Due to unfavorable climatic conditions in the field during the summer of 1985, the mortality rate of trisomic plants was high. Nevertheless, 19 male-fertile trisomic plants from five plant progenies were tagged on the basis of pollen abortion analysis. Three of the male-fertile plant progenies with a maximum of surviving trisomic plants (16 plants in all) were analyzed cytologically. Each plant progeny was analyzed separately. All three progenies were primary trisomics, so the cytological data were combined and the totals were as follows. Out of 50 analyzable PMCs at diplotene to M1, 36 showed a

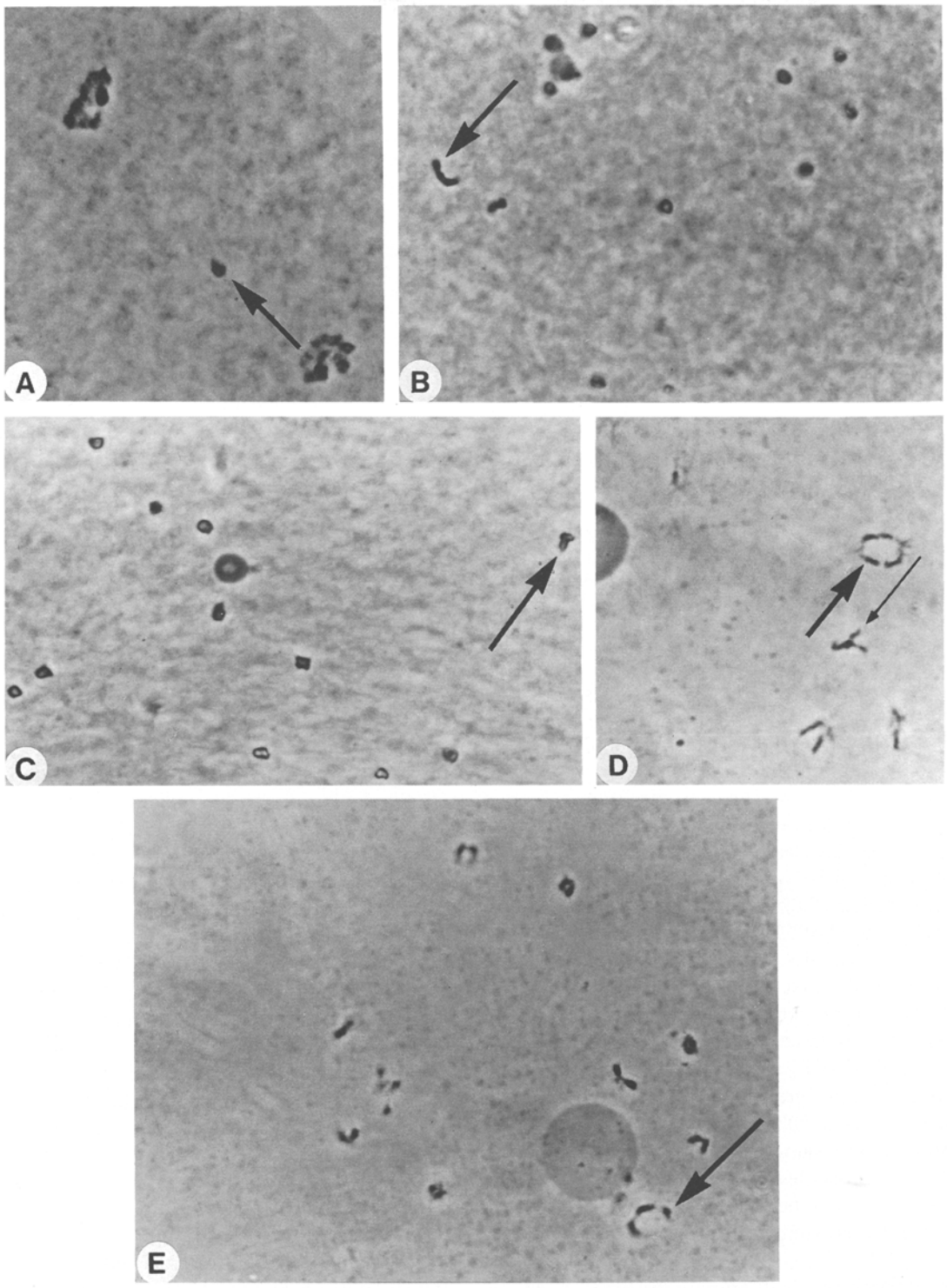

Fig. 2 A–E. Meiotic diplotene to anaphase I as observed in the trisomics of common bean (× 1,000). **A** a PMC at anaphase I, showing a laggard (*arrow*); **B** diakinesis showing a trivalent association (*arrow*). One bivalent is still associated with the nucleolus. Nine bivalents are spread apart; **C** diakinesis showing a trivalent association (*arrow*) along with 10 bivalents; **D** a PMC at diplotene. The thicker arrow points to a ring of 4 chromosomes. The thinner arrow points to a trivalent; **E** diplotene with a trivalent (*arrow*) and 10 bivalents

