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Assessing flue-cured tobacco crop growth and biomass Response to nitrogen application levels using canopy Reflectance

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A B S T R A C T

Visual seedling health monitoring can be done through the use of optical sensors that rely on light to assess the physiological status either at the leaf or canopy level using vegetation indices. Plant reflectance properties that are indicative of crop N status can be useful for directing in-season variable-rates of N applications and help interpret if there are any nitrogen related problems. In this study a multispectral Radiometer (MSR5) was used to monitor tobacco varieties response to N application levels. A 3 x 4 factorial experiment in a Randomized Complete Block Design, with four replications was used. Three tobacco varieties and four N levels were established in a conventional seedbed experiment at Kutsaga research station in Harare. MSR 5 readings, biomass and total nitrogen data were collected and analysed to investigate Normalised Difference vegetation Index (NDVI) response to these canopy characteristics. The results showed a significant two way interaction between fertiliser and varieties ($P < 0.05$). Significant differences ($P < 0.05$) were recorded on KE1, differing from both KRK 26 and T66 and also on all the N treatments. Crop biomass and total nitrogen were also highly correlated to NDVI ($R^2 > 0.8$), in an N dependent pattern. The NDVI values increased with an increase in N levels up to, and above the recommended rates. The results indicated that NDVI can be a useful tool in N management in tobacco. The fact that different varieties showed differing NDVI values makes the application of this technique variety-specific.

Key words: *plant monitoring vegetation indices NDVI Crop biomass N management*

Background

The production of high quality tobacco seedlings starts in the seedbeds, with the selection of variety and crop management practices. Tobacco varieties perform differently in seedbeds in response to nitrogen application and subsequently ripening rates, yield and quality attributes (Tobacco Facts, 2009). Some Zimbabwean tobacco varieties like KE1 are fast ripening and have darker green leaves compared to the others like T 66 and K RK 26. For this reason seedlings of different varieties will all have a different chemical composition depending on how efficient they utilize plant mineral nutrition (Ikisan, 2000). An ideal seedling for transplanting should have a stem length of around 15-17 cm, 6-10 mm thickness and should be well hardened (TRB, 2010). Nitrogen (N) is a limiting nutrient element in crop production (Fageria, 2014) and is considered as one of the valuable inputs a producer can invest to make significant profit under an appropriate management system.

Nitrogen deficiency in tobacco seedlings decreases chlorophyll and soluble protein content, leading to a progressive loss in green colour particularly in the older leaves and subsequent poor establishment in the field (Moran et al, 2000). Excessive nitrogen application results in large, dark-green leaves which take a long time to harden in the seedbed (Fageria, 2014). Nutrient stress adversely affects seedling growth vigour, productivity, and overall quality. Early detection of nutrient stress in seedlings facilitates the timely application of corrective cultural practices before it severely affects seedling yield and quality (Jackson, 1986). Tobacco growers visually monitor seedlings for nutrient stress symptoms (Mazarura, 2004). Traditionally, nitrogen application rates are mainly based on years of experience, blanket recommendations and soil sampling.

A possible alternative to visual seedling health monitoring is the use of optical sensors that rely on light to assess the physiological status either at the leaf or canopy level using vegetation indices. Vegetation indices (VIs) are combinations of surface reflectance at two or more wavelengths designed to highlight a particular property of vegetation (Jackson et al, 1983). Plant reflectance properties that are indicative of crop N status can be useful for directing in-season variable-rates of N applications and help interpret if there are any nitrogen related problems.

Leaf nitrogen concentration is an important indicator of plant nutrient requirements (Muñoz-Huerta et al, 2013). This nutrient is a constituent of chlorophyll, the green pigment in plants that plays a role in absorbing light for photosynthesis. The molecular structure of the chlorophyll incorporates a large proportion of total leaf nitrogen and, several studies have found that foliar chlorophyll concentration provides an accurate, indirect estimate of plant nutrient status (Ikisan, 2000). Remote sensing for leaf N characteristics can thus reduce surplus N in the crop production system without significantly reducing crop yield, and would in turn reduce N losses to surface and ground waters (Enfors et al, 2011).

