HETEROSIS STUDIESON YIELD AND QUALITY PARAMETERS IN BIDI TOBACCO (*Nicotianatabacum* L.)

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ABSTRACT

Tobacco is one of the important commercial crops in the world. It has various types such as Flue Cured Virginia, Hookah, Cigar type, Lanka type Bidi type etc . All these are categorized based on their quality and consumption purpose. Among these Bidi tobacco is more widely grown in Pakistan and in India . The genetic work on the bidi tobacco quality parameters is very much limited. In this connection this work has been carried out in Agriculture research Station Nipaniat Belgaum district in Karnataka . In this investigation six lines and eight testers were used to generate the F1 hybrids in all possible cross combination with line x tester design . Thus total experimental materials consists of 62 genotypes . All these genotypes were grown in lattice design with three replication .Various characters observed for their genetic analysis viz Days to flower, Plant height (cm), Inter nodal length (cm), Numbers of leaves ,Leaf length (cm), Leaf breadth (cm) Total dry weight (g/plant), stalk weight (gg/plant) and Leaf yield (g/plant) these quantitative traits were recorded from the field at vegetative stage.The quality traits such as nicotine content, spangles scrore(sort of puckering on the leaves indicates more quality), reducing sugar and chloride content in percentage is recorded using auto analyser readings. The investigation found out Vairam, MS NPN-190, MS A-119 and Kunkumatri are best combiners MS PL-5 ×Vairam and MS GT-4 ×Thangam are hybrids performed better compared to check on yield and quality parameters , MS NPN-190 ×Kunkumatriand MS A-119 ×Kunkumatri were best lines for nicotine content .

INTRODUCTION

One of the main objectives of genetic analysis is to know the gene action operating in the genotypes which in turn is an indication of the possibility of exploiting available heterotic advantage in a crop under study. The term heterosis was first coined by Shull (1914). He defined heterosis as the superiority of F_1 hybrid over both its parents in terms of yield and some other characters. Heterosis is manifested as increase in vigour, size, growth rate and yield or some other traits. It can be best explained as superiority or inferiority of the hybrid over both the parents. Heterosis is often estimated over the average of the two parents (mid parent) and is called as mid parental heterosis. More generally heterosis estimated over the superior parent which is referred to as hetero beltiosis.Heterosis manifestation for yield is expressed in the form of increased yield which in turn depend on heterosis in the component traits. All the component traits of yield are studied together with regard to heterosis manifestation in order to assess the worth of crosses.

MATERIAL AND METHODS

The field experiment was carried out at Agricultural Research Station, Nipani, Karnataka in randomized block design with two replications. The materials consisted of 14 cultivars and 48 F_1 hybrids, which were generated by crossing them in LXT fashion. These hybrids along with parents were grown in plots consisting of two rows following 100 x 100 cm spacing. The recommend cultivation practices were followed to raise the crop. Five competitive plants in each treatment were selected for recording the observations on Days to flower, Plant height (cm), Internodal length (cm), Numbers of leaves ,Leaf length (cm), Leaf breadth (cm) Total dry weight (g/plant), The quality parameters were analyzed at Central tobacco research institute at Rajhamundry Nicotine content (%),Reducing sugar content (%), Chloride content (%), Leaf yield (g/plant).Techniques used to record the various characters is as follows, Days to flower :The number of days taken from the date of transplanting to the date of first flower opening in each entry was counted as days to flower. Plant height : Height of the main stalk from the ground level to the tip was recorded as plant height in centimeter (cm) at the time of maturity. Internodal length :It was measured in cm as the distance between fourth and fifth leaves of plant. Number of leaves per plant :Total number of leaves present in a plant was recorded at the time of maturity. Leaf length :Length of the eighth leaf was measured in cm and recorded as leaf length. Leaf breadth :Leaf breadth in the fifth leaf was measured in cm. Total dry weight :After harvesting, plants were sun dried for 15 days and later weighed in grams (g). Leaf yield :Leaf yield was recorded as average weight of dried leaves in grams (g) after removing from stalks. For nicotine and reducing sugar were recorded on the auto analysing readings and conversion factor as per the procedures of Harvey *et al.* (1969) .ie0.01 × AAR (Auto Analyzer Readings)/weight of the sample

RESULTS AND DISCUSSIONS

Significant heterosis over mid parent is observed in majority of the crosses, it indicates non-additive gene action for such characters. Assuming that epistasis is absent, the cause of heterosis can only be attributed to dominance gene action. The inference drawn about gene action based on heterosis for different characters are presented below.

