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SCREENING OF ELITE TOBACCO (*NICOTIANA TABACUM* L.) GENOTYPES FOR THEIR PHYSIOLOGICAL TRAITS AND RESISTANT TO TOBACCO BUD WORM (*HELIOTHIS VIRESCENS* F.)

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Abstract

Varietal resistance is an effective pest control strategy, if used in combination with other control measures, taking this the present experiment was conducted at Philip Morris Pak. Ltd formally known as Lakson Tobacco Company Limited Mardan Khyber Pakhtunkhwa during 2006-2007; to investigate the growth and yield parameters and comparative natural resistance against tobacco budworm, for this purpose four tobacco genotypes comprising Speight G-28, NC- 606, K-399 and RGH-4 were tested. The experiment was laid out in Randomized complete block (RCB) design with 4 replications and 5 treatments including check, results revealed that for each cultivar statistical differences were observed and recorded for parameters i.e. damaged plants (%), plant height (cm), number of leaves per plant, leaf area (cm^2), yield per hectare (kg), Grade index (%) that were at par for RGH-4 followed by Speight G-28 and K-399 in comparison with NC-606. We concluded from this experiment that genotypes RGH-4 and Speight G-28 were recorded comparatively more resistant against the pest and for other traits so in case of unavailability of RGH-4 the Speight G-28 may also be cultivated for good performance in terms of comparatively resistant cultivar against tobacco budworm.

Introduction

Tobacco (*Nicotiana tabacum*) belongs to the family Solanaceae which also includes some other important crop species such as tomatoes, potatoes peppers etc (Bakht *et al.*, 2012). It is cultivated on about 0.27% area of Pakistan is of great economic importance and a source of revenue, employment and foreign exchange (Anon., 2005). As far as the yield in Pakistan is concerned, it is higher than mostly tobacco growing countries of the world like China, India, Brazil, America and Greece. But when it comes to quality, our tobacco is inferior and do not fetch good price in international market (Badshah, 2005). A lot of factors are responsible for its low quality. Among these, damages caused by the insect pests and the chemicals applied for their control are the serious constraints (Anon., 1979). Among insect pests the most important are cutworms (*Agrotis ipsilon, A. segetum, A. flammatra*), budworms (*Heliothis virescens* (F.) (Lepidoptera: Noctuidae) and aphids (*Myzus persicae* and *Aphis tabaci*) (Homoptera: Aphididae). These pests adversely affect the crop growth and yield (Sajjad *et al*., 2011). Insect pest attack start right from the nursery and continue till crop maturity. Among these pests the most damaging one is the budworm, *Heliothis virescens* (F). (Badshah *et al*., 2011).

The moths generally appear during March-April, and lay white or cream color, spherical eggs on the leaves. After hatching, the young larvae start feeding on leaves. Larvae have 5-6 instars and most of the damage is done in larval stage. During development the larvae may go from one plant to another one. Late in August the pest pupate in the top 4 cm of soil. There are 3-4 generations per year of this pest. Usually a single caterpillar can damage up to 12 leaves the insect attacks all portions and all growth stages of Tobacco (Atwal & Dhaliwal (2009). Due to pest attack the quality of the tobacco is highly affected and has low market price ultimately the farmers bear great financial losses (Patil & Chari, 1977).

Indiscriminate application of pesticides during 1980s and 1990s has contributed a lot in heavy out breaks of *H. armigera* (Ahmad *et al*., 1997; Wage, 1989). There are now several pesticides resistant biotypes active in various cropping systems worldwide (Johnson *et al.*, 1997a and b). The insect has developed resistance to even the most modern insecticides like endosulfan, profenofos, thiodicarb, cypermethrin, alphacypermethrin, deltam-ethrin, lambdacyhalothrin, bifenthrin and cyfluthrin (Maitland, 1996) besides, due to excessive use of these pesticides, residues in tobacco and other crops is getting greater attention. Tobacco cured leaf is highly ideal for residues due to more leaf surface area to weight ratio. Therefore, there are greater chances of pesticides residues accumulation in tobacco (Sreedhar *et al*., 2004). Various control measures are used to minimize crop losses caused by this pest (Kharboutli *et al.*, 1999). Varietal Resistance is an effective pest control strategy, if used in combination with other control measures like biological, cultural, plant extracts and chemical control (Johnson *et al*., 1992), moreover extracts from wild species of plants for insecticidal properties could lead to the discovery of new agents for pest control (Manzoor *et al*., 2011). The concept of Varietal /insect resistance is not particularly new and some important practical progress has been made in other crops. For example jassid resistance in cotton is related to leaf hairiness and is a controllable factor in breeding programs. In tobacco there has been little investigation. There are some unexplained preferences, such as that of Thrips tabaci in Turkey for Malaya tobaccos over bursa types, even when the two are grown

in the same locality (Akehurst, 1981). There are possibilities for further investigation. It was found by Burk & Stewart (1969., 1971) that the species of *Nicotiana* resistant to aphids, budworms and hornworm showed, in each case, phylogenetic relationship, which suggests that the respective mechanism might have a common origin.