Table 3. Transmission of the extra chromosome in two trisomics of common bean after crossing $[(2n+1)\times 2n]$ (second greenhouse planting, spring, 1985)

Translocation stock	Trisomic plant phenotype	No. progeny classified	No. trisomic segregates	% transmission on crossing $(2n+1)\times 2n$
II-70	Puckered leaf	91	46	50
I-97	Weak stem	71	31	44

Table 4. Segregation for trisomic progeny from selfed $2n+1$ or cross-pollinated $[(2n+1)\times 2n]$ trisomics originally derived from four different translocation heterozygote progenies (second field planting, summer, 1985)

Translocation stock	Trisomic phenotype	No. of plants grown		No. trisomic progeny	Progeny phenotype	% transmission
		$2n+1$ self	$(2n+1)\times 2n$			
II-70	Puckered leaf	5,567		2,025	Puckered leaf	36
			528	201	Puckered leaf	38
I-99	Puckered leaf	147		57	Puckered leaf	39
				37	Chlorotic leaf[a]	0.6
I-97	Weak stem	2,373		851	Weak stem	36
			85	35	Weak stem	41
II-121	Weak stem	4		Seeds did not germinate		

[a] Chlorotic leaf is a primary trisomic (relationship undetermined) that originated in all the above progenies

trivalent association with 10 bivalents (Fig. 2C, E); 12 PMCs showed 1 univalent with 11 bivalents (Fig. 1B). Also, 2 pentavalent associations were observed and were probably due to overlapping of chromosomes.

Transmission rates of the extra chromosome after selfing $(2n+1)$ or crossing $[(2n+1)\times 2n]$ have been given in Table 4, for progenies classified in the second field generation. A total of 5,567 progeny plants from selfed $(2n+1)$ parents yielded 2,025 trisomics, giving a 36% transmission rate. A total of 528 F_1 progeny plants from crosses yielded 201 trisomics, giving a 38% transmission rate. Similarly in the first greenhouse generation (Table 2), a total of 100 progeny plants from selfed trisomic parents yielded 26 trisomics, giving a 26% transmission rate. A total of 91 F_1 progeny plants from crosses (Table 3) in the second greenhouse generation yielded 46 trisomics, giving a 50% transmission rate. Here again we observe the same trend, i.e., transmission is higher in cross progenies than self progenies. Higher rates from crossing have been attributed to the heterozygosity created by crossing (Khush and Rick 1967).

The phenotype of puckered leaf primary trisomic can be distinguished 15–20 days after planting or after the second true leaf emerges. Leaves are puckered along the midrib and have a glazed appearance after attaining full size. Plants are weaker than diploid sibs during the early stage of growth but attain the same size later (Fig. 3B). Also, puckered plants show a tendency for an accentuated semi-vining growth habit. They flower nearly at the same time as diploids. There is no difference in flower color. The pods are broader and shorter with an unfilled basal portion, and they have slightly longer stylar tips with a pronounced curvature. Seeds are larger and have slight wrinkles on the testa. Because this trisomic can be identified easily at the seedling stage, all or most of the undesired diploids can be rogued from stocks or F_1 in early stages, thus saving greenhouse space. This trisomic is sensitive to heat and drought which affects pod and seed set adversely. It sets well in the greenhouse where mean seeds per pod in trisomic plants is 3.35 ± 1.63 compared to 5.61 ± 1.46 in normal diploids (means from 10 plants), i.e., 43% reduction in seed set. Trisomic plants may have improved seed set when developed on a homozygous normal chromosome background.

Weak stem trisomic

For this study 19 S_1 seeds recovered from two plants of the weak stem trisomic originating in the selfed progeny of a translocation heterozygote in the first field generation were planted in the first greenhouse generation. Then 6 plants were identified as trisomic from their phenotype (Table 2). Pollen fertility analysis showed that they were all semisterile. Pollen abortion rates ranged from 60% to 75% in these trisomic plants. These data gave an indication that these plants were either primary or tertiary trisomics and still had a

