Research Article

Heterotic Studies in Flue-Cured Tobacco across Environments

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Abstract | This research was conducted to determine heterosis for various agronomic and biochemical traits in Flue Cured Virginia tobacco. Seven tobacco varieties/lines, 'NC606', 'K399', 'Spt G 126', 'Spt G 28', 'KHG21', 'KHG22' and 'KHG24' with contrasting traits were crossed in all possible combinations to generate 7 x 7 diallel crosses. In 2008 and 2009, all F_1 hybrids along with their parent cultivars were planted in randomized complete block (RCB) design with four replicates at Tobacco Research Station Mardan (plain) and Tobacco Research Substation Mansehra (hilly). Data for all traits were analysed in pooled analysis and individual environments. Heterosis over better parent was determined for all traits across environments. Heterobeltiosis estimates reflected both positive and negative values, suggesting additive as well as non-additive gene actions in the expression of traits. Performance of hybrids was environment-specific. However, heterobeltiosis estimates ranged between -7.9 and 25.90 for leaf area, -6.76 and 130.8 for number of cured leaves kg^{-1}, -10.8 and 17.1 for yield, -16.0 and 9.94 for grade index, -10.4 and 68.66 for nicotine and -26.89 and 31.25 for reducing sugar, respectively. The current study identified KHG24/Spt G 28; KHG21/NC606 and Spt G 126/KHG24 as best crosses for yield while KHG22/KHG21 was the only best cross for grade index.

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Introduction

The Flue Cured Virginia (FCV) tobacco (*Nicotiana tabacum* L.) is commonly known as cigarette tobacco. It is one of the cultivated types grown worldwide for smoking. Botanically it is known as *Nicotiana tabacum* and belongs to a nightshade family Solanaceae along with 76 other members of the same genus of Nicotiana. Tobacco is the major cash crop of Pakistan and mostly cultivated in province of Khyber Pakhtunkhwa (Ahmed et al., 2014). It is one of the major sources of income in government revenues due to taxes and levies upon it worldwide. Being one of the highly self-pollinated crops in nature, it maintains its genetic purity relatively longer. But inbreeding methods practiced with tobacco breeding in the latest decades have almost exhausted the variability existed in old and native cultivars. Artificial hybridization proved to be one of the most effective tools for restoring a range of new recombination's (Dean, 1974). There is great probability that new lines with preferred characters can be obtained from the varieties with high heterotic effect (Aleksoski, 2010). The objectives of tobacco breeding programmes include improvement in yield, quality and consequently income per hectare; disease resistance; ease of handling and curing; and chemical constituents while meeting the demands of the grower, the manufacturer and the

consumer (Mulekano, 1999).

Table 1: *Seven tobacco varieties/parents and their characteristics*

Variety/ line	Pedigree	Main features
NC 606	NC729 / NC 82	Good cured-leaf quality Tall plants with about 30 leaves and longer internodal length.
K 399	(Coker139x Coker 319) and NC 95	Dwarf plants with about 26 leaves. Somewhat late flowering. Broader leaves.
Spt. G 126	K 326 x Speight G-96	Produces average yields of less than average quality Produces nearly 20 to 25 leaves on a stalk of average height Flowers later than most varieties and good holding ability.
Spt, G 28	(Coker 139 x Oxford 1-181) and NC 95	The recommended variety for last 30 years in Pakistan by Pakistan Tobacco Board. Moderate yield and medium quality tobacco. Short plants having more than 25 leaves. Flowers medium to late.
KHG 21	Locally selected accession	Based on long-term data it has tall plants, more than 30 leaves per plant and intermediate internodal length. It has more leaf area than KHG 22and KHG 24.
KHG 22	Locally selected accession	Based on long-term data, it possesses an average of 24 leaves per plant with intermediate leaf area. It has more internodal length than KHG21 and KHG 24.
KHG 24	Locally selected accession	Based on long-term data, it has an average of 25 leaves per plant with intermediate internodal length. It is dwarf, having smaller leaf area than KHG21 and KHG 22.

