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# DEVELOPING TOBACCO CULTIVARS FOR HIGH SEED YIELD, HIGH BIOMASS AND OTHER PHYTO-CHEMICALS

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**Tobacco is a one of the important high value commercial crop grown in India. It is the economic lifeline of millions of people, including six million farmers and traditionally used for smoking and chewing purposes. However, worldwide anti-tobacco campaign posing a serious challenge to continuation of tobacco for its traditional uses. In view of this, research efforts are made at ICAR-CTRI, Rajahmundry towards exploring tobacco for its non-conventional and economically viable alternative uses. In the present study, two sets of 28 crosses each and their parents were evaluated for exploiting them for alternative uses of tobacco. Variability was observed for various characters recorded in both set one and set two crosses and parents. In set one, minimum values for number of primary branches, secondary branches, weight of single capsule and seed yield were found to be lower and maximum values were higher in crosses than parents suggesting epistatic effects. With around 21% improvement in total seed weight per ha, the cross EC.554900 X A-145 can be a choice cross in set one for seed yield improvement through its direct use or pedigree breeding for selecting high yielding sergeants. In set two, both minimum and maximum values under green leaf and total biomass were high in crosses than parents indicating dominance or positive epistasis. Reduction was observed in both minimum and maximum values for contents of nicotine, solanesol and protein in set two indicating negative epistasis or additive effects. However, minimum values for various parameters were observed to be lower and maximum values were higher for per hectare yields of nicotine, total solanesol and total protein (marginally) in set two crosses than parents. In set two, the crosses NP-19 X VDH-3 (24351 kg/ha) HDBRG X Abirami (24220 kg/ha) and HDBRG X NP-19 (24099 kg/ha) can be exploited for high biomass yields. While, the crosses, HDBRG X VDH-3 (33.51 kg/ha) and Abirami X NP-19 (33.98 kg/ha) with higher total solanesol may be promising crosses. Thus, the present study indicated the existence of variability for biomass yield, seed yield and phytochemicals such nicotine, solanesol and protein content which can be exploited through appropriate breeding programmes.**

## INTRODUCTION

Tobacco is one of the important commercial crops of India and is being grown in 0.45 M ha of area (accounting for 0.31% of net cultivated area in the country) with 750 M kg production. It is the economic lifeline of six million farmers. Traditionally, tobacco is used for smoking and chewing purposes. In recent times, the perceived health hazards associated with this traditional form of tobacco consumption is posing a serious challenge to tobacco production, commerce and industry in India. In order to protect the livelihood of the people dependent on tobacco, this threat needs to be converted into an opportunity. It is in this context, it is imperative to intensify the efforts towards channelizing tobacco into non-conventional and economically viable alternative uses through policy initiatives, re-orientation of research efforts and forging effective collaboration with the industry.

Tobacco is found to be an excellent source of phyto-chemicals viz. nicotine, solanesol, seed oil, edible proteins (green leaf) that have pharmaceutical, agricultural and industrial uses (Chida *et al.*, 2005; Deo Singh and Narasimha Rao 2005; Sarala *et al.*, 2013). Nicotine and nicotine like compounds may slow down or ameliorate the symptoms of certain diseases like Tourette's

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syndrome, Alzheimer's disease, Parkinson's disease, Ulcerative Colitis and Attention Deficit Disorder (ADD) (Sarala *et al.*, 2013). Pure nicotine is being used in the production of Tobacco Cessation Products (TCP) such as chewing gum, nicotine patches, nicorette tablets.

Tobacco is the richest plant source of solanesol (Rowland *et al.*, 1956). Solanesol possesses anti-inflammatory, neuroprotective, and antimicrobial properties (Yan *et al.*, 2019). It is a key intermediate in the synthesis of Vitamin K2 (anti-hemorrhagic vitamin), Vitamin E (anti-sterility vitamin), Coenzyme-Q9, Coenzyme Q10 and the anticancer agent synergiser N-solanesyl-N,N2 -bis(3,4-dimethoxybenzyl) ethylenediamine. Other applications of solanesol include solanesol derivative micelles for hydrophobic drug delivery, solanesol-derived scaffolds for bioactive peptide multimerization, and solanesol-anchored DNA for mediating vesicle fusion.

