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Triple test cross and six-population techniques for partitioning the components of genetic variance in faba bean (*Vicia faba*)

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SUMMARY

The efficiency of the triple test cross (TTC) and the six-population biometrical analyses was compared in terms of assessing and quantifying the components of genetic variance for two faba bean crosses: Triple White × Giza 843 and NA112 × Giza 429. Several traits were studied including days to first flower, plant height, branches/plant, pods/plant, seeds/pod, 100-seed weight and seed yield/plant. The results supported the triple test cross biometrical approach as it uses first degree statistics and can be applied to any population irrespective of its genetic architecture. Absence of a scalar relationship between triple test cross families (orthogonality) ensures independence between means and variance with no restrictive assumptions. Both methods provided evidence for epistasis, and both additive and dominance genetic components in the genetic control of the studied traits.

INTRODUCTION

Biometrical procedures are applied to assess and quantify components of genetic variance in breeding populations. Each procedure has its merits and limitations, and a method suitable for a particular situation may not yield valid genetic information under different conditions. Biometrical procedures must make few assumptions and provide reliable estimates of genetic variance. A critical assumption is the absence of epistasis, which may result in biased estimates of components of genetic variance. Therefore, breeders are encouraged to apply biometrical approaches which take account of epistasis. In this respect the triple test cross (TTC) is a powerful design (Kearsey & Jinks 1968). The six-population technique, based on a number of related generations, is considered adequate to provide some genetic information (Mather & Jinks 1982). There is no review of the literature concerning the relative efficiency of triple test cross and six-population methods for faba bean.

The present investigation compared the six-population and triple test cross approaches in generations derived from two separate crosses of faba bean, to estimate the components of genetic variance for morphological traits and yield and its component characters.

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MATERIALS AND METHODS

The investigation was carried out in the Agricultural Experimental Farms of Assiut and South Valley (Sohag) Universities, Egypt during four seasons; 1994/95, 1995/96, 1996/97 and 1997/98. Two separate crosses of faba bean were used as a basic for genetic analysis: 1 – Triple White × Giza 843 and 2 – NA112 × Giza 429. The description and origin of parental genotypes is given in Table 1. From 1994/95 to 1996/97 the three types of families, comprising a triple test cross, were derived from each of the parental crosses, i.e. L_{1i} ($F_{2i} \times P_{1i}$), L_{2i} ($F_{2i} \times P_{2i}$) and L_{3i} ($F_{1i} \times F_{2i}$).

In November 1997, the 72 triple test cross families (L_{1i}, L_{2i} and L_{3i}) were grown in single row plots; each row was 3 metres long and 60 cm wide with single-seed hills spaced 20 cm apart on one side of the ridge. Also, the six populations (P_1, P_2, F_1, F_2, Bc_1 and Bc_2) for each cross were grown as single plants. A randomized complete block design with three replications was used. At harvest, data were recorded on 10 competitive plants in each of the TTC families, while for the six populations, data were collected on 30

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Table 1. *Description and origin of four faba bean* (Vicia faba *L.*) *parental genotypes*

Genotypes	Sub species	Origin	Flowering	Seed		Hilum colour	Yielding ability	100-seed weight (g)
				Size	Colour			
Giza 429	Eu-faba Equina	Egypt, selection from Giza 402	Early	Medium	Buff	Black	High	70–75
Giza 843	Eu-faba Equina	Egypt, through hybridization	Early	Medium	Buff	Black	High	80–85
Triple White	Eu-faba Equina	An introduction from Sudan	Early	Medium	White	Colourless	Medium	45–55
NA112	Paucijuga	An introduction from Pakistan	Late	Small	Black	Black	Low	10–20

individual plants for P_1, P_2 and F_1s, 25 plants in each of Bc and 70 plants for F_2 in each replicate.

The number of days to first flower was estimated on a plot basis, and plant height (cm), number of branches/plant, number of pods/plant, number of seeds/pod, 100-seed weight and seed yield/plant were recorded for each plant.