Maximum yield potential can be exploited through hybrid vigour (Akbar et al., 2010) which is the increased performance of hybrid over its parents. There are two types of vigour performances. Increase of hybrid over mean performance of its parents is called mid-parent heterosis while increase in performance over the best parent is known as high parent heterosis or heterobeltiosis. Mackey (1976) described genetic principles of expression of heterobeltiosis, which may result from one or two of the following situations; (i) the accumulated action of favourable dominant or semi-dominant genes dispersed amongst two parents i.e. dominance; (ii) the complementing interaction of additive dominant on recessive genes at different loci i.e., non-allelic interactions or epistasis; (iii) favourable interaction between two alleles at the same locus i.e. intra-locus or inter-allelic interactions referred to as over dominance. Significant values of heterosis for several economic traits have been reported by Legg et al. (1970), Matzinger et al. (1971), Dean (1974) and Butorac et al. (1999a). Tobacco genotypes differ in performance across locations (Gupton et al., 1974) suggesting that genotypic ranking gets changed with change in environment. Most of the production traits are highly influenced by environment (Legg et al., 1970), signifying indirect selection for quantitative traits such as yield. Keeping in view the demands of growers and tobacco industry, this study was initiated with the objective to estimate heterosis over better parents in diallel combinations for identifying crosses with high level of heterobeltiosis for some production and quality traits than the existing commercial varieties.

Materials and Methods

The present study was conducted to determine heterosis for various agronomic and biochemical traits in FCV tobacco. Seven tobacco varieties/lines, NC606, K399, Spt G 126, Spt G 28, KHG21, KHG22 and KHG24, with contrasting traits were crossed in all possible combinations to generate 7x7 diallel at the Tobacco Research Sub Station (TRSS) Mansehra, Pakistan Tobacco Board, in 2007 (Table 1). In 2008 and 2009, all F_1 hybrids along with their parent cultivars were planted in randomized complete block design with four replicates at Tobacco Research Station (TRS) Mardan (plain) and Tobacco Research Substation (TRSS) Mansehra (hilly). The experiments conducted at Mardan during 2008, Mansehra during 2008, Mardan during 2009 and Mansehra during 2009 were referred as environment-1, environment-2, environment-3 and environment-4, respectively (Table 2). Cultural practices including weeding, hoeing, earthing-up, fertilizer application etc. were applied as recommended in general. The properties related to soil and climate across environments are given as in Table 2.

Collection of data

Five random competitive plants in each plot were used for morphological, yield and biochemical parameters. Length and broadest width of 5th, 10th and 15th leaves were averaged for leaf area (cm^2). Mean value was further adjusted as proposed by Suggs et al. (1960).

Table 2: *Description of the experimental environments*

		Environment-1	Environment-2	Environment-3	Environment-4
Altitude (meters)		283	975.36	283	975.36
Annual Rainfall (mm)		480.98	926.00	275	605.50
Rainfall during March to August (mm)		339.68	524.50	114	388.00
Mean annual temperature (Min and Max °C)		13.4-30.1	13.3-27.5	15.5-32.6	13.0-27.8
Mean temperature, March to August (Min and Max °C)		18.1-35.1	17.8-32.5	18.9-36.2	16.9-32.4
Soil texture		Silt loam	Silt loam	Silt loam	Silt loam
Soil chemical nature	pH	7.70-7.83	7.2	7.78	7.2
	Chlorides %	0.001	0.006	0.002	0.006
Soil nutrients	N %	0.04-0.08	0.185	0.048	0.179
	P %	0.0008	0.00098	0.001	0.00096
	K %	0.0115	0.018	0.0115	0.0174
	Organic matter %	0.6	2.1	0.6	2.0

As soon as the leaves got matured were picked in 5 or 6 phases. Aggregate of all pickings was taken as weight of mature leaves. Leaves were picked from field and tied to sticks, loaded in flue-curing barns for one week. After complete process of curing, the cured leaves were weighed. Number of cured leaf kg^{-1} was taken as leaves in one kilogram after curing. Yield was recorded as weight of leaves after curing. Grade index (%) was assessed visually as per standard procedure, prevailing in the market. Nicotine and reducing sugar contents were determined in a composite sample of all pickings as outlined by Cundiff and Markunas (1964).

Statistical analysis

Analyses of variance for various traits across environments were worked out to know the significance of interaction due to genotype by environment. Significant genotype by environment interaction (GEI) implies that response of genotypes to various environments was different and thus it necessitates considering analysis of genotypes in individual environments.

