Optimal Production of Paddy Fields Using Modified GSTAR Models

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Abstract

The optimal paddy harvested in paddy fields of Boyolali is discussed here. The given data are varied by increasing the number of data by random normal generator. Basic model of GSTAR is evaluated and the model must be improved due to the error greater than 20%. The same time is used to model of critical region and the amount of rainfalls instead of using previous data in the usual GSTAR model. The errors are reduced.

The amount of paddy is model as a function of critical region and rainfalls from different 2 surrounding locations. This model contains 7 variables. An other modification is done by reducing the number of variables. As a result, a simpler optimization problem arises which is beneficial for computational point of view. Based on the given data, the amount of paddy in Selo and Ampel are possible to improve for future.

Keywords

Paddy Fields; Critical Region; Rainfalls; Modified GSTAR

Introduction

Central Java province contains critical regions about 982.9 thousand hectares indicated by 63% its region potentially crisis, 34% conditionally crisis and 3% extremely crisis (information was obtained by local government). The regency of Boyolali (with 19 districts) contains the biggest critical regions among other regencies in Cental Java since 39.49% (about 43,241 hectares) of its area considerably crisis due to illegal logging. Figure 1 is a map of Boyolali where dominated paddy fields are not shaded.

One way to evaluate a region for its economic productivity is determining the used region for agriculture where some factors are taken into account, i.e. the area of critical region and its rainfalls in some period of time for each district since the amount of rainfalls is natural source for agriculture, typically productivity of paddy fields.

Modeling for better cropping in paddy fields have been concerned by many authors. In agricultural economy of Canadian for instance, the TRACE model is used to forecast some important sectors (Chan , et.al. 1981). To assess the risk of pesticide pollution in Vietnam, a new model developed for simulating short-term pesticide dynamics in combined paddy rice field–fish pond farming systems. The model was calibrated using the Gauss–Marquardt–Levenberg algorithm and validated against measured pesticide concentrations of a paddy field–fish pond system typical for northern Vietnam (La, et.al. 2014). Irrigation on paddy field has also been studied (Maeda et.al. 2011) with water saving practices , paddy fields are considered for flood reduction under different water saving irrigation techniques (Unami & Kawachi 2005:191-199).

Since rice are the major food for Indonesian, productivity of paddy fields for each district will be considered to be the main factor affecting economic productivity of Boyolali for its food sustainability. Though some regions have no significant paddy fields, paddies may produced in these regions for individuals planting. The optimal paddy fields production is the interest of this paper.

The paper will be organized as follows. Small regions are considered for implementing GSTAR (Generalized Spatial Time Autoregressive) model. One should consider that the data must support the underlying theory in this method, i.e. data must be stationer in the sense of variance and mean (Borovkova,et.al 2008) and (Wutsqa, et.al.

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2010). The Box-Cox transformation and trend analysis will be employed for testing these conditions respectively before GSTAR model is proceeded. Since original model of GSTAR can not be implemented for the studied data, therefore the model is modified. This is the objective of this paper.

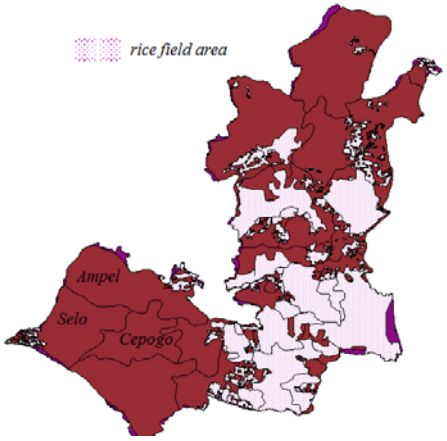

FIGURE 1. BOYOLALI MAP, A REGENCY IN CENTRAL JAVA WHERE THE SHADED AREA CONTAIN NO PADDY FIELDS

GSTAR Model and its Modification

Standard GSTAR

Accoroding to Borovkova et.al (2008) and Wutsqa et.al (2010) The standard GSTAR model is a regression based on position and time in the form

$$Z(t) = \sum_{k=1}^{p} (\varphi_{k0} + \varphi_{k1} W) Z(t-k) + e(t) \tag{1}$$

where $\varphi_{k0} = \text{diag}(\varphi_{k0}^1, ..., \varphi_{k0}^n)$ and

$$\varphi_{k1} = \text{diag}(\varphi_{k1}^1, ..., \varphi_{k1}^N)$$

W = weighted matrix are chosen such that $w_{ii} = 0$ dan $\sum_{1 \neq j} w_{ij} = 1$.

Using 3 different locations, one wrote the Equation (1) into a matrix-vector notation, i.e.

$$\begin{bmatrix} Z_1(t) \\ Z_2(t) \\ Z_3(t) \end{bmatrix} = \begin{bmatrix} \varphi_{10} & 0 & 0 \\ 0 & \varphi_{20} & 0 \\ 0 & 0 & \varphi_{30} \end{bmatrix} \begin{bmatrix} Z_1(t-1) \\ Z_2(t-1) \\ Z_3(t-1) \end{bmatrix} + \begin{bmatrix} \varphi_{11} & 0 & 0 \\ 0 & \varphi_{22} & 0 \\ 0 & 0 & \varphi_{33} \end{bmatrix} \begin{bmatrix} 0 & w_{12} & w_{13} \\ w_{21} & 0 & w_{23} \\ w_{31} & w_{32} & 0 \end{bmatrix} \begin{bmatrix} Z_1(t-1) \\ Z_2(t-1) \\ Z_3(t-1) \end{bmatrix} + \begin{bmatrix} e_1(t) \\ e_2(t) \\ e_3(t) \end{bmatrix} . \tag{2}$$

Equation (2) is more easily understood compared to Equation (1) and we will use the vector-matrix notation for further discussion. The usual weighted matrix in a standard GSTAR is defined by homogeneous weighted matrix and cross correlation matrix based on the number of locations related to the considered locations. Borovkova that the weighed matrix depends on spatial correlations between locations called semivariogram. The strong consistency and asymptotic normality of the least squares estimator in generalized STAR models are then shown (Borovkova et.al., 2008) applied to a multivariate time series of monthly tea production in west Java, Indonesia. This paper will not study analysis aspect of the model (2). Its modification will be the purpose of this research.

Equation (2) describes that each quantity on each location at time t as a linear combination of this quantity of previous time or t-1 , and other 2 locations at time t-1 . One reads that the model relates linearly only for the same quantity for 3 different locations. We may explore the Equation (2) for regression in classical sense, i.e describes a dependent variable depends linearly to some independent variables.

The study is based on the production of paddy fields (in ton) from some districts in Boyolali, i.e Selo, Ampel and Cepogo in 2008-2010. Though these districts are not dominant locations in Boyolali for producing paddies, were are interested in searching the possibilities of these districts to improve the production of paddy fields in these

locations.

There are 5 sets data in 3 locations. These data are considered too few to employ the GSTAR model (since the number of samples/ the time variation too small compared to the number of locations). By considering that the variation of data will not vary too big in the whole 3 years, one may simulate data to be 100 for each set. Moreover the correlation between Ampel and Selo and between Ampel and Cepogo are weak. On the other hand, the strong correlation between two locations is needed to employ GSTAR model.

Equation (2) however is not always true, particularly, in the case that the quantity at time t may not depend on the same quantity at time t-1. First research in this case has shown for critical region modeling of Selo, Ampel and Cepogo using standard GSTAR. Two kinds of parameter approximation in GSTAR,i.e uniformly weighted and cross sectional correlation (Parhusip and Winarso ,2014).

Comparison between data and the approximation of of critical region using these two kinds of weighted parameters have been studied (Parhusip & Winarso,2014) and some results are shown in Figure 2. Cross corellation matrix is also used for weighted matrix of Equation (2) to model rainfalls in Selo, Ampel and Cepogo. One result is shown in Figure 2. The standard GSTAR shows big errors for all cases.

The errors in Figure 2 for each district is still big enough (more 20%). One reason is due to incorrect construction of weighted matrix. Again, the model must be modified.

