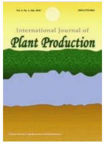

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Optimizing plant traits to increase yield quality and quantity in tobacco using artificial neural network

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Abstract

There are complex inter- and intra-relations between regressors (independent variables) and yield quantity (W) and quality (Q) in tobacco. For instance, nitrogen (N) increases W but decreases Q; starch harms Q but soluble sugars promote it. The balance between (optimization of) regressors is needed for simultaneous increase in W and Q components [higher potassium (K), medium nicotine and lower chloride (Cl) contents in cured leaf]. This study was aimed to optimize 10 regressors (content of N and soluble sugars in root, stem and leaf, leaf nicotine content at flowering and nitrate reductase activity (NRA) at 3 phenological stages) for increased W and Q components, using an artificial neural network (ANN). Two field experiments were conducted to get diversified regressors, Q and W, using 2 N sources and 4 application patterns in Tirtash and Oromieh. Treatments and 2 locations produced a wide range of variation in regressors, W and Q components which is prerequisite of ANN. The results indicated that configuration of 12 neurons in one hidden layer was the best for prediction. The obtained optimum values of regressors (1.64%, 2.12% and 1.04% N content, 4.32%, 13.04% and 9.54% soluble sugar content for leaf, stem and root, respectively; 2.31% nicotine content and NRA of 13.11, 4.74 and 4.70 μmol.NO_2.g^{-1}.h^{-1} for pre-flowering, flowering and post-flowering stages, respectively) increased W by 3% accompanied by 4.75% K, 1.87% nicotine and 1.5% Cl in cured leaf.

Keywords: Artificial neural network; Optimization; Tobacco; Quality.

Introduction

In tobacco, the leaf yield quantity and its quality including ability to burn with a glow, thereby influencing aroma, flavor and character of the ash are very important. Many factors tend to affect these properties of leaf. The chloride (Cl) is known to be closely related to the combustibility, moisture absorption and flexibility of tobacco leaves. Excessively high or excessively low Cl content affects the quality of the tobacco leaves and the optimum Cl level of cured tobacco should be 0.3%-0.8% (Zeng et al., 2014). Chari (1995) suggested that the threshold level for Cl content in the tobacco leaf is less than 1.5% because large amounts of it tends to slow the burning process. The thickness of tobacco leaf, retardation in leaf burn and decrease in fire-holding capacity are attributed to high accumulation of Cl in leaves. Leaves with more than 2.5% Cl content are nearly noncombustible (Akehurst, 1981). Generally, it should be noted that the amount of Cl absorbed by tobacco plant is substantial. Therefore, in arid and semi-arid regions like Iran, the Cl accumulation in leaves tends to be considerable.

Potassium (K) is an essential element for tobacco nutrition. Its concentration in tobacco leaves ranges from 2 to 8% and sometimes reaches 10%. This element plays a key role in controlling important quality parameters such as leaf color, texture, hygroscopic properties, combustibility, sugar and alkaloid contents (Farrokh and Farrokh, 2012). The leaves that do not have enough K, after curing are brittle and straw like while those accumulated enough K are bright yellow.

Nicotine and sugars are other important factors affecting the quality of tobacco leaves. It is believed that the aroma and smoke flavor are best when the nicotine content falls between 2.5 and 3.5% (Akehurst, 1981). The presence of sugars in the form of starch has an adverse effect on the quality of smoke for the user because it affects the rate and completeness of burning. In addition, it produces a burning smell, harming the aroma of the tobacco (Gao et al., 2006 cited from Zeng et al., 2014). The total amounts of water-soluble and reducing sugars are the primary factors determining smoking quality.

