Theor Appl Genet (1994) 89: 1027–1031

H. S. Pooni · P. S. Virk · D. T. Coombs
M. K. U. Chowdhury

The genetical basis of hybrid vigour in a highly heterotic cross of *Nicotiana tabacum*

Received: 13 April 1994 / Accepted: 28 July 1994

Abstract The genetical control of F_1 heterosis, observed in a cross of desirable *Nicotiana tabacum* varieties, was investigated by analysing the data of the basic generations, triple test cross-families and random samples of doubled haploids (DH) and single-seed descent (SSD) lines. Analyses of the first-degree statistics revealed a complex control underlying the genetic variation, including the presence of epistasis, linkage, maternal effects and their interactions, in addition to the additive and dominance effects of the genes segregating in the cross. These analyses identified gene dispersion, directional dominance, and duplicate epistasis, as the main causes of heterosis. The triple test-cross analysis also confirmed the presence of non-allelic interactions and indicated that the dominance ratio, although inflated by epistasis, is consistently partial for all the traits. The extent of transgression in the recombinant inbred lines finally established unequivocally that, as in numerous other crosses, gene dispersion and unidirectional, but partial, dominance are the true causes of heterosis in this cross too.

Key words Basic generations · Epistasis
Maternal effects · Recombinant inbreds · Triple test cross

Introduction

Jinks (1983) outlined the genetical basis of heterosis and expounded the theory that gene dispersion and directional, but partial, dominance are its main causes. While his theory is generally accepted, it has been applied mostly to those crosses that show heterosis for one or two characters only (e.g., Pooni et al. 1978; Tapsell and Thomas 1983; Thomas and Tapsell 1983). Further, such studies have endeavoured to demonstrate the applicability of both the theory and the requisite biometrical procedures to specific experimental situations (e.g., Jinks and Pooni 1976, 1980; Pooni and Jinks 1978, 1981) and the approach is rarely applied to explain the results of an entire breeding programme. In this paper, we present the results of a series of biometrical analyses that have been applied to study the genetical basis of hybrid vigour in a highly heterotic cross of *Nicotiana tabacum* which shows significant heterosis for eight of the fifteen characters measured on it, and test whether Jinks' theory of gene dispersion/directional dominance is as applicable to this cross as it was to others.

Communicated by J. W. Snape

H. S. Pooni (✉) · P. S. Virk · D. T. Coombs · M. K. U. Chowdhury
School of Biological Sciences, The University of Birmingham, Birmingham B15 2TT, England, UK

Materials and methods

The cross under investigation was of *N. tabacum* lines SCR and S3. SCR is a German flue-cured variety which was derived from an old US variety called "Golden Harvest" while S3 is an air-cured Burley variety of Swiss origin (Coombs 1980). Seeds of both pure breeding lines were obtained from Carreras Rothmans Limited during the late seventies and the plants were crossed to obtain the F_1 seed. In consecutive seasons, the SCR × S3 cross was selfed to obtain the F_2 seed and a random sample of 23 F_2 plants were both individually selfed and crossed to each of the SCR, S3, and F_1 (SCR × S3) genotypes to produce the F_3, L_{1i}, L_{2i} and L_{3i} sets of families respectively. These families/generations, supplemented with the parental and the reciprocally produced F_1, F_2, B_1 and B_2 generations of the SCR × S3 cross, formed the material of the first experiment.

This experiment had two blocks which were raised at different U.K. sites some 17 miles apart; viz., the University of Birmingham (normal density) and Avoncroft near Bromsgrove (high density). Individual plant randomisation was applied in both blocks and the experiment was initiated in the glasshouse to avoid the problems of failed/slow germination during early summer. Further details of the experimental structure are given in Table 1.

Parallel to the above breeding/assessment work, a single-seed descent programme was initiated from the F_2 of the SCR × S3 cross to extract a large sample of inbred lines as quickly as possible. The material was put through two cycles of selfing per year and a mixture of F_6, F_7 and F_8 families was produced 3 years later. In addition, 60 doubled haploid (DH) lines were also produced from the F_1 generation of the cross by the method of haploidy/diploidy (Chowdhury 1984). These materials and the basic generations of the original cross were assessed in another experiment. Again the experiment was raised in two blocks, one at high density and the other at normal density.

