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TOBACCO LEAF BIOMASS ENHANCEMENT FOR ALTERNATIVE USES BY MANAGEMENT PRACTICES IN DIFFERENT LINES

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Tobacco is a rich source of phytochemicals having pharmaceutical, agricultural and industrial importance like nicotine an alkaloid, solanesol a trisesquiterpene alcohol and organic acids (malic and citric) etc. With the objective of maximizing leaf biomass production for optimum recovery of nicotine, solanesol and proteins in tobacco, a field experiment was conducted during 2013-15 seasons at BSR farm Katheru with five varieties/ breeding lines (HDBRG, GT-7x A-145, TI-163 X A-145, RT 46-1, RT 51-1), three spacings (60 X 40 cm, 70 X 40 cm, 80 x 40cm) and two fertiliser levels (100:50:50 and 150:75:75 kg NPK/ha) tested in split- plot design with three replications. After harvesting green leaf analysed for nicotine solanesol and protein content. HDBRG with 60 x 40cm spacing and 150:75:75 NPK kg/ha recorded higher biomass leaf yields of 392.05 q/ha followed by 60 x40cm spacing and 100:50:50 NPK kg/ha with an yield of 370.64 q/ha with same spacing. Line RT 46-1 followed by TI-163 X A-145 recorded higher nicotine yields with 80 x40 cm spacing and 150:75:75 NPK kg/ha. HDBRG followed by TI-163 X A-145 with a spacing of 70 x40cm and 150:75:75 NPK kg/ha gave higher solanesol yield. HDBRG with 70 x 40cm spacing and 150:75:75 NPK kg/ha recorded higher protein yield which was on par with TI-163 X A-145 at same spacing and fertilizer level.

INTRODUCTION

Conventionally tobacco is used in the manufacture of cigarettes, *bidis*, chewing mixtures, cigars, cheroots, *hookah* tobacco paste, snuff, gutka, zarda and quiwam. However, tobacco is an excellent source of phytochemicals having pharmaceutical, agricultural and industrial importance. Tobacco plant is unique in its accessibility to genetic modification, its excellent reproducibility and big biomass. Thus, alternatively, tobacco is a source for extraction of nicotine an alkaloid, solanesol, a trisesquiterpene alcohol and organic acids (malic and citric). Apart from these chemicals, tobacco green leaf is a source of protein, oil from seed, rutin (Vitamin P) from cured leaf and furfural (industrial solvent) from stalk. Genetically modified tobacco may be used for the production of proteins, e.g. vaccines and diagnostics, or for secondary metabolites, which cannot be produced by bacteria, due to the lack of secondary plant metabolism features in such simple organisms (Weggmann, 2008). Keeping all these facts in view, the present experiment was conducted with the objective of increasing biomass production for nicotine, protein and solanesol recovery as they have commercial importance.

MATERIALS & METHODS

Field experiment was conducted during 2013-15 at BSR farm Katheru during *rabi* season. The soils are silty clay (sand, silt and clay content in 0-22.5 cm soil layer was 17, 32 and 51% and in 22.5-45 cm was 16, 31 and 53%, respectively). These Godavari Deltaic alluvium-derived Vertisols are slightly alkaline (pH 7.75), low electrical conductivity (0.30 dS/m), available N (230 kg/ha), organic C (0.44 %) and high in available P (32.0 kg/ha) and K (437 kg/ha). In the study, five varieties/ breeding lines (HDBRG, GT-7x A-145, TI-163 X A-145, RT 46-1, RT 51-1), three spacings (60 X 40 cm , 70 X 40 cm, 80 x 40) and two fertiliser levels (100:50:50 and 150:75:75 kg NPK/ha) were tested in split- plot design with three replications. Nitrogen and potassium were applied in two splits 15 days before planting and 30 days after planting and phosphorus was applied 15 days before planting as per the treatment. One irrigation was given at 30 days after planting with good quality water to all the plots for leaf improvement after second dose of fertilizer application.

