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Full Length Research Paper

Evaluation of insolvency in mutual credit unions by the models of artificial neural networks and support vector machines

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This study aimed to develop and compare statistical models using the techniques of artificial neural networks (ANNs) and support vector machines (SVMs) to investigate which one offers the best results in evaluating insolvency of mutual credit unions. The information required to build the models were obtained with a sample of 62 credit unions (31 solvent and 31 insolvent) to which financial indicators of the PEARLS (Protection, Effective financial structure, Asset quality, Rates of return and cost, Liquidity and Signs of growth) system were calculated. The RBF network, multilayer perceptron, multilayer perceptronCS and LibSVM algorithms were used to obtain the ANNs and SVMs; for each algorithm, the ANNs were built with three groups of indicators (27, 11 and 10 indicators). This is the first study done with ANNs in Brazilian credit unions. When analyzing the results of ANNs and SVMs, the superiority of the SVMs as binary classifier for evaluating insolvency was evidenced, since its LibSVM algorithm showed the best results in all assessments of performance proposed in this study. The only LibSVM indicator with performance inferior to ANNs was the error rate of the negative class which indicates those negative class data that were classified incorrectly.

Key words: Insolvency, credit unions, artificial neural networks, support vector machines.

INTRODUCTION

Cooperatives are currently an alternative means to make credit accessible and include people who are at the boundaries of the National Financial System in the financial market; especially to benefit those small urban

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and rural entrepreneurs by raising funds at lower interest rates than the average rates charged on the market.

According to Bressan et al. (2011), one of the biggest challenges of the credit union is to build management models adjusted to the particularities of the business segment meeting all requirements and doctrinal principles of the regulatory agency, in this case of Central Bank of Brazil (BACEN).

Studies making use of well-designed statistics to predict insolvency in cooperatives have been performed long ago. Initially, univariate techniques were applied in this type of study, and then, multivariate analyzes appeared bringing more suitable solutions. Currently, techniques that make use of artificial intelligence and need a great capacity for computational processing have been largely adopted (Azmathullah et al., 2005; 2006; 2008; Hsiao and Whang, 2009; Jardin, 2010; Azmathullah and Wu, 2011; Karra and Krichene, 2012).

By observing this progress, we see continuous improvements in the results achieved on this theme. Today, it is possible to affirm that the financial statements of the cooperatives when properly analyzed are sources of information for predicting insolvency more accurately (Hsiao and Whang, 2009).

Quantitative models for insolvency prediction may be built from financial indicators obtained from the financial statements of a sample of solvent or insolvent cooperatives. Statistical models work to distinguish the characteristics of solvent and insolvent cooperatives. The final result of this processing is a mathematical formula that predicts the future situation of a cooperative within a certain margin of error.

This study aimed to evaluate the state of insolvency of the mutual credit unions in the Paraná State (Brazil) by the methods of artificial neural networks (ANNs) and support vector machines (SVMs). This is the first study done with ANNs in Brazilian credit unions.

Theoretical backgrounds

Bressan et al. (2004a) consider that any method to evaluate the risks of bankruptcy or insolvency of a company should meet the following stages: i) obtaining a sample containing solvent and insolvent companies; ii) choosing variables from financial statements to indicate company insolvency which will be called predictor variables; iii) choosing a mathematical or statistical model to fit the variables obtained from financial statements; and iv) validating the model in order to check the model ability to discriminate categorical variables.

In Brazil, most studies on models for insolvency prediction have proposed to analyze the model variables. Normally, it is done based on a statistical technique where the researchers aim to find a set of appropriate financial indicators to predict accurately the company's financial health in a given period in the future (Wu et al., 2007; Fernandez et al., 2013). The statistical modeling commonly used with this purpose is the discriminant analysis. On the same line, the insolvency models have showed increasingly relevance in financial analysis mainly by taking into account the credit and life insures risks (Wu et al., 2007; Hsiao and Whang, 2009; Jardin, 2010; Ribeiro et al., 2012).

With respect to credit unions, Table 1 shows logistic regression analysis performed in four works, the Cox proportional-hazard regressions applied in three works, and discrimant analysis in one work (Araújo, 2011). Note that no study with ANNs was found within the analyzed period for Brazilian credit unions.

With respect to studies on insolvency of credit unions, three studies are worth mentioning. The first by Bressan et al. (2004a) describes an economic-financial evaluation of rural credit cooperatives in the Minas Gerais State (Brazil) made by logistic regression on a 1998 to 2001 sample of the rural credit cooperatives of Minas Gerais. The second, also by Bressan et al. (2004b), evaluates the insolvency of rural credit cooperatives of the Crediminas system by using the Cox proportional-hazard model. The third work, developed by Bressan (2009; 2011), uses PEARLS (Protection; Effective Financial Structure; Assets Quality; Rates of Return and Costs; Liquidity; and Signs of Growth) indicators with Logit Model to predict the possibility of insolvency of credit unions that participate in the SICOOB-Brasil and SICCOB-Crediminas systems.

According to Bressan (2009, 2011), the PEARLS system is one acronym of a group of indicators used by the Word Council of Credit Unions (WOCCU) (2002) since 1990, which originates from the following key-operating areas of the credit union.

Monitoring the credit union performance is the main objective of the PEARLS system. It was designed to go beyond the identification issue, helping managers to find significant solutions to solve institutional problems. The PEARLS system can identify if a credit union is based on weak capital and indicate the causes of such weakness. This system allows managers to identify problems and solutions quickly and accurately, and the best actions to be taken before the problems becoming serious.

Another objective of the PEARLS system is to standardize financial indicators and formulas to eliminate criteria used by local credit unions to evaluate operations. The system also creates a universal financial language to evaluate credit unions worldwide – with an easy-access language that can improve the communication and information uniformity (Richardson, 2002).

The PEARLS system is adopted by about 97 countries in Africa, Asia, Caribe, Europe, North America, Latin America and Oceania. However, it is not used to analyze credit unions in Brazil (Bressan et al., 2011).

Bressan et al. (2011) have created 39 financial indicators from the PEARLS system to evaluate the Brazilian credit unions. These indicators have enabled

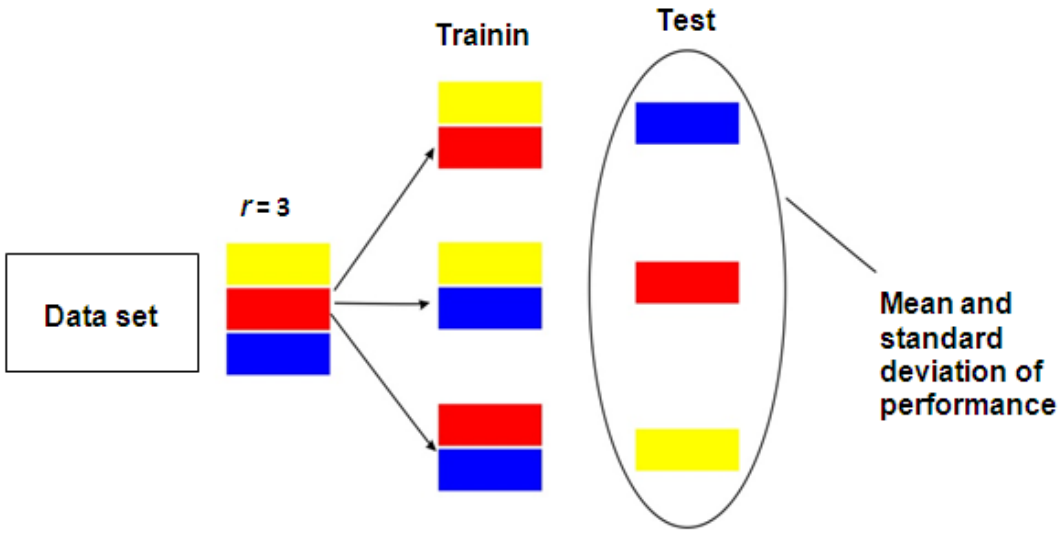

Figure 1. Cross Validation Method. Source: Carvalho (2011).

comparisons and financial analysis among credit unions in Brazil and abroad (Karaa and Krichene, 2012).

The indicators were grouped into key-operating areas of the credit unions and linked in the *Plano Contábil das Instituições Financeiras do Sistema Financeiro Nacional – COSIF* (Accounting Plan of National Institutions of the Financial System) (Bressan et al., 2010).

METHODS

Data gathering was made based on information from financial statements of a sample of 62 credit unions from which 31 are solvent and the other 31 are insolvent. The cooperatives that did not send their financial statements to BACEN in a period of at least 10 years were considered insolvent since it characterizes an operational disruption.

According to Araújo (2011), this concept suggests that interrupting the sending of financial statements implies lack of interest of the cooperative managers in being accountable to the regulator, and this usually occurs before the formal closure of the cooperative – a typical profile of insolvency.

The evaluation of insolvency of cooperatives was based on the financial indicators of the PERALS system, as mentioned in the theoretical background of this study. Twenty-seven indicators were selected from 39 proposed by the PEARLS system according to the availability of information on the financial statements prepared and provided by the BACEN website.

To obtain the best model of data classification by artificial neural networks (ANNs) and support vector machines (SVMs), the re-sampling technique by the cross validation method was used (Figure 1) (Carvalho, 2011) together with the evaluation of the generated models by visualizing points in two-dimensional space (*x, y*) on receiver operating characteristics (ROC) curve (Figure 2) (Kohonen, 1988).

In the *r - fold cross-validation* method, the data set is partitioned into *r* subsets of similar sizes. The objects of *r-1* partitions are used for training a predictor variable which is tested in the remaining partitions. This process is repeated for *r* times, each cycle with a different partition to be tested. The final performance is taken from the averaged performance observed in each subset of test (Carvalho, 2011).

The re-sampling is indicated when the sample is not representing the population well and then an estimate closer to the study population is needed. The *k - fold cross-validation* consists of randomly selecting a group of data extracted from the dataset to train and test predictors. The iteration was made 10 folds changing the number of test samples and tests, but keeping the same training dataset (Han and Kamber, 2006).

In addition to compute algorithms by the ANNs and SVMs, a ROC curve was also designed. According to Han and Kamber (2006), it is important to validate the results in order to quantify the discriminating power for prediction and identify the accuracy of a method or procedure in certain analysis. However, it should be considered that only a single quantification of “misses and hits” in a given test group is not necessarily reflecting the efficiency of the process. The quantification is also highly depending on the quality of data distribution through the test group. On the ROC curve, a perfect classifier is showed by a horizontal line on the top of the graph. However, it would be hardly achieved. ROC curves are considered good when positioned between the diagonal and the perfect lines, that is, the farther from the diagonal line, the better the algorithm.

According to Carvalho (2011), a classification conflict in the two classes, one positive (+) and the other negative (-), generates a confusion matrix as shown in Table 2, where: TP = the number of true positives, which is the positive data correctly classified; TN = the number of true negatives, which is the negative data correctly classified; FP = the number of false positives, data whose the class is negative but were incorrectly classified as positive; and FN = the number of false negatives, data whose the class is positive but were incorrectly classified as negative.

According to Monard and Baranauskas (2003), it is possible to calculate other performance measures from the confusion matrix:

a) Error rate in the positive class: The proportion of positive class data that were incorrectly classified by the predictor $\hat{f}$ also known as false negative rate (FNR):

$$(\hat{f}) = \frac{\mathrm{FN}}{\mathrm{TP}+\mathrm{TN}}$$

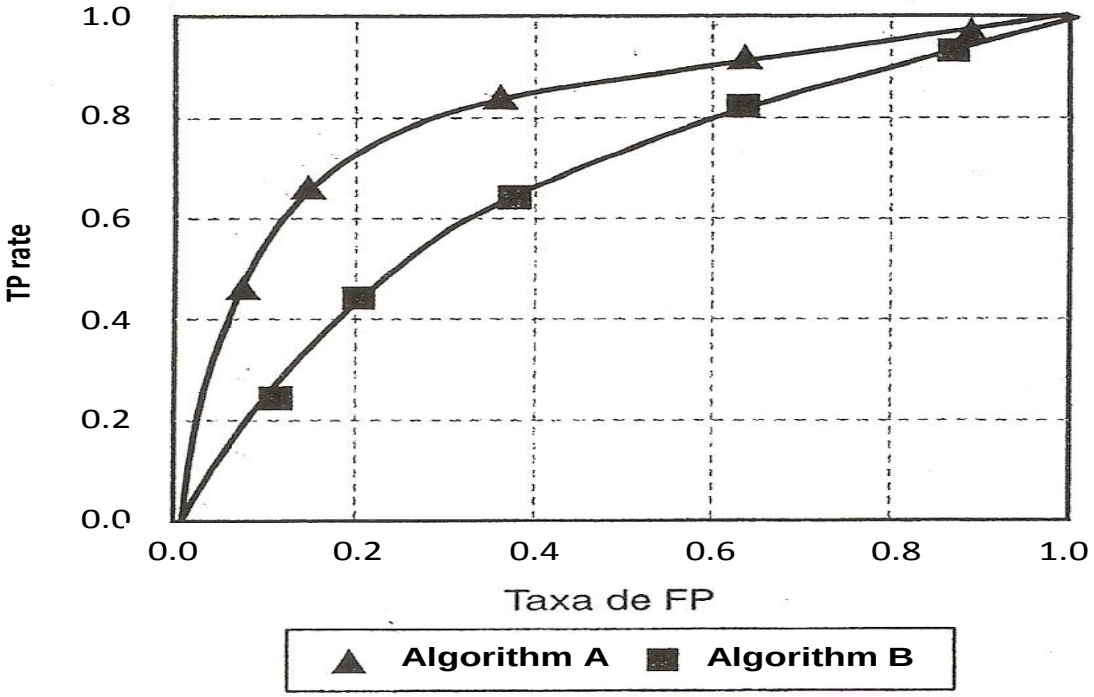

Figure 2. Example of a receiver operating characteristics (ROC) curve. Source: Carvalho (2011).

Table 1. Studies on credit union in solvency in Brazil.

Method	References
Logistic regression and cox proportional hazard	Bressan (2002)
Discriminant analysis	Pinheiro (2003)
Logistic regression and cox proportional hazard	Bressan et al. (2004a)
Cox proportional hazard	Bressan et al. (2004b)
Cox proportional hazard	Ribeiro (2008)
Logit model	Bressan (2009)
Logistic regression	Ferreira (2010)

Source: Araujo (2011).

b) Error rate in the err_+ negative class: Proportion of negative class data incorrectly classified by $\hat{f}$, also known as false positive rate (FPR): $err_{-}\ (\hat{f}) = \frac{\text{FP}}{\text{FP+TN}}$,

c) Total error rate: Given by the sum of the values of the secondary diagonal of the matrix divided by the sum of values of all components of the matrix: $err\ (\hat{f}) = \frac{\text{FP+FN}}{n}$,

d) Precision rate or overall precision: Obtained by adding the values of the main diagonal of the matrix divided by the sum of values of all components of the matrix: $prec_{-}\ (\hat{f}) = \frac{\text{TP+TN}}{n}$,

e) Precision: Proportion of positive data correctly classified from those predicted as positive by $\hat{f}$:
$prec\ (\hat{f}) = \frac{\text{TP}}{\text{TP+FP}}$,

f) Sensibility or recall: It is the hit rate in the positive class also called the true positive rate (TPR): $sens(\hat{f}) = rec(\hat{f}) = \text{TPR}(\hat{f}) = \frac{\text{TP}}{\text{TP+FN}}$,

g) Specificity: It is the hit rate in the negative class. The complement corresponds to the TFP rate: $spec\ (\hat{f}) = \frac{\text{TN}}{\text{TN+FP}} = 1 - \text{TFP}(\hat{f})$,

The accuracy can be considered as one measure of the model, and the sensitivity or recall as measures of its completeness. The precision and recall are not analyzed separately, but combined into a single measure, which is the weighted harmonic mean of precision and recall (Tafner, 1998):

$$F_1\ (\hat{f}) = \frac{2 \text{ x prec } (\hat{f}) \text{ x rec } (\hat{f})}{\text{prec } (\hat{f}) + rec\ (\hat{f})}.$$

The ROC curve is a bi-dimensional graph plotted in a called ROC dimension, with (*x,y*) axes representing the rates of false positives (FPR; x) and of true positives (TPR; y). The performance of a

Table 2. Confusion matrix.

Predicted value (obtained by test)	True value (proved by test)	
	Positive	**Negative**
Positive	TP – True positive	FP – False positive
Negative	FN – False negative	TN – True negative

Source: Carvalho (2011).

classifier can be plotted to form the curve and represented by a dimensional point (*x, y*) in the ROC dimension (Carvalho, 2011).

In Figure 1, Carvalho (2011) showed a perfect classification by the point (0, 1) where all positive and negative data are correctly classified, and then it is called ROC heaven. Conversely, the point (1, 0) represents the ROC hell. The point (1, 1) are the classifications always positive and the point (0, 0), those always negative. A classifier is considered superior to others when its point is positioned above and to the left of the points of the others classifiers. Given the above, it is common to compare the performance of algorithms in terms of a single measure extracted from the ROC curve: the area under the ROC curve. The area of the ROC curve produces values between 0 and 1; values closer to 1 are considered best, but it is still advisable to calculate the full ROC area through a cross-validation procedure.

The ROC graph used in this study holds the false positives rate (FP Rate) on the horizontal axis (x) and the true positives rate (TP Rate) on the vertical axis (y). The evaluation criterion for a good model of classification is to find the highest true positives rates and the lowest false positive rates in a 0-1 scale. The optimal model is the one that had the ability to hit all data classification which means 100% true positives and 0% false positive (Tahin, 2010).

The ANNs algorithms used in this study were the radial basis function – RBF network, and the multilayers: Multilayer Perceptron and the multilayer perceptronCS (Karaa and Krichene, 2012; Fernandez et al., 2013) and the SVM algorithm selected was the LibSVM (Chang and Lin, 2011). All algorithms in this study belong to the Weka software, which is largely used in data mining and machine learning (Karaa and Krichene, 2012).

The LibSVM is a library of SVM implementations developed by Chang and Lin for several purposes: classification, regression and distribution estimation. The version 3 was used in present study (Chang and Lin, 2011).

The information obtained in the decision tree was also used to build the ANNs. Three ANNs were built, the first with 27 indicators selected from a total of 39 proposed by the PEARLS system; the second was built with 10 indicators selected by market analysts as suitable to analyze the insolvency of credit unions; and the third was made with the R13 indicator from the second neural network added to those 10 indicators, which showed the best performance in building the decision tree.

Eleven PEARLS indicators selected by market analysts as the most adequate to analyze insolvency in credit unions are presented in Table 3. They are: one for protection (P), one for effective financial structure (E), one for asset quality (A), three for rates of return and cost, one for liquidity (L) and four for signals of growth (S).

RESULTS

As previously described, the ANNs were built with 3 algorithms: RBF network, Multilayer perceptron and multilayer perceptronCS. For each algorithm, three ANNs were built: the first with 27 indicators, the second with 10 indicators, and the third with the R13 indicator extracted from the second neural network and added to the group of 10 indicators. For the SVMs model only the LibSVM algorithm was used.

The ANN with RBFNetwork algorithm and 27 indicators

The ANN with 27 indicators and RBF network algorithm obtained a 0.677 Kappa statistic showing that 83.87% of total credit unions were correctly classified by the ANN, while 16.13% were not (Table 4). That means ANN has classified 27 credit unions correctly and 4 incorrectly from the set of 31 solvent credit unions. From the insolvent set, the ANN has classified 25 credit unions correctly and 6 incorrectly (Table 5).

The ANN with multilyer perceptron and multilayer perceptronCS algorithms and 27 indicators

The ANN with multilyer perceptron and multilayer perceptronCS algorithms and 27 indicators had a 0.839 Kappa statistic showing that 91.94% of total credit unions were correctly classified while 8.06% were not (Table 4). That means ANN has classified 27 credit unions correctly and 4 incorrectly from the set of 31 solvent credit unions. From the insolvent set, the ANN classified 30 credit unions correctly and only 1 incorrectly (Table 5).

The SVM with LibSVM algorithm and 27 indicators

The SVM with LibSVM algorithm and 27 indicators obtained a 0.903 Kappa statistic indicating that 95.16% of total credit unions were correctly classified by the SVM, while 4.84% were not (Table 4). The algorithm has classified all the 31 solvent credit unions correctly. From the insolvent set, the SVM classified 28 credit unions correctly and 3 incorrectly (Table 5).

The ANN with RBF network algorithm and 11 indicators

The ANN with RBF network algorithm and 11 indicators had a 0.774 Kappa statistic implying that 88.71% of total credit unions were correctly classified, while 11.29% were

Table 3. Indicators selected by market analysts.

Indicator	Purpose
P1 = Allowance for loan and lease losses / Total Portfolio classified	Measure the amount of allowance for loan and lease losses relative to the total portfolio classified.
E5 = Revenues from financial intermediation / Total Average Assets	Measure the proportion of income from financial intermediation relative to total adjusted assets.
A1 = Fixed assets + Assets not intended to the purpose of cooperative / Adjusted equity	Measure the degree of utilization of own funds with fixed assets and assets not directed to the purpose of the cooperative. The higher the value, the less the focus of the institution on its core business.
R7 = Leftovers / Average Total Assets.	Measure the extent of earnings and also the ability to build social capital. This is an indicator of return on assets.
R8 = Leftovers / Average Adjusted Equity	Measuring the return on equity capital. This is an indicator of return on equity.
R13 = Administrative Costs / Average Total Assets	Measure the percentage of administrative expenses in relation to total assets.
L2 = Short-term Assets / Total Deposits	This indicator is a *proxy* for the liquidity flow.
S1 = Operating Income Growth = (Operating income of the current month / operating income of the previous month) - 1	Measure the rate of growth in operating income.
S6 = Administrative Costs Growth = (administrative cost of the current month / administrative cost of the previous month) -1	Measure the rate of growth of administrative expenses.
S8 = Total Assets Growth = (Total Assets of the current month / total assets of the previous month) -1	Measure the rate of growth of total assets.
S9 = Loan Operations Growth = (Loans of this month / Loans from the previous month) -1	Measure the monthly increase of investments in loans. The higher the index, the more the institution is expanding loan operations.

not (Table 6). The ANN has classified 27 credit unions correctly and 4 incorrectly, from the set of 31 solvent credit unions. From the insolvent set, the ANN classified 28 credit unions correctly and 3 incorrectly (Table 7).

The ANN with multilyer perceptron and multilayer perceptronCS algorithms and 11 indicators

The ANN with multilyer perceptron and multilayer perceptronCS algorithms and 11 indicators had a 0.710 Kappa statistic showing that 85.48% of total credit unions were correctly classified while 14.52% were not (Table 6). The ANN has classified 30 credit unions correctly and only 1 incorrectly, from the set of 31 solvent credit unions. From the insolvent set, ANN also classified 30 credit unions correctly and 1 incorrectly (Table 7).

The SVM with LibSVM algorithm and 11 indicators

The SVM with LibSVM algorithm and 11 indicators had a 0.968 Kappa statistic indicating that 98.39% of total credit unions were correctly classified while 1.61% was not (Table 6). SVM has classified all the 31 solvent credit unions correctly. From the insolvent set, ANN classified 30 credit unions correctly and only 1 incorrectly (Table 7).

The ANN with RBF network algorithm and 10 indicators

The ANN with RBF network algorithm and 10 indicators had a 0.742 Kappa statistic showing that 87.09% of total credit unions were correctly classified, while 12.91% were not (Table 8). That means, ANN has classified 26 credit

Table 4. Summary of the RBF Network, the Multilyer Perceptron and Multilayer PerceptronCS, and the LibSVM algorithms with 27 indicators.

RBF network	Total credit unions	
	No.	%
Correctly classified instances	52	83.87
Incorrectly classified instances	10	16.13
Kappa statistic	0.677	
Multilyer perceptron and multilayer perceptronCS		
Correctly classified instances	57	91.94
Incorrectly classified instances	5	8.06
Kappa statistic	0.839	
LibSVM		
Correctly classified instances	59	95.16
Incorrectly classified instances	3	4.84
Kappa statistic	0.903	

Table 5. The confusion matrix for the RBF network, the multilyer perceptron and the multilayer perceptronCS, and LibSVM algorithms with 27 indicators. Classified as a = INSOLVENT and b = SOLVENT.

Classified as	a	b	<-- classified as
RBF network	27	4	| a = INSOLVENT
	6	25	| b = SOLVENT
Multilyer perceptron and multilayer perceptronCS	27	4	| a = INSOLVENT
	1	30	| b = SOLVENT
LibSVM	31	0	| a = INSOLVENT
	3	28	| b = SOLVENT

unions correctly and 5 incorrectly, from the set of 31 solvent credit unions. From the insolvent set, ANN classified 28 credit unions correctly and 3 incorrectly (Table 9).

The ANN with multilyer perceptron and multilayer perceptronCS algorithms and 10 indicators

The ANN with multilyer perceptron and multilayer perceptronCS algorithms and 10 indicators obtained a 0.710 Kappa statistic indicating that 85.48% of total credit unions were correctly classified while 14.52% were not (Table 8). The ANN has classified 23 credit unions correctly and 8 incorrectly, from the set of 31 solvent credit unions. From the insolvent set, ANN classified 30 credit unions correctly and only 1 incorrectly (Table 9).

The SVM with LibSVM algorithm and 10 indicators

The ANN with LibSVM algorithm and 10 indicators had a 0.968 Kappa statistics indicating that 98.39% of total credit unions were correctly classified, while 1.61% were not (Table 8). SVM has classified all 31 solvent credit unions correctly. From the insolvent set, SVM classified 30 credit unions correctly and only 1 incorrectly (Table 9). According to Table 10, the LibSVM showed superior performance for the error rate of the positive class (amount of false negatives). The same performance is observed when using 10 or 11 indicators, that is, the number of hits within this class does not increase with the presence of the R13 indicator.

The error rate of the negative class which is the portion of the negative class data that were incorrectly classified (amount of false positives) (Table 10). Based on these results, the ANN with the multilayer perceptron and multilayer perceptron algorithms presented superior performance for the three groups of indicators. LibSVM presented the same performance as the multilayer perceptron and multilayer perceptron algorithms for either 10 or 11 indicators (Table 10). Again, the presence of the R13 indicator did not interfere on the evaluation of

Table 6. Summary of the RBF network, the multilyer perceptron and multilayer perceptronCS, and the LibSVM algorithms with 11 indicators.

RBF network	Total credit unions	
	No.	%
Correctly classified instances	55	88.71
Incorrectly classified instances	7	11.29
Kappa statistic	0.774	
Multilyer perceptron and multilayer perceptronCS		
Correctly classified instances	53	85.48
Incorrectly classified instances	9	14.52
Kappa statistic	0.710	
LibSVM		
Correctly classified instances	61	98.39
Incorrectly classified instances	1	1.61
Kappa statistic	0.968	

Table 7. The Confusion Matrix for the RBFNetwork, the Multilyer Perceptron and the Multilayer PerceptronCS, and LibSVM algorithms with 11 indicators. Classified as a = INSOLVENT and b = SOLVENT.

Classified as	a	b	<-- classified as
RBF network	27	4	| a = INSOLVENT
	3	28	| b = SOLVENT
Multilyer perceptron and multilayer perceptronCS	23	8	| a = INSOLVENT
	1	30	| b = SOLVENT
LibSVM	31	0	| a = INSOLVENT
	1	30	| b = SOLVENT

Table 8. Summary of the RBFNetwork, the Multilyer Perceptron and Multilayer PerceptronCS, and the LibSVM algorithms with 10 indicators.

RBF network	Total credit unions	
	No.	%
Correctly classified instances	54	87.09%
Incorrectly classified instances	8	12.91%
Kappa statistic	0.742	
Multilyer perceptron and multilayer perceptronCS		
Correctly classified instances	53	85.48%
Incorrectly classified instances	9	14.52%
Kappa statistic	0.710	
LibSVM		
Correctly classified instances	61	98.39%
Incorrectly classified instances	1	1.61%
Kappa statistic	0.968	

insolvency of the credit unions sampled for this study. Table 11 shows the precision rates for the built models. The LibSVM algorithm showed the best results for the three groups of indicators, without difference in precision between the groups with 10 and 11 indicators.

The sensibility of algorithms used for building the models proposed in this study is displayed in Table 11. According to these results, the LibSVM algorithm was superior to others for the three groups of indicators with higher values for the groups of 10 and 11 indicators.

Table 9. The Confusion Matrix for the RBF network, the multilyer perceptron and the multilayer perceptronCS, and LibSVM algorithms with 10 indicators. Classified as a = INSOLVENT and b = SOLVENT.

Classified as	a	b	<-- classified as
RBF network	26	5	| a = INSOLVENT
	3	28	| b = SOLVENT
Multilyer perceptron and multilayer perceptronCS	23	8	| a = INSOLVENT
	1	30	| b = SOLVENT
LibSVM	31	0	| a = INSOLVENT
	1	30	| b = SOLVENT

Table 10. Mean values of TP and FP rates of solvent and insolvent credit unions by the ANN and the LibSVM algorithms.

Indicator	RBF network	Multilayer perceptron and multilayer perceptronCS	LibSVM
	TP rates		
27	0.839	0.919	0.952
11	0.887	0.855	0.984
10	0.871	0.855	0.984
	FP rates		
27	0.194	0.032	0.097
11	0.097	0.032	0.032
10	0.097	0.032	0.032

Table 11. Mean precision, recall, F – Measure (the weighted harmonic mean of precision and recall) and mean ROC area for insolvent and solvent credit unions by the ANN and the LibSVM algorithms.

Indicator	RBF network	Multilayer perceptron and multilayer perceptronCS	LibSVM
	Mean precision		
27	0.841	0.923	0.956
11	0.888	0.874	0.984
10	0.873	0.874	0.984
	Mean recall		
27	0.839	0.919	0.952
11	0.887	0.855	0.984
10	0.871	0.855	0.984
	F – Measure		
27	0.839	0.919	0.951
11	0.887	0.853	0.984
10	0.871	0.853	0.984
	Mean ROC area		
27	0.869	0.947	0.952
11	0.879	0.870	0.984
10	0.889	0.879	0.984

As previously described in methods, precision and sensibility should not be analyzed separately, but jointly by the F- Measure (the weighted harmonic mean of precision and recall). As shown in Table 11, the LibSVM algorithm presented the highest values for the three groups of indicators with superior result when working with 10 or 11 indicators instead of 27 indicators.

The ROC area as evidenced by the ROC graph analysis is larger by the LibSVM algorithm for the three groups of indicators (Table 11). The groups containing either 10 or 11 indicators showed the highest LibSVM value.

DISCUSSION

SVM model was included in this work because currently is one of the machine-learning algorithms most used to evaluate binary systems suitably. The algorithms of the ANNs and SVMs were used to build models of classification suited to select one financial standard for identifying status of insolvency of credit unions, bank and life insures (Hsiao and Whang, 2009; Karra and Krichene, 2012; Ribeiro et al., 2012). To this end, we used some indicators proposed by the PEARLS system, which were grouped into 3 sets of 27, 11 and 10 indicators previously defined.

Comparing the results of ANNs and SVMs, it is evident that superiority of the SVMs as binary classifier of solvency since its LibSVM algorithm showed the best results in all evaluations of performance proposed in this study, except for the error rate of the negative class which indicates the incorrect classifications in the negative class.

From the ROC curve, it was possible to observe the highest TP and FP rates for the ANNs algorithms, which resulted in more liberal models. On the other hand, the LibSVM algorithm generated more conservative models since it showed good performance with respect to the FP rates, but few high TP rates. The performance of the ANNs by the multilayer perceptron, multilayer perceptronCS and the RBF network algorithms in classifying data was inferior to the LibSVM.

Even though only with a single ANN algorithm, the performance would be better likely by classifying a new credit union as true positive (INSOLVENT). It can be deduced just by observing separately the performance curves in the ROC graph.

It is still early to say that the ANNs and the SVMs algorithms can replace other methods for evaluating insolvency. However, it can be still helpful as tools of support for detecting signs and risks of imminent insolvency in credit unions and banks (Karaa and Krichene, 2012).

With respect to the number of indicators of the PEARLS system, appropriate to evaluate insolvency of credit unions, there is no need to use all those 39 indicators previously proposed. It can be confirmed by the same high efficiency of the group with 10 indicators elected by market analysts for this evaluation. And still, adding the R13 indicator, prominently in the decision tree, to the group of the 10 indicators did not alter the amount of hits by the models proposed in this study.