There are complex multilateral interactions between above 4 factors. Moreover, these interactions tend to be affected by many other factors. Some of these inter- and intra-relations are available in literature. For example, the nicotine content tends to decrease with increasing K content (Shamel Rostami, 1996). N increases the yield quantity (Marchetti et al., 2006), but decreases the leaf quality via enhancing nicotine content, declining K content (Kena, 1990) and diminishing sugar concentration (Ryding, 1981). The higher tissue ammonia level, say N, increases the ratio of nicotine/nicotine salts delivered on smoking. This causes the sensory and physiological perception of increased nicotine strength (and harshness) (Davis and Nielson, 1999). Tissue ammonia interactions with sugars or carbonyl compounds can form a variety of chemical entities, thereby affect the flavor and aroma of smoke. For instance formation of fructosazines and deoxyfructosazines is known to be formed by the interaction of ammonia with glucose and fructose (Davis and Nielson, 1999). Tobaccos with high nitrate, say N, content are assisted in the burning process as nitrate is itself a combustible material (Leffingwell and Leffingwell, 1988).

Considering the complexity of inter- and intra-relations between regressors [independent variables that affect dependent variables (yield quantity and quality; output)] and output variables, the balance between each of regressors for increased output is crucial. The ordinary mathematical equations and techniques are not capable of optimizing (making balance between) the regressors for enhanced output. Problems of this kind can be solved with an alternative approach involving the artificial neural network (ANN) technique (Rohani et al., 2011). This method has been used for optimization of some attributes in barley (Gholipoor et al., 2013) and sugar beet (Gholipoor et al., 2012). This study was aimed to optimize (find the balanced values of) the N and sugar contents of root, stem and leaf, nitrate reductase activity in leaf at 3 different phenological stages and leaf nicotine content at flowering for increased both quantity and quality (higher K, medium nicotine and lower Cl contents) of cured leaf in tobacco, using ANN.

Materials and Methods

Field experiment

Two field experiments were conducted on cultivar Burley 21 of tobacco in Tirtash (36.75 °N, 53.73 °E and 14 m asl) and Oromieh (37.54 °N, 45.04 °E and 1332 m asl) Research Centers in 2014. Each experiment was based on complete block design with 3 replications. Treatments were factorial arrangement of 2 N sources (urea and nitrate ammonium) and 4 application patterns [basal, 2/3 basal and 1/3 after initiation of rapid growth (AIRG), 1/2 basal and 1/2 AIRG, 1/3 basal and 2/3 AIRG)]. The required N fertilizer was calculated based of soil samples test. The tissue concentration of N and soluble sugars was measured for root, stem and leaf 100 days after transplanting. The activity of nitrate reductase enzyme in leaf was determined at the stages of vegetative growth, flowering and after flowering. The nicotine content of leaf was measured at flowering. These attributed were considered as input layer (regressors) for ANN. After harvesting the leaves, the cured leaf yield (yield quantity) and contents of K, Cl and nicotine in cured leaf (yield quality) were measured and taken as output layer for ANN.

Artificial Neural Network Analysis

This approach is a computing system made up of a number of simple, highly interconnected processing elements, which process information by their dynamic state response to external inputs. The data set was randomly shuffled and split into a training set (70% of the total data) and a test set (30%). These percentages were determined by trial and error (Bateni et al., 2007) using QNET software. Among ANN models, the multilayer perceptron (MLP) approach was used in this study. This approach offers the highest practical significance (Emamgholizadeh et al., 2015; Rohani et al., 2011). The transfer function used in ANN serves to normalize a node's output signal strength to values between 0 and 1. Each node multiplies every input by its interconnection weight, sums the product and then passes the sum through a transfer function to produce its result. This transfer function is usually a steadily increasing S-shaped curve.

Different transfer functions, such as Sigmoid, Gaussian, Hyperbolic Tangent and Hyperbolic Secant, were used in this study. Among many MLP training methods, the back-propagation method was applied. In this algorithm, neural networks process the information in interconnecting processing elements (often termed neurons, units or nodes). To compare the performance of various ANN configurations, 4 statistical parameters including correlation coefficient (r), mean absolute error (MAE) (equation 1), root mean square of error (RMSE) (equation 2) and relative standard error (RSE) (equation 3) were used:

$$MAE = \frac{1}{n}\sum_{i=1}^{n}\left|Y_i - \hat{Y}_i\right| \tag{1}$$

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(Y_i - \hat{Y}_i)^2} \tag{2}$$

$$RSE = \frac{\sqrt{\frac{1}{n}\sum_{i=1}^{n}(Y_i - \hat{Y}_i)^2}}{Y_{bar}} \tag{3}$$

Where Y_i observed output, Y_{bar} mean observed output, $\hat{Y}_i$ predicted output and $\hat{Y}_{bar}$ mean predicted output.