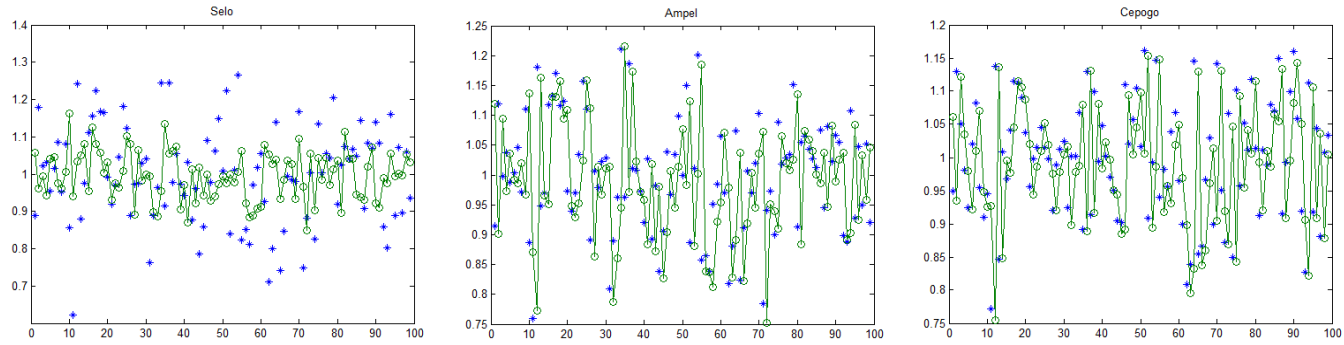

FIGURE 2. RESULT OF GSTAR AND DATA OF CRITICAL REGION USING WEIGHTED UNIFORMLY OF PARAMETERS IN GSTAR (* : DATA) (Parhusip and Winarso 2014)

Modified GSTAR on Rainfalls Data , Critical Regions and Production of Paddy Fields

The simple correction is obtained by using classical regression, i.e

$$Z_1(t) = \beta_0 + \beta_1 Z_1(t-1) + \beta_2 Z_2(t) + \beta_2 Z_3(t)\,. \tag{3.a}$$

Equation (3.a) is written for each location. In matrix-vector notation for all locations as shown in Equation (2), the model (3.a) becomes

$$\begin{bmatrix} Z_1(t) \\ Z_2(t) \\ Z_3(t) \end{bmatrix} = \begin{bmatrix} \varphi_{10} & 0 & 0 \\ 0 & \varphi_{20} & 0 \\ 0 & 0 & \varphi_{30} \end{bmatrix} \begin{bmatrix} Z_1(t-1) \\ Z_2(t-1) \\ Z_3(t-1) \end{bmatrix} + \begin{bmatrix} 0 & w_{12} & w_{13} \\ w_{21} & 0 & w_{23} \\ w_{31} & w_{32} & 0 \end{bmatrix} \begin{bmatrix} Z_1(t) \\ Z_2(t) \\ Z_3(t) \end{bmatrix} + \begin{bmatrix} e_1(t) \\ e_2(t) \\ e_3(t) \end{bmatrix}. \tag{3.b}$$

Employing this regression will give small error as depicted in Figure 3. This result shows that the chosen independent variables play important role. If $Z_2(t-1), Z_3(t-1)$ are also added the right hand side of Equation (3.a), then the error is big, i.e about 80%. On the other hand the error is only in the order 10^{-3} % for Equation (3.a). Using Equation (3.a) for $Z_2(t)$ and $Z_3(t)$, one observes that the error behaves similarly. Note that $Z_k(t)$ and $Z_k(t-1)$ does not interact linearly since the parameter β_1 approaches to zero for all cases. Thus one concludes that 3 variables have linear interaction which indicate interaction among the locations at the same time. Furthermore in time variant, there exists no significant correlation since β_1 tends to zero in Equation(3.a). In other words, Equation (3.a) becomes

$$Z_1(t) = \beta_0 + \beta_2 Z_2(t) + \beta_2 Z_3(t)\,. \tag{3.c}$$

Thus this result is implemented to other set data, i.e critical region. Equation (3.b) simplifies the GSTAR into classical regression.

Critical regions on 3 locations are modeled using Equation (3.a). Improvements are also obtained since all errors only 0.4178%, 0.4023 %, 0.9230% for 3 locations respectively.

However, in the case of production of paddy fields, the given data behave differently. Using similar approximation (i.e regression with the data given at the same time for different locations) give big errors, i.e. 25.5821% 11.5124% 21.7046% respectively. Therefore the standard model of GSTAR is not used to predict the optimal paddy harvested in Boyolali.

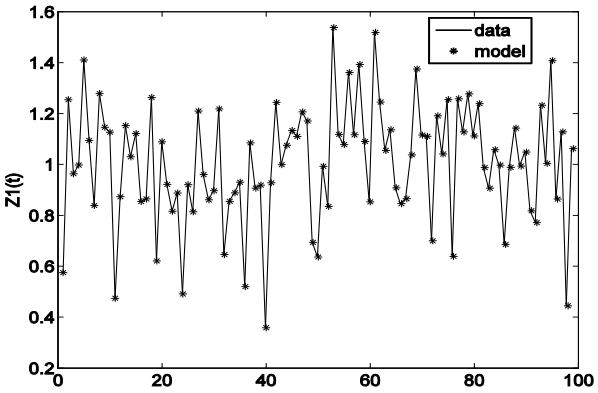

FIGURE 3. APPROXIMATION OF CLASSICAL REGRESSION ON THE RAINFALLS MODEL FOR 3 DIFFERENT LOCATIONS SIMULTANEOUSLY. HORIZONTAL : INDEX, VERTICAL : THE AMOUNT OF RAINFALLS (STATIONER DATA)

Optimization Method on the Amount of Paddy Harvested from Selo, Ampel and Cepogo

Optimization of paddy fields have been considered by several authors. Optimization drying paddy for minimum drying time and maximum head yield (Jha et.al 2012). The best period of 3 periods of planting paddy in rice field in Indonesia has been analyzed using quadratic function (Dewi et.al. 2013) by considering paddy harvested is a function of the planting area and harvested area. The optimization is done by Singular Value Decomposition and Ant Colony Algorithm (Dewi, et.al. 2013).

This research concerns the productivity of paddy fields due to characteristics of lands in Boyolali which are considerable in crisis and the amount of rainfalls dominating the productivity of rice fields naturally. The linear model obtained by GSTAR will be optimized by standard linear programming provided by MATLAB. This approach is well known in the study of paddy production, such as by (Chiu, and Fon,-) and (Tzimopoulos , et.al. 2011) for optimization on irrigation of paddy field. The problem arises for having a good linear objective functions before proceeding an optimization on the amount of paddy.

Analysis and Discussion

Modified GSTAR on Critical Region as the Dependent Variable

One may assume that a critical region depends on the rainfall around it. Thus a critical region on Selo depends on the amount of rainfall in this region, the area of critical regions from other districts, and the amount of rainfalls of these districts at the same time *t*. One may write as

$$Z_1(t) = \beta_0 + \beta_2 Z_2(t) + \beta_2 Z_3(t) + \beta_3 Y_1(t) + \beta_4 Y_2(t) + \beta_5 Y_3(t) \tag{4}$$

where

$Z_k(t) :=$ the area of critical region in the k-th location at time t; $Y_k(t) :=$ the amount of rainfalls at location k at time t, for k=1,2,3.

Equation (4) gives approximation with 0.4064 % error and the vector of parameters is

$$\vec{\beta} = \left[205.2286\ 1.0435\ 0.4484\ 283.9430\ -613.7395\ 124.0760\right]^T .$$

On the other hand, $\det(X'X)$ closes to 0 indicating the matrix is nearly singular. However, since the error is small enough , we can rely on this vector of parameters to proceed other analysis based on the Equation (4).

Equation (4) can be applied similarly for other location. In similar fashion with Equation (2), one yields

$$\begin{bmatrix} Z_1(t) \\ Z_2(t) \\ Z_3(t) \end{bmatrix} = \begin{bmatrix} \beta_{10} & 0 & 0 \\ 0 & \beta_{20} & 0 \\ 0 & 0 & \beta_{30} \end{bmatrix} \begin{bmatrix} 1 \\ 1 \\ 1 \end{bmatrix} + \begin{bmatrix} 0 & \beta_{12} & \beta_{13} \\ \beta_{21} & 0 & \beta_{23} \\ \beta_{31} & \beta_{32} & 0 \end{bmatrix} \begin{bmatrix} Z_1(t) \\ Z_2(t) \\ Z_3(t) \end{bmatrix} + \begin{bmatrix} \gamma_{11} & \gamma_{12} & \gamma_{13} \\ \gamma_{21} & \gamma_{22} & \gamma_{23} \\ \gamma_{31} & \gamma_{32} & \gamma_{33} \end{bmatrix} \begin{bmatrix} Y_1(t) \\ Y_2(t) \\ Y_3(t) \end{bmatrix} + \begin{bmatrix} e_1(t) \\ e_2(t) \\ e_3(t) \end{bmatrix}. \quad (5)$$

The first vector on the ride hand side describes the constant on each regression. The second vector illustrates the dependent of the same quantities from other two nearby locations, and the third vector describes the dependent of other quantities from other two locations. Each parameter is found by classical regression.

Note that the standard GSTAR model here is valid for the data observed from 3 locations and previous time such as oil production where production of each reservoir relates to other reservoirs in previous time. Drilling of oil from a reservoir affects the production rate of other nearby reservoirs. Oil production at time *t* may depend on the oil production at time *t*-1 as shown in the model of GSTAR (Equation (2)). On the other hand, rainfalls produced not from the location itself. Thus rainfalls at time *t*-1 may not affect significantly for the amount of rainfalls at time *t* to the nearby locations. Therefore the standard model of GSTAR needs to be modified.