Conclusions

The major implication of this work is reducing the gap existing on this theme by suggesting new financial tools to evaluate the health of credit unions which are very important to the socioeconomic development of the regions where they are located.

This work proposes to help credit unions to meet solid financial structures in order to comply with its business mission, valuing relationships, offering financial solutions for adding income and improving the life quality of their members and the society.

Conflict of Interests

The authors have not declared any conflict of interests.

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Pruning management of cassava for animal feeding: Parameters of the root

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To evaluate the effects of pruning at different data on the parameters of the root, a randomized complete block experiment involving the IAC-14 cassava variety was conducted between years 2008/2010, at UNESP, Botucatu, Brazil. Plants were pruned monthly from the 7th to the 15th month after planted. The chemical analyses were performed at the time of harvest, that is, 22 months after planting (MAP). Production and yield plus the starch, crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), total digestible nutrients (TDN), nitrogen-free extract percentage (NFE) and total sugars (TS) contents of the root were analyzed. Pruning shoots of IAC-14 variety before the physiological rest affects positively yield and the percentage of root, and do not affect the root starch content.

Key words: Food science, starch, fiber, ruminant, monogastric.

INTRODUCTION

Brazil is one of the world's major producers of cassava and the most important one in productivity and industrialization of cassava starch (FAO, 2010). While cassava in other producing countries are harvested between 8 and 12 months of cultivation regardless of the final destination of the root, as a rule, Brazilian cassava grown for the processing industry is harvested between 18 and 24 months after planting (MAP) (Lorenzi, 2003),

Another unique aspect is that the Brazilian cultivation of cassava plant is clearly divided into two sectors, table and industry. Such distinction applies from the variety to the farming techniques. The table varieties have by principle low hydrocyanic acid content in fresh pulp roots. Varieties for industries can be divided into two segments: flour production and starch extraction. Besides root yield, bark color is critical in flour production and thus clear barks are preferred in order to avoid tainting the final product with dark pigments which is a consumption

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disqualification factor. For starch extraction or alcohol production, the factor of primary importance is dry matter (DM) content (Lorenzi, 2003), which has a highcorrelation with starch content (Borges et al., 2002), regardless of bark color, pulp or format.

Oliveira, (2010) commented that the pruning of the shoots of cassava can be incorporated as a seemingly efficient and viable strategy, although its recommendation will require deeper study about the interaction between pruning and environmental conditions.

The effects on the yield components and nutritive value of roots are influenced by pruning data, however the results found in the literature are controversial, and it depends on the phonological stage that plant is pruned (Conceição, 1981; Sagrilo et al., 2003; Aguiar, 2011).

According to Aguiar, (2011) and Andrade et al. (2011), pruning managed after the first physiological rest causes reduction of roots in DM content and in their starch content (Borges et al., 2002).

Considering the possibility of using the root of cassava as a food source for animals of economic interest and starting from its productive and nutritive characteristics, this study was designed to evaluate potential changes to these characteristics in plants pruned at ten different data.

MATERIALS AND METHODS

The experiment was conducted at the Lageado Experimental Farm (22°51' S, 48°26' W, altitude 786 m) owned by UNESP, Botucatu, during the years 2008 to 2010. The local climate is humid with some water deficit in April, July and August (Cunha and Martins, 2009).

The soil of the experimental area is classified as clayey structured distroferic red latosol (Carvalho et al., 1983), and with the following chemical characteristics: pH ($CaCl_2$) = 5, organic matter (dm^{-3}) = 21, P resin ($mg \cdot dm^{-3}$) = 15, H + Al ($mmolc \cdot dm^{-3}$) = 44 , K ($mmolc \cdot dm^{-3}$) = 2.9, Ca ($mmolc \cdot dm^{-3}$) = 30, Mg ($mmolc \cdot dm^{-3}$) = 13, base saturation = 46, cation exchange capacity = 90 and V% = 52. Between 20 to 40 cm deep the soil showed pH ($CaCl_2$) = 5, organic matter (dm^{-3}) = 16, P resin ($mg\ dm^{-3}$) = 8, H + Al ($mmolc \cdot dm^{-3}$) = 38, K ($mmolc \cdot dm^{-3}$) = 1.5, Ca ($mmolc \cdot dm^{-3}$) = 28, Mg ($mmolc \cdot dm^{-3}$) = 14, base saturation = 43, cation exchange capacity = 81 and V% = 53.

The experiment was carried out in a complete randomized block design with 10 treatments and 4 replications. The treatments were composed of 9 different pruning times and one control, without pruning. Plants were pruned monthly from April to December, 2009. The crop was harvested 22 months after planting and the chemical analyzes were performed on the root material sampled at harvest.

Planting was manual with a density of 12,500 plants ha^{-1}. Considering the results of soil chemical analysis and the recommendations of Lorenzi et al. (1996), 200 kg ha^{-1} of NPK 08-28-16 + 0.5 Zn fertilizer were applied at planting. Treatments were set up in plots of 48 plants, 4 rows of 12 plants each. The useful plots consisted of the 20 plants in the two central lines of each plot. On 24 September, 2008, 0.20 m long IAC-14 cassavas were planted. Roots were harvested between 21 and 29 July, 2010.

Plants were pruned to 0.10 m at the previously defined times and at harvest. Root fresh weight was determined immediately after the harvest of each plot. Ten roots were randomly picked from each plot and then mechanically chopped to pieces. A sample of approximately 400 g was taken from each treatment and dried in an oven with forced air circulation at 60°C until attaining constant weight. Root DM yield in t ha^{-1} was computed from the root DM and fresh matter yield of each plot.

Computation of the total DM yield in t ha^{-1} was performed from the DM and the total yield of fresh root in each plot.

Root samples underwent chemical parameter analysis. DM, starch (Instituto Adolfo Lutz, 1985), total sugars (TS), ash (AOAC, 1984) and crude protein (CP) (AOAC, 1984) were analyzed at the Chemical Analysis Laboratory of the *Centro de Raízes e Amidos Tropicais* – CERAT, FCA, UNESP, Botucatu. Ether extract (EE) (Van Soest, 1991), crude fiber (CF) (AOAC, 1990), neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest, 1991) analysis were carried out at the Laboratory Of Food Science of the *Faculdade de Medicina Veterinária e Zootecnia*, UNESP, Botucatu. The percentage of total digestible nutrients (TDN) was calculated using the methodology proposed by Kearl (1982): %TDN = 40.2625 + 0.1969 + 1.1903 × EE-0.1379 × CF.

The nitrogen-free extract percentage (NFE) was calculated according to Winton and Winton (1947): %NFE = 100 − (CP + EE + Ash + CF).

Data were subjected to analysis of variance and means were separated using Tukey's test at the 0.05 probability level.

RESULTS AND DISCUSSION

The DM content (%) of cassava root was affected by pruning time (Table 1). Productivity in tons of fresh matter per hectare was not affected and the experimental mean value was 27.85 t ha^{-1}. Root DM yield had similar results to those of DM content in each treatment.

Andrade et al. (2011) found no difference in DM yield of roots of cassava plants pruned at different dates however, the no pruning treatment showed lower productivity when compared with the pruning ones, in his experiment pruning started at 8 MAP and was repeated every month up to 20 MAP. In a similar study Oliveira, (2010) found differences in root yield between treatments pruned and without pruning, and Sagrilo et al. (2003) found a cubic effect on the DM concentration of IAC-14 cassava roots, pruned monthly from 12 to 21 MAP.

The reduction in root mass is due to the cassava plant consuming the reserves stored in the tuberous roots for overcoming vegetative buds dormancy and for leaf growth, always when the plant has environment conditions to develop (Andrade et al., 2011; Oliveira et al., 2010). This explains the fact that when pruning is done at 7, 8 and 9 MAP a lower percentage of dry roots results as well as pruning done farther from harvest having higher percentages of DM and yield. Pruning at 12, 13 and 15 MAP resulted in lower productivity due to total restructuring of shoots in the second growth stage, associated with the occurrence of rain and temperatures adequate for vegetative growth (Sagrilo et al., 2003; El-Sharkawy, 1990).

Unlike the other pruning times, effects of pruning were detected in the cassava root starch contents (Table 2). The lowest cassava root starch content (71.06%) was recorded when the shoot was pruned in June, that is, 9 MAP.

Table 1. Content (%) and yield on fresh and dry basis (t ha^{-1}) of cassava roots harvested 22 MAP from plants pruned at different dates.

Pruning dates	MAP	Fresh matter	Dry matter	Dry mass
		t ha^{-1}	%	t DM ha^{-1}
Apr/09	7	26.98^{a}	39.94^{ab}	10.82^{ab}
May/09	8	30.43^{a}	42.80^{a}	13.03^{a}
Jun/09	9	30.59^{a}	40.93^{a}	12.52^{ab}
Jul/09	10	31.78^{a}	41.42^{a}	13.17^{a}
Aug/09	11	27.95^{a}	40.86^{ab}	11.48^{ab}
Sep/09	12	24.15^{a}	39.50^{ab}	9.54^{ab}
Oct/09	13	24.71^{a}	35.01^{b}	8.74^{b}
Nov/09	14	27.21^{a}	37.11^{ab}	10.10^{ab}
Dec/09	15	24.61^{a}	37.60^{ab}	9.28^{ab}
No pruning	-	30.12^{a}	41.39^{a}	12.48^{ab}
Average		27.85	39.66	11.12
CV (%)		12.65	6.12	15.42
MSD		8.57	5.91	4.17

Different letters in columns differ by Turkey test (0.05), CV - coefficient of variation, MSD - minimum significant difference.

Table 2. Content (%) and yield in dry matter (t ha^{-1}) of starch from cassava roots harvested 22 MAP from plants pruned at different times of the year.

Treatment	MAP	Starch	Production of starch
		% DM	t ha^{-1}
Apr/09	7	87.53^{a}	9.53^{a}
May/09	8	82.88^{a}	10.77^{a}
Jun/09	9	71.06^{b}	8.88^{a}
Jul/09	10	90.61^{a}	11.94^{a}
Aug/09	11	88.07^{a}	10.21^{a}
Sep/09	12	79.54^{a}	7.63^{a}
Oct/09	13	87.39^{a}	7.71^{a}
Nov/09	14	90.00^{a}	9.09^{a}
Dec/09	15	90.45^{a}	8.42^{a}
No pruning	-	90.88^{a}	11.32^{a}
Average		85.84	9.55
CV (%)		7.50	18.81
MSD		15.66	4.37

Different letters in the columns differ by Turkey test (0.05), CV - coefficient of variation, MSD - minimum significant difference.

Marques et al. (2000), in a study on the replacement of corn by cassava root and other types of food found 82.5% starch in DM, but just as Michelan et al. (2007), who observed 55.35% starch in DM, those authors did not specify the range studied. The IAC-14 variety was selected because of its greater percentage and yield of starch in roots which makes it more suitable to industrial uses. Sagrilo et al. (2003) found effects of pruning time and root DM starch contents varying from 56 to 75% for the IAC-14 variety. Plants pruned between 14 and 18 MAP had lower means when compared to plants pruned from 21 to 22 MAP which was explained by the fact that the demand for carbohydrates accumulated in the roots is outweighted by its photosynthesis production.

There were no differences in the percentage of ash in root's DM. The coefficient of variation was 15.77% and the least significant difference 2.38. The average root ash content was 6.21%.

The content of EE in the roots was not affected by the pruning times of treatments and showed a mean value of

Table 3. Content (%) of crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF) and total digestible nutrients (TDN) of cassava roots harvested 22 MAP from plants pruned at different dates.

Treatment	MAP	CF	NDF	ADF	TDN
		% DM			
Apr/09	7	1.64^{d}	3.18^{c}	2.25^{cde}	79.51^{a}
May/09	8	2.14^{bc}	3.71^{bc}	2.58^{bc}	79.37^{a}
Jun/09	9	1.71^{cd}	3.51^{c}	2.06^{de}	79.22^{a}
Jul/09	10	1.80^{cd}	3.34^{c}	2.17^{cde}	79.19^{a}
Aug/09	11	1.85^{cd}	3.79^{bc}	2.22^{cde}	78.98^{a}
Sep/09	12	2.75^{a}	4.92^{ab}	3.31^{a}	78.97^{a}
Oct/09	13	2.14^{bc}	3.91^{abc}	2.58^{bc}	78.91^{a}
Nov/09	14	1.50^{d}	3.04^{c}	1.81^{e}	78.77^{a}
Dec/09	15	2.50^{ab}	5.13^{a}	2.90^{ab}	78.60^{a}
No pruning	-	2.10^{bc}	3.73^{bc}	2.53^{bcd}	78.57^{a}
Average		2.01	3.83	2.44	79.01
CV (%)		8.73	13.65	8.10	0.75
MSD		0.42	1.27	0.48	1.44

Different letters in the columns differ by Turkey test (0.05), CV - coefficient of variation, MSD - minimum significant difference.

0.80% of EE. Azevedo et al. (2011) in a study of the use of co-products in ruminant feed, found 0.59% EE in cassava bark, above the ones found in a study by Olafadehan (2011) who working with rabbits detected 0.14% EE in unprocessed cassava barks.

The low content of EE may be related to the root's chemical composition and to the extraction method. Besides lipids, other compounds such as waxes, carotenoids, chlorophyll and vitamins A and D are measured when petroleum ether is used. It is well known (Valdivié and Bicudo, 2011) that cassava root is deficient in those components.

Treatments affected the CF content of cassava root (Table 3). The highest value of CF was obtained when the shoot was pruned at 12 and 15 MAP (2.75 and 2.5%), respectively, which corresponded to the months of September and December. Also, the least means were obtained for pruning at 7, 9, 10, 11 and 14 MAP. Intermediate values were observed for the remaining months.

In an experiment carried out in Egypt, Abd El-Baki et al. (1993) found higher values of CF in cassava roots for the feeding of rabbits, averaging 3.97% of DM. Gil and Buitrago (2002), in a chapter on the use of cassava in animal feed, cite that Buitrago (1990) used as recommendation 2.8% of CF in cassava roots DM and that in the Netherlands van Poppel (personal communication) has found higher values, 4.10% CF in DM, and that analyzes made at CIAT revealed 3.08% of CF in cassava root DM. The values obtained by this work are lower than those found in literature; however, none of those studies reported either the variety employed or the harvest period.

The difference with respect to CF may be related to the bark ratio of cassava roots. This element is richer in fibers than the root pulp (Valdivié and Bicudo, 2011) and the treatment applied may have influenced the amount of bark.

The content (%) of NDF was affected by the treatments applied (Table 3), the average value being highest (5.13% NDF in DM) when the aerial part of the cassava plant was pruned 15 MAP, which corresponds to the month of December. Similar figures were found for September and October. Valdivié and Bicudo, (2011) reported that the average value of NDF in cassava roots is 5.1% for the fresh base and 2.1% for the dry one. Zeoula et al. (2003), in an experiment where corn for ruminants was replaced by alternate energy sources including cassava, found an NDF value of 8.12% of DM. Works on the inclusion of cassava root in animal feed use cassava root meal as a replacement for maize; these works found higher NDF values. Zeoula et al. (2003) reported that the value of NDF from cassava root meal for the experiment with sheep was 12.31% in DM; Caldas Neto et al. (2007) found 11.1% NDF when composing diets for growing steers and Abrahão et al. (2005) for cassava residue, which the author believe to be the mass resulting from the industrial starch removal, found 30.50% NDF in DM, a value almost three times greater than the one for cassava root meal and 8 times as high as the one for whole cassava root. Perhaps such difference can be explained by the industrial extraction of starch causing fiber concentration in the resulting slurry.

Pruning time affects the ADF content in DM (Table 3). Plants whose shoots were removed from 12 to 15 MAP yield more detergent acid fibers in roots. Pruning in November, 2 months later, leads to a lower average of 1.82%.

Table 4. Content (%) on dry basis (t ha^{-1}) of nitrogen free extract (NFE) of cassava roots harvested 22 MAP from plants pruned at different dates.

Treatment	MAP	NFE
		% DM
Apr/09	7	90.31^{ab}
May/09	8	89.03^{abc}
Jun/09	9	90.43^{ab}
Jul/09	10	90.06^{ab}
Aug/09	11	90.74^{the}
Sep/09	12	88.11^{bc}
Oct/09	13	88.92^{abc}
Nov/09	14	89.11^{abc}
Dec/09	15	87.35^{c}
No pruning	-	88.41^{abc}
Average		89.25
CV (%)		1.17
MSD		2.54

Different letters in the columns differ by Tukey test (0.05), CV - coefficient of variation, MSD - minimum significant difference.

Michelan et al. (2007) found an average of 8.57% ADF in whole cassava root scraps for feeding rabbits between weaning and slaughter. Abrahão et al. (2005) found 22.66% ADF in DM of cassava residues. In neither article the authors have specified the variety or the harvest time. The difference in ADF can also be explained by the amount of bark since the root pulp is not rich in ADF constituents (cellulose, lignin and silica).

There was no difference in the content (%) of TDN among treatments which showed average of 79.01% with a 0.75% coefficient of variation.

Regarding cassava root meal, Caldas Neto et al. (2007) and Ferreira Filho et al. (2007) reported values of 80 and 90.40% TDN in DM, respectively.

Root CP content (% DM) did not differ among treatments. DM content found in roots of the plants pruned at different times of the year as well as on those left without pruning averaged 1.72% for a 14.24% coefficient of variation.

According to Valdivié and Bicudo, (2011), in general cassava root has 1.1% CP in DM and 2.40 to 3.19% in fresh matter. Several balanced experimental diets from root, CP figures even though the cassava root is poor in that. Evaluating feed for ruminants, Caldas Neto et al. (2007) found 1.9% CP in DM of cassava root meal, while Ferreira Filho et al. (2007) and Jorge et al. (2002) found 1.62 and 1.8%, respectively. Abd El-Baki et al. (1993) found an average of 5.15% of whole cassava root, while for mashed whole cassava root Cruz et al. (2006) found 2.43%. Abrahão et al. (2005) studying cassava for feeding of young bulls obtained 1.92% CP. The bark of the cassava root, according to Faria et al. (2011), contains 3.94 and 4.38% CP, respectively for dry and fresh matter.

Once the roots are processed into pellets or chips, CP value reaches 2.5% on average (Garcia and Dale, 1999). It is observed that CP content is reduced in the grinding of cassava flour (Caldas Neto et al., 2007; Ferreira Filho et al., 2007; Jorge et al., 2002) higher values being found only in the root bark (Faria et al., 2011) and intermediate ones in whole dry root, ground or not.

The treatments caused differences in NFE values (Table 4). The treatment with pruning in August, 11 MAP, obtained the highest value, 90.74%, not much different from values for 7, 8, 9, 10, 13, 14 and 22 MAP. Root NFE (% DM) values were below those for the other treatments when cassava shoots were pruned at 15 MAP, together with treatments May/2009, Sep/2009, Oct/2009, Nov/2009 and with the no pruning group. Intermediate averages were found for the rest of the treatments.

No differences were observed ($p < 0.05$) in the percentages of root TS. On average IAC-14 cassava roots contained 0.43% of TS in whole roots.

The percentage of root production, relative to the total biomass produced by the plant, shoot plus root, was greater in the absence of pruning where about 54.34% of the total root biomass was relative to the root, that is, in non pruned plants accumulates more in the root, with respect to the total biomass produced. Pruning in other months hardly interfered with the percentage of root production. Pruning before the physiological rest negatively affects the concentration of starch in the root. Although no difference was found in starch yield, probably the plant reorganizing its photosynthetic device after pruning and then having it fresh leaves falling in consequence of the physiological rest causes an energy or starch reserve expenditure higher when compared to treatments that just translocated root assimilates to the post pruning shoot development.

Conclusion

Pruning shoots of IAC-14 variety before the physiological rest affects positively yield and the percentage of root, and do not affect the root starch content.

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Conflict of interest

The author(s) have not declared any conflict of interests.

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Spore density and diversity of Arbuscular mycorrhizal fungi in medicinal and seasoning plants

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Arbuscular mycorrhizal fungi (AMF) set mutualistic symbiosis with most plants. Understanding this association and meet the diversity of AMF in both the medicinal and the seasoning herbs is very important, since these plants have increasingly contributed to improving the quality of human life. The aim of this study was to assess the spore density, taxonomic diversity, and root colonization by AMF in experimental beds of rosemary (*Rosmarinus officinalis* L.), nasturtiums (*Tropaeolum majus*), mint (*Mentha crispa* L.), boldo (*Peumus boldus*), oregano (*Origanum vulgare*) and chamomile (*Matricaria chamomilla*), all planted in the Medicinal Plant Nursery of the Paranaense University - UNIPAR, Umuarama – PR. Soil samples (0 to 10 cm depth) and plant roots were collected in two periods, June and November 2011. Colonization of plant roots by AMF ranged 17 to 48%. The rosemary treatment was highly responsive to the sampling periods, with only 17% of root colonization in June compared with 48% in November. The AMF spore density was higher in June than in November for all species of plants studied. Among the AMF identified within this study, the dominant genus was *Glomus sp*., followed by *Acaulospora sp*. in all plants analyzed. Greater knowledge over diversity and density of AMF spores can strongly contribute to the sustainable management of nutrition for medicinal and seasoning plants, particularly on phosphorus supply.

Key words: Diversity of mycorrhizal fungi, symbiosis, mycorrhizae, medicinal and seasoning plants.

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) compose a key functional group of the soil biota that can substantially contribute to plant yields and ecosystem sustainability in crop production strategies. Presently, applications of beneficial microbial inoculants (biofertilizers) are increasingly attracting attention toward sustainable agriculture and life quality as a consequence of the need to solve health and environmental problems resulting from the excessive use of agrochemicals through conventional farming practices (Gianinazzi et al., 2010).

The AMF are commonly found in nature and very important as biofertilizers. They belong to Phylum *Glomeromycota*, Class *Glomeromycetes* and form a monophyletic group of fungi classified into four orders, thirteen families, and nineteen genera, with somewhat 215 species already described (Siqueira et al., 2010).

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AMF form mutualistic symbiosis with the roots of most plants. Through this symbiosis, the fungus gets carbohydrates and other elements essential to their development from the host plant, forming new spores by sporulation processes. In contrast, the host plant obtains from the soil, with the help of the fungus, water and inorganic nutrients such as phosphorus (P), benefits by getting long and bulky roots, and acquires resistance to pathogens and abiotic stress, such as the presence of heavy metals and water shortage (Carrenho et al., 2007; Smith and Read, 2008).

Studies on mycorrhizal symbiosis with medicinal and seasoning plants are scarce. However, some of these few studies have shown that AMF can increase the production of secondary compounds containing medicinal active ingredients in plants under mycorrhizal symbiosis, in addition to promoting their growth (Faria et al., 2000; Russomano et al., 2008).

The aim of this study was to assess the content of soil organic matter (SOM), spore density and AMF root colonization in plants of rosemary (*Rosmarinus officinalis* L.), nasturtiums (*Tropaeolum majus*), mint (*Mentha crispa* L.), boldo (*Peumus boldus*), oregano (*Origanum vulgare*) and chamomile (*Matricaria chamomilla*) cultivated in experimental beds (plots) in the Medicinal Plant Nursery of the Paranaense University - UNIPAR, Umuarama – PR, in two periods, June and November, 2011.

MATERIALS AND METHODS

Experimental field: Soil and root sampling

Root and soil samples were collected at the Medicinal Plant Nursery of the Paranaense University - UNIPAR - Campus II, in the Umuarama city, northwestern Paraná State at coordinates S 23° 46' 11.34'' and WO 53° 16' 41.78''.

For each plant of rosemary, nasturtiums, mint, boldo, oregano and chamomile were assigned three experimental beds. Roots and rhizosphere soil were sampled in three points of each bed, giving a total of 9 replications per plant species in a completely randomized design. The beds received organic compost (coffee leaf straw transformed by fermentation process of composting) before being planted. Then, as with plants, the beds were irrigated daily by spraying when needed. Plants with the exception of boldo and rosemary, were at the phenological stage of pre-flowering.

The roots and soil sampling was performed at 0 to 10 cm, about 10 cm away from the stem of each plant, into two periods: June and November 2011. Sampling was done at the same point in each bed for the two periods. In each plot, three samples were collected for approximately 0.5 kg of soil, placed in plastic bags and stored in a refrigerator (4°C) until laboratory analyzes.

A soil sample was collected for chemical and granulometric analyses. One portion of that sample was utilized to determine the soil chemical characteristics at *Solo Fértil* Laboratory in the city of Umuarama, Paraná, Brazil. The characteristics determined were: pH in $CaCl_2$, Ca^{2+}, Mg^{2+} and Al^{3+} extracted in KCl (1 Mol L^{-1}), and P and K^+ extracted in Mehlich-1. All the analyses followed the CELA/PR standards to obtain a greater reliability of the results (Table 1). The other portion of the soil sample was intended for identification of the density and taxonomy of AMF spores. In both periods, the thinner roots of the plants were collected at three points of each bed (n = 9), washed in water, placed in flasks with preserving solution containing ethyl alcohol, acetic acid and formaldehyde (1:1:1) and stored in a refrigerator (4°C) (Souza, 2000) until laboratory analyzes for determining the percentage of AMF root colonization.

Spore density of arbuscular mycorrhizal fungal

The spores were extracted from 50 g of soil subsamples using the wet sieving method (Gerdemann and Nicolson, 1963). Each sample was suspended in 1 L of water and agitated in a beaker, kept at rest for 1 min so that the rougher particles of the soil were decanted, and then the content was poured on two juxtaposed sieves with 0.710 mm and 0.053 mm opening; the procedure repeated for four times. The material remained at the 0.053 mm sieve was transferred to 50 mL Falcon tubes, centrifuged in distilled water (3000 rpm, 3 min), and supernatant discarded. Next, saccharose solution (50%) was added into the tubes and they were agitated and centrifuged (2000 rpm, 2 min). The spores in the supernatant were transferred to the 0.053 mm sieve, washed to eliminate saccharose excess, transferred to Petri dishes and then counted under stereoscopic lens (40X).

Characterization and diversity of AMF

In Glomeromycota, taxonomy can be performed through morphological analysis of the formation, structure and germination of AMF spores. Spores were fixed on semi-permanent slides in two separate groups: one group with PVLG (polyvinyl alcohol and glycerol) resin and the other with PVLG resin + Melzer, and counted under a microscope (Morton et al., 1993). The sporocarps were carefully broken and the spores were counted.

Species taxa of AMFs were identified using Schenck and Pérez (1988) and INVAM - International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu) in addition to other species descriptions. From the number of individuals of each genus, the indexes of dominance (Simpson) and diversity (Shannon-Wiener) were estimated according to Souza et al. (2010). They were calculated according to the equations:

$$C = -\Sigma (X_I/X_0) \times \log (X_I/X_0) \quad \text{Simpson,}$$

$$H' = -\Sigma (X_I/X_0)^2 \quad \text{Shannon-Wiener,}$$

where X_I is the spore density of each genus in 100 g of soil, X_0 is the total spore density of all AMF genera.

AMF root colonization

To determine AMF root colonization, six plants with roots were collected from each subplot (beds) and washed in running water. Plant roots were freshly cut at the length of ±1.5 cm so that they can represented as the whole radicular system. The lab procedure was done according to Phillips and Hayman (1970), where sample roots are placed in 10% KOH and closed in plastic Falcon Tubes. After heating the tubes with roots in water bath at 90°C for 1 h, the KOH solution was removed and the roots were washed in running water. A solution of 1% HCL was added in the tubes with roots and agitated for acidification for 5 min; next, the solution was removed. Then, roots were stained by adding 0.05% trypan blue to the tubes, which were heated in water bath at 90°C for 30 min. At the end of the process, the roots were preserved in lactoglycerol. The root segments were examined in stereoscopic microscope (100x) for AMF structures and percentage root length colonization was

Table 1. Chemical properties of the experimental soil (0 – 10 cm) sampled in the experiment area during 1st and 2nd sampling period – June and November of 2011.

Plant	pH	P	C	Al^{3+}	$H^{+}+Al^{3+}$	$Ca^{2+}+Mg^{2+}$	Ca^{2+}	Mg^{2+}	K^{+}	SB	CEC	V
	$CaCl_2$	mg dm^{-3}	g dm^{-3}	---------- $Cmol_c$ dm^{-3} ----------								(%)
						1st sampling – June						
Rosemary	4.63	52.40	7.60	0.0	4.96	3.50	2.00	1.50	0.15	3.65	8.61	42.42
Boldo	5.24	21.00	6.04	0.0	3.68	4.38	3.38	1.00	0.21	4.58	8.26	55.45
Chamomile	4.72	90.00	7.21	0.0	4.96	7.63	3.88	3.75	0.21	7.83	12.79	61.22
Nasturtiums	5.47	173.60	8.77	0.0	3.68	5.75	3.25	2.50	0.21	5.96	9.64	61.81
Mint	5.32	282.80	8.57	0.0	4.28	6.25	4.25	2.00	0.21	6.46	10.74	60.13
Oregano	5.20	57.40	8.57	0.0	4.28	5.50	3.25	2.25	0.21	5.71	9.99	57.14
						2nd sampling – November						
Rosemary	5.17	60.10	6.62	0.0	3.97	4.50	2.75	1.75	0.15	4.65	8.62	53.96
Boldo	5.30	23.70	7.21	0.0	3.97	6.88	4.00	2.88	0.10	6.98	10.95	63.74
Nasturtiums	5.69	248.20	10.91	0.0	3.42	6.25	3.50	2.75	0.26	6.51	9.93	65.55
Mint	5.46	207.20	7.60	0.0	3.97	5.25	4.25	1.00	0.15	5.4	9.37	57.65
Oregano	5.08	69.90	7.60	0.0	4.28	4.88	2.63	2.25	0.15	5.03	9.31	54.02

P – Phosphorus; C – Carbon; Al^{3+}– Aluminium; $H^{+}+Al^{3+}$ – Potential Acidity; Ca^{2+} – Calcium; Mg^{2+} – Magnesium; K^{+} – Potassium; SB – Sum of Bases; CEC –Cation Exchange Capacity; V – Bases saturation

estimated according slide method (Giovannetti and Mosse, 1980) for each replication of each treatment.

Statistical analysis

Data was subjected to one-way ANOVA using general linear model with mixed-effects and balanced design, considering each plant species as one treatment, and compared with the Duncan's test ($p \leq 0.05$), by using SPSS version 16.0 for Windows (SPSS Inc., Chicago, IL, USA). To comply with ANOVA assumptions, the data was previously checked with the Levene´s test. In the two periods June and November, *t*-test was done with independent bilateral averages.

RESULTS AND DISCUSSION

Soil chemical analysis from the first sampling period (June, 2011) presented the highest P level in soil with mint (282.8 mg dm^{-3}), followed by soil with nasturtiums (173.6 mg dm^{-3}) and boldo (21 mg dm^{-3}) (Table 1).

Both the P fixation and the natural P levels of soils vary according to plant variety or cultivar. Changes also may occur in the AMF activity depending on the conditions of soil fertility (Siqueira et al., 2010). However, the amount of organic matter from composting added to the beds is not exactly known for evaluating fertility within this study.

In general, stable levels of P were observed in the analysis of soils collected from the second sampling (November, 2011), with an increase in P levels observed only in the soil cultivated with nasturtiums (Table 1).

The availability of nutrients is affected by the soil pH. In this study, results of soil pH (Table 1) are according to the literature, which indicates values of 6 to 7 as ideal to grow most of plant species (Corrêa Junior and Scheffer, 2009); remembering that there are plants that can tolerate lower pH. On the other hand, the low pH, as of the soil with rosemary (4.63 – Table 1), may affect the mycorrhizal association with plants. This is due to the variation of the solubility of elements such as Al, Fe, Mn and Cu, which at toxic levels may reduce the germination of spores and germ tubes, reducing the sporulation of AMF (Lambais and Cardoso, 1989). Studies show that soil pH regulates the mycorrhizal condition and controls the distribution of AMF species (Moreira et al., 2003).

The C levels in the soil differed between sampling periods (Table 1). For example, the bed with nasturtiums had 8.77 g dm^{-3} C in June and started having 10.91 g dm^{-3} C in November. According to Kaschuk et al. (2010; 2011), the C level in soils can also be used as indicator of their fertility and quality as it supplies biological activity, maintains environmental quality and promotes the health of plants and animals.