The training of the ANN models was ended when either the acceptable level of error was achieved or the number of iterations exceeded a prescribed maximum of 8000. The model that minimized the error, i.e., RMSE, MAE and RSE, but maximized r values were selected as the appropriate model. After the satisfactory performance of the ANN model was confirmed, the relative contributions of the regressors to the determination of output were estimated.

Optimization Analysis

There was no linear relationship between output and any of the 10 regressors (data not shown). Therefore, the following 3-stage approach was used (Gholipoor et al., 2012):

Stage 1

Initially, 4 of the 10 regressors including leaf N and nicotine contents, root sugar content and nitrate reductase activity at post-flowering stage, which appeared to have a more obvious direct effect on output, were used with an ANN having 4 inputs (cured leaf yield, content of Cl, K and nicotine of cured leaf) and 1 output (each of above mentioned 4 regressors). Seventy percent of the original data (the values obtained from the 2 field experiments) were used to train the ANN model and 30% were used to test the model. The satisfactory performance of the ANN was confirmed and the values of above mentioned 4 regressors were then predicted. Each of these values could potentially be the optimum value for increased yield quantity and quality. From these values, those that were within the range of the original data were selected and used as the sample data set.

Stage 2

This stage was aimed to include the remaining regressors, i.e., N content of stem and root, sugar content of leaf and stem, nitrate reductase activity at pre-flowering and flowering stages, in the ANN model using the original data and sample data set obtained from the previous stage. An ANN with 8 inputs (observed values of cured leaf yield, content of Cl, K and nicotine of cured leaf plus values of leaf N and nicotine contents, root sugar content and nitrate reductase activity at post-flowering stage which were predicted in previous stage) and 1 output (each of above mentioned remaining regressors) was used. As in the previous stage, 70% of the data were used for the training phase and the remaining data for the testing phase. The trained neural network was then used to find the values of N content of stem and root, sugar content of leaf and stem, nitrate reductase activity at pre-flowering and flowering stages thereby complete the estimation of the values of the 10 regressors. These predicted values do not necessarily correspond to the desired quantity and quality of tobacco yield. Hence, in the next stage the values obtained were examined.

Stage 3

The multi-layer ANN which previously developed using the original data was used in this stage to assess the regressors. The regressors were predicted values obtained during previous 2 stages of optimization. The output of the network for each group of inputs was the predicted values of cured leaf yield, contents of Cl, K and nicotine in cured leaf.

Results

The results showed a wide range of variation in the values of the regressors and output (Table 1) due to the substantial differences in soil and weather conditions (data not shown) and treatments. For instance, although the value of N fertilizer was calculated based on the soil samples test and it was tried to amend the soil with optimum amount of N fertilizer, the accumulated N in leaves varied as much as 4.18 fold over locations and treatments. The lowest and highest variation was for stem sugar content (1.82 fold) and leaf nicotine content (7.67 fold), respectively.

Table 1. Some statistical properties of the attributes, including the input layer (regressors) and output layer (last 4 rows) used in the ANN.

Attribute	Min.	Max.	Mean	Range
Leaf N content (%)	0.90	3.76	2.16	2.86
Stem N content (%)	1.50	4.00	2.47	2.50
Root N content (%)	0.50	2.90	1.70	2.40
Leaf nicotine content (%)	0.60	4.60	3.01	4.00
Leaf sugar content (%)	2.60	6.90	4.56	4.30
Stem sugar content (%)	9.00	16.40	13.14	7.40
Root sugar content (%)	5.80	20.30	12.08	14.50
Nitrate reductase activity in leaf before flowering (μmol NO_2 g^{-1} h^{-1})	9.70	18.00	13.61	8.30
Nitrate reductase activity in leaf at flowering (μmol NO_2 g^{-1} h^{-1})	1.50	8.05	5.15	6.55
Nitrate reductase activity in leaf after flowering (μmol NO_2 g^{-1} h^{-1})	1.20	7.20	3.53	6.00
Cured leaf yield (Ton ha^{-1})	1.68	5.8	3.74	4.12
Cured leaf K content (%)	1.90	4.80	3.57	2.90
Cured leaf Cl content (%)	0.70	3.20	1.64	2.50
Cured leaf nicotine content (%)	1.50	4.20	2.99	2.70