Production of Paddy Fields with Modified GSTAR

1) First Modification

Similarly, one may use the Eqation (3.b) for the data of production of paddy fields for each location. The error is not greater than 20% indicating the model is considerable. The result is shown in Table 1. In this table that dependency of previous production is not significant due to small value of parameters β_1 for 3 locations.

Due to the area of critical regions, Equation (3.b) is applied to the amount of paddy(left hand side Equation(3.b)). For each location, one yields Table 2.

TABLE 1. MODIFIED GSTAR RESULT FOR PRODUCTION OF PADDY FIELDS

Parameters Eq.(3.b)			Error (%)		
Selo	Ampel	Cepog	Selo	Ampel	Cepogo
0.8864	0.8329	-0.8210	18.4265	9.8391	15.7379
0.0044	0.0185	-0.0622			
0.8946	-0.2192	0.7518			
1.0043	0.3676	1.1318			

TABLE 2. MODIFIED GSTAR RESULT FOR PRODUCTION OF PADDY FIELDS BASED ON THE AREA OF CRITICAL REGIONS

Parameters Eq.(5)			Error (%)		
Selo	Ampel	Cepogo	Selo	Ampel	Cepogo
1.7377	-0.8678	-0.2519	18.0635	9.3511	15.3837
-0.9467	-0.2174	0.7437			
1.0220	0.3705	1.1741			
1.9717	-3.5047	1.0758			
-2.5233	3.2739	-0.5562			
-0.2612	1.9451	-1.1849			

By implementing Equation (5) for all locations, one yields the linear system with parameters given by Table 2, i.e

$$\begin{bmatrix} Z_1(t) \\ Z_2(t) \\ Z_3(t) \end{bmatrix} = \begin{bmatrix} 1.7377 & 0 & 0 \\ 0 & -0.8678 & 0 \\ 0 & 0 & -0.2519 \end{bmatrix} \begin{bmatrix} 1 \\ 1 \\ 1 \end{bmatrix} + \begin{bmatrix} 0 & -0.9467 & 1.0220 \\ -0.2174 & 0 & 0.3705 \\ 0.7437 & 1.1741 & 0 \end{bmatrix} \begin{bmatrix} Z_1(t) \\ Z_2(t) \\ Z_3(t) \end{bmatrix} + \begin{bmatrix} 1.9717 & -2.5233 & -0.2612 \\ -3.5047 & 3.2739 & 1.9451 \\ 1.0758 & -0.5562 & -1.1849 \end{bmatrix} \begin{bmatrix} Y_1(t) \\ Y_2(t) \\ Y_3(t) \end{bmatrix}. \quad (6)$$

Note that GSTAR is modified by using data at the same time *t*, whereas standard GSTAR using data at time *t*-1 on the right hand side to obtain parameters of matrix. The right hand side of the modification model depends on paddy harvested by surrounding locations, the surrounding of critical regions and itself, the amount of rainfalls at *t*-1 on itself, and the amount of rainfalls at time *t* from other surrounding locations. One symbolizes

the model into

$$\begin{bmatrix} Z_1(t) \\ Z_2(t) \\ Z_3(t) \end{bmatrix} = \begin{bmatrix} \beta_{10} & 0 & 0 \\ 0 & \beta_{20} & 0 \\ 0 & 0 & \beta_{30} \end{bmatrix} \begin{bmatrix} 1 \\ 1 \\ 1 \end{bmatrix} + \begin{bmatrix} 0 & \beta_{12} & \beta_{13} \\ \beta_{21} & 0 & \beta_{23} \\ \beta_{31} & \beta_{32} & 0 \end{bmatrix} \begin{bmatrix} Z_1(t) \\ Z_2(t) \\ Z_3(t) \end{bmatrix} + \begin{bmatrix} \gamma_{11} & \gamma_{12} & \gamma_{13} \\ \gamma_{21} & \gamma_{22} & \gamma_{23} \\ \gamma_{31} & \gamma_{32} & \gamma_{33} \end{bmatrix} \begin{bmatrix} Y_1(t) \\ Y_2(t) \\ Y_3(t) \end{bmatrix} + \begin{bmatrix} \varsigma_{11} & 0 & 0 \\ 0 & \varsigma_{22} & 0 \\ 0 & 0 & \varsigma_{33} \end{bmatrix} \begin{bmatrix} W(t-1) \\ W(t-1) \\ W(t-1) \end{bmatrix}$$

$$+ \begin{bmatrix} 0 & \varphi_{12} & \varphi_{13} \\ \varphi_{21} & 0 & \varphi_{23} \\ \varphi_{31} & \varphi_{32} & 0 \end{bmatrix} \begin{bmatrix} W_1(t) \\ W_2(t) \\ W_3(t) \end{bmatrix} + \begin{bmatrix} e_1(t) \\ e_2(t) \\ e_3(t) \end{bmatrix}. \quad (7)$$

Compared to Equation (5), the Equation (7) includes natural behavior that rice production depends on the characteristics of land given by the area of critical region, and the amount of rainfalls dominating the rice production field. However if data provides singular matrices in Equation (7), the model should be reduced into Equation (5). Fortunately the model in Equation (7) gives similar error as if one uses Equation (5).

Though the errors considerable good enough, one may observe by R's software that the variables of paddies from other 2 locations contribute more significantly to the model Equation(7) under 95% tolerance or 0.05 *p*-value for each set of parameters. In this research ,we decide to choose Equation (7) since natural phenomena are forced to consider here to satisfy the modified GSTAR model. Figure (4) illustrates the result of modified GSTAR model. Compared to previous model, the errors in Table 2 and Table 3 do not differ significantly. Fortunately, modification can be done again for reducing the number of variables in optimization procedure. This is shown in the next subsection.

TABLE 3. MODIFIED GSTAR RESULT FOR PRODUCTION OF PADDY FIELDS BASED ON THE NUMBER OF PADDIES CREATED BY SURROUNDINGS, THE AREA OF CRITICAL REGIONS BY 3 LOCATIONS AT THE SAME TIME *T*, THE AMOUNT OF RAINFALLS AT TIME *T*-1 IN ITSELF AND THE AMOUNT OF RAINFALLS IN THE 3 LOCATIONS AT THE SAME TIME *T*

Parameters in Equation(7)			Error (%)		
Selo	Ampel	Cepogo	Selo	Ampel	Cepogo
1.3873	-1.2763	-0.3545	17.9354	9.2006	15.2895
-0.9290	-0.2083	0.7384			
1.0143	0.3583	1.1703			
1.1743	-4.1075	0.6320			
-1.7223	3.9220	-0.1186			
0.1062	2.2199	-0.9679			
-0.0850	0.0186	-0.0496			
0.1602	0.0499	-0.1400			
-0.1060	0.0228	0.0906			

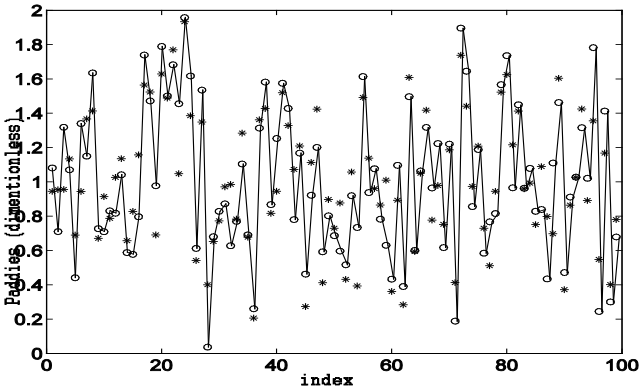

FIGURE 4. GSTAR MODEL (DENOTED BY '-O') AND DATA (DENOTED BY '*') FOR PADDIES PRODUCTION DUE TO THE AREA OF CRITICAL REGION AND THE AMOUNT OF RAINFALLS AT THE SAME TIME ; USING EQUATION (7)

2) *Second Modification*

The area of critical region and the amount of rainfalls of Selo is modeled each quantity by Equation (3), i.e. $Z_1(t) = \beta_0 + \beta_2 Z_2(t) + \beta_2 Z_3(t)$. Finally the amount of paddy produced in Selo is a function of rainfalls and critical region obtained by the models. This approach reduces the number of variables compared to model in Equation (7). Using this model , one achieves 18.42% error. Thus the complete model of the amount of paddy harvested in Selo is

$$Z_1(t) = \beta_0 + \beta_2 Z_2(t) + \beta_2 Z_3(t) \quad (P.1)$$

$$Y_1(t) = \alpha_0 + \alpha_1 Y_2(t) + \alpha_2 Y_3(t) \quad (P.2)$$

$$P_1(t) = \varphi_0 + \varphi_1 P_2(t) + \varphi_2 P_3(t) + \varphi_3 Z_1(t) + w_1 Y_1(t) \quad (P.3)$$

The Equations (P.1),(P.2),(P.3) represent the critical region model, the amount of rainfall model and the amount of paddy respectively.