The roots of all plants analyzed were colonized by AMF. The AMF root colonization in June was significantly lower than in November (Table 2), with averages of 25.72 and 35.36%, respectively. Boldo, mint, oregano and nasturtiums had no significant differences in AMF root colonization (Table 2). Rosemary had a significant increase in AMF root colonization in November compared to June. It indicates that AMF root colonization was maximum in winter season and lowest in early summer season. Kumar et al. (2010) observed similar results for *Spilantes acmella*, *Withania somnifera*, *Salvia officinalis*, *Mentha spicata* when AMF root colonization was significantly higher in November than in June.

Among all plants analyzed within this study, mint

Tabela 2. Means of AMF soil spore density (nº g^{-1} of dry soil) and AMF root colonization (%) (± standard deviation, n = 9) in June and November, 2011.

Plant	AMF root colonization		AMF spore density	
	June	November	June	November
Rosemary	17.40±4.83 Bb	48.04±8.26 Aa	21.15±4.41 BCa	2.84±0.70 Ab
Boldo	30.74±4.92 Aa	45.67±8.82 Aa	16.80±3.11 Ca	3.01±1.09 Ab
Chamomile	20.80±4.82 B	ND	37.30±8.60 A	ND
Nasturtiums	21.88±4.31 Ba	18.35±4.45 Ba	16.52±2.37 Ca	3.05±0.47 Ab
Mint	42.37±7.47 Aa	45.35±7.01 Aa	19.96±2.65 BCa	3.84±0.77 Ab
Oregano	19.28±2.65 Ba	16.55±3.29 Ba	32.11±4.75 ABa	3.18±0.46 Ab
p value	> 0.001	> 0.002	> 0.001	0.902

ND = Not determined. Means followed by the same capital letter in the column are not significantly different by the Duncan test ($p \leq 0.05$) and by the same minor letter in the line did not differ by the t-test ($p \leq 0.05$).

showed the highest percentage of colonization, with 42.37% (June) and 45.35% (November), followed by boldo with 30.74% (June) and 45.67% (November). This result indicates that both the mint and the boldo are the species that are more depending on the AMF associations.

As AMF started establishing on the thinnest roots, temperature and humidity were likely to influence AMF root colonization as well as the nutrients intake by plants in this study (Smith and Read, 2008). According to Carrenho et al. (2007), the process of root colonization can be influenced by changes in seasonal periods. It was also observed in other studies in which the best root colonization was in the rainy season (Kumar et al., 2010). However, Radhika and Rodrigues (2010) states that mycorrhizal root colonization is present in all seasons, suggesting a plant dependence on AMF throughout the year.

A root can be colonized by more than one species of mycorrhizal fungus (Dood et al., 2000) and a fungus species can grow at different rates when associated to different species of plants (Smith and Read, 2008). Still, there may be several colonizing rates across genotypes of the same plant species (Grahman and Eissenstat, 1994).

Gupta et al. (2002) observed an increased percentage of root colonization in plants inoculated with AMF compared with respective controls of non-inoculated plants. The authors also noted a possible difference in response to mycorrhizal colonization across varieties of the same plant, as observed in the three cultivars of mint inoculated with *G. fasciculatum* in their study.

The major density of AMF spores (number of spores g^{-1} of dry soil) was observed in the period of June, with emphasis on soils with oregano (32.11) and chamomile (37.29), which showed densities significantly higher than the soils of other plants studied (Table 2). However, AMF spores were present in November with minor density, but with no significant difference when compared to June (Table 2). It can be explained here by a wetter weather (data not shown) affecting directly fungus sporulation. Kumar et al. (2010) observed similar results for soils with *S. acmella* and *Mellisa officinalis*, in which AMF spores density was significantly lower in November than in June. Radhika and Rodrigues (2010) reported a density of AMF spores varying in function of seasons with a higher number of spores in August than in January. On the other hand, Coppetta et al. (2006) developed a study suggesting that the density of spores in soils is most dependent on the extent of root colonization between the AMF and the plant.

Negative and significant correlation ($p = 0.035$) was observed between AMF root colonization and spore density. Similar results were found by Radhika and Rodrigues (2010) when they studied thirty-six medicinal plant species.

Studies demonstrate that the seasonality of mycorrhizal colonization is usually a function of environmental conditions as temperature, humidity, phenology and physiological condition of the plant (Mohammad et al., 1998; Brundrett, 2002) and in this way, significant differences in both the root colonization and the density of AMF spores were observed in this study (Table 2).

Phyla Glomeromycota is identified mainly from the analysis of the formation, structure and germination of spores. In this study, *Glomus* was the most predominant genus within the diversity of AMF in the two sampling periods (June and November - Tables 3 and 4, respectively). *Acaulospora, Gigaspora, Scutellospora* and *Pacispora* were others genera found in this study, but with lower frequency (Table 5). Similar results were found by Radhika and Rodrigues (2010) in samples of soils cultivated with thirty-six medicinal plant species. The great diversity of AMF found in this study indicates that the plants studied form a symbiotic-mandatory association with the AMF, regardless of the period analyzed.

The indexes of Shannon diversity and Simpson dominance (Table 5) revealed great spore diversity among species of plants regardless of the sampling

Table 3. Taxonomy and number of spores per species of mycorrhizal fungi (Phylum *Glomeromycota*) in the 1st sampling period (June, 2011) determined according to Siqueira et al. (2010).

Plant	Order	Family	Genus	Species	Nº
Rosemary	Glomerales	Glomeraceae	*Glomus*	*Glomus aff. Lamellosum*	23
				Glomus mosseae	11
				Glomus microaggregatum	30
				Glomus aff. Tortuosum	3
				Gigaspora margarita	2
				Glomus claroideum	3
				Glomus etunicatum	7
	Diversispolares	Acaulosporaceae	*Acaulospora*	*Acaulospora sp.*	1
				Acaulospora koskei	2
Boldo	Glomerales	Glomeraceae	*Glomus*	*Glomus macrocarpum*	2
				Glomus aff. Lamellosum	27
				Glomus claroideum	1
				Glomus microaggregatum	5
				Glomus mosseae	1
				Glomus aff. Deserticola	9
				Glomus constrictum	1
				Glomus etunicatum	10
	Diversispolares	Acaulosporaceae	*Acaulospora*	*Acaulospora delicata*	5
				Acaulospora koskei	27
				Acaulospora morrowiae	13
				Acaulospora (Entrophospora) colombiana	3
				Acaulospora sp. (scro-reticulata)	1
		Gigasporaceae	*Gigaspora*	*Gigaspora margarita*	1
		Scutellosporaceae	*Scutellospora*	*Scutellospora aff. Verrucosa*	1
Chamomile	Glomerales	Glomeraceae	*Glomus*	*Glomus aff. Lamellosum*	7
				Glomus mosseae	7
				Glomus claroideum	10
	Diversispolares	Gigasporaceae	*Gigaspora*	*Gigaspora margarita*	1
		Scutellosporaceae	*Scutellospora*	*Scutellospora calospora*	1
Nasturtiums	Glomerales	Glomeraceae	*Glomus*	*Glomus mosseae*	4
				Glomus macrocarpum	13
				Glomus aff. Lamellosum	4
				Glomus tortuosum	31
				Glomus aff. Deserticola	5
				Glomus claroideum	2
				Glomus geosporum	1
				Glomus invermaium	5
				Glomus etunicatum	9
	Diversispolares	Acaulosporaceae	*Acaulospora*	*Acaulospora sp. (scro-reticulata*	2
				Acaulospora koskei	2
				Entrophospora infrequens	4
		Gigasporaceae	*Gigaspora*	*Gigaspora margarita*	1
Mint	Glomerales	Glomeraceae	*Glomus*	*Glomus claroideum*	3
				Glomus aff. lamellosum	3
				Glomus mosseae	1
				Glomus macrocarpum	1
				Glomus constrictum	1
				Glomus etunicatum	3
	Diversispolares	Acaulosporaceae	*Acaulospora*	*Acaulospora scrobiculata*	4
				Acaulospora delicata	1
				Acaulospora koskei	1
				Acaulospora delicata	1
		Gigasporaceae	*Gigaspora*	*Gigaspora margarita*	2
				Gigaspora decipiens	1
Oregano	Glomerales	Glomeraceae	*Glomus*	*Glomus macrocarpum*	4
				Glomus aff. lamellosum	1
				Glomus claroideum	1
	Diversispolares	Acaulosporaceae	*Acaulospora*	*Acaulospora koskei*	2
		Gigasporaceae	*Gigaspora*	*Gigaspora decipiens*	1

Table 4. Taxonomy and number of spores per species of mycorrhizal fungi (Phylum *Glomeromycota*) in the 2nd sampling period (November, 2011) determined according to Siqueira et al. (2010).

Plant	Order	Family	Genus	Species	Nº
Rosemary	Glomerales	Glomeraceae	*Glomus*	*Glomus tortuosum*	5
				Glomus aff. lamellosum	5
				Glomus mosseae	2
				Glomus aff. luteum	3
				Glomus claroideum	1
	Diversispolares	Acaulosporaceae	*Acaulospora*	*Acaulospora (Entrophospora) colombiana*	2
		Gigasporaceae	*Gigaspora*	*Gigaspora decipiens*	4
Boldo	Glomerales	Glomeraceae	*Glomus*	*Glomus margarita*	1
				Glomus aff. lamellosum	5
				Glomus aff. luteum	1
				Glomus claroideum	3
	Diversispolares	Acaulosporaceae	*Acaulospora*	*Acaulospora koskei*	3
				Acaulospora (Entrophospora) colombiana	4
		Scutellosporaceae	*Scutellospora*	*Scutellospora rubra*	1
				Scutellospora heterogama	1
Nasturtiums	Glomerales	Glomeraceae	*Glomus*	*Glomus tortuosum*	6
				Glomus aff. lamellosum	7
				Glomus geosporum	1
				Glomus aff. luteum	1
				Glomus constrictum	1
				Glomus mosseae	2
				Glomus microaggregatum	30
				Gigaspora decipiens	1
Oregano	Glomerales	Glomeraceae	*Glomus*	*Glomus aff. lamellosum*	3
				Glomus mosseae	4
	Diversispolares	Acaulosporaceae	*Acaulospora*	*Acaulospora sp. (com espinhos)*	1
		Gigasporaceae	*Gigaspora*	*Gigaspora gigantea*	1
Mint	Glomerales	Glomeraceae	*Glomus*	*Glomus aff. lamellosum*	3
				Glomus geosporum	2
				Glomus macrocarpum	1
				Glomus etunicatum	1
				Glomus margarita	1
				Glomus aff. luteum	1
		Gigasporaceae	*Gigaspora*	*Gigaspora ramisporophora*	2
	Diversispolares	Acaulosporaceae	*Acaulospora*	*Acaulospora scrobiculata*	4
		Pacisporaceae	*Pacispora*	*Pacispora robiginia*	1

period. Shannon index for rosemary increased from 0.066 in June to 0.329 in November, indicating greater diversity of AMF in the period of June compared to November. However, the opposite was observed for nasturtiums (Table 5).

The plants of mint, oregano and bold had similar AMF indexes of Shannon diversity and Simpson dominance in the two sampling periods studied, thus these plants were efficient in their symbiosis in both seasons.

Among all AMF genera found, *Glomus sp.* was the most frequent in all species and in both periods analyzed in this study (Table 5). The dominance of *Glomus* sp. in soil cultivated with rosemary and sampled in June was 0.931 whereas in soil under nasturtiums and sampled in November was 0.96 (Table 5). Spores of *Glomus sp.* were observed in soils cultivated with all species studied. Their frequency was higher than 52%, reaching 98% in soils cultivated with nasturtiums.

Conclusions

All plants showed levels of AMF root colonization, and spore density of AMF decreased in June when compared

Table 5. AMF genera frequency, indexes of Shannon diversity and Simpson dominance (June and November, 2011).

Plantas	*Glomus* sp.	*Acaulospora* sp.	*Gigaspora* sp.	*Scutellospora* sp.	*Pacispora* sp.	Shannon	Simpson
---------- Relative frequency (%) ----------							
June							
Rosemary	96.47	3.53	0	0	0	0.066	0.931
Boldo	51.88	46.22	0.95	0.95	0	0.341	0.482
Chamomile	92.30	0	3.85	3.85	0	0.141	0.854
Nasturtiums	89.15	9.65	1.20	0	0	0.165	0.804
Mint	54.55	31.82	13.63	0	0	0.419	0.417
Oregano	66.67	22.22	11.11	0	0	0.368	0.506
November							
Rosemary	72.72	9.10	18.18	0	0	0.329	0.570
Boldo	52.63	36.84	0	10.53	0	0.409	0.423
Nasturtiums	97.96	0	2.04	0	0	0.043	0.960
Mint	56.25	25	12.50	0	6.25	0.403	0.394
Oregano	77.78	11.11	11.11	0	0	0.296	0.629

to November. The taxonomic diversity of AMF varied among species of medicinal and seasoning plants studied. Spores of *Glomus* sp. were observed in all species studied. Their frequency was higher than 52%, reaching 98% in soils cultivated with nasturtiums.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Genetic variability among traits associated with grain yield of rice (*Oryza sativa* L.) exposed to drought at flowering stage

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Morphometric and biochemical characterization of eighteen rainfed upland rice genotypes were studied under drought stress situation. Relation of the studied traits was also observed with grain yield under drought stress situation. A significant variation among the rice genotypes were observed and a significant genotype × environment interaction for all the traits was seen indicating better scope for drought tolerance and improvement in yield. The different biochemical parameter that is, shoot starch at maturity, leaf's starch, upper root's and lower root's carbohydrate under both the conditions were positively increased. However, proline in irrigated condition was the only trait which possessed very high estimates of phenotypic and genotypic coefficient of variation, heritability (h^2_b) and genetic advance (Ga). Traits like, chlorophyll a, nitrate reductase activity, carbohydrate at flowering, starch at maturity, leaf's carbohydrate, leaf's starch and lower root's starch showed positively high (desired) genotypic correlation as well as direct effect on grain yield. SDS-PAGE profiling in control and drought condition was conducted and 25 kDa proteins was found to be induced in resistance rice genotypes. Drought tolerance of well-known cultivars- Vandana, NDR-359, Azucena, Moroberekan, P-0326 and TN-1 (DSI<1 & DTE>75%) was validated through biochemical as well as physiological characterization in the study. These traits showed a promise for selection parameters for the drought stress situation.

Key words: Correlation, drought susceptibility index, drought tolerance efficiency, path coefficient, genetic advance, upland rice and variance.

INTRODUCTION

Rice is the most important food crop for more than one third of world's population. To meet the needs of the growing population, the present annual rice production of 560 million tons must be increased to 850 million tons by

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2025 (Khush, 1997). Rice is probably the most diversely cultivated crop under varied environments including: (i) irrigated, (ii) rainfed sloping uplands, (iii) rainfed plain upland, and (iv) rainfed lowland to deepwater conditions. Among various abiotic stresses, drought is one of the basic factors for restricting crops production (Vallivodan and Nguyen, 2006; Sinclair, 2011). As being semi-aquatic plant, it consumes huge amount of water for its life cycle that is, two to three times more water than other food crops such as wheat or maize (Barker et al., 2003). According to an estimate, approximately 3000 to 5000 l of water are required to produce one kg of rice. Irrigated rice is the most common rice ecosystem by occupying 55% of the total 158 Mha of cultivated rice area, while rainfed lowland rice harbours (34%) 54 Mha, rainfed upland (9%) 14 Mha and flood-prone rice areas (7%) 11 Mha (Bouman et al., 2007). Rainfed rice occupies 40% area of the total rice area in Asia. South Asia alone holds 37% of the world's rice area, 50% of which is rainfed (Dawe et al., 2010). The upland ecosystem presents 12% of global rice area, which is the lowest yielding rice ecosystem (Khush, 1997). Rainfed rice accounts for 84% of total rice area in Sub-Saharan Africa (Gauchan and Pandey, 2011).

The recent scenario of global climate change and unpredictable rainfall patterns lead to severe drought spells in rain-fed areas. Even though water occupies almost 70% of our planet, freshwater resources are limited. According to an estimate, amount of crop water consumption will increase by 70 to 90% in 2050 and will reach 12,050 to 13,500 cubic kilometers from the present 7,130 cubic kilometres (de Fraiture et al., 2007). Therefore, it is highly likely that in the future, rainfed rice-growing areas will face severe spells of drought stress, consequently with high yield decline. To ensure the food security and reduce the water requirement for crop growth, development of drought tolerant and water-saving rice varieties has become increasingly important particularly to upland conditions in African as well as Asian countries (Levitt, 1980 and Graff, 1980). Plant water status has a major impact on plant function and adaptive processes under water limited environments. Several studies of past have reported that the major control of plant water status to crop plants subject to drought is exerted mainly by constitutive traits (Blum, 2005). These traits may help to maintain high relative water content and tend to retain higher leaf area and low leaf rolling. Multidisciplinary approach involving genetics, biochemistry, biotechnology, physiology, plant breeding and crop science will be appropriate to assess the complicated and integrated responses of plants to drought and to evolve superior drought resistant genotypes (Mitra, 2001).

It is reported that biochemical and physiological changes occur in response to low water condition in different plants. There is increase in several biochemical components like increase of free proline occurs in decrease in water supply (Zhang et al., 2006). The amount of proline in rice (*Oryza sativa* L.) was also increased steadily in salt stress using 24-epibassinolide which causes proline gene expression (Ozdemir et al., 2004). Although plant resistant mechanisms are not known clearly, new proteins accumulation and stress genes expression that code biosynthetic enzymes against osmotic stress were investigated (Vallivodan and Nguyen, 2006). Generally, drought stress reduces growth (Levitt, 1980) and yield of various crops (Dhillon et al., 1995) by decreasing chlorophyll pigments and photosynthetic rate, and stomatal conductance as well as transpiration rates. Drought stress reduces the nutrient uptake in plants (Kamran et al., 2009).

To improve rice production under water stress productivity, it is necessary to understand the mechanism and changes in the biochemical and molecular component of plant responses to drought conditions. Furthermore, the functional significance of the physiological and biochemical traits and their relationship to sustain grain yield are still not clearly established in rice. Therefore, present study was undertaken to analyze the biochemical traits which confer grain yield of rain fed lowland rice genotypes under irrigated as well as drought exposed at flowering stages in order to identify drought tolerant genotypes as well as their genotypic and phenotypic inter-relationships with grain yield.

MATERIALS AND METHODS

Experimental sites, genotypes and years of screen

The present investigation was carried out in wet season under normal (E_1) as well as flowering stage drought (E_2) condition during 2005 to 2006 and 2006 to 2007 at the instructional farm of Department of Crop Physiology, N. D. University of Agriculture and Technology, Faizabad (U.P.), India. The eighteen genotypes of upland rice from different geographical regions were screened for drought tolerance (Table 1).

Experimental design

The genotypes were seeded in dry beds and one seedling per hill transplanting was done at 21 days after seeding in randomized block design with three replications in 20 × 15 cm spacing of 3 m row length. All recommended agronomic practices were followed for a good crop at optimum level.

Water management

The experiments were conducted with well defined protocol for water management under two environmental conditions (E_1 and E_2) in the wet season as follows:

Irrigated (E_1)

The experimental field was left uncovered to receive natural rainfall as well as also irrigated with normal water, as and when required, to maintain appropriate moisture levels as recommended for irrigated rice.

Table 1. The place of origin and salient features of genotypes.

S/No.	Cultivar	Group	Salient feature
1	Moroberekeen	Japonica	Upland cultivar, coarse grain, high grain yielder, broad leaf, selection landraces
2	Azucena	Japonica	Highly green broad leaf, drought tolerant, coarse grain
3	TN-1	Japonica	Susceptible for multi disease and insect dwarf plant, low grain yield, short gold grain
4	IR-64	Indica	Highly susceptible for drought, tiny fine grain semi dwarf
5	Vandana	Indica	Upland cultivar, tall plant and drought tolerant
6	NDR-359	Indica	Irrigated (ecology) long gold high grain yielder semi dwarf plant
7	NDR-97	Indica	Upland cultivar, dwarf plant, short duration, drought tolerant (escaping fine grain, eating quality good)
8	Saita	Indica	Highly susceptible for drought and sheath blight, semi dwarf plant, land races
9	DGI-21	Indica × Japonica	Double haploid
10	DGI-75	Indica × Japonica	Double haploid
11	DGI-138	Indica × Japonica	Double haploid
12	DGI-152	Indica × Japonica	Double haploid
13	DGI-379	Indica × Japonica	Double haploid
14	DSU-18-6	Indica × Japonica	Double haploid
15	P 0088	IR64 introgression line	Introgression line
16	P 0090	IR64 introgression line	Introgression line
17	P 0326	IR64 introgression line	Introgression line
18	P 0397	IR64 introgression line	Introgression line

Reproductive stage drought stress (E_2)

The experiments were laid out in rainout shelter at a height of 10 to 12 feet using polythene sheets to exclude any possibility of natural rainfall falling in the experimental plots with proper drainage channel. Care was taken to check the inflow or seepage of water from the adjoining areas by making adequate bunds around the experiment and covered with polythene in drought condition. The heading stage drought was created with holding the irrigation for 15 days up to 80 K Pa at 0 to 15 cm soil profile and 60 K Pa at 30 cm soil depth. Soil moisture content (SMC) during stress period was monitored through fabricated soil tensio meter, periodically.

Observation and evaluation

Observations were recorded on five competitive plants of the middle row of each plot for 18 biochemical traits and grain yield on the basis of plot grain yield (gram m^{-2}). The biochemical traits that is, chlorophyll content (mg g^{-1} fresh weight), protein content (mg g^{-1} fresh weight), carbohydrate content (mg g^{-1} dry weight), starch content (mg g^{-1} dry weight), proline content (mg g^{-1} fresh weight), nitrate reductase activity (µmol NO_2^- g^{-1} fresh weight h^{-1}) and α-amylase activity (Unit g^{-1} fresh weight h^{-1}) were estimated according to Arnon (1949), Lowery et al. (1951), Yemm and Willis (1954), Mc Cready et al. (1950), Bates et al. (1973), Asada et al. (1974), Jowarski (1971) and Chance and Maechly (1955), respectively. The total protein was isolated in sodium phosphate buffer (0.5 M, pH 7.0) and protein was separated 12% SDS-PAGE as method described by Laemmli (1970).

The pooled data of two year under E_1 and E_2 were analyzed by appropriate statistical analysis (Gomez and Gomez, 1984) using Crop Stat 7.2 (IRRI, 2009) programme. Phenotypic (PCV) and genotypic (GCV) coefficient of variation, path coefficient, heritability (broad sense) and genetic advance in percent of mean were analysed according to Singh and Chaudhary (1985). Drought tolerance efficiency [DTE (%) = (Grain yield under stress/Grain yield under non-stress) x 100] and drought susceptibility index (DSI) was computed according to Fischer and Wood (1981) and Fischer and Maurer (1978) to identify the promising genotypes for limited irrigation, respectively.

RESULTS

Analysis of variance (ANOVA)

Significant differences were observed among the genotypes and also among the G × E (Genotypes × Environments) interactions for all the traits under both the conditions. The results of analysis of variance have been presented in Table 2.

Means and range of biochemical traits under observations

The grand mean of all traits for two environments over the seasons showed lower value under E_2 compared to E_1 except proline and α-amylase which represents the sensitivity of these traits to the stress. However, differences in mean values of all the characters were higher except carbohydrate and chlorophyll, which showed marginal differences between the two environments (Table 3).

Table 2. Analysis of variance (ANOVA) for 18+1 traits in upland rice under irrigated (E_1) as well as flowering stage drought (E_2) environment (E).

Source of variation	d.f	E	Chl a	Chl b	Proline	NR	α Amylase	CHO at 50% F	Starch at 50% F	CHO at M	Starch at M	Leaf's CHO	Leaf's starch	Upper root's CHO	Lower root's CHO	Lower root's starch	Grain yield
Environments (Years)	1	E_1	0.007	0.00126	7.92	2966.25*	584.00	42.50	11.50	44.25	151.7	83.56	7.78	0.0004	0.0018	0.0000008	0.0007
		E_2	0.000005	0.0009	1.39	1776.37**	3512.00	36.75	8.87	228.25	20.43	34.43	5.78	0.00003	0.000001	0.00001	0.0001
Replications (R)	2	E_1	0.00002	0.00002	0.0117	9.00	8.00	45.00	31.87	7.75	84.37	2.68	106.25	0.00008	0.0001	0.00006	0.0004
		E_2	0.00003	0.00006	0.171	28.25	30.00	88.12	7.12	10.37	129.00	16.78	45.44*	0.000008	0.00001	0.00004	0.00004
E x R	2	E_1	0.0001	0.000008	0.0351	15.62	3.00	1.500	20.62	6.12	0.812	5.34	2.48	0.000008	0.00002	0.00001	0.0001
		E_2	0.00001	0.00002	0.0703	7.12	10.00	28.00	44.50	9.78	2015	24.17	3.37	0.000001	0.000003	0.000006	0.00001
Genotypes (G)	17	E_1	0.0133**	0.0216**	173.86**	597.39	14437.76**	3068.30**	3149.95**	4636.74**	8124.90**	666.16**	835.64**	0.01**	0.008**	0.0109**	0.00285**
		E_2	0.0635**	0.0164**	59.38**	1530.07**	10614.98**	9814.20**	6475.24**	4965.41**	10261.33**	875.91**	519.49**	0.0039**	0.005**	0.00291**	0.00318**
G x E	17	E_1	0.00300**	0.00290**	1.88**	441.65**	1365.64**	200.24**	78.15**	560.03**	94.85**	35.32**	30.53*	0.00009	0.00028**	0.0000120	0.0004**
		E_2	0.00187**	0.00148**	1.20**	207.95**	1885.01**	209.29**	40.13**	505.76**	42.57**	13.92**	13.27**	0.0001**	0.0001**	0.000015*	0.00008**
Error	68	E_1	0.00009	0.00002	0.033	328.34	13.79	6.73	19.23	8.00	9.05	8.82	5.65	0.00001	0.0006	0.000019	0.00043
		E_2	0.00004	0.00002	0.105	12.19	16.47	19.62	6.68	6.38	6.32	6.52	5.21	0.000006	0.00002	0.000007	0.000005

* Significant at 5% level, ** Significant at 1% level, Chl a = Chlorophyll a, Chl b = Chlorophyll b, NR = Nitrate reductase, CHO = Carbohydrate, F = Flowering stage, M = Maturity stage, d.f = degree of freedom.

Phenotypic (PCV) and genotypic (GCV) coefficient of variation

The phenotypic and genotypic coefficients of variations for all biochemical and physiological traits observed in this study have been presented in Table 3. Differences, between PCV and GCV were quite low for all the characters except nitrate reductase activity, upper and lower root's starch under E_1 (Table 3).

Heritability (h^2_b) and genetic advance (Ga)

High heritability coupled with high genetic advance (determining scale from Johnson et al., 1955a) were estimated for chlorophyll b, proline, carbohydrate and starch at flowering, carbohydrate and starch at maturity, leaf's carbohydrate and starch, upper and lower root's carbohydrate under both the conditions. Simultaneously, upper root's starch, nitrate reductase and grain yield under E_1 and lower root's starch and chlorophyll a under E_2 showed high heritability (>75.0) and genetic advance (>30.0) while rest traits exhibited indefinite combinations (Table 3).

Drought tolerance parameters

There was the wide range of corresponding variations of "drought susceptible index (DSI) with drought tolerant efficiency (DTE) in all the genotypes for all the traits e.g. from 0.42 (Vandana) to 1.99 (Saita) of DSI while correspondingly from 46.49 (Saita) to 88.66 (Vandana) of DTE for grain yield. Out of 18 genotypes; six, eleven and one genotype (s) showed below average (ADSI<1), above average (ADSI>1) and average (ADSI~1) to drought susceptibility for grain yield, respectively (Table 6). Drought susceptible index with high grain yield potential can be used to identify parents to improve the performance of rice under variable moisture conditions (Ouk et al., 2006). The genotypes with high DTE and low DSI recorded minimum grain yield reduction (Bahar and Yildirim, 2010 and Parameshwarappa et al., 2010). Drought tolerant parameters- DTE and DSI have been presented in Table 4a,b.

Table 3. Estimates of grand mean, range, phenotypic (PCV) and genotypic (GCV) coefficients of variation, heritability in broad sense (h^2_b) and genetic advance in per cent of mean (Ga) for 18+1 characters in rice germplasm lines under irrigated (E_1) and flowering stage drought (E_2) conditions.

Characters	Environments	Grand mean	Range	Coefficient of Variation		h^2_b (%)	Ga (in % of mean)
				PCV	GCV		
Chlorophyll a	Irrigated	0.783 ±0.025	0.73-0.87	5.45	5.30	94.60	10.21
	Drought	0.521 ±0.024	0.25-0.66	19.51	19.47	99.60	40.30
Chlorophyll b	Irrigated	0.259 ±0.023	0.21-0.45	18.97	18.91	99.30	42.47
	Drought	0.209 ±0.010	0.13-0.35	24.02	23.91	99.10	47.84
Proline	Irrigated	23.03 ±2.11	17-34	23.26	23.25	99.90	47.85
	Drought	33.68 ±1.62	27-38	9.30	9.25	98.90	18.44
Nitrate reductase	Irrigated	168.00 ±5.78	148-181	14.87	14.48	94.80	30.03
	Drought	102.52 ±4.22	76-128	4.56	3.03	44.20	4.14
α Amylase	Irrigated	512.2±17.10	410-581	9.14	9.11	99.40	18.70
	Drought	655.82 ±22.22	568-735	5.85	5.82	98.90	11.99
Carbohydrate at 50% flowering	Irrigated	210.11 ±6.48	166-245	10.95	10.87	98.60	21.28
	Drought	145.31 ±3.77	78-218	27.70	27.54	98.80	56.37
Starch at 50% flowering	Irrigated	151.60 ±3.12	113-192	15.20	14.93	96.40	30.18
	Drought	112.10 ±2.09	60-158	29.31	29.22	99.40	60.00
Carbohydrate at maturity	Irrigated	140.62 ±2.66	119-214	18.65	18.54	98.80	37.96
	Drought	94.31 ±1.53	38-140	29.03	28.91	99.10	59.29
Starch at maturity	Irrigated	131.24 ±3.26	83-195	27.97	27.87	99.30	57.23
	Drought	88.88 ±2.09	33-180	46.52	46.43	99.60	75.47
Leaf's Carbohydrate	Irrigated	68.63 ±2.66	43-85	15.55	14.94	92.30	29.56
	Drought	48.00 ±1.53	35-71	25.53	24.97	95.70	50.30
Leaf's starch	Irrigated	46.95 ±3.54	32-74	25.19	24.67	96.00	49.79
	Drought	34.14 ±2.25	23-53	27.72	26.91	94.20	53.78
Upper root's Carbohydrate	Irrigated	0.175 ±0.018	0.12-0.24	25.15	25.06	99.30	51.42
	Drought	0.123 ±0.016	0.09-0.19	20.55	20.45	99.00	40.75
Upper root's Starch	Irrigated	0.214 ±0.007	0.14-0.32	23.72	23.37	97.10	49.18
	Drought	0.122 ±0.004	0.05-0.21	21.26	17.57	68.30	28.03
Lower root's Carbohydrate	Irrigated	0.129 ±0.008	0.06-0.21	33.26	33.12	99.00	69.76
	Drought	0.079 ±0.006	0.03-0.11	27.84	27.61	98.40	50.63
Lower root's Starch	Irrigated	0.104 ±0.009	0.08-0.16	27.91	19.32	47.90	28.84
	Drought	0.068 ±0.005	0.04-0.11	33.81	33.64	99.00	73.83
Grain yield	Irrigated	520.69 ±7.12	390-664	16.70	16.69	99.90	34.36
	Drought	371.57 ±6.68	228-550	25.37	25.35	99.90	52.19

Table 4(a). Drought tolerance parameters [i.e. drought susceptible index (DSI), drought tolerance efficiency (DTE) as well as per cent increase in α-amylase, proline and leaf water potential (LWP)] of 18 upland rice genotypes including Azucena (DT Check) and IR 64 (DS Check) under flowering stage drought condition.