Generally, the performance of the MLP tended to be improved by an increase in the number of hidden neurons. However, too many neurons in the hidden layer caused over-fitting problems, which resulted in good network learning and data memorization, but an inability to generalize. On the other hand, the network could not learn, as only a small number of neurons in the hidden layer were used. For this data set, the MLP model with the configuration of 12 neurons in 1 hidden layer appeared to be appropriate for the prediction and optimization of output (Figure 1). This optimal number of hidden neurons was attained when the value of the learning rate and the iteration process reached 0.089 and 8000, respectively.

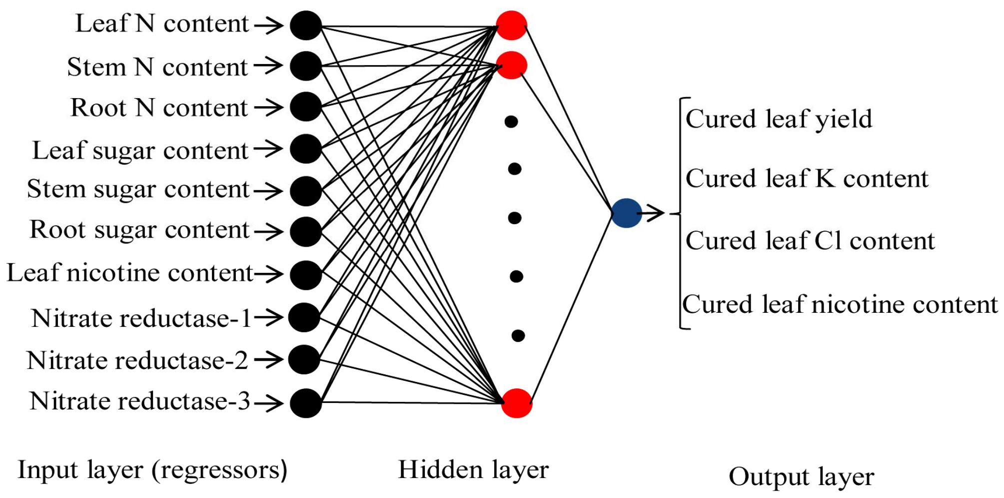

Figure 1. Multilayer neural network used for optimization of regressors to increase tobacco yield quantity and quality (K, Cl and nicotine contents of cured leaf). In input layer, the nitrate reductase-1 to 3 represent the activity of nitrate reductase enzyme at pre-flowering, flowering and post-flowering stages, respectively.

The performance of the ANN with 4 different transfer functions is shown in Table 2. The lower values of MAE, RMSE, RSE, but higher value of r proved the capability of above mentioned MLP configuration for generating accurate estimates for sigmoid transfer function by ANN. The suitability of this choice was also confirmed by the acceptable coincidence of the target network with the output network for different pattern sequences (Figure 2). The predicted and observed values were evenly distributed throughout the entire range (Figure 3). Although the results of the training phase were generally better than the test phase, the test phase demonstrated the ability of the MLP neural network to predict the values of output from new data. The high R^2 demonstrated that the trained network was reliable and accurate and could therefore be used to predict the cured leaf yield and contents of Cl, K and nicotine in cured leaf.

Table 2. Some statistical properties of the results of the MLP model with different transfer functions for 4 output variables at the training phase.