Out of 48 crosses, 36 showed significant heterosis over mid parent for days to flower indicating non-additive gene action in controlling this trait. Fifty per cent of these crosses were in desired direction so that these crosses could be used for developing late maturing types. These results are in accordance with the reports of Kher *et al.*(2001). Thirty two crosses showed significant heterosis over better parent for this trait. Among them 6 showed positive heterosis and 26 negative heterosis over better parent indicating dominance gene action involved in the control of days to flower. In 22 crosses, heterosis over mid parent was significantly positive and 2 crosses had (MS PL-5 ×Bhagyashree and MS PL-5 × NPN-22) showed significant negative heterosis indicating the role of non-additive gene action for plant height. Thirty nine crosses exhibited significant negative heterosis over check indicating dominance gene action in controlling the trait. These crosses could be used for development of dwarf genotypes.

Inter nodal length an important trait in bidi tobacco to get good quality leaves, as dwarf types are desirable. Forty five crosses showed significant heterosis over mid parent and 46 crosses over check NPN-22 indicating non-additive gene action involved in the control of this trait. These results are further confirmed by the fact that GCA variance was relatively less than SCA variance. The magnitude of GCA vs parents was less, when compared to SCA vs crosses, which also indicated non-additive gene action. The highest hetero beltiosis (80.00%) and mid parent heterosis (62.58%) was exhibited by the hybrids MS NPN190 ×Bhagyashree and MS GT4 × DWFC, respectively for this trait, which indicated dominance gene action for inter nodal length in these crosses. Similar significant heterosis over respective better parent was reported by Kara and Essendal (1995), Number of leaves is an important yield contributing trait in tobacco. The hybrid MS PL-5 ×Vairam recorded the highest hetero beltiosis (21.68%) indicating a possible relationship among *per se* performance of sca effects and observed heterosis for the trait. Thirty three crosses showed significantly positive mid parent heterosis indicating importance of non-additive gene action for the trait. Two crosses showed significant positive heterosis over check NPN-22 indicating these two crosses (MS PL-5 ×Vairam and MS PL-5 ×Thangam) could be used for developing genotypes with more number of leaves. The present findings are in conformity with Kara and Esendal (1995) and Kher *et al.* (2001).

The highest leaf length was found in NPN-22 (72.67 cm) among the parents but with respect to crosses MS NPN-190 × NPN-22 (79.27 cm) was found to record the highest leaf length. In 27 crosses positive mid parental heterosis was found and of these only two (MS A-2 ×Maragadham and MS PL-5 × NPN-22) crosses showed significant negative heterosis, which indicated the role of non-additive gene action. MS NPN-190 × NPN-22 cross recorded 8.60 per cent heterosis. Fifteen crosses recorded positive better parental heterosis which suggested that the trait was under control of dominance gene action. Similar findings on heterosis were documented by Amarnath *et al.* (1995). The highest leaf breadth was found in NPN-22 (37.67 cm) among parents, whereas the crosses MS A-119 × DWFC and MS GT-4 × NPN-22 recorded minimum (24.93 cm) and maximum (39.67 cm) leaf breadth, respectively. In 29 crosses, positive mid parental heterosis was found and of these nine crosses showed significant negative heterosis, indicating the role of non-additive gene action. Similar reports on heterosis over mid parent and better parent on this trait reported by Wilkinson *et al.* (1994).Six crosses showed significant positive heterosis over mid parent for leaf yield. The range of heterosis varied from -29.91 per cent in MS PL-5 × NPN-22 to 80.00 per cent of MS PL-5 ×Vairam over mid parent. One cross recorded significant heterosis and maximum heterosis over better parent was 60.00 per cent in MS PL-5 × Vairam hybrid. The cross MS GT-4 × Thangam (1.38%) and showed positive heterosis over check NPN-22 for leaf yield per plant. The correlation between scaeffects and heterosis was 0.55 indicating considerable amount of heterosis was due to non-additive gene action in the crosses and which could be exploited. The correlation between sca effects (-0.55) and mean of crosses was higher than that of gca effects (0.93) and parents which indicated non-additive gene action in these hybrid combinations. Similar results were found by Amarnath*et al.* (1995) and Kher *et al.* (2001).