Keeping in view the above facts the experiments were initiated to find out the most resistant variety against this pest and to determine the growth and yield parameters of these tobacco genotypes.

Materials and Methods

Four Flue cured Virginia tobacco FCV varieties/hybrids viz., Speight G-28, NC- 606, K-399 and RGH-4 (hybrid) were sown on raised seed beds under polythene shelter on December 15, 2006. Seed beds were irrigated twice a day with sprinkler till germination. Germination completed in 22 days after sowing. Proper weeding and thinning were done. Healthy seedlings about 5-6 inches in length with pencil size thickness were selected and transplanted on March 5, 2007. Irrigation was given immediately after plantation and fertilizer application. The experiments were laid out in Randomized complete block (RCB) design with four replications and five treatments including check, and in each replication with four rows per treatment. Plant-to-plant and row-to-row distance was kept 60 cm and 90 cm, respectively. There were at least 60 plants per treatment (15 plants/row). The parameters tested in the course of experiments included Damaged plants (%), Plant height (cm), Number of leaves per plant, Leaf area (cm²), Yield per hectare (kg), Grade index (%), Reducing sugar (%), Nicotine content (%) and the methodology adopted to determine these parameters were as under:

A. Damaged plants (%): The numbers of damaged plants by *H. virescens (F)* in each treatment were counted throughout the season and the percentages were calculated.

B. Plant height (cm): After the plants attained maturity, 10 randomly selected plants from the central two rows in each treatment were measured (cm) from soil level to tip of the upper most leaf of plant by a measuring rod.

C. Number of leaves per plant: Number of leaves per plant was recorded by selecting 10 plants randomly in each treatment. The number of leaves from bottom to top of the main stalk of each plant was counted after topping the plants (flowers removal).

D. Leaf area (cm²): Leaf Area was calculated through a measuring rod by selecting 10 plants randomly in each treatment. All the four plant positions (Lugs, Cutter, Bodied leaf and tips) were measured.

Total leaf area= (leaf length x leaf width) 0.643

E. Yield (kg ha^{-1}): Total weight (kg) of cured leaves in each treatment after each picking were summed and yield per hectare for each treatment were obtained as under:

$$\text{Cured leaf yield (kg/ha)} = \frac{\text{Total cured leaf weight (kg)}}{\text{Net area harvested (m}^2\text{)}} \times 1000$$

F. Grade index (%): Grade index was calculated on grade turn out of the total yield according to the grade description of each grade by Pakistan Tobacco Board.

G. Reducing sugar (%): Reducing sugar was calculated by collecting and analyzing samples of each plant position (Lugs, cutter, bodied leaf and Tips) in Lakson Tobacco company central analytical services laboratory, Karachi. Generally reducing sugar ranges from 10.00 to 18.00 % depending on various factors. Ideally we prefer reducing sugar of 14 to 16 % for better taste and after smoke effects. Maximum reducing sugar is found in Cutters (Middle) plant portion.

H. Nicotine content (%): Same procedure was adopted as mentioned above for reducing sugar. Nicotine % also depends on various factors and ranges from 1.00 to 3.20%. Ideally we prefer Nicotine percentage of 2 to 2.50% for better taste and smooth burning. Maximum Nicotine content is found in Tips (Top part) of the FCV Tobacco plant.

No preventive measures were adopted for the control of *H. virescens.*, throughout the whole experiment. Percent plant damage/infestation was recorded throughout the season while the other parameters i.e. Plant height (cm), Number of leaves per plant, Leaf area (cm²) were recorded when the crop was de-flowered (topped) and de-suckered manually, Similarly yield per hectare (kg) data was recorded at the end of the season when the last curing was done. The data for individual parameters were analyzed according to appropriate statistical procedure for RCB design using DMR-test at 0.01% level of probability.

Results

Four genotypes of tobacco were tested for their relative resistance to tobacco budworm, *Heliothis virescens* (F.). Estimates of percent infestation, plants height, number of leaves per plant and leaf area were recorded in the field when the crop de-topped while total yield of specific cultivars/hybrid was made by taking the average weight of cured leaf of the total plot at the end of the season. The observations for yield were recorded at an interval of one week after each curing. Results of percent infestation, total yield and other parameters of particular cultivars/hybrids are described in different Tables from 1 to 5.

