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Heterosis for Chiasma Frequency and Quantitative Traits in Common Beans (*Phaseolus vulgaris* L.)

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Summary. Ten genotypes, including inbreds, hybrids, and advanced populations, were examined in order to elucidate the relationship between position and frequency distribution of chiasmata and quantitative traits, including yield heterosis in common beans. The hybrid and advanced population groups were determined to possess 83% and 54% increased chiasma frequency, respectively in contrast to inbred lines. The increase in chiasma frequency of these populations was further manifested in a high number of interstitial chiasmata. The regular and superior chromosome behaviour of the hybrids was found to be positively associated with quantitative measures on bean yield, harvest index and bean yield efficiency. The results were discussed from the point of view that: a) increased interstitial chiasmata may provide an effective mechanism for maintaining genetic diversity and heterosis in hybrid populations; and b) heterosis for chiasma frequency and quantitative traits may be due to dispersed genes on the chromosomes having combined intra- and interallelic interactions. The data provide evidence for the existence of positive associations between interstitially localized chiasmata with its recombination potential and regular chromosome behaviour to bean yield heterosis. The role of enhanced interstitial chiasmata to promote higher levels of genetic variation and heterozygous advantage is discussed.

Key words: Heterosis – Chiasma frequency – Quantitative traits – Genetic diversity – Interallelic interaction

Introduction

In a highly self-pollinated population, like common beans, with a more restricted range of phenotypic variation, an important aspect of genetic control may be through the regulation of chiasma frequency since, given sufficient heterozygosity, a raised chiasma frequency will lead to an increase in the range of gametic phenotypes. Normal residual heterozygosity and a chiasma frequency dependent heterozygote advantage may potentiate the importance of maintaining a panmictic gene pool and a proper level of heterosis in such inbreeding cultivars. An increased chiasma frequency in hybrid populations of pearl millet (*Pennisetum typhoides* Rich.) and its subsequent positive correlation to grain yield heterosis has been observed (Srivastava and Balyan 1977). Significant genetic associations of chiasma frequency in relation to heterosis (Rees and Thompson 1956; Dayal 1977), sex differentiation (Sarma et al. 1976), and male sterility (Singh et al. 1977) have recently been demonstrated. Some variation in the distribution of chiasmata between and within nuclei in irradiated and non-irradiated populations in *Phaseolus* species (Sinha and Roy 1976) has also been enumerated. In these studies a greater emphasis has been given to the differences in mean chiasma frequencies between populations rather than the differences of localised position and frequency distribution of chiasmata. The amount of genetic recombination depends not only on chiasma frequency but also on chiasma position; thus counting of chiasma position (interstitial and terminal) is a technique which could yield more information than studies of chiasma frequencies alone.

Genetic control over recombination by means of position and frequency distribution of chiasmata could be expected to affect the release of variability of progenies and of populations. An inbred population of rye (*Secale cereale* L.) with a relatively low chiasma frequency, about 13 per cell, but which has an unusually high frequency of bivalents with interstitial chiasmata, has been reported (Jones 1967, 1974). In view of this and of the pronounced differences in chiasma frequency among hybrid and inbred populations found by Srivastava and Balyan (1977) it seemed worthwhile to elucidate whether there is in fact any association between position and frequency distribution of chiasmata and yield and yield contributing

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quantitative traits in genetically different populations of common beans, and in particular whether interstitial chiasmata frequencies in hybrid populations are different from those in inbred populations. The cytogenetical data presented here reveal that: a) the inbred populations are characterized to possess low chiasma frequency while the chiasmata are mostly localized in terminal regions of the bivalents; b) in the cases of the hybrids and advanced populations, respectively, there is a spreading of the chiasmata to interstitial regions as the chiasmata frequency increases; and c) the hybrid populations exhibit heterosis; there is a positive association between increased interstitial chiasmata frequency and key morphological and physiological traits, including those of bean yield and harvest index.

Materials and Methods

Seeds from ten genotypes of common beans (*Phaseolus vulgaris* L., 2n = 2X = 22) including inbreds, hybrids and advanced populations, as listed below, were grown in the field under normal conditions in a randomized block design with three replications.