The variance of the comparison $(\bar{L}_{1i}+\bar{L}_{2i}-2\bar{L}_{3i})$ was used to test for the presence of epistasis following Kearsey & Jinks (1968). The sum of squares due to epistasis was partitioned into the (i) additive × additive, the (j) additive × dominance and (l) dominance × dominance types of interaction. The variances of $(\bar{L}_{1i}+\bar{L}_{2i}+\bar{L}_{3i})$ and $(\bar{L}_{1i}-\bar{L}_{2i})$ were used to detect and estimate additive (D) and dominance (H) genetic components according to Jinks & Perkins (1970). The average degree of dominance was estimated as $(H/D)^{1/2}$. The direction of dominance was determined according to the correlation coefficient (r) of $(\bar{L}_1+\bar{L}_2)$ sums and $(\bar{L}_1-\bar{L}_2)$ differences (Mather & Jinks 1982). Broad and narrow sense heritability values were computed using the components of genetic variance computed from TTC. Predicting the properties of recombinant $d/\sqrt{D}$% lines were computed according to Jinks & Pooni (1976), where d is the additive genetic component of line performance and D is the additive genetic variance.

For the six-populations analysis method, the scaling tests A, B and C were applied according to Mather & Jinks (1982) to test the appropriate genetic model. The six-parameters genetic model outlined by Jinks & Jones (1958) was used to obtain the main gene effects and the different gene interactions. Components of genetic variance, additive (D), dominance (H) and environmental (E) variances were estimated according to Mather and Jinks (1982). Heritability in broad (Tb) and narrow senses (Tn) was estimated from the variance components.

RESULTS AND DISCUSSION

Triple test cross (*TTC*)

The mean squares testing for epistasis (Table 2) are significant for all studied characters in the two crosses, except for number of seeds/pod in TTC_1 and 100-seed weight in TTC_2. Further partitioning of the epistasis mean squares revealed significant type (i) epistasis (additive × additive) for all characters except number of days to first flower in TTC_2. The (j+l) type of epistasis was significant for number of days to first flower, plant height (both crosses), number of branches/plant, pods/plant, 100-seed weight (TTC_1), seeds/pod and seed yield/plant (TTC_2). The results also revealed that type (i) epistasis was larger in magnitude than the (j+l) type for all the studied characters in the two crosses, except number of days to first flower in TTC_2. The predominance of additive interactions suggests that selection in early segregating generations would be effective in improving these traits.

The analysis of variance for sums and differences between the test cross families (Table 3) was used to detect additive and dominance effects respectively even in the presence of epistasis. Significant mean squares for additive and dominance effects were obtained for all the studied characters in the two crosses.

Six populations

Tests for non-allelic interactions using the A, B and C scaling tests (Table 4) indicated that at least one of the scaling tests was significant for all the studied characters in both crosses, suggesting the presence of epistasis. Additive gene effects (d) were significant for all the studied characters in the crosses except for number of days to first flower, plant height and number of pods/plant in cross 1 only. Also, dominance gene effects (h) were significant for all characters in both crosses, except for number of branches/plant (cross 2) and number of seeds/pod (cross 1 and 2). With respect to epistatic interactions the results indicated that type (i) effects (additive × additive) were significant for number of days to first flower, plant height, 100-seed weight and seed yield/plant (both crosses), number of branches/plant (cross 1) and number of seeds/pod (cross 2), while type (j)

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Table 2. *Mean squares for test of epistasis in the two sets of triple test crosses*

Epistasis ($\bar{L}_{1i}+\bar{L}_{2i}-2\bar{L}_{3i}$)	D.F.	Number of days to first flower	Plant height	Number of branches/ plant	Number of pods/plant	Number of seeds/pod	100-seed weight	Seed yield/ plant
TTC_1								
Overall epistasis	24	60·51**	287·08**	4·20**	425·97**	0·438	507·86**	1759·56**
[i] type	1	483·02**	2560·00**	30·00**	2541·40**	7·400**	7535·90**	30337·10**
[j+l] types	23	42·14**	188·26*	3·08*	333·99**	0·135	202·29**	517·06
Error (within families)	648	19·98	109·83	2·01	182·70	0·350	89·02	534·73
TTC_2								
Overall epistasis	24	32·48**	855·76**	12·41**	1341·01**	0·275**	42·58	1609·37**
[i] type	1	1·22	11616·70**	159·30**	17717·10**	2·900**	347·20**	5933·70**
[j+l] types	23	33·84**	387·90**	6·03	629·00	0·161**	29·33	1421·36**
Error (within families)	648	12·93	169·53	4·62	442·47	0·087	30·02	538·93

*, ** significant at 0·05 and 0·01 levels of probabilities, respectively.