Similar model can be designed for the other two locations (Ampel and Cepogo). Finally modified GSTAR model

contains 3 linear systems ,i.e

$$\begin{bmatrix} Z_1(t) \\ Z_2(t) \\ Z_3(t) \end{bmatrix} = \begin{bmatrix} \beta_{10} & 0 & 0 \\ 0 & \beta_{20} & 0 \\ 0 & 0 & \beta_{30} \end{bmatrix} \begin{bmatrix} 1 \\ 1 \\ 1 \end{bmatrix} + \begin{bmatrix} 0 & \beta_{12} & \beta_{13} \\ \beta_{21} & 0 & \beta_{23} \\ \beta_{31} & \beta_{32} & 0 \end{bmatrix} \begin{bmatrix} Z_1(t) \\ Z_2(t) \\ Z_3(t) \end{bmatrix} \quad \text{(P.4)}$$

$$\begin{bmatrix} Y_1(t) \\ Y_2(t) \\ Y_3(t) \end{bmatrix} = \begin{bmatrix} \alpha_{10} & 0 & 0 \\ 0 & \alpha_{20} & 0 \\ 0 & 0 & \alpha_{30} \end{bmatrix} \begin{bmatrix} 1 \\ 1 \\ 1 \end{bmatrix} + \begin{bmatrix} 0 & \alpha_{12} & \alpha_{13} \\ \alpha_{21} & 0 & \alpha_{23} \\ \alpha_{31} & \alpha_{32} & 0 \end{bmatrix} \begin{bmatrix} Y_1(t) \\ Y_2(t) \\ Y_3(t) \end{bmatrix} \quad \text{(P.5)}$$

$$\begin{bmatrix} P_1(t) \\ P_2(t) \\ P_3(t) \end{bmatrix} = \begin{bmatrix} \varphi_{10} & 0 & 0 \\ 0 & \varphi_{20} & 0 \\ 0 & 0 & \varphi_{30} \end{bmatrix} \begin{bmatrix} 1 \\ 1 \\ 1 \end{bmatrix} + \begin{bmatrix} 0 & \varphi_{12} & \varphi_{13} \\ \varphi_{21} & 0 & \varphi_{23} \\ \varphi_{31} & \varphi_{32} & 0 \end{bmatrix} \begin{bmatrix} P_1(t) \\ P_2(t) \\ P_3(t) \end{bmatrix} + \begin{bmatrix} \varphi_{11} & 0 & 0 \\ 0 & \varphi_{22} & 0 \\ 0 & 0 & \varphi_{33} \end{bmatrix} \begin{bmatrix} Z_1(t) \\ Z_2(t) \\ Z_3(t) \end{bmatrix} + \begin{bmatrix} w_{11} & 0 & 0 \\ 0 & w_{22} & 0 \\ 0 & 0 & w_{33} \end{bmatrix} \begin{bmatrix} Y_1(t) \\ Y_2(t) \\ Y_3(t) \end{bmatrix} \quad \text{(P.6)}$$

TABLE 4. THE VALUE OF EACH PARAMETER IN THE MODEL. EACH ROW IN FULL MATRIX OF PARAMETER BELONGS TO EACH LOCATION ; FIRST, SECOND, AND THIRD BELONG TO SELO, AMPEL , CEPOGO RESPECTIVELY ACCORDING TO EQUATION (P.4)-(P.6)

No	Name of parameters	Values of parameters	Error (%) [Selo Ampel Cepogo]
1.	$\begin{bmatrix} \beta_{10} & \beta_{12} & \beta_{13} \\ \beta_{21} & \beta_{20} & \beta_{23} \\ \beta_{31} & \beta_{32} & \beta_{30} \end{bmatrix}$	$\begin{bmatrix} -0.4911 & 1.0435 & 0.4477 \\ 0.4698 & 0.9550 & -0.4248 \\ 1.0806 & 2.1839 & -2.2646 \end{bmatrix}$	[0.4209 0.4041 0.9333]
2.	$\begin{bmatrix} \alpha_{10} & \alpha_{12} & \alpha_{13} \\ \alpha_{21} & \alpha_{20} & \alpha_{23} \\ \alpha_{31} & \alpha_{32} & \alpha_{30} \end{bmatrix}$	$\begin{bmatrix} -0.7245 & 2.1615 & -0.4370 \\ 0.3352 & 0.4626 & 0.2022 \\ -1.6580 & -2.2884 & 4.9464 \end{bmatrix}$	10^{-3} x [0.2381 0.1119 0.5380]
3.	$\begin{bmatrix} \varphi_{10} & \varphi_{12} & \varphi_{13} \\ \varphi_{21} & \varphi_{20} & \varphi_{23} \\ \varphi_{31} & \varphi_{32} & \varphi_{30} \end{bmatrix}$	$\begin{bmatrix} 0.8674 & 0.9055 & -0.9084 \\ -0.8913 & -0.2106 & 0.7526 \\ 1.0040 & 0.3548 & 1.1168 \end{bmatrix}$	[18.4260 9.6524 15.9238]
	$\begin{bmatrix} \varphi_{11} & 0 & 0 \\ 0 & \varphi_{22} & 0 \\ 0 & 0 & \varphi_{33} \end{bmatrix}$	$\begin{bmatrix} 0.0184 & 0 & 0 \\ 0 & -0.1350 & 0 \\ 0 & 0 & 0.0101 \end{bmatrix}$	
	$\begin{bmatrix} w_{11} & 0 & 0 \\ 0 & w_{22} & 0 \\ 0 & 0 & w_{33} \end{bmatrix}$	$\begin{bmatrix} 0.0020 & 0 & 0 \\ 0 & 0.0849 & 0 \\ 0 & 0 & 0.0290 \end{bmatrix}$	

TABLE 5. PRODUCED PADDY FROM EACH LOCATION BY MODIFIED GSTAR

Location	Model	Min (data) (dimension-less)	Maximum (data) (dimension-less)	optimal (dimensional) Obtained by model (in ton)
Selo	1.9987	0.0361	1.9566	102.1506
Ampel	1.4120	0.6703	1.3502	7.0383
Cepogo	1.7069	0.0592	2.0323	71.3926

Compared to the Equation (7), Equation (P.6) has less number of variables which is good for computational point of view, i.e applying the result of GSTAR in (P.4-P.5) into Equation (P.6). The last model gives the smaller number of variables to the optimization later on. Hence this model is more acceptable to proceed for having an optimization problem. Fortunately the linear programming designed here may achieve reasonable results as shown in Table 4 (though is not yet proven formally). In order to have comparison result due to the given data, Table 5 provides the optimization result, i.e. the maximum paddy produced on each paddy field on each location.

We observe that the optimal values approximate to the given data. For practical purpose, Selo and Ampel are still able to increase their productivities about 1 ton. This result can be used to local government how to predict the optimal of paddy field for each location in Boyolali for further research. Other possible research can be done, i.e. to solve the optimization problem by linear stochastic optimization for more reliable solutions since the solutions are given in some intervals.

Conclusions

This paper has shown the used of GSTAR model and its modification to present the models of critical region among 3 locations (Selo, Ampel and Cepogo) , the amount of rainfalls and the harvested paddy fields in these 3 locations. The standard GSTAR is not good enough to simulate the given data. Therefore modifications are proposed in 3 kinds by studying the resulting errors. Though the last modification can not improve the error, the number of variables can be reduced without ignoring the most important parts for paddy cropping, i.e. by considering the critical region on each location due to the present of 2 surrounding locations, by taking into account the amount of rainfalls in the same year and the amount paddy produced by neighboring districts.

The optimal harvested paddy from 3 locations can be obtained. This research suggests that Selo and Ampel could have obtained larger 1 ton in the year 2008-2010. If optimal productivity of each location can be known, (due to formal optimization procedures), the local government may have prediction more formally of producing rice for the people in Boyolali based on local capacity. Finally, this idea can be applied for other consecutive locations for all districts in Boyolali and the amount of optimal paddy harvested in entire Boyolali can be approximated. This will give better planning of food sustainability of Boyolali, especially rice as staple food and paddy is major cropping object.

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Characterization of the *Solanum Nigrum* Complex of Kenya by AFLP Markers

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Abstract

Species of the *Solanum nigrum* complex are known to have a long tradition as alternative food- or fodder plants and a significant medical value in parts of Africa, India, Indonesia and China. Mature berries are often edible and cooked leaves are used as herbs and vegetables. The taxonomy of the *S. nigrum* complex is difficult, especially because of extensive synonymy. Amplified fragment length polymorphism (AFLP) technique was used to assess the genetic relationship of twelve accessions from Kenya and Germany. Distances on the basis of molecular markers were only partially correlated to morphological descriptions. Intermixing of *S. nigrum* and *S. scabrum* accessions suggests close relationship of both species.

Keywords

Solanum Nigrum Complex; AFLP; Genetic Distance; Dissimilarity; Germany; Kenya

Introduction

The Solanaceae family is worldwide distributed and includes more than 2000 species (D'Arcy, 1986). Among them are economically important vegetables and fruits such as potato, tomato, aubergine and pepper but also tobacco, belladonna, stramonium and black henbane. Most of them are toxic at least for special organs or developmental stages. But the content of glycoalcaloides, glycoproteines and polysaccharides is also responsible for their significant medical value.