Among 48 hybrids, 28 recorded significant positive heterosis over mid parent, whereas 19 were found to have negative heterosis for *Cercospora* leaf spot disease. The more resistant hybrid was MS F_7-127×Vairam and most susceptible one MS S-20 × DWFC. Nearly 12 crosses showed resistance against *Cercospora* leaf spot disease. About forty seven crosses showed heterosis over both mid parent and better parent, respectively, indicating the dominance gene action in controlling the disease resistance. Eight crosses recorded better resistance to *Cercospora* leaf spot over check NPN-22, whereas 40 crosses had positive heterosis for the *Cercospora*leaf spot incident. Similar results were observed by Laxmisha (1997). Root knot nematode infestation reduce the leaf yield to a large extent. All the crosses showed heterosis over mid parent indicating the role of non-additive gene action for this character. Forty three crosses out of 48 recorded heterosis over better parent which suggested over dominance gene action in controlling the root knot nematode. The root knot nematode infestation was found to be lowest in Vairam followed by MS A-119, but maximum disease was recorded in Kunkumatri. More susceptible crosses were MS S-20 ×Vairam and MS A-119 ×Thangam. Forty one crosses recorded resistance to root knot nematode over check NPN-22. The correlation between SCA and heterosis, SCA and crosses and GCA and parents clearly confirmed the role non-additive gene action in controlling this trait

For spangle score 39 out of 48 hybrids showed significant heterosis over mid parent suggesting non-additive gene action. Significant positive better heterosis over better parent was observed in two out of 48 crosses, suggesting dominance gene action. Thirty-three crosses registered negative heterosis of which the cross MS A-2 x Vairam, MS A-119 x Vairam, MS PL-5 x Vairam and MS S-20 x DWFC were highly heterotic. Twenty two crosses registered positive heterosis of which MS GT-4 × Maragadham, MS GT-4 × DWFC and MS-GT-4 × Kunkumatri were highly heterotic over check NPN-22. So both additive and dominance genes were involved in these crosses. Similar observations made by Kher *et al.* (2001). Twenty eight out of 48 hybrids showed positive significant heterosis over mid parent suggesting non-additive gene action for nicotine content. Twenty out of 48 (52.33%) hybrids recorded better parental heterosis in desirable direction. So, these 20 crosses could be used to develop high nicotine content genotypes as higher nicotine and reducing sugar content in tobacco give desired kick and sweetness, respectively with good satisfaction to the smokers. All 48 crosses studied were recorded positive heterosis over check NPN-22. Similar conclusions were drawn by Patel*et al.* (2001). Almost all the hybrids except MS A-2 x NPN-22 and MS GT-4 x DWFC registered significant heterosis over mid parent, out of which 64.58 per cent crosses were in desired direction, while 20 hybrids were superior over better parent for reducing sugar. These deviations indicated the existence of dominance for reducing sugar. Thirty nine crosses recorded significant positive heterosis and seven crosses showed significant negative heterosis over check NPN-22. These results are in agreement with Kara and Esendal (1995) and Patel *et al.* (2001). . All the hybrids exhibited significant heterosis over both mid and better parent for chloride content. Twenty-three crosses (62.25%) and 12 crosses (29.16%) were in desirable direction over mid and better parental level, respectively. The highest negative heterosis over better parent was observed in cross MS A-119 ×Bhagyashree followed by MS S-20 × NPN-22. The highest negative heterosis over better parent was observed in crosses *viz.,* MS A-119 ×Maragadham and MS A-119 ×Bhagyashree. Hence, these hybrids may be useful in isolating the genotypes with less chloride content. Twenty five crosses showed significant positive heterosis over mid parent for reducing sugar to nicotine ratio. It was observed that MS NPN-190 could be used to develop genotypes with optimum reducing sugar to nicotine ratio. Twenty hybrids recorded significant positive heterosis over better parent, 13 crosses registered significant heterosis over check NPN-22 indicating the dominance gene action in controlling reducing sugar to nicotine ratio. Similar observations were made by Laxmisha (1997).