Table 3. *Analysis of variance for sums* (*additive*) *and differences* (*dominance*) *for triple test crosses*

	D.F.	Number of days to first flower	Plant height	Number of branches/ plant	Number of pods/plant	Number of seeds/pod	100-seed weight	Seed yield/ plant
TTC_1								
Additive ($\bar{L}_1+\bar{L}_2+\bar{L}_3$)								
Between	23	382·92**	440·42**	6·64**	1015·78**	0·522**	340·66**	2454·73**
Within	648	17·45	84·92	1·68	128·43	0·065	49·47	424·92
Dominance ($\bar{L}_1-\bar{L}_2$)								
Between	23	35·08**	171·73**	3·13**	188·86**	0·130**	185·97**	641·41*
Within	432	17·02	76·19	1·67	120·59	0·067	43·56	380·13
TTC_2								
Additive ($\bar{L}_1+\bar{L}_2+\bar{L}_3$)								
Between	23	249·23**	3362·36**	41·41**	2444·75**	0·852**	203·64**	4053·62**
Within	648	12·17	140·03	3·33	281·04	0·067	21·82	413·14
Dominance ($\bar{L}_1-\bar{L}_2$)								
Between	23	41·00**	227·06**	9·10**	466·79**	0·235**	65·01**	1428·78**
Within	432	12·89	136·01	3·29	245·91	0·074	19·88	418·37

*, ** significant at 0·05 and 0·01 levels of probabilities, respectively.

effects (additive × dominance) were significant in both crosses for number of days to first flower and plant height and in cross 2 for number of branches/plant, pods/plant, 100-seed weight and seed yield/plant. The type (l) effects (dominance × dominance), were significant for number of days to first flower, 100-seed weight and seed yield/plant (both crosses), branches/plant (cross 1), plant height and pods/plant (cross 2).

Six-populations v. *triple test cross methods*

The 'six populations' and triple test cross analyses of the two faba bean crosses were compared for their ability to detect types of gene action. The results in Table 5 reveal that the relative importance of additive and dominance components of variance in the six populations analysis is in complete agreement with the results of TTC analysis for plant height and 100-seed weight in both crosses. However, while all the studied characters showed a larger magnitude of additive compared with dominance effects in the triple test cross analysis (Table 5) for number of days to first flower, number of branches/plant, number of pods/plant, number of seeds/pod and seed yield/plant the dominance effect was greater than the additive effect in the 'six-population' analysis. Similar conclusions are indicated by the average degree of dominance $(H/D)^{1/2}$. This discrepancy in the relative importance of additive and dominance gene effects may be attributed to bias in estimating the two components

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Table 4. *Scaling test and gene effects components in the two crosses*