Within the genus *Solanum* the section *Solanum* (referred often as the *Solanum nigrum* complex) is the most diverse one. Plants of this section are known as weeds in horticulture and agriculture, competing with the main crops for moisture, nutrients and light. But in developing countries of Africa, Asia and South America they are used also as important edible leafy vegetables (Keller *et al*., 1969). In Kenya fruits of both, wild plants and cultivated varieties of black nightshade are used for nutrition (Edmonds and Chweya, 1997; Masinde *et al*., 2009).

Black nightshade is a fairly common herb or short-living perennial shrub, found in many wooded areas as well as in rural habitats from temperate to tropical regions. It has a height range from 30 to 120 cm. The leaves are alternate, dark green, soft, rather thin, and often riddled with bug holes like those of amaranth. The size of the leaves is quite variable, while the shape is ranging from ovate and lanceolate to diamond-shaped, ovate to heart-shaped, with wavy or large-toothed edges. Both surfaces can be haired or hairless. Petioles are 1 to 3 cm long with a winged upper portion (Edmonds, 1983). The flowers have prominent bright yellow anthers surrounded by greenish to nearly white petals, which recurve when aged. The berries have in most cases a diameter of 6 to 8 mm and dull black, purple-black, red or orange color (Olet *et al*, 2011; Edmonds and Chweya 1997). Black nightshade varieties are believed to have a high nutritional value (ERA, 2008).

43 accessions of vegetable black nightshade were collected by the gene bank of Kenya at 650 to 2200 meters above sea level from diverse habitats including undulating landscapes, plains, hillside and floodplains. 9 of the samples were classified as wild, 13 as weedy and 12 as landraces; 9 accessions are without any such data (Edmonds and Chweya, 1997). In Kenya different ethnic communities have given black nightshade various names: mnavu (Kiswahili), managu (Kikuyu), kitulu (Kamba), momoi (Maasai). In Africa the name *Solanum nigrum* is often used for almost all species of section *Solanum* with black fruits, including *S. scabrum*. This confusion is probably aggravated by the use of vernacular names whereby one name can apply to several species, or several names to the

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same species. *S. scabrum* is often confused with *S. americanum*, but the later one can be distinguished by more slender stems, narrower leaves and smaller flowers and fruits (Stevels, 1990).

Amplified fragment length polymorphism (AFLP), is a polymerase chain reaction-based technique for DNA fingerprinting (Vos *et al*., 1995). It has been widely used for studying genetic relationships among cultivars of different species or genera. Advantages of this technique are good reproducibility, high level of polymorphism, genome wide distribution of the markers, and no requirement of prior knowledge of the genome being studied (Mueller and Wolfenbarger, 1999; Prabhu and Gresshof, 1994). The AFLP technique is particularly valuable for studying intraspecific variation also for polyploidy plants (Meudt and Clarke, 2007). The usefulness for Solanaceae was demonstrated by Kardolus *et al*., (1998) for 19 taxa of *Solanum* and 3 taxa of *Lycopersicum*. AFLP studies for the *Solanum nigrum* complex have been performed by Jacoby *et al*., (2003), Dehmer and Hammer (2004), Manoko *et al*., (2007, 2008) and Olet *et al*., (2011).

This study was undertaken to characterize twelve accessions of Black nightshade (*Solanum nigrum* complex) from Kenya and Germany, using morphological and AFLP markers, in order to evaluate the genetic distance of these accessions and to detect redundancies inside the collection.

Materials and Methods

Plant Material

Twelve accessions of the *Solanum nigrum* complex from both Kenya and Germany were analyzed. Three to ten plants were used to represent each accession (Table 1). Seeds of ten accessions were from Kenya and two accessions were from Germany. The seeds were germinated and grown in the green house under identical conditions.

TABLE 1 NUMBER OF PLANTS ANALYSED PER ACCESSION OF THE *SOLANUM NIGRUM* COMPLEX

Accession	Origin	Number of plants
1) *Solanum scabrum*	Kabras, Kakamega	5
2) *Solanum scabrum*	Bungoma	5
3) *Solanum scabrum*	Botsotso, Kakamega	5
4) *Solanum scabrum*	AMPATH	5
5) *Solanum scabrum*	Eldoret	5
6) *Solanum scabrum*	Kisumu, Ahero	5
7) *Solanum villosum*	Eldoret	5
8) *Solanum villosum*	Bungoma	4
9) *Solanum nigrum*	Kabras, Kakamega	3
10) *Solanum nigrum*	Bungoma	10
11) *Solanum nigrum*	Quedlinburg, Germany	3
12) *Solanum nigrum*	Quedlinburg, Germany	3
Total		58

Morphological Analysis

Morphological studies were made on fresh samples of the *Solanum nigrum* complex. The studies were made on fruits, flowers and leaves of the species. Due to the small number of the plants available, no statistical analysis was done but interesting characteristics were recorded.

DNA Isolation

Very young and healthy leaves from individual plants were collected, transferred to ice and stored at -80 °C. For DNA extraction was used the protocol of Porebski *et al*., (1997) with some modifications: 100 mg of fresh leaves were placed in an Eppendorf tube together with 400 µl of preheated (65 °C) extraction buffer and one titanium ball. The leaf tissue was disrupted for 5 min with a frequency of 30/s by MM300 mixer mill (Retsch, Haan, Germany). The tubes were incubated for 1 hour at 65 °C in a water bath and cooled down to room temperature before adding 500 µl of chloroform: isoamylalcohol (24:1). After intensive mixing to form an emulsion, the tubes were centrifuged at 13,000 rpm for 20 min at room temperature (RT). After centrifugation the top aqueous solution was transferred

to a new tube to repeat the chloroform: isoamylalcohol extraction step to remove all protein from the aqueous phase. The aqueous solution was transferred to a new tube and 0.5 volumes of 5M NaCl were added. For DNA precipitation two volumes of ice cold 96% ethanol were added. After intensive mixing samples were placed in a freezer (-20 °C) for 10 min. After centrifugation at 13,000 rpm for 10 min at RT the supernatant was poured off and the resulting pellets were washed twice with 70% ethanol and air dried in the vacuum for 10 min. The pellets were then re-dissolved in 100 µl TE buffer overnight. To remove RNA from the preparation, 1 µl RNase A (10 mg/ml) was added, and the tubes were incubated at 37 °C for 1 h followed by Proteinase K (10 mg/ml) treatment for 1 hour at 37 °C. Once more an equal volume of chloroform-isoamyl alcohol was added and the mixture was centrifuged at 13,000 rpm for 10 min. To the supernatant was added 1/10 volume of 3 M NaCl and 2 volumes of ice cold absolute ethanol. The mixture was placed in the freezer at -20 °C for 1 h followed by centrifugation at 13,000 rpm for 10 min. The resulting pellets were washed with 70% ethanol, air dried and resuspended in 100 µl of 0.1x TE buffer. Measurement of amount and purity of DNA was done by using a UV-VIS Spectronic Genesys 5 (Milton Roy) spectrophotometer at 260/280 nm.

The final concentration of the DNA samples ranged from 200 to 632 ng/µl. The individual DNA samples were diluted with the appropriate amount of TE buffer to obtain a final DNA concentration of 10 ng/µl. The equimolar pools were created by combining the same amount of the diluted DNA samples of each accession (which originated from 3, 4, 5 or 10 plants) producing the final pooled DNA sample with the concentration of 10 ng/µl.

AFLP Analysis

The AFLP analysis followed the protocol of Vos *et al*. (1995). 250 ng of genomic DNA were digested for 4 hours with the restriction endonucleases *Mse*I and *Eco*RI following suppliers recommendations. Restricted DNA fragments were ligated to pre-annealed adaptors and preamplification was carried out using *Eco*RI+1 and *Mse*1+1 primers. Sixteen *Eco*RI+3 / *Mse*1+3 primers combinations were screened. The following twelve primer combinations showed good amplification and were selected for further analysis: E-AAG/MCAA, E-AAG/M-CAG, E-AAG/MCAT, E-AAG/M-CTA, E-AGC/MCAA, E-AGC/M-CAC, E-AGC/M-CAG, E-AGC/MCAT, E-AGC/M-CTA, E-AGC/M-CTT, E-AGG/M-CAC and E-AGG/M-CTT. The forward primer of each selective amplification reaction was labeled by IRD 700 or IRD 800, respectively (Eurofins MWG Operon, Ebersberg, Germany). PCR products generated from selective amplification with IRD 700 and IRD 800 were mixed and separated together on a 6.5% polyacrylamide gel run in a 1X TBE buffer on LI-COR 4300 automatic sequencer (LI-COR Biosciences, Lincoln, NE, USA). For size determination the corresponding IRD 700 or IRD 800 fluorescence labeled 700 bp size ladder was applied.