Heterosis over better parent

Heterosis values for various traits were calculated in terms of percent increase (+) or decrease (-) of the F_1 hybrids against its better parent value. The term heterobeltiosis was coined by Fonseca (1965) for heterosis over better parent. It was estimated in terms of percent increase or decrease of the F_1 hybrid over its better parent.

The data were further subjected to "t" test to determine whether F_1 hybrid means were statistically different from mid and better parental values. The "t" values were calculated by following formula as used by Wynne et al. (1970).

Results and Discussion

Test of significance

Data recorded for traits i.e. leaf area, number of cured leaves kg^{-1}, yield, grade index, nicotine and reducing sugar on seven FCV tobacco varieties and their 42 F_1 hybrids i.e 42 F1 hybrids over four environments (environment-1, environment-2, environment-3 and environment-4) were subjected to combined analysis of variance. Genotypes and GEI were significant ($P \leq 0.01$) for all traits (Table 3). Significant GEI implied that response of genotypes to various environments was different and thus it necessitated considering analysis of genotypes in individual environments. Analysis of variance revealed significant differences among genotypes for all traits in four environments (Table 3). The significant results suggested that further statistical analysis for heterotic studies could be carried out successfully over all environments. Utilization of heterosis in commercial crops is of significance only when the vigour is in excess of the better parent (Fonesca and Patterson, 1968). Heterobeltiosis estimates are presented for each trait across environments as under.

Leaf area

Larger leaf area is desirable for reception of considerable sunlight to carry out photosynthetic and biochemical processes resulting in enhancing the quality of leaf besides better yield. Positive heterosis is desirable

Table 3: *Mean squares for leaf area (LA), number of cured leaf (NCL), yield (YLD), grade index (GI), nicotine contents (NI) and reducing sugars (RS) in 7x7 diallel of FCV tobacco during 2008 & 2009*

Source	Df	LA	NCL	YLD	GI	NI	RS
Combined analysis across 4 environments							
Environment	3	17446049.21**	238732.6**	188711246.1**	2080.7**	30.74**	27.6**
Error	12	73869.60	1094.1	985170.4	31.3	0.32	5.8
Genotype	48	35269.68**	439.3**	231918.3**	193.6**	0.76**	69..3**
G x E	144	20280.99**	445.5**	187534.1**	44.5**	0.01**	2.7**
Error	576	10079.94	140.8	65608.0	14.9	0.007	0.151
CV %		10.08	9.38	8.49	5.38	3.90	2.04
Environment-1							
Block	3	231405.37**	24.49	27788.29	73.23**	0.008	0.34
Genotype	48	46547.29**	196.78**	149720.86**	75.03**	0.192**	18.11**
Error	144	13781.32	36.97	15743.95	13.34	0.006	0.18
CV %		8.53	6.99	3.06	5.33	4.37	2.16
Environment-2							
Block	3	60263.79	3720.37**	3066832.48**	36.80	1.161**	18.59**
Genotype	48	42313.96**	306.59**	293584.52**	79.09**	0.239**	20.65**
Error	144	23548.28	126.47	139874.94	17.84	0.008	0.19
CV %		14.11	10.49	10.54	6.06	3.58	2.27
Environment-3							
Block	3	1299.57	252.73	50066.82	3.23	0.018*	0.67**
Genotype	48	4031.52**	756.51**	127951.84**	77.57**	0.187**	19.88**
Error	144	1657.47	219.09	23242.27	13.50	0.006	0.14
CV %		5.62	9.45	7.73	4.86	4.41	1.99
Environment-4							
Block	3	2659.01	379.51	795994.18**	11.50	0.110**	3.50**
Genotype	48	3186.07**	516.43**	223263.48**	95.31**	0.171**	18.79**
Error	144	1331.96	180.69	83570.70	15.00	0.007	0.10
CV %		4.59	8.67	11.82	5.29	3.48	1.66