Transfer function	Output	RSE	MAE	r	RMSE
Sigmoid	Cured leaf yield	0.068124	0.157515	0.978797	0.192461
	Cured leaf K	0.038730	0.099357	0.973595	0.139804
	Cured leaf Cl	0.070707	0.083541	0.963912	0.117739
	Cured leaf nicotine	0.036818	0.096014	0.975364	0.109479
Gaussian	Cured leaf yield	0.135385	0.411804	0.916159	0.382486
	Cured leaf K	0.106393	0.372745	0.785077	0.384043
	Cured leaf Cl	0.189038	0.267023	0.780665	0.314779
	Cured leaf nicotine	0.116225	0.282445	0.800129	0.345601
Hyper. Tan.	Cured leaf yield	0.138517	0.406149	0.908178	0.391334
	Cured leaf K	0.089171	0.326828	0.857283	0.321878
	Cured leaf Cl	0.175995	0.277535	0.800828	0.293060
	Cured leaf nicotine	0.133645	0.392570	0.706755	0.397400
Hyper. Sec.	Cured leaf yield	0.202151	0.613573	0.786436	0.571110
	Cured leaf K	0.123146	0.465656	0.669292	0.444518
	Cured leaf Cl	0.171513	0.263725	0.780038	0.285597
	Cured leaf nicotine	0.115125	0.324038	0.752107	0.342328

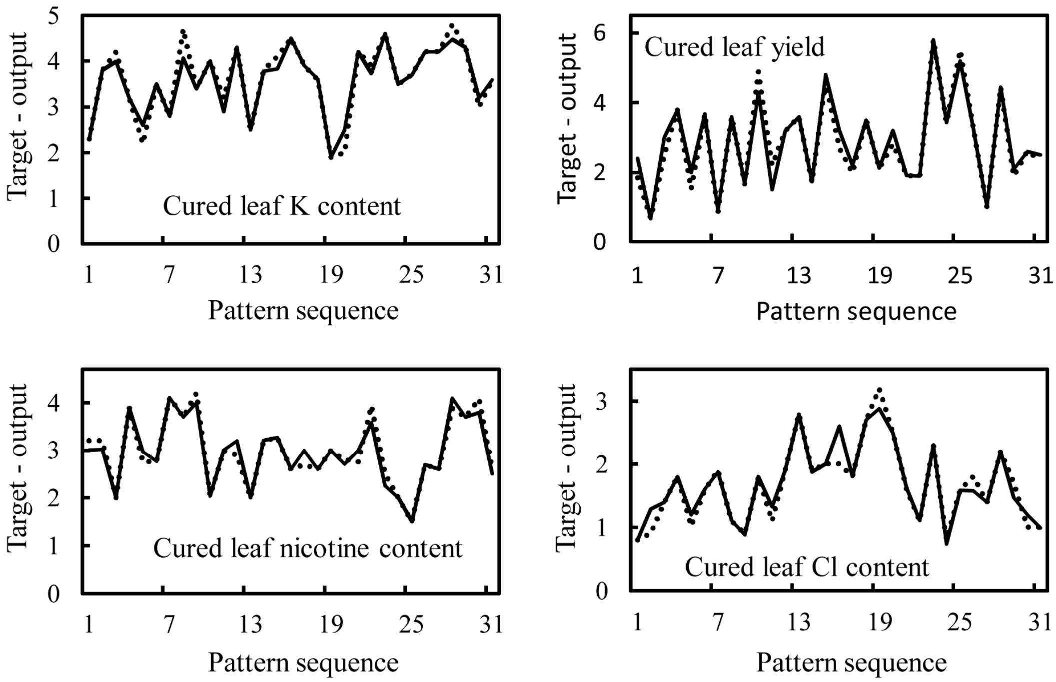

Figure 2. Targets and output network vs. pattern sequence for quantity (cured leaf yield) and quality of tobacco (contents of K, Cl and nicotine in cured leaf) at the training phase when Sigmoid transfer function was used.