Selective AFLP amplification was done two times in parallel for each sample. Only bands with consistent results were counted as present (1) or absent (0). The genetic distance between each pair of genotypes was calculated by SIMGEND analysis of the NTSYSpc software package version 2.1 (Rohlf, 2000). For cluster analysis the software package TREECON for Windows version 1.3 (van de Peer and de Wachter, 1994) in UPGMA algorithm was used. Bootstrap analysis was performed with 100 replications.

Results and Discussion

Morphological Analysis

Morphological analysis of the twelve accessions was performed in the green house and showed in some characteristics a remarkable variation within the *Solanum nigrum* complex. *S. villosum* leaves were obtuse with conspicuously dentate margins (Figure 1). The flowers had white petals and yellow stamens. The unripe berries were green and turned yellow when ripe. The stem was angular with dentate ridges.

S. nigrum from Quedlinburg (Germany) resembled those from Kenya but tended to have slightly dentate leaf margins and dull dark berries (Figure 2). In *S. nigrum* from Kenya the leaves were lanceolate (elliptic) and had entire margins (Figure 3). The flowers had light purple petals and yellow stamens. The berries were green with dark tiny spots at the bottom when unripe while the ripe berries were small like in *S. villosum* but shining dark with bend pedicels.

The leaves of *S. scabrum* were obtuse with undulate margins (Figure 4). The unripe berries were green with

translucent cuticles while the ripe berries were large, dark and having straight pedicels. The flowers had white petals and yellow stamens. On the other hand, there were other *S. scabrum* species from different locations which had straight pedicels with drooping fruits (Figure 5 and 6).

FIGURE 1. *SOLANUM VILLOSUM* FLOWERS, LEAVES, FLOWER BUDS AND FRUITS FROM BUNGOMA, KENYA

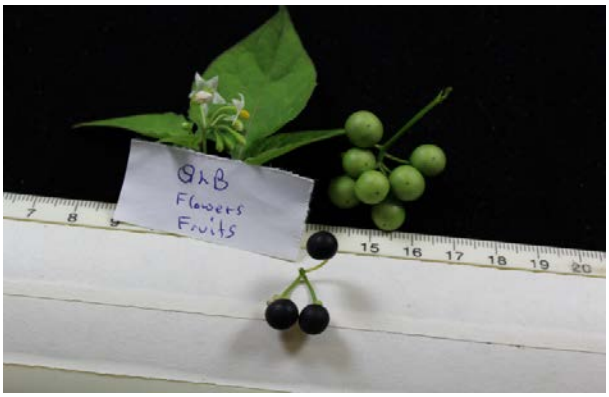

FIGURE 2. *SOLANUM NIGRUM* FLOWERS, FLOWER BUDS AND FRUITS FROM QUEDLINBURG, GERMANY

FIGURE 3. *SOLANUM NIGRUM* LEAVES, FLOWERS, AND FRUITS FROM BUNGOMA, KENYA

FIGURE 4. *SOLANUM SCABRUM* OF AHERO, KISUMA, KENYA

FIGURE 5. *SOLANUM SCABRUM* LEAVES, FLOWERS AND FRUITS FROM AMPATH, ELDORET, KENYA

FIGURE 6. *SOLANUM SCABRUM* FROM KABRAS, KAKAMEGA, KENYA

Stem form, leaf shape, flower color as well as fruit size and color were critical morphological characteristics that distinguished the species of the *Solanum nigrum* complex. According to Edmonds and Chweya (1997) *Solanum* species exhibit considerable genetic variation, both florally and vegetatively. This variation may occur in different populations of the same species, or even different infraspecific categories of a species. Sometimes, the character may be genetically controlled in one species, but phenotypically plastic in another. Edmonds and Chweya (1997) further indicated that in *Solanum* most common species in Africa are also found in Europe and Asia.

Morphologically, *S. villosum* showed greater divergence from the other accessions. *S. villosum*, is considered to be a Eurasian taxon (Edmonds and Chweya, 1997; Hunziker, 2001) and was most probably introduced to Uganda in recent years. Similarly, the greater divergence of *S. villosum* can be attributed to it being an introduction to Kenyan. *S. nigrum* and *S. scabrum* seemed to have speciated recently. The findings by Dehmer (2003) suggest a recent speciation of these two taxonomic units due to observed small genetic differences between them.

AFLP Analysis

The number of polymorphic DNA fragments detected by the individual primer combinations ranged from 8 (E-AAG/M-CAA) to 95 (E-AAG/M-CAG). In total 417 polymorphic bands were used for further analysis. All twelve *Solanum* accessions were clearly distinguishable from each other with different distance values (Table 2). By distance analysis were shown two main and well differentiated clusters within the *Solanum nigrum* complex (Figure 7). The first main cluster is composed of two indigenous Kenyan *S. villosum* accessions from Eldoret and Bungoma that tended to be the most divergent from the other accessions. The other main cluster is composed of both *S. nigrum and S. scabrum* accessions. In the second cluster indigenous *S. nigrum* from Bungoma formed a different sub-cluster from the other *S. nigrum* and *S. scabrum* accessions. *S. scabrum* accessions from Ahero differed from the other *S. scabrum* accessions. Also, *S. scabrum* tended to be more diverse within it by having more sub clusters. *S. nigrum* from Quedlinburg were closely related but slightly differed from the other *S. nigrum* accessions from Kenya. Overall, the neighbor joining dendrogram tended to show how the different *S. nigrum* accessions were related. These results seemed to be in concurrence with that of Olet *et al* (2011) that indicated that *S. villosum* was the most divergent accession compared to the other accessions. The data from dissimilarity analysis of the species and the morphological analysis seem to be consistent by indicating that *S. villosum* accessions were more divergent from the other accessions. This confirms reports by Edmonds and Chweya (1997) and Hunziker, (2001) that *S. villosum* is Eurasian taxon, and is an introduction to Uganda and probably Kenya, the two countries being close

neighbours. Further, genetic data show intermixing between *S. scabrum* and *S. nigrum* in some instances. Edmonds (1979) indicated that the intermixing of *Solanum* genotypes, irrespective of geographic locality points to the predominantly autogamous nature of members. *S. scabrum* subsp. *laevis* was previously considered to be *S. nigrum* (Olet *et al*., 2006). This probably explains the fact that genetic data of both *S. nigrum* and *S. scabrum* seemed to show overlap in some instances.

TABLE 2 DISSIMILARITY OF SPECIES AND ACCESSIONS OF *SOLANUM NIGRUM* COMPLEX FROM KENYA AND GERMANY

	acc.1	acc.2	acc.3	acc.4	acc.5	acc.6	acc.7	acc.8	acc.9	acc.10	acc.11	acc.12
acc.1	0,000											
acc.2	0,111	0,000										
acc.3	0,145	0,152	0,000									
acc.4	0,119	0,102	0,119	0,000								
acc.5	0,248	0,267	0,245	0,247	0,000							
acc.6	0,575	0,517	0,579	0,519	0,654	0,000						
acc.7	0,847	0,853	0,895	0,822	0,894	1,455	0,000					
acc.8	0,893	0,938	0,974	0,878	0,933	1,524	0,547	0,000				
acc.9	0,194	0,190	0,232	0,200	0,314	0,527	0,924	1,007	0,000			
acc.10	0,575	0,558	0,586	0,542	0,637	1,017	0,749	0,730	0,621	0,000		
acc.11	0,343	0,348	0,373	0,328	0,448	0,788	0,636	0,628	0,388	0,551	0,000	
acc.12	0,338	0,331	0,328	0,324	0,420	0,754	0,659	0,651	0,349	0,519	0,074	0,000

Key: Acc. 1 *S. scabrum* (Kakamega, Kabras); Acc. 2 *S. scabrum* (Bungoma); Acc. 3 *S. scabrum* (Kakamega, Butsotso); Acc. 4 *S. scabrum* (AMPATH); Acc. 5 *S. scabrum* (Eldoret); Acc. 6 *S. scabrum* (Kisumu, Ahero); Acc. 7 *S. villosum* (Eldoret); Acc. 8 *S. villosum* (Bungoma); Acc. 9 *S. nigrum* (Kakamega, Kabras); Acc. 10 *S. nigrum* (Bungoma); Acc. 11 *S. nigrum* (Germany, Quedlinburg); Acc. 12 *S. nigrum* (Germany, Quedlinburg).