** and ** : $P \leq 0.05$ and $P \leq 0.01$, respectively*

for leaf area. Five crosses were found with significant positive heterotic values over respective better parent in environment-1 (Table 4). Maximum positive heterobeltiosis was shown by the cross combination NC606/KHG21 (19.91%) followed by KHG21/KHG22 (19.59%). Heterotic values ranged from -9.13 (Spt G 28/KHG22) to 19.91 (NC606 /KHG21) percent (Table 4). In environment-2, four crosses showed significant positive heterobeltiosis (Table 4). Maximum increase over better parent was expressed by the cross combination KHG21/Spt G 126 (25.9%) followed by Spt G 28/Spt G 126 (22.89%). The heterotic values ranged from -19.83 (K399/KHG21) to 25.90 (KHG21/Spt G 126) percent in environment-2 (Table 4). In environment-3 and environment-4 (Table 4), none of the crosses showed significant positive heterobeltiosis. However, the heterotic range was from -12.40 (NC606/KHG21) to 5.36 (Spt G 28/Spt G 126) percent in environment-3 and from -7.9 (Spt G 126/KHG24) to 6.1 (KHG21/KHG24) percent environment-4.

Leaf area is very important component of yield in tobacco. This study identified few crosses with remarkable increase over better parent for leaf area at environment-1 and environment-2. It may be the result of non-additive type of inheritance. However, high performing hybrids in environment-1 and environment-2 could not perform up to the mark in environment-3 and environment-4, which may be due to differences in seasons and the resulting genotype x environment interaction. Thus the environment-1 and environment-2 were more discriminative than environment-3 and environment-4. However, recording

Table 4: *Heterotic range, number of significant crosses, number of positive or negative heterotic crosses and best crosses in four environments for FCV tobacco*

	LA	NCL	YLD	GI	NC	RS
Environment-1						
Heterotic range	-9.13 to 19.91	-6.76 to 23.2	-10.80 to 7.81	-20.9 to 9.94	-11.17 to 68.66	-26.89 to 27.39
No of Significant desirable crosses	5	0	5	1	21	13
No of positively heterotic crosses	25	36	7	5	29	17
No of negatively heterotic crosses	17	6	35	37	13	25
Best heterotic crosses	NC 606/ KHG21		KHG24/Spt G 28	KHG22/ KHG21	KHG22/NC 606	KHG24/NC 606
	KHG21/ KHG22		KHG21/NC 606		KHG22/ K399	NC 606/ KHG24
	Spt G 28/ Spt G 126		Spt G 28/ KHG 24		NC 606/ KHG21	KHG24/ KHG22
Environment-2						
Heterotic range	-19.83 to 25.9	-13.7 to 40.54	-22.40 to 7.89	-23.3 to 6.79	-10.91 to 42.06	-31.62 to 25.05
No of Significant desirable crosses	4	0	0	0	21	12
No of positively heterotic crosses	24	29	10	4	26	16
No of negatively heterotic crosses	18	13	32	38	15	26
Best heterotic crosses	KHG21/Spt G 126				KHG22/ K399	KHG21/NC 606
	Spt G 28/ Spt G 126				Spt G 126/ KHG 21	KHG21/Spt G 28
	K399/Spt G 126				KHG22/NC 606	KHG24/Spt G 126
Environment-3						
Heterotic range	-12.4 to 5.36	-17.7 to 130.8	-25.0 to 9.38	-16.0 to 1.6	-14.2 to 66.7	-27.5 to 31.25
No of Significant desirable crosses	0	2	0	0	19	15
No of positively heterotic crosses	8	28	2	2	25	17
No of negatively heterotic crosses	34	14	40	40	16	25
Best heterotic crosses		KHG24/ K399			KHG22/NC 606	NC 606/ KHG24
		KHG22/Spt G 126			KHG22/ K399	KHG24/NC 606
					NC 606/ KHG21	KHG24/ KHG22
Environment-4						
Heterotic range	-7.9 to 6.1	-13.3 to 28.6	-28.0 to 17.1	-18.9 to 5.8	-10.4 to 43.4	-31.8 to 29.4
No of Significant desirable crosses	0	1	1	0	18	9
No of positively heterotic crosses	5	30	10	9	24	11
No of negatively heterotic crosses	37	12	32	33	18	31
Best heterotic crosses		Spt G 126/ KHG 24	Spt G 126/ KHG 24		KHG22/NC 606	KHG24/ KHG22
					KHG22/ K399	KHG24/Spt G 126
					NC 606/ KHG21	KHG21/NC 606

LA: *leaf area;* **NCL**: *number of cured leaves kg*$^{-1}$*;* **YLD**: *yield; GI: grade index,* **NC**: *nicotine and* **RS**: *reducing sugars*

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of heterobeltiosis values both in positive and negative directions reflects additive as well as non-additive gene actions for the expression of leaf area. Findings of the current study validated earlier reports of Woras et al. (1993) who revealed significant heterosis in their breeding material. Non-significant heterosis was observed in leaf length and width in tobacco (Legg et al., 1970). Small amount of heterosis was also reported for leaf size by Gopinath et al. (1967) and Matzinger et al. (1971).