Inbreds: ICA Duva, ICA Pijao, ICA Tui
Hybrids (F_1 generation):
F_{1a} = (ICA Duva X P.I. 312004)
F_{1b} = (ICA Pijao X P.I. 613653)
F_{1c} = (ICA Tui X Preto 141)
Advanced populations (F_4, F_5, and F_6 generations)
F_{4a} = (ICA Duva X P.I. 312004)
F_{4b} = (ICA Pijao X P.I. 613653)
F_5 = (ICA Duva X P.I. 312004)
F_6 = (ICA Duva X P.I. 312004)

The three inbred genotypes were the products of selection from local materials and represented relatively old commercial varieties. The term advanced populations refers to segregating population groups in F_4, F_5 and F_6 generations which were produced after the respective crosses were first made. Bean seeds were planted at a density of 100 plants/m². Young flowers from as many plants at the appropriate stage per genotype as was possible were taken between 8 A.M. until 10 A.M. and fixed in Carnoy's solution (6 parts absolute alcohol: 3 parts chloroform: 1 part glacial acetic acid). In order to obtain better transparency of the cytoplasmic background, the anthers were subjected to a short pretreatment in 1N HCL (24°C for 1-2 min.) and thereafter washed thoroughly to remove excess acid and acid soluble materials. Pollen mother cells (PMC's) from pretreated anthers were examined using the 2% acetocarmine squash technique. PMC's only in diplotene stage were scored for chiasmata; all chiasmata were scored terminal in bivalents with frequencies of 1 and 2; interstitial chiasmata were recorded in bivalents with 3 or more. The procedures of scoring chiasma distribution with respect to interstitial and terminal types were similar to those described by Rees and Jones (1977). Differentiation was also made between rod bivalents with one and two chiasmata and ring bivalents with two and three chiasmata. Diakinetic cells were not used since chiasma frequency is sometimes difficult to score at this stage. Meiotic irregularities were scored as number of univalents at metaphase 1 (M1), laggards at anaphase 1 (A1) and anaphase 2 (A2), and micronuclei at telophase 2 (T2). A minimum of 3500 PMC's from twenty five randomly selected plants per genotype were studied for cytogenetic analysis.

A minimum of 50 plants per genotype per replication were selected for quantitative measurements. The data on the following quantitative traits: bean yield, maximum leaf area (postflowering), total dry matter, and days to maturity were recorded under normal field and laboratory conditions. Estimates for other characters were made as follows: harvest index = bean yield/total dry matter X 100; bean yield efficiency = bean yield/days to maturity; and total dry matter efficiency = total dry matter/days to maturity. Total dry matter was equal to weight of stem, petioles, pods and beans at maturity plus maximum leaf dry matter at post-flowering.

Results and Discussion

Results obtained on position and frequency distribution of chiasmata in ten genotypes representing three groups of populations are summarized in Table 1. The hybrid populations (F_{1a}, F_{1b}, F_{1c}) showed high mean chiasma frequency per cell relative to the inbred populations. The advanced populations (F_{4a}, F_{4b}, F_5, F_6) also exhibited marked increase in mean chiasma frequency per cell. In contrast to the inbred populations (ICA Pijao, ICA Duva, and ICA Tui), both the hybrid and advanced populations demonstrated 83% and 54% increased chiasma frequency, respectively. The data on the distribution of bivalents with different numbers of chiasmata for the three groups of populations revealed that the hybrids were additionally characterized to possess bivalents with four chiasmata; this feature was never observed in the other two population groups. Bivalents with three chiasmata were, however, a common feature in both hybrid and advanced populations. The inbred populations on the contrary possessed a majority of bivalents with two chiasmata. More important were the results regarding position distribution of chiasmata in these population groups also presented in Table 1. The hybrid and advanced populations showed a relatively high frequency of interstitial chiasmata. The superiority in the amount of interstitial chiasmata in hybrid and advanced populations over inbred populations were 300% and 155%, respectively.

There have been some reports (Hazarika and Rees 1967; Crowley and Rees 1968; Ellis et al. 1973; Hussain 1976) from higher plants indicating that an increase in chiasma frequency improves meiotic regularity. It is not clear whether the meiotic improvement was due to an increase in chiasma frequency or due to position distribution of chiasmata. It is important in this context to bear in mind that bean chromosomes are small (Mok and Mok 1977), and structural configurations may be difficult to characterize. Some of the frequently observed structural configurations of bean chromosomes were ring bivalents at diplotene with 2, 3, and 4 chiasmata in hybrid popula-

Table 1. Cytogenetical data obtained on position and frequency distribution of chiasmata in 10 genotypes of common beans

	Mean chiasma frequency per cell ± S.D.	Bivalent distribution with different number of chiasmata (%)				Position distribution of chiasmata (%)	
		1	2	3	4	Terminal	Interstitial
ICA Pijao	7.82 ± 1.67	22.0	68.2	9.8	–	96.74	3.24
ICA Duva	5.96 ± 1.57	30.2	59.4	10.4	–	92.59	7.41
ICA Tui	5.68 ± 2.10	19.5	63.2	17.3	–	94.24	5.76
F1a	10.06 ± 2.40	12.7	31.3	45.8	10.2	79.60	20.36
F1b	11.24 ± 2.12	14.0	22.1	58.2	5.7	77.70	22.30
F1c	12.45 ± 2.34	4.9	28.2	62.7	4.2	77.0	23.0
F4a	9.62 ± 3.21	27.2	38.3	34.5	–	88.5	11.5
F4b	9.56 ± 3.42	24.1	35.7	40.2	–	86.6	13.4
F5	10.31 ± 2.45	18.2	55.4	36.4	–	87.8	12.1
F6	8.62 ± 2.70	20.6	48.2	31.2	–	85.7	14.3