CONCLUSION

Two best hybrids have been listed with their mean performance and extent of standard heterosis. As expected though hybrids differed from character to character some best hybrids which are promising for more than one character can be identified. Such hybrids deserve attention for future handling in the breeding programme. MS PL-5 ×Vairam was promising in respect of number of leaves, total fresh weight, total dry weight, stalk weight also resistant to *Cercospora* leaf spot. Likewise, MS NPN-190 × NPN-22 hybrid was found to be promising for leaf length, leaf area, total fresh weight and total dry weight. The data therefore indicate that these two hybrids were generally desirable in the point of view of productivity with regard to quality MS NPN-190 × F_7-127 deserved mention. This hybrid was excellent in chloride content and reducing sugar to nicotine ratio.

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Table1: *Analysis of variance (mean squares) of parents and F_1 hybrids for yield and quality characters in bidi*

Source of variation	Df	Days to flower	Plant height (cm)	Internodal length (cm)	Numbers of leaves	Leaf length (cm)	Leaf breadth (cm)	Leaf yield (g/plant)	Frog eye leaf spot (%)	Root knot nematode (%)	Spangle score (%)	Nicotine content (%)	Reducing sugar content (%)	Chloride content (%)	Reducing sugar to nicotine (ratio)
Replications	2	0.66	115.82	0.70	55.11**	112.52*	262.22**	5376.25*	2.21**	1.97**	27.62**	6.99**	10.30**	0.157**	0.29**
Treatments	61	107.91**	856.66**	2.37**	8.07**	65.44**	40.89**	3818.08**	19.22**	9.14**	3.10**	0.81**	1.15*	0.028	0.045**
Parents	13	82.30**	1709.38**	2.79**	13.54**	74.61**	65.99**	5712.08**	0.5**	0.087	0.0064	1.35**	0.99	0.069**	0.041
Females	5	51.04**	2123.63**	2.85*	11.92**	19.90	39.29**	6223.33**	0.4**	0.098	0.083**	1.20*	1.35	0.008	0.055
Males	7	80.17**	1218.93**	2.80*	8.37**	120.04	78.97**	5213.68**	0.53**	0.091	0.04	1.66**	0.81	0.091**	0.035
Females vs males	35	253.58**	3026.34**	2.41	56.66**	30.21	108.65**	6644.68*	0.76**	0.003	0.13*	0.004	0.39	0.219**	0.014
Hybrids	47	116.38**	579.23**	1.89	6.74*	46.56	33.98	3089.30	0.34**	0.121	0.06	0.64	1.16	0.016	0.04
Parents vs Hybrids	1	42.64**	2811.00**	19.50**	0.84	833.82**	39.28	13448.7**	0.81**	0.069	0.26**	1.87*	2.39	0.049	0.19
Error	122	5.83	91.47	1.18	1.65	26.00	11.17	1227.70	0.04	0.085	0.02	0.48	0.84	0.022	0.026
C. D at 5%	-	1.97	7.80	0.88	1.05	4.16	2.72	28.60	1.55	1.99	0.87	0.56	0.74	0.12	0.13

Tobacco (Nicotiana tabacum L.)

Table 2: *Standard heterosis for leaf yield and chemical composition in bidi Tobacco (Nicotiana tabacum L).*

SNo	Parents and hybrids	Mean performance for yield	Heterosis for yield (%)	Mean performance for nicotine content	Heterosis for nicotine (%)
1	MS A-2 × Bhagyashree	140	-41.66	5.69	28.15**
2	MS A-2 × NPN-22	233.33	-2.77	5.95	34.00**
3	MS A-2 × Maragadham	173.33	-27.77	6.7	50.90**
4	MS A-2 × DWFC	143.33	-40.27	6.06	36.48**
5	MS A-2 × Kunkumatri	150	-37.5	6.86	54.50**
6	MS A-2 × Thangam	183.33	-23.61	5.74	29.27**
7	MS A-2 × Vairam	180	-25	6.07	36.71**
8	MS A-2 × F_7-127	176.67	-26.38	5.82	31.08**
9	MS A-119 × Bhagyashree	166.67	-30.35	5.52	24.32**