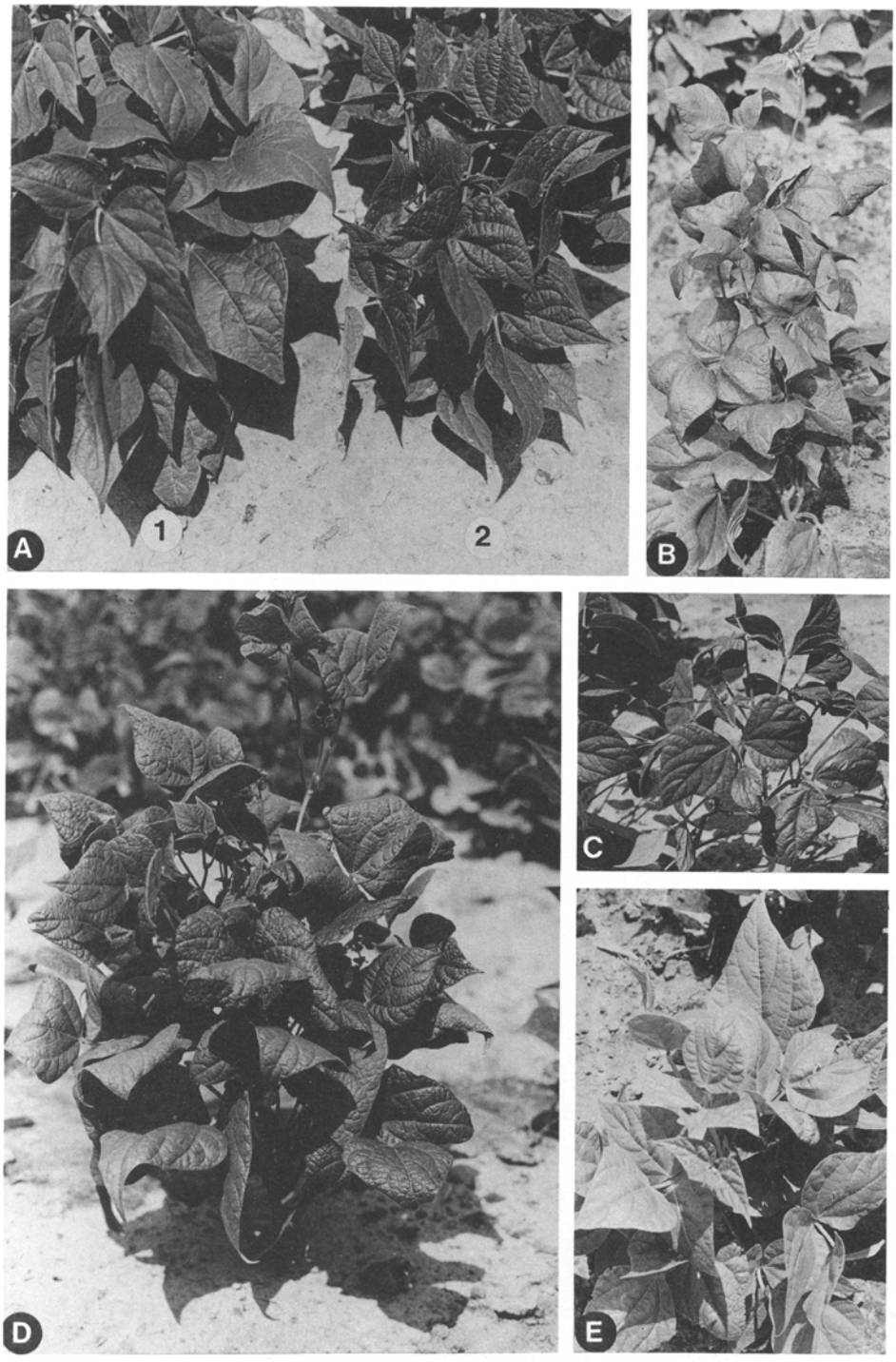

Fig. 3A–E. Photographs of five types of primary trisomic plants. **A** a normal disomic plant, 30 days after planting (*1*), and dark green leaf trisomic of the same age (*2*); **B** puckered leaf trisomic, 30 days after planting. The glossy leaf surface gives a light color (pseudo-chlorosis) in black and white photographs; **C** weak stem trisomic, 30 days after planting; **D** convex leaf trisomic, 45 days after planting; **E** chlorotic leaf trisomic, 30 days after planting

Table 5. The frequency of PMCs with different chromosome configurations at diplotene to MI of meiosis in different plant progenies of weak stem trisomic

Progeny code	Frequencies of different chromosome configurations		
	$11_{II}+1_{I}$	$10_{II}+1_{III}$	$9_{II}+1_{V}$
WS No. 19	25	67	8
WS No. 22	15	75	10
WS No. 23	22	34	44
WS No. 25	17	11	72

translocation heterozygote background. To convert them to homozygous normal chromosome background, they were crossed with pollen from normal ($2n$) plants. It should be emphasized that out of six trisomic segregates the five that set seed were all from one of the two original weak stem trisomic plants. The sixth trisomic plant, derived from the other original parent, set no seed.