Table 1 Numbers of families and sibs raised per density in each of the two assessment experiments

Generation	Fams	Sibs	Total per density	Total per experiment
Experiment-1				
SCR, S3, F_1 and RF_1	4	40	160	320
F_2	1	160	160	320
$F_1 \times SCR$ and $SCR \times F_1$	2	80	160	320
$F_1 \times S3$ and $S3 \times F_1$	2	80	160	320
F_3 families	23	20	460	920
$F_2 \times SCR$ families	23	20	460	920
$F_2 \times S3$ families	23	20	460	920
$F_2 \times F_1$ families	23	20	460	920
Total			2480	4960
Experiment-2				
SCR, S3, F_1 and RF_1	4	25	100	200
F_2	4	25	100	200
$F_1 \times SCR$ and $SCR \times F_1$	4	25	100	200
$F_1 \times S3$ and $S3 \times F_1$	3	25	75	150
F_6 families	129	9	1161	2322
Doubled haploids	60	9	540	1080
Total			2076	4152

Table 2 A list of characters scored on individual plants

Character	Description
H1	Plant height (cm) at 12 weeks after sowing
H2	Plant height (cm) at 14 weeks after sowing
LL	Length (cm) of the largest leaf blade 15 weeks after sowing
LW	Breadth (cm) of the largest leaf blade 15 weeks after sowing
FT	Flowering time in days from sowing
HFT	Height (cm) at the time of flowering
FH	Height (cm) at the end of the season
TY	Total plant weight (g)
SY	Stem weight (g)
LN	Number of leaves on the main stem of the plant
LY	Leaf weight (g), calculated as (TY - SY)
INL	Internode length (cm), calculated as FH/LN
LR	Leaf ratio, calculated as LL/LB
LSR	Leaf stalk ratio, calculated as LY/SY
HAF	Height (cm) gained after flowering, measured as FH−HFT

Altogether 15 characters were scored in both experiments, out of which ten were measured directly on the plants. A description of the characters is provided in Table 2.

Results

Heterosis and reciprocal effects

Table 3 shows the extent of variation displayed by the means of the parental and the F_1 families for the various traits. These means are the averages of the two densities and, therefore, are equivalent to the mean performance of each genotype in the terminology of Jinks and Pooni (1980). It is apparent from the table that SCR and S3 have contrasting scores for many traits. SCR is fast growing, early flowering, tall, and high yielding, while S3 has more and larger leaves and consequently produces more leaf weight and achieves a better leaf/stalk ratio. When tested against the appropriate standard errors, these differences are significant for all the traits (except LY) suggesting that the parental lines possess markedly diverse genotypes.

Table 3 Overall means of SCR, S3, F_1(SCR×S3) and RF_1(S3×SCR) families for the various traits

Character	$\overline{SCR}$	$\overline{S3}$	$\bar{F}_1$	$\overline{RF}_1$
H1	18.31	7.39	18.84	15.66
H2	66.00	30.68	77.44	66.18
LL	51.45	57.10	58.69	54.18
LB	26.33	27.94	30.58	27.43
FT	44.17	76.69	34.30	42.04
HFT	203.81	172.98	221.43	208.11
FH	212.99	187.69	240.53	224.15
TY	1108.02	803.35	1454.44	1179.28
SY	774.46	520.63	1020.38	792.34
LN	18.61	21.28	18.49	18.42
LY	339.20	355.64	442.55	386.95
INL	11.61	9.01	13.21	12.32
LR	2.09	1.98	1.94	2.00
LSR	0.44	0.65	0.43	0.48
HAF	10.25	18.08	19.43	17.50

Table 4 Test of reciprocal effects between the F_1 families and the significance of heterosis for the various traits