Results indicated from Table 1 that minimum percent plant damage was recorded in RGH-4 (26.57%) followed by Speight G-28 (30.77%) and K-399 (34.70%) which

were significantly different from NC-606 (43.68%). Maximum percent plant damage was recorded in NC-606.

Table 1. Percent damaged plants by *Heliothis virescens* (F.) in different tobacco genotypes at Shergarh/ Mardan in crop 2007.

Variety/Treatments	Percent infestation /damaged plants
Speight G-28	30.775 bc
NC-606	43.688 a
RGH-4	26.57 5 c
K-399	34.700 b

Means in a column followed by the same letters are not significantly different at 0.1 % level of probability (DMR-test)

Similarly Table 2 showed that plant height was statistically not significant between the two cultivars of Speight.G-28 and K-399 i.e., 98.78 cm and 97.86 cm respectively, while it was significantly different for RGH-4 cultivars 103.24 cm, the lowest plant height was recorded in NC-606 cultivar (93.42).The data of number of leaves per plant was also found exactly like recorded for plant height that was not significantly different between Speight G-28 and K-399 cultivars however, maximum number of leaves per plants were recorded in RGH-4 (20.60) cultivar that was statistically different from all other cultivars. Similarly in NC-606 the lowest number of mean leaves were recorded i.e., 14.6 per plant. The data of leaf area (cm2) was also found significantly not different among the different three cultivars i.e. Speight.G-28, RGH-4 and K-399 while significantly different from leaf area of NC-606 i.e., 612.46 cm2, the lowest leaf area was recorded in NC-606 cultivar.

Table 2. Effect on plant height, number of leaves per plant and leaf area of different genotypes at Shergarh/Mardan in Crop 2007.

Variety/Treatment	Plant height (cm)	Number of leaves per plant	Leaf area (cm^2)
Speight.G-28	98.78 b	17.60 b	697.99 a
RGH-4	103.24 a	20.60 a	702.14 a
K-399	97.86 b	16.60 b	684.64 a
NC-606	93.42 c	14.60 c	612.46 b

Means in columns followed by the same letters are not significantly different at 0.1 % level of probability (DMR-test)

Data in Table 3 indicated that maximum mean percent grade index was recorded in cultivar RGH-4 (72.2%) that was significantly different from the other three treatments the lowest grade index was found in NC-606 while in the rest two cultivars grade index was statistically non significant with each other.

The results given in Table 4 indicated that maximum mean percent reducing sugar was found in RGH-4 ranged from 10.70 to 15.47% in different plant positions while it was 7.19 to 8.828% in NC-606 cultivar, in case of speight.G-28 and K-399 it was found as 9.78 to 11.78% and 7.99 to 14.90% respectively. It was also found that reducing sugar was in maximum quantity in cutters in comparison with other parts. Similarly, Nicotine content (%) in different tobacco cultivars was recorded from 1.66 to 2.42% in RGH-4 while it was 1.65 to 2.19% in Speight. G-28, 1.48 to 2.94 in K-399 while in NC-606 it was found as 1.87 to 3.00% as clear from NC-606 it was found as 1.87 to 3.00% as clear from Table 5.

The maximum and minimum yields of four cultivars are presented in Table 6. As evident from the results, there was significant difference among the cultivars for the total yield. The maximum yield was recorded for the cultivar RGH-4 (2300.70 Kg/ha) which was significantly different from, Speight G-28 (2000.50 Kg/ha and NC-606 (1400.70 Kg/ha). RGH- 4 was followed by Speight G-28 in terms of yield while with a non-significant variation to cultivar K-399 while significantly different from cultivars NC-606, and no significant difference was found between these cultivars at 5% level of probability but they were significantly different than cultivar RGH-4 and Speight G-28. Minimum yield was recorded on NC-606 (1400.7 Kg/ha), which was significantly lower than rest of tobacco cultivars.

Table 3. Plant position wise grade index in different tobacco genotypes at Shergarh, Mardan in crop 2007.

Variety	Plant position wise grade index				
	Lugs	Cutters	Leaf	Tips	Mean
Speight-G-28	50.9 b	51.95 b	52.30 b	53.8 b	52.23 b
RGH-4	67.9 a	71.8 a	73.5 a	75.6 a	72.2 a
NC-606	36.8 c	43.6 d	45.8 c	47.8 c	43.5 c
K-399	47.30 b	49.60 c	51.6 b	53.8 b	50.57 b

Means in columns followed by the same letters are not significantly different at 0.1 % level of probability (DMR-test)

Table 4. Plant position wise nicotine content in different tobacco genotypes at Mardan in crop 2007.