10	MS A-119 × NPN-22	230	-4.16	5.1	14.86**
11	MS A-119 × Maragadham	193.33	-19.44	5.61	26.35**
12	MS A-119 × DWFC	100	-58.33*	5.23	17.79**
13	MS A-119 × Kunkumatri	166.67	-30.55	5.88	32.43**
14	MS A-119 × Thangam	180	-25	5.61	26.35**
15	MS A-119 × Vairam	200	-16.66	5.83	31.30**
16	MS A-119 × F_7-127	153.33	-36.11	5.33	20.04**
17	MS PL-5 × Bhagyashree	150	-37.55	5.51	24.09**
18	MS PL-5 × NPN-22	136.67	-43.05	5.3	19.36**
19	MS PL-5 × Maragadham	170	-29.16	5.99	34.90**
20	MS PL-5 × DWFC	160	-33.33	5.78	30.18**
21	MS PL-5 × Kunkumatri	140	-41.66	5.95	34.00**
22	MS PL-5 × Thangam	183.33	-23.61	6.23	40.31**
23	MS PL-5 × Vairam	240	0	5.26	18.46**
24	MS PL-5 × F_7-127	153.33	-36.11	5.86	31.98**
25	MS GT-4 × Bhagyashree	176.67	-26.38	5.68	27.92**
26	MS GT-4 × NPN-22	206.67	-13.88	6.15	38.51**
27	MS GT-4 × Maragadham	176.67	-26.38	6.52	38.51**
28	MS GT-4 × DWFC	146.67	-38.88	5.54	24.77**
29	MS GT-4 × Kunkumatri	133.33	-44.44	5.94	33.78**
30	MS GT-4 × Thangam	243.33	1.38	5.81	30.85**
31	MS GT-4 × Vairam	183.33	-23.61	5.94	33.78**
32	MS GT-4 × F_7-127	160	-33.33	5.6	26.12**
33	MS NPN-190 × Bhagyashree	183.33	-23.61	4.56	2.70**
34	MS NPN-190 × NPN-22	216.67	-9.72	5.66	27.47**
35	MS NPN-190 × Maragadham	190	-20.83	5.96	34.23**
36	MS NPN-190 × DWFC	133.33	-44.44	6.5	46.39**
37	MS NPN-190 × Kunkumatri	150	-37.5	7.08	59.45**
38	MS NPN-190 × Thangam	203.33	-15.27	6.44	45.04**
39	MS NPN-190 × Vairam	180	-25	5.64	27.02**
40	MS NPN-190 × F_7-127	166.67	-30.55	4.85	9.23**
41	MS S-20 × Bhagyashree	150	-37.55	6.54	47.29**
42	MS S-20 × NPN-22	166.67	-30.55	5.81	30.85**
43	MS S-20 × Maragadham	150	-37.5	5.93	33.55**
44	MS S-20 × DWFC	116.67	-51.38	5.42	22.07**
45	MS S-20 × Kunkumatri	113.33	-52.77	6.2	39.63**
46	MS S-20 × Thangam	183.33	-23.61	6.27	41.21**
47	MS S-20 × Vairam	143.33	-40.27	6.62	49.07**
48	MS S-20 × F_7-127	136.67	-43	5.96	34.23**
49	CD (0.05)	-	56.05		1.11
50	CD (0.01)	-	73.81		1.46

Table 3: *Potential hybrids identified for yield and quality parameter in bidi tobacco (Nicotiana tabacum L.)*

Characters	Hybrids	Mean performance	Standard heterosis (%)
Days to flower	MS A-119 × Thangam	69.30	7.99
	MS A-119 × DWFC	67.90	5.80
Plant height (cm)	MS A-119 × DWFC	89.07	-39.27
	MS GT-4 × Maragadham	90.47	-38.31
Internodal length (cm)	MS S-20 × Vairam	4.83	-40.14
	MS S-20 × F_7-127	5.3	-34.32
Number of leaves	MS PL-5 × Vairam	18.33	7.38
	MS PL-5 × Thangam	17.42	2.05