Number of cured leaves kg^{-1}

The heavier leaf gives more yield and counts lesser in a unit weight so negative heterosis values become desirable for this parameter. The results of environment-1 and environment-2 revealed that no cross combination had desirable heterosis over better parent in negative direction (Table 4). The crosses in environment-1 exhibited heterotic values ranging between -6.76 (K399/KHG21) and 23.20 (KHG21/Spt G 126) percent and in environment-2 heterotic range was noticed from -13.73 (KHG21/Spt G 126) to 40.54 (Spt G 126/KHG22) percent (Table 4). The heterobeltiosis in positive direction in environment-1 and environment-2 indicate non-additive type of gene action for leaf number. Two crosses manifested desirable heterobeltiosis in negative direction while some crosses with undesirable heterobeltiosis in positive direction for cured leaf number in environment-3, pointing both to the presence of additive and non-additive gene actions (Table 4). The cross KHG24/K399 exhibited maximum value, followed by KHG22/Spt G 126. The heterotic range observed from -17.74 (KHG24/K399) to 130.80 (KHG24/Spt G 28) percent. At environment-4, only one hybrid with desirable significant heterotic value in negative direction for cured leaf number was Spt G 126/KHG24. The heterotic range, however, was observed between 28.60 (Spt G 28/KHG22) and -13.29 (Spt G 126/KHG24) percent.

The results across different environments are indicative of non-additive gene actions in most crosses but additive gene action in few crosses. However, few crosses viz. KHG24/K399, KHG22/Spt G 126 and Spt G 126/KHG24 had shown encouraging better parent heterosis in negative direction which could be used in developing commercial hybrids. Sheng (2007) reported significant heterosis in F_1s for the character of single leaf weight.

Yield

Results depicted in Table 4 are the percent increase over better parent and their level of significance across all Environments. It is clear that only 5 F_1 hybrids had significant positive heterobeltiosis for yield in environment-1 (Table 4), where values for heterobeltiosis ranged from -10.80 (Spt G 126/KHG21) to 7.81 (KHG24/Spt G 28) percent (Table 4). In environment-2 and environment-3, none of the hybrid had significant positive heterobeltiosis. However, many crosses exhibited heterobeltiosis in negative direction, indicating additive gene inheritance for yield in the material. The heterotic values ranged from -22.40 (KHG24/Spt G 28) to 7.89 percent (Spt G 126/KHG24) and -25.00 (NC606/K399) to 9.38 percent (KHG22/Spt G 126) in environment-2 and environment-3, respectively (Table 4), while in environment-4 there was only one hybrid (Spt G 126/KHG24) with 17.1 percent heterobeltiosis (Table 4). The range of heterobeltiosis values was -28.0 to 17.1 percent in environment-4 (Table 4).

Yield of cured leaf in FCV tobacco is a complex polygenic trait in nature. Its inheritance has been characterised as the most fluctuated one. Most of the hybrids could not surpass better parent which indicated absence of over-dominance and involvement of additive type of gene action in the inheritance of this important trait. The environment-1 appeared to have favourable conditions for complementary gene action in the breeding material. The hybrids KHG24/Spt G 28 with its reciprocal and KHG21/NC606 were most important in manipulating the better parent heterosis to achieve higher yields over commercial varieties. The results of the current study authenticate the results of Marani and Sachs (1966), who reported 21 percent higher yield in their hybrids as compared to parents. Significant positive heterosis for yield in tobacco had been reported by Vandenberg and Matzinger (1970). Legg et al. (1970) found small but positive and significant heterosis in yield of tobacco. Like results of environment-1, Matzinger et al. (1971) also reported 9.8 percent more yield in F_1 hybrids than the parent varieties. Heterosis in yield of tobacco has been endorsed by several other researchers including Dean (1974), Fan and Aycock (1974), Ibrahim et al. (1984), Woras et al. (1993), Butorac (1999b), Sheng (2007), Zeng (2008), Feng et al. (2009). In contrast to all aforementioned findings Aleksoski (2010) found negative but poor heterosis for yield in tobacco and opined that applying of low heterosis was economi-

cally unjustified. In the current study, the reason for manifestation of heterobeltiosis in very few hybrids may be due to narrow genetic gap in the parent material and prevalence of additive type of gene action for yield which is also reported by earlier researchers in their breeding material.