The tiny tobacco seed contains 35 per cent oil and the oil is classified as linoleic oil, as it is the major fatty acid (66 – 76%) (Raju *et al.*, 2015). Tobacco seed oil is having nutritive, pharmaceutical and industrial uses (Awolola *et al.*, 2010). The tobacco seed oil is comparable to sunflower oil and is superior to groundnut oil. Another important feature of tobacco seed oil is it contains 1.30% of linolenic acid which is an essential fatty acid for human body. Refined tobacco seed oil has been used as edible oil in countries like Bulgaria, Turkey, Tunisia and Greece. However, the tobacco seed oil is not being used for edible purpose in India, but finds extensive use in paints, varnishes, lubricants and soap industries. An estimated 1300- 1500 tonnes of tobacco seed oil is expelled and exported from India to other countries for utilization in paint industry (Deo Singh and Narasimha Rao, 2005). It is used as raw material in coating industries, preparation of printing inks, dyes (Zlatanov *et al.*, 2007), production of soaps, shoe polish, varnishes (Purseglove, 1991), and an alternative to diesel fuel (Giannelos *et al.*, 2002) in addition to potential use in food and coating industries (Mukhtar *et al.*, 2007).

So far attempts have been made only to develop lines/varieties for higher yield and superior leaf quality suitable for traditional forms of tobacco consumption like cigarette, bidi, hookah, chewing etc. (Ramana Rao et al., 1993; Deo Singh *et al.*, 2003; Sarala *et al.*, 2005; Sarala *et al.*, 2013).In order to utilise tobacco for its alternative uses, research initiated to develop lines having high seed yield, oil content and other phyto-chemicals like nicotine, solanesol and high biomass through a systematic breeding programme utilising parents having variability for the said characters. The efforts in this direction are presented in the paper.

## MATERIALS AND METHODS

Two sets of 28 crosses, each, were made using eight different parents in half diallel method [n (n-1) number of crosses]. Set I crosses were generated using eight high seed yielding parents namely TI-1112, EC-554900, A-145, TI-163, GT-7, A-119, GT-8 and NP-19 in half diallel method (n (n-1) number of crosses). While crosses under Set II were generated utilising high phytochemical and biomass yielding tobacco genotypes viz. Connecticut-7-D, Speight G-140, Lanka spl, Chemical Mutant, HDBRG, Abirami, VDH-3 and KV-1. Both sets of $F_1$s (28 each) were evaluated in replicated trials during 2013-14 and 2014-15 crop season along with their respective parents. The first set genotypes were assessed for seed yield and biometric observations and the second evaluated for green leaf, total biomass and phyto chemicals such as nicotine, solanesol and protein content. The recorded data was subjected to statistical analysis (Panse and Sukhatme, 1957).

## RESULTS AND DISCUSSION

Variability observed for various characters recorded in both set one and set two crosses and parents (Table 1, 2 and 3). In set 1, minimum values for various parameters found to be lower and maximum values higher in crosses than parents suggesting epistatic effects (Table 1). In set two, both minimum and maximum values under green leaf and total biomass are high in crosses than parents indicating dominance or positive epistasis. Reduction observed in both minimum and maximum values for contents of nicotine, solanesol and protein in set two indicating negative epistasis or additive effects. However, minimum values for various parameters observed to be lower and maximum values higher for per hectare yields of nicotine, total solanesol and total protein (marginally). This may be due to the combined effects for the contents of various chemicals and biomass yields in crosses compared to parents.

In the first set, significant differences were noticed for all the characters studied indicating variability for the characters. Among the parents of first set, TI-163 recorded higher number of primary branches (3.26), GT-8 secondary branches (6.36), TI-163 high weight of single capsule (280) and A-145 higher total seed weight (770 kg). Significantly higher number of primary branches was observed in the crosses, GT-7 x GT-8 and TI-163 x GT-7 (3.60 and 3.53, respectively) and secondary branches in TI-1112 X GT-7 (7.13) than better parents. Higher single capsule weight was recorded in GT-7 X NP-19 (300 mg) and total seed weight in EC.554900 X A-145 (930 kg) than better parents. Palanichamy *et al.* (2009) also reported wide variability for seed yield in chewing tobacco germplasm. With around 21% improvement in total seed weight per ha, the cross EC.554900 X A-145 can be a choice cross for seed yield improvement through its direct use or pedigree breeding for selecting high yielding sergeants.

In the second set, the differences among parents and crosses were significant for green leaf yield and total biomass. Among the parents, GT-7 recorded higher green leaf and biomass (16049 kg/ha; 22227 kg/ha, respectively). The crosses NP-19 X VDH-3 (17076 kg/ha), GT-7 X A-119 (16299 kg/ha), GT-7 X Abirami (16188 kg/ha) and HDBRG X NP-19 (16076 kg/ha) exhibited higher green leaf yield than all the parents indicating dominance effects and/or additive effects in these crosses for yield. The crosses NP-19 X VDH-3 (24351 kg/ha) HDBRG X Abirami (24220 kg/ha) and HDBRG X NP-19 (24099 kg/ha), GT-7 X Abirami (23113 kg/ha), Abirami X NP-19 (22824 kg/ha) and GT-7 X A-119 (22516 kg/ha) found to have higher total biomass than parents and can be useful for exploitation.