Variety	Plant positions wise Nicotine content % age.			
	Lugs	Cutters	Leaf	Tips
Speight G 28	1.65 a	1.93 b	2.19 b	2.175 b
RGH-4	1.66 a	1.96 b	2.42 b	2.87 a
NC-606	1.87 a	2.06 a	3.00 a	2.33 b
K-399	1.48 a	1.56 b	2.73 ab	2.94 a

Means in columns followed by the same letters are not significantly different at 0.1 % level of probability (DMR-test)

Table 5. Plant position wise reducing sugars in different tobacco genotypes at Mardan in crop 2007.

Variety/Hybrids	Plant positions wise reducing sugars % age			
	Lugs	Cutters	Leaf	Tips
Speight G 28	9.78 a	11.93 b	9.77 b	9.87 b
RGH-4	10.70 a	15.47 a	10.67 a	11.26 a
NC-606	7.19 c	9.50 c	8.49 c	8.828 c
K-399	7.99 b	14.90 a	10.16 ab	11.95 a

Means in columns followed by the same letters are not significantly different at 0.1 % level of probability (DMR-test)

Table 6. Yield (Kg/ha) of different tobacco genotypes at Shergarh, Mardan in crop 2007.

Variety	Total Yield
Speight G-28	2000.525 b
NC-606	1400.725c
RGH-4	2300.70 a
K-399	1800.80 ab

Means in columns followed by the same letters are not significantly different at 0.1% level of probability (DMR-test)

Discussion

Significant differences were found among the different cultivars. Comparatively most resistant cultivars were RGH-4 and Speight-G 28, followed by K-399. The most susceptible cultivar was NC-606 found significantly different from all three other cultivars. It is clearly evident that different tobacco cultivars had different response towards tobacco budworm, *Heliothis virescens* (F.) resulting in increased/decreased percent infestation. In high resistant cultivars, low infestation of tobacco budworm was found, that also affected the other physiological characteristics i.e. plant height, mean number of leaves per plant and leaf area these are the parameters that may play vital role in the total yield of the plant, which may be due to the natural resistance found in the different cultivars (Ramaswamy, 1987; Thurston, 1972). While in most susceptible cultivars highest infestation minimum plant height, minimum mean number of leaves, small leaf area, minimum grade index, low reducing sugars while high amount of nicotine content was recorded may be the high infestation level and other parameters in NC-606 is due to this chemical constituent that *Heliothis virescens* (F.) prefers in comparison with other cultivars.

Total yield of four different cultivars showed that there were significant differences among the different cultivars at 0.1% level of probability. The cultivar NC-606 gave the minimum yield of 1400.70 Kg/ha, which was significantly lower than the rest of cultivars. The cultivars Spt-G-28 and K-399 showed significantly no difference but significantly at par with the cultivars RGH-4, which produces the highest yield due to tall plants, maximum number of leaves and broad leaf area in comparison with NC-606 cultivar (Juba *et al.,* 2000, Burk & Stewart. 1971). The present studies were designed for a general screening of different tobacco cultivars for resistance against tobacco bud worm. We did not study the mechanisms responsible for such genetic resistance. Leaf hairs, cuticular waxes, plant phenols, temperature of the area and tannin have been reported as different mechanisms responsible for resistance and yield traits in different plants i.e. Maize crop (Akber *et al.,* 2009), but specific literature is not available so far related to specific resistance in tobacco except (Almaas & Mustaparta, 1990).

Due to indiscriminate use of insecticides, development of resistance has been observed in number of pest insects of various crops in Pakistan and the cited trend now established for the food preference, consumption and utilization might get changed after a short interval or span of time for *H. virescens* (Ahmad *et al.,* 1997). In the light of above discussion and keeping in view all the results presented here, it is suggested that different parts of tobacco like lugs, cutters, leaf, tips and stems should also be tested for further investigations and such findings can go a long way in studies for developing the use of plant, microbial and biological derivatives in the pest control research.

Conclusions and recommendations: Based upon the different characters showed by each individual genotype among the four tested genotypes against the tobacco budworm throughout the experiments it is concluded that RGH-4 is the most promising one followed by Speight G-28. So in case of unavailability of RGH-4, growers are advised to plant Speight G-28, that will show best performance and least affected by tobacco budworm that will lead to lower cost of production.

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