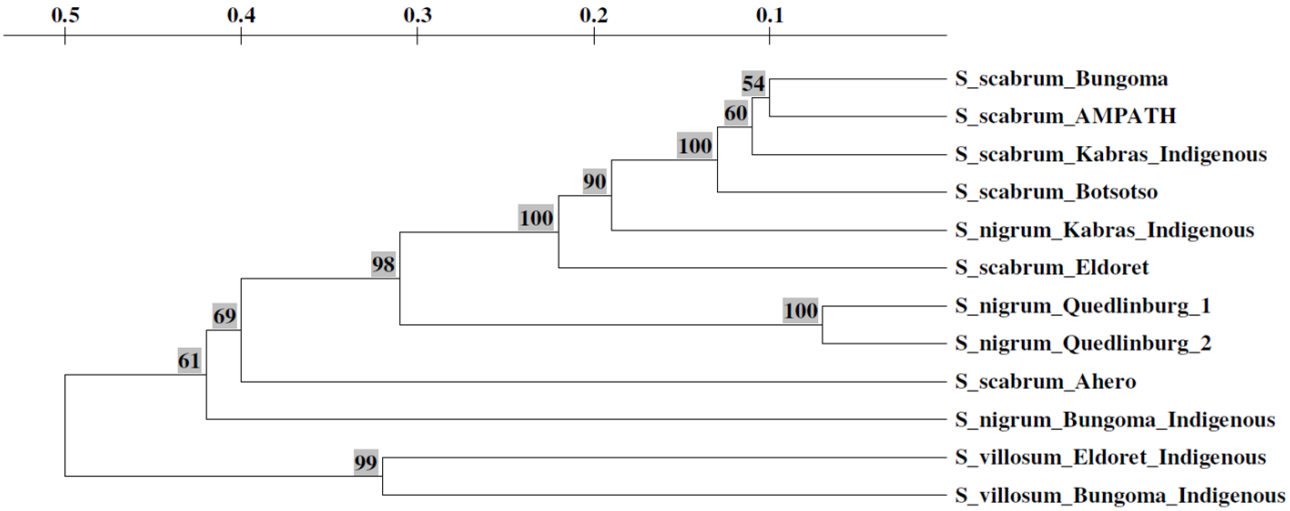

FIGURE 7. DISTANCE ANALYSIS OF 12 ACCESSIONS FROM *SOLANUM NIGRUM* COMPLEX

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Anti-oxidative Activity in Some Tropical Fruits during Refrigerated Storage and its Relationship to Cold Tolerance

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Abstract

Fruits contain compounds which are secondary metabolites with antioxidant activity that could be beneficial to cope with stress by cold. The aim of this work is to study the antioxidant capacity of papaya, mango, vinagrillo (Averrhoa bilimbi) and calamondin fruits and its relationship to cold tolerance during cold storage under refrigerated conditions at 4-6 °C and 90-95% RH. The activity and total antioxidant capacity were determined comparatively by three methods: ABTS (2, 2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), DPPH (1,1-diphenyl-2-picrylhydrazyl) and DMPD (N, N-dimethyl-p-phenylenediamine). Ascorbic acid was used as a reference antioxidant. The antioxidant capacity obtained by DPPH, ABTS and DMPD methods is correlated to cold tolerance in refrigerated fruits. Results showed a relationship between the antioxidant capacity and the days of symptom onset of chilling injury during cold storage. Vinagrillo and calamondin fruits with lower antioxidant capacity were more susceptible to cold in relation to papaya and mango fruits with higher one.

Keywords

Chilling Injury; Papaya; Mango; Averrhoa Bilimbi and Calamondin Fruits; Cold Storage

Introduction

To reduce post-harvest losses as a result of natural decay processes like respiration and transpiration, the fruits should be stored in appropriate conditions. One technique universally known to prolong post-harvest life of the fruit is cooling which the respiration rate of the fruit is reduced by the effect of low temperatures, affecting their metabolic activity. However, in the case of tropical fruits there is a limit in reducing the temperature of cold storage, due to the sensitivity of these fruits to chilling injury, altering its organoleptic characteristics and modifying its external appearance, making them undesirable for the consumer (Guadarrama, 2001). Most fruits originated from the tropical or subtropical regions are chilling sensitive. These crops are injured after a period of exposure to chilling temperatures below 10 to 15°C but above their freezing points (Gross et al., 2002).

It has been reported in some fruits that cold tolerance during refrigerated storage is related to its antioxidant ability measured by the activity of certain enzymes such as peroxidase, polyphenoloxidase, catalase and superoxide dismutase, which have the capacity to remove species reactive oxygen, which can cause oxidation of lipids, proteins and DNA, consequently resulting physiological disorders in the fruits (Omata et al., 2010). Furthermore, the antioxidant capacity of the fruit is related to the presence of bioactive compounds in fruits such as phenols, ascorbic acid, carotenoids, anthocyanins and among others. These antioxidants present in fruits help prevent some chronic diseases such as cancer, arthritis and heart disorders, since these compounds protect the cellular processes, both internal and external oxidative stress. The plant products such as fruits and vegetables, represent an important alternative as a potential source of antioxidants they contain a variety of highly active chemical compounds at low concentrations, mainly polyphenols such as flavonoids and carotenoids (anthocyanins, catechins, quercetinas, lutein, b-carotene, α-carotene and lycopene, among others (Huang and Prior, 2005).

Antioxidants are substances that lower the oxidation reactions caused by atmospheric oxygen in different biomolecules. Oxidation causes changes in quality attributes, decreasing the life of many horticultural products (Lim et al., 2007). Interest in the antioxidant properties of fruits has been increased. Some authors have assessed the

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capacity to remove free radicals and phenolic content of some tropical fruits like blackberry, mango, guava, passion fruit, strawberry, gooseberry and pineapple, among others (Sellappan et al., 2002; Heinonen et al., 1998; Halvorson et al., 2002 and Shivashankara et al., 2004). The most used methods are ABTS, DPPH and DMPD, which exhibit excellent stability under certain conditions, but also differences. The free radical DPPH is obtainable directly without preparation, while the ABTS has to be generated after a chemical reaction that can be (manganese dioxide and potassium persulfate), enzyme (peroxidase and myoglobin), or Electrochemical. With ABTS can measure the activity of compounds of hydrophilic and lipophilic nature, while the DPPH can only be dissolved in organic medium and the aqueous medium only DMPD. The radical ABTS • + also has the advantage that their spectrum shows absorbance maxima at 414, 654, 754 and 815 nm in an alcoholic medium, while the DPPH has an absorbance peak at 515 nm, and DMPD at 505 nm (Huang et al., 2005).

Currently the ABTS method has been widely used for both biological materials, pure compounds or plant extracts hydrophilic or lipophilic nature. ABTS chromogen compound presents blue / green with absorption maximum at 342nm, is highly water soluble and chemically stable. The ABTS • + radical once generated by enzymes (peroxidase for example) or chemically (manganese dioxide, potassium persulfate or ABAP [2,2'-azobis (2-amidinopropeno), passes forward new features with maxima absorption at 414, 645, 734 and 815 nm (Arnao, 2000 and Re et al., 1999).

Various physiological and biochemical alterations and cellular dysfunctions occur in chilling-sensitive species in response to chilling stress. These alterations include stimulation of ethylene production, increase in respiratory rate, interference in energy production, increase in activation energy, slowing of protoplasmic streaming, increase in permeability, reduction in photosynthesis, inactivation in enzyme activity, membrane dysfunction, and alteration of cellular structure. If chilling stress is prolonged, these alterations and dysfunctions will lead to the development of a variety of chilling injury symptoms such as surface lesions, internal discoloration, water-soaking of the tissue, off-flavor, decay and failure to ripen normally (Saltveit and Morris, 1990).

The aim of this work was evaluate the antioxidant capacity of some tropical fruits under refrigerated storage and its relationship to cold tolerance.

Materials and Methods

Sample Preparation

Fruits of different cultivars of mango (Mangifera indica) and papaya (Carica papaya), vinagrillo (Averrhoa bilimbi) and calamondin (Citrofortunella sp) were stored in refrigerated conditions (4-6 ° C and 90-95% RH). Five replications were used in different analyzes to be performed each five days for 20 days under refrigerated storage conditions mentioned above. Fruit samples to be analyzed for antioxidant activity were prepared using 50 g of pulp, which is homogenized with 100 ml of distilled water and then diluted with 100 ml of ethanol and centrifuged at 14,000 rpm for 15 min. The supernatant was used for subsequent analysis.

Methods

The activity and total antioxidant capacity are determined comparatively by three methods:

ABTS Method (3-Ethylbenzothiazoline-6-Sulfonic Acid)

According to the methodology developed by RE et al. and the radical ABTS •+ is obtained after reaction of ABTS (7 mM) with potassium persulphate (2.45 mM final concentration) and incubated at room temperature (± 25 ° C) and in the dark for 16 h. Once formed the radical ABTS • + is diluted with ethanol to obtain an absorbance ranging from 0.70 (± 0.1) at 754 nm (wavelength of maximum absorption). The filtered samples were diluted with ethanol. The absorbance was measured continuously after 7 minutes. Results are expressed in VCEAC (equivalent to vitamin C or ascorbic acid antioxidant activity).

DPPH Method (1, 1-Diphenyl-2-Picrylhydrazyl)

It is based on reducing the absorbance at 515 nm of DPPH • radical by antioxidants (Brand-Williams, 1995). DPPH radical • 100 uM (3.9 mL) dissolved in 80% methanol. 0.1 mL of sample or standard is added, the mixture was

thoroughly homogenized, and kept in the dark for 30 minutes. The absorbance measurements at 517 nm are performed before adding the sample after 30 and 60 minutes. • DPPH concentration in the reaction medium was calculated from a calibration curve obtained by linear regression. Results are expressed in VCEAC (equivalent to vitamin C or ascorbic acid antioxidant activity).