The data represent mean values from a range of 2500 to 3500 PMC's studied per genotype

tion groups. The inbred populations, in contrast, showed a few rod bivalents in addition to ring bivalents with 2, or occassionally three, chiasmata. The chromosomal irregularities were scored at M1, A1, A2, and T2 in the three groups of populations and the respective results are condensed in Table 2. Most commonly observed chromosomes irregularities in the inbred and advanced populations were M1 univalents, A1 laggards, A2 laggards and T2 micronuclei, whereas the hybrid population groups showed improved meiotic regularities. While there was some trace of M1 univalents and A2 laggards in the two hybrid populations, the third hybrid (F$_{1c}$) showed exceptionally regular meiosis. In general, chromosome behaviour in case of hybrid populations seemed more regular because there was no trace of instabilities at advanced A2 or T2 stages.

The data presented so far indicate that the hybrid populations possess markedly increased mean chiasma frequency per cell and this increased chiasma frequency is reflected in a high number of interstitial chiasmata per cell and meiotic chromosome regularity. Many physiological and morphological traits related to bean yield in *Phaseolus vulgaris* have been reported to be governed by one or a few genes (Bliss 1971; Ibrahim and Coyne 1975; Coyne and Stadman 1977). To this end, quantitative traits of ten genotypes of common beans were analysed to further elucidate whether there is a positive association between position and frequency distribution of chiasmata to heterosis in hybrid populations. The hybrids exhibited superiority (72%-100%) in terms of bean yield, harvest index, and bean yield efficiency when compared to the mean values of the three inbred lines (Table 3). The advanced populations were also found to exhibit hybrid superiority (35%-50%) over the inbred populations in regard to bean yield, harvest index and bean yield efficiency. These morphological and physiological observations are in conformity with the data on the position and frequency distribution of chiasmata and chromosome behaviour in the three population groups as illustrated in Tables 1 and 2.

Table 2. Data on mean and range of different meiotic chromosome irregularities in 10 genotypes of common beans

	M1 univalents per cell	A1 laggards per cell	A2 laggards per cell	T2 micronuclei per quartet
ICA Pijao	2.1 (0-4)	2.3 (0-4)	1.4 (0-5)	0.42 (0-3)
ICA Duva	2.7 (0-4)	1.2 (0-2)	1.2 (0-4)	0.34 (0-3)
ICA Tui	3.1 (0-5)	3.3 (0-7)	2.1 (0-4)	0.44 (0-4)
F1a	0.8 (0-2)	0.45 (0-2)	–	–
F1b	0.6 (0-2)	0.35 (0-1)	–	–
F1c	–	–	–	–
F4a	2.1 (0-4)	1.9 (0-3)	1.2 (0-3)	0.18 (0-3)
F4b	2.5 (0-7)	2.3 (0-4)	1.5 (0-5)	0.32 (0-4)
F5	1.5 (0-2)	1.2 (0-2)	1.3 (0-4)	0.29 (0-3)
F6	2.6 (0-8)	3.2 (0-6)	2.4 (0-4)	0.37 (0-4)

Approximately 500 PMC's/meiotic stage/genotype were studied

It is of interest that the hybrid and advanced populations, besides being heterotic for bean yield and other key quantitative traits, exhibit a higher degree of interstitial chiasmata than the inbred populations. In terms of recombination it could be interpreted that in bivalents with two or fewer chiasmata the gene sequences at all but the ends of the chromosomes are unaffected; with three or four chiasmata per bivalent, gene sequences in the middle regions of the chromosomes are vulnerable to disruption by crossing over. It is suggested, therefore, that increased chiasma frequency as a result of physically spreading of the chiasmata to interstitial regions is important in recombining gene segment of homologous chromosomes, and if gene loci are heterozygous, they may lead to effective genetic recombination and population variability. An examination of chiasma frequency in a predominantly poly-

Table 3. Bean yield and key quantitative traits in 10 genotypes of common beans.