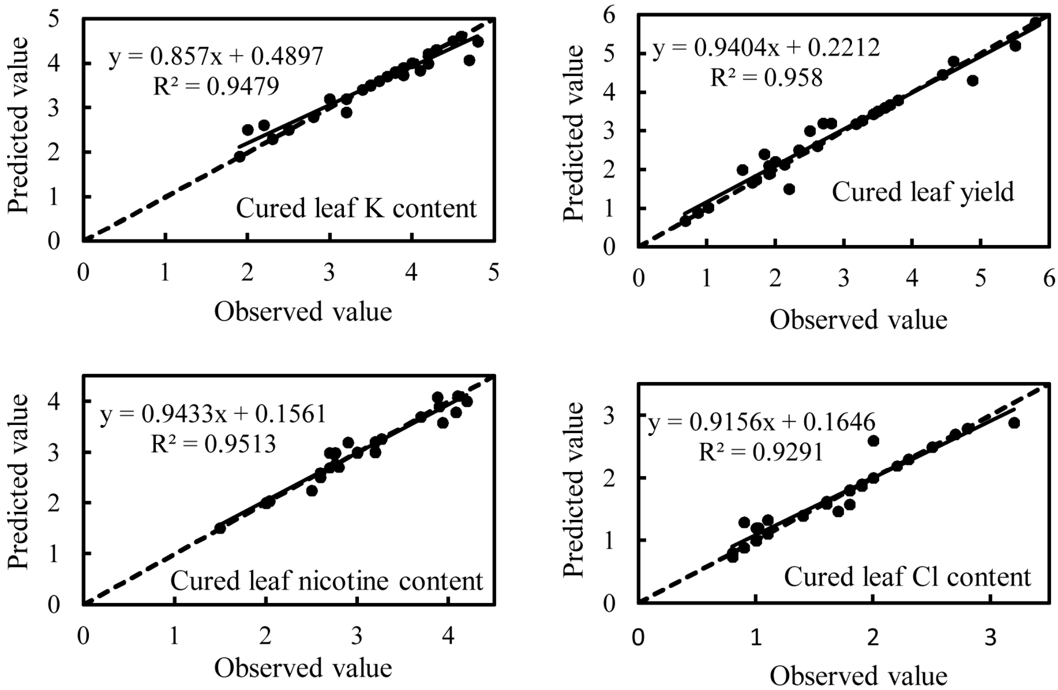

Figure 3. Plot of network output vs. training targets for quantity (cured leaf yield) and quality of tobacco (contents of K, Cl and nicotine in cured leaf) at the training phase when Sigmoid transfer function was used.

The relative contributions of the regressors to the prediction of the yield quantity and quality are shown in Figure 4. This figure clearly shows how important the regressors are in affecting the amount of yield quantity and quality. Expectedly, the tested regressors are different for affecting the quantity and quality of yield. The highest contribution (about 20%) was found for leaf N in affecting the Cl content in cured leaf. The stem N content appeared to show the lowest contribution (4.25% to cured leaf nicotine content). Despite the other regressors, leaf sugar content tended to contribute almost equally to the 4 components of output layer.