Genotypes	Proline		Chlorophyll a		Chlorophyll b		α Amylase		Upper root starch		Lower root starch		Upper root sugar		Lower root sugar		Leaf starch		Shoot starch	
	DSI	DTE %	DSI	DTE %	DSI	DTE %	DSI	DTE %	DSI	DTE %	DSI	DTE %	DSI	DTE %	DSI	DTE%	DSI	DTE%	DSI	DTE%
Azucena	0.38	114.0	0.69	76.74	0.78	76.67	0.41	109.6	0.34	85.71	0.67	76.92	0.33	89.47	0.51	80.95	0.51	85.76	0.45	85.77
DGI-138	0.82	130.7	1.00	66.23	0.79	76.19	1.76	141.0	0.69	70.59	1.28	56.25	0.45	85.71	0.67	75.00	0.41	88.39	0.80	74.40
DGI-152	1.61	159.9	0.98	67.09	1.18	64.71	1.09	125.5	1.18	50.00	1.22	58.33	1.33	58.33	0.89	66.67	1.28	63.95	1.49	52.59
DGI-21	1.83	168.4	0.75	74.67	0.73	78.26	1.77	141.5	1.01	57.14	0.32	88.89	0.21	93.33	1.23	53.85	1.29	63.75	1.34	57.40
DGI-379	0.64	123.7	1.13	62.07	0.90	72.97	0.07	101.6	1.30	45.00	1.25	57.14	0.68	78.57	0.97	63.64	1.03	70.97	1.88	39.96
DGI-75	1.73	164.5	1.27	57.14	0.90	73.08	1.49	134.9	0.82	65.00	1.86	36.36	1.43	55.00	1.33	50.00	1.39	61.05	0.94	70.12
DSU-18-6	1.50	156.1	1.29	56.58	0.64	80.77	1.99	146.6	1.02	56.52	0.65	77.78	1.51	52.63	0.41	84.62	0.91	74.31	0.69	77.95
IR-64	2.20	182.0	1.56	47.67	1.73	48.15	1.49	134.9	0.74	68.42	0.67	76.92	1.36	57.14	1.40	47.62	1.52	57.36	2.13	32.16
Moroberekan	0.31	111.4	0.59	80.00	0.56	83.33	0.87	120.2	0.96	59.09	1.62	44.44	0.91	71.43	0.82	69.23	1.13	68.22	0.31	90.22
NDR-359	1.79	166.6	0.63	78.95	0.92	72.41	0.99	123.1	1.74	26.32	0.37	87.50	0.53	83.33	0.59	77.78	0.90	74.72	0.40	87.34
NDR-97	0.33	112.3	0.52	82.67	0.76	77.14	0.93	121.7	0.50	78.57	0.27	90.91	0.49	84.62	1.23	53.85	0.84	76.31	0.23	92.55
P-0088	0.18	106.7	1.25	58.11	1.04	68.97	1.52	135.5	0.74	68.42	0.80	72.73	1.12	64.71	0.24	90.91	0.77	78.47	0.85	72.82
P-0090	0.57	121.3	1.21	59.26	1.36	59.26	1.15	126.8	0.81	65.63	1.62	44.44	1.36	57.14	0.67	75.00	0.43	87.85	1.40	55.43
P-0326	0.55	120.6	0.70	76.39	0.74	77.78	0.47	111.0	0.45	80.95	0.65	77.78	0.49	84.62	0.44	83.33	0.89	75.01	0.48	84.87
P-0397	2.34	187.3	1.13	62.03	1.84	44.83	1.13	126.5	1.18	50.00	1.62	44.44	0.93	70.83	1.33	50.00	1.73	51.46	0.79	74.88
Saita	2.22	182.9	1.97	33.78	1.23	63.16	1.88	144.1	1.27	46.15	1.62	44.44	0.80	75.00	1.14	57.14	0.87	75.56	1.81	42.43
TN-1	2.18	181.3	0.71	76.19	1.03	69.23	0.58	113.6	1.01	57.14	0.97	66.67	1.46	54.17	1.33	50.00	1.18	66.75	1.36	56.60
Vandana	0.37	113.7	0.58	80.52	0.74	77.78	0.75	117.5	0.90	61.90	0.29	90.00	1.59	50.00	0.59	77.78	0.55	84.45	0.40	87.35

Genotypic correlation coefficients under irrigated (r_{gE1}) and flowering stage drought (r_{gE2}) condition

Amongst various component traits, the traits namely chlorophyll A, chlorophyll B, nitrate reductase activity, carbohydrate at flowering stage and leaf's carbohydrate exhibited significant positive association with grain yield. While, some other traits namely starch at flowering, carbohydrate at maturity, starch at maturity, upper root's carbohydrate and lower root's starch exhibited significant positive association with above mentioned traits other than grain yield. Results of the genetic correlations have been presented in Table 4.

Direct and indirect effects under irrigated (E_1) and flowering stage drought (E_2) condition

The traits namely chlorophyll A, proline content, carbohydrate at flowering, starch at maturity, leaf's carbohydrate and lower's root starch showed low to high (determined from the scale of Lenka and Mishra, 1973) positive direct effect on grain yield under both environment. All the traits exhibited somehow indirect effect on grain yield through other traits (Table 5).

Plant water status and proline accumulation

Strong positive regression coefficient were obtained between grain yield and RWC (r = 0.52) and almost all the genotypes recorded low DSI for RWC in present investigation. Grain yield was also positively and significantly correlated (r = 0.78) with proline under water stress situation. Leaf water potential (LWP) was positively associated with accumulation of proline and grain yield under drought stress. Similarly, we found strong negative correlation between RWC *vs.* sterility and LWP *vs.* sterility.

Protien profiling

SDS-PAGE analysis revealed differences between the protein patterns of drought stressed

Table 4(b). Drought tolerance parameters that is, drought susceptible index (DSI), drought tolerance efficiency (DTE) as well as per cent increase in α-amylase, proline and leaf water potential (LWP)] of 18 upland rice genotypes including Azucena (DT Check) and IR 64 (DS Check) under flowering stage drought condition.

Genotypes	Nitrate reductase		Leaf carbohydrate		Shoot carbohydrate		RWC		Biomass		Harvest index		Test Weight		Grain Yield		Per cent increase		
	DSI	DTE%	DSI	DTE%	DSI	DTE%	DSI	DTE%	DSI	DTE%	DSI	DTE%	DSI	DTE%	DSI	DTE%	αAmylase	Proline	LWP
Azucena	0.35	86.07	0.41	87.78	0.19	91.64	1.05	79.47	0.84	75.77	0.83	82.42	0.58	91.42	0.55	85.08	20.26	11.46	66.10
DGI-138	1.18	52.91	0.70	78.84	0.56	74.74	1.24	75.85	0.53	84.93	1.12	76.35	1.01	84.90	1.17	68.61	41.08	30.78	27.94
DGI-152	1.17	53.43	1.61	51.70	1.21	45.65	0.75	85.30	1.43	58.95	1.00	78.80	1.19	82.28	1.50	59.58	25.49	59.94	23.36
DGI-21	0.54	78.68	0.95	71.46	0.55	75.38	1.39	72.86	1.11	68.18	0.88	81.49	0.62	90.69	1.34	63.99	41.54	68.49	15.73
DGI-379	0.77	69.52	1.52	54.33	0.96	56.89	0.08	98.51	1.10	68.36	0.74	84.30	1.26	81.17	1.45	60.90	5.60	23.73	47.83
DGI-75	0.84	66.48	1.51	54.72	1.43	35.44	1.21	76.46	0.87	74.99	0.97	79.60	0.41	93.96	1.46	60.61	34.98	64.56	79.31
DSU-18-6	1.11	55.81	0.86	74.29	0.27	87.75	0.90	82.51	0.92	73.48	0.88	81.47	0.92	86.26	1.09	70.72	46.68	56.17	31.34
IR-64	0.98	61.12	1.26	62.10	1.16	47.67	0.30	94.08	1.44	58.58	1.41	70.18	1.09	83.78	1.27	65.74	34.96	82.00	73.91
Moroberekan	0.82	67.24	0.62	81.41	0.84	62.08	0.79	84.56	0.41	88.10	0.46	90.34	0.81	87.86	0.60	83.88	9.66	55.00	12.16
NDR-359	0.63	75.03	0.51	84.71	0.29	86.75	0.95	81.47	0.47	86.49	0.70	85.22	1.23	81.61	0.47	88.45	23.16	66.67	25.42
NDR-97	0.60	76.09	0.59	82.28	0.20	91.01	0.63	87.69	1.38	60.27	1.19	74.89	0.71	89.38	1.28	60.38	21.75	12.31	19.57
P-0088	1.38	44.94	1.19	64.30	1.70	23.40	1.79	65.07	1.48	57.48	1.30	72.51	1.21	81.96	1.47	60.38	35.52	6.72	87.67
P-0090	1.74	30.90	1.37	58.77	0.44	79.98	0.64	87.57	0.89	74.44	0.89	81.25	0.95	85.85	1.41	62.11	26.81	21.38	28.79
P-0326	1.00	60.02	0.49	85.38	0.36	84.00	1.14	77.69	1.14	67.17	0.70	85.14	0.50	92.50	0.74	80.10	10.99	20.69	23.77
P-0397	1.12	55.48	1.45	56.44	1.98	11.00	1.33	74.10	1.14	67.31	1.23	74.08	1.20	82.09	1.36	63.40	26.53	87.32	80.30
Saita	1.34	46.75	1.83	44.94	1.75	21.20	1.67	67.50	1.63	53.16	1.53	67.65	2.72	59.52	1.99	46.49	44.10	82.99	92.65
TN-1	1.26	49.95	0.59	82.30	0.32	85.71	1.85	63.88	0.30	91.43	1.25	73.60	1.17	82.61	0.81	78.26	1.91	81.30	16.88
Vandana	0.67	73.16	0.34	89.80	0.19	91.50	0.33	93.66	0.59	82.97	0.93	80.46	0.24	96.47	0.42	88.66	17.52	43.74	17.86

and controlled condition's rice leaves proteins (Figure 1). A protein band of 25+2 kDa molecular weight was observed under control as well as drought stressed environment; while, this particular protein band was not observed in susceptible rice varieties exposed to drought stress. A specific protein band of 25+2 kDa was found in the stressed condition in NDR-97 and NDR-359, this protein was not observed in the controlled condition. Drought tolerant variety Vandana, showed constitutive expression of this particular protein in control as well as stress condition. However, this novel protein band was not present in the susceptible rice variety IR-64 in both control and drought condition.

DISCUSSION

The high and significant differences among genotypes as well as G × E reveals the existence of sufficient variability among the genotypes and over whelming effect of environment on genetic performance, respectively (Table 2). A significant range of variation was observed for the biochemical traits observed among different genotypes. However, widest range of variability was recorded for proline, nitrate reductase, α-amylase, carbohydrate and starch at flowering and maturity under E_1 and Chlorophyll B under E_2 (Table 3). The low difference between GCV and PCV of the traits except nitrate reductase activity, upper and lower root's starch indicates towards true genetic variability and this is also supported by higher values of heritability (Table 3). Therefore, selection on the basis of phenotype alone can be effective for the improvement of these traits. Girish et al. (2006) have also reported the influence of environment on the characters if the PCV is higher than GCV. Blum (1988) also reported the reduction in genetic variance under severe stress condition.

Table 5. Genotypic correlation coefficient among 15+1 traits in upland rice grown under irrigated (upper diagonal) as well as flowering stage drought (lower diagonal) condition.

Traits	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	r_{gE2}	0.115	-0.079	0.402	0.206	0.376	0.610*	-0.245	-0.344	0.164	0.144	0.480	0.012	0.414	0.255	-0.085
2	0.291		0.280	0.319	0.211	-0.237	-0.017	-0.159	-0.267	0.416	0.017	0.073	0.033	-0.014	-0.241	0.061
3	-0.330	0.065		-0.284	0.243	0.239	0.221	0.003	-0.008	0.162	-0.240	-0.301	-0.033	-0.121	-0.251	0.249
4	0.627*	0.473	0.060		-0.168	0.903**	0.493	0.444	-0.145	0.006	0.014	0.865	0.465	0.130	0.642**	-0.120
5	-0.096	0.164	0.358	-0.011		0.286	0.495	0.074	0.146	0.237	-0.274	-0.125	-0.284	0.510	0.147	-0.107
6	0.604*	0.165	0.111	0.432	-0.222		0.742**	0.131	0.005	0.246	0.310	0.551*	0.433	0.083	0.307	0.061
7	0.579*	-0.055	0.127	0.430	-0.285	0.844**		0.036	-0.016	0.234	0.204	0.328	0.227	0.172	-0.068	-0.375
8	0.356	-0.007	-0.032	0.234	-0.167	0.453	0.612*		0.712**	0.028	-0.156	0.221	-0.249	0.110	0.152	-0.253
9	0.355	0.121	-0.024	0.258	0.324	0.459	0.525*	0.827**		-0.228	-0.087	0.086	-0.360	0.234	-0.052	0.003
10	0.752**	0.432	0.000	0.818**	0.102	0.624*	0.627*	0.655**	0.486		0.387	0.089	-0.019	-0.106	-0.014	0.094
11	0.242	0.197	-0.188	0.116	-0.434	0.394	0.432	0.407	0.041	0.356		0.703**	0.348	-0.333	0.014	0.171
12	0.038	-0.535*	0.016	0.134	-0.303	-0.128	0.037	0.109	-0.098	-0.041	-0.157		0.361	-0.083	0.319	0.107
13	-0.250	-0.214	0.244	-0.497	-0.235	0.064	0.118	0.099	-0.137	-0.194	0.471	0.047		-0.152	-0.332	-0.318
14	-0.029	-0.157	0.267	-0.191	0.344	0.072	0.099	0.113	0.102	0.014	-0.239	0.026	0.174		0.226	-0.405
15	0.411	0.200	-0.097	0.463	0.010	0.191	0.173	0.448	0.282	0.594*	0.088	0.419	-0.170	0.222		0.333
16	0.726**	0.546*	-0.163	0.669**	-0.135	0.559*	0.333	.413	0.512	0.741**	0.207	-0.180	-0.252	-0.133	0.513	

1=Chlorophyll a; 2= Chlorophyll b; 3=Proline; 4=Nitrate reductase; 5= α Amylase; 6=Carbohydrate at 50% flowering; 7=Starch at 50% flowering; 8=Carbohydrate at maturity; 9=Starch at maturity; 10=Leaf's carbohydrate; 11=Leaf's starch; 12=Upper root's carbohydrate; 13=Upper root's starch; 14= Lower root's carbohydrate; 15=Lower root's starch; 16=Grain yield; * and ** Significant at 5% and 1% level [i.e. 0.514 and 0.641 r value from Fisher & Yates (1963)], respectively; r_{gE2} = Genotypic coefficient under irrigated condition; r_{gE2} = Genotypic coefficient under drought condition.

Heritability of some of the physiological, biochemical and root related traits were higher in present analysis as revealed in Table 3. In general, the character that shows high heritability with high genetic advance is controlled by additive gene action and selection is always effective only for that trait (Warkad et al., 2008). Starch at maturity, leaf's starch, upper root and lower root's carbohydrate in both the environments while proline in E_1 were the only traits which possessed very high estimates of phenotypic (PCV) and genotypic (GCV) coefficient of variation, heritability (h^2_b) and genetic advance (Ga) (Table 3). These characters could be considered as preferred selection criteria for irrigated and drought condition. Further, characters showing high heritability along with moderate or low genetic advance can be improved by inter mating superior genotypes of population developed from combination breeding (Samadia, 2005).

A close agreement between genotypic and phenotypic correlation in almost all the traits (data not presented in this paper) were found, which indicates least environment influence on the degree of association. Henceforth, reference is being made only to genotypic correlation (r_{gE2}). r_{gE2} were, in general, similar in nature and higher than the corresponding r_{gE1}; which reveals true genetical correlation of the traits with each other. The significant and positive correlation of chlorophyll a, chlorophyll b, nitrate reductase activity, carbohydrate at flowering stage and leaf's carbohydrate with grain yield was observed. Interestingly a significant positive correlation of above mentioned traits (starch at flowering, carbohydrate and starch at maturity, upper root's carbohydrate and lower root's starch) was observed with chlorophyll A, chlorophyll B, nitrate reductase activity, carbohydrate at flowering stage and leaf's carbohydrate indicating that grain yield and these traits has the same biochemical/genetical basis for their expression (Table 4). Since chlorophyll a, proline and carbohydrate content at flowering, starch at maturity, leaf's carbohydrate and lower's root

Table 6. Genotypic direct and indirect effects of biochemical traits on grain yield under irrigated (E_1) as well as flowering stage drought (E_2) condition.

Traits	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 E_1	1.123	-0.031	-0.021	-0.202	0.046	0.028	-0.609	0.289	-0.617	0.147	-0.18	0.27	0.011	-0.594	0.253
E_2	0.697	-0.138	-0.248	-0.085	0.071	-0.068	-0.816	-0.549	0.398	1.349	0.214	-0.005	0.079	-0.005	0.045
2 E_1	0.129	-0.266	0.074	-0.160	0.047	-0.018	0.017	0.188	-0.478	0.373	-0.022	0.041	0.029	0.344	-0.239
E_2	0.203	-0.475	0.049	-0.064	-0.123	-0.018	0.077	0.012	0.135	0.774	0.174	0.074	0.068	-0.027	0.022
3 E_1	-0.088	-0.074	0.264	0.143	0.054	0.018	-0.220	-0.003	-0.015	0.146	0.299	-0.170	-0.029	0.173	-0.249
E_2	-0.230	-0.031	0.750	-0.008	-0.267	-0.012	-0.180	0.049	-0.027	0.001	-0.166	-0.002	-0.077	0.046	-0.011
4 E_1	0.452	-0.085	-0.075	-0.503	-0.037	0.067	-0.492	-0.524	-0.260	0.005	-0.018	0.488	0.413	-0.186	0.636
E_2	0.437	-0.225	0.045	0.135	0.008	0.048	-0.607	-0.360	0.289	1.467	0.102	-0.019	0.157	-0.033	0.051
5 E_1	0.231	-0.056	0.064	0.085	0.222	0.021	-0.494	-0.087	0.262	0.212	0.341	-0.070	-0.252	-0.732	0.146
E_2	-0.067	-0.078	0.269	0.001	-0.745	0.025	0.402	0.258	-0.363	0.183	-0.384	0.042	0.074	0.060	0.001
6 E_1	0.422	0.063	0.063	-0.454	0.064	0.074	-0.740	-0.155	0.010	0.221	-0.387	0.311	0.385	-0.120	0.304
E_2	0.428	-0.078	0.083	-0.058	0.166	0.111	-1.190	-0.698	0.514	1.119	0.348	0.018	-0.020	0.013	0.021
7 E_1	0.686	0.004	0.058	-0.248	0.110	0.055	-0.998	-0.004	-0.028	0.210	-0.254	0.185	0.202	-0.247	-0.067
E_2	0.403	0.026	0.096	-0.058	0.212	-0.093	-1.411	-0.944	0.588	1.124	0.382	-0.005	-0.037	0.017	0.019
8 E_1	-0.275	0.042	0.001	-0.223	0.016	0.010	-0.036	-1.181	1.278	0.025	0.195	0.125	-0.221	-0.158	0.150
E_2	-0.248	0.004	-0.024	-0.032	0.125	-0.050	-0.864	-1.541	0.927	1.175	0.359	-0.015	-0.031	0.020	0.049
9 E_1	-0.386	0.071	-0.002	0.073	0.032	0.000	0.016	-0.841	1.794	-0.205	0.109	0.049	-0.320	-0.336	-0.052
E_2	0.247	-0.057	-0.018	-0.035	0.245	-0.051	-0.740	-1.275	1.120	0.871	0.037	0.014	0.043	0.018	0.031
10 E_1	0.184	-0.111	0.043	-0.003	0.053	0.018	-0.234	-0.033	-0.410	0.897	-0.482	0.050	-0.017	0.152	-0.014
E_2	0.524	-0.205	0.000	-0.110	-0.076	-0.069	-0.885	-1.009	0.544	1.793	0.315	0.006	0.061	0.003	0.065
11 E_1	0.164	-0.005	-0.063	-0.007	-0.061	0.023	-0.203	0.184	-0.157	0.347	-1.247	0.396	0.309	0.478	0.014
E_2	0.169	-0.094	-0.141	-0.016	0.324	-0.044	-0.609	-0.626	0.046	0.638	0.884	0.022	-0.148	-0.041	0.010
12 E_1	0.539	-0.019	-0.079	-0.435	-0.028	0.041	-0.327	-0.261	0.155	0.079	-0.877	0.564	0.321	0.119	0.316
E_2	0.026	0.254	0.012	-0.018	0.226	0.014	-0.052	-0.167	-0.110	-0.074	-0.139	-0.039	-0.015	0.004	0.046

Table 6. Contd.

13	E_1	0.014	-0.009	-0.009	-0.234	-0.063	0.032	-0.227	0.294	-0.645	-0.017	-0.434	0.204	0.888	0.218	-0.329
	E_2	-0.174	0.102	0.183	-0.067	0.175	-0.007	-0.166	-0.153	-0.154	-0.348	0.416	-0.007	-0.315	0.030	-0.019
14	E_1	0.465	0.064	-0.032	-0.065	0.113	0.006	-0.172	-0.130	0.420	-0.095	0.415	-0.047	-0.135	-1.435	0.224
	E_2	-0.020	0.075	0.201	0.026	-0.256	-0.009	-0.140	-0.175	0.114	0.026	-0.211	-0.004	-0.055	0.174	-0.024
15	E_1	0.287	0.064	-0.066	-0.323	0.033	0.023	0.068	-0.179	-0.093	-0.013	-0.018	0.180	-0.295	-0.324	0.990
	E_2	0.286	-0.095	-0.073	-0.062	-0.008	-0.021	0.244	-0.690	0.316	1.065	0.078	-0.058	0.053	0.039	0.110

1=Chlorophyll a; 2= Chlorophyll b; 3=Proline; 4=Nitrate reductase; 5=α Amylase; 6=Carbohydrate at 50% flowering; 7=Starch at 50% flowering; 8=Carbohydrate at maturity; 9=Starch at maturity; 10=Leaf's carbohydrate; 11=Leaf's starch; 12=Upper root's carbohydrate; 13=Upper root's starch; 14= Lower root's carbohydrate; 15=Lower root's starch; Note: Under lined digits denote the direct effects; Residual effect is -0.231 and 0.149in irrigated and flowering stage drought condition, respectively.

starch showed low to high positive direct effect on grain yield under E1 and E2 these traits could also be focused. Following, Singh and Chaudhary (1985) some conclusions from correlation coefficient (Table 4) and path coefficient (Table 5) α analyses were drawn- (i) Chlorophyll A, nitrate reductase activity, carbohydrate at flowering, starch at maturity, leaf's carbohydrate, leaf's starch and lower root's starch showed positive and high (desired) genotypic correlation as well as direct effect on grain yield. These traits could therefore be considered as a preferred one for direct selection; (ii) Proline content and lower root's carbohydrate showed negative correlation and positive direct effect. Here restricted selection index is required in which only selected traits will be considered (Singh and Kakar, 1977) and (iii) Less amount of residual effect (that is, 0.149 and -0.231 under drought and irrigated condition, respectively) reveals that the sufficient yield contributing traits have been included in the study. The mean values of DSI, for most of the characters were close to or below one, indicated the relative tolerance of these traits to drought. Higher DSI values, observed for proline (DSI=1.20), amylase (DSI=1.13) and grain yield (DSI=1.13), indicated that these traits were relatively more prone to drought stress. In parallel, most of findings (Ouk et al., 2006) showed that lowest DSI values were more tolerant than with the highest DSI. In this study, statistically significant correlations were obtained between grain yield and DSI under both the conditions. Thus, positive correlation (r= 0.511**) was shown between grain yield under irrigated and DSI while negative correlation (r= -0.771**, $p < 0.05$) between grain yield under drought and DSI. Results of this study have showed a parallelism with Ouk et al. (2006). Similarly, grain yield under drought was significantly correlated with DTE (0.757**); while negative and significant correlation (-0.903**) was found between DSI and DTE. These results are similar with that of Bahar and Yildirim (2010). Similar trends, of correlation between *per se* performance and drought resistance parameters (DSI and DTE), were found for most of the characters under study. Plant breeders may select varieties capable of producing relatively high grain yields in both favourable and unfavourable environments/years (Bernier et al., 2008).

Positive correlation between grain yield and RWC was observed in the present study. Previous reports suggested RWC to be an important parameter though not sufficient to ensure good grain yield (Lafitte, 2002). Nguyen et al. (1997) reported the consistent differences in osmotic adjustment among rice genotypes at a RWC of 75 percent. Similar to RWC, grain yield was positively and significantly correlated (r = 0.78) with proline accumulation under water stress in the present study. It was also observed that genotypes *viz.,* TN 1, Vandana, Azucena, NDR 359, DSU 18-6 and Moroberekan, recorded highest RWC, accumulated more proline (in percent) and had a lower DSI values for grain yield whereas the genotypes, recorded lowest RWC, had *vice versa* results. Similar results were reported by Bayoumi et al. (2008). The involvement of proline in the response to water deficit has been demonstrated and suppression of proline synthesis resulted in increased sensitivity to water deficit (Valliyon and Nguyen, 2006). In present study, leaf water potential (LWP) was positively associated with accumulation of proline and grain yield under drought stress. Similarly, we found strong negative correlation between RWC *vs.* sterility and LWP *vs.* sterility. O'Toole and Namuco (1983)

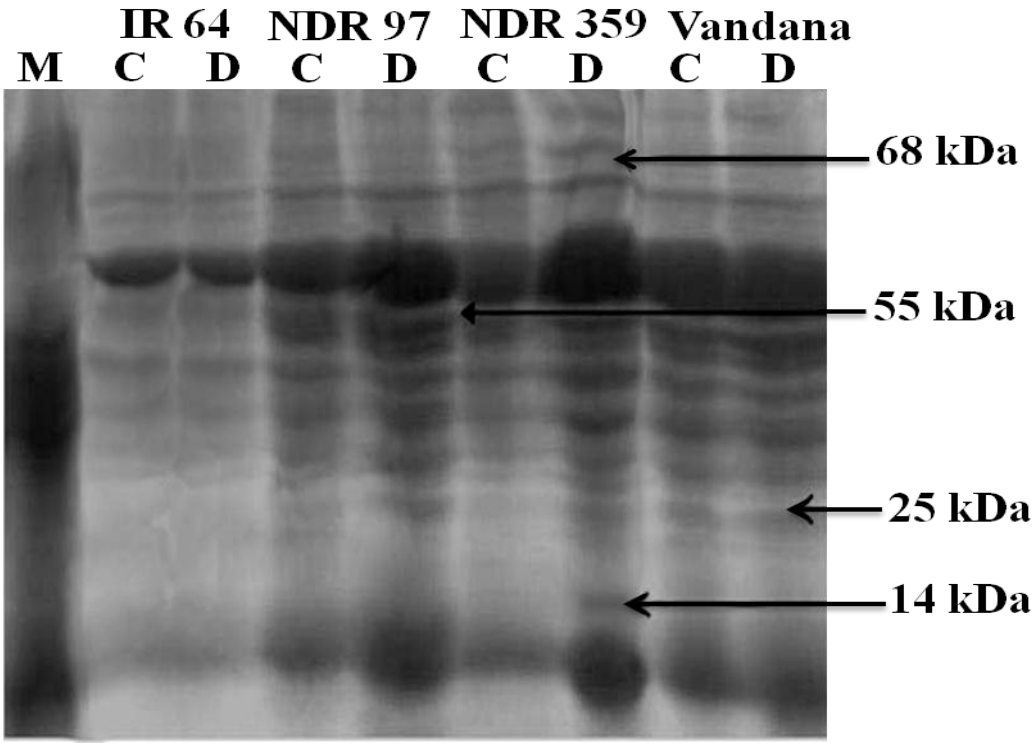

Figure 1. SDS-PAGE profiling of the susceptible(IR64), and drought resistance rice varieties (NDR97, NDR-359 and Vandana) in control (c) and drought (D) condition.

found that panicle exertion rate was decreased linearly with decrease in water potential and subsequently grain yield also. However, genotypic differences in proline accumulation may be simply a reflection of respective differences in leaf water potential (Dingkuhn et al., 1991). Thus, studies indicated that capacity to maintain high LWP is promising traits for selection to improve tolerance against late season drought in rainfed upland rice. In above view, genotypes selected as drought resistance for grain yield *viz*., NDR 359, DSU 18-6, Vandana and Moroberekan were considered as best among the top genotypes with low DSI (<1) for all other biochemical and physiological traits under study.

The presence of protein band of 25+2 kDa in drought tolerant cultivars- NDR-97, NDR-359 and Vandana and its absence in IR64 can be due to induction of specific proteins involved in stress tolerance/response in the resistance rice varieties. Beside their specific functions, proteins which are accumulated in the plants by stress exposure may provide a storage form of nitrogen that is reutilized when stress is over and probably play a role in osmotic adjustment (Niknam et al., 2006; Ahmad et al., 2007). The disappearance of a protein band/expression under waters stress seems to be due to ionic component which is a conserved response among different rice cultivars. The observed difference between the intensity of a protein band suggests a probable role of this protein in drought tolerance. Also, the induction of some protein bands under stress treatments, which exclusively occurred in NDR 369, may play a role in higher osmotic stress tolerance of NDR357 compared to IR64.

The establishment of managed drought conditions by rainout shelter allows rice research workers to select drought tolerant genotypes by following drought susceptibility index and drought tolerance efficiency as the important parameters. A comprehensive screening of physiological and biochemical traits during drought stress will advance our fundamental understanding of these traits and provide direction for future strategies for drought tolerance in rice. Breeding procedures like pureline selection, pedigree breeding method would be effective for development of drought tolerant genotypes. Vandana, as drought tolerant cultivar (Bernier et al., 2008 and Acuna et al., 2008), is grown in drought prone upland of eastern India. Thus, improvement in it's *per se* performance and drought tolerance could be of significant for food security under target environments. Saita, as displaying concerned opposite traits, must be used to develop mapping population. Complex nature of upland drought suggested that multi environment testing and selection is necessary for NDR 359, DSU 18-6, Vandana and Moroberekan to develop high yield potential with drought tolerance.

Abbreviations: DTE, Drought tolerance efficiency; **DSI,** drought susceptibility index; **GCV,** genotypic coefficient of

variation; **G × E,** Genotypes × Environments; **PCV,** phenotypic coefficient of variation; **RWC,** relative water content; **SDS-PAGE:** sodium dodecyl sulphate poly acrylamide gel electrophoresis; **SMC,** soil moisture content.

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Review

Biocontrol potentiality of plant growth promoting bacteria (PGPR) - *Pseudomonas fluorescens* and *Bacillus subtilis*: A review

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Plant growth in agricultural soils is influenced by many abiotic and biotic factors. There is a thin layer of soil immediately surrounding plant roots that is an extremely important and active area for root activity and metabolism which is known as rhizosphere. The rhizosphere concept was first introduced by Hiltner to describe the narrow zone of soil surrounding the roots where microbe populations are stimulated by root activities. A large number of microorganisms such as bacteria, fungi, protozoa and algae coexist in the rhizosphere. Bacteria are the most abundant among them. Plants select those bacteria contributing most to their fitness by releasing organic compounds through exudates creating a very selective environment where diversity is low. Since, bacteria are the most abundant microorganisms in the rhizosphere, it is highly probable that they influence the plants physiology to a greater extent, especially considering their competitiveness in root colonization. The present review deals with the following topics: Plant growth promoting rhizobacteria (PGPR), occurrence of PGPR, nitrogen fixation by PGPR, *Bacillus* species, *Pseudomonas* species, Production of plant growth promoting substances by PGPR isolates, PGPR as biocontrol agent and antagonistic activity of PGPR isolates against phytopathogens.

Key words: Biocontrol, plant growth promoting rhizobacteria (PGPR), *Pseudomonas fluorescens*, *Bacillus subtilis*.

INTRODUCTION

Agriculture is heavily dependent on the use of chemical fertilizers and pesticides to achieve higher yields. This dependence is associated with problems such as environmental pollution, health hazards, interruption of natural ecological nutrient cycling and destruction of biological communities that otherwise support crop production. Hence, crop production and pest and disease management have to be achieved in shorter intervals of time with fewer detrimental inputs. The use of bioresource to replace chemical fertilizers and pesticides is growing. In this context, plant growth promoting microorganisms are often novel and potential tools to provide substantial benefits to agriculture (Sivasakthi et al., 2013).