Character	$\bar{F}_1$-$\overline{RF}_1$	Heterosis ($\bar{F}_1$)	Heterosis ($\overline{RF}_1$)
H1	3.18***	0.53	–
H2	11.26***	11.44***	0.18
LL	4.51***	1.59	–
LB	3.15***	2.64***	–
FT	−7.74**	−9.87***	−2.13
HFT	13.32***	17.62***	4.30
FH	16.38***	27.54***	11.16***
TY	275.16***	346.42***	71.26
SY	228.04***	245.92***	17.88
LN	0.07	−0.12	−0.19
LY	55.60*	86.91**	31.31
INL	0.89***	1.60***	0.71**
LR	−0.06	−0.04	–
LSR	−0.05*	−0.01	–
HAF	1.93	1.35	–

* $0.05 \geq P > 0.01$; ** $0.01 \geq P > 0.001$; *** $P \leq 0.001$
–, No heterosis

The reciprocal F_1s also show marked differences in performance, indicating the presence of maternal effects (Table 3). Clearly, the performance of the SCR × S3 cross is much better than that of the reciprocal, S3 × SCR, and the mean of the former is larger for 12 of the 15 traits under investigation. These differences between the reciprocal crosses are highly significant for all the characters except LN, LR and HAF (Table 4). Consequently, the SCR × S3 cross shows highly significant heterosis for nine charac-

Table 5 Summary of the best-fit models of the generation means and the corresponding χ^2 values

Character	Components in the model					
	Additive/ dominance	Epistasis	Linkage	Maternal effects	Genetic × mat. interaction[a]	$\chi^2(df)$[b]
H1	yes	yes	no	yes	no	11.64 (6)
H2	yes	yes	no	yes	yes	9.15 (5)
LL	yes	yes	yes	yes	yes	3.82 (2)
LB	yes	yes	yes	yes	yes	2.62 (2)
FT	yes	yes	yes	yes	yes	5.39 (3)
HFT	yes	yes	no	yes	no	6.05 (8)
FH	yes	yes	yes	yes	no	4.78 (5)
TY	yes	no	no	yes	yes+[lm][c]	14.18 (8)
SY	yes	yes	no	yes	yes	7.23 (6)
LN	yes	yes	no	yes	yes+[lm]	10.63 (5)
LY	yes	yes	yes	yes	yes	4.55 (4)
INL	yes	yes	yes	yes	yes	4.02 (3)
LR	yes	yes	no	no	no	9.95 (10)
LSR	yes	yes	yes	yes	no	1.05 (3)
HAF	yes	yes	yes	yes	no	2.52 (5)

[a] d.dm etc., see Mather and Jinks (1982) for further details
[b] χ^2 is non-significant for each trait
[c] [lm] is interaction of the $hm_a \times hm_b$ type

ters while the reciprocal, S3 × SCR is heterotic for only two.

The conclusive evidence that the cross is highly heterotic, however, comes from the comparison of the averaged F_1 score with that of the better parent because the hybrid still shows significant heterosis for eight characters. The general level of heterosis is quite high too, both on the measured scale and as a percentage. For instance, as a proportion of the best-parent's score, the F_1 performance is better by 23–32% for the yield characters (TY, SY and LY), by 22% for flowering time, and by between 9% and 17% for the remaining traits.

Components of means

In the present study, the components of means were estimated by the weighted least squares method (see Mather and Jinks 1982 for the procedure) but only after the significance of each source was established a priori by the scaling tests. These analyses were carried out by Pooni et al. (1987) and a summary is presented in Table 5 with the χ^2s of goodness of fit which show that an adequate model was obtained for each trait. It is apparent that the inheritance of various characters is rather complex. With the exception of total yield (TY), epistasis is detected for all the traits irrespective of the presence of heterosis. Similarly, linkage of interacting genes is detected for eight characters and maternal effects are significant for all the traits except leaf/stalk ratio (LR). Furthermore, a large proportion of differences between the parental varieties is accounted for by the additive maternal component [dm], while the dominance component [h], or the dominance and the dominance × dominance interaction components ([h]+[l]), always take a larger value than the additive component, [d], or the additive and the additive × additive interaction components ([d]+[i]) (see Mather and Jinks 1982 for definitions). This indicates that either the genes are highly dispersed in the parents or that dominance and its interactions are more important.

Opposing signs of the [h] and [l] components reveal that the cross displays predominantly duplicate interaction for most of the traits under study.