F_1 seed from the five trisomic plants was planted in the second greenhouse generation (Table 3). A total of 71 self progeny segregated for 31 trisomic phenotype

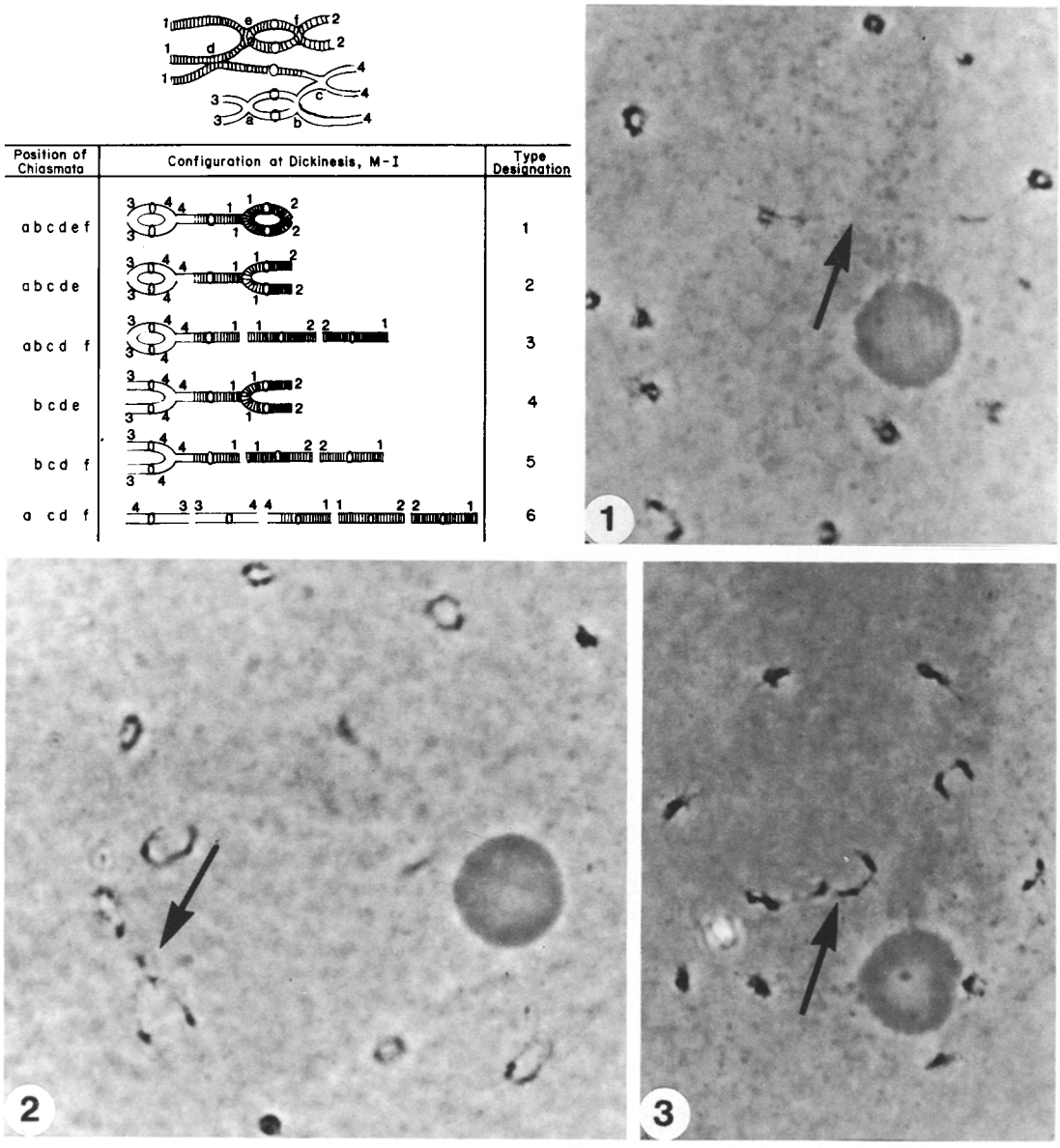

Fig. 4. Pentavalent configurations expected in a tertiary trisomic according to various positions of chiasmata (modified from Khush and Rick 1967). Numbers in the photomicrographs (×1,000) represent the type designation in the illustrative drawing

plants. These were tested for pollen fertility; seven trisomic plants with pollen abortion rates between 25% and 40% were classified as fertiles, whereas the rest with 50% to 70% pollen abortion were classified as semisterile. The fertile trisomic plants were tagged and allowed to self-pollinate.

F_2 seed from the fertile trisomic segregates produced 450 plants in the second field generation, out of which 160 plants were classified as trisomics. As this trisomic is the most sensitive to unfavorable climatic conditions, nearly 40% of the plants died at the seedling stage; however, it probably did not affect our transmission data because of a desirable characteristic of this trisomic group. It can easily be classified at an early seedling stage, as early as 10 days after planting. Under greenhouse conditions no mortality of trisomic seedlings was observed in this group.

Four out of seven fertile trisomic plant progenies with the maximum number of trisomic survivors were analyzed cytologically (24 plants in all). Fifty analyzable PMCs from each plant progeny were scored (Table 5).