Key words: Leaf Biomass, Protein, Nicotine, Solanesol, Alternate uses, SEm±

Two years data was pooled and statistical analysis was done for biometric observations, yield and chemical analysis data. Standard Processes developed at ICAR-CTRI were used for solanesol, protein and nicotine estimation from the immature green leaf at 70 days after planting. Leaves were harvested when the canopy of plant leaves completely covered the ground and the leaves near the ground start to turn yellow. Leaves were harvested three times while the first priming was done 65-70 days after planting and later two harvests were done at 10 days intervals. Green leaf was immediately processed for analysis of protein, solanesol and nicotine.

RESULTS AND DISCUSSION

Biometric observations: Observations were taken during crop growth stage showed significant treatment variations with regard to plant height and no. of leaves (Table 1). Line RT 51-1 recorded significantly higher plant height followed by

Table 1: Biometric observations & leaf yield, nicotine, solanesol and protein yields of tobacco as influenced by breeding lines, spacing levels and fertiliser doses

Treatment	Plant height (cm)	No. of leaves	Leaf Yield (Q/ha)	Nicotine (kg/ha)	Solanesol (kg/ha)	Protein (kg/ha)
Breeding Lines						
1. HDBRG	167.7	28.64	604.85	44.28	42.09	822.3
2.GT-7x A-145	129.8	18.36	291.75	46.35	29.67	715.4
3. TI-163 X A-145	121.8	15.44	273.74	59.08	38.96	746.2
4. RT 46-1	146.5	23.17	315.35	54.62	36.96	756.9
5. RT 51-1	202.6	23.33	295.23	57.65	39.59	734.1
SEm ±	**2.8**	**0.43**	**3.66**	**0.62**	**0.25**	**8.1**
CD (P=0.05)	**8.42**	**1.30**	**11.03**	**1.86**	**0.69**	**24.3**
Spacing (cm x cm)						
1.60x40	153.6	22.3	317.59	46.94	33.59	702.4
2.70x40	156.3	21.82	455.31	50.25	39.09	794.4
3. 80x40	151.1	21.93	295.65	60.00	39.69	768.1
SEm ±	**1.89**	**0.27**	**5.18**	**0.45**	**0.30**	**6.2**
CD (P=0.05)	**NS**	**NS**	**15.6**	**1.25**	**0.84**	**17.2**
Fertiliser doses NPK (kg/ha)						
1.100:50:50	150.1	21.36	394.29	47.12	33.91	689.2
2.150:75:75	157.3	22.22	318.08	57.67	41.0	820.8
SEm ±	**1.54**	**0.22**	**2.04**	**0.37**	**0.25**	**5.1**
CD (P=0.05)	**NS**	**NS**	**5.65**	**1.02**	**0.69**	**14.1**
Seasons						
2013-14	146.1	19.26	382.96	53.54	45.59	928.8
2014-15	161.2	24.32	329.4	51.26	29.32	587.2
SEm ±	**2.52**	**0.34**	**4.68**	**0.75**	**0.60**	**11.3**
CD (P=0.05)	**NS**	**NS**	**NS**	**NS**	**2.34**	**44.4**

HDBRG. HDBRG produced more no. of leaves which was on a par with RT 51-1 and RT 46-1 lines. Though significant differences were not observed between treatments, 80x40 cm recorded higher plant height and 70x40 cm recorded more no. of leaves. Significant differences were not observed for fertiliser doses but at higher dose of 150:75:75 NPK kg /ha, more plant height was recorded. Interactions between treatments were not significant.