Analyses of the L_{1i}, L_{2i} and L_{3i} sets of families

The procedures of Kearsey and Jinks (1968) and Jinks and Perkins (1970) were employed to analyse these data. Variation between the L_{1i}, L_{2i} and L_{3i} sets of families was partitioned into three components, viz., $L_{1i}+L_2i-2L_{3i}$, $L_{1i}+L_{2i}+L_{3i}$ and $L_{1i}-L_{2i}$, which provided independent tests of the epistatic, additive and dominance effects of the loci that were segregating in the cross. Each trait was analysed separately and the densities/site were treated as fixed effects.

Analysis of the $L_{1i}+L_{2i}-2L_{3i}$ values showed that epistasis is an important source of variation in this cross. The 'between-sets' mean squares were significant for eight traits showing that additive × dominance and dominance × dominance interactions were present for most of the height and leaf measurements (see Kearsey and Jinks 1968 for expectations). The significance of the correction term for seven traits also pointed to the presence of additive × additive interaction. Clearly, these results are in broad agreement with those of the first-degree statistics described earlier.

Analyses of $L_{1i}+L_{2i}+L_{3i}$ and $L_{1i}-L_{2i}$ comparisons also showed that both the additive and dominance components of variance are highly significant for all but two traits, LR and HAF, for which the non-additive effects are statistically non-existent. More important, however, is the rela-

Table 6 Estimates of the additive genetic (D) and dominance (H) components of variation and of the dominance ratio, obtained from the triple test-cross analysis

Character	D	H	$\sqrt{H/D}$
H1	17.99	12.90	0.85
H2	182.00	115.12	0.80
LL	15.67	5.12	0.57
LB	3.87	2.52	0.81
FT	229.01	73.68	0.57
HFT	618.64	190.96	0.56
FH	482.57	186.98	0.62
TY	69652.00	44936.00	0.80
SY	32943.00	26126.00	0.89
LN	10.28	1.44	0.37
LY	11780.00	3622.00	0.55
INL	2.03	0.52	0.51
LR	0.028	–	–
LSR	0.008	0.002	0.50
HAF	19.60	–	–

Table 8 Estimates of r_d, the coefficient of gene association/dispersion, calculated from the parental difference and the range of the extreme scores among the recombinant lines, and of narrow heritability (h_n^2), obtained from the recombinant inbred lines experiment, for the various characters

Character	r_d	h_n^2(ND)[a]	h_n^2(HD)[a]
H1	0.44	0.21	0.16
H2	0.50	0.17	0.13
LL	0.18	0.22	0.18
LB	0.17	0.15	0.15
FT	0.36	0.26	0.25
HFT	0.19	0.59	0.29
FH	0.18	0.56	0.18
TY	0.09	0.13	0.12
SY	0.25	0.11	0.08
LN	0.08	0.39	0.23
LY	0.19	0.15	0.15
INL	0.12	0.15	0.15
LR	0.02	0.29	0.56
LSR	0.18	0.12	0.10
HAF	0.19	0.38	0.12

[a] ND=normal density, HD=high density

Table 7 Frequencies of recombinant inbred lines that have means higher or lower than that of the F_1 and the degree of improvement achieved in a single cycle of inbreeding for each character

Character	$>\bar{F}_1$	$<\bar{F}_1$	$\bar{P}_{large}-\bar{F}_1$	$\bar{P}_{small}-\bar{F}_1$
H1	78	108	27.92	–17.94
H2	44	142	23.39	–71.17
LL	1	185	0.38 ns[a]	–30.79
LB	0	186	–0.58 ns	–17.05
FT	118	68	63.78	–33.61
HFT	17	169	18.88	–108.07
FH	10	176	12.02	–121.42
TY	0	186	–13.40 ns	–616.60
SY	0	186	–2.60 ns	–407.00
LN	74	112	6.22	–9.39
LY	1	185	1.40 ns	–212.20
INL	31	155	1.76	–3.92
LR	163	23	0.77	–0.23
LSR	101	85	0.35	–0.16
HAF	40	145	32.67	–11.55

[a] ns=non-significant, all others significant at $P \leq 0.05$

tionship between the additive genetic variance D and the dominance variance H, because it determines whether over- or super-dominance is the main cause of heterosis. In the present experiment, $\sqrt{H/D}$ values of between 0.0 and 0.89 (Table 6) lend support to the results of the first-degree statistics and indicate that heterosis is caused jointly by the unidirectional dominance and gene dispersion.