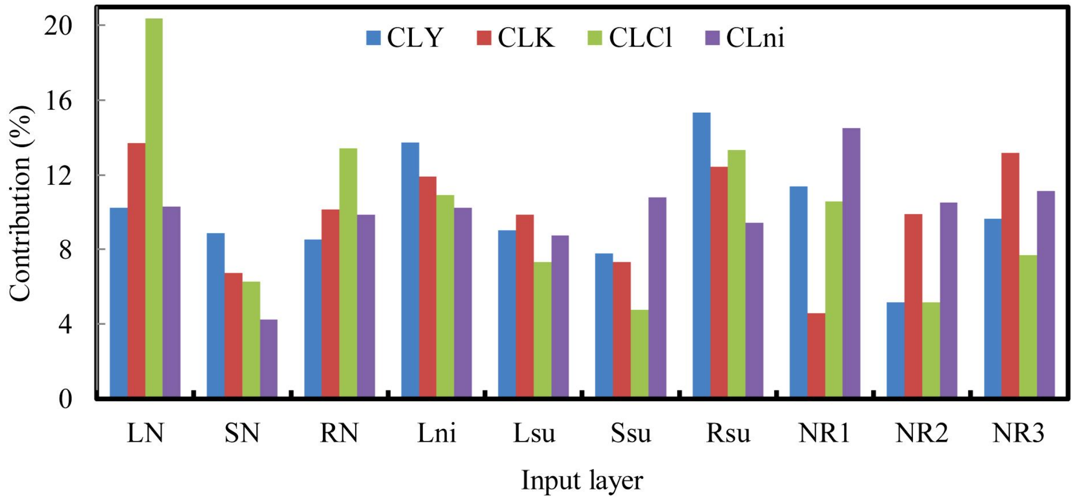

Figure 4. The relative contribution of input layer [nitrogen content of leaf (LN), stem (SN), root (RN), sugar content of leaf (Lsu), stem (Ssu), root (Rsu), leaf nicotine content (Lni),Nitrate reductase activity in leaf before flowering (NR1), at flowering (NR2) and after flowering (NR3)] to the changes in the output layer [Cured leaf yield (CLY), cured leaf K content (CLK), cured leaf Cl content (CLCl) and cured leaf nicotine content (CLni)]. CLK, CLCl and CLni are yield quality.

Discussion

The high variation in input and output layers which is prerequisite of reliable optimization by ANN was obtained in this study (Table 1). So that the average variation in nitrogen content of plant tissue, as a most important nutrient affecting quality and quantity of yield of tobacco (Allen and Raven, 1987; Maw et al., 1995; Haghighi et al., 2011), passed 4.2 fold. Part of the considerable variation in tissue N content and other attributes is due to different source of N fertilizer used in the experiment. This is in accordance with Skogley and McCants (1963) who reported how remarkable variation in growth and nutrient uptake of tobacco is achieved by changing the ratio of the ammonium to nitrate in the soil. For example they found that the growth of tobacco plants fertilized with ammonium was 30% less than those fertilized with nitrate. The high ratio of the ammonium to nitrate in the soil is followed by decrease in uptake of cations such as K, Ca and Mg (Skogley and McCants, 1963). Expectedly, the effect of this ratio tends to vary with changing climatic factors like rainfall. Results of Williams and Miner (1982) have shown that in years receiving higher than normal rainfall, higher amounts of ammonium yielded up to 26% higher than nitrate nitrogen; in years receiving less rainfall, treatments with 100% nitrate nitrogen increased yield by 6% compared to treatments which included ammonium. The data reported by Islamic

Republic of Iran Meteorological Organization (IRIMO) shows that the tested locations gathered data from differ considerably for rainfall (Oromieh: 341 mm; Tirtash: 789 mm).

The optimization of regressors, such as tested ones in this study, which there are complex inter- and intra- relation between them and output is a complicated problem whose solution requires an efficient technique like ANN. The ANN produced good predictive ability (Table 1; Figures 2 and 3) and an effective optimization of the regressors when its configuration included single hidden layer. This result is in agreement with the universal approximation theorem, which states that a neural network with a single hidden layer and a sufficiently large number of neurons can well approximate any arbitrary continuous function (Haykin, 1994). The high contribution of regressors to the changes in output (Figure 4) proves the importance and crucial effect of optimization for higher quantity and quality of tobacco in this study.

In literature it is common that the evaluation of effect of nutrients on plant is being based on the plant response to levels of fertilizers used and/or concentrations of nutrients in the soil. In some extent, this pattern may not lead to precise conclusion. This is because of the fact that so many factors other than soil nutrient concentration tend to interfere in nutrient uptake and accumulation into the plant tissue. For instance, transpiration rate affects the mass flow of nutrients to root surfaces and their influx (Havlin et al., 2005). The transpiration rate itself tends to be influenced by many factors including vapor pressure deficit (Gholipoor and Sinclair, 2011). Therefore, in this study it was focused on N concentration in plant tissue rather than in the soil. The similar pattern has been also used for sugar beet (Gholipoor et al., 2012). The optimized N concentration obtained here was 1.64% for leaf, 2.12% for stem and 1.04% for root. In terms of the average participation to changes in yield quality and quantity, leaf N concentration was more noticeable than stem N concentration (Figure 4).