Leaf length (cm)	NPN-190 × NPN-22	79.27	9.08
Leaf breadth (cm)	MS GT-4 × NPN-22	39.67	5.30
	MS GT-4 × Maragadham	39.27	4.24
Leaf area (sq. cm)	MS NPN-190 × NPN-22	1854.27	7.91
	MS GT-4 × NPN-22	1852.57	7.81
Total fresh weight (kg)	MS PL-5 × Vairam	2.00	30.43
	MS NPN-190 × NPN-22	1.58	3.26
Total dry weight (g)	MS PL-5 × Vairam	0.823	15.42
	MS NPN-190 × NPN-22	816.33	14.43
Stalk weight (g)	MS PL-5 × Vairam	0.583	21.52
	MS NPN-190 × Maragadham	0.506	5.55
Leaf yield (g)	MS GT-4 × Thangam	0.243	1.38
	MS PL-5 × Vairam	0.240	0.00
Frog eye leaf spot (%)	MS PL-5 × Vairam	1.00	-24.81
	MS GT-4 × Thangam	1.00	-24.81
Root knot nemtode (%)	MS A-119 × Bhagyashree	1.24	-40.47
	MS A-119 × Maragadham	1.49	-24.40
Spangle score (%)	MS S-20 × Kunkumatri	2.45	13.45
	MS GT-4 × Maragadham	2.45	13.25
Nicotine content (%)	MS NPN-190 × Kunkumatri	7.08	59.45
	MS A-119 × Kunkumatri	6.86	54.50
Reducing sugar content (%)	MS NPN-190 × F_7-127	4.93	98.79
	MS NPN-190 × DWFC	3.97	60.08
Chloride content (%)	MS NPN-190 × F_7-127	0.30	-31.81
	MS GT-4 × Vairam	0.33	-25.00
Reducing sugar to nicotine ratio	MS NPN-190 × F_7-127	1.02	82.14
	MS S-20 × DEFC	0.64	14.28

REFERENCE

AMARNATH, S., MURTHY, N. S. AND SINGH, K. D., 1995, Heterosis in chewing tobacco.*Tobacco Research*, **21**(1&2) : 93-96.

BUTORAC, C. J., VASILIJ, D., KOZUMPLIK, V. AND BELJO, J., 2000, Inheritance of certain economic and agronomic traits in burley tobacco.*Bodenkulur*, **51**(3) : 151-156.

HARVEY, W. R., STAHR, H. M. AND SMITH, W. C., 1969, Automated determination of reducing sugars and nicotine alkaloids on the same extract of tobacco leaf. *Tobacco Science*, **XIII** : 13-15.

KARA, S. M. AND ESENDAL, E., 1995, Heterosis and combining ability analysis of some quantitative characters in turkish tobacco. *Tobacco Research*, **21**(1-2) : 16-22

KHER, H. R., PATHAK, H. C. AND PATEL, A. D., 2001, Heterosis for yield components in bidi tobacco (*Nicotianatabacum*L.) over diverse cytoplasm. *Tobacco Research*, **27**(2): 98-102.

LAKSHMISHA, K. J., 1997, Assessment of genetic diversity and histological characterization of frog eye leaf spot disease (*Cercosporanicotianae*Ell. and Ev.) in diverse collections of FCV tobacco *Nicotianatabacum*L. *M. Sc. (Agri.) Thesis*, University of Agricultural Sciences, Dharwad.

PATEL, B. K., CHAVDA, J. C., PARMAR, N. B. AND MEHATA, K. G., 2001, Heterosis for chemical and biochemical parameters in bidi tobacco (*Nicotianatabacum*L.). *Tobacco Research*, **27**(1): 58-62.

RAO, G. S. B. P. S., 1989, Heterosis and combining ability studies in intervarietal crosses of chewing tobacco (*Nicotianatabacum*L.). *Madras Agricultural Journal*, **76**(11) : 616-620.

SHULL, G. H., 1908, Wheat in Heterosis. *Genetics*, **33** : 439-446

WILKINSON, C. A., JONES, J. L. AND TILSON, W. M., 1994, Diallel analysis of crosses among virgenia fire-cured tobacco cultivars.*Tobacco Science*, **38** : 21-24