Recombinant inbred lines

Table 7 shows the magnitude and rate of transgression that is displayed by the recombinant inbred lines. Clearly, superior lines that surpass the F_1 performance by a significant margin are obtained from the cross for all the traits except LL, LB, TY and SY. For LL, only one inbred had longer leaves than the F_1 but the difference was not significant. Similarly, the scores of the best recombinant lines fall short of the F_1 score for LB, TY and SY but only by a non-significant margin. Thus, we can safely assume that recombination has yielded novel lines that either surpassed the F_1 mean significantly or are as good as the hybrid for all the traits.

Comparisons of the SSD and DH lines, on the other hand, revealed no critical differences between the samples for most of the traits (see Jinks et al. 1985a). This suggested that neither are the doubled haploids less vigorous than the SSD lines nor is there any clear evidence of linkage disequilibrium in many traits.

The present sample of inbred lines is also large enough to provide realistic estimates of the coefficient of gene association/dispersion (r_d) for the parents, which we estimate as $r_d=(\bar{P}_1-\bar{P}_2)/(\bar{P}_{large}-\bar{P}_{small})$ assuming that the extreme scoring recombinant lines are *the most associated* ($r_d=1$) that are possible from the cross for each trait. However, these estimates of r_d must be considered as maximum because P_{large} and P_{small} may not be *the most extreme* that are extractable from the cross.

The r_d values given in Table 8 further show that the increasing and decreasing alleles are highly dispersed in the parents for all the traits, and in no case is the coefficient of gene association/dispersion greater than 0.50. When translated into percentages, this shows that the P_1 possesses decreasing alleles at 25% to 49% of the loci that are segregating in the cross for the various traits.

Finally, the heritability of several traits seems to be rather low, both in the triple-test cross experiment and the doubled haploids/single-seed descent families. When estimated from the second experiment (for each density) as $\frac{1}{2}D/\text{Variance}(F_2)$, the h_n^2 values (Table 8) indicate clearly that many traits are highly sensitive to the microenvironmental variation encountered in our experimental fields. A

greater affinity of the h_n^2 values across the densities also suggests that increased plant density generally has had little effect on the overall expression of genetic variability in the material for all the traits except HFT, FH and HAF.

Discussion and conclusions

It is apparent that the genetic control of variability in the SCR × S3 cross is very complex indeed. All the major sources that can affect the expression of genetic variation, e.g., additive, dominance, epistatic and maternal effects, are detected for most of the traits. Thus, it is only logical to assume that most, if not all, of these sources are involved in the expression of heterosis too.

Like many other cases, dominance plays an important role in the expression of heterosis in this cross. For most of the traits, dominance is unidirectional and the dominance ratio is moderately high. Therefore, dominance is instrumental in shifting the F_1 mean away from the mid-parent. However, in no case is $\sqrt{H/D}$ greater than, or even equal to, unity which shows categorically that over- or super-dominance is not involved in the control of heterosis in any of the characters under investigation (Table 6).

Presence of partial dominance for all the traits further indicates that, on its own, it is unlikely to generate even a modest level of heterosis for any trait. This clearly implicates gene dispersion, which then becomes essential for reducing the parental performance and making the parents look poorer than the F_1. This indirect evidence for gene dispersion, of course, is supplemented by the experimental data which come in the form of the modest to high rates of transgression that are observed among the SSD/DH derived lines for almost all the traits (Table 7).

More evidence of gene dispersion in the parents of the SCR × S3 cross is provided by a comparison of the $\sqrt{H/D}$ and the rd values. According to Pooni (1993), whenever gene dispersion is the main cause of heterosis, not only is the dominance ratio ($\sqrt{H/D}$) expected to be less than one but it should also take a larger value than rd (the degree of allele association/dispersion in the parents). Further, the ratio $\sqrt{H/D} \div rd$ should be much larger for traits that show high levels of heterosis compared to those that either show little or no hybrid vigour. The results in Tables 6 and 8 follow this pattern unequivocally and reveal that the $\sqrt{H/D} \div rd$ ratio is comparatively much larger (average =6.02) for the five characters (LL, LB, TY, SY and LY) that show a markedly higher level of heterosis than for the others (average of ratio=3.6).