Remote sensing is the acquisition of information about an object or phenomenon, without making physical contact with the object (Adamchuk, 2003). When electromagnetic energy from the sun strikes the vegetative material, the energy is reflected, absorbed, or transmitted depending upon the wavelength of the energy and characteristics of individual plants. (Moran, 1997) Reflected energy bounces off leaves and is readily identified by human eyes as the green colour in plants. Sunlight that is not reflected or absorbed is transmitted through the leaves to the ground. Every substance emits, absorbs, transmits or reflects electromagnetic radiation in a manner characteristic of the substance (Balaselvakumar, 2006).

By measuring the quantity of radiation in each of the wavelengths, the characteristics of vegetation can be defined. The differences in leaf colours, textures, shapes or even how the leaves are attached to plants, determine the amount of reflected, absorbed or transmitted energy and such relationships are used to determine spectral signatures of individual plants, which are unique to each plant species (Blackburn, 2002). Remote sensing can show differences in vegetation types and among crop varieties (Sabins and Floyd, 1997). Spectral signatures make it possible to use remote sensing in studying changes in specific crop conditions in the field like fertilizer management.

The comparison of the reflectance values at different wavelengths, called a vegetative index, is commonly used to determine plant vigour (Fitzgerald et al, 2005). The most common vegetative index is the normalized difference vegetative index (NDVI), defined as the difference between the visible (RED) and near-infrared (NIR) bands, over their sum (Lamb, 2005). The index is associated with vegetation canopy characteristics such as biomass, leaf area index and percentage of vegetation cover.

The reflectance of crop canopies are similar, but modified by the non-uniformity of incident solar radiation, plant structures, leaf areas, shadows, and background reflectivity (Eitel et al, 2010). Radiometers receive an integrated view of all these effects, and each variety/ vegetation type tends to have a characteristic signature which permits its discrimination. This shape of the reflectance spectrum according to Liew (2001) can be used for identification of varieties. Incident radiation has a spectral range of 400-2 700 nanometres of which the visible part of the spectrum is between 400 and 700 nanometres. The blue and red regions have the lowest reflectance from plants with green considered low but much greater than either red or blue. NIR has the greatest.

Although reflectance in the visible region there is typically low because of the absorption properties of pigments, the infrared region from (700-1 300nm) is characterised by high reflectance because individual leaves and whole plant canopies strongly scatter NIR energy (Gates *et al.*, 1965; Knipling, 1970, Endo, 2000,).

The physical, biochemical and morphological characteristics of a canopy are determined by the variety and the environmental conditions it is growing in (Hatfield et al, 2008). These optical properties of leaves influence the availability of photosynthetically active radiation (PAR) at the cellular level, the penetration of light through plant canopies, the transmission of light to the lower canopies and ultimately the reflection of light from the canopy (Thomas, 2008). For the same vegetation type, the spectral signature also depends on other factors such as the leaf moisture content and health of the plants.

The multispectral radiometer is a hand held instrument which measures quantity of radiation at different wavelengths, and has 5 wavebands of which, the absorption bands are related to the amount of concentration of foliar chemicals and leaf architecture at the cellular level. The ground-based active-light sensor suitably developed to non-destructively evaluate N status in crops (Lamb, 2005). It provides the remote sensing community with a hand held tool capable of measuring reflectance data in the same wavebands as the Landsat Thematic Mapper satellite. The multispectral radiometer has customized data logger firmware and measurement and analysis software.

The Cropscan MSR5 has been tried very successfully in experiments for measuring severity of nitrogen stress and its effects on crop yield and quality. The radiometer has proven to be a more efficient and accurate means of estimating severity of nitrogen related problems on crops than conventional visual rating methods (Schepers, 2006).The Cropscan MRS5 was found useful in detecting nitrogen stress and its effects on dry matter production (Mahey et al., 1991).Spectral data were correlated with plant height, leaf area index, total fresh and total dry biomass and yield. The instrument has been used to estimate crop biomass prior to harvest while NDVI values were used to link ground biomass via regression equations (Liew, 2001).

This study sought to assess flue cured tobacco crop growth and health status, N application level and canopy N using canopy reflectance. It was hypothesized in this study that (1) tobacco varieties and fertiliser application levels could be distinguished at different growth stages, using reflectance from the crop canopy and that (2) there is a positive relationship among canopy reflectance, seedling biomass, and leaf total N.