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which is known as rhizosphere. The rhizosphere concept was first introduced by Hiltner to describe the narrow zone of soil surrounding the roots where microbe populations are stimulated by root activities. The original concept has now been extended to include the soil surrounding a root in which physical, chemical and biological properties have been changed by root growth and activity (McCully, 2005; Sivasakthivelan and Saranraj, 2013).

A large number of microorganisms such as bacteria, fungi, protozoa and algae coexist in the rhizosphere. Bacteria are the most abundant among them. Plants select those bacteria contributing most to their fitness by releasing organic compounds through exudates creating a very selective environment where diversity is low (Gracia et al., 2011). Since, bacteria are the most abundant microorganisms in the rhizosphere; it is highly probable that they influence the plants physiology to a greater extent, especially considering their competitiveness in root colonization (Barriuso et al., 2008).

Plant growth promoting rhizobacteria (PGPR) are free - living, soil - borne bacteria, which enhance the growth of the plant either directly or indirectly (Kloepper et al., 1980; Glick and Ibid, 1995). The direct mechanisms involve nitrogen fixation, phosphorus solubilization, HCN production, production of phytohormones such as auxins, cytokinins and gibberellins and lowering of ethylene concentration (Glick and Ibid, 1995; Glick et al., 1999). Bacteria belonging to the genera *Azospirillum*, *Pseudomonas*, *Xanthomonas* and *Rhizobium* as well as *Alcaligenes faecalis*, *Enterobactercloacae*, *Acetobacter diazotrophicus* and *Bradyrhizobium japonicum* have been shown to produce auxins which help in stimulating plant growth (Patten and Glick, 2002).

There are many reports on plant growth promotion and yield enhancement by plant growth promoting rhizobacteria (PGPR) (Lugtenberg et al., 2001). The mechanisms of plant growth promotion by PGPR include: the ability to produce phytohormones, N_2 fixation, antagonism against phytopathogens and solubilization of insoluble phosphates (Lugtenberg and Kamilova, 2009). It was also suggested that PGPR can also prevent the deleterious effects of stresses from the environment (Paul and Nair, 2008).

Bacteria associated with plants can be either harmful or beneficial plant growth promoting rhizobacteria (PGPR) may promote growth directly, by fixation of atmospheric nitrogen, solubilization of minerals such as phosphorus, production of siderophores that solubilize and sequester iron or production of plant growth regulators, phytohormones (Kloepper, 1997). Some bacteria support plant growth indirectly by improving growth restricting conditions either *via* production of antagonistic substances or by inducing host resistance towards plant pathogens. Since, associative interactions of plant and microorganisms must have come into existence as a result of convolution; the use of either former or latter groups as bioinoculants forms one of the vital components for a long - term sustainable agriculture system (Tilak et al., 2005; Usharani et al., 2013).

Rhizospheric bacterial communities have efficient systems for uptake and catabolism of organic compounds present in root exudates (Barraquio et al., 2000). Several bacteria have the ability to attach to the root surfaces (rhizoplane) making them to derive maximum benefit from root exudates. Few of them are more specialized, as they possess the ability to penetrate inside the root tissues (endophytes) and have direct access to organic compounds present in the apoplast. By occupying this privileged endophytic location, bacteria do not have to face competition from their counterparts as encountered in the rhizosphere or in soil (Kanchana et al., 2013a, b).

The use of PGPR offers an attractive way to replace chemical fertilizer, pesticides and supplements; most of the isolates result in a significant increase in plant height, root length and dry matter production of shoot and root of plants. PGPR help in the disease control in plants. Some PGPR, especially if they are inoculated on the seed before planting, are able to establish themselves on the crop roots. PGPR as a component in integrated management systems in which reduced rates of agrochemicals and cultural control practices are used as biocontrol agents. Such an integrated system could be used for transplanted vegetables to produce more vigorous transplants that would be tolerant to nematodes and other diseases for at least a few weeks after transplanting to the field (Kloepper et al., 2004).

PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR)

The rhizosphere, representing the thin layer of soil surrounding plant roots and the soil occupied by the roots, supports large active groups of bacteria known as plant growth promoting rhizobacteria (PGPR). Plant growth promoting rhizobacteria are known to rapidly colonize the rhizosphere and suppress soil borne pathogens at the root surface (Rangarajan *et al*., 2003). These organisms can also be beneficial to the plant by stimulating growth (Bloemberg and Lugtenberg, 2001). Among these organisms, Fluorescent *Pseudomonas* are considered to be the most promising group of plant growth promoting rhizobacteria involved in biocontrol of plant diseases. They produce secondary metabolites such as antibiotics, phytohormones, volatile compound hydrogen cyanide and siderophores. Plant growth promoting ability of these bacteria is mainly because of the production of indole – 3 - acetic acid, siderophores and antibiotics.

The genera of PGPR include *Azotobacter*, *Azospirillum*, *Pseudomonas*, *Acetobacter*, *Burkholderia*, *Bacillus, Paenibacillus* and some are members of the Enterobacteriaceae. Direct use of microorganisms to

promote plant growth and to control plant pests continues to be an area of rapidly expanding research. Rhizosphere colonization is one of the first steps in the pathogenesis of soil borne microorganisms. It is also crucial for the microbial inoculants used as biofertilizers, biocontrol agents, phytostimulators and bioremediators. *Pseudomonas* spp. is often used as model root colonizing bacteria (Lugtenberg et al., 2001).

The beneficial effects of these rhizobacteria have been variously attributed to their ability to produce various compounds including phytohormones, organic acids, siderophores, fixation of atmospheric nitrogen, phosphate solubilization, antibiotics and some other unidentified mechanisms (Glick and Ibid, 1995). Motile rhizobacteria may colonize the rhizosphere more profusely than the non - motile organisms resulting in better rhizosphere activity and nutrient transformation. They also eliminate deleterious rhizobacteria from the rhizosphere by niche exclusion thereby better plant growth. Induced systemic resistance has been reported to be one of the mechanisms by which PGPR control plant diseases through the manipulation of the host plant's physical and biochemical properties.

The recognition of plant growth promoting rhizobacteria (PGPR), a group of beneficial plant bacteria, as potentially useful for stimulating plant growth and increasing crop yields has evolved over the past several years to where today researchers are able to repeatedly use them successfully in field experiments. Increased growth and yields of potato, sugar beet, radish and sweet potato have been reported. Commercial applications of PGPR are being tested and are frequently successful. However, a better understanding of the microbial interactions that result in plant growth increases will greatly increase the success rate of field applications (Farzana et al., 2009).

PGPR, root - colonizing bacteria are known to influence plant growth by various direct or indirect mechanisms. Several chemical changes in soil are associated with PGPR. Plant growth promoting bacteria (PGPB) are reported to influence the growth, yield and nutrient uptake by an array of mechanisms. Some bacterial strains directly regulate plant physiology by mimicking synthesis of plant hormones, whereas others increase mineral and nitrogen availability in the soil as a way to augment growth.

OCCURRENCE OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR)

The rhizosphere is defined as the zone of soil in which the micro flora is influenced by the root (Hiltner, 1904). The mechanism by which PGPR species exert their beneficial effect on plants can be very diverse. PGPR produce plant hormones which promote root growth (Brown, 1972). The establishment of beneficial bacteria on roots systems *via* seed inoculation (that is, seed bacterization) has long been a major interest of agricultural researches (Brown, 1974). Ever since, the concept of the rhizosphere was first introduced, soil microbiologists have attempted to characterize and quantify microorganisms that inhabit this zone. Several methods including direct observation with light or electron microscopy have been used to demonstrate the rhizosphere effect.

BIOLOGICAL NITROGEN FIXATION BY PGPR ISOLATES

A number of bacterial species belonging to genera *Azospirillum*, *Alcaligenes*, *Arthrobacter*, *Acinetobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Pseudomonas*, *Rhizobium* and *Serratia* are associated with the plant rhizosphere and are able to exert a beneficial effect on plant growth (Tilak et al., 2005). The important role is played by plants in selecting and enriching the types of bacteria by the constituents of their root exudates. Thus, the bacterial community in the rhizosphere develops depending on the nature and concentrations of organic constituents of exudates and the corresponding ability of the bacteria to utilize these as sources of energy. There is a continuum of bacterial presence in soil rhizosphere, rhizoplane and internal of the plant tissues (Hallmann et al., 1997). Rhizospheric bacterial communities however have efficient systems for uptake and catabolism of organic compounds present in root exudates (Barraquiro et al., 2000).

Several bacteria help to derive maximum benefit from root exudates by their ability to attach to the root surfaces. Since, associative interactions of plants and microorganisms must have come into existence as a result of co-evolution, the use of latter group as bioinoculants must be pre-adapted, so that it fits into a long term sustainable agricultural system. PGPR are commonly used as inoculants for improving the growth and yield of agricultural crops and offers an attractive way to replace chemical fertilizers, pesticides and supplements (Ashrafuzzaman et al., 2009).

The use of biofertilizer and bioenhancer such as N_2 fixing bacteria and beneficial microorganism can reduce chemical fertilizer applications and consequently lower production cost. Utilization of PGPR in order to increase the productivity may be a viable alternative to organic fertilizers which also helps in reducing the pollution and preserving the environment in the spirit of an ecological agriculture (Stefan et al., 2008). Thus, rhizospheric bacteria can be a promising source for plant growth promoting agent in agriculture (Chaiharn et al., 2005) and are commonly used as inoculants for improving the growth and yield of agricultural crops.

The use of PGPR isolates as inoculants biofertilizers is beneficial for rice cultivation as they enhance growth of

rice and by inducing other plant growth promoting traits. Applying the combined inoculation of PGPR as biofertilizer affects beneficially the yield and growth of chickpea in field conditions (Rokhzadi et al., 2008). Biological nitrogen fixation contributes 180×10^6 metric tons/year globally, out of which symbiotic associations' produces 80% and the rest comes from free-living or associative systems. The ability to reduce and derive such appreciable amounts of nitrogen from the atmospheric reservoir and enrich the soil is confined to bacteria and Archaea. These include symbiotic nitrogen fixing forms, *viz*., *Rhizobium,* the obligate symbionts in leguminous plants and *Frankia* in non - leguminous trees, and non - symbiotic N_2 -fixing forms such as cyanobacteria, *Azospirillum*, *Azotobacter*, *Acetobacter diazotrophicus*, *Azoarcus,* etc.

Non - symbiotic nitrogen fixation has a great agronomic significance. One main limitation that it faces is the availability of carbon and energy source for the energy intensive nitrogen fixation process. However, this limitation can be compensated by moving closer to or inside the plants, *viz*., in diazotrophs present in rhizosphere, rhizoplane or those growing endophytically. Some important non - symbiotic nitrogen - fixing bacteria include *Azoarcus* sp., *Gluconacetobacter diazotrophicus*, *Herbaspirillium* sp., *Azotobacter* sp. (Vessey, 2003; Barriuso et al., 2008), *Achromobacter*, *Acetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azomonas*, *Bacillus*, *Beijerinckia*, *Clostridium*, *Corynebacterium*, *Derxia*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Rhodospirillum, Rhodopseudomonas* and *Xanthobacter* (Saxena and Tilak, 1998).

Bacillus species

Bacillus is the most abundant genus in the rhizosphere, and the PGPR activity of some of these strains has been known for many years, resulting in a broad knowledge of the mechanisms involved. There are a number of metabolites that are released by these strains (Charest et al., 2005), which strongly affect the environment by increasing nutrient availability of the plants. *Bacillus* species are naturally present in the immediate vicinity of plant roots.

Bacillus subtilis is able to maintain stable contact with higher plants and promote their growth. In a micro propagated plant system, bacterial inoculation at the beginning of the acclimatization phase can be observed from the perspective of the establishment of the soil microbiota rhizosphere. *Bacillus licheniformis* when inoculated on tomato and pepper shows considerable colonization and can be used as a biofertilizer without altering normal management in green houses (Garcia et al., 2004).

Bacillus species used as biofertilizers probably have direct effects on plant growth through the synthesis of plant growth hormones (Amer and Utkhede, 2007). Phosphate solubilizing *Bacillus* spp. stimulates plant growth through enhanced P nutrition increasing the uptake of N, P, potassium (K) and iron (Fe). Phosphorus biofertilizers could help increase the availability of phosphates accumulated in the soil and could enhance plant growth by increasing the efficiency of biological nitrogen fixation and the availability of iron (Fe) and zinc (Zn) through production of plant growth promoting substances.

Growth promotion and disease control by *Pseudomonas* and *Bacillus* are complex interrelated processes involving direct and indirect mechanisms that include synthesis of some metabolites (auxin, cytokinin and gibberellins), induction of 1 - amino cyclopropane – 1 -carbocylate (ACC) deaminase, production of siderophore, antibiotics, hydrogen cyanide (HCN), and volatile compounds. Others include mineral solubilization (e.g phosphorus), competition, and induced systemic resistance (Hamid Abbasdokht; Ahmad Gholam, 2010).

Jaizme-Vega et al. (2004) evaluated the effect of a rhizobacteria consortium of *Bacillus* spp. on the first developmental stages of two micropropagated bananas and concluded that this bacterial consortium can be described as a prospective way to increase plant health and survival rates in commercial nurseries. *Bacillus* is also found to have potential to increase the yield, growth and nutrition of raspberry plant under organic growing conditions (Orhan et al., 2006).

Bacillus megaterium is very consistent in improving different root parameters (rooting performance, root length and dry matter content of root) in mint (Kaymak et al., 2008). The *B. megaterium* var. *phosphaticum* and Potassium solubilizing bacteria *B. mucilaginosus* when inoculated in nutrient limited soil showed that rock materials and both bacterial strains consistently increased mineral availability, uptake and plant growth of pepper and cucumber, suggesting its potential use as fertilizer (Han et al., 2006; Supanjani et al., 2006). The *B. pumilus* can be used as a bioinoculant for biofertilizer production to increase the crop yield of wheat variety Orkhon in Mongolia (Hafeez et al., 2006).

Pseudomonas species

Pseudomonas sp. is ubiquitous bacteria in agricultural soils and has many traits that make them well suited as PGPR. The most effective strains of *Pseudomonas* have been Fluorescent *Pseudomonas* spp. Considerable research is underway globally to exploit the potential of one group of bacteria that belong to fluorescent *Pseudomonas* (FLPs). FLPs help in the maintenance of soil health and are metabolically and functionally most diverse (Lata et al., 2002). The presence of *Pseudomonas fluorescence* inoculant in the combination of microbial fertilizer plays an effective role in stimulating

yield and growth traits of chickpea. Isolates of FLPs from roots, shoots and rhizosphere soil of sugarcane provides significant increases in fresh and dry masses (Mehnaz et al., 2009). Field trials of a *Pseudomonas* strain lead to a great increase in yield of legumes (Johri, 2001).

Specific strains of the *Pseudomonas fluorescens* group have recently been used as seed inoculants on crop plants to promote growth and increase yields. These *Pseudomonas*, termed PGPR, rapidly colonize plant roots of potato, sugar beet and radish and cause statistically significant yield increased upto 44% in field tests. The occurrence and activity of soil microorganisms are affected by a variety of environmental factors (e.g. soil type, nutrient abundance, pH, moisture content) as well as plant related factors (species, age). So, while working on two winter wheat cultivars it was found that the genus *Pseudomonas* show higher counts, thus the population size of bacteria of the genus *Pseudomonas* depends on the development phase of wheat plants (Wachowska et al., 2006).

Pseudomonas spp. are important plant growth promoting rhizobacteria (PGPR) used as biofertilizers and are able to enhance crop yield by direct and indirect mechanisms (Walsh et al., 2001). Several researchers have shown that fluorescent *Pseudomonas* is abundant in the rhizosphere of different crops (Kumar and Sugitha, 2004). Effectively, they produce a variety of biologically active substances among which growth promoting compounds represent a keen interest (Rodriguez, 2006).

The strains of *Pseudomonas* are able to solublize phosphorous in soil and increase its availability to plants (Sundara et al., 2002). Some strains of *Pseudomonas* produce chelating agents called siderophores with high affinity for iron absorption. Microbial siderophores can enhance plant growth through increasing iron solubility in the plant rhizosphere. Such products are also able to alleviate the unfavorable effects of pathogens on plant growth.

Plant growth promoting rhizobacterial strains belonging to fluorescent *Pseudomonas* were isolated from the rhizosphere of rice among 30 strains, that were confirmed as *Pseudomonas fluorescens*. These *P. fluorescens* strains were characterized by PCR-RAPD analysis and biochemical methods. Ten exhibited strong antifungal activity against *Pyricularia oryzae* mainly through the production of antifungal metabolites. Chanway et al. (1989) reported that 32 bacterial strains representing *Pseudomonas putida, Pseudomonas fluorescens* and *Serratia* sp. were isolated from soil and were seen to colonize soya been roots in laboratory, green house and field assays when applied as seed inoculants. The colony forming units (CFU) was ranged from 1 - 9 to 6.1 CFU/g of root.

Reddy et al. (2007) obtained thirty isolates of *P. fluorescens* from rice rhizosphere and were tested for antifungal activity against *Magnaporthe grisea, Dreschelaria oryzae*, *Rhizoctonia solani* and *Sarocladium oryzae* that are known to attack rice plants. One *P. fluorescens* isolate effectively inhibited the mycelial growth in all these fungi in dual culture tests (62 - 85%). The antifungal compounds were extracted with equal volume of ethyl acetate. The antifungal compounds from *Pseudomonas fluorescens* at 5% completely inhibited the pathogens. The antifungal compounds were tentatively identified on thin layer chromatography (TLC) at Rf 0.22, 0.35, 0.42 and 0.51. These compounds were individually purified by Column chromatography and retested for antifungal activity.

Egamberdieva (2010) analyzed the plant growth promoting bacteria for their growth-stimulating effects on two wheat cultivars. The investigations were carried out in pot experiments using calcarous soil. The results showed that bacterial strains *Pseudomonas* sp. and *P. fluorescens* were able to colonize the rhizosphere of both wheat cultivars. Their plant growth-stimulating abilities were affected by wheat cultivars. The bacterial strains *Pseudomonas* sp. and *P. fluorescens* significantly stimulated the shoot and root length and dry weight of wheat.

Maleki et al. (2010) isolated 144 bacteria from cucumber rhizosphere and screened as potential biological control agents against *Phytophthora drechsleri,* causal agent of cucumber root rot, *in vitro* and greenhouse condition. On the basis of dual culture assays, eight isolates were selected for root colonization, PGPR and greenhouse studies. Among these isolates, isolate CV6 exhibited the highest colonization on the roots and significantly promoted plant growth under *in vitro* condition.

Ramette et al. (2006) revealed that *Pseudomonas* have plant growth promoting properties. Isolated strains showed high ability of IAA production, phosphate solubilization and siderophore production, while genotyping analysis showed that *Pseudomonas* isolated from the rhizosphere of rice are genetically diverse. Nevertheless, the strains were distributed into 11 genotypes, including five groups of fluorescent *Pseudomonas*.

PRODUCTION OF PLANT GROWTH PROMOTING SUBSTANCES BY PGPR ISOLATES

Plant hormones are chemical messengers that affect a plants ability to respond to its environment. Hormones are organic compounds that are effective at very low concentration; they are usually synthesized in one part of the plant and are transported to another location. They interact with specific target tissues to cause physiological responses, such as growth or fruit ripening. Each response is often the result of two or more hormones acting together. Because hormones stimulate or inhibit plant growth, many botanists also refer to them as plant growth regulators. Botanists recognize five major groups

of hormones: auxins, gibberellins, ethylene, cytokinins and abscisic acid.

Saranraj et al. (2013) collected the paddy rhizosphere soil sample from ten different locations in Cuddalore district of Tamil Nadu. The population of bacteria, fungi and actinomycetes in the rhizosphere soil sample of paddy was estimated by Serial dilution and Pour plating method. The total bacterial population ranged from 15.8 to 24.3 × 10^6 cfu g^{-1} of soil and the highest population of 24.3 × 10^6 cfu g^{-1} was observed in soil of Sivapuri. The total fungal and actinomycetes population were ranged between 8.9 to 13.3 × 10^3 cfu g^{-1} and 10.5 to 19.3 × 10^5 cfu g^{-1} of soil respectively. The occurrence of *Pseudomonas fluorescens* also examined and the population ranged between 7.71 × 10^6 cfu g^{-1} and 7.21 x 10^6 cfu g^{-1} of soil. The *P. fluorescens* was isolated and characterized by gram staining, motility test, plating on King's B medium and bio-chemical tests. The ten *P. fluorescens* isolates obtained from the rhizosphere of paddy were tested for their efficiency of IAA and siderophore production. The maximum IAA production was recorded by the isolate PF-8. The minimum production of IAA was found in PF - 4 isolates. The isolate *P. fluorescens* (PS-8) showed maximum siderophore production and the least siderophore production was showed by the *P. fluorescens* isolate PS- 4.

Indole - 3- Acetic acid

Indole – 3 - acetic acid (IAA) is a member of the auxin family of phytohormones that influence many cellular functions in plants and therefore are important regulators of plant growth and development. In addition to production in plant tissues, IAA synthesis is widespread among plant-associated bacteria (Patten and Glick, 1996) and provides bacteria with a mechanism to influence plant growth (Patten and Glick, 2002).

Indole – 3 - acetic acid is the member of the group of phytohormones and is generally considered the most important native Auxin (Ashrafuzzaman et al., 2009). It functions as an important signal molecule in the regulation of plant development including organogenesis, tropic responses, cellular responses such as cell expansion, division and differentiation and gene regulation. Diverse bacterial species possess the ability to produce the auxin phytohormone IAA. Different biosynthesis pathways have been identified and redundancy for IAA biosynthesis is widespread among plant - associated bacteria. Interactions between IAA - producing bacteria and plants lead to diverse outcomes on the plant side, varying from pathogenesis to phytostimulation.

The isolates producing IAA have stimulatory effect on the plant growth. When the crop is inoculated with the isolates capable of IAA production significantly increases the plant growth by the N, P, K, Ca and Mg uptake of sweet potato cultivar (Farzana and Radizah, 2005). There is a significant increase in rooting and root dry matter of cuttings of eucalypts when grown on IAA producing rhizobacteria inoculated substrate. Some rhizobacterial isolates stimulates the rhizogenesis and plant growth, maximizing yield of rooted cuttings in clonal nurseries (Teixeria et al., 2007). When cucumber, tomato and pepper are inoculated with different strains of PGPR which produce IAA, there is a significant increase in the growth of the vegetables (Kidoglu et al., 2007).

The IAA of microbial origin plays a major role in promotion of orchid germination, at least when the bacterial strains are in tight association with the seeds. *Azospirillum brasilense* strain Az39 and *Brayrhizobium japonicum* strain E109 both are able to excrete IAA into the culture medium, at a concentration sufficient to produce morphological and physiological changes in young seed tissues of Corn (*Zea mays* L.) and Soybean (*Glycine max* L.) and are responsible for their early growth promotion (Cassana et al., 2009). The use of PGPR isolates is beneficial for rice cultivation as they enhance the growth of rice by inducing IAA production.

Some microorganisms produce auxins in the presence of a suitable precursor such as L - tryptophan. The tryptophan increases the production of IAA in *Bacillus amyloliquefaciens*. Tien et al. (1979) showed that *Azospirillum* is able to produce auxins when exposed to tryptophan. Plants inoculated with the rhizobia together with Ag^+ ion and L - tryptophan (Trp), give the highest root dry weight, and significantly increase the uptake of N, P and K compared to non - inoculated control plants.

Beyeler et al. (1999) explained that the biocontrol strain CHA0 of *P. fluorescens* produces small amounts of indole-3-acetic acid *via* the tryptophan side chain oxidase and the tryptophan transaminase pathways. A recombinant plasmid (pME3468) expressing the tryptophan monooxygenase pathway was introduced into strain CHA0; this resulted in elevated synthesis of indole-3-acetic acid *in vitro*, especially after addition of L - tryptophan. In natural soil, strain CHA0/pME3468 increased fresh root weight of cucumber by 17 to 36%, compared to the effect of strain CHA0; root colonization was about 10^6 cells per g of root. However, both strains gave similar protection of cucumber against *Pythium ultimum*.

Shino et al. (2002) investigated the IAA biosynthesis in strain *P. fluorescens* HP72. After several repeated subcultures, the spontaneous IAA low - producing mutant HP72LI was isolated. The IAA low production of the strain HP72LI was due to the low tryptophan side chain oxidase (TSO) activity. Colonization of strain HP72 on the bent grass root induced root growth reduction, while strain HP72LI did not induce such growth reduction. The colonization ability of strain HP72 on the bent grass root is higher than that of strain HP72LI. However, as for biocontrol ability, a significant difference in both strains was not detected.

Khakipour et al. (2008) evaluated the auxin productivity potential in studied *Pseudomonas* strains through

chromatography, using HPLC devise; comparing the methods used and appointing IAA synthesize method by the studied strains in the applied cultivars. In fact, a variety of auxins like indole-3-acetic acid (IAA), indole-3-pyruvic acid, indole-3-butyric acid and indole lactic acid; cytokinins and gibberellins are detected, with auxin production being quantitatively most important. *Azospirillum brasilense* strain SM has the potential to be a competent rhizospheric bacterium as it triggers the IAA accumulation under nutrient stresses, likely environmental fluctuations and long - term batch cultures and beneficially influences the growth of sorghum.

Prassana Battu and Reddy (2009) isolated twenty *P. fluorescens* strains from rice growing soil samples and characterized. One of the *P. fluorescens* isolated and identified from the dual culture test. It was fermented for secondary metabolite in a small scale and extracted with ethyl acetate. The isolated metabolite tested against rice fungal pathogens. The structure of the compound was elucidated by high resolution NMR spectroscopy.

Karnwal (2009) obtained 30 fluorescent *Pseudomonas* isolates from different plant rhizosphere and were characterized on the basis of biochemical tests and plant growth promoting activities. *P. fluorescens* and *Pseudomonas aeruginosa* showed the best plant growth promoting activity. These isolates were tested for their ability to produce indole acetic acid in pure culture in the absence and presence of L-tryptophan at 50, 100, 200 and 500 µg/ml. For both strains, indole production increased with increases in tryptophan concentration *P. aeruginosa* was less effective in production of indole acetic acid than *P. fluorescens*. Inoculation of rice seeds with *P. fluorescens* and *P. aeruginosa* showed a good level of indole acetic acid compared to uninoculated seeds.

Sivasakthivelan and Saranraj et al. (2013) analyzed the biocontrol strain *P. fluorescens* Psd for indole-3-acetic acid (IAA) biosynthesis and studied the effect of its consequent manipulation on its plant-growth-promoting (PGP) potential. While the indole pyruvic acid (IPyA) pathway commonly associated with PGP bacteria was lacking, the indole acetamide (IAM) pathway generally observed in phytopathogens was expressed in strain Psd. Over expression of IAM pathway genes *iaaM-iaaH*, from *Pseudomonas syringae* subsp. *savastanoi* drastically increased IAA levels and showed a detrimental effect on sorghum root development.

Siderophore production

Iron is an essential growth element for all living organisms. The scarcity of bioavailable iron in soil habitats and on plant surfaces foments a furious competition (Whipps, 2001). Under iron limiting conditions PGPB produce low molecular weight compounds called siderophores to competitively acquire ferric ion. Siderophores (Greek: "iron carrier") are small, high - affinity iron chelating compounds secreted by microorganisms such as bacteria, fungi and grasses (Miller and Marvin, 2009). Microbes release siderophores to scavenge iron from these mineral phases by formation of soluble Fe^{3+} complexes that can be taken up by active transport mechanisms. Many siderophores are non - ribosomal peptides (Miethke and Maraheil, 2007), although several are biosynthesized independently.

Siderophores are also important for some pathogenic bacteria for their acquisition of iron. Siderophores are amongst the strongest binders to Fe^{3+} known, with enterobactin being one of the strongest of these (Raymond et al., 2003). Distribution of siderophore producing isolates according to amplified ribosomal DNA restriction analysis (ARDRA) groups, reveals that most of the isolates belong to Gram negative bacteria corresponding to the *Pseudomonas* and *Enterobacter* genera and *Bacillus* and *Rhodococcus* genera are the Gram positive bacteria found to produce siderophores (Tian et al., 2009).

Although, various bacterial siderophores differ in their abilities to sequester iron in general, they deprive pathogenic fungi of this essential element since the fungal siderophores have lower affinity. Some PGPB strains go one - step further and draw iron from heterologous siderophores produced by cohabiting microorganisms. *Pseudomonas* sp. have the capacity to utilize siderophores produced by diverse species of bacteria and fungi and *Pseudomonas putida* can utilize the heterologous siderophores produced by rhizosphere microorganisms to enhance the level of iron available to it in the natural habitat (Loper and Henkels, 1999). The two strains of *P. fluorescens* along with *Pseudomonas putida* produce maximum yield of hydroxamate type of siderophore in the modified succinic acid medium (SM).

Soil bacteria isolates including *Azotobacter vinelandii* and *Bacillus cereus* produces siderophores and they can be used as efficient PGPR to increase the yield of the crop (Husen, 2003). *Bacillus megaterium* from rhizosphere is able produce siderophore and thus it helps in the plant growth promotion and reduction of disease intensity. Specific strains of the *P. fluorescens* group have recently been used as seed inoculants on crop plants to promote growth and increase yields of various crops. These results prompted Kloepper et al. (1980) to investigate the mechanism by which plant growth was enhanced.

A previous study indicated that PGPR increase plant growth by antagonism to potentially deleterious rhizoplane fungi and bacteria, but the nature of this antagonism was not determined. They presented evidence that PGPR exert their plant growth promoting activity by depriving native microflora of iron. PGPR produces extracellular siderophores which efficiently complex environmental iron, making it less available to certain native microflora. The siderophores production by

Bacillus and *Pseudomonas* when assessed both in the presence and in absence of technical grade of herbicides show that the metabolic activities of plant growth promoting rhizobacteria decline following herbicides application (Munees and Mohammad, 2009).

Siderophores are low molecular weight (<10 kD) iron binding compounds synthesized by microbes in large quantity under iron limited conditions. Siderophores chelate the ferric ions with a high specific activity and serve as vehicles for the transport of iron (Fe^{3+}) into the microbial cell. Most of the siderophores have either hydroxamate, catechol or carboxylate ligands (Hofte, 1993).

Djibaoui and Bensoltane (2005) tested the ability of *Pseudomonas* to grow and to produce siderophores is dependent on the iron content and the type of carbon sources in the medium. Under conditions of low - iron concentration the *Pseudomonas* isolates studied produced yellow - green fluorescent iron - binding peptide siderophores and the biosynthesis of this siderophores was affected by several different environmental parameters. Four basal media, supplemented with different concentration of iron were employed to study the effect of iron and different organic carbon sources on siderophore production in *P. fluorescens*. The highest siderophores concentration was obtained in succinate medium. Ferric iron increased the growth yield and completely repressed siderophores production above 200 g/l, but had a positive effect below 160 g/l.

Urszula (2006) tested the ability of six strains belonging to the genus *Pseudomonas* isolated from the rhizosphere of wheat to produce pyoverdin. The studied strains demonstrated a varied level of production of the siderophore, depending on the culture conditions. The highest level of pyoverdin was determined after 72 h of growth at 20 - 25°C in iron - free medium supplemented with succinate. The synthesis of pyoverdin by all the strains studied was strongly repressed by the addition of iron ions (III) to the growth medium. Calcium, cadmium and magnesium ions stimulated the synthesis of the siderophore examined, whereas zinc and lead ions partially decreased its level. Enrichment of the growth medium in cobalt ions completely inhibited the synthesis of siderophores as well as growth of the bacteria.

Phosphate solubilization

Phosphorous is one of the major nutrient second only to nitrogen in requirement for plants. Most of the phosphorous in soil is present in the form of insoluble phosphates and cannot be utilize by plants (Pradhan and Sukla, 2006). The ability of bacteria to solublize mineral phosphates has been of interest to agricultural microbiologists as it can enhance the availability of phosphorous for plant growth PGPR has been show to solublize precipitated phosphates and enhance phosphate availability to rice that represent a possible mechanism of plant growth promotion under field condition (Verma et al., 2001).