Items	Number of days to first flower	Plant height	Number of branches/plant	Number of pods/plant	Number of seeds/pod	100-seed weight	Seed yield/ plant
TTC_1							
A	−3·72** ± 0·677†	4·03* ± 1·894	1·43** ± 0·368	1·74 ± 2·408	0·04 ± 0·071	−3·30 ± 2·281	−3·29 ± 3·262
B	2·15** ± 0·583	−3·26 ± 2·055	0·90* ± 0·424	−0·85 ± 2·327	0·07 ± 0·075	−4·65* ± 2·160	0·59 ± 2·342
C	−7·35** ± 0·845	−7·39** ± 2·867	0·6 ± 0·543	−9·05** ± 3·206	0·21* ± 0·101	−31·55** ± 3·338	−38·92** ± 4·515
d	0·75 ± 0·412	0·34 ± 1·244	−0·61* ± 0·248	1·37 ± 1·516	−0·23** ± 0·045	−16·16** ± 1·444	−11·61** ± 2·115
h	−2·88* ± 1·131	10·19** ± 3·493	2·20** ± 0·675	9·02* ± 4·150	−0·06 ± 0·125	34·51** ± 4·214	39·26** ± 5·794
i	5·78** ± 1·113	8·16* ± 3·403	1·70** ± 0·654	6·46 ± 4·059	−0·10 ± 0·121	23·60** ± 4·145	36·22** ± 5·658
j	−2·94** ± 0·434	3·65** ± 1·322	0·27 ± 0·260	−0·45 ± 1·599	−0·02 ± 0·048	0·68 ± 1·508	−1·94 ± 2·206
l	−4·21* ± 1·855	−8·93 ± 5·712	−4·03** ± 1·132	−3·87 ± 6·860	−0·01 ± 0·207	−15·65* ± 6·672	−33·52** ± 9·588
TTC_2							
A	−10·64** ± 0·811	2·48 ± 1·997	−3·27** ± 0·527	−45·93** ± 4·579	−0·10 ± 0·071	−1·01 ± 1·127	17·59** ± 2·613
B	−6·92** ± 0·799	−12·98** ± 2·087	2·46** ± 0·498	20·20** ± 4·186	−0·02 ± 0·076	5·56** ± 1·708	31·50** ± 2·957
C	−1·58 ± 1·071	20·44** ± 2·975	−1·17 ± 0·695	−11·73 ± 6·243	−0·48** ± 0·099	−17·63** ± 2·014	−16·73** ± 3·942
d	8·47** ± 0·517	−8·27** ± 1·302	1·14** ± 0·319	13·96** ± 2·941	0·14** ± 0·045	−32·27** ± 0·923	−21·81** ± 1·748
h	−14·89** ± 1·399	−22·38** ± 3·732	1·18 ± 0·870	−18·97* ± 8·298	0·16 ± 0·125	11·49** ± 2·591	71·60** ± 4·849
i	−15·98** ± 1·368	−30·94** ± 3·655	0·36 ± 1·845	14·00 ± 8·203	0·36** ± 0·121	22·18** ± 2·543	65·82** ± 4·700
j	−1·86** ± 0·543	7·73** ± 1·381	−2·87** ± 0·342	−33·07** ± 3·005	−0·04 ± 0·050	−3·29** ± 0·999	−6·96** ± 1·820
l	33·54** ± 2·329	41·44** ± 5·998	0·45 ± 1·454	39·73** ± 12·317	−0·24 ± 0·207	−26·73** ± 4·207	−114·91** ± 8·027

*, ** significant at 0·05 and 0·01 levels of probabilities, respectively.

† S.E.

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Table 5. *Estimates of genetic components of variation for two crosses of faba bean using the six-populations and triple test cross designs*

Characters	Days to first flower		Plant height		Branches/plant		Pods/plant		Seeds/pod		100-seed weight		Seed yield/plant	
Cross number†	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Six-populations														
D	3·78	2·12	51·38	91·20	0·24	0·76	37·26	419·24	0·027	0·028	151·10	32·58	70·60	60·00
H	11·12	17·50	32·76	22·60	2·81	3·88	126·11	526·92	0·052	0·053	24·40	25·44	247·36	96·75
E	2·65	5·12	36·96	35·13	1·55	2·66	45·36	87·77	0·057	0·057	34·35	17·47	88·21	75·38
$\sqrt{H/D}$	1·72	2·87	0·80	0·50	3·60	2·26	1·84	1·12	1·38	1·37	0·40	0·88	1·87	1·27
F	−2·61	0·14	12·93	6·66	0·60	0·08	−4·31	−52·72	0·008	0·012	−11·17	21·21	7·36	27·02
h^2 (b)%	63·80	51·52	47·83	59·33	34·68	33·67	52·51	79·55	31·66	32·38	70·39	56·47	52·41	41·82
h^2 (n)%	25·82	10·05	36·26	52·79	5·06	9·48	19·90	48·85	16·25	16·73	65·13	40·61	19·05	23·15
$d/\sqrt{D}$%	35·20	–	48·40	19·49	10·75	9·51	41·29	24·83	9·01	20·33	9·51	–	8·38	0·25
Triple test cross														
D	98·26	63·21	94·80	859·29	1·32	10·16	23·63	576·99	0·122	0·209	77·65	48·48	541·28	970·79
H	7·23	11·24	38·21	36·42	0·58	2·32	27·31	88·35	0·025	0·064	56·97	18·05	104·51	404·16
E	3·96	3·59	59·56	24·04	1·43	1·73	87·60	162·74	0·049	0·040	26·73	11·56	299·40	246·50
$\sqrt{H/D}$	0·27	0·42	0·63	0·21	0·66	0·48	0·34	0·39	0·45	0·55	0·80	0·61	0·44	0·64
r (sums/diff.)	0·0034	−0·3225	−0·0177	0·0266	−0·3961	0·4407*	0·1975	0·6879**	−0·3700	−0·2573	−0·4186*	−0·8268**	−0·2857	−0·0382
H^2 (b)%	92·78	90·56	48·88	94·80	36·04	76·58	58·82	65·55	57·85	75·08	66·50	71·41	49·78	70·41
H^2 (n)%	89·49	83·16	40·68	92·84	29·52	68·72	55·61	60·95	52·47	65·11	48·65	59·93	45·40	58·28
$D/\sqrt{D}$%	37·45	10·03	32·28	36·32	8·23	22·06	49·60	11·70	25·78	18·67	1·10	–	21·77	7·78