Materials and methods

Site

The study was carried out at the Tobacco Research Board's Kutsaga Research Station. The experiment was done in a conventional seedbed between June and September during the 2011 and 2012 season. Kutsaga lies in Natural Region II at an altitude of 1 479 meters above sea level. The station is found on latitude 17`55``S, longitude 31`08``E and receives a mean annual rainfall of 800-1000mm. Average temperature is 18^0C in winter and 32^0C in summer. The area is dominated by light, well drained sandy soils and they are position two on the soil catena. These are typically moderately deep to deep well drained soils. The site previously had Katambora Rhodes grass for three years.

Cultural Practices

The site was ploughed deeply using a tractor drawn plough to a depth of 50 cm and a month later in May fumigated. The soil was watered to field capacity a week before fumigation, the upper 30 cm was cultivated and some undecomponsed matter removed. Fumigation was done using Ethylene dibromide water- miscible concentrate on 1 June 2011. The chemical was applied using a gun at a rate of $35mlm^{-2}$ on the soil and mulched. Clomazone, a herbicide, was sprayed a week before sowing at a rate of $1.5litresha^{-1}$ diluted in 200 litres of water. Beds were constructed with a width of 1.2m and 3 m length raised up to 10cm above pathways. Three tobacco varieties KE1, KRK 26 and T66 were sown on 13 June 2011 using a watering can fitted with a fine rose continuously agitated to distribute seeds evenly. Grass mulch was used and the beds were watered twice daily before germination and once after germination till hardening. Aphids were controlled using Acephate.

Fertilizer Treatments

Basal fertilizer application of 1kg of compound S 7:21:7 (N: P: K)10^{-1} square meters was broadcasted and incorporated into the upper 5 cm with a rake. The first top dressing fertilizer treatment was applied when the seedling leaves were 1-2 cm in diameter using 10 g ammonium nitrate m^{-2} which is half of the full rate. The fertilizer was first dissolved in water and the solution was irrigated on the beds before watering, to prevent the leaves from developing fertilizer burn symptoms. A week later, 10g ammonium nitrate was applied and this repeated twice subsequently every seven days following the same method.

Procedure

Radiometric measurements were done on 3m x 1.2m plots, using a hand held multispectral radiometer (Cropscan MSR-5, 450–1750 nm; resolution 0.06 %). The radiometer simultaneously measures irradiance and radiance to provide canopy surface reflectance. Normalized Difference Vegetation Index (NDVI) was calculated from red (Channels 3 = 630-690) and Near Infra Red (Channel 4 = 760-900).

NDVI = (NIR – R/ NIR + R)

Radiometric measurements were done on 3m x 1.2m plots, using a hand held multispectral radiometer (Cropscan MSR-5, 450–1750 nm). The radiometer simultaneously measures irradiance and radiance to provide canopy surface reflectance. Normalized difference vegetation index (NDVI) was calculated from the spectral bands.

The Multispectral Radiometer (MRS 5) was positioned facing vertically downward at 1 m above seedling canopies, and measurements taken around solar noon to minimize the effect of diurnal changes in solar zenith angle. Ten measurements were taken per sampling plot and reflectance measurements were averaged for each sampling plot to estimate a single reflectance value. The seedlings were harvested after 80 days and total nitrogen determined in the laboratory using the Kjedjal method and expressed as a percentage of the total dry weight.

A 3x4 factorial experiment in a Randomized Complete Block Design was used with three Kutsaga varieties (KE1, KRK 26 and T66) and four Nitrogen levels replicated four times.

Experimental Design

A 3x4 Factorial experiment in a Randomized Complete Block Design was used with three varieties (KE1, KRK 26 and T66) and four Nitrogen levels replicated four times.

The fertilizer treatments were applied as shown in Table 1 below.