The improvement of soil fertility is one of the most common strategies to increase agricultural production. The biological nitrogen fixation is very important in enhancing the soil fertility. In addition to biological nitrogen fixation, Phosphate solubilization is equally important. Phosphorus (P) is major essential macronutrients for biological growth and development. Microorganisms offer a biological rescue system capable of solubilizing the insoluble inorganic P of soil and make it available to the plants. The ability of some microorganisms to convert insoluble phosphorus (P) to an accessible form, like orthophosphate, is an important trait in a PGPB for increasing plant yields (Rodriguez et al., 2006). The rhizospheric phosphate utilizing bacteria could be a promising source for plant growth promoting agent in agriculture.

The use of phosphate solubilizing bacteria as inoculants increases the phosphorous uptake by plants (Chen et al., 2006). Among the heterogeneous and naturally abundant microbes inhabiting the rhizosphere, the Phosphate solubilizing microorganisms (PSM) including bacteria have provided an alternative biotechnological solution in sustainable agriculture to meet the phosphorous demands of plants. These organisms in addition to providing phosphorous to plants also facilitate plant growth by other mechanisms.

Current developments in our understanding of the functional diversity, rhizosphere colonizing ability, mode of actions and judicious application are likely to facilitate their use as reliable components in the management of sustainable agricultural systems (Zaidi et al., 2009). PSM include largely bacteria and fungi. The most efficient PSM belong to genera *Bacillus, Rhizobium* and *Pseudomonas* among bacteria, and *Aspergillus* and *Penicillium* among fungi. Within rhizobia, two species nodulating chickpea, *Mesorhizobium ciceri* and *Mesorhizobium mediterraneum*, are known as good phosphate solubilizers (Rivas et al., 2006). However, it is known that every aspect of the process of nodule formation is limited by the availability of phosphorous.

PGPR AS BIOCONTROL AGENT

PGPR are indigenous to soil and the plant rhizosphere and play a major role in the biocontrol of plant pathogens. They can suppress a broad spectrum of bacterial, fungal and nematode diseases. PGPR can also provide protection against viral diseases. The use of PGPR has become a common practice in many regions of the world. Although, significant control of plant pathogens has been demonstrated by PGPR in laboratory and greenhouse studies, results in the field have been inconsistent.

Recent progress in our understanding of their diversity, colonizing ability and mechanism of action, formulation and application should facilitate their development as

reliable biocontrol agents against plant pathogens. Some of these rhizobacteria may also be used in integrated pest management programmes. Greater application of PGPR is possible in agriculture for biocontrol of plant pathogens and biofertilization (Siddiqui, 2006). The bacterial strains isolated from *Lolium perenne* rhizosphere are capable of acting as plant growth promoting bacteria and as biocontrol agents as they show various plant growth promoting activities (Shoebitz et al., 2007).

A major group of rhizobacteria with potential for biological control is the *Pseudomonades* (Kremer and Kennedy, 1996). *Pseudomonas* sp. is ubiquitous bacteria in agricultural soils. Tremendous progress has been made in characterizing the process of root colonization by *Pseudomonas*, the biotic and abiotic factors affecting colonization, bacterial traits and genes contributing to rhizosphere competence and the mechanisms of pathogen suppression (Weller, 2007). *Pseudomonas* possesses many traits that make them well suited as biocontrol and growth promoting agents. These include the ability to (i) grow rapidly *in vitro* and to be mass produced; (ii) rapidly utilize seed and root exudates; (iii) colonize and multiply in the rhizosphere and spermosphere environments and in the interior of the plant; (iv) produce a wide spectrum of bioactive metabolites (that is, antibiotics, siderophores, volatiles and growth promoting substances); (v) compete aggressively with other microorganisms; and (vi) adapt to environmental stresses.

The major weakness of *Pseudomonas* as biocontrol agents is their inability to produce resting spores, which complicates formulation of the bacteria for commercial use. Fluorescent *Pseudomonas* spp. has been studied for decades for their plant growth promoting effects through effective suppression of soil borne plant diseases. Among various biocontrol agents, Fluorescent *Pseudomonas*, equipped with multiple mechanisms for biocontrol of phytopathogens and plant growth promotion, are being used widely (Banasco et al., 1998) as they produce a wide variety of antibiotics, chitinolytic enzymes, growth promoting hormones, siderophores, HCN and catalase, and can solublize phosphorous (Seong and Shin, 1996). *P. fluorescens* MSP-393, a plant growth - promoting rhizobacteria is an efficient biocontrol agent in rice grown in saline soils of coastal ecosystems (Paul et al., 2006).

Bacillus subtilis is also used as a biocontrol agent. This prevalent inhabitant of soil is widely recognized as a powerful biocontrol agent. In addition, due to its broad host range, its ability to form endospores and produce different biologically active compounds with a broad spectrum of activity, *B. subtilis* as well as other *Bacilli* are potentially useful as biocontrol agents (Nagorska et al., 2007). *Bacillus megaterium* from tea rhizosphere is able to solublize phosphate, produce IAA, siderophore and antifungal metabolite and thus it helps in the plant growth promotion and reduction of disease intensity (Chakraborty et al., 2006). Two strains (*Bacillus thuringiensis* and *Bacillus sphaericus*) have the ability to solublize inorganic phosphates and help in the control of the lepidopteron pests.

Antagonistic activity of pgpr isolates against phytopathogens

PGPR improve plant growth by preventing the proliferation of phytopathogens and thereby support plant growth. Some PGPR synthesize antifungal antibiotics, e.g. *P. fluorescens* produces 2, 4-diacetyl phloroglucinol which inhibits growth of phytopathogenic fungi. Certain PGPR degrade fusaric acid produced by *Fusarium* sp. causative agent of wilt and thus prevents the pathogenesis (Nowak et al., 1994).

Some PGPR can also produce enzymes that can lyse fungal cells. For example, *Pseudomonas stutzeri* produces extracellular chitinase and laminarinase which lyses the mycelia of *Fusarium solani*. In recent years, fluorescent *Pseudomonas* has been suggested as potential biological control agent due to its ability to colonize rhizosphere and protect plants against a wide range of important agronomic fungal diseases such as black root - rot of tobacco, root - rot of pea, root - rot of wheat, damping - off of sugar beet and as the prospects of genetically manipulating the producer organisms to improve the efficacy of these biocontrol agents. A concern was shown on the use of FLPs in crop plants as the antifungal substances released by the bacterium, particularly 2, 4 - diacetylphloroglucinol (DAPG) could affect the arbuscular mycorrhizal fungi (Kumar et al., 2002).

Gaur et al. (2004) confirmed that DAPG producing *Pseudomonas* recovered from wheat rhizosphere did not adversely affect AM colonization. However, given the toxicity of DAPG, such an inhibition may probably be dependent on the amounts released by the bacterium. Fluorescent *Pseudomonas* exhibit strong antifungal activity against *Pyricularia oryzae* and *Rhizoctonia solani* mainly through the production of antifungal metabolites. One of the isolate of a *fluorescent Pseudomonas* spp. EM85 is found to be strongly antagonistic to *Rhizoctonia solani*, a causal agent of damping-off of cotton. The *P. oryzihabitans* and *X. nematophila* strains produce secondary metabolites and suppress *Pythium* and *Rhizoctonia* species which also causes damping - off of cotton.

Fluorescent *Pseudomonas* also exhibits strong antifungal activity against *Rhizoctonia bataticola* and *Fusarium oxysporum* found in rice and sugarcane rhizosphere, mainly through the production of antifungal metabolites (Kumar et al., 2004). *Xanthomonas oryzae* and *Rhizoctonia solani* – the bacterial leaf blight (BB) and sheath blight (ShB) pathogens of rice (*Oryza sativa*) are

suppressed by indigenous *Pseudomonas* strains isolated from rhizosphere of rice cultivated in the coastal agri - ecosystem under both natural and saline soil conditions (Reddy et al., 2008). Isolates of *P. fluorescens* from rice rhizosphere are also shown to exhibit strong antifungal activity against *Pyricularia oryzae* and *Rhizoctonia solani* mainly through the production of antifungal metabolites. 50 - 60% of fluorescent *Pseudomonas* recovered from the rhizosphere and endorhizosphere of wheat grown in Indo-gangetic plains are antagonistic towards *Helminthosporium sativum* (Gaur et al., 2004).

Zadeh et al. (2008) worked to show the antagonistic potential of non - pathogenic rhizosphere isolates of fluorescent *Pseudomonas* in the biocontrol of *Pseudomonas savastanoi* which is the causative agent of Olive knot disease. *Pseudomonas corrugata*, a form that grows at 4°C under laboratory conditions, produces antifungals such as diacetylphloroglucinol and phenazine compounds. *P. fluorescens* CHA0 suppresses black root rot of tobacco, a disease caused by the fungus *Thielaviopsis basicola* and contributes in the biocontrol of *Meloidogyne javanica*, the root-knot nematode, *in situ* (Siddiqui et al., 2005). In addition, certain soils from Morens, Switzerland, are naturally suppressive to *Thielaviopsis basicola* - mediated black root rot of tobacco, and fluorescent *Pseudomonas* populations producing the biocontrol compounds (Ramettee et al., 2006).

Pseudomonas shows biocontrol potential against phytopathogenic fungi *in vivo* and *in vitro* conditions from chickpea rhizosphere. *Pseudomonas putida* has potential for the biocontrol of root - rot disease complex of chickpea by showing antifungal activity against *Macrophomina phaseolina*. It has also been shown that anaerobic regulator ANR - mediated cyanogenesis contributes to the suppression of black root rot (Saraf et al., 2008).

Pseudomonas strains acts as the effective candidates in suppressing *Pseudomonas capsici* in all seasons of plant growth as fluorescent *Pseudomonas* antagonizes all the reproductive phases of the *Phytophthora capsici*, the causal organism of foot rot disease (Paul and Sarma, 2006). Some metabolites produced by *Pseudomonas aeruginosa* Sha8 produces toxic volatile compound which reduces the growth of both *Fusarium oxysporum* and *Helmithosporium* sp. while, *Aspergillus niger* is not affected (Hassanein et al., 2009). *Bacillus luciferensis* strain KJ2C12 reduces *Phytophthora blight* of pepper by protecting infection courts through enhanced effective root colonization with protease production and an increase of soil microbial activity. Lima bean (*Phaseolus lunatus* L.) plants release hydrogen cyanide (HCN) in response to damage caused by natural enemies, thereby directly defending plant tissue. The bacteria *P. fluorescens* CHA0 shows biocontrol against the ciliated protozoa *Tetrahymena pyriformis* which feeds on it (Kim et al., 2009).

The nutritional superiority of more vigorous AM plants has been proposed to be a mechanism in reduction of root diseases. Wild rhizobial cultural filtrates and AM plants are found to have a significant antagonistic effect against soil born pathogenic fungi and therefore enhance the plant resistance to diseases. Siderophore mediated antagonism by *Acinetobacter calcoaceticus* was observed against common phytopathogens *viz*., *Aspergillus flavus*, *Aspergillus niger, Colletotrichum capsicum* and *Fusarium oxysporum* (Jousset et al., 2009).

Soil application of bacterial PSMs manages the wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (Khan et al., 2007). Inoculation of pepper with the phosphate solubilizing bacteria significantly reduces the *Phytophthora blight* or crown blight of peppers and increases the yield compared to untreated controls. *Azotobacter* isolates, *Pseudomonas* and *Bacillus* showed broad spectrum antifungal activity on Mueller Hinton medium against *Aspergillus*, one or more species of *Fusarium* and *Rhizoctonia bataticola* (Akgul and Mirik, 2008).

Wafaa and Haggag (2007) investigated the effect of *Paenibacillus polymyxa* (syn. *Bacillus polymyxa*) which produces an exopolysaccharide (EPS) on control of *Aspergillus niger*. In an *in vitro* assay, two strains of *Paenibacillus polymyxa* were tested against *Aspergillus niger*. Both strains showed inhibitory effect against *Aspergillus niger.* When these strains of *Paenibacillus polymyxa* were applied to seed and sowed in soil infested with *Aspergillus niger*, they significantly suppressed crown rot disease development and decreased survival of the *Aspergillus niger* pathogen. Over a period of 60 days, the population of bacteria was greatly increased. The bacterium colonized plant roots and were able to migrate downward with the root as it elongated.

Carissimi et al. (2009) isolated the bacteria strains with antifungal activity against *Bipolaris sorokiniana* to evaluate the best growth conditions for the antifungal production; and to test its action *in vivo*. The bacterial strains were pre-screened against four *Bipolaris sorokiniana* isolates on plates containing Sabouraud maltose agar. The isolate that showed the best result was grown on different culture media, cells were filtrated and the filtrates were tested against *Bipolaris sorokiniana* on plates with PDA medium. The *in vivo* test was done on wheat seeds, infected with *Bipolaris sorokiniana* isolate on a chamber with controlled temperature. *Bacillus* was chosen among the 86 bacterial isolates tested against the phytopathogen. The filtrate from *Bacillus* grown on tryptic casein soy broth (TSB) and straw culture media showed a similar degree of inhibition against the phytopathogen, the same result was not observed with malt extract broth media.

Reddy and Rao (2009) isolated plant growth promoting rhizobacterial strains belonging to fluorescent *Pseudomonas* from the rhizosphere of rice. Among 30

strains that were confirmed as *P. fluorescens*, this *P. fluorescens* strain was characterized by PCR-RAPD analysis and biochemical methods. Ten exhibited strong antifungal activity against *Pyricularia oryzae* and *Rhizoctonia solani* mainly through the production of antifungal metabolites.

Ningthoujam et al. (2009) screened for activity against some major rice fungal pathogens such as *Curvularia oryzae*, *Pyricularia oryzae* and *Fusarium oxysporum* showed potent antagonistic activities in dual culture assay. Among 33 indigenous actinomycetes isolates, LSCH-10C isolated from Loktak lake sediment on chitin agar, was found most promising to be developed as biocontrol agent (BCA) for rice. The nature of the activity in terms of fungitoxic or fungistatic nature was also determined. This report presents the preliminary results of these bio-control actinomycetes. Some of the strains have been selected for further studies towards application as rice BCAs.

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Flower-visiting insect pollinators of Brown Mustard, *Brassica juncea* (L.) Czern and Coss and their foraging behaviour under caged and open pollination

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Honey bees are the primary pollinators of mustard crop because it is highly attractive to bees and provides both nectar and pollen. To analyze the diversity and foraging behavior of insect pollinators under caged and open pollination, different varieties/ lines (Kranti, Varuna, Marigold, Vardan, Vaibhav, EC399299, EC399301, EC399312 and EC399313) of *Brassica juncea* were planted during Rabi season 2010-2011 at Apiary Garden, G. B. Pant University of Agriculture and Technology, Pantnagar. It was revealed that 30 species of insect pollinators belonging to ten families under four orders visited mustard flowers. In open pollination, *Trigona laeviceps* had maximum average abundance (4.51 bees/m^2/2 min) and foraging speed (18.55s) in Vardan and EC399313, respectively whereas *Apis mellifera* showed highest foraging rate (10.68 flower/min.) in EC399313 as compared to other bees in different varieties. Under caged pollination, line EC399313 was most preferred by *A. mellifera* with maximum abundance (6.24 bees/m^2/2 min), foraging speed (2.33 s) and foraging rate (7.69 flower/min). The results indicated that *A. mellifera* visited all nine varieties/lines in both open and caged plots. Overall average abundance (4.09 bees/1 m^2/2 min) and foraging speed (1.97s) of *A. mellifera* was found maximum under caged pollination while highest foraging rate (8.52 flower/min) was recorded in open pollination per day.

Key words: *Brassica juncea,* foraging behavior, Insect pollinators, caged pollination, open pollination.

INTRODUCTION

The Brassicaceae, which contains about 338 genera and 3,709 species currently recognized (Warwick et al., 2006), is one of the 10 most economically important plant families. The oleiferous *Brassica* species, commonly known as rapeseed-mustard comprising eight different species viz. Indian mustard, toria, yellow sarson, brown sarson, gobhi sarson, karan rai, black mustard and taramira are being cultivated in 53 countries spreading all over the globe. *Brassica juncea* is the dominant species grown for oil-seed usage in India. *B. juncea* is self compatible crop but also cross pollinated by insects. Plants of the genus *Brassica*, as with many others crucifers, are known to be very attractive to insect pollinators, serve mainly as a source

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Figure 1. Open pollinated plots.

of nectar and pollen. The amount of nectar secreted per 100 flowers of *B. juncea* ranged from 11.76 to 165 mg (79.85 mg on average). Sugar concentration in nectar was high enough to attract pollinating insects, reaching a maximum of 52% (Masierowska, 2003). Insect pollinators play vital role to increase productivity as proved by different reports from various parts around the world.

For a pollinating agent to be effective, its foraging behaviour should favour the transportation of anther pollen to flower stigmas on the same plant or different target species plants (Freitas and Paxton, 1996). It has been reported that there are more than 25000 described species of bees in the world and account for 70% pollination of various valuable flowering crops. Bees are the important components of agro-ecosystem as they provide free ecosystem services in the form of pollination which not only enhance the productivity of agricultural crops but also help in conservation of biological diversity. We rely on bees to pollinate 87 of the 124 (70%) most valuable crops used directly for human consumption (Klein et al., 2007).

Insect-mediated pollen transfer in mass-flowering *Brassica* crops has been particularly well studied, as insect pollinator activity can contribute significantly to pollination (Sihag 1985; Hayter and Cresswell 2006; Rader et al., 2009). A large number of insect species visit *Brassica* flowers (Howlett et al., 2009a, b, 2011), and absolute visitation rate is thought to play a central role in the resulting quality and yield of seed (Bhalla et al., 1983). Honey bees are included in this context, whose foraging behaviour is favourable to increase the crop productivity (D'Ávila and Marchini, 2005). In Asia, the domesticated honeybee, *Apis mellifera* have been utilized to provide managed pollination systems. The present investigation was undertaken to study the diversity and foraging behaviour of honey bee pollinators visiting mustard crop, *B. juncea* under open and caged condition.

MATERIAL AND METHODS

The experiment was conducted with 5 varieties and 4 lines of *Brassica juncea* viz., Kranti, Varuna, Vaibhav, Margold, Vardan, EC399299, EC399301, EC399312 and EC399313 respectively, during Rabi season and diversity of insect species were observed during 2010 to 2011 at G. B. Pant University of Agriculture and Technology, Pantnagar, U. S. Nagar, Uttarakhand, India. Six plots of size 3×4 m^2 were used to study the effect of Bee pollination (plots caged with *A. mellifera*, Figure 2) and open pollination (control, Figure 1) on foraging behavior of bees with three replications for each variety/line. Plant-plant and row-row spacing for *B. juncea* were taken 20 and 30 cm, respectively. Sowing of mustard was done on 18 November 2010 whereas the plots were caged on 24 January 2011 before plants of different variety/lines started flowering. To facilitate caged pollination, the fine muslin cloth and nylon net with the size of 4 m × 3 m × 2.5 m were used.

Abundance of insect species visiting *B. juncea* flowers was recorded during peak blooming period per 1 m^2 of plot for 2 min at

Figure 2. Caged pollinated plots.

two hours interval. Insects visiting these plots were collected by making five sweeps per plot with a hand net with 30 cm ring diameter Foraging rate was recorded in terms of number of flowers visited by an individual forager per minute of time whereas foraging speed is the time spent on a single flower by the insect forager collecting either nectar or pollen. The observations were recorded at two hour interval starting from 10AM to 4PM during the peak blooming period of different varieties/lines of *B. juncea*. The data collected from field experiments were subjected to the analysis of variance following Randomized Block Design.

RESULTS AND DISCUSSION

Diversity of insect pollinators on *B. juncea*:

A total of 30 species belonging to four orders, Hymenoptera (23), Diptera (5), Lepidoptera (1) and Coleoptera (1) visited mustard flowers (Table 1 and Figure 3). Hymenopteran visitors belonged to six families namely Apidae (5), Andrenidae (1), Anthophoridae (3), Megachilidae (4), Halictidae (4) and Xylocopidae (6). From the family Apidae, honey bees (*A. cerana, A. florea, A. mellifera, A. dorsata,* and stingless bee *Trigona laeviceps*) were observed on the flowers of *B. juncea* whereas Andrenidae *(Andrena* sp.), Anthophoridae (*Amegilla violaeca*, *A. zonata* and *Anthophora* sp.), Megachilidae *(Megachile lanata, M. disjuncta, M. hera* and *M. bicolor*), Halictidae *(Halictus gutturosus, Halictus* sp*., Nomia iridescence* and *Nomia* sp.), Xylocopidae *(Ceratina sexmaculata*, *Xylocopa iridipennis, X. pectinifrons, X. aestuans, X. latipes* and *X. amethystine)* were also observed during mustard blossoms. From Diptera, four species from family Syrphidae (*Episyrphus balteatus, Syrphus corollae, Melanostoma orientale and Spherophoria* sp.) and one species from Muscidae (*Musca domestica*) were recorded. From Lepidoptera order (*Pieris brassicae*) of family Pieridae and from Coleoptera order (*Coccinela septumpunctata*) Coccinellidae was also found to visit mustard flowers.

In the past study, Mahindru et al. (1995) found *Apis dorsata*, *A. florea*, A*. mellifera* and *Andrena* sp. on brown sarson at Ludhiana, Punjab and Chakravarty (2000) reported *Eristalis, Syrphus* sp., *A. cerana indica, A. dorsata, A. mellifera, Mellipona* sp*., Bumbus* sp*., Haliothis armigera, Plusia orichalcea* and *Pieris brassicae* as the visitors of *Brassica napus* at Pantnagar, Uttarakhand. On the other hand, Chaudhary (2001) reported that honey bees (58%), leaf-cutter bee (*Megachile hera*; 14.4% proportion), alkali bee (*Nomia curvipes*; 14.3%), *Chalcidoma creusa* (7.8%), *Andrena sacrissima* (2.0%), *Sphicodes fumipennis* (0.3%), *Braunaspis moderata* (0.1%), bumble bee (*Bombus sp*.; 0.1%) carpenter bee (*Xylocopa sp*.; 0.1%) syrphid fly (1.3%), house fly (0.1%) and butterfly (*Danais sp.*; 0.2%) were insect visitors on *Brassica campestris* var. Brown Sarson (cv. BSH-1), *Brassica carinata* cv. Carinata and Indian mustard cultivars RH-30, Laxmi and T-59 whereas

Table 1. Diversity of different insect visitors on flowers of mustard (*Brassica juncea*) at Pantnagar.

S/N	Common name	Scientific name	Family	Order
1	Indian honey bee	*Apis cerana* Fab.	Apidae	Hymenoptera
2	Little honey bee	*Apis florea* Fab.	Apidae	Hymenoptera
3	European honey bee	*Apis mellifera* L.	Apidae	Hymenoptera
4	Giant honey bee	*Apis dorsata* Fab.	Apidae	Hymenoptera
5	Sting less bee	*Trigona laeviceps* Smith	Apidae	Hymenoptera
6	Sand bee	*Andrena* sp.	Andrenidae	Hymenoptera
7	Digger bee	*Amegilla violacea* (Lepe.)	Anthophoridae	Hymenoptera
8	Digger bee	*Amegilla zonata* (Lin.)	Anthophoridae	Hymenoptera
9	Digger bee	*Anthophora* sp.	Anthophoridae	Hymenoptera
10	Leaf cutter bee	*Megachile lanata* (Fab.)	Megachilidae	Hymenoptera
11	Leaf cutter bee	*Megachile disjuncta* (Fab.)	Megachilidae	Hymenoptera
12	Leaf cutter bee	*Megachile hera* Bingham	Megachilidae	Hymenoptera
13	Leaf cutter bee	*Megachile bicolor* (Fab.)	Megachilidae	Hymenoptera
14	Sweat bee	*Halictus gutturosus* Vachal	Halictidae	Hymenoptera
15	Sweat bee	*Halictus* sp.	Halictidae	Hymenoptera
16	Alkali bee	*Nomia iridescence*	Halictidae	Hymenoptera
17	Alkali bee	*Nomia* sp.	Halictidae	Hymenoptera
18	Small carpenter bee	*Ceratina sexmaculata* Smith	Xylocopidae	Hymenoptera
19	Carpenter bee	*Xylocopa iridipennis* (Lepe.)	Xylocopidae	Hymenoptera
20	Carpenter bee	*Xylocopa pectinifrons*	Xylocopidae	Hymenoptera
21	Carpenter bee	*Xylocopa aestuans* (L.)	Xylocopidae	Hymenoptera
22	Carpenter bee	*Xylocopa latipes* (Drury)	Xylocopidae	Hymenoptera
23	Carpenter bee	*Xylocopa amethystine* (Fab.)	Xylocopidae	Hymenoptera
24	Hover fly	*Episyrphus balteatus* De Geer	Syrphidae	Diptera
25	Hover fly	*Syrphus corollae* Fab.	Syrphidae	Diptera
26	Hover fly	*Melanostoma orientale* L.	Syrphidae	Diptera
27	Hover fly	*Spherophoria* sp. L.	Syrphidae	Diptera
28	House fly	*Musca domestica* L.	Muscidae	Diptera
29	Cabbage butterfly	*Pieris brassicae*	Pieridae	Lepidoptera
30	Ladybird beetle	*Coccinella septumpunctata*	Coccinellidae	Coleoptera

Ahmad (2005) reported 22 and 16 hymenopterans and 7 and 5 dipteran species visiting mustard flowers in Diriyah and Derab (Saudi Arabia) respectively. They observed honey bees as the dominant hymenopteran pollinators followed by other bees such as *Andrena, Hexachysis, Halictus, Osmia, Pompilus* and *Dieles* and wasps. More abundant dipteran genera on the other hand were *Agromyza, Chrysoma, Drosophila* and *Syrphus*.

Foraging behaviour of insect pollinators under open pollination

It was observed that *A. mellifera* was most abundant/dominant species in all the varieties/lines of *B. juncea* than other bees under open pollination (Table 2). During the observation period at different interval hours maximum number of bees, *A. mellifera* (3.08 bees/1 m^2/2 min), *A. cerana* (0.16 bees/1 m^2/2 min), *A. dorsata* (0.60 bees/1 m^2/2 min) and *Trigona laeviceps* (1.07 bees/1 m^2/2 min) were recorded at 2.00 pm and minimum at 10.00 am with *A. mellifera* (1.50 bees/1 m^2/2 min), *A. dorsata* (0.19 bees/1 m^2/2 min) and *T. laeviceps*. (0.35 bees/1 m^2/2 min) while *A. cerana* abundance (0.10 bees/1 m^2/2 min) was found minimum at 4.00 pm. Among the varieties/lines of *B. juncea* average abundance of *A. mellifera* was found highest in Varuna and Vaibhav with 3.33 bees/1 m^2/2 min each whereas *A. cerana* was observed in EC399312 (0.68 bees/1 m^2/2 min, *A. dorsata* in EC399313 (2.05 bees/1 m^2/2 min) and *T. laeviceps* in Vardan (4.51 bees/1 m^2/2 min). In the present investigation peak activity of the most frequent pollinator *A. mellifera* on *Brassica* was recorded in the afternoon hours (2.00 h) whereas in an earlier study conducted by Thakur et al. (1982) reported higher number of *A. cerana indica* bees foraging mustard in the morning than *A. mellifera* but both species had a similar peak in the noon at Palampur, Himachal Pradesh. Brunel et al. (1994)

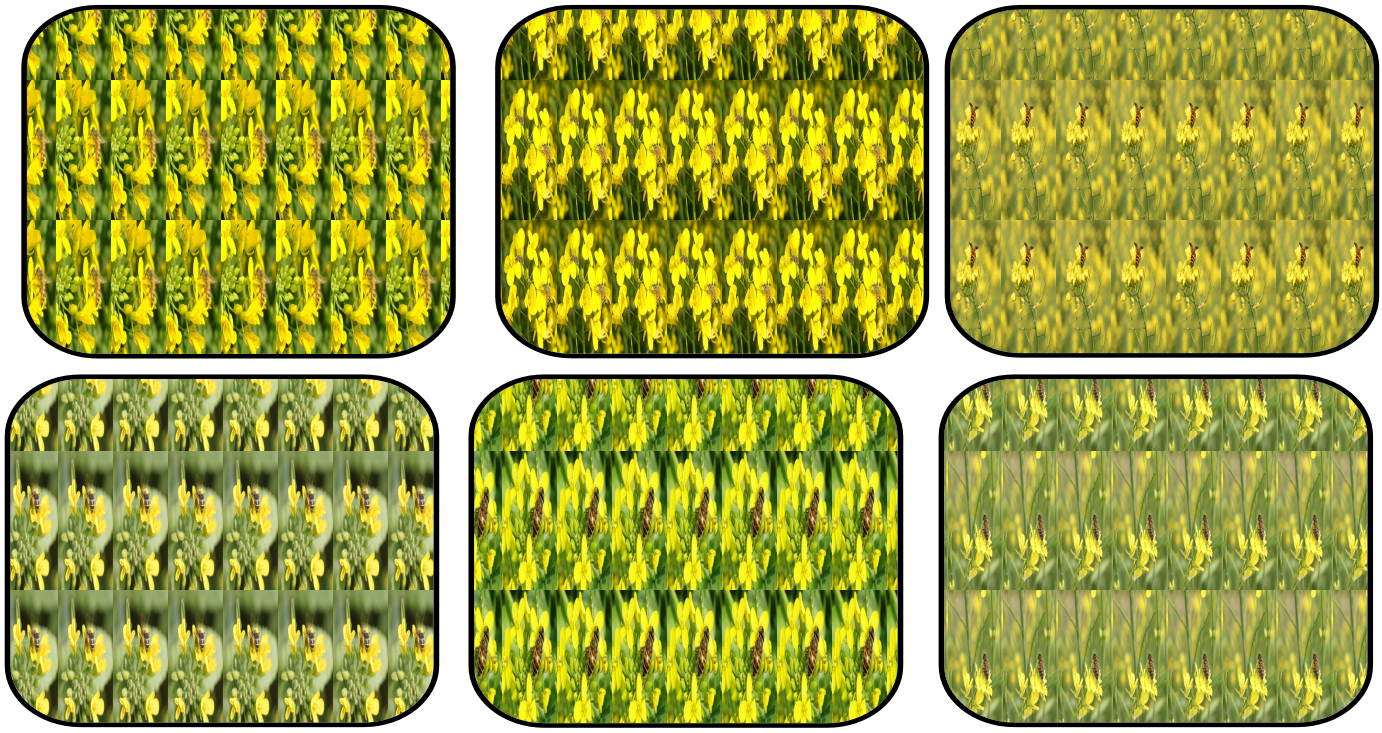

Figure 3. Foraging activity of insect pollinators on *Brassica juncea* crop.

reported that *A. c. indica* bees were the dominant (34 to 43 per cent) visitors on mustard, *Brassica juncea* L. followed by *A. dorsata* (20 to 26%).

Foraging speed was observed during the peak blooming period in different varieties/lines of *B. juncea* at different time intervals. *A. mellifera* showed maximum foraging speed which was more or less similar at 12.00 pm and 2.00 pm, 2.25 s and 2.24 s respectively while *A. cerana* at 4.00 pm (0.74 s). For *A. dorsata* it was observed highest at 2.00 pm (1.05 s) whereas *T. laeviceps* had maximum foraging speed of 9.88 s at 12.00 pm. The maximum average time spent by different insect pollinators in variety/lines of *B. juncea* was observed in EC399313 with *A. mellifera* (2.23 s), *A. dorsata* (3.03 s) and *T. laeviceps* (18.55 s) whereas *A. cerana* (3.10 s) was found highest in Kranti. Our results showed that *T. laeviceps* was found to spend maximum time on mustard flower but in an earlier study carried out by Kumar et al. (1994) revealed that *A. mellifera* exhibited higher foraging speed than solitary bees on mustard flower. More recently Sharma et al. (2001) reported that *A. mellifera* spent 1.64 s/flower followed by *A. dorsata* 2.18 s/flower and *A. florea* 3.54 s/flower on the *Brassica campestris* var. sarson.