Leaf biomass: HDBRG variety produced significantly higher yield due to more no. of leaves followed by RT 46-1, RT 51-1, GT-7x A-145, TI-163 X A-145. Line RT 51-1 though recorded higher plant height, more no. of leaves but recorded lower yields than RT 46-1 due to lower leaf length, width and harvest index. Significant differences were observed among spacings, 60x40 cm (41,666 pl/ ha) showed significantly higher yields, where as lower yields were observed (Table 1) with 80x40 cm spacing (31,250 pl/ha) which might be due to more no.of plants per unit area than other spacings. Fertiliser dose of 150:75:75 NPK kg/ha recorded significantly higher leaf yield than lower dose. Interaction effects showed that HDBRG with 60x40cm spacing and 150:75:75 NPK kg/ha recorded higher leaf yields followed by 60 x40 cm spacing and 100:50:50 NPK kg/ha compared to other lines, the reason being with more no. of leaves in HDBRG coupled with higher dose of fertilizers. Planting densities of upto 6,00,000 plants/ha showed optimium yields of biomass in tobacco for various purposes (Campbell et al ,1982). Mahendra et al (2011) reported that about 384.5 kg of crude protein, 41.2 kg of nicotine [97.8 kg of nicotine sulphate (40%)], 151.8 kg of crude solanesol, 18.9 kg of pure solanesol (90%) could be extracted from one hectare crop grown under the modified agronomic practices, with a green leaf yield of 22,450 kg/ha. Wildman (1979) suggested that direct seeding of tobacco to establish a stand of more than 3,70,000 plants per hectare with multiple harvests in one growing season, which would yield about 3000 kg of protein.

Nicotine yield: Significant differences were observed in nicotine yield due to breeding lines, spacings and fertiliser levels (Table 1). Line TI-163 X A-145 being on par with RT 51-1 line recorded significantly higher nicotine followed by , RT 51-1 and RT 46-1. There is considerable variation in nicotine in different of tobacco types produced in different agro-ecological situations (Prabhu and Narasimha Rao, 2005). Wider spacing of 80X 40cm recorded higher nicotine yield of 60 kg/ha followed by 70x40cm and 60x40 cm spacing. Fertiliser dose of 150:75:75 NPK kg/ha recorded significantly higher nicotine than lower dose. Interaction effects show that Line RT 46-1 with 80 x40 spacing and 150:75:75 NPK kg/ha recorded higher nicotine yields followed by TI-163 X A-145 line with 80 x40 spacing and 150:75:75 NPK kg/ha (Table 2).

Table 2: Nicotine yield (kg/ha) as influenced by interaction between breeding lines, spacing levels and fertiliser doses for alternative uses

Varieties(V)	Spacing levels (S)x fertiliser dose (F)					
	S1F1	**S1F2**	**S2F1**	**S2F2**	**S3F1**	**S3F2**
1. HDBRG	36.78	40.76	44.82	44.51	45.35	53.46
2. GT-7x A-145	36.01	48.14	37.96	51.66	47.37	56.96
3. TI-163 X A-145	41.10	59.40	50.78	67.51	61.40	74.26
4. RT 46-1	38.00	49.12	41.99	58.78	65.18	74.65
5. RT 51-1	52.90	67.18	49.83	54.63	57.30	64.09
	SEm ±	CD (P=0.05)	Interactions	SEm ±	CD (P=0.05)	
Variety	0.62	1.86	VS	1.00	2.78	
Spacing	0.45	1.25	VF	0.82	2.27	
Fertiliser doses	0.37	1.02	FS	0.64	NS	
			VSF	1.42	3.94	

Solanesol yield : Significantly higher solanesol recovery was recorded by HDBRG (42.09 kg/ha) than other lines; Wider spacing of 80X 40 cm recorded higher solanesol followed by 70x40cm; Fertiliser dose of 150:75:75 NPK kg/ha recorded higher solanesol yield than lower dose (Table 1). Interaction effects (Table 3) showed that HDBRG followed by TI-163 X A-145 with a spacing of 70 x40cm and 150:75:75 NPK kg/ha showed higher solanesol. It is possible to control solanesol concentration through genetic selection and more importantly through production practices (Burton et al., 1989). The potential of FCV and non FCV tobacco germplasm lines can be explored for extraction of solanesol (Narasimha Rao and Prabhu, 2005).