Table 1**:** Variety X Fertilizer treatments

Variety	Fertilizer levels of the recommended rate	N/ m^2
KRK 26	0%	0g N
	50%	6g N
	100%	12g N
	150%	18g N
T66	0%	0g N
	50%	6g N
	100%	12g N
	150%	18g N
KE 1	0%	0g N
	50%	6g N
	100%	12g N
	150%	18g N

Figure 1: The spatial arrangement of the treatment blocks

Measurements

Cropscan (MSR5) was used to measure seedling canopy reflectance measurements at day 49, 56, 63, 70 and 77 after sowing in the wavelength ranges of 450–1750 nm. Above ground samples were collected at harvesting which are seedling dry mass, seedling countm^{-2} was done at pulling and the samples were dried and ground for laboratory total nitrogen analysis. Seedling dry mass was measured using an electric digital scale and the seedlings were physically counted in each plot and it was averaged out. The seedlings were dried and a 2kg sample taken to the Chemistry department for total nitrogen analysis.

Data analysis

Data was subjected to analysis of variance and regression analysis, statistically significant treatment effects were separated using least significant difference (LSD's). The data was analyzed using the Genstat 9.2 statistical package at 5% level of significance.

Results

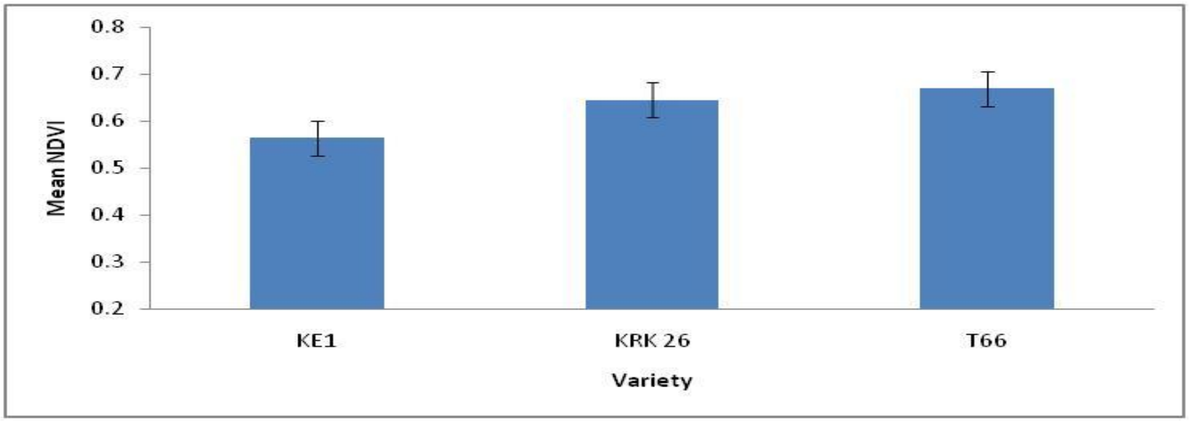

Figure 4.1 Effect of three different flue cured tobacco varieties on mean seasonal average NDVI

The average NDVI values for the entire seedling growing season for each variety (Figure 4.2) indicate KE1 as having a significantly ($p<0.05$) lower vegetative reflectance compared to both T66 and KRK26 with a seasonal average of 0.56. There were no significant differences ($p>0.05$) between the KRK26 and T66 and with seasonal average NDVI values of 0.645 and 0.67 respectively.