Weak stem (WS) No. 22, 23 and 25 progenies were classified as tertiary because of their pentavalent configurations (Fig. 4). WS No. 19 was identified as primary because none of its pentavalent configurations gave an indication of its being a tertiary trisomic. Most of the pentavalents appeared to be the result of overlapping chromosomes. Some of the cells showing a univalent or trivalent configuration, also showed a quadruple association of four chromosomes appearing as a ring, a characteristic of translocation heterozygosity (Fig. 2D). When trisomic plants in this progeny were checked for pollen fertility, four out of nine plants were fertile, with pollen abortion rates ranging from 30% to 40%. The remaining five semisterile plants had 70% to 85% pollen abortion rates. Under the same conditions, a normal plant had 15% to 20% pollen abortion. From these data, it was inferred that the parent plant, which was originally classified as fertile in F_1 progeny of the second greenhouse planting, was not actually a fertile but a semisterile that again segregated into nearly 1 semisterile : 1 fertile trisomics. The presence of a quadruple at MI also indicated semisterility. Hence, in the progeny WS No. 19, fertile trisomic segregates were tagged as primary trisomics with a homozygous normal chromosome background, whereas the semisteriles with trisomic phenotype were classified as primary trisomics with a translocation heterozygote chromosome background. Due to unfavorable climatic conditions, seed was not recovered from any of the plants in the WS No. 19 progeny; however, this trisomic can be recovered in the greenhouse from remnant F_2 seed.

Some of the photomicrographs show a ring of four chromosomes + 1 III (Fig. 2D). The extra chromosome is either associated or merely appears associated with another bivalent pair. Usually, the extra chromosome of a trisomic derived from the nondisjunction progeny of a translocation heterozygote is one of the four chromosomes forming a quadruple. It is difficult to explain why it is associated with another pair with which it has no homology. A primary trisomic still having a translocation heterozygote background may also appear in the form of a straight chain of five chromosomes, which if it gets twisted, can appear in strange shapes. This may be why we observed pentavalents (Table 5, WS No. 19) at such a frequency in this class.

All weak stem trisomic progeny were derived from only one trisomic plant. This plant could, theoretically, have been either a primary or a tertiary trisome. If it was a primary trisomic, then all the progeny trisomics should be primary with rare unrelated primaries. If it was tertiary, the progeny generally should segregate into parental class tertiaries and one or both related primaries (Khush and Rick 1967). Proceeding with only one plant progeny, the segregating plants were searched for distinctive primary trisomics in case the parent was a tertiary trisome. The primary trisomic plants isolated on the basis of genetic tests and cytological analysis discussed above did not show any morphological dissimilarities from the parental tertiary phenotype.

This trisomic can be distinguished from diploid sibs in the very early seedling stage, usually 10 days after planting and sometimes even before the first true leaf emerges. The hypocotyl and the stem from the cotyledons to the primary leaves are longer than in diploid sibs, and the hypocotyl usually bends aside because it is weaker than in normal plants. Plant internodes and leaf petioles are longer than normal segregates. The entire plant grows taller than normal segregates and has a spindly appearance. The weak stem needs support. If the plant apex is broken or support is not provided, it produces many branches and becomes bushy. Leaf petioles have a wider angle with the stem than normal plants. The terminal leaflet of the trifoliolate leaf has an angular shape. The plant has a wider or more open plant canopy (Fig. 3C). This trisomic is more sensitive to heat, drought, lodging and sunburn effects than normal plants, and these effects are very obvious under Florida field conditions. It is late in maturity, the delay being about 15 days more than normal diploid sibs. It has poor pod set under high-temperature field conditions, but pods set adequately in the greenhouse. Pods are not well filled towards the basal portion. Constrictions in the pods represent the areas where ovules aborted and seeds are missing. The pods are broader and their stylar tips are longer than normal plant pods. Seeds are comparatively longer and wider than normal diploids. The data collected from 10 plants chosen at

random from the spring, 1985, greenhouse planting shows that the average number of seeds per pod is 2.13 ± 1.13 compared to 5.64 ± 1.46 seeds in diploid sibs. Part of this difference can be attributed to translocation heterozygosity. An improvement in seed set is expected when the trisomics with translocation heterozygote background are replaced with a homozygous normal chromosome background. Transmission rates of the extra chromosome on selfing or crossing have been presented in Table 4. Among 2,373 total plants in the progeny of selfed trisomics, there were 851 weak stem segregates, i.e., 36% of the total. Among a total of 85 progeny plants from crossing $(2n+1)\times 2n$, there were 35 trisomic and 50 disomic segregates giving 41% transmission through the female. Transmission rates from selfing are less than from crossing the same trisomic plants with normal disomics, which can be attributed to the heterozygosity created by crossing (Khush and Rick 1967).

Dark green leaf and convex leaf trisomics and tetrasomics

Both of these trisomic phenotypes originated from the nondisjunction progenies of one translocation heterozygote I-52. S_1 seed from six off-type plants with dark green leaf phenotype and two off-type plants with convex leaf phenotype was planted in the first greenhouse generation (Table 2). S_2 and F_1 $[(2n+1)\times 2n]$ seed from these plants was grown in the second field generation. All the progenies fell into one of four types.