Protein yield: Significantly higher protein yield was observed in HDBRG (822 kg/ha) than other lines. Fertiliser dose of 150:75:75 NPK kg/ha and 70x40 cm spacing recorded higher protein yield than lower dose (Table 1) which might be due to higher biomass that contributed for higher protein yield. Interaction effects (Table 4) showed that HDBRG with 70 x40cm spacing and 150:75:75 NPK kg/ha recorded higher protein yield which was on par with TI-163 X A-145 at same spacing and fertiliser level (Table.4). Under normal cultural

Table 3: Solanesol yield (kg/ha)as influenced by interaction between breeding lines, spacing levels and fertiliser doses for alternative uses

Varieties(V)	Spacing levels (S)x fertiliser dose (F)					
	S1F1	**S1F2**	**S2F1**	**S2F2**	**S3F1**	**S3F2**
1. HDBRG	30.77	37.66	41.29	51.45	43.18	48.20
2. GT-7x A-145	27.32	29.20	25.50	33.01	28.71	34.29
3. TI-163 X A-145	31.75	40.86	32.65	48.48	33.95	46.09
4. RT 46-1	28.65	41.77	37.36	39.41	35.72	38.88
5. RT 51-1	30.52	37.36	39.12	42.61	42.20	45.71
	SEm ±	CD (P=0.05)		Interactions	SEm ±	CD (P=0.05)
Variety	0.41	1.23		VS	0.68	1.88
Spacing	0.30	0.84		VF	0.55	1.54
Fertiliser doses	0.25	0.69		FS	0.43	NS
				VSF	0.96	2.66

Table 4 : Protein yield (kg/ha) as influenced by interaction between breeding lines, spacing levels and fertiliser doses for alternative uses

Varieties(V)	Spacing levels (S)x fertiliser dose (F)					
	S1F1	**S1F2**	**S2F1**	**S2F2**	**S3F1**	**S3F2**
1. HDBRG	722	876	824	976	720	822
2. GT-7x A-145	616	751	677	776	69	778
3. TI-163 X A-145	582	722	648	950	672	900
4. RT 46-1	576	771	751	846	749	846
5. RT 51-1	696	707	735	766	681	818
	SEm ±	CD (P=0.05)		Interactions	SEm ±	CD (P=0.05)
Variety	8.10	24.3		VS	13.9	38.5
Spacing	6.20	17.2		VF	11.3	31.4
Fertiliser doses	5.10	14.1		FS	8.80	NS
				VSF	19.6	54.5

practices burley tobacco yielded 888, 470 and 281 kg/ha soluble protein at immature plant stage, about one week before topping and at the time of harvest, respectively. Immature plants of NC95 produced 1387 kg/ha of both soluble and insoluble protein. The differences in the protein yield also depend on the genetic variability including plants (Lowe, 1982.). The extractable per cent of protein from close grown flue-cured tobacco were about 8 and 9.2 to 10.8% and no significant differences in the varieties for extractable protein per cent were reported (Woodlief *et al.,* 1981). With modified cultural practices, the close-grown tobacco (3 lakh plant/ha) of *bidi* type exhibited maximum crude protein potential (25 q/ha) at 90 days growth (Patel *et al., 1985*). Ushasri *et al.,* (1986) reported a recovery of 203 kg/ha crude protein from the left over seedlings of FCV tobacco with a plant population of 2,50,000 plants/ha and harvested at 45 days of growth with 4 ratoons.

From the field experiments it can be concluded that HDBRG with 60 x40 spacing and 150:75:75 NPK kg/ha recorded higher tobacco green leaf yield of 392.05 q/ha followed by 60 x40 cm spacing with 100:50:50 NPK kg/ha with an yield of 370.64 q/ha. HDBRG followed by TI-163 X A-145 with a spacing of 70 x40cm and 150:75:75 NPK kg/ha gave higher solanesol and protein yield. Line RT 46-1 followed by TI-163 X A-145 line recorded higher nicotine yields with 80 x40 cm spacing and 150:75:75 NPK kg/ha. Possibility of commercial cultivation of these lines can be explored for alternative use of tobacco.

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