Tobacco Research 37 (1) : 1-17, 2011

NITROGEN NUTRITION OF FLUE - CURED TOBACCO

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Nitrogen is the key nutrient for plant growth, yield and quality of plants. In tobacco, leaf being economic product, too excess or too short a supply of N fertilizer leads to low yields, poor quality and very low market price. Further, the balanced nutrition of N and K holds key for successful tobacco production and higher net returns. Since soil test methods often fail to predict the N-availability to plant, plant tissue analysis is the only sure way to assess the N fertilizer requirement of crop plants. The nitrogen fertilization should be made in such a way that the plant would get enough available nitrogen during the growth period to ensure vigorous plant growth and at the same time soil nitrogen should be depleted soon after topping so as to harvest at correct stage to obtain quality leaf. Since nitrogen is the master nutrient controlling the plant growth, yield and quality of leaf, a rational N fertilization along with potassium based on soil/ plant analysis and also visual observations of the plant can only help the farmer to realize the best quality leaf and higher net returns.

INTRODUCTION

In India, tobacco is grown on 0.45 M ha of area with 750 M kg production. The world tobacco production is ~7 billion kg, China occupying the first place with 2.35 billion kg. There are ten types of tobacco grown in India, out of which flue-cured Virginia (FCV) tobacco is an important type used for manufacturing cigarettes. In India, FCV tobacco is grown in an area of 2,40,000 ha by about 85,000 registered growers in Andhra Pradesh and Karnataka states with an annual production of 300 million kg of leaf out of which nearly 200 M kg is exported to different countries. Tobacco is a leading commercial crop valued for its leaf containing nicotine in which the main constituent is nitrogen.

Indian soils are low in organic matter and nitrogen status due to tropical temperature which oxidises soil organic matter (Jenny and Raychaudhuri, 1960). Hence, nitrogen fertilization is essential for obtaining higher yields. Tobacco is remarkably sensitive to nitrogen application. It is an indispensable constituent of numerous organic compounds viz., amino acids, proteins, nucleic acids and the metabolic processes involved in the synthesis and transfer of energy. It is part of chlorophyll, the green pigment of the plant which is responsible for photosynthesis. Nitrate and ammonium ions are the major forms of inorganic nitrogen taken up by the roots of higher plants. Most of the ammonium is incorporated into organic compounds in roots whereas the nitrate freely moves into above ground parts of the plant. Nitrate ion is important for osmoregulation and ionic balance in plant cells and its reduction and assimilation is as important as CO_2 reduction and assimilation in photosynthesis. As early as in 1940's, Vickery *et al.*, reported that ^{15}N from ammonium salt was incorporated into protein within four hours after N application (Fig. 1).

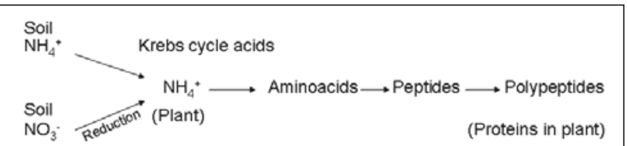

Fig.1: Synthesis of proteins in plants

Further, Calvin and Benson (1948) demonstrated that ^{14}C from $^{14}CO_2$ was incorporated into amino acids in thirty seconds. However, in a living system, utilization of nitrogen or carbon is not an isolated process; it affects the total nitrogen-carbon balance of the organic metabolism. The relationship between carbon and nitrogen in tobacco plant is of special importance as the metabolism of these two fractions essentially decides leaf quality or usability. Organically bound nitrogen from glutamate and glutamine is utilized for the synthesis of other amides, amino acids and proteins. Low molecular weight nitrogen compounds play a vital role in plant metabolism and their content in plant parts depend on the age of the plant, source of nitrogen, water supply and concentration of other nutrients. In general, the total N concentration in the cured leaf varies between 2-3% while protein nitrogen is around 1%. In this paper, the authors have made a humble attempt to review the research work done on nitrogen nutrition of flue-cured tobacco from 1940 to 2010 and tried to trace the N movement from soil to tobacco plant/leaf and ultimately to atmosphere through cigarette smoke.

Influence of N on growth and development

Balanced and desirable tobacco crop growth can only be achieved with an adequate and well-timed supply of nutrients. Nitrogen is vitally important plant nutrient, the supply of which can be controlled by the farmer. The supply of nitrogen is related to carbohydrate utilization. When nitrogen supplies are insufficient, carbohydrates will be deposited in vegetative cells which will cause them to thicken. When the nitrogen supply is adequate, and conditions are favourable for growth, proteins are formed from the manufactured carbohydrates. Less carbohydrate is thus deposited in the vegetative portion, more protoplasm is formed and because protoplasm is highly hydrated, a more succulent plant results (Tisdale and Nelson, 1975). From the seedling stage to final leaf harvest, the soil nitrogen regime greatly affects the process of plant development more than any other element (Mc Cants and Woltz, 1967).

Growth curve in tobacco

Typical growth curve for the tobacco crop is sigmoid in shape (Fig.2). After transplanting, the crop growth is slow (lag phase) and three weeks after transplanting, the plant starts growing vigorously in the development phase (exponential or active growth phase) which follows inflorescence emergence and expansion (Papenifus and Quin, 1984). Nitrogen availability to the plant during the development phase determines the rate of growth and dry matter accumulation in tobacco. With adequate moisture, an increase in the supply of nitrogen from deficiency to excessive results in an increase in