Grade index

The perceptible quality of FCV tobacco leaf is noticed by visual grades. The optimum mature leaf fetches high prices. The produced leaves from each cross were grouped into grades and then their value was converted in terms of percentage to compare the differences among various hybrids. The greater percentage meant higher economic return. The breeding material projected only one cross with significant heterosis over better parent for grade index in environment-1 (Table 4), showing the trend of additive gene action for grade index. The heterotic range was observed between -20.89 (KHG22/Spt G 126) and 9.94 percent (KHG22/KHG21). Whereas, the data observed in environment-2, environment-3 and environment-4 revealed none of the crosses with better parent heterotic values for grade index (Table 4), validating that the trait is governed by additive type of gene action. However, the heterotic ranges in environment-2 and environment-3 were observed between -23.29 (KHG22/Spt G 126) and 6.79 (KHG22/KHG21) percent and -16.0 (KHG22/KHG24) and 1.63 percent (KHG24/K399), respectively (Table 4). Heterobeltiosis for grade index in environment-4 ranged from -18.9 (Spt G 28/KHG21) to 5.8 (KHG24/K399) percent.

The better parent heterosis in positive direction for grade index was rarely found in the current crosses in all environments. The heterotic range observed was also not very much dispersed in all environments. Majority of crosses expressed heterosis in negative direction. It is indicative of additive type of gene action involved in the inheritance of grade index in the current breeding material. Only in environment-1, a single cross KHG22/KHG21 showed significant heterotic value. Due to narrow genetic base in the current breeding material for grade index, chance of its improvement is dim. Actually, grade index is cultural practices driven trait such as harvesting ripe leaf, expert curing practices, etc. which were managed fully in the handling of this breeding material. In contrast to our findings Dean (1974), Woras et al. (1993) and Butorac (1999b) found significant heterosis for the trait in their studies, which might be due to genetic and handling differences in breeding material.

Nicotine

Heterobeltiosis results for nicotine contents revealed 21 crosses with significant heterobeltiosis in positive direction in environment-1 and environment-2 (Table 4), showing a predominant role of non-additive gene action. The F_1 KHG22/NC606 exhibited maximum value, followed by KHG22/K399 in environment-1 whereas KHG22/K399 showed highest heterotic value, followed by Spt G 126/KHG21 in environment-2. The heterotic range exhibited in environment-1 lied between -11.17 (KHG24/KHG22) and 68.66 (KHG22/NC606) percent and in environment-2 it ranged from -10.91 (NC606/K399) to 42.06 (KHG22/K399) percent. Under environment-3 and environment-4, 19 and 18 crosses displayed significant positive heterobeltiosis, respectively (Table 4), confirming the presence of non-additive gene action for nicotine content. Maximum heterotic expression was shown by the cross KHG22/NC606 followed by KHG22/K399 at environment-3 with a heterotic range of -14.2 (KHG22/Spt G 28) to 66.7 (KHG22/NC606) percent. The results of environment-4 expressed the crosses KHG22/NC606 and KHG22/K399 as the first and second performers in heterotic values for nicotine content. Here the heterotic range was from -10.4 (KHG22/Spt G 28) to 43.4 (KHG22/NC606) percent (Table 4).

It is interesting to note that in all environments a number of crosses displayed significant positive heterotic values for nicotine contents. Higher increase over better parent is an indication of non-additive type of gene action in the expression of nicotine content. It was noticed that the parents KHG22, NC606 and K399 appeared to be the best combiners for nicotine contents in FCV tobacco. The results of the current study corroborate the findings of Pan et al. (2011), who found an increase in the tendency of heterosis for nicotine contents in their breeding material.