Though variability is observed for contents of nicotine and solanesol, and their total yields, the differences were non-significant. Parents, A-119 (3.27% and 46.10 kg/ha, respectively) and GT-8 (3.02% and 39.07 kg/ha) reported higher nicotine content as well as total nicotine yields per hactare. However, none of the set two crosses recorded higher nicotine than any of the parental lines. Parent, HDBRG recorded highest solanesol percentage than all the parents and crosses. HDBRG X VDH-3 (33.51 kg/ha), Abirami X NP-19 (33.98 kg/ha) and HDBRG X Abirami (30.41 kg/ha) recorded higher total solanesol than high

**Table 1: Minimum and maximum values recorded under various Characters in parents and crosses of both the sets**

| Character | Parents | | Crosses | |
|---|---|---|---|---|
| | Minimum | Maximum | Minimum | Maximum |
| **Set one** | | | | |
| No of Primary branches | 3.13 | 3.26 | 3.06 | 3.6 |
| No of Secondary branches | 5.53 | 6.36 | 3.4 | 7.13 |
| Weight of Single capsule (mg) | 270 | 280 | 160 | 300 |
| Total Seed weight (kg/ha) | 537 | 770 | 369 | 930 |
| **Set two** | | | | |
| Green Leaf (kg/ha) | 8441 | 16049 | 9746 | 17076 |
| Total Biomass (kg/ha) | 13841 | 22227 | 15180 | 24351 |
| Nicotine (%) | 2.65 | 3.27 | 1.34 | 2.52 |
| Total Nicotine (kg/ha) | 27.00 | 46.10 | 20.95 | 45.23 |
| Solanesol (%) | 1.49 | 2.09 | 0.86 | 1.97 |
| Total Solanesol (kg/ha) | 14.53 | 27.25 | 13.59 | 33.98 |
| Protein (%) | 18.04 | 31.71 | 12.42 | 26.09 |
| Total Protein (kg/ha) | 147.93 | 453.45 | 182.57 | 458.7 |

yielding parent, HDBRG (27.25 kg/ha). These crosses may be promising for achieving high solanesol yields.

The protein content in parents ranged from 13% (VDH-3) to 32% (Abirami) and total protein from 148 kg/ha (A-145) to 453 kg/ha (Abirami).