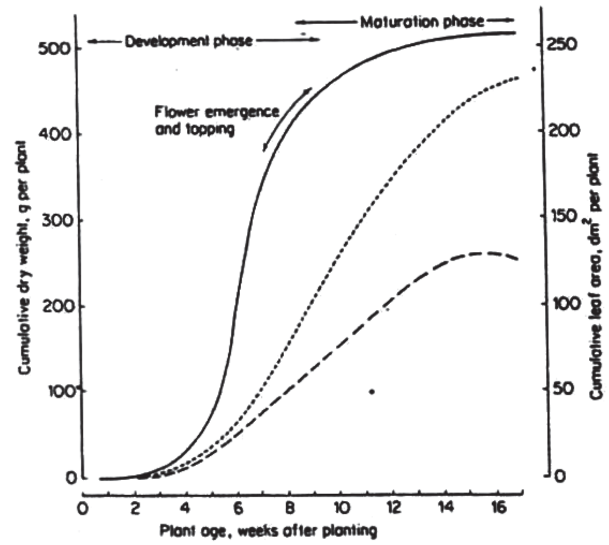

Fig.2: Schematic representation of the increase in leaf area per plant (—), the total dry matter accumulation (··········) and the leaf dry matter accumulation (——)

the area of the leaf but a decrease in the weight per unit area, the latter effect being due primarily to a decrease in the thickness of the leaf (Raper, 1966).

Morphological developments of plants greatly affect the physical and chemical characteristics of tobacco leaf and thus its quality and usability. The economic part being the leaf, it contributes to good yields and income to the farmer. The crop growth and dry matter accumulation is increased with increase in nitrogen fertilizer application (Krishnamurthy *et al.*, 1997). Nitrogen application helps vigorous crop growth, rapid gain in dry weight and leaf expansion during the developmental phase and it must be depleted during the end of the phase. If there is excess nitrogen, the nitrogen uptake is prolonged and maturation is delayed with detrimental effect on quality of the leaf. On the other hand, deficiency of nitrogen during the developmental phase results in small chlorophyll deficient leaves that cure to a pale colour, having poor texture, aroma and low nicotine content.

Hunger signs of N

When nitrogen supply is sub-optimal, growth is retarded, re-translocation of nitrogen from older leaves to newly growing leaves occur and nitrogen deficiency symptoms first appear on older leaves. Nitrogen deficiency results in reduction of plant growth, thin stems and small leaves. There will be decrease in normal green colour of the leaf. The lower leaves turn yellow, followed by drying up (Anuradha *et al.*, 2005). Typical nitrogen deficiency symptoms such as enhanced senescence of older leaves were evident as shown in Fig. 3a.

Symptoms of excess N

Higher nitrogen supply during early stages of plant growth retards root growth and enhances shoot growth, which is unfavorable for nutrient absorption and water uptake in the latter stages of plant growth. Plants with excess nitrogen fertilization will have larger, dark-green, succulent, sappy leaves with the signs of drooping and wilting during mid-day (Krishnamurthy *et al.*, 1994). Due to excess nitrogen, the leaf area increases, thickness decreases and become thicker as they mature. Excess nitrogen also results in mid-day wilting of the plants because of higher transpiring area and lower root volume for absorption of water (Fig. 3c). Excess N in tobacco induces K deficiency in plants.


Fig. 3a: Nitrogen starved crop


Fig. 3b: Healthy crop


Fig. 3c: Excess nitrogen crop

Corrective measures

Nitrogen deficiency can be corrected by application of nitrogenous fertilizers at recommended dose at the appropriate stage of plant growth for achieving maximum yield and desirable leaf quality. In tobacco fields, nitrogen deficiency is not usually observed except under heavy rainfall conditions because tobacco growers generally apply sufficient nitrogen fertilizers. Judicious use of nitrogenous fertilizers and their choice for use in tobacco govern the leaf yield and quality.

Crop response to nitrogen fertilization

Nitrogen supply has great influence on growth, curability and usability of FCV tobacco (Collins, 1989). Soil available N status, rate of application, source of N and time of availability to the plant can have a definite influence on the growth and usability of cured leaf. For production of high quality FCV tobacco, it is necessary to have a soil which is free-draining and low in organic matter content. Available nitrogen status should not be high and nitrogen starvation condition is desirable at the time of maturation of leaf. The soil should have extremely limited quantities of residual N. An excess or deficiency of N supply can substantially lower yield and quality (Gopalachari, 1984).

Two common sources of nitrogen are organic and mineral. Organic nitrogen is not absorbed in significant quantity by the tobacco plant. Use of organic manures alone for raising FCV tobacco is generally considered to be undesirable chiefly because nitrogen from such manures become available to the crop even late during the season, when the crop would be ripening (Garner, 1946). Tobacco utilizes and absorbs both mineral forms of nitrogen viz., ammonical and nitrate forms. Plant response to ammonia or nitrate forms of nitrogen rests essentially on the extent and speed of the conversion of ammonical form to nitrate form. Conditions of slow conversion are unfavourable to ammonical form as a source of nitrogen. Hence soil conditions determining nitrification and leaching are to be taken into consideration while selecting nitrogenous fertilizers (Collins, 1989).

In India FCV tobacco is mainly grown in different soils referred to as traditional black soils (TBS), northern light soils (NLS), southern light soils (SLS) and Karnataka light soils (KLS) producing different types of tobacco. The nitrogen fertilizer requirement of popular varieties grown in different tobacco zones are presented in Table 1.

Traditional black soils of Andhra Pradesh

FCV tobacco is grown with conserved soil moisture conditions in Northern black soils of East Godavari, West Godavari, Krishna and Guntur districts and under semi-monsoonic conditions in Southern black soils of Prakasam and Nellore districts. Recent findings revealed that one life saving irrigation with good quality water containing chlorides < 50 ppm is found to be beneficial for realizing high yields.