The overall mean of foraging rate of insect pollinators observed in the present study was highest with 11.48 flowers/min by *A. mellifera*, 2.09 flowers/min by *A. cerana*, 4.03 flowers/min by *A. dorsata* and 1.93 flowers/min by *T. laeviceps* at 12.00 pm. Amongst the varieties/lines of *B. juncea* highest number of flower visited by *A. mellifera* (10.6 flowers/min), *A. dorsata* (9.40 flowers/min) and *T. laeviceps* (3.67 flowers/min.) was observed in EC399313 whereas foraging rate of *A. cerana* (7.13 flowers/min) was found maximum in Kranti. Benedek et al. (1972) recorded that individual honey bee (*A. mellifera*) took on an average of 4.4 min to visit 39 flowers on oilseed rape. One bee visited 342 flowers on 186 plants in 35.5 min. while Abrol and Kapil (1996) at Hisar, India, found that the foraging rates of different bee species varied greatly in different oilseed crops. On an average, *A. florea* visited 4.2 to 8.5 flowers per min in different cruciferous crops and 10.2 in sunflower, whereas *A. dorsata* visited 4.9 to 13.5 flowers per min on cruciferous crops. Rana et al. (1997) observed higher foraging activity at 12.00 h of both *A. mellifera* and *A. c. indica* than at 09.00 h. However, in both the species, there were no significant differences of the population of bees between 12.00 and 15.00 h.

Foraging behaviour of *A. mellifera* under caged condition

Data presented in Table 3 revealed that under caged pollination the average abundance of *A. mellifera* was maximum (5.04 bees/1 m^2/2 min) at 2.00 pm and minimum (2.19 bees/1 m^2/2 min) at 10.00 am. The maximum number of *A. mellifera* was recorded in the line

Table 2. Foraging behaviour of pollinators in different varieties/lines of *B. juncea* under open pollination.

Varieties /Lines	Abundance of bees at different hours per day (no. of bees/1 m²/2 min)																			
	A. mellifera					*A. cerana*					*A. dorsata*					*T. laeviceps*				
	10 am	12 pm	2 pm	4 pm	Avg.	10am	12pm	2pm	4 pm	Avg.	10am	12 pm	2pm	4pm	Avg.	10am	12pm	2pm	4 pm	Avg.
Kranti	1.74	4.09	3.33	3.46	3.16	0.66	0.68	0.68	0.36	0.59	0.00	0.00	0.00	0.00	0.00	0.76	0.76	0.94	0.98	0.86
Varuna	1.88	3.59	3.99	3.86	3.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Margold	1.85	1.96	2.42	1.77	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Vardan	1.18	3.01	3.42	3.48	2.77	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.82	5.74	6.14	5.35	4.51
Vaibhav	1.70	3.45	4.41	3.76	3.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
EC399299	1.39	1.90	1.95	1.68	1.73	0.00	0.00	0.00	0.00	0.00	0.68	0.85	0.85	0.63	0.75	0.00	0.00	0.00	0.00	0.00
EC399301	1.38	2.99	2.82	2.52	2.43	0.00	0.00	0.00	0.00	0.00	0.68	1.58	1.75	1.40	1.35	0.80	0.99	1.16	0.65	0.90
EC399312	0.52	2.34	1.68	1.47	1.50	0.70	0.68	0.80	0.56	0.68	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
EC399313	1.88	3.36	3.70	3.28	3.05	0.00	0.00	0.00	0.00	0.00	0.37	2.35	2.88	2.60	2.05	0.74	1.56	1.46	1.15	1.23
GM	1.50	2.97	3.08	2.81	2.58	0.15	0.15	0.16	0.10	0.14	0.19	0.53	0.60	0.52	0.46	0.35	1.00	1.07	0.90	0.83
SEm±	0.15	0.22	0.15	0.13	0.21	0.03	0.03	0.02	0.06	0.02	0.02	0.05	0.18	0.02	0.19	0.05	0.13	0.15	0.10	0.41
CD at 5%	0.43	0.66	0.46	0.38	0.64	0.09	0.11	0.06	0.18	0.08	0.07	0.18	0.57	0.07	0.57	0.16	0.38	0.44	0.30	1.19
Foraging speed of bees at different hours per day (time (s)/flower)																				
Kranti	1.43	2.51	2.65	2.11	2.17	2.58	3.18	3.17	3.47	3.10	0.00	0.00	0.00	0.00	0.00	11.33	17.62	14.48	13.10	14.13
Varuna	1.29	2.08	2.26	2.14	1.94	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Margold	0.96	2.07	1.92	2.02	1.74	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Vardan	1.02	2.15	1.98	1.84	1.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	14.06	22.08	17.40	15.37	17.22
Vaibhav	1.22	2.29	2.36	1.80	1.91	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
EC399299	0.99	1.76	1.93	1.68	1.59	0.00	0.00	0.00	0.00	0.00	2.11	2.85	3.11	2.88	2.73	0.00	0.00	0.00	0.00	0.00
EC399301	1.30	2.39	2.16	2.11	1.99	0.00	0.00	0.00	0.00	0.00	2.09	3.01	2.85	2.78	2.68	15.34	25.73	15.00	13.56	17.41
EC399312	1.20	2.39	2.35	2.33	2.06	2.68	3.25	3.11	3.25	3.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
EC399313	1.44	2.64	2.57	2.30	2.23	0.00	0.00	0.00	0.00	0.00	2.36	3.32	3.56	2.85	3.03	16.15	23.48	18.60	15.96	18.55
GM	1.21	2.25	2.24	2.04	1.93	0.58	0.71	0.06	0.74	0.69	0.72	1.02	1.05	0.94	0.93	6.32	9.88	7.27	6.44	7.48
SEm±	0.02	0.35	0.04	0.04	0.06	0.03	0.04	0.01	0.04	0.07	0.06	0.77	0.23	0.15	0.12	0.55	0.67	0.28	0.36	1.09
CD at 5%	0.79	0.10	0.15	0.14	0.18	0.09	0.12	0.05	0.12	0.21	0.18	0.23	0.69	0.43	0.34	1.66	1.99	0.84	1.06	3.19
Foraging rate of bees at different hours per day (flowers visited/min.)																				
Kranti	5.50	14.79	12.95	8.90	10.54	4.38	9.92	7.89	6.33	7.13	0.00	0.00	0.00	0.00	0.00	2.93	4.47	3.42	3.13	3.49
Varuna	4.60	12.67	11.62	6.97	8.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Margold	4.27	8.57	7.58	5.81	6.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Vardan	4.98	12.33	11.64	8.11	9.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.95	4.67	3.15	3.08	3.46
Vaibhav	3.44	10.45	10.55	7.22	7.92	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
EC399299	2.93	7.74	6.31	4.68	5.42	0.00	0.00	0.00	0.00	0.00	2.93	9.36	7.67	5.81	6.44	0.00	0.00	0.00	0.00	0.00
EC399301	4.30	12.80	13.16	8.98	9.81	0.00	0.00	0.00	0.00	0.00	3.77	12.27	13.00	7.18	9.06	2.22	3.59	4.49	2.85	3.29

Table 2. contd.

EC399312	4.01	9.03	10.41	6.76	7.55	3.70	8.96	7.15	6.23	6.51	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
EC399313	5.34	14.96	13.27	9.15	10.68	0.00	0.00	0.00	0.00	0.00	4.00	14.67	10.02	8.89	9.40	3.57	4.69	3.62	2.78	3.67
GM	4.37	11.48	10.83	7.40	8.52	0.89	2.09	1.67	1.39	1.51	1.19	4.03	3.41	2.43	2.76	1.29	1.93	1.63	1.31	1.54
SEm±	0.25	0.28	0.28	0.22	0.51	0.09	0.10	0.11	0.09	0.50	0.06	0.19	0.22	0.20	1.00	0.18	0.14	0.13	0.17	0.24
CD at 5%	0.77	0.85	0.85	0.66	1.51	0.27	0.31	0.34	0.27	1.46	0.20	0.58	0.66	0.62	2.92	0.56	0.43	0.38	0.51	0.70

Table 3. Foraging behaviour of *A. mellifera* in different varieties/lines of *B. juncea* under caged condition.

Varieties /Lines	**Abundance of bees at different hours per day (no. of bees/1 m^2/2 min)**					**Foraging speed of bees at different hours per day (time (s)/flower)**					**Foraging rate of bees at different hours per day (Avg. no. of flower visited/ min.)**				
	10am	**12pm**	**2pm**	**4pm**	**Avg.**	**10am**	**12pm**	**2pm**	**4pm**	**Avg.**	**10am**	**12pm**	**2pm**	**4pm**	**Avg.**
Kranti	3.19	6.86	7.62	5.23	5.73	1.49	2.60	2.72	2.09	2.23	3.40	10.27	9.61	7.43	7.68
Varuna	2.09	4.65	5.50	4.49	4.18	1.36	2.08	2.39	2.08	1.98	2.51	9.88	8.53	5.65	6.64
Margold	1.05	2.31	3.09	3.53	2.49	0.98	2.02	1.86	2.11	1.74	1.86	5.60	4.43	3.24	3.78
Vardan	1.87	3.31	3.81	3.74	3.18	1.15	2.12	2.02	1.94	1.80	3.00	9.36	9.63	5.69	6.92
Vaibhav	1.71	4.44	6.41	5.29	4.46	1.27	2.36	2.39	1.73	1.94	2.67	9.63	8.44	5.89	6.66
EC399299	1.80	2.41	2.44	2.71	2.34	0.88	1.83	1.97	1.68	1.59	2.02	5.98	4.63	3.93	4.14
EC399301	2.05	4.15	4.27	4.47	3.73	1.34	2.62	2.26	1.97	2.05	2.93	6.09	7.02	5.40	5.36
EC399312	2.47	5.99	4.74	4.72	4.48	0.97	2.47	2.41	2.33	2.05	2.98	9.53	8.51	5.87	6.72
EC399313	3.48	7.29	7.47	6.74	6.24	1.53	2.72	2.63	2.43	2.33	3.75	10.55	9.64	6.83	7.69
GM	2.19	4.59	5.04	4.54	4.09	1.22	2.31	2.29	2.04	1.97	2.79	8.54	7.82	5.54	6.17
SEm±	0.09	0.21	0.15	0.18	0.36	0.14	0.04	0.04	0.042	0.08	0.45	0.91	0.30	0.40	0.42
CD at 5%	0.29	0.62	0.46	0.55	1.04	0.42	0.13	0.15	0.13	0.25	1.35	2.75	0.90	1.20	1.23

EC399313 (6.24 bees/1 m^2/2 min) and minimum in EC399299 (2.34 bees/1 m^2/2 min). Average time of foraging speed 2.31s was recorded highest at 12.00 pm and lowest 1.22 s at 10.00 am. On the other hand foraging speed of 6.24 s in EC399313 was found maximum among different varieties/lines of *B. juncea* and minimum in EC399299 with 2.34 s. In caged condition the highest number of flowers visited per minute by *A. mellifera* was 8.54 at 12.00 pm while it was minimum 2.79 flowers/min. at 10.00 am. Maximum foraging rate was observed in EC399313 (7.69 flowers/min) which was more or less similar to Kranti (7.68 flowers/min) whereas minimum flowers visited by *A. mellifera* was found 3.78 flowers/min in Margold.

After recorded open and caged pollination data, it was concluded that *A. mellifera* visited all the nine varieties/lines of *B. juncea* both in open and caged plots but with variable preference. Under open pollination, varieties Varuna and Vaibhav (3.33 bees/1 m^2/2 min) were more preferred by *A. mellifera* while in caged highest abundance was recorded in line EC399313 (6.24 bees/1 m^2/2 min). In caged pollination overall average abundance of *A. mellifera* 4.09 bees/1 m^2/2 min was more as compared to open pollination which was only 2.58 bees/1 m^2/2 min per day. Foraging speed of *A. mellifera* was recorded 1.97 s in caged and 1.93 s in open pollinated plots. However, foraging rate of *A. mellifera* was found more (8.52 flower/min) in open pollination when compared to caged pollination which was calculated as 6.17 flower/min per day. Under open and caged pollination, line EC399313 was most preferred by *A. mellifera* with foraging speed of 2.33 s in both and foraging rate of 10.6 flower/min and 7.69 flower/min, respectively.

The aim of this study were to measure diversity and foraging behaviour of important insect pollinators to help plant pollination, including mustard an important oilseeds crop in Uttarakhand district. Enhancement of insect pollinators as part of crop management should be considered by farmers. Our result indicates that diversity and foraging behaviour on mustard will help in finding the most efficient pollinators for enhancing the crop yield, especially *Apis* and non *Apis* bees, plays a significant role to enhancing the productivity of mustard crop.

Conflict of Interests

The author(s) have not declared any conflict of interests

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Combining ability of maize (Zea mays) inbred lines resistant to *Striga* hermonthica (Del.) Benth evaluated under artificial *Striga* infestation

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The parasitic weed *Striga* affects maize on an estimated 20 million ha in Africa, making it a major cause of maize yield reduction from near world average of 4.2 t/ha few decades ago to the present 1.5 t/ha. The objectives of this study were to examine the combining ability of 20 inbred lines and identify single crosses which can be used to develop other hybrids resistant to *Striga* hermonthica (Del.) Benth. Fourteen female lines were mated using North Carolina Design II with all six males. The resulting 84 F_1s along with six commercial checks were evaluated in four separate trials for two rainy seasons during 2010. The trials were conducted on station under both artificial *Striga* infestation and *Striga* free environments using standard procedures at the Kibos and Alupe sites, both in the Kenya's Lake Victoria Basin. Data were recorded on *Striga* counts, *Striga* damage rating (SDR), grain yield and other agronomic traits. General combining ability (GCA) and specific combining ability (SCA) effects were computed using SAS. The new F1 hybrids outperformed the commercial checks in grain yield and reaction to Striga. Single crosses JI-30-3/TESTR 151, JI-30-18/TESTR 151, CML206//56/44-6-3-7-1/TESTR 149 and JI-30-18/TESTR 156 gave the highest yield while single cross JI10-28-#/TESTR 136 gave the lowest yield. The ratio of GCA: SCA mean squares exhibited a predominance of additive gene effects in the inheritance of *Striga* resistance traits as opposed to dominance gene effects. Inbred lines with good GCA for yield and *Striga* resistance traits were identified as TESTR 151, TESTR 156 and OSU231//56/44-6-4-17-3. The high GCA inbred lines and the superior single crosses will provide a basis for future use perse and also development of three-way and double cross hybrids to be grown in *Striga* prone areas of the Lake Victoria Basin in eastern Africa.

Key words: Maize, *Striga* hermonthica, general combining ability (GCA), specific combining ability (SCA), host plant resistance, sub-Saharan Africa.

INTRODUCTION

Many African countries often produce less maize than what they consume making them net importers of maize as maize is an important food crop in sub-Saharan Africa (SSA) and it provides the bulk of the calories in diet

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(Vivek, 2009). The average maize yield is 1.5 t/ha much below the world average of 4.2 t/ha (FAO, 2009). This results to malnourishment due to shortage which affects about 300 million people in Africa (Kim and Adetimirin, 1997). Solutions are needed to various maize production constraints, including low grain yield, susceptibility to pests and diseases, adaptation to the specific growing ecologies and yield loss that result from the devastating effects of *Striga* parasitic weed (Kim, 1994).

Many *Striga* control approaches have been developed, without much success when used singly (Kiruki et al, 2006). The major control strategies are; 1) hand weeding which is probably the oldest and also requires intensive work for several seasons before any benefits can be noticed in the field, 2) use of herbicide resistant maize which has a risk of *Striga* developing resistance to the chemicals, and 3) use of host plant resistance which is the plants ability to prevent attachment of the parasite or to kill or impair development of the attached parasite (Badu-Apraku et al., 2007). Host plant resistance offers the most effective and economical *Striga* control for the resource poor farmers in sub-Saharan Africa.

Host plant resistance (HPR) to *Striga* spp. constitutes two complementary mechanisms: resistance and tolerance (Rodenburg and Bastiaans, 2011). Resistance refers to the ability to reduce or prevent infection and reproduction (Shew and Shew, 1994) while tolerance refers to withstand infection with lower or minimum yield loss (Caldwell et al., 1958). The use of HPR has been limited, though it is the most economically feasible and environmentally friendly means of *Striga* control for the farmer (Rodenburg and Bastiaans, 2011). Series of studies at International Institute for Tropical Agriculture (IITA) found some maize varieties that were tolerant to *Striga* (Kim, 1994). The results from these studies concluded that the genetic control for tolerance and resistance of maize genotypes tested to S. hermonthica was polygenic and the inheritance quantitative. Twenty inbred lines and seven synthetics which were found to be tolerant and resistant to S. hermonthica were developed from diverse germplasm through artificial infestation with seeds obtained from various host crops (Kim, 1994). Some of these lines were used in the present study to determine their usefulness in variety development.

Combining ability of inbred lines determines the usefulness of the lines in hybrid combinations as the value of the line can best be expressed through the performance of crossing combinations (Hallauer and Miranda, 1981). Sprague and Tatum (1942) introduced the terms general combining ability (GCA) and specific combining ability (SCA). The general combining ability can be determined by using a broad base heterogeneous population as tester, while differences in the SCA can be revealed using a tester with a narrow genetic base (inbred line or single cross (Spitko et al., 2010). Identification of inbred lines with good GCA and SCA effects rely on the availability of genetic diversity among genotypes involved in the breeding program (Legesse et al., 2009). GCA expresses the mean performance of a parental line in hybrid combinations, while the SCA is a measure of the value of individual combinations as a function of the mean performance of the parental components. GCA and SCA are always relative values and depend greatly on the performance of the specific inbred lines involved in the crosses (Spitko et al., 2010). The value of GCA tends to express additive gene effects, while SCA is more indicative of dominant and epistatic gene effects.

In SSA, maize is grown over a diverse range of environments starting from the lowlands, mid- altitude to the highland ecologies (Derek and Carl, 1997). Some of these regions are infested with S. hermonthica which cause a loss of 40 to 60% in grain yield but can go up to 100%. The grain lost is estimated at seven billion tons annually, affecting about 100 million people (Kanampiu and Friesen, 2003). Enhancement of maize production in the *Striga* prone areas can be achieved by identifying elite *Striga* resistant lines which can be used to develop high yielding resistant varieties. The objective of this study were to: 1) estimate the combining ability effects of maize inbred lines from IITA, Kenya Agricultural Research Institute (KARI), and CIMMYT for *Striga* resistance traits, grain yield and foliar diseases, 2) identify promising hybrid crosses which may be used directly or be used in the formation of three way and double cross hybrids which can be grown by the resource poor farmers.

MATERIALS AND METHODS

Genotypes

There were 20 maize inbred lines sourced from three different institutions (Table 1). Materials involved eight *Striga* resistant inbred lines from IITA, the Kenya Agricultural Research Institute (KARI) contributed nine resistant inbred lines, while the International Maize and Wheat improvement Center (CIMMYT) contributed three well adapted inbred lines. Fourteen inbred lines designated as females were factorially mated with six IITA lines using North Carolina Design II to form 84 single cross hybrids in a line x tester mating design.

Field evaluation

Eighty-four single crosses along with six commercial checks were evaluated under both artificial *Striga* infestation and in *Striga* free environments. The F1 hybrids and the parents were tested for two long-rain seasons during 2009 and 2010 each at Kibos (0°40'S, 34°48'E) and Alupe (0°29'N, 34°20'E) in Kenya. Inoculum was prepared by mixing 5 kg of fine sand with 10 grams of *Striga* seeds. Infestation was done by applying the inoculum into an expanded hill of 7 to 10 cm during planting, this way deploying about 3,000 viable *Striga* seeds per hill.

The maize seed was placed on top of the inoculum and covered with soil. The experimental materials were planted in an alpha (0, 1) lattice design with three replications (Patterson and Williams, 1976). The spacing was 75 cm between rows and 25 cm within rows in both sites. The hybrids were over sown with two seeds per hill and later thinned to one to attain a plant density of 53,333 plants per

Table 1. The list of maize inbred lines tested for the combining ability.

Entry	Pedigree	Source	Remarks
1	CML 444	CIMMYT	Adapted
6	CML204	CIMMYT	Adapted
8	CML312	CIMMYT	Adapted
9	CML206//56/44-6-3-7-1	KARI	Adapted
2	TESTR 153	IITA	*Striga* resistant
3	JI-30--4	KARI	*Striga* resistant
4	JI-30--3	KARI	*Striga* resistant
5	JI-30-18	KARI	*Striga* resistant
7	TESTR 132	IITA	*Striga* resistant
10	F1-14-14-24-4-5-4	KARI	*Striga* tolerant
11	F1-14-79-4-1-3	KARI	*Striga* tolerant
12	OSU231//56/44-6-4-17-3	KARI	*Striga* tolerant
13	JI10-76-#	KARI	*Striga* tolerant
14	JI10-28-#	KARI	*Striga* tolerant
15	TESTR 136	IITA	*Striga* resistant
16	TESTR 139	IITA	*Striga* resistant
17	TESTR 149	IITA	*Striga* resistant
18	TESTR 150	IITA	*Striga* resistant
19	TESTR 151	IITA	*Striga* resistant
20	TESTR 156	IITA	*Striga* resistant

ha. The six checks included KSTP94 and UA Kayongo as the resistant checks and PHB3253, WH505, H513 and DH04 as susceptible checks.

Trial management practices including fertilization and weeding were done differently for each of the *Striga* infested and *Striga* free environment. For the *Striga* infested trials, the first weeding was done mechanically using a hoe but subsequent weedings were done by hand to uproot only other weeds but not Striga. Data for all agronomic traits were recorded on a per plot basis for each experiment. Data recorded included *Striga* counts at 6, 8, 10, and 12 weeks after planting (WAP) by counting *Striga* plants which emerged in each plot.

Striga damage rating was recorded at the 10th WAP using a 1-9 scale (1 = clean with no damage and 9 = heavily damaged). Days to 50% pollen shed and days to 50% silking were recorded when half of the total maize plants in a plot produced pollen or silked, respectively. Disease data was recorded on maize streak virus (MSV), gray leaf spot (GLS), rust and *Exserohilum turcicum* using a 1-5 scale (1 = no disease and 5 = severely diseased). Grain yield (tha^{-1}) was computed from unshelled cobs by assuming 0.8 shelling percent and adjusting it to 12.5% moisture content.

Statistical analysis

Combined analyses of variance were conducted for all the traits measured for each environment separately. Log_{10} transformation was done on the *Striga* counts. Using:

$$Y = Log_{10}(x+1)$$

Where, Y = Transformed data and x = actual *Striga* counts. Line x tester analyses of variance were performed to estimate (GCA) and (SCA) effects and variance according to the factorial model by Comstock and Robinson (1948).

$$Y_{hijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + R_h + \varepsilon_{hijk}$$

Where Y_{hijk} = the observation of the k-th full-sib progeny in a plot in h-replication of the i-th paternal parent and the j-th maternal parent; μ = the general mean; α_i = the effect of the i-th male parent; β_j = the effect of the j-th female parent; $(\alpha\beta)_{ij}$ = the interaction of paternal and maternal genotypes; R_h + the effect of h-th replication and ε_{hijk} = the environment effect and remainder of the genetic effect between full sibs on the same plot.

The SAS software (SAS, 2003) was used with the effects of environment and replicates considered as random while those of the genotype were considered as fixed. The mean squares of variance for the lines (females) and the testers (males) and their interaction effects were determined. The GCA effects of all 20 lines and SCA effects of the 84 single cross hybrids were determined. Test for significance of GCA and SCA effects were performed by computing the standard error for lines, testers and crosses and then tested against the t-test by taking the degree of freedom of the pooled error mean square.

RESULTS

Agronomic performance under artificial *Striga* infestation

Highly significant differences (P≤0.001) were observed in grain yield, days to 50% silking, SDR, *Striga* emergence counts (6th, 8th, 10th and 12th WAP), and disease severity caused by MSV, *Turcicum*, GLS and leaf rust. The grain yield of the crosses ranged 2.3 to 6.8 tha^{-1} and the trial mean was 5 tha^{-1} while the yield of the six commercial checks ranged 3.8 to 4.1 tha^{-1} with a mean of 4 tha^{-1}

Table 2. Performance of selected F1 hybrids under artificial *Striga* infestation.

Rank	Genotype	Grain yield (t/ha)	50% days to anthesis	*Striga* damage rating (Score 1-9)	*Striga* count 6 WAP/M^2	*Striga* count 8 WAP/M^2	*Striga* count 10 WAP/M^2	*Striga* count 12WAP/M^2	*E. turcicum* (score 1-5)	Gray leaf spot (Scored 1-5)
1	CML 444/ TESTR 136	6.8	66.0	1.3	0.03	0.33	0.67	0.93	2.3	1.3
2	TESTR 153/TESTR 136	6.8	66.0	1.7	0.03	0.27	0.75	0.96	2.3	1.2
3	JI-30--4/TESTR 136	6.4	65.4	1.8	0.04	0.52	1.01	1.18	1.8	1.3
4	JI-30--3/ TESTR 136	6.4	65.8	2.3	0.13	0.79	1.23	1.38	1.9	1.6
5	JI-30-18/ TESTR 136	6.4	65.7	1.7	0.08	0.49	1.04	1.25	1.9	1.2
6	CML204/TESTR 136	6.4	66.3	1.7	0.08	0.57	1.13	1.31	2.1	1.5
7	TESTR 132/TESTR 136	6.3	64.3	1.4	0.06	0.67	1.19	1.34	2.3	2.0
8	CML312/TESTR 136	6.2	64.5	2.7	0.04	0.47	1.02	1.17	1.8	1.4
9	CML206//56/44-6-3-7-1/TESTR 136	6.2	64.5	1.8	0.01	0.53	0.94	1.09	2.1	1.2
10	F1-14-14-24-4-5-4/ TESTR 136	6.2	65.4	1.6	0.08	0.48	0.97	1.26	2.6	1.4
11	F1-14-79-4-1-3/ TESTR 136	6.1	63.8	1.7	0.02	0.37	0.78	0.98	2.4	1.4
12	OSU231//56/44-6-4-17-3/ TESTR 136	6.1	64.8	1.5	0.04	0.41	0.73	1.02	2.3	1.4
13	JI10-76-#/ TESTR 136	6.0	64.4	3.2	0.04	0.55	1.03	1.20	2.3	1.3
14	JI10-28-#/TESTR 136	6.0	64.9	1.4	0.11	0.48	0.88	1.08	2.2	1.2
15	CML 444/TESTR 139	6.0	64.5	1.5	0.03	0.42	0.75	0.91	2.4	1.2
16	TESTR 153/TESTR 139	5.9	62.6	2.5	0.06	0.47	0.90	1.06	2.1	1.2
17	JI-30--4/TESTR 139	5.9	67.3	2.2	0.08	0.65	1.15	1.33	2.7	1.5
18	JI-30--3/ TESTR 139	5.9	67.2	1.6	0.06	0.45	0.96	1.07	2.8	1.2
19	JI-30-18/TESTR 139	5.8	64.9	2.1	0.07	0.57	1.04	1.18	2.3	1.7
20	CML204/TESTR 139	5.8	66.5	1.7	0.02	0.32	0.79	0.93	2.5	1.6
90	COMMERCIAL CHECK-1	2.1	68.5	4.6	0.04	0.54	1.00	1.23	2.4	1.4
87	COMMERCIAL CHECK-2	2.5	62.3	3.3	0.08	0.65	1.09	1.26	2.8	1.8
81	COMMERCIAL CHECK-3	3.0	68.9	2.7	0.01	0.09	0.36	0.65	2.3	1.4
89	COMMERCIAL CHECK-4	2.3	67.0	4.7	0.13	0.76	1.22	1.35	2.5	1.4
82	COMMERCIAL CHECK-5	3.0	64.7	5.3	0.07	0.65	1.07	1.20	2.7	2.0
85	COMMERCIAL CHECK-6	2.9	65.3	5.0	0.10	0.53	1.00	1.15	2.8	1.9
	Mean (Trial)	2.48	64.63	4.10	0.07	0.53	0.97	1.12	3.14	1.57
	CV (%)	39.26	3.95	36.07	195.42	65.24	33.01	28.1	14.59	31.1
	LSD(0.05)	1.29	2.13	1.25	0.11	0.29	0.26	0.26	0.31	0.42
	Significance	**	***	***	NS	***	***	***	**	**

*, **and *** indicates significance differences at P<0.05, P<0.01 and P<0.001, respectively.

(Table 2). F1 hybrids were in the same maturity bracket as the commercial checks under. It also exhibited a lower mean score for the SDR (2.4) as opposed to the commercial checks (4.3) (Table 2). The F1 single cross hybrids (registered low disease incidences) than commercial checks

Table 3. Coefficient of phenotypic correlation between agronomic and the *Striga* resistance traits of 84 maize hybrids under artificial *Striga* infestation.

Parameter	AD	ASI	PH	EH	EPP	YLD	SDR	STR6	STR8	STR10	STR12	MSV	TURC
AD													
ASI	0.25**												
PH	0.33***	-0.20*											
EH	0.38***	-0.23*	0.87***										
EPP	0.22*	-0.22*	0.24*	0.36***									
YLD	0.30***	-0.17	0.51***	0.55***	0.51***								
SDR	-0.24*	0.37***	-0.76***	-0.76***	-0.42***	-0.67***							
STR6M2TR	0.03	0.02	0.14	0.12	0.14	0.22*	-0.01						
STR8M2TR	0.01	0.18	-0.03	-0.01	0.18	0.29**	0.06	0.47***					
STR10M2TR	0.1	0.12	0.01	0.0002	0.21*	0.35***	0.01	0.44***	0.87***				
STR12M2TR	0.21*	0.1	0.07	0.06	0.27**	0.44***	-0.06	0.37***	0.81***	0.94***			
MSV	0.25**	0.31***	0.19	0.09	-0.001	0.30***	-0.01	0.31***	0.36***	0.38***	0.43***		
TURC	-0.48***	-0.16	-0.28**	-0.35***	-0.44***	-0.82***	0.44***	-0.21*	-0.29**	-0.34***	-0.44***	-0.36***	
GLS	-0.13	0.20*	-0.25	-0.24**	-0.02	-0.18	0.32**	-0.01	0.17	0.28**	0.26**	0.18	0.01

*, **and *** indicates significance differences at P<0.05, P<0.01 and P<0.001, respectively. Yld = Yield, AD = 50% days to anthesis, SD = 50% days to silking, ASI = anthesis silking interval, PH = Plant height, EH = ear height, GLS= Gray leaf spot, *Turc* = *E.turcicum*, SDR = *Striga* damage rating, STR8 = *Striga* counts 8WAP, STR10 = *Striga* counts 10WAP and STR12 = *Striga* counts 12 WAP

(Table 2). The genotypes differed significantly for reaction to leaf blight caused by E. turcicum and gray leaf spot (GLS) caused by Cercospora zeae-maydis. The mean score for E. turcicum was 3.14 and the range was 1.8 to 4.2 on a scale of 1-5. For gray leaf spot the mean was 1.57 while the range was 1.2 to 2.2 (Table 2).

The relationship between yield performance and the *Striga* resistance traits of the hybrids was investigated by a simple linear phenotypic correlation in a combined analysis for the two sites (Table 3). A highly significant (P<0.001) and negative correlation was observed between grain yield and SDR (r = -0.67***). A positive and significant correlation was observed between *Striga* counts per m^2 and yield 6 WAP, r = 0.22, and 8 WAP. *Striga* counts 10 WAP and 12 WAP was highly significantly correlated to yield across sites (r = 0.44) and (r = 0.30), respectively.

Combining ability analysis

Significant GCA mean squares (P < 0.001) were observed in most of the traits except ears per plant (EPP), indicating that there were some differences in the agronomic performance of the inbred lines used as the parents of the hybrid combinations in a line x tester experiment (Table 4). The site x GCA interaction was highly significant (P < 0.001) for grain yield, EPP, ear aspect, days to 50% anthesis, days to 50% silking, GLS and E. turcicum, indicating that the GCA effects were specific to the sites. This underlines that selection based on performance across the sites.

The ratio of the GCA: SCA mean squares were higher than unit in all the traits observed, suggesting that the additive gene action effects could be more important than the dominance gene action for the agronomic traits. The GCA mean squares under the *Striga* infested environment were highly significant (P≤0.001) for most of the traits except for SDR suggesting that dominance effects were more important (Table 5). The GCA mean squares for the *Striga* resistant traits were 1.78, 1.96, 5.31, 13.04 and 14.98 times larger than the SCA mean squares. This also suggested that the additive gene action was more important than dominance gene action for *Striga* resistance for these genotypes.

Significant GCA effects were observed on yield, days to 50% anthesis, SDR and *Striga* counts 6, 8, 10 and 12 WAP. Inbred lines TESTR

Table 4. Analysis of variance (mean squares) of a 14x6 factorial cross of maize inbred lines in a *Striga* free environment.