**Table 2: Seed yielding ability of set one tobacco crosses and parents (2013-15)**

| S. No. | Treatments | No of Primary branches | No of Secondary branches | Weight of Single capsule (mg) | Total Seed weight kg/ha |
|---|---|---|---|---|---|
| 1 | TI-1112 X EC. 554900 | 3.26 | 5.26 | 270 | 447 |
| 2 | TI-1112 X A-145 | 3.20 | 5.00 | 220 | 494 |
| 3 | TI-1112 X TI-163 | 3.20 | 5.60 | 220 | 517 |
| 4 | TI-1112 X GT-7 | 3.06 | 7.13 | 230 | 382 |
| 5 | TI-1112 X A-119 | 3.26 | 5.93 | 240 | 551 |
| 6 | TI-1112 X GT-8 | 3.20 | 6.20 | 220 | 522 |
| 7 | TI-1112 X NP-19 | 3.20 | 5.53 | 220 | 565 |
| 8 | EC.554900 X A-145 | 3.40 | 4.60 | 200 | 930 |
| 9 | EC.554900 X TI-163 | 3.13 | 3.46 | 200 | 369 |
| 10 | EC.554900 X GT-7 | 3.20 | 4.73 | 260 | 718 |
| 11 | EC.554900 X A-119 | 3.13 | 4.20 | 240 | 736 |
| 12 | EC.554900 X GT-8 | 3.20 | 3.86 | 190 | 653 |
| 13 | EC.554900 X NP-19 | 3.13 | 4.26 | 220 | 482 |
| 14 | A-145 X TI-163 | 3.20 | 5.20 | 200 | 620 |
| 15 | A-145 X GT-7 | 3.33 | 5.66 | 200 | 573 |
| 16 | A.-145 X A-119 | 3.46 | 5.60 | 220 | 707 |
| 17 | A-145 X GT-8 | 3.06 | 5.66 | 180 | 516 |
| 18 | A-145 X NP-19 | 3.13 | 4.93 | 280 | 580 |
| 19 | TI-163 X GT-7 | 3.53 | 5.26 | 280 | 505 |
| 20 | TI-163 X A-119 | 3.06 | 5.06 | 210 | 427 |
| 21 | T1-163 X GT-8 | 3.40 | 4.53 | 200 | 513 |
| 22 | TI-163 X NP-19 | 3.06 | 4.13 | 270 | 566 |
| 23 | GT-7 X A-119 | 3.20 | 6.33 | 160 | 450 |
| 24 | GT-7 X GT-8 | 3.60 | 5.53 | 190 | 500 |
| 25 | GT-7 X NP-19 | 3.06 | 5.13 | 300 | 563 |
| 26 | A-119 X GT-8 | 3.20 | 4.80 | 220 | 563 |
| 27 | A-119 X NP-19 | 3.36 | 3.40 | 270 | 522 |
| 28 | GT-8 X NP-19 | 3.26 | 4.00 | 240 | 434 |
| 29 | TI.1112 | 3.13 | 5.53 | 270 | 537 |
| 30 | EC.554900 | 3.13 | 3.40 | 210 | 408 |
| 31 | A-145 | 3.06 | 5.06 | 220 | 770 |
| 32 | TI-163 | 3.26 | 4.20 | 280 | 404 |
| 33 | GT-7 | 3.20 | 6.33 | 220 | 342 |
| 34 | A.119 | 3.13 | 5.26 | 200 | 489 |
| 35 | GT-8 | 3.20 | 6.36 | 130 | 344 |
| 36 | NP-19 | 3.20 | 3.80 | 230 | 315 |
| S.EM | | 0.09 | 0.15 | 18.87 | 77.15 |
| CD 5% | | 0.25 | 0.44 | 52.32 | 213.83 |
| 1% | | 0.33 | 0.58 | 68.76 | 281.02 |
| CV% | | 4.91 | 5.51 | 14.17 | 25.28 |