The role of epistasis in the expression of heterosis, on the other hand, is not very clear. Apparently, there is a strong association between the presence of heterosis and that of epistasis and this usually implies that the latter is affecting the former in some way. The type (duplicate) of epistasis that we have observed for most of the traits, however, restricts the fuller expression of the extreme genotypes and therefore is not expected to boost heterosis in most cases ([h] and [l] take opposite signs, see Mather and Jinks 1982; Pooni and Treharne 1994).

Finally, covariance analyses of the triple test-cross families and the SSD/DH-derived lines have revealed that several traits are tightly linked and therefore show correlated expression, both for heterosis and transgression (see Jinks et al. 1985b). This is perhaps the main reason why the SCR × S3 cross shows significant heterosis for so many characters.

References

Chowdhury MKU (1984) Dihaploids of *Nicotiana tabacum* and *Nicotiana rustica*. PhD thesis, The University of Birmingham

Coombs DT (1980) Biometrical genetics of tobacco. PhD thesis, The University of Birmingham

Jinks JL (1983) Biometrical genetics of heterosis. In: Frankel R (ed) Heterosis. Monographs on theoretical and applied genetics. Springer-Verlag, Berlin, pp 1–46

Jinks JL, Perkins JM (1970) A general method of detecting additive, dominance and epistatic components of variation. III. F_2 and backcross populations. Heredity 25:419–429

Jinks JL, Pooni HS (1976) Predicting the properties of recombinant inbred lines derived by single seed descent. Heredity 36:253–266

Jinks JL, Pooni HS (1980) Comparing predictions of mean performance and environmental sensitivity of recombinant inbred lines based upon F_3 and triple test-cross families. Heredity 45:305–312

Jinks JL, Chowdhury MKU, Pooni HS (1985a) Comparison of the inbred lines derived from a hybrid of tobacco (burley×flue cured) by dihaploidy and single-seed descent. Heredity 55:127–133

Jinks JL, Pooni HS and Chowdhury MKU (1985b) Detection of linkage and pleiotropy between characters of *Nicotiana tabacum* using inbred lines produced by dihaploidy and single-seed descent. Heredity 55:327–333

Kearsey MJ, Jinks JL (1968) A general method of detecting additive, dominance and epistatic variation for metrical traits. I. Theory. Heredity 23:403–409

Mather K, Jinks JL (1982) Biometrical genetics (3rd edn.). Chapman and Hall, London

Pooni HS (1993) Genetics of heterosis and its implications for crop improvement. Annal Biol 9:323–332

Pooni HS, Jinks JL (1978) Predicting the properties of recombinant inbred lines derived by single-seed descent for two or more characters simultaneously. Heredity 40:349–361

Pooni HS, Jinks JL (1981) Sources of predictions and their reliability in predicting the properties of recombinant inbred lines which can be obtained from a cross by single-seed descent. IVth Int Barley Genet Symp, Edinburgh University Press, Edinburgh, Scotland, pp 73–78

Pooni HS, Treharne AJ (1994) The role of epistasis and background genotype in the expression of heterosis. Heredity 72:628–635

Pooni HS, Jinks JL, Jayasekara NEM (1978) An investigation of gene action and genotype×environment interaction in two crosses of *Nicotiana rustica* by triple test-cross and inbred-line analysis. Heredity 41:83–92

Pooni HS, Coombs DT, Virk PS, Jinks JL (1987) Detection of epistasis and linkage of interacting genes in the presence of reciprocal differences. Heredity 58:257–266

Tapsell CR, Thomas WTB (1983) Cross prediction studies on spring barley. 2. Estimation of genetical and environmental control of yield and its component characters. Theor Appl Genet 64:353–358

Thomas WTB, Tapsell CR (1983) Cross prediction studies on spring barley. 1. Estimation of genetical and environmental control of morphological and maturity characters. Theor Appl Genet 64:345–352