	Bean yield (gr/plant)	Maximum leaf area (mm^2/plant)	Total dry matter (gr/plant)	Harvest index (%)	Days to maturity	Bean yield efficiency (gr/day)	Total dry matter efficiency (gr/day)
ICA Pijao	8.46 ± 0.98	105 ± 8	28.2 ± 2.46	30	75	0.113	0.376
ICA Duva	9.56 ± 1.28	95 ± 12	34.1 ± 1.82	20	68	0.141	0.502
ICA Tui	9.40 ± 2.30	97 ± 9	36.2 ± 2.34	26	70	0.131	0.516
F1a	14.56 ± 1.80	130 ± 12	30.34 ± 1.35	48	62	0.234	0.489
F1b	16.28 ± 1.40	128 ± 8	36.17 ± 1.81	45	64	0.254	0.565
F1c	17.56 ± 2.10	118 ± 7	33.76 ± 1.42	52	60	0.292	0.560
F4a	13.3 ± 1.92	108 ± 12	33.25 ± 2.42	40	67	0.198	0.496
F4b	10.9 ± 2.87	118 ± 14	25.95 ± 2.72	42	68	0.160	0.382
F5	12.5 ± 1.80	124 ± 10	32.89 ± 2.82	38	65	0.192	0.506
F6	11.6 ± 2.10	121 ± 9	30.45 ± 1.78	38	60	0.194	0.508

The data represent mean values ± S. D. from observations on 150 plants per genotype

morphic population of *Cepaca nemoralis* L. (Bantock 1972) revealed the occurrence of a limited number of interstitial chiasmata in its bivalents. This led to the hypothesis that the localized chiasmata may be a reflection of the need to preserve 'effective epistatic gene complexes' in this population. Considering the fact that interstitial and terminal chiasmata are under separate genetic control (Zarchi et al. 1972; Price 1974), it could be suggested that interstitial chiasmata are more important in producing effective recombination and polymorphism in populations.

Although the heritability of position and frequency distribution of chiasmata in *Phaseolus vulgaris* remains to be worked out, genetic control of chiasma frequency (Rees 1961), variance (Price 1974), and distribution along bivalents (Jones 1967) by 'polygenes' have been shown in other species, and several investigators have argued that chiasma variation is adaptive (Rees and Ahmad 1963; Crowley 1969). However, direct evidence is lacking that high frequencies in hybrids or, conversely, low chiasma frequencies in inbreds, are a result of selection. It is possible that they are merely a second-order effect due to greater homology of the chromosomes. It is generally considered that there are some localized points ('the Zygomere hypothesis') along the chromosome length where pairing is initiated (Sybenga 1966). In the case of a single point of pairing initiation along the entire length of the chromosome, as per the requirement of the zygomere hypothesis, only bivalents with total pairing would be formed since partner exchange leading to incomplete pairing is excluded. One of the possible cytological consequences of this would be more complete zygotene pairing which would perhaps facilitate the formation of interstitial chiasmata. It has been emphasized that the failure of pairing of zygotene could result in both a reduction and a localization of chiasmata and that the distribution of chiasmata may be determined by the position at which the 'synaptinemal complex' forms between homologues (Henderson 1969).

The experience of plant breeders indicates that there is a certain residual heterozygosity in almost every autogamous cultivar and that the response to directional selection of progeny of individual plants is often improved ideotypes. Within population variability has actually been demonstrated in experimental populations of *Phaseolus lunatus* (Allard et al. 1968) and in wild oats (Imam and Allard 1965). The important phenotypic expressions of heterotic hybrids, taking into consideration all the physiological and genetic analyses, are increased height, leaf area, growth, dry matter accumulation, early flowering, higher yield and higher harvest index (Bennett et al. 1977; Rosielle and Frey 1977). The results described so far lead to the suggestions that: 1) there is a positive genetic association between increased interstitial chiasmata frequency and bean yield heterosis in hybrid populations; and 2) enhanced interstitial chiasmata frequency may be an effective mechanism for the maintenance of genetic diversity and heterosis. Such inferences are further supported by the recent results of Tehrani and Wricke (1977) where direct positive correlations between chiasma frequency – particularly the amount of interstitial chiasmata, and three agronomically important characters, namely plant height, 1000 kernel weight and kernel yield in rye (*Secale cereale* L.), have been observed.

How could the results on heterosis for chiasma frequency and quantitative traits be interpreted in genetic terms? The genes controlling for chiasma frequency and several quantitative traits can not be considered common. Diallel analysis carried out to study the genetic nature of heterosis for chiasma frequency revealed average overdominance for this character and the overdominance was found to be partly due to interallelic interaction (Manga and Pantulu 1974). The whole chromosome analysis in substituted wheat lines also indicates 'chiasma heterosis' of hybrids to be the result of a greater number of dispersed genes with directional dominance derived from the substituted chromosome of the parents, combined with between chromosome interaction (Snape et al. 1977). The heterosis for chiasma frequency, as observed in hybrid and advanced populations of beans, is most likely due to dispersed genes on the chromosomes possessing combined overdominance and epistatic interactions. More data on the genetic basis for position and frequency distribution of chiasmata in both homozygous and heterozygous populations is required to fully establish the relationship between dispersed genes having intra- and interallelic interactions and chromosome heterosis.

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