*, ** significant at 0·05 and 0·01 levels of probability, respectively.
† 1: Triple White × Giza 843; 2: NA112 × Giza 429.

of genetic variation because of the presence of epistasis. However, the triple test cross analysis is expected to provide more reliable estimates of additive and dominance components even if epistasis is present. Similar conclusions have been drawn by Chahal & Singh (1974) in *Gossypium*, Pooni *et al.* (1978) in *Nicotiana* and Chaudhary (1997) in *Vigna angularis*.

The six populations method provides only crude estimates of genetic variance components (additive and non-additive) even when epistasis is absent. On the other hand, TTC analysis not only provides an independent test of epistasis but, in the absence of epistasis, an independent and equally precise estimate of additive and dominance genetic components. The partitioning of epistasis in the TTC analysis can also indicate what proportion may be fixable through selection. The type (i) epistasis (additive × additive) is fixable and the relative proportion of type (i) epistasis to the (j) and (l) sub-components can help in determining breeding strategy.

The triple test cross analysis also provides additional information about the direction of dominance based on the correlation between the sums and differences of the test cross families. In the present investigation, the correlation of sums and differences (r) was significant for number of branches/plant and number of pods/plant in one cross and 100-seed weight in both crosses (Table 5). This suggests unidirectional dominance for these traits and ambidirectional dominance for the others.

Narrow sense heritability estimates in the six-populations method were high (> 50%) for plant height (cross 2) and 100-seed weight (cross 1), moderate (30–50%) for plant height (cross 1), number of pods/plant and 100-seed weight (cross 2), and low (< 30%) for the remaining characters. However, in the TTC analysis heritability was high for most characters, i.e. days to first flower (two crosses), plant height (cross 1), 100-seed weight (cross 2) and seed yield/plant (cross 2). Moderate values were obtained for plant height, 100-seed weight and seed yield in cross 1 (Table 5). These results again reflect a higher efficiency of genetic analysis in the TTC than in the six-populations approach.

With respect to predicting the performance of recombinant lines the six-populations method is in agreement with the TTC analysis in obtaining high values (> 30%) for days to first flower, plant height and number of pods/plant in cross 1, a moderate value (30–15%) for number of seeds/pod in cross 2 and low values (< 15%) for number of days to first flower and seed yield/plant in cross 2, branches/plant in cross 1 and 100-seed weight in both crosses (Table 5).

Several studies have been carried out to compare the TTC design with other designs. Fulker (1972) compared TTC with full and half diallel analysis and suggested that TTC is powerful in providing a valid test for epistasis and better estimates for the level of dominance. Chaudhary (1997) found that the TTC design is advantageous in providing an unambiguous test for the presence of epistasis, while the line × tester design provides additional information, particularly with regard to the effects of general and specific combining ability and variances, thus helping breeders in the choice of better parents.

A conclusion of the present study is that the highest proportion of recombinant lines was obtained for plant height, number of pods/plant and 100-seed weight in the NA112 × Giza 429 cross. Therefore, it is a promising source of material for faba bean breeding programmes.

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