Time and method of application

As the crop is grown with conserved soil

Table 1: Manures and nutrient requirement of FCV tobacco grown in different tobacco zones

Tobacco zone	Popular varieties	Organic manures	Recommended dose of nutrients (kg/ha)		
			N	P_2O_5	K_2O
Traditional black soils (TBS)					
Northern Black Soils (NBS)	Siri, VT-1158, Hema	FYM @ 7.5 tonnes/ha	50	-	-
Central Black Soils (CBS)	Siri, VT-1158, Hema	FYM @ 7.5 tonnes/ha	50	-	-
Southern Black Soils (SBS)	Siri, VT-1158, Hema	FYM @ 7.5 tonnes/ha	60	60	60
Light soils					
Northern light soils (NLS)	Kanchan	Sunnhemp green manuring and application FYM or FPC @ 10-12 tonnes/ha	115	60	120
Southern light soils (SLS)	N-98, VT-1158, Hema	FYM @ 5 tonnes/ha	60	60	60
Karnataka light soils (KLS)	Kanchan, Ratna	FYM @ 8-10 tonnes/ha	60	40	120

moisture, it is not possible to give side-dressings of fertilizers in TBS without irrigation; the entire dose of nitrogen is given as basal dose before planting. Early application of fertilizers three weeks before planting in plant row-plough furrow (PRPF) method resulted in significant increase in cured leaf yield (Anonymous, 1980). Preliminary trials on foliar nutrition indicated that application of nitrogen as foliar spray during grand growth period either in the form of urea or calcium ammonium nitrate (CAN) or potassium nitrate did not increase cured leaf yield but increased seed yield (Bangarayya *et al.*, 1975). The quality of leaf assessed by Total Bright Grade Leaf Equivalent (TBLE) yield was adversely affected by foliar spray.

Nitrogen level

Application of 20 kg N/ha was found sufficient for dark-cast varieties that were in vogue till early sixties (Gopalachari, 1984). Dark-cast varieties gave cured leaf with low grades. A slight increase in nitrogen level drastically reduced bright grade yield. With the introduction of medium and light-cast varieties and preference for high production level, the nitrogen application is increased.

Dhanadai, Kanakaprabha, HR-65-35 and CTRI special varieties were found to give better yields at 35 kg/ha than 20 kg/ha (Krishnamurthy *et al.*, 1977). Nitrogen @ 30 kg/ha for Jayasri and Godavari special (Tripathi *et al.*, 1987) and 40 kg/ha for Hema and L-621 were found superior. Optimum dose of N for Gouthami was reported to be 40.8 kg/ha, but under conditions of delayed planting, it requires 50 kg N/ha to get maximum yields (Anonymous, 1992). The nitrogen requirement of the breeding lines V-3189, L-1158 which gave higher yield than Hema and VT-1158 were also found to be 50 kg N/ha (Harishukumar *et al.*, 1996). Later, the Siri variety which responded well to nitrogen fertilization gave higher yield at 50 kg N/ha.

Nitrogen source

Majority of the FCV tobacco farmers in TBS use ammonium sulphate (AS) as the source of nitrogen. Several experiments conducted at Rajahmundry have indicated that application of N in the form of ammonium phosphate, potassium nitrate, ammonium nitrate, Chilean nitrate, ammonium sulphate nitrate, urea, nitrophoska blue, calcium nitrate, complex fertilizers viz.,28-28-0, 24-24-0, 20-20-0, 17-17-0, calcium ammonium nitrate (CAN), and diammonium phosphate (DAP) gave cured leaf and bright grade yields comparable to ammonium sulphate (AS). Experiments conducted at Guntur indicated that DAP, large granule urea, prilled urea and AS did not significantly influence nitrogen, nicotine and leaf burn, and all the quality parameters are within the acceptable limits (Gopalchari *et al.*, 1987).

At present, in traditional black soils, application of 50 kg N/ha as AS in plant row-plough furrow (PRPF) method 3 weeks before planting is recommended. In PRPF method, the fertilizer is applied by opening furrows at 70 cm distance by covering it by a plank. Later, planting is done on the rows where fertilizers are applied.

Dry matter production and nitrogen uptake

The dry matter production and nitrogen uptake were less during quiescent phase (0-30 days after transplanting). By 90th day after transplanting (DAT), plants have accumulated 80% of total dry matter and 88% of total N uptake (Gopalachari *et al.*, 1978). Sriramamurthy and Gopalachari (1987) reported that total N concentration in the leaf was highest at 30th day and gradually decreased from 30th to 105th day. The protein nitrogen was highest at 30th day and gradually decreased from 60th to 105th day of plant growth. Higher levels of applied N increased nitrogen and nicotine content and decreased the reducing sugars in the leaf. There was an increase in thickness and filling value with increasing level of nitrogen from 20 to 60 kg N/ha. Neither pore volume nor equilibrium moisture content (EMC) were affected by nitrogen level. Among the chemical constituents, there was significant reduction in soluble carbohydrates with increasing levels of nitrogen. The quality ratios were also affected by high nitrogen (Sriramamurthy and Gopalachari, 1987).

Northern light soils (NLS) of Andhra Pradesh

FCV tobacco is grown in light soils during *rabi* season with assured irrigations. As the soils are coarse textured, low in organic carbon and residual nitrogen, nitrogen management plays a decisive role in the production of quality FCV tobacco. This zone is known for the production of semi-flavouraful tobacco. Northern light soils being lighter in texture (sandy to sandy loam) with low water holding capacity are essentially irrigated and are prone to leaching of nitrogen. Hence nitrogen application is preferred in splits. Nitrogen application in 3 splits depending on soil texture is recommended to minimise the leaching losses in the early growth stages. Since first harvest starts by 65-75 days after transplanting (DAT), nitrogen availability should decrease for getting normal ripening. In NLS, the fertilisers are applied by Dollop method.

Time and method of application

Investigations on the efficacy of N-fertilizer indicated that the application of N in three splits i.e., 25%, 50% and 25% at 10^{th}, 25^{th} and 40^{th} after planting, respectively, is quite effective in meeting the plant requirements and minimizes the leaching losses. Development of leaf from bottom pick depends on the first split, while leaf from middle pick utilizes N from the two succeeding picks and the top position leaves and suckers depend mostly on native N. The plant utilizes only 46.2% of fertilizer N and is distributed among different plant parts (lamina 21.3%, stem 7%, roots, 7.6% midrib, 4.3% and suckers 6%) (Rao *et al.*, 1992).

Nitrogen level

Exploratory trials in NLS area were started with 30 kg N/ha. At this level, cured leaf yields, N and nicotine in leaf were low and colours varied from yellow to lemon with higher percentage of bright grades. Fertilizer trials in NLS on different varieties (HR 70-57, Mc Nair-133, Line 1784, 16/ 103, Jayasri, Speight G-28) indicated linear response in cured leaf yields up to 60 kg N/ha. Research results also indicated that 50 to 60 kg N/ha produced significantly higher TBLE yield (Sannibabu *et al.*, 1981). For higher TBLE yields, optimum dose of N was found to be 48 kg/ha and economic dose of N was 47.71 kg/ha (Ramachandram *et al.*, 1984). With the change in preference for leaf colour and style, optimum nitrogen level of 60 kg/ha was recommended. Ramachandram *et al.* (1995) reported that closer spacing of 1.0 X 0.5 m produced significantly higher cured leaf and top grade equivalent with increase in N levels from 50 to 90 kg/ha. They also stated that variety Mc Nair released during 1986 for NLS was found to realize higher yield at 90 kg N/ha. Later with the advent of high yielding variety Kanchan (>2500 kg/ha), the nitrogen requirement was found to be 115 kg/ha applied in three splits in 1:2:1 ratio at 10, 25 and 40 DAT. At 10 DAT, nitrogen is applied as DAP and at 25 and 40 DAT, N is applied as CAN.

Nitrogen source

Application of straight fertilizers produced significantly more cured leaf and medium grade leaf yield over mixed fertilizer 17-17-17 in NLS. However, bright grade and TBLE yields were not affected. Physical (filling value, EMC and leaf burn) and chemical (nicotine, nitrogen, reducing sugars and chloride) quality parameters were also not significantly influenced by source of nitrogen (Anonymous, 1982, 1983). Later work indicated that various nitrogen sources AS, CAN, DAP, 28-28-0, 24-24-0, 20-20-0 and urea) did not significantly influence leaf yields. However, CAN gave 7.9, 12.8, 11.6% increase in cured leaf yield, bright leaf and TBLE yields over AS, respectively (Anonymous, 1992). In an experiment conducted with different forms of nitrogen revealed that application of total N in the form of nitrate increased cured leaf yield and grade index over other N sources supplying lesser proportion of nitrate N. These results indicated the advantage of nitrate nitrogen over ammonical form of N (Anonymous, 1992). CAN contains 50% N in nitrate form, 50% in ammonical form, 8.1% Ca and 4.5% Mg which are needed by light soil tobacco crop and hence it is preferably used for side-dressing in tobacco. DAP is used as source of N and P as basal dose.

In NLS, application of FYM or FPC @10-12 tonnes/ha or green manuring with sunnhemp (@ 50 kg seed/ha for sandy soils is beneficial. All the inorganic fertilsers recommended to be applied by Dollop method. Nitrogen 115 kg/ha in 1:2:1 ratio in three splits at 10, 25 and 40 DAT is to be applied. If crop receives >12.5 cm rain after side dressing, an adjustment dose of 6.0 kg N and 7.5 kg K_2O N has to be applied and if crop receives

25.0 cm rain fall an adjustment dose of 15.0 kg N and 20 kg K_2O is necessary based on soil type and crop growth (Rao and Reddy, 1998).

Dry matter production and nitrogen uptake

Dry matter production and nutrient uptake were minimum during quiescent phase and it was maximum by 90 DAT. From the field experiments conducted during 1998-2001, Krishnareddy *et al.* (2003) reported that application of 110 kg N/ha and topping at 24-26 leaves at bud stage would be optimum for the variety Kanchan in terms of yield, grade index and quality.

In a field experiment conducted at CTRI RS Jeelugumilli during 2008-10 with different levels of nitrogen viz., 0, 40, 80, 120, 160, 200 kg N/ha, (Fig. 4) The cured leaf yield increased with increase

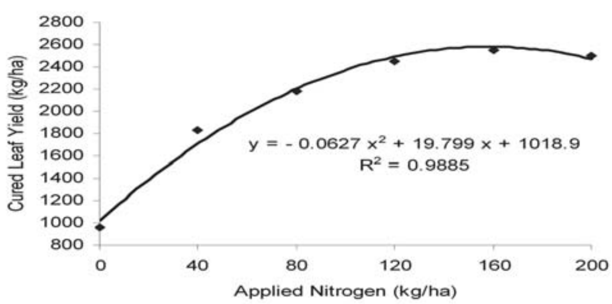

Fig. 4: Response curve for N in NLS

in applied nitrogen up to 120 kg N/ha significantly which is on par with 160 kg N/ha, showing declined trend thereafter.

Parker *et al.* (1993) reported that nitrogen in leaf lamina, midrib and stalks represent 47, 14 and 38%, respectively of the total N in tobacco at harvest, not including the amount lost in topping (flowers and the upper 3-4 leaves). Increasing N levels influence the physical and chemical quality parameters of cured leaf. With increase in nitrogen levels there was increase in total nitrogen and decrease in sugars in cured leaf.

Trials conducted at CTRI research station Jeelugumill revealed that with increased applied nitrogen level, total N concentration increased. The nitrogen uptake increased with increase in the duration of the crop up to harvest and the mean per day nutrient uptake was highest between 60-75 DAT. The N uptake was marginal between 90 DAT and harvest. At harvest, average N uptake by lamina, midrib, stem and root was 19, 13, 48 and 20%, respectively (Anonymous, 2010).

Southern light soils (SLS) of Andhra Pradesh

In SLS, tobacco is grown by using North-East monsoon rains and the area is prone to droughts and cyclones. Thus the crop faces both drought and excess soil moisture situations in the same season. Green manuring with sunnhemp or cowpea is feasible and economical under SLS conditions (Anonymous, 1988). Field trials at CTRI RS, Kandukur showed that combination of 25% N in organic form and 75% N in inorganic form was superior to 100% N in inorganic form. FYM was superior to castor cake or groundnut cake in improving yield and bright grade out-turn (Anonymous, 1983).

Time and method of application

Application of entire N as basal dose in SLS was significantly superior to split application. Further, plant row-plough furrow (PRPF) method of fertilizer application was more beneficial than Dollop and broadcast method as soil hardening and crusting is a problem. Hence, application of entire dose of nitrogen as basal dose in PRPF is recommended (Singh *et al.*, 1992).

Source and level of N

Different N-fertilsers viz., AS, DAP, CAN, Pushkal (12-6-6) and Suphala (20-20-0) applied on equivalent nutrient basis, did not show significant differences in respect of cured leaf yield for three seasons and bright grade out-turn for two seasons.

Increase in level of nitrogen increased cured leaf yield up to 50 kg N/ha whereas improvement in TBLE and bright leaf yields increased up to 40 kg N /ha only. Closer spacing of 75 x 45 cm require 50 kg N/ha (Anonymous, 1991 and 1992). FCV tobacco crop preceded by non-leguminous *Kharif* crops need higher level of nitrogen compared to legume crops. With the advent of high yielding varieties, the nitrogen requirement is increased to 60 kg N/ha.

Karnataka Light Soils (KLS)

In KLS, crop is grown as a monsoon crop during *Kharif* season from May-June to August-September. As the soils are coarse textured, low in organic carbon and residual nitrogen, the nitrogen management plays a decisive role in the production of quality tobacco. Erratic rainfall and moisture stress are common during the crop growth period, affecting the yield and quality. Under such circumstances application of organic manures help to conserve soil moisture apart from supply of nutrients.

Time and method of application

Either basal or side dressing did not significantly influence the cured leaf yield in KLS. Application of 50% N at 10 DAT and 50% N at 21 DAT is recommended to prevent leaching losses of nitrogen

Source and level of N

Among the different sources tested, CAN was at par with AS and prilled urea as basal dressing. Nitrate form of fertilizers showed superiority over ammonical form of fertilizers as side dressing (Anonymous, 1992). In KLS, majority of the farmers are applying DAP, CAN, AS. Invariably, CAN is used as a side-dresser.

Fertiliser trials at CTRI Research Station, Hunsur and AINRPT Centre, Shimoga showed that increasing levels of N up to 60 kg N/ha increased green leaf yield significantly but differences in cured leaf yield and bright leaf yield were not significant beyond 40 kg N/ha. For varieties Bhavya, FCH 107 and FCH 6248, application of 40 kg N/ha + 80 kg P_2O_5 + 100 kg K_2O significantly increased cured leaf yield and grade index over 30 kg N/ha + 80 kg P_2O_5 + 100 kg K_2O (Anonymous, 1992). Heavy rains during the early part of the season leading to nitrogen leaching may necessitate application of an additional 5 to 10 kg N/ha over the recommended dose of 40 kg N/ha.

Neem cake and pressmud were found to reduce the nematode infestation.Combination of pressmud @ 6 tonnes and 50 kg N/ha produced maximum cured leaf yield of 1769 kg/ha and net profit (Giridhar *et al.*, 2003). Naik *et al.* (2004) reported that in an integrated management involving 25% N supply through pressmud and 75% through inorganic fertilizers improve both cured leaf yield and quality of FCV tobacco significantly.

Diagnosis of N requirement

Assessment of N status in tobacco leaves requires tissue analysis as the present soil test methods for N estimation fail to predict N availability to tobacco plant (Krishnamurthy *et al.*, 1996). In the absence of reliable method for estimation of nitrogen in soil, the plant nitrogen status is the only measure to find out the N requirements of the tobacco crop. Nitrogen is estimated in the plant tissue by micro-Kjeldal distillation or by digesting the plant tissue in H_2SO_4 – HgO - K_2SO_4 digestion mixture followed by feeding to Auto-anayser. Monitoring of plant N status is important in improving the balance between crop N demands and N supply from soil and applied nitrogen (Cassmann *et al.*, 1994). As leaf N content is closely related to photosynthetic rate (Peng *et al.*, 1995) and biomass production (Kropff *et al.*, 1993), N in plant is a sensitive indicator for the dynamic changes in crop N demand with the growing season. The direct measurement of leaf N concentration by laboratory procedures is laborious and time consuming. Such procedures have limited use as a diagnostic tool for optimizing N supply to the plant. The chlorophyll meter provides a simple, rapid and non-destructive method for estimating leaf chlorophyll content. As the chlorophyll content in a leaf is closely related to leaf N concentration (Blackmer and Schepesrs, 1994), the measurement of chlorophyll provides an indirect assessment of nitrogen status. From the experimental results, it was found that Chlorophyll Content Index (CCI) measured using Chlorophyll Meter may be a valuable tool for non-destructive assessment of nitrogen content in the FCV tobacco plant (Anonymous, 2010, Follin *et al.*, 2007)

Nitrogen distribution in tobacco plant

Nitrogen is a dynamic nutrient which moves continuously within the plant. Total nitrogen concentration of leaves increases from bottom to top in ascending order in FCV tobacco plant. The FCV tobacco plant showing different leaf positions is shown in Fig. 5. Krishnamurthy *et al.* (1994) reported that the total N concentration was 20% lower in the midrib compared to its lamina. The distribution pattern of the total N in the midrib followed a normal trend. It increased steadily from P to T position in cured leaf in ascending order reaching the highest value in T position (> 2% N at all levels) (Krishnamurthy *et al.,* 1996). As no

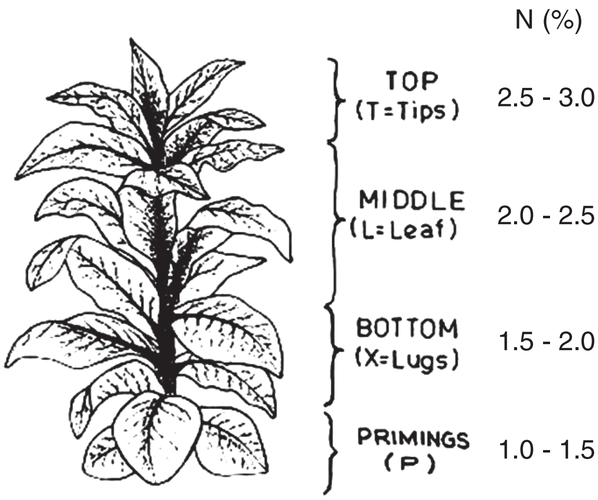

Fig. 5: Diagram showing different plant positions in FCV tobacco plant grown in Northern light soils

nitrogen will be supplied after topping (removal of terminal bud), N absorbed before topping was translocated from the lower parts of the plant to the younger plant tissue as the nitrogen is relatively mobile within the plant.

Nitrogen is a constituent of nicotine, an alkaloid, which is synthesized in tobacco roots and nicotine synthesis is regulated more by nitrogen supply than by any other nutrient (Hawks, Jr. and Collins, 1983). The distribution pattern of nitrogen within the individual mature leaf was investigated by analyzing the nitrogen content in the different segments of lamina and midrib in healthy and excess N supplied leaves. FCV tobacco leaf showing different segments of lamina and midrib is shown in Fig. 6.

The tobacco growers generally apply excess N that often induces K deficiency. The excess N-

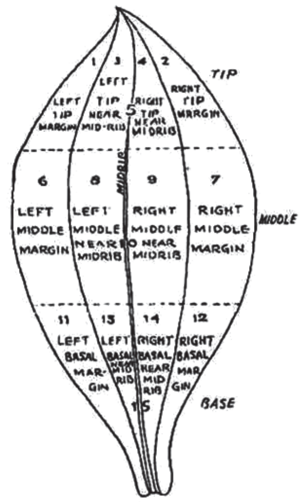

Fig. 6: Diagram of FCV tobacco leaf showing different segments of lamina and midrib (Krishnamurthy *et al.*, 1997)

induced-K-deficiency is often observed in tobacco fields in NLS and KLS. Krishnamurthy *et al.* (2000) studied the distribution of nitrogen within the mature leaf of healthy tobacco and tobacco crop grown with excess levels of N in NLS.

Nitrogen distribution in healthy leaf

The healthy leaf which is supplied with sufficient quantity of N accumulated 2.42 to 2.77% N on dry weight basis and the N distribution pattern in different segments of mature healthy green leaf is presented in Fig. 7. From the figure it is clear that there is slight increase in N content from tip to basal portion of the lamina in

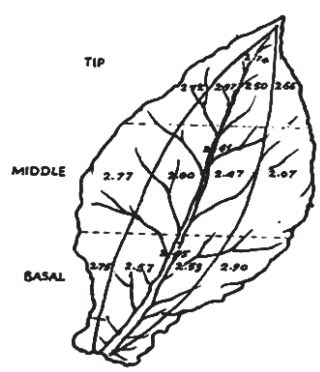

Fig.7: Nitrogen distribution pattern (N %) in different segments of healthy green leaf. (Krishnamurthy *et al.*, 2000)

descending order. The marginal portion of the lamina has slightly higher N concentration than the central portion of the lamina adjacent to the midrib. The N concentration of the midrib decreased from tip to base and was always lesser than the central portion of the lamina adjacent to the mid-rib.

N distribution in green leaf with excess N

In contrast, the excess build up of N (3.07-3.80%) was observed in all the lamina segments of the leaf compared to the corresponding values of the healthy crop in the samples showing excess N-induced-K-deficiency. The K content in these leaves varied from 0.62-0.75% as against 2.36 to 3.92% in healthy leaves. In these leaves, the N content of the leaf tissue (lamina and midrib) showed a gradual decrease from leaf tip to base (Fig. 8). The marginal portion of the leaf lamina always contained more N than the corresponding central portion of the lamina adjacent to the mid rib. Mid-rib contained lesser N content than the corresponding lamina portion in both healthy and excess N contained leaves.

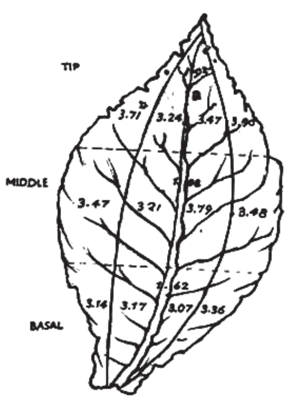

Fig. 8: Nitrogen distribution pattern of (N %) in different segments of green leaf with excess nitrogen ((Krishnamurthy *et al.*, 2000)

Interaction of N with other nutrients

An interaction occurs when the level of one production factor influences the response to another factor. Deficient and excess levels of nitrogen influence concentration and uptake of other nutrients. Increase in the level of nitrogen increased P uptake up to 80 kg N/ha; K, Mg and S uptake up to 120 kg N/ha and Ca uptake up to 160kg N/ha in Alfisols (Anonymous, 2010). Mc Evoy (1951) reported that low nitrogen application accelerated the maturity and decreased the content of other macronutrients, except phosphorus in the leaf. There occurs a close relationship between N and K in the plant. Nitrogen uptake and utilization is considerably enhanced by potassium application and excess use of N fertilizers led to K-deficiency (Krishnamurthy *et al.*, 2003). Krishnamurthy *et al.* (1997) reported that higher levels of nitrogen increased the protein N and soluble N fractions whereas the higher levels of potassium resulted in an increase in the protein-N content and a decrease in the soluble N fraction in the cured leaf of FCV tobacco grown in Northern Light Soils. Percentage of total alkaloids, total N, Ca and Mg increased significantly as applied N increased (Raper *et al.*, 1977). Krishnamurthy *et al.* (1989) reported that an N:K ratio of 1:2 is ideal for FCV tobacco production in irrigated Alfisols. Further Krishnamurthy and Ramakrishnayya (1994) showed that the concentration of 3.0% N and 2.5% to 3.0% K in green leaf lamina of 60-70 days old crop in NLS were optimum levels for normal growth, maturity, higher yield and better leaf quality.

The Cl^- content and uptake was less with NO_3^- than NH_4^+-N showing the antagonistic effect of NO_3^- - N on the Cl^- ion (Bangarayya *et al.*, 1976). Gopalachari *et al.* (1968) also observed a reduction in Cl uptake by the plant due to the NO_3^- - N than NH_4^+- N, but the chloride concentration in the leaf was not reduced to the extent required to have desired beneficial effect.

Effect of N on leaf maturity and harvesting

Nitrogen is the most critical element in determining the rate of maturity and leaf growth after topping. In maturation phase, individual leaves continue to accumulate dry weight but change is little in leaf area but their specific leaf weight increases. In plants, generally leaf ripening or senescence begins in the bottom leaves and proceeds to top. Generally, the leaf imports nitrogen during its expansion and then exports it steadily during its senescence. However, per unit of leaf dry weight the nitrogen content declines throughout, from emergence to ripening. When the leaf becomes net exporter of nitrogen, its nitrate reductase activity would decline to near zero. At the beginning of ripening process, as the leaf becomes a net exporter of nitrogen, its rate of accumulation of carbohydrates increases (Papenifus and Quin, 1984).

The supply of nitrogen must be controlled within narrow limits; an adequate amount is required for good growth, but excess nitrogen affects the quality. To get fully mature tobacco, the available nitrogen in the soil must be depleted by the time the maximum growth is attained. If the nitrogen is still available in the later part, maturity and ripening processes are delayed and the leaves remain green and become dark green and continue to grow. Elliot (1975) estimated that crop duration will be extended by one day for each 1 kg excess nitrogen applied. Typical cured leaf from such over-fertilsed crop will have low sugar content, high nitrogen and nicotine contents, and too low sugar: nicotine ratio reflecting poor leaf chemistry.

Some times, senescence begins and then re-greens because of mineralization of nitrogen in the soil or extension of the root system into the sub-soil. Then the crop growth response is similar to that of an over fertilized crop but occurs at a later stage. This means that the leaves switch over the export to import of nitrogen with arrested starch accumulation. If the re-greening occurs early during the ripening phase, the cured leaf will have low carbohydrate content and the other undesirable characteristics set in. However, if it occurs at a later stage, the carbohydrate content might have already reached satisfactory level and as such the nitrogen-carbohydrate balance is less affected.

In a study on the pattern of distribution of chemical constituents in the different regions of the leaf, it was found that the tips and margins which yellowed first contained more total N, protein N and nicotine and less moisture and starch than the areas near the base and midrib (Sastry and Ramakrishnayya, 1961). Bangarayya *et al.* (1985) reported that ethrel can counteract the adverse effects of higher levels of nitrogen on the yield and quality of tobacco. At higher levels of nitrogen, 5.0 mg ethereal/leaf improved the quality of tobacco.

Effect of N on cured leaf quality

Flue-curing is a controlled process of leaf drying. During the process of curing, some of the chemical changes begin at senescence, continue and other processes are initiated to allow the development of the desired smoking quality of the leaf and to preserve it. During the flue-curing there is little change in total nitrogen content of the tobacco leaf (Tso, 1972) although starch and protein N may have changes.

The acceptable limits for the important quality constituents and quality indices for FCV tobacco are presented in Table 2.

N effects on physical properties

Excessive soil nitrogen will generally produce cured leaves which are dark brown to black colour, dry and chaffy and have a strong and pungent smoke. N deficiency causes premature yellowing of leaves, which when cured are generally pale in colour, close-grained and thick-bodied and their smoke is flat and insipid. An increase in nitrogen will increase the yield of tobacco, but quality is often reduced at higher levels (Court *et al.*, 1984). Krishnareddy *et al.* (2003) reported that application of N did not induce discernible variation in equilibrium moisture content whereas filling value increased with enhanced N level.

N effects on chemical constituents

As there are two nitrogen atoms in each nicotine molecule, availability of nitrogen to the plant is an important factor in nicotine synthesis. Nicotine, thus synthesised in the roots is moved to leaves by transprational pull through xylem vessels.

Collins and Hawks Jr. (1994) also reported that nicotine is a key index for evaluating the quality of tobacco and it is closely related to the amount of N supplied since N is 17.3% of the total molecular weight of the nicotine. Nitrogen has a marked effect on the sugar: nicotine ratio, an important quality criterion for FCV tobacco. Many workers reported that increased level of N increased the concentration of N and nicotine in cured leaves and decreased the concentration of reducing sugars.

Table 2: Quality Indices for FCV tobacco*

Constituent/Quality Index	Acceptable Limits
Total nitrogen (%)	1.0-3.0
Nicotine (%)	0.7-3.0
Total Sugars (%)	10.0-26.0
Reducing Sugars (%)	8.0-24.0
pH	4.6-5.5
Reducing sugars/Total Nitrogen	7-13
Reducing sugars/Nicotine	7-13
Total N/Nicotine	<1.2
Chloride (%)	<1.5
Filling value at 60% R.H. and 20^0C	3.3-3.8 cc/g shreds
Equilibrium moisture content at 60% R.H. and 20^0C	11-15%
Pore Volume	0.13-0.18 ml/g
Combustibility	2.5-3.5 mm/minute
Leaf burn	3-6 seconds
Shatterability Index	>3

* The individual chemical constituents alone should not be taken into consideration for quality evaluation. The ratios of the constituents are also very important and should be taken into consideration for quality appraisal of tobacco.

Increased nitrogen levels increased the nitrate nitrogen, chlorogenic acid and decreased the starch and rutin contents. The biochemical constituents viz., nitrate nitrogen, starch, chlorogenic acid and rutin increased from top to bottom position of the plant (Sivaraju *et al.*, 2005). Increased rate of nitrogen has been found to increase the sand free ash and protein nitrogen (Elliot, 1970). Increasing rate of nitrogen increased the petroleum ether extractives (PEE). Nitrogen deficiency has been shown to increase scopolin and chlorogenic acid concentrations (Armstrong *et al.*, 1970) and the total polyphenol content was found to decrease with increased fertilization (Anderson *et al.*, 1970). However Tso *et al.* (1967) showed that the sum of chlorogenic acid, rutin, scopoletin and scopolin increased with increased nitrogen fertlilsation. An increase in N supply increases the leaf area but decreases the weight per unit area which is expressed as higher filling value. N fertilization increased the levels of total alkaloids, total N and scopoletin in cured leaf. Chlorogenic acid, neo-chlorogenic acid, 4-caffeoyl quinic acid, rutin and scopolin were inversely related to the rate of N (Court and Elliot, 1978). Increasing available nitrogen increases the level of nitrogen in the leaves, but this is not manifested by an equivalent increase in yield. Nitrogen beyond that required for maximum yield can reduce grade and leaf quality. Increasing applied nitrogen increased the smoke nicotine but did not affect the levels of tar (Elliot, 1975). Tso and Gori (1972) found a positive correlation between total N in the leaf and dry total particulate matter (TPM).

The ability of the leaf to burn continuously, evenly and gradually is one of the most important characteristics of tobacco. Potassium is related positively and nitrogen and chloride negatively to rate of leaf burn (Peterson and Tibbits, 1963, Pal *et al.*, 1966).

Nitrogen from soil to smoke

Soil nitrogen regimes affect the plant development more than any other nutrient from seedling stage to the time of leaf harvest. Nitrate and ammonical forms are the two major forms of inorganic nitrogen taken up by the roots of higher plants. During the developmental phase, as a constituent of organic compounds like amino acids, proteins, nucleic acids and metabolic processes involved in the synthesis and transfer of energy, nitrogen plays vital role in plant metabolism. The role of nitrogen in the development and quality of tobacco is of major importance with respect to time of absorption, form of nitrogen absorbed, rate of application, concentration in the leaf and numerous other aspects.

Soil nitrogen must be sufficiently available during early and mid-season growth stages to ensure vigorous growth, but not excessive growth, and it should be nearly depleted by flowering to facilitate the plant to mature and ripen properly to get quality tobacco leaf. After harvesting, during flue-curing, there is little change in total nitrogen content and nicotine content of the leaf. In general, as total N in the plant increases more than the required for maximum growth, quality of the tobacco tends to decrease. During the process of curing, degradation of chlorophyll, hydrolysis of starch to sugars and proteins to amino acids and subsequent reaction of free amino acids with sugars to form aromatic compounds, conversion of nitrate to nitrite and its subsequent reaction with alkaloids to form tobacco specific nitrosamines (TSNA) are some of the important changes. After curing the cured leaf is processed and used for cigarette manufacture. When the end user smokes the cigarette, the nitrogen again moves to the smoke in the form of constituents like nicotine, TSNA, nitrogen oxides etc.

Thus nitrogen moves from the soil to roots then to plant parts, cured leaf, cigarette and finally reaches atmosphere through smoking. The schematic representation of nitrogen movement from soil to tobacco smoke is shown in Fig. 9.

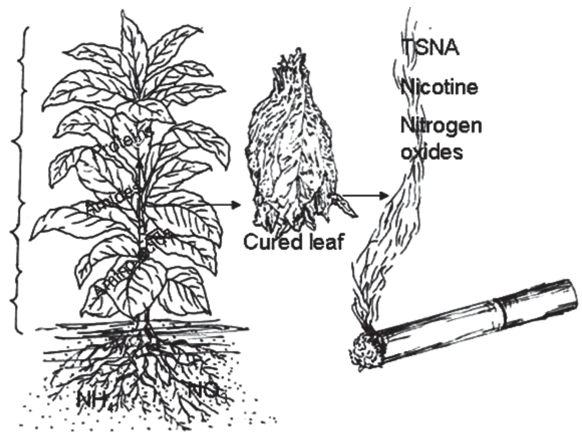

Fig. 9: N from soil to tobacco plant and smoke

CONCLUSIONS

Balanced and desirable tobacco crop growth can only be achieved with an adequate and timely supply of nutrients. Nitrogen is the most important element that influences the growth and development of tobacco plant and leaf quality. Tobacco utilizes and absorbs both mineral forms of nitrogen viz., ammonical and nitrate forms and nitrate is the preferred source of nitrogen fertilizer. The growth of the tobacco plant mainly depends on nitrogen availability to the plant. In the absence of reliable method for soil nitrogen estimation, plant nitrogen status is the measure of N status. As leaf nitrogen content is closely related to chlorophyll content index, measured using chlorophyll meter, it can be used as a valuable tool for evaluation of nitrogen status. The nitrogen fertilization should be in such a way that the plant should get enough available nitrogen during the growth phase to ensure vigorous growth and soil nitrogen should be depleted soon after topping so that leaves ripen correctly.

In India, as FCV tobacco is grown in different soils viz., traditional black soils with conserved soil moisture, northern light soils as irrigated crop, southern light soils using North-East monsoon and Karnataka light soils as monsoon crop; nitrogen fertilizer requirement depends on zone where it is grown. Nitrogen level also affects the uptake of other essential nutrients. It is the critical element in determining the rate of maturity and synthesis of nicotine which is the key index for cured leaf quality. Thus rational application of nitrogen is essential for proper growth, yield and quality of FCV tobacco.

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