**Table 3: Biomass and photochemical yields of set two tobacco crosses and parents (2013-15)**

| S. No. | Treatments | Green Leaf (kg/ha) | Total Biomass (kg/ha) | Nicotine (%) | Total Nicotine (kg/ha) | Solanesol (%) | Total Solanesol (kg/ha) | Protein (%) | Total Protein (kg/ha) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | A-145 X GT-5 | 11773 | 16779 | 2.30 | 32.29 | 1.72 | 24.40 | 22.94 | 337.22 |
| 2 | A-145 X HDBRG | 12134 | 16729 | 2.29 | 33.22 | 1.84 | 26.85 | 13.93 | 215.92 |
| 3 | A-145 X Abirami | 11245 | 17359 | 2.08 | 27.79 | 1.51 | 20.38 | 22.74 | 295.62 |
| 4 | A-145 X A-119 | 10357 | 14744 | 2.52 | 29.73 | 1.42 | 17.50 | 14.68 | 221.67 |
| 5 | A-145 X GT-8 | 10773 | 16940 | 2.18 | 27.40 | 1.61 | 19.67 | 17.02 | 268.92 |
| 6 | A-145 X NP-19 | 9746 | 15180 | 2.13 | 25.73 | 1.32 | 15.84 | 21.19 | 264.88 |
| 7 | A-145 X VDH-3 | 12911 | 20728 | 2.00 | 31.12 | 1.16 | 17.61 | 12.42 | 182.57 |
| 8 | GT-7 X HDBRG | 15827 | 21491 | 2.30 | 43.59 | 1.60 | 30.39 | 18.95 | 369.53 |
| 9 | GT-7 X Abirami | 16188 | 23113 | 1.71 | 33.26 | 1.34 | 26.12 | 19.77 | 385.52 |
| 10 | GT-7 X A-119 | 16299 | 22516 | 1.34 | 26.02 | 1.06 | 20.34 | 19.00 | 393.30 |
| 11 | GT-7 X GT-8 | 14133 | 19728 | 2.09 | 36.75 | 1.05 | 17.57 | 18.38 | 264.67 |
| 12 | GT-7 X NP-19 | 16021 | 21630 | 1.81 | 36.93 | 1.20 | 24.38 | 16.49 | 275.38 |
| 13 | GT-7 X VDH-3 | 14855 | 21374 | 1.54 | 28.03 | 0.98 | 18.45 | 23.05 | 458.70 |
| 14 | HDBRG X Abirami | 15577 | 24220 | 1.61 | 30.13 | 1.58 | 30.41 | 12.91 | 196.23 |
| 15 | HDBRG X A-119 | 14355 | 20852 | 2.19 | 38.48 | 1.29 | 24.83 | 17.59 | 272.65 |
| 16 | HDBRG X GT-8 | 12439 | 19478 | 1.79 | 28.16 | 0.86 | 13.59 | 23.26 | 458.22 |
| 17 | HDBRG X NP-19 | 16076 | 24099 | 1.53 | 28.77 | 0.97 | 17.12 | 22.06 | 304.43 |
| 18 | HDBRG X VDH-3 | 15188 | 20594 | 2.29 | 41.35 | 1.85 | 33.51 | 13.48 | 200.85 |
| 19 | Abirami X A-119 | 12356 | 18936 | 2.49 | 34.87 | 1.42 | 20.26 | 13.47 | 222.26 |
| 20 | Abirami X GT-8 | 11884 | 18645 | 2.38 | 33.79 | 1.60 | 22.48 | 24.43 | 410.42 |
| 21 | Abirami X NP-19 | 14938 | 22824 | 2.39 | 41.74 | 1.97 | 33.98 | 13.80 | 200.10 |
| 22 | Abirami X VDH-3 | 13744 | 20922 | 1.93 | 45.23 | 1.34 | 22.24 | 24.79 | 421.43 |
| 23 | A-119 X GT-8 | 13716 | 19492 | 2.45 | 39.32 | 1.31 | 21.10 | 24.12 | 402.80 |
| 24 | A-119 X NP-19 | 14133 | 19520 | 2.37 | 40.71 | 1.45 | 23.67 | 26.09 | 318.30 |
| 25 | A-119 X VDH-3 | 11162 | 17146 | 1.62 | 20.95 | 1.19 | 15.76 | 18.31 | 265.50 |
| 26 | GT-8 X NP-19 | 14438 | 20481 | 1.88 | 32.86 | 1.14 | 19.82 | 24.96 | 399.36 |
| 27 | GT-9 X VDH-3 | 14555 | 20741 | 1.73 | 29.29 | 0.98 | 16.79 | 20.96 | 358.42 |
| 28 | NP-19 X VDH-3 | 17076 | 24351 | 1.91 | 40.22 | 1.09 | 24.39 | 21.92 | 357.30 |
| 29 | A-145 | 8441 | 13841 | 2.65 | 27.00 | 1.49 | 14.53 | 18.04 | 147.93 |
| 30 | GT-7 | 16049 | 22227 | 2.35 | 45.94 | 1.16 | 22.70 | 21.77 | 431.05 |
| 31 | HDBRG | 10968 | 14550 | 2.85 | 36.67 | 2.09 | 27.25 | 23.25 | 344.10 |
| 32 | Abirami | 11245 | 17509 | 2.64 | 34.25 | 1.56 | 20.49 | 31.71 | 453.45 |
| 33 | A-119 | 11551 | 17037 | 3.27 | 46.10 | 1.44 | 20.83 | 22.85 | 260.49 |
| 34 | GT-8 | 10912 | 16535 | 3.02 | 39.07 | 1.89 | 24.09 | 23.27 | 302.51 |
| 35 | NP-19 | 8746 | 15355 | 2.01 | 20.42 | 1.63 | 16.12 | 25.11 | 241.06 |
| 36 | VDH-3 | 12411 | 17280 | 2.05 | 30.54 | 1.45 | 21.78 | 13.24 | 221.11 |
| | S.EM | | 1478 | 2015 | 0.38 | 07.11 | 0.30 | 4.93 | -- |
| | CD 5% | | 4097 | 5585 | NS | NS | NS | NS | -- |
| | 1% | | 5385 | 7340 | NS | NS | NS | NS | -- |
| | CV % | | 19.44 | 18.08 | 31.03 | 36.41 | 36.95 | 39.05 | -- |

None of the crosses recorded higher protein content than parents indicating negative epistasis. The total protein yield in crosses ranged from 183 kg/ ha to 459 kg/ha. Hence, the crosses may not be effective in obtaining higher protein yields. Existence of variability in rustica tobacco for various physio-chemical traits were reported in earlier studies (Saiyad *et al.*, 1984).

The present study indicated the existence of variability for biomass yield, seed yield, and phytochemicals like nicotine, solanesol and protein content which can be exploited through appropriate breeding programmes. As the present market value and demand for the phytochemicals is high, tobacco crop can be exploited for alternative uses in addition to its traditional uses.

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