Source	Degrees of freedom	Grain yield (t/ha)	Ears per plant (no.)	Ear aspect (Score 1-5)	50% days to anthesis (days)	Plant height (Cm)	Ear height (Cm)	Gray leaf spot (score 1-5)	*Exserohilum turcicum* (score 1-5)
REP	2	1.566	0.028*	0.404	38.727***	15043.742***	9102.257***	0.311**	0.226
SITE	3	370.593***	0.321***	19.784***	1411.408***	224643.525***	78776.281***	39.257***	13.268***
LINE (GCA)	13	71.975***	0.111***	17.628***	204.825***	3383.093***	1898.636***	1.253***	20.100***
TESTER (GCA)	5	31.089***	0.018	6.973***	136.556***	11817.577***	5689.251***	3.068***	1.243***
SITE*LINE	39	4.402***	0.032***	0.755***	4.558***	150.906	161.930	0.114***	0.981***
SITE*TESTER	15	10.825***	0.030***	0.656***	4.854***	278.214	308.920**	0.202***	0.898***
LINE*TESTER (SCA)	65	2.258***	0.018***	0.587***	3.069***	309.510**	151.837	0.140***	0.335***
SITE*LINE*TESTER	195	1.168***	0.012	0.308***	2.134	204.635	172.945*	0.078**	0.165
GCA/SCA		31.88	6.17	30.03	66.74	10.93	12.50	8.95	60.00
ERROR	670	0.781	0.011	0.176	2.078	199.566	138.3	0.061	0.147
CV		19	11.01	14.9	2.2	6.79	10.61	17.01	13.66

*, **and *** indicates significance differences at P<0.05, P<0.01 and P<0.001, respectively.

Table 5. Analysis of variance (mean squares) of a 14x6 factorial cross of maize inbred lines under artificial *Striga* infestation.

Source	Degrees of freedom	Grain yield (t/ha)	50% days to anthesis (days)	Plant height (Cm)	Ear height (Cm)	Ears per plant (no.)	*Striga* damage rating (Score 1-9)	*Striga* count per M2 (6 WAP)	*Striga* count per M2 (8 WAP)	*Striga* count per M2 (10 WAP)	*Striga* count per M2 (12 WAP)	Gray leaf spot (score 1-5)	*Exserohilum turcicum* (score 1-5)
REP	2	1.493**	149.738	1850.131**	127.797	0.005	0.371	0.005	1.004***	1.772***	1.230***	0.210	0.354
SITE	3	3.568***	806.996***	220507.063***	53380.479***	0.362***	6.567***	1.374***	12.397***	8.004***	8.240***	12.953***	2.186***
LINE GCA	13	5.749***	192.822***	2412.596***	1941.884***	0.089***	0.060	0.015*	0.381***	0.787***	0.999***	1.854***	19.491***
TESTER GCA	5	1.965***	58.461	11400.264***	7264.425***	0.033*	0.248	0.017*	0.538***	1.593***	1.553***	5.891***	1.295***
SITE*LINE(GCA)	32	0.429	81.043	327.452	230.885	0.022*	0.148	0.015**	0.071	0.099	0.097	0.199***	0.564***
SITE*TESTER(GCA)	15	0.495	80.595	403.742	186.636	0.025*	0.223	0.014*	0.106**	0.168**	0.116	0.554***	0.509*
LINE*TESTER(SCA)	65	0.466*	77.840	254.836	190.080	0.017	0.034	0.008	0.072	0.060	0.067	0.206***	0.409**
SITE*LINE*TESTER(SCA)	123	0.368	76.810	312.302	187.551	0.014	0.073	0.008	0.055	0.056	0.062	0.112	0.285
GCA/SCA		12.337	2.477	9.467	10.216	5.144	1.780	1.955	5.308	13.041	14.979	9.000	47.655
ERROR	245	0.33	77.7	344.305	199.62	0.015	0.226	0.009	0.059	0.078	0.078	0.109	0.284
CV		21.09	13.62	9.1	12.36	12.2	289.62	95.66	54.53	30.74	25.78	21.54	20.15

*, **and *** indicates significance differences at P<0.05, P<0.01 and P<0.001, respectively.

151, TESTR 156 and OSU231//56/44-6-4-17-3 exhibited significant positive GCA effects for yield (Table 6). However, inbred line TESTR 156 exhibited significant positive GCA effects for the *Striga* resistance traits. Inbred lines JI10-76-# and JI10-28-# were the best general combiners for the *Striga* resistance traits as they had significant ($P \leq 0.001$) negative GCA effects for SDR and *Striga* counts although they had significant negative

Table 6. The GCA effects of the parental materials under artificial *Striga* infestation.

Entry	Genotype	Grain yield (t/ha)		50% days to anthesis (d)	*Striga* damage rating (Score 1-9)	*Striga* counts 8 WAP	*Striga* counts 10 WAP	*Striga* counts 12 WAP	*E. turcicum* (Score 1-5)	Gray leaf spot (Score 1-5)
		INF	No-INF							
1	CML 444	1.7	0.3	1.7	-1.06***	-1.06***	0.1**	0.08*	0.01	-0.1*
2	TESTR 153	-0.14	-0.66**	-0.14	0.13	0.13	0	0	0.38***	0.25***
3	JI-30--4	0.04	0.37	0.04	-0.09	-0.09	-0.05	-0.04	-0.31***	0.07
4	JI-30--3	1.04	0.85***	1.04	0.23	0.23	-0.06	-0.06	-0.3***	0
5	JI-30-18	0.7	0.66**	0.7	0.2	0.2	0.05	0.05	-0.48***	-0.01
6	CML204	1.63	0.33	1.63	0.19	0.19	0.03	0.07	-0.23**	0.19***
7	TESTR 132	1.48	-1.55***	1.48	0.88***	0.88***	-0.11**	-0.15***	0.53***	-0.29***
8	CML312	0.08	0.91***	0.08	0.01	0.01	0.23***	0.26***	-0.08	0.07
9	CML206//56/44-6-3-7-1	-0.12	1.12***	-0.12	-0.04	-0.04	0.07*	0.07*	-0.62***	-0.25***
10	F1-14-14-24-4-5-4	-1.12	0.63**	-1.12	0.23	0.23	0.1**	0.06	-0.25**	0.14**
11	F1-14-79-4-1-3	-2.78**	0.11	-2.78**	0.03	0.03	-0.04	-0.03	-0.22*	-0.12*
12	OSU231//56/44-6-4-17-3	2.2*	0.36	2.2*	-0.24*	-0.24*	-0.23*	-0.54**	-0.37***	-0.05
13	JI10-76-#	-3.03**	-1.41***	-3.03**	-0.97***	-0.97***	-0.14***	-0.17***	0.77***	0.16**
14	JI10-28-#	-1.65	-2.01***	-1.65	0.03	0.03	-0.15***	-0.18***	1.18***	-0.06
15	TESTR 136	-0.17	-0.37	-0.99	-0.29*	-0.29*	-0.03	-0.05*	0.11*	-0.19***
16	TESTR 139	-0.44**	-0.52*	0.36	0.61***	0.61***	-0.01	-0.02	-0.1*	0.24***
17	TESTR 149	-0.11	0.16	0.36	-0.53***	-0.53***	0	0.01	0.1*	0.03
18	TESTR 150	-0.05***	0.06	0.14	0.18	0.18	0.03	0.02	-0.06	0.08
19	TESTR 151	0.41**	-0.02	0.57	-0.23*	-0.23*	-0.14***	-0.13***	0.01	-0.26***
20	TESTR 156	0.36**	0.7**	-0.43	0.26*	0.26*	0.16***	0.16***	-0.06	0.08

WAP = Weeks after planting, INF- Artificially infested with *Striga*, NO-INF- *Striga* free environment. *, **and *** indicates significance differences at P<0.05, P<0.01 and P<0.001, respectively.

GCA effects for yield. These two inbred lines were also found to be very susceptible to E. turcicum.

Significant (P ≤ 0.01) positive SCA effects for yield were observed among the F1 hybrids. These were found out in crosses involving parents 7x2, 13x4, and 14x2. Hybrids 13x4 and 14x2 also had favorable SCA effects for *Striga* resistance traits (Table 7), making them the best F1 hybrids which could be grown in *Striga* infested fields. Hybrid 7x6 had favorable SCA effects for *Striga* resistance and diseases but a significant negative SCA effects for yield.

DISCUSSION

Host plant resistance with reduced *Striga* emergence is considered as the best strategy for long term control of *Striga* in sub-Saharan Africa. *Striga* resistant F1 hybrids with low *Striga* emergence were identified. However, susceptible F1 hybrids which supported few and many *Striga* plants were also present. Resistant maize cultivars should be able to support few emerged parasites, and sustain low *Striga* damage symptoms and produce high grain yields (Rodenburg and Bastiaans, 2011). In this case an inbred line which supports few *Striga* plants and finally succumbs to the effect of the parasite is considered not useful in the development of host plant resistance materials (Kim and Adetimirin, 1997).

The usefulness of the inbred lines in hybrid combinations is determined through studying their combining ability (Hallauer and Miranda, 1981). For the present study therefore, the desirable and *Striga* resistant lines would show negative GCA effects for SDR and *Striga* counts and a positive

Table 7. The SCA effects of the best performing F1 hybrids under artificial *Striga* infestation.

Cross	Grain yield (t/ha)	50% days to anthesis (d)	*Striga* counts 6 WAP	*Striga* counts 8 WAP	*Striga* counts 10 WAP	*Striga* counts 12 WAP	*E. turcicum* (Score 1-5)	Gray leaf spot (Score 1-5)
1X2	-0.09	-0.09	-0.16	0.7**	0	0.01	-0.31*	-0.05
2X5	-0.33	-0.22	0.48	0.42	0.1	0.06	0.05	-0.31***
6X5	-0.13	-0.32	0.17	0.09	0.07	0.03	0.07	-0.26**
6X6	0.34	2.27	-0.14	0.08	-0.02	-0.03	0.02	-0.22**
7X2	0.74**	-1.21	-0.36	0.06	0.06	0.16*	-0.2	-0.15
7X3	0.34	0.29	-0.14	0.02	-0.04	-0.07	-0.31*	0.02
7X4	0.39	-0.74	0.66*	0.05	0.08	0.08	-0.29*	-0.11
7X6	-0.99	2.75	0.08	0.01	-0.2***	-0.19**	-0.43**	-0.07
8X1	0.41	0.04	0.17	-0.04	-0.12*	-0.04	-0.13	-0.17*
9X3	0.36	0.47	0.11	-0.11	0.03	0.01	-0.33*	-0.02
11X3	0.06	3.54	-0.46	-0.22	-0.12*	0.02	-0.03	-0.03
13X4	0.83**	0.68	0.09	-0.4	-0.06	-0.66*	-0.44**	-0.06
14X2	0.89**	-0.25	-0.09	-0.43	0.01	-0.72*	-0.19	-0.09
14X5	-0.07	0.13	0.16	-0.62*	-0.09	-0.14*	-0.29*	0.2*

WAP = Weeks after planting. *, **and *** indicates significance differences at P<0.05, P<0.01 and P<0.001, respectively.

GCA effects for grain yield under *Striga* infested conditions. In our study TESTR 151 and OSU231//56/44-6-4-17-3 would be considered superior and desirable. Inbred line TESTR 156 exhibited a significant positive GCA effects for grain yield and a positive GCA effects for *Striga* resistance traits making it not suitable for S. hermonthica resistance. Inbred lines JI10-76-# and JI10-28-# exhibited high GCA effects for *Striga* resistance traits but negative GCA effects for grain yield. These lines can therefore be utilized only as source of resistance to *Striga* in maize breeding. The importance of additive gene action was observed for grain yield and the *Striga* resistance traits as opposed to non-additive gene action. Similar findings were reported by Yallou et al. (2009) who reported the importance of additive gene effects while studying combining ability of maize inbred lines containing genes from *Zea diploperennis*.The relative importance of GCA and SCA variance was examined by expressing it as the ratio of additive to total genetic variance. The closer this ratio is to unity, the greater the predictability based on GCA alone (Baker, 1978). In our study, the additive gene effects were found to be more important than the dominance effects. The importance of the GCA effects was 12% under *Striga* infested environment and 31% under *Striga* free environment. Makumbi et al. (2010) reported GCA effects of 51 to 79% in well watered environment and 40 to 64% under water stressed environment.The significant GCA effects as opposed to SCA effects for the SDR and *Striga* counts indicate that the genetic variation for resistance to S. hermonthica among the lines was mainly controlled by additive type of gene action. This is in agreement with findings of Gethi and Smith (2004) findings who reported significant GCA mean squares for *Striga* counts but contrary on SDR. However, these results are in contrast to that of Kim (1994), who found higher SCA mean squares than GCA mean squares for *Striga* counts and higher GCA mean squares than SCA mean squares for SDR. *Striga* infestation coupled with other stresses such as foliar diseases including E. turcicum and gray leaf spot are major causes that has hampered maize production in the sub-Saharan Africa over the last two decades, making it to remain at 1.5 t/ha well below the world average of 4.2 t/ha (FAO, 2003, Kanampiu et al., 2007). It is therefore important to develop genetic materials with good levels of resistance to these diseases. Quite a good number of F1 hybrids with good and acceptable scores were identified. In the development of maize hybrids, resistance to *Striga* should be tested under both *Striga* free and *Striga* infested environments following procedures developed by Kim (1991) and Haussmann et al. (2000). This helps in identifying superior inbred lines in both environments which would be ideal for the farmers as *Striga* infestation in the field is not uniform and the parasite infestation in the field is erratic. In *Striga* free environments TESTR 156 and OSU231//56/44-6-4-17-3 had positive and significant GCA effects for yield making them superior under both environments. Inbred lines JI-30-3, JI-30-18, CML 312, CML 206, and F1-14-14-24-4-5-4 showed positive and highly significant GCA effects for grain yield under *Striga* free environments.

Conclusion

The outcome of the present studies confirms the

availability of inbred lines for developing maize hybrids with good levels of resistance to S. hermonthica. The importance of additive gene action was demonstrated in breeding for *Striga* resistance as opposed to non-additive gene action. Inbred lines with good GCA for yield and *Striga* resistance traits were identified as TESTR 151, TESTR 156 and OSU231//56/44-6-4-17-3. These inbred lines would be of great importance in the breeding for *Striga* resistance in maize. Single crosses 7x2, 13x4, and 14x2 were identified as the best performing hybrids under *Striga* infested environment. They would therefore be recommended to be grown by farmers in the *Striga* prone areas. Inbred lines JI10-76-# and JI10-28-# which are mutants from KARI Muguga might be a very good source of resistance as they gave very good GCA effects for the *Striga* resistance traits.

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Full Length Research Paper

Comparative analysis of phenolic profile of *Monodora myristica* and *Monodora tenuifolia*

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This study sought to identify phenolics present in the seeds of *Monodora myristica* and *Monodora tenuifolia* by Gas chromatography (GC) coupled to Flame ionization detector (FID). GC-FID analysis identified fifty-three different types of phenolics in both *M. myristica* and *M. tenuifolia* seeds. Predominant phenolics are Myristicin (RT: 13.78), Caffeic acid (RT: 14.16), Safrole (RT: 11.37), Methyl Eugenol (RT: 12.32), Catechin (RT: 3.63), Elemicin (RT: 13.69), Quercetin (RT: 25.05), Kaempherol (RT: 21.52), Methyl Isoeugenol (RT: 12.69) and Eugenol (RT: 11.70). It was observed that *M. myristica* is rich in Myristicin (42.60%), Caffeic acid (23.39%), Elemicin (3.82%) and Eugenol (1.02%), while *M. tenuifolia* is rich in Safrole (11.86%), Methyl Eugenol (6.28%), Catechin (5.27%), Quercetin (2.97%), Kaempherol (2.27%) and Methyl Isoeugenol (1.45%). However, the two species of *Monodora* contained virtually the same kinds of phenolics but in varying quantities with *M. myristica* having a higher total phenolic content (1478.32 mg/100 g) than *M. tenuifolia* (1171.52 mg/100 g). Both species are promising sources of phenolics.

Key words: *Monodora myristica*, *Monodora tenuifolia,* phenolics.

INTRODUCTION

Phenolics are secondary metabolites that are synthesized by plants during development, and in response to biotic and abiotic stresses such as infections, wounding, ultraviolet (UV) radiation (Lattanzio et al., 2006; Putrussa et al., 2013). They are present in all plants and contribute to the development of color, taste and palatability, as well as the defense system of plants (Tarnai et al., 1994). They share a common structural feature: an aromatic ring bearing at least one hydroxyl substituent (Croteau et al., 2000). Historically, some of these phenolics were considered as antinutrients (Fattouch et al., 2007) but recent reports on the antioxidant and antimicrobial properties of phenolics lead to a rethinking among food scientists (Mercy et al., 2009).

Phenolics have an array of health promoting benefits; they are of current interest due to their important biological and pharmacological properties, especially the antiinflamatory, antioxidants, antimutagenic and anticarcinogenic activities (Ehsan et al., 2012). It is therefore important to analyze the composition of phenolic compounds in foods before their health-promoting properties can be adequately studied. The analysis of phenolic compounds in plant samples is difficult because of the great variety of their structure and the lack of appropriate standards (Magalhães et al., 2009; Huang et al., 2007). Although extracts of spices

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Figure 1. Photographs of *Monodora myristica* (a) and *Monodora tenuifolia* (b).

have been shown to be rich in phenolics (Wojdjlo et al., 2007), literature is scarce on the nature of phenolics present in these spices, therefore, this study sort to report the different types of phenolics present in the plants *Monodora myristica* and *Monodora tenuifolia*.

M. myristica (Figure 1a) and *M. tenuifolia* (Figure 1b) belong to the custard apple family of flowering plants Annonaecea. Their generic name was derived from the Greek word meaning “single gift”. *M. myristica* is widely distributed from Africa to Asia, Central and South America and Australia (Omobuwajo et al., 2003). It is native to West, Central and East Africa extending from Sierra leone to Uganda, Kenya, Kongo and Angola (Keay, 1989). It grows well in the evergreen forest of West Africa and most prevalent in the Sothern part of Nigeria (Adegoke and Akinsanya, 1970). Its local name include: Ehuru or Ehiri (Igbo), Ariwo (Yoruba), Jamaica nutmeg, African nutmeg, Calabash nutmeg, and Airama. *M. myristica* is used in Ivory Coast to treat hemorrhoids, stomach ache and febrile pains. *M. myristica* seeds are aromatic and are used after grinding to powder as condiments in food providing a flavour resembling that of nutmeg (Ekeanyanwu et al., 2010). The seeds are also used as an aromatic and stimulating addition to medicines and to snuff (Burkill, 1985; Ekeanyawu et al., 2010). When ground to powder, the kernel is used to prepare pepper soup as stimulant to relieve constipation and control passive uterine hemorrhage in women immediately after child birth (Okafor, 1987; Udeala, 2000; Iwu, 2002).

M. tenuifolia is found in the forest region of East Indies, West Indies, Malaysia, Sirlanka and Africa (Talaji, 1965). In Africa, *M. tenuifolia* is widely distributed along the West Coast (Adeoye et al., 1986) and occurs in Nigeria, Guinea, Cameroun, Gabon and Zaire (Congo Democratic Republic) where it is used as an ornamental plants in food and as medicines (Burkhill, 1985). In traditional medicine, it is widely used to relieve toothache, dysentery, (Nelson, 1979; Adeoye et al., 1986), dermatitis, headache and as cermifuge (Adeoye 1986). *M. tenuifolia* seeds (Figure 1b) are aromatic and are used as ingredient in herbal medicine in Southern Nigeria and as spices and flavour (Ogutimein et al., 1989). When roasted, the ground seed of *M. tenuifolia* are rubbed on the skin for skin diseases (Irvine, 1961).

MATERIALS AND METHODS

Collection of plant materials

Seeds of *M. myristica* were purchased from a local market in Ile-Ife, while the seeds of *M. tenuifolia* were collected from the Zoological garden, Obafemi Awolowo University. These seeds were identified and authenticated at Ife herbarium Obafemi Awolowo University, Ile-Ife.

Preparation of plant material

The seeds were air-dried in the laboratory for a period of 7 days after which they were decocted to free the kernel which were later ground to powder with an electronic blending machine.

Extraction of phenolics

About 50.0 mg of the sample was extracted with 5 ml of 1M NaOH

for 16 h on a shaker at ambient temperatures as described by Kelley et al. (1994). After extraction, the sample was centrifuged (5000 × g), rinsed with water, centrifuged again, and the supernatants were combined and placed in a disposable glass test tube and heated at 90°C for 2 h to release the conjugated phenolic compounds as supported by Whitehead et al. (1983). The heated extract was cooled, titrated with 4M HCL to pH < 2.0, diluted to 10 ml, with deionised water, and centrifuged to remove the precipitate. The supernatant was kept for subsequent purification and the residue was further extracted with 5 ml of 4M NaOH, heated to 160°C in Teflon. After cooling, the mixture was filtered. Supernatant was collected and the residue was washed with water (deionised). The supernatants were combined and adjusted to pH < 2.0 with 4M HCL. The filtrates were combined for further purification.

Purification of extracted phenolics

An aliquot (5 to 15 ml) of various supernatants was passed through a conditioned Varian (Varian Assoc., Harbor City, CA) Bond Elut PPL (3 ml, size with 200 mg packing) solid phase extraction tube at ~1 ml min^{-1} attached to a Visiprep (Supelco, Bellefonte, PA). The tubes were then placed under a vacuum (-60 KPa) until the resin was thoroughly dried after which the Pas were eluted with 1 ml of ethyl acetate into Gas chromatography (GC) auto sampler vials. The PPL tubes were conditioned by first passing 2 ml of ethyl acetate followed by 2 ml water (Ph < 2.0).

Derivatisation

The concentrated extract of about 2 ml in the GC vials was derivatized by adding BSTFA (bis (trimethylsily) trifluoroacetamide). The silicone septum corked vial was lowered into the water bath with hanger to stand upright in the water bath with a magnetic stirrer at 45°C for the derivitization period of 10 min.

Chromatographic conditions

A GC-Flame ionization detector (FID) (GC-FID) HP 6890 series equipped with HP INNOWax capillary column (30 m × 0.25 mm × 0.25 µm) was used. The injector in split mode (20:1) was set at 250°C, injection volume was 1 µl and the detector temperature was 320°C. Nitrogen was used as the carrier gas. The oven was initially set at 50°C, raised to 210°C at 8°C/min and maintained for 4 min. The temperature was again increased to 260°C at a rate of 12°C/min and held for 4 min.

RESULTS AND DISCUSSION

The total phenolic content of seeds of *M. myristica* was estimated to be 1478.32 mg/100 g, while the phenolic content of the seed of *M. tenuifolia* was estimated to be 1171.52 mg/100 g. The total phenolic content of *M. tenuifolia* was lower than that of *M. myristica*. The levels of total phenolics in both plant seeds were higher than the levels of total phenolics in some vegetables and spices reported by previous works of Lee et al. (2003), Abdou et al. (2010), and George and Osioma (2011). Variations in phenolic content as reported in the literature could partially be associated with the method of extraction employed. It has been noted that extraction yield of phenolics using ethanol was 2 to 3-fold lower than that with methanol (Abdou et al., 2010). It might also be due to differences in the analytical techniques employed as well as maturity of the plant (Howard et al., 2000).

About fifty-three different types of phenolics were identified in the seeds of *M. myristica* (Table 1) and *M. tenuifolia* (Table 2). Both seeds contained virtually the same kinds of phenolics but in varying amounts. Table 3 shows the ten prominent phenolics (Myristicin, Caffeic acid, Safrole, Methyl Eugenol, Catechin, Elemicin, Quercetin, Kaempherol, Methyl Isoeugenol and Eugenol) identified in the seeds of *M. myristica* and *M. tenuifolia* and their percentage composition, while Figure 2 shows the structures of prominent phenolics identified in seeds of *M. myristica* and *M. tenuifolia*. It was observed that *M. myristica* is richer in Myristicin, Caffeic acid, Elemicin and Eugenol compared to *M. tenuifolia* that is richer in Safrole, MethylEugenol, Catechin, Quercetin, Kaempherol and Methyl Isoeugenol. Plants have been known to vary within and among species in the types and concentrations of phenolics due to variables in plant growth, soil, weather condition and the age of the plant (Scalbert et al., 2005). It is known that amongst other factors such as maturity stage or light exposure, phenolic composition also varies with the cultivar and their different structures or levels are likely to have different functional properties (Huang et al., 2007; Magalhaes et al., 2009).

Phenolics, which are present in foods, have attracted a great deal of attention recently due to reports of the role they play in preventing diseases (Shahidi and Naczk, 2004; Fattouch et al., 2007; Sara et al., 2010). They are important due to their ability to serve as antioxidants which are widely found in secondary products of medicinal plants (Wang et al., 2008; Ehsan et al., 2012). The antioxidant activity of phenolics is attributed to their redox properties which play a role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994; Rice-Evans et al., 1997; Aranya et al., 2013). To the best of our knowledge information is scarce on the nature of phenolics present in the spices *M. myristica* and *M. tenuifolia*. However, several researchers (Abdou et al., 2010; George and Osioma, 2011; Lovet and Enebi, 2012) have reported the presence of phenolics in *M. myristica* and *M. tenuifolia* seeds.

Conclusion

The results obtained in this present work suggest that *M. myristica* and *M. tenuifolia* are good sources of phenolics, indicating that inclusion of these spices in human diet could contribute to potential health benefits.

Conflict of Interests

The authors have not declared any conflict of interests.

Table 1. Phenolic compounds identified in the seed of *Monodora myristica* by GC-FID showing their retention time (RT), area pA*s, amount (mg/100 g) and names.

pK	Retention time (min)	Area (pA*s)	Amount (mg/100 g)	Name
1	3.63	207.97	72.21	Catechin
2.	6.78	18.53	1.48e-4	Phenol
3.	7.35	9.52	7.59e-5	Phenylacetic acid
4.	7.65	21.18	1.69e-4	Salicylic acid
5.	7.96	8.61	6.86	Myrcene
6	8.78	4.00	3.19e-5	Cinnamic acid
7	9.69	48.59	13.21	Protocatechuic acid
8.	10.01	1.91	1.28e-2	Carvacrol
9.	10.11	2.97	2.36e-5	Gentisic acid
10.	10.79	1.64	1.98e-2	p-coumaric acid
11.	11.17	3.38	4.00e-2	Vanillic acid
12.	11.37	101.88	165.39	Safrole
13.	11.70	3.75	15.08	Eugenol
14	11.96	4.11	3.27e-5	Isoeugenol
15.	12.32	4.28	79.76	Methyly Eugenol
16.	12.69	3.44	20.49	Methyl Isoeugenol
17.	13.21	10.02	7.98e-5	Gallic acid
18.	13.69	23.50	56.50	Elemicin
19.	13.78	5.24	629.72	Myristicin
20.	14.16	42.60	345.79	Caffeic acid
21	14.94	15.48	5.60e-2	Ferulic acid
22.	15.39	35.18	2.80e-4	Syringic acid
23.	15.50	4.87	7.87e-3	Piperic acid
24.	16.14	16.35	1.17e-2	Sinapinic acid
25.	16.55	24.36	9.82e-3	Daidzein
26.	17.52	4.06	1.10e-3	Coumestrol
27	18.39	3.33	3.25e-3	Genistein
28.	18.77	5.36	1.10e-2	Apigenin
29.	19.00	6.35	5.06e-5	Naringenin Chalcone
30.	19.31	3.77	3.77e-5	Naringenin
31.	19.67	9.39	2.88e-2	Shogaol
32.	20.61	2.34	1.92e-3	Glycitein
33.	21.52	3.40	32.95	Kaempferol
34.	21.83	1.03	1.11e-3	Luteolin
35.	22.39	1.92	2.98e-3	Capsaicin
36.	22.70	2.06	1.64e-5	Epicatechin
37.	23.22	3.37	2.92e-5	Epigallocatechin
38.	23.34	9.30e-1	9.01e-4	Gingerol
39.	24.14	9.74e-1	9.44e-3	Myricetin
40.	24.50	8.44e-1	2.50e-3	Isorhamnetin
41.	25.05	49.42	40.11	Quercetin
42.	25.26	3.63	2.96e-4	3-o-caffeoylquinic
43.	25.53	3.90	6.25e-3	Chlorogenic acid
44.	26.24	4.46	7.01e-3	Rosmarinic acid
45.	26.93	3.49	2.83e-3	Curcumin
46.	27.20	1.72	2.76e-3	Miquelianin
47.	27.48	9.09e-1	1.44e-3	Eriocitrin
48.	28.25	2.48	4.03e-3	Rutin
49.	29.00	5.27	4.29e-6	Papain
50	29.31	4.08	3.25e-5	Phenyl-6-o-malonyl-beta-D- glucoside

Table 1. Contd.

51.	29.48	9.21	7.33e-5	4-o-methyl-epi-gallocatechin
52.	30.06	48.07	3.83e-4	Epi-gallocatechin-3-O-gallate
53	30.26	19.03	1.52e-4	Lupeol

Table 2. Phenolic compounds identified in the seed of *Monodora myristica* by GC-FID showing their retention time (RT), area (pA*s), amount (mg/100g) and names.

pK	Retention time (min)	Area (pA*s)	Amount (mg/100 g)	Name
1.	3.63	232.21	61.76	Catechin
2.	6.94	74.18	5.91e-4	Phenol
3.	7.37	26.50	2.11e-4	Phenylacetic Acid
4.	7.65	34.60	2.75e-4	Salicylic Acid
5.	7.95	12.71	5.06	Myrcene
6.	8.77	23.44	1.87e-4	Cinnamic Acid
7.	9.69	11.25	8.79	Protocatechuric Acid
8.	10.13	17.92	1.43e-4	Gentisic Acid
9.	10.28	14.00	9.39e-2	Carvacrol
10	10.79	11.47	1.39e-1	p-coumaric Acid
11	11.14	17.79	2.11e-1	Vanillic Acid
12	11.37	68.89	138.80	Safrole
13	11.70	18.79	11.13	Eugenol
14	12.02	15.21	1.21e-4	Isoeugenol
15	12.25	8.67	73.54	Methyl Eugenol
16	12.69	5.69	16.93	Methyl Isoeugenol
17	13.19	$9.47e^{-1}$	7.54e-6	Gallic Acid
18	13.61	5.06	39.51	Elemicin
19	13.83	31.00	490.55	Myristicin
20	14.16	32.44	263.30	Caffeic Acid
21	14.97	61.00	2.21e-2	Ferulic Acid
22	15.25	16.61	1.32e-4	Syringic Acid
23	15.54	23.92	3.86e-3	Piperic Acid
24	16.25	17.59	1.26e-2	Sinapinic Acid
25	16.55	42.96	1.73e-3	Daidzein
26	17.45	18.27	4.96e-3	Coumestrol
27	18.40	31.58	3.08e-3	Genistein
28	18.78	21.81	4.46e-2	Apigenin
29	19.03	10.38	8.26e-5	Naringenin Chalcone
30	19.35	10.59	1.06e-5	Naringenin
31	19.67	23.70	7.23e-2	Shogaol
32	20.51	25.74	2.12e-3	Glycitein
33	21.53	13.82	26.62	Kaempferol
34	21.85	10.94	1.19e-3	Luteolin
35	22.32	7.47	1.16e-3	Capsaicin
36	22.86	5.22	4.16e-5	Epicatechin
37	23.08	2.25	1.80e-5	Epigallocatechin
38	23.41	3.44	3.34e-4	Gingerol
39	24.03	1.55	1.50e-3	Myricetin
40	24.57	4.43e-1	1.31e-3	Isorhamnetin
41	25.05	2.20	34.82	Quercetin
42	25.26	1.74	1.42e-4	3-o-caffeoylquinic

Table 2. Contd.

43	25.54	1.95	3.12e-3	Chlorogenic Acid
44	26.38	1.84	2.89e-3	Rosmarinio Acid
45	26.94	6.57	5.32e-3	Curcumin
46	27.08	8.12	1.30e-3	Miquelianin
47	27.48	18.83	2.93e-3	Eriocitrin
48	28.32	35.66	1.62e-4	Rutin
49	29.00	9.01	7.96e-7	Papain
50	29.30	25.94	8.00e-7	4-o-methyl-epi-gallocatechin
51	29.69	17.38	7.96e-7	Phenyl-6-o-malonyl-beta-D-glucoside
52	29.84	18.67	7.96e-6	Lupeol
53.	30.00	36.71	7.96e-6	Epigallocatechin-3-o-gallate