Trisomics in the third and fourth types described above were classified as tertiaries on the basis of their phenotypic characteristics and genetic segregations. Out of 50 PMCs of dark green leaf trisomics of the first type, 17 showed a univalent with 11 bivalents (Fig. 1 A, B) and the remaining 33 showed a trivalent and 10 bivalents at diakinesis to MI in each PMC (Fig. 2 B, C, E). In 50 PMCs from convex leaf trisomics of the second type, the frequencies of univalents and trivalents at diakinesis to MI were equal. On the basis of cytological evidence, morphological characteristics, and genetic segregations, the two classes described in the first and second types were identified as primary trisomics. These plant progenies might have translocation heterozygote or homozygous translocation background, but no cytological evidence is available. One or two backcrosses with normal disomics need to be made to develop them on homozygous normal chromosome background. Transmission rates of both these classes have been given in Table 6. There is no clear cut trend of improvement in transmission by crossing $[(2n+1\,T\times 2n]$ compared to selfing. This may be due to the segregations taking place among the progenies of tertiary trisomics.

PMCs of convex leaf tetrasomics showed 11 bivalents and two univalents at diakinesis (Fig. 5 A), 24 chromosomes at early anaphase I (Fig. 5 B) and 12 vs 12 at the two poles at late anaphase I (Fig. 5 C) of meiosis. The dark green leaf tetrasomic class was identified from its phenotypic relationship with the seed parent phenotype and was not analyzed cytologically. None of the tetrasomic plants from any class set seed. The extra chromosome has not yet been physically identified in any of the trisomics reported in this study.

Phenotype of dark green leaf

In the field, the trisomic plants are late germinating and slow growing at the early stage, but they attain the same size as disomics at maturity. Segregating progenies can easily be classified 15 to 20 days after planting. Trisomic plant leaves are glossy dark green and smaller in size compared to the normal, but the primary trisomic has bigger leaves compared to the dark green leaf tertiary. Leaf veins are slightly recessed (Fig. 3 A). Flower color in trisomics is more intense than in disomics. The pod set is a bit late with many missing seeds under field conditions but less so in the greenhouse. The data collected from 10 trisomic plants picked at random from the field showed the mean number of seeds per pod to be 2.09 ± 1.05, compared to 5.85 ± 1.61 in normal plants under the same conditions, a reduction in seed set of 64%. This may be due partly to translocation heterozygosity, which is probably still present in the background complement. Pod and seed size are slightly smaller than those of diploid segregates. Pods possess straight stylar tips that are nearly the same size as in normal pods. The phenotype is easily distinguishable in the field, but very difficult to distinguish in the greenhouse.

Phenotype of convex leaf trisomic

This trisomic is difficult to identify at the seedling stage. Nearly 20 days after planting, the leaves start turning to a convex shape with recessed veins. Thirty days after planting, the phenotype becomes quite obvious in the field but is still indistinct in the greenhouse. Leaf color is normal and the leaf surface is slightly rough. This class sets seed and pods equally well under field or greenhouse conditions. Primary trisomics have slightly longer internodes compared to normal disomics and to tertiary trisomics, and the branches are weak, needing support. They grow even taller than normal diploids, and, due to late pod set, senesce late (Fig. 3 D). Pods are smaller than normal and stylar tips are normal in length without pronounced curvature. The average number of seeds per pod (from 10 plants) in trisomics under field conditions is 3.84 ± 1.76 compared to $5.85 \pm$

Table 6. Progeny tests of two tertiary trisomics originally derived from translocation heterozygote I-52 (second field planting, summer, 1985)

Progeny[y] code	No. plants grown ($2n+1$) self	Tertiary trisomic parent phenotype	Classes of progeny	No. aneuploids in the progeny	% transmission
a	1,758[z]	Dark green leaf	Dark green leaf (primary)	718	41
			Chlorotic leaf (primary, unrelated)	23	1
b	211	Dark green leaf	Convex leaf (primary)	64	30
			Chlorotic leaf (primary, unrelated)	3	1
			Convex leaf tetrasomics	3	1
c	523	Dark green leaf	Dark green leaf (tertiary with rare primary)	209	40
			Dark green leaf tetrasomics	7	1
d	1,032	Dark green leaf	Dark green leaf (tertiary with rare primary)	365	35
			Convex leaf (primary)	53	5
			Chlorotic leaf (primary, unrelated)	22	2
			Convex leaf tetrasomics	16	2
			Dark green leaf tetrasomics	10	1
e	1,530	Convex leaf	Convex leaf (tertiary)	428	28
			Chlorotic leaf (primary, unrelated)	9	1
			Convex leaf tetrasomics	94	6
f	434	Convex leaf	Convex leaf (tertiary)	87	20
			Dark green leaf (primary)	27	6
			Chlorotic leaf (primary, unrelated)	6	1
			Convex leaf tetrasomics	26	6
g	266[z]	Dark green leaf	Dark green leaf (primary)	73	32
h	67	Dark green leaf	Dark green leaf (tertiary and primary)	15	22
			Convex leaf (primary)	11	16
			Chlorotic leaf (primary, unrelated)	2	3
i	101	Convex leaf	Convex leaf (tertiary)	24	24
			Chlorotic leaf (primary, unrelated)	3	3
			Convex leaf tetrasomics	2	2
j	25	Convex leaf	Convex leaf (tertiary)	4	16
			Dark green leaf (primary)	7	28
			chlorotic leaf (primary, unrelated)	3	12