DMPD Method (N, N-Dimethyl-P-Phenylenediamine)

The antioxidant activity is determined by applying the proposed method by Fogliano et al., 1999. This is based on adding 1 mL of 100 mM DMPD solution to 100 mL of acetic acid buffered solution / 0.1 M sodium acetate (pH 5.25). After addition of 0.2 mL of a 0.05 M ferric chloride (0.1 mM final concentration) colored radical cations (DMPD •) are formed. One milliliter of this solution was transferred to a cuvette for absorbance measured, ranging from 0.90 (± 0.1) at 506 nm. 50 uL of a standard antioxidant solution or diluted samples were added and after ten minutes (at 25). Another measure of absorbance at 506 nm was made. Results were expressed in VCEAC equivalent to Vitamin C (mg / L or mg / 100 g) activity.

Results and Discussion

The Figures 1, 2 and 3 show antioxidant capacity of fruits by DPPH, ABTS and DMPD, respectively.

The antioxidant capacity obtained by and DPPH, ABTS and DMPD methods is correlated to cold tolerance in these fruits. Results showed a relationship between the antioxidant capacity and the onset day of symptom of chilling injury during cold storage. Vinagrillo and Calamondin fruits were most susceptible to cold (Table 1).

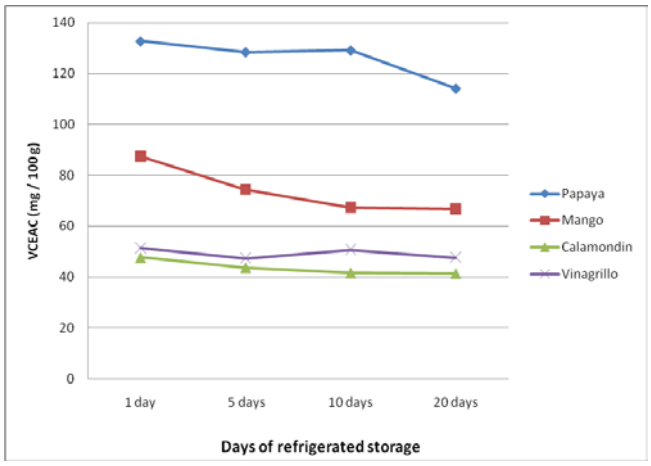

FIGURE 1. ANTIOXIDANT CAPACITY OF FRUITS, BY THE DPPH METHOD (EXPRESSED AS VCEAC (mg / 100 g) DURING 20 DAYS OF REFRIGERATED STORAGE (6 ^{0}C AND 90-95% RH)

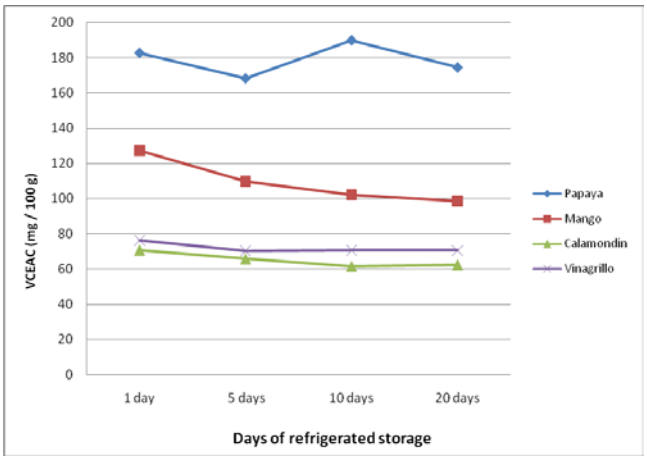

FIGURE 2. ANTIOXIDANT CAPACITY OF FRUITS, BY THE ABTS METHOD (EXPRESSED AS VCEAC (mg / 100 g) DURING 20 DAYS OF REFRIGERATED STORAGE (6 ^{0}C AND 90-95% RH)

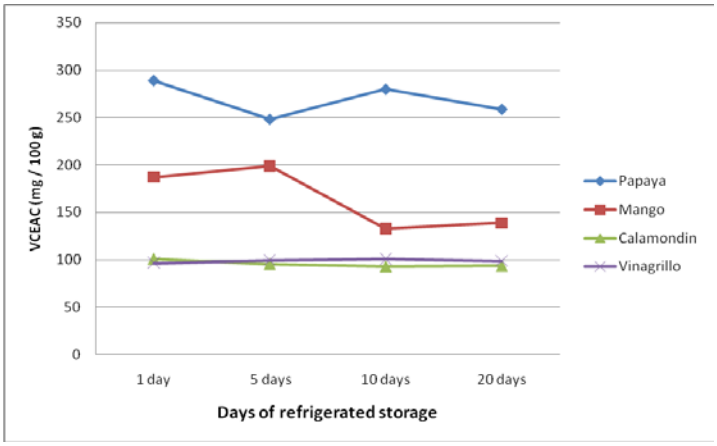

FIGURE 3. ANTIOXIDANT CAPACITY OF FRUITS, BY THE DMPD METHOD (EXPRESSED AS VCEAC (mg / 100 g) DURING 20 DAYS OF REFRIGERATED STORAGE (6 ^{0}C AND 90-95% RH)

TABLE 1. TIME OF ONSET OF THE FIRST SYMPTOMS OF CHILLING INJURY DURING REFRIGERATED STORAGE

Fruit	Main external symptom of chilling injury	Onset time of symptom (Days)
Papaya	Darkening of skin	14-15
Mango	Epidermal depressions	11-12
Calamondin	Darkening of skin	08-09
Vinagrillo	Opaque epidermis	06-07

Cold induces numerous physiological and biochemical alterations at cellular level: it stimulates the respiratory rate, alters the production of energy, increases membrane permeability, inactivates some enzymes and alters the cell structure (Inze and Van Montagu, 1995). In abiotic stress conditions, reactive oxygen species (ROS) are generated by metabolic processes, acting as change signals that regulate the expression of genes (Pei et al., 2000; Desikan et al., 2004; Pastori and Foyer, 2002; Mittler et al., 2004 and Shin and Schachtman., 2004) and ion channel activity (Foreman et al., 2003). In higher plants, excessive production of ROS is an intrinsic feature of a stressed metabolism under various types of stress. Response mechanisms to stress are complemented by the production of antioxidant enzymes. These are a group involved in the elimination of ROS. The main anti-stress enzymes are: superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR). Insufficient elimination of ROS produces oxidative stress, which is characterized by harmful reactions of ROS with biologically important macromolecules such as proteins, lipids and DNA, and this can cause cell damage (Inze and Van Montagu, 1995).

The accumulation of certain metabolites and changes in the activity of antioxidant enzymes such as catalase and peroxidase may be related to the potential of a plant to counteract the harmful effect of abiotic stress in different physiological and morphological processes. Vega-Garcia et al. (2010) looked at changes in protein expression associated with chilling injury in tomato fruit. The proteins identified were involved in carbon metabolism, oxidative stress, photosynthesis, protein processing, and degradation, and two protein (thioredoxin peroxidase and glycine-rich RNA binding protein) were related to cold stress. The mechanisms which these proteins operate and how any enzyme performs under chilled storage show that there are several mechanisms needed to maintain cellular homeostasis under cold stress. Variation in the proteins involved in these pathways could provide insights into why one variety is more cold tolerant than another. Characterization of these cold-stress-associated pathways and the proteins involved can contribute to the identification of potential markers to detect the disorder at early stages of chilling injury.

The most common of chilling injury in papaya fruit is darkening and have small areas of subsidence shell symptoms. In addition, the fruit ripening is delayed and have a blemish color (Couey, 1982). Analysis of subjective color and photographic records indicates that there has been an incidence of chilling injury expressively in the face of the fruit that remained exposed to sunlight in the field during ontogeny, which are like those found by Silva et al. (2003). According to these authors, the exposure to determined papaya region in the sun, contributed to the emergence of the incidence of chilling injury. The collapse of small areas papaya peel was after eighteen days of cold storage at 6°C observed, coinciding with a flesh firmness of the downward trend. These results can be attributed to an increase in polygalacturonase enzyme activity, which hydrolyzes components of the cell wall such as pectin substances, responsible for firmness (Karakurt and Huber, 2002).

Antioxidant molecules and enzymes protect cellular membranes and organelles from the damaging effects of reactive oxygen species (ROS), which are formed both during normal cellular metabolism and unwanted environmental conditions. Among the non-enzymatic antioxidants, which are generally small molecules, ascorbic acid and flavonoids are widely distributed in plant cells, playing important role in the destruction of ROS. The enzymatic system involves a wide range of enzymes such as superoxide dismutase, catalase and ascorbate peroxidase which play an important role in formation and degradation of ROS. A high reduced/oxidized ratio of ascorbate forms seems to be vital for the efficient detoxification of ROS and for the acclimation of plants to environmental condition and/or for the enhancement of resistance to biotic and abiotic environmental stresses (Lee and Kader, 2000).

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