Reducing sugars

Thirteen crosses in environment-1 manifested significant heterobeltiosis in positive direction for reducing sugar (Table 4), revealing non-additive as well as additive gene actions. Among these crosses, KHG24/NC606 was on the top, followed by NC606/KHG24. The heterotic range was between -26.89 (K399/KHG22) and 27.39 (KHG24/NC606) percent (Ta-

ble 4). Under environment-2 twelve crosses appeared to have significant better parent heterosis in positive direction and 24 crosses exhibited heterobeltiosis in negative direction (Table 4), setting up trend for additive and non-additive type of gene actions. The cross KHG21/NC606 expressed maximum value of heterobeltiosis, followed by KHG21/Spt G 28. Heterotic range of -31.62 (Spt G 28/K399) to 25.05 (KHG21/NC606) percent was observed in environment-2 (Table 4). In environment-3 fifteen crosses manifested significant values of heterobeltiosis in positive direction while 24 crosses in negative direction (Table 4), confirming additive and non-additive gene actions. The cross NC606/KHG24 and its reciprocal were having maximum heterotic values. The heterotic range was observed between -27.5 (K399/KHG22) and 31.25 (NC606/KHG24) percent in environment-3 (Table 4). Nine hybrids realized significant heterobeltiosis in positive direction and 26 crosses in negative direction in environment-4 (Table 4). Here the cross KHG24/KHG22 was on the top, followed by KHG24/Spt G 126. The range of heterotic values was between -31.8 (Spt G 28/KHG24) and 29.4 (KHG24/KHG22) percent in environment-4 (Table 4).

For reducing sugars in FCV tobacco, the current breeding material had a number of hybrids that manifested heterobeltiosis in all four environments for increasing as well as decreasing trend of reducing sugar. The magnitude of heterotic values is high both in positive and negative directions. This indicates additive and non-additive type of gene actions in the inheritance of reducing sugar. Results of the reducing sugar are encouraging, as it has prime importance in the development of aroma in smoke of cigarette tobacco. Similar to the findings of the current research, Pan et al. (2011) reported a decreasing tendency in contents of sugars in hybrids as compared to parents in their tobacco lines.

Conclusions

The pattern of inheritance of different agronomic traits in the studied hybrids were both additive and non-additive types as heterobeltiosis estimates reflected both positive and negative values in the studied environments. However, disparate performances of hybrids were observed across environments, suggesting the hybrids were sensitive to varying environments. The current study discerned KHG24/Spt G 28; KHG21/NC606 and Spt G 126/KHG24 as best crosses for yield while KHG22/KHG21 was the only best cross for grade index. The mentioned crosses could be used as such or further selection could be made to evolve even a better and stable variety.

Authors Contributions

Qaizar Ahmed (QA) and Fida Mohammad (FM) designed the research, QA conducted the research. Hidayat-ur-Rahman (HUR) reviewed and made critical corrections in the first draft. Sheraz Ahmed (SA) contributed in the data collection and analysis. SA and Fakharuddin (FU) equally contributed in the first draft.

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References

Ahmed, S., F. Mohammad, Q. Ahmed and M.A.U. Khan. 2014. Assessing genetic variation for morpho-agronomic traits of some native and exotic FCV tobacco genotypes in Pakistan. Am-Euras. J. Agri. Environ. Sci. 14(5):428-433.

Akbar, M., J. Anwar, M. Hussain, M.M. Iqbal and W. Sabir. 2010. Heterosis and heterobeltiosis for grain yield improvement in bread wheat. J. Agri. Res. 48(1):15-23.

Aleksoski, J. 2010. Estimation of the heterotic effect in F_1 generation of various tobacco genotypes and their diallel crosses. Second Balakan Conference on Biology 21-23 May 2010, Plovdiv. Bulgaria. p. 407-411. http://dx.doi.org/10.1080/13102818.2010.10817873

Butorac, J. 1999a. Inheritance of some leaf parameters in burley tobacco. Agriculture Conspectus Sci. 64(2):87-96.

Butorac, J., D. Vasili, V. Kozumplik and J. Beljo. 1999b. Quantitative parameter of some burley tobacco traits. Rostlinna Vyroba. 45(4):149-156.

Cundif, R.H. and P.C. Markunas. 1964. Determination of alkaloids. Tobac. Sci. 8:136.