[y] Each code refers to a group of S_2 trisomic plant progenies derived from S_1 trisomic plant progenies in the first greenhouse planting, fall, 1984. The members of each group of progenies had similar segregation patterns

[z] One plant in each progeny, *a* and *g*, was extreme lanceolate leaf (ELL) type. Both plants were sterile

1.61 in normal diploids. The reduction in seed set is 34% which is less than the other trisomics. Seeds are nearly of the same size as normal seeds, but slightly different in shape.

Chlorotic leaf

Trisomic plants with this phenotype originated in three other types of trisomic plant progenies, namely dark green leaf, convex leaf and puckered leaf, always at a very low frequency in each progeny (Tables 4 and 6). A total of 108 chlorotic leaf plants were scored in the second field generation, which was less than 1% of the total population. Twenty PMCs of these plants showed a trivalent and 10 bivalents at diakinesis and MI of meiosis (Fig. 2C, E). This and the fact that unrelated primaries regularly appear among the progenies of trisomics at low frequency (Khush 1973) helped us to identify this phenotypic class as a primary trisomic. We are sure that the chlorotic leaf is an unrelated primary trisomic segregating in convex leaf and dark green leaf trisomic progenies because both of the latter are related primaries recovered only from translocation stock I-52. For puckered leaf, further evidence is needed to determine whether the chlorotic leaf is a related or unrelated primary. None of the plants in this class set any pods