A balance between tissue N content, nitrate reductase activity and soluble sugar content plays major role in yield quality and quantity of tobacco. Nitrate is the major source of organic N, including nicotine ($C_{10}H_{14}N_2$), in tobacco (Li et al., 2010). It is converted to ammonium via the glutamate synthesis cycle in two successive steps catalyzed by nitrate reductase and nitrite reductase in the cytosol and chloroplasts of leaves (Stitt et al., 2002). The nitrate reductase activity is regulated at the transcriptional level by the availability of the substrate nitrate, photosynthetic carbon metabolism (soluble sugars) and by the end product of the N assimilation pathway, glycine. In many conditions, the carbon that is required during nitrate assimilation can be obtained by reducing the rate of starch [undesirable for tobacco quality (Scheible et al., 1997)] accumulation or by remobilizing it (Fichtner and Schulze, 1992).

The mentioned close relationship between these 3 regressors does not necessarily imply that the value of one regressor could be used as an index for values of other 2 ones. This is due to (1) there is no linear relation between activity of nitrate reductase and N content of tissue; it's activity shows increasing trend with increasing tissue N content, but subsequently gets decreasing trend (Sharifi-Rad et al., 2013), (2) several factors other than tissue N content including day length, temperature and the plant age tend to largely affect the activity of this enzyme (Roth-Bejerano and Lips 1970) and (3) an increase in tissue N content causes the decline in total non-structural carbohydrates; the magnitude of tissue N effect on these carbohydrates is highly dependent on environmental factors (Reddy et al., 1996; Druege et al., 2000). Therefore these 3 regressors together were included in the ANN. The optimum value of nitrate reductase activity predicted by ANN was 13.11 µmol NO_2 g^{-1} h^{-1} for pre-flowering stage, 4.74 µmol NO_2 g^{-1} h^{-1} for flowering stage and 4.70 µmol NO_2 g^{-1} h^{-1} for post-flowering stage. The optimum sugar content obtained by ANN was 4.32%, 13.04% and 9.54% for leaf, stem and root, respectively.

Nicotine is mainly synthesized in root and represents 90-97% of the total alkaloid content of tobacco leaf (Saitoh et al., 1985). During curing and processing of tobacco products, nicotine content of leaf may change. Part of the nicotine may be converted to other organic materials like nornicotine [detrimental alkaloid for human being (Hecht, 1998; Williams, 1999)] by the enzyme nicotine N-demethylase. Here, it was only focused on finding the best nicotine content of leaf at flowering for getting medium nicotine content of leaf after curing. Optimization of several physiological and environmental factors for decreased nornicotine seems necessary in future studies. Based on ANN model prediction, the optimal value of leaf nicotine content at flowering was 2.31% which led to appropriate nicotine content of leaf after curing (1.87%).

Conclusion

The results of this study demonstrated that the precise optimization of tested regressors could simultaneously increase the potential quantity (about 3%) and quality (1.87% nicotine, 1.5% Cl and 4.75% K contents) of cured leaf in tobacco. It should be noted that the optimum values of regressors predicted by ANN are all within the range of observed values of field experiments (Table 1). It is necessary to be mentioned that, for instance, the minimum Cl content of cured leaf was 0.7 in field experiment which in terms of cured leaf quality is better than the value predicted by ANN (1.5%); but this better Cl content was counterbalanced by accompanied higher nicotine content and lower yield of cured leaf in observed values. One of the capabilities of ANN is to optimize the regressors on the basis of the zero counterbalancing effect between them. Agronomic management and breeding programs should be used to obtain these optimum values of regressors in tobacco. Fortunately there is genetic variation among tobacco cultivars for nitrate reductase activity (Ostrem and Collins, 1983), N accumulation in different parts of root, stem and leaf (Mohsenzadeh, 2015) and leaf nicotine content (Saitoh et al., 1985) which could be used for breeding purposes.

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