Dean, C.E. 1974. Heterosis, inbreeding depression, and combining ability in diallel crosses of cigar-wrapper tobacco. Crop Sci. 14(3):482-484. http://dx.doi.org/10.2135/crops-

ci1974.0011183X001400030043x

Fan, C.J., and M.K. Aycock. 1974. Diallel crosses among Maryland cultivars of tobacco. Crop Sci. 14(5):679-682. http://dx.doi.org/10.2135/cropsci1974.0011183X001400050021x

Feng, T., C. Qianfeng, L.V. Qisong, T. Xiaoyun, L. Yueping, C. Zhifeng, S. Haitao, T. Minghui and P. Fangfang. 2009. Analysis on heterosis of major agronomic and yield traits of red sun-cured tobacco. Chinese Tobac. Sci. 1:77-81.

Fonseca, S. 1965. Heterosis, heterobeltiosis, diallel analysis and gene action in crosses of *Triticum aestivum* L. PhD Thesis. Purdue University USA.

Gopinath, D.M., R. Lakshminaray and C.L. Narayana. 1967. The mode of gene action in flue-cured tobacco. Euphytica. 16(2):293-299. http://dx.doi.org/10.1007/BF00043464

Gupton, C.L., P.D. Legg, L.A. Link and H.F. Ross. 1974. Genotype x environment interactions in burley tobacco variety tests. Crop Sci. 14(6):811-814. http://dx.doi.org/10.2135/cropsci1974.0011183X001400060010x

Ibrahim, H., B. Sliavik and N. Avratovscukova. 1984. Yield and yield components in flue-cured tobacco and their genetic analysis. Biologia Plantrarum. 26(4):285-292. http://dx.doi.org/10.1007/BF02902910

Legg, P.D., G.B. Collins and C.C. Litton. 1970. Heterosis and combining ability in diallel crosses of burley tobacco, *Nicotiana tabacum* L. Crop Sci. 10(6):705-707. http://dx.doi.org/10.2135/cropsci1970.0011183X001000060033x

Mackey, I. 1976. Genetics and evolutionary principles of heterosis. In: Heterosis of Plant Breeding Proc 8th Congr. Eucarpia. Elsevier, (Eds.): A. Janossy and F.G.H. Lupton. p. 17-33.

Marani, A., and Y. Sachs. 1966. Heterosis and combining ability in a diallel cross among nine varieties of oriental tobacco. Crop Sci. 6(1):19-22. http://dx.doi.org/10.2135/cropsci1966.0011183X000600010005x

Matzinger, D.F., E.A. Wernsman and H.F. Ross. 1971. Diallel crosses among burley varieties of Nicotiana tabacum L. in the F_1 and F_2 generations. Crop Sci. 11(2):275-279. http://dx.doi.org/10.2135/cropsci1971.0011183X001100020033x

Mulekano, O.L.P. 1999. Evaluation of elite breeding lines of flue-cured tobacco for field and market performance. Master Thesis. Sustainable Plant-Genetic Resource Management, University of Pretoria, Pretoria.

Pan, Z., L. Dai, W. Cheng-lin, Z. Xi and S. Cuihua. 2011. Analysis on heterosis of chemical constituents and dynamic of agronomic traits in flue tobacco. Crop Research, China.

Sheng, H.R. 2007. Genetic studies on heterosis of flue cured tobacco ms hybrids. PhD Thesis. Hunan Agriculture University.

Suggs, P.W., J.E. Beeman and W.E. Splinter. 1960. Physical properties of green Virginia type's tobacco leaves in relation to length and breadth of leaf area. Tobac. Sci. 4:194-197.

Vandenberg, P., and D.F. Matzinger. 1970. Genetic diversity and heterosis in Nicotiana. III. crosses among tobacco introductions and flue-cured varieties. Crop Sci. 10(4):437-440. http://dx.doi.org/10.2135/cropsci1970.0011183X001000040037x

Woras, G., E.A. Hashmi, M.Z. Qazi, B. Ali and Z. Ahmad. 1993. Performance of different Virginia tobacco (*Nicotina tabacum* L.) hybrid strains and their parents. Pak. Tobac. 28(1-2):5-8.

Wynne, J.C., D.A. Emery and P.H. Rice. 1970. Combining ability estimation in Arachis hypogaea L. II. Field performance of F_1 hybrids. Crop Sci. 10(6):713-715. http://dx.doi.org/10.2135/cropsci1970.0011183X001000060036x

Zeng, H.Y. 2008. Genetic studies on heterosis of economic characters in flue cured tobacco. Master's thesis, Hunan Agriculture University, China.