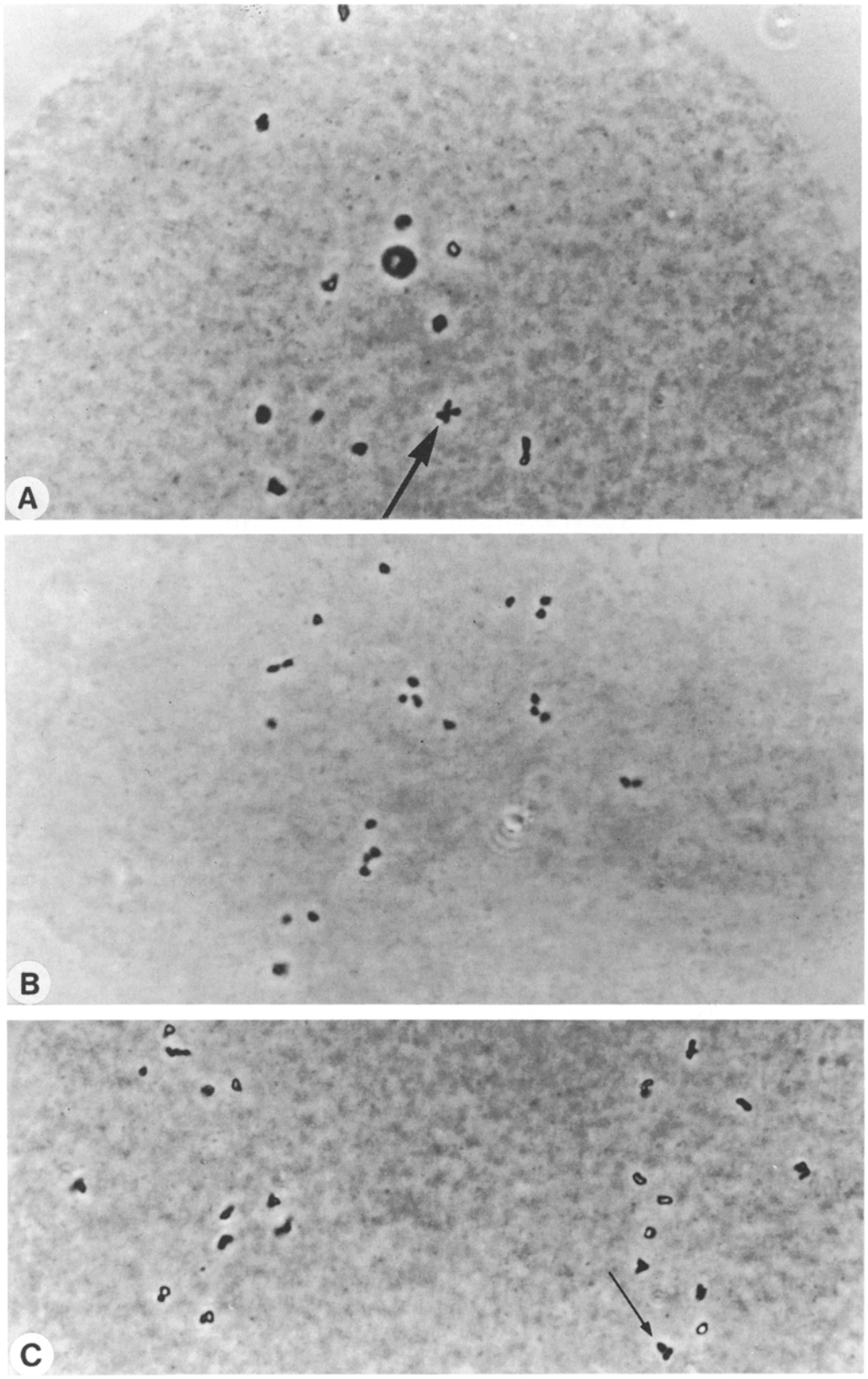

Fig. 5A–C. Meiotic diakinesis to anaphase I as observed in a tetrasomic of common bean (× 1,000). **A** a PMC at diakinesis showing 11 bivalents and 2 univalents that overlap (*arrow*); **B** early anaphase showing 24 univalents; **C** late anaphase showing 12 univalents at each pole. The *arrow* points to two chromosomes lying close to each other

or seeds. Heterozygosity and genetic background have been reported to improve the transmission of and tolerance to the extra chromosome (Khush 1973). If it reappears in the future, it may be pollinated with a bean line having diverse genetic origin compared to 7-1404, which was the original genotype used for developing chromosome translocations and trisomic stocks. The leaves of this trisomic are slightly chlorotic. All other morphological aspects are similar to the normal plants (Fig. 3 E). It blooms profusely, fails to set any pods under hot field conditions, and consequently senesces very late.

Overview of trisomic segregation from translocation stock I-52

To fully understand the segregation data in Table 6, it is necessary to review the origin and development of these progenies. In the first field generation, 16 off-type plants falling into two phenotypic groups, dark green leaf and convex leaf, were tagged in F_2 progeny of the translocation heterozygote I-52. Selfed progenies from 8 of these 16 plants, 6 of dark green leaf and 2 of convex leaf, were tested in the 1st greenhouse generation (Table 2). On the basis of information we recorded from the second field generation (Table 6), it is hypothesized that all eight of these plants were tertiary trisomics of the two types, i.e., dark green leaf tertiary and convex leaf tertiary. In the first greenhouse generation, there was segregation for the tertiary trisomics and the related primaries. If the dark green leaf trisomic progenies are examined (Table 6), it seems that some of the plants segregated into both related primaries in S_1 and produced uniform primary trisomics in S_2 (Table 6, a and b). They also produced identical tertiaries in their segregating progenies in S_1. These tertiaries either produced identical tertiaries and one related primary in S_2 (Table 6, c), or they produced identical tertiaries along with both the related primaries in S_2 (Table 6, d). A similar explanation seems true for convex leaf tertiary trisomic. The only difference is that the convex leaf did not segregate for either primary in S_1; but in S_2, it segregated for one related primary trisomic, i.e., dark green leaf (Table 6, f). The phenotype was very difficult to differentiate in the greenhouse, and so these could not be identified in S_1.

One of the unique features of the breeding behavior by which tertiary trisomics differ from other types of trisomics is the regular appearance of the two related primary trisomics in their progenies (Khush and Rick 1967). This is illustrated by the segregating progenies of dark green leaf and convex leaf tertiary trisomics (Table 6). Translocation heterozygote I-52 was the only stock yielding all four trisomic phenotypes expected from a translocation heterozygote, i.e., two primaries and two tertiaries. Similar results have been obtained in other species. In tomato, Khush and Rick (1967) obtained tertiary trisomics from four translocation stocks. None of the four stocks yielded all four expected trisomic categories. T5-7 and T7-11 each produced one tertiary and two related primaries, whereas T9-12 yielded both tertiaries but one related primary.

Chromosome identity in translocation stocks

Translocation stock II-121 has produced the weak stem trisomic as did I-97 (Table 4), indicating that these translocation stocks involve a common chromosome in the interchanges. A triple dose of this chromosome gives rise to the weak stem phenotype. The presence of a common chromosome has already been confirmed cytologically. When translocation stock II-121 was crossed with I-97, F_1 cytology showed a chain of six chromosomes and eight bivalents (Ashraf and Bassett 1986). Two other translocation stocks, I-99 and II-70, segregated for the puckered leaf trisomic (Table 4), indicating that they also have one common chromosome involved in their interchanges. In a triple dose, this chromosome presumably is responsible for alteration of the normal phenotype to that of puckered leaf. Our previous cytogenetic analysis of translocation stocks did not include I-99 (Ashraf and Bassett 1986) and no direct cytological test was possible.

Five primary trisomics reported in this study have directly or indirectly been isolated from the progenies of translocation heterozygotes. Work is in progress to develop them on homozygous normal chromosome background and recover additional primary trisomics to complete the full series for use in linkage studies of common bean.

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