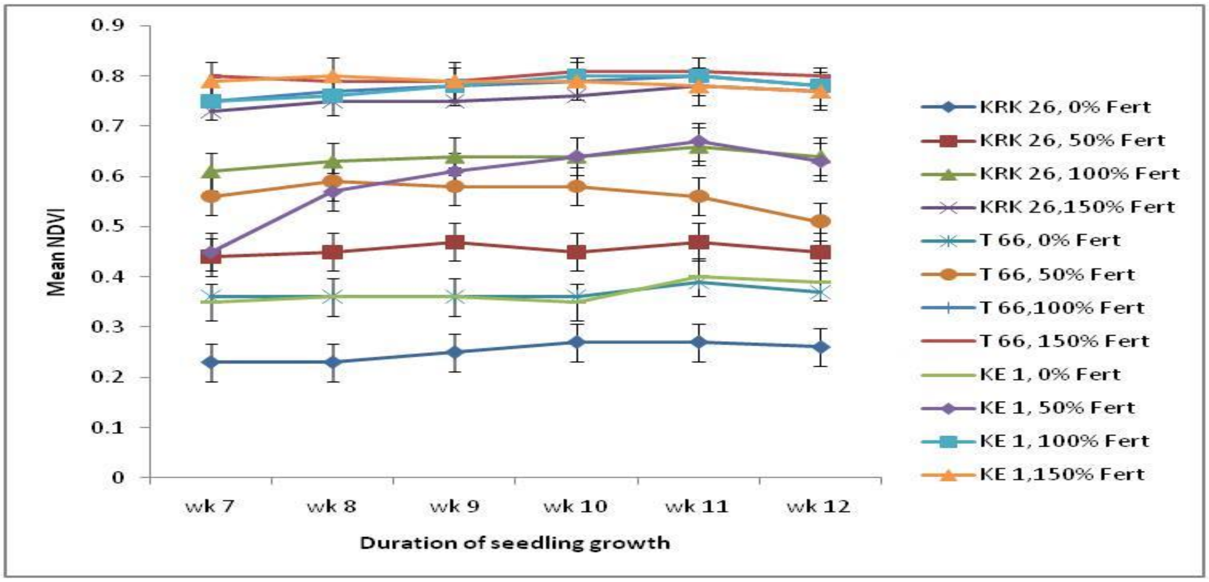

Figure 4.2 The combined effects of different fertiliser levels on NDVI of three tobacco varieties in a seedling growing season.

Measurements taken 7 weeks after sowing (Figure 4.1) showed that the mean NDVI for the varieties increased with crop growth up to week 11 (about 84 days after sowing). This characteristic trend of increase in the NDVI values with time, growth and development of the plants, was observed in all the three varieties and treatments. KE1 at 50% showed the highest increase in NDVI from week 7 to week 10 where it had the same NDVI value with KRK 26 at 100%. The highest NDVI value of 0.81 was recorded for T66 at 150% variety in week 11 and KRK 26 had the lowest NDVI values at 0%. High fertilizer rate treatments had the highest NDVI throughout the season and the 0% fertiliser treatments had lowest NDVI values. KRK 26 showed low NDVI in all the treatments except the 150%. Each variety had a characteristic trend which is different from others In all the weeks there were no significant differences between the 100% and 150% except for KRK 26 at 100%. The NDVI started falling in week 12 for all the varieties and all treatments.

Between week 9 and 11, three NDVI categories could be identified. All the 0 % fertiliser treatments ranged between 0.2 and 0.4, while those for the 50 % fertiliser treatments were between 0.4 and 0.6. The NDVI for the all the 100 and 150 % fertiliser treatments except that for K RK 26, were above 0.7.

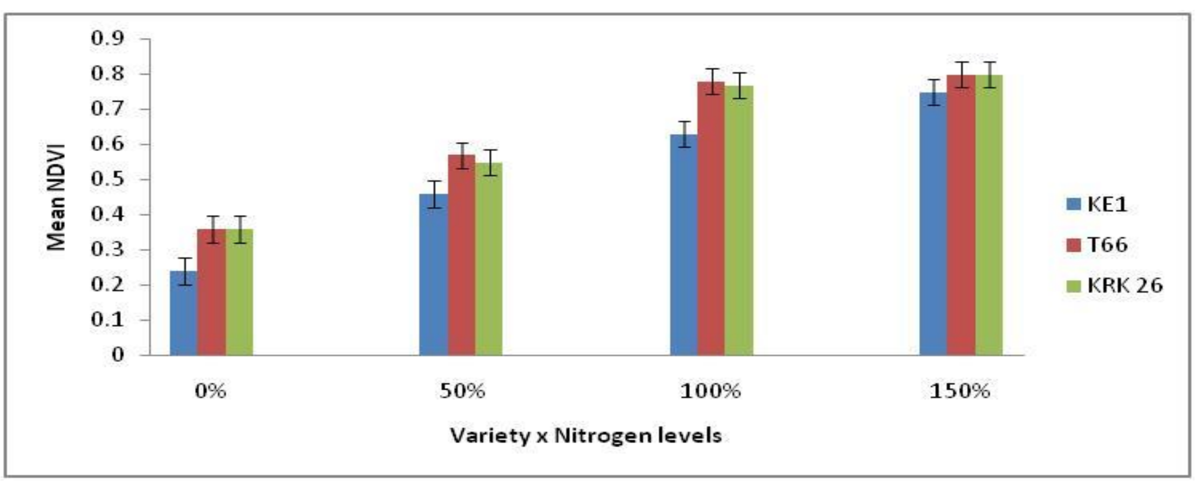

Figure 4.3 Effect of nitrogen levels on NDVI of different flue-cured tobacco varieties (mean seasonal average).

There was significant 2-way interaction ($p<0.05$) in the seedling canopy reflectance due to different fertiliser application rates (Figure 4.3). For each variety, canopy reflectance increased as the nitrogen application rate increased from 0% to 150%. KE1 had the significantly lower NDVI with no significant differences between KRK 26 and T66 but all varieties had no significant difference at 150%. For both KRK 26 and T66 there were no significant differences in the 100% and 150% fertiliser treatments.

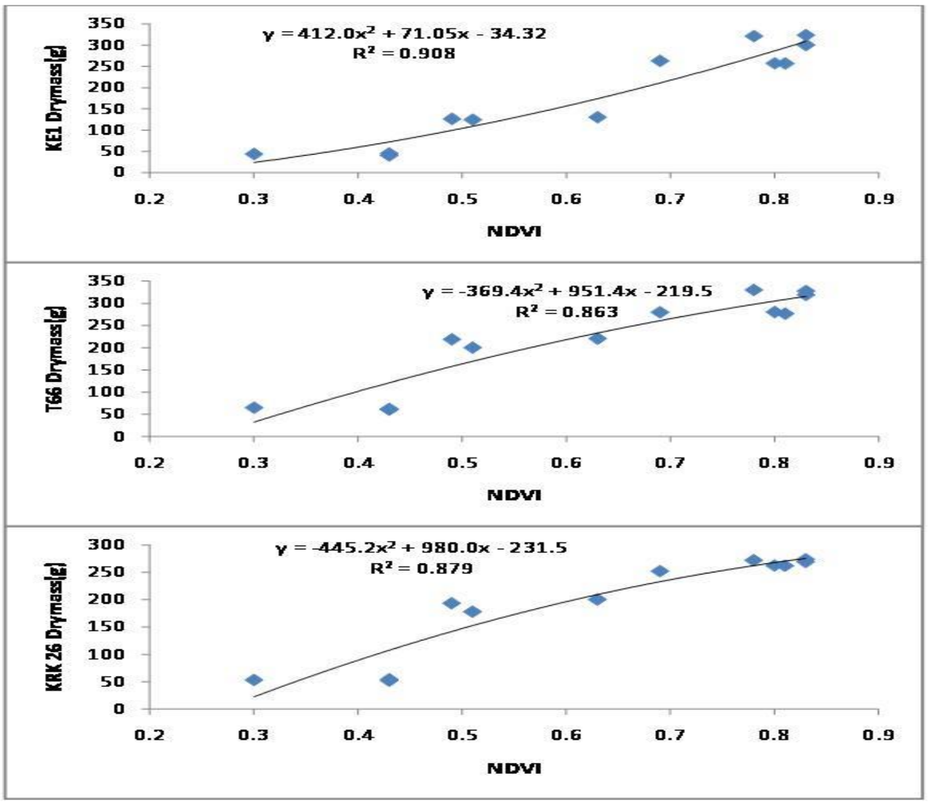

Figure 4 .Relationship between NDVI and dry mass of different flue-cured tobacco varieties tobacco.

The correlations between seedling dry mass with NDVI is high with KE1 showing the strongest relationship (R^2= 0.899). The graphs show that as NDVI is increasing so does the dry mass. T66 and KRK 26 varieties are also positively correlated with dry mass (R^2=0.855 and 0.864) respectively.
A regression of pooled seedling drymass and NDVI had an equally strong coefficient of determination (Figure 5).

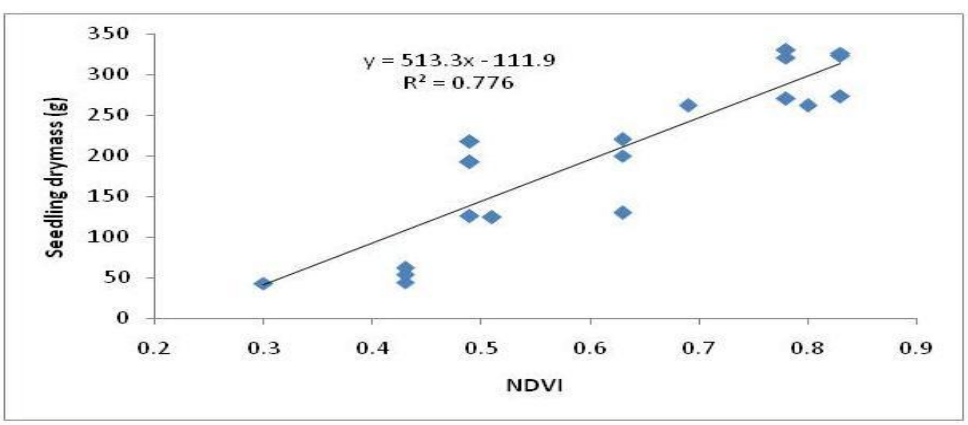

Figure 5. Relationship between seedling dry mass and NDVI for all varieties pooled together.

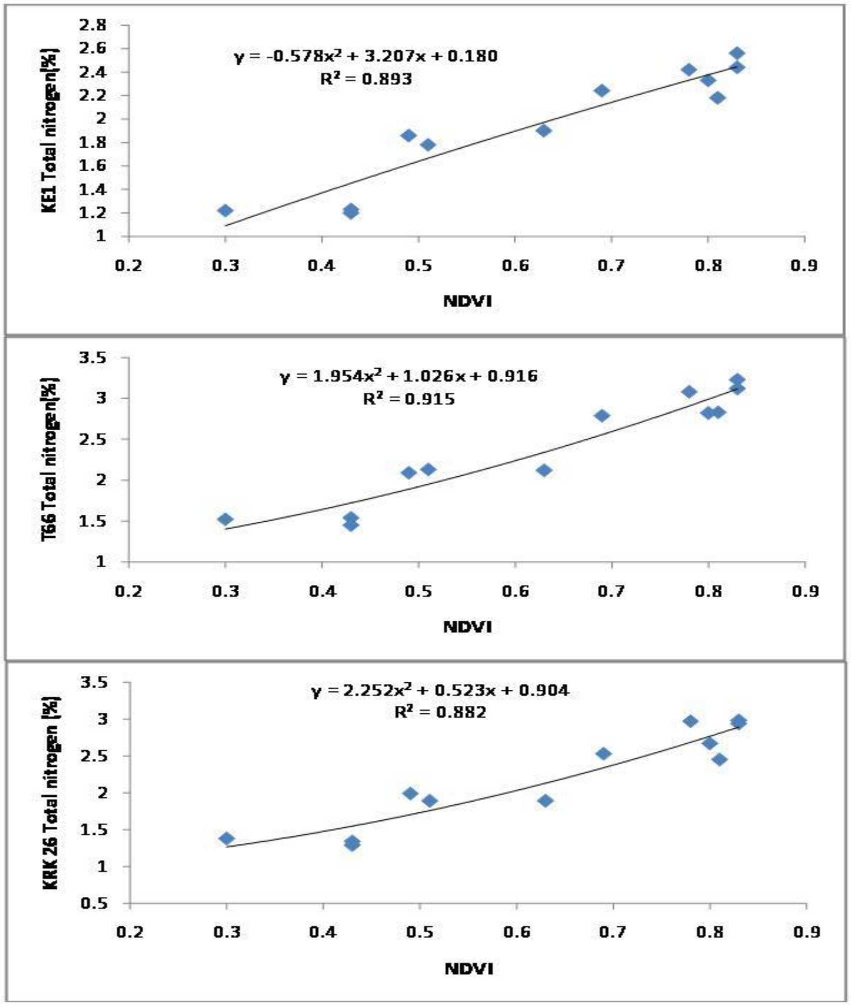

Figure 4.6 Relationship between NDVI and total nitrogen of different flue-cured tobacco varieties tobacco

T66 had the highest correlation with total nitrogen it showed the most positive correlations with NDVI (R^2=0.910). The other varieties KE1 and KRK 26 also had positive correlation with NDVI (R^2=0.892 and 0.874) respectively.

Discussion

At 7 weeks after sowing, all varieties displayed different NDVI values though T66 and KRK 26 are almost similar, the differences in reflectance makes it possible to monitor and differentiate the varieties in the seedbed. All the three varieties differ in maturation rates, leaf orientation, and pigmentation hence they also differ in their response to canopy reflectance. Varieties that begin senescing at different times and rates result in significant differences in NDVI (Carter, 2000). KE1 is a fast ripening variety and it flowers early in the seedbed thus it differs significantly with other varieties. For tobacco, the genetic material of the cultivar, environmental conditions of production and the growing practice defines the quality and yield of the tobacco (Jure et al, 1999). KRK 26 and T66 share a similar parental heritage but extensive selection processes during breeding have resulted in them differing in photosynthetic capacity thus their canopies will absorb and reflect light differently. T66 is a more superior variety than KRK 26 and KE1 respectively in terms of their yielding ability (Nyoka, 2005).

NDVI was also shown to be dependent on the stage of the vegetation growth with denser canopies having higher NDVI values. For every variety, the NDVI values tended to reach a plateau at high leaf area index(LAI) levels and the trend indicates a temporary saturation of reflectance, which remains almost steady for a long time until the subsequent senescence of the foliage before it starts to fall (Gemtos et al, 2003). This explains the trend in this experiment where in week 12, NDVI started to fall after the seedlings have matured.

The was a significant interaction between nitrogen concentrations and varieties on NDVI showing that vegetation has a unique spectral signature and though plants has similar canopy reflectance it is modified by non uniformity of incident solar radiation, plant structures, leaf areas, and chemical composition (Svotwa, 2012). The differences among varieties and at different fertiliser rates suggest varietal differences in the ability to exploit environmental resources for biomass production (Nyoka 2005). During the growing season the same trend was noted such that corrective measures could be taken if the detection has been done early enough, while at an advanced phenological stage, this response could be used to determine the size of well fertilized seedbed area that could be available for planting for each variety. The higher NDVI is also due to structure and composition of chemicals in the leaves, which in this experiment, was to a combination of different nitrogen levels and the genotype of the varieties.

KE1 is an inferior variety even in nutrient use efficiency thus it performs poorly even when resources are not limiting (TRB, 2010). This showed that due to differences in the genotype and different N levels the NDVI values will differ such that each variety have its own NDVI value for monitoring nitrogen in tobacco production. However both KRK 26 and T66 have almost same genetic material thus their performance is more or less similar. There are no significant differences between the 100% and 150% which suggest that N application higher than 100% the recommended could be considered as over-fertilization since there are no additional benefits in N-uptake.

NDVI can be used to estimate biomass and total nitrogen because of the high dry mass and total nitrogen correlations with NDVI. This is indicative of possible application of these parameters tobacco seedling quality and quantity assessment using remote sensing (Svotwa, 2012). Studies in maize shows that vegetation indices developed from spectral reflectance wavebands tend to correlate highly with plant physical parameters such as leaf area index (LAI), nitrogen content, dry biomass and percent ground cover (Cassanova et al, 1998).

Conclusions and Recommendations

The study shows that different tobacco varieties have different NDVI values. It further reveals that NDVI values increase with increase in N fertilisation for each variety it further reveals that 150% rate and the 100% N rate are not statistically different. There is a relationship between NDVI and biomass and or total nitrogen of different tobacco varieties. Farmers growing the T66 and KRK 26 varieties can use similar NDVI values for the management of N fertilisation. The strong correlation between the NDVI values with different fertilisation rates suggests the possibility of it being a useful tool in N management.

Based on the results of this study, it can be deduced that the estimation of biomass for tobacco varieties dry mass and total nitrogen are best related to NDVI. However, further research is required to validate the precision of the use of NDVI in estimating biomass and other related yield parameters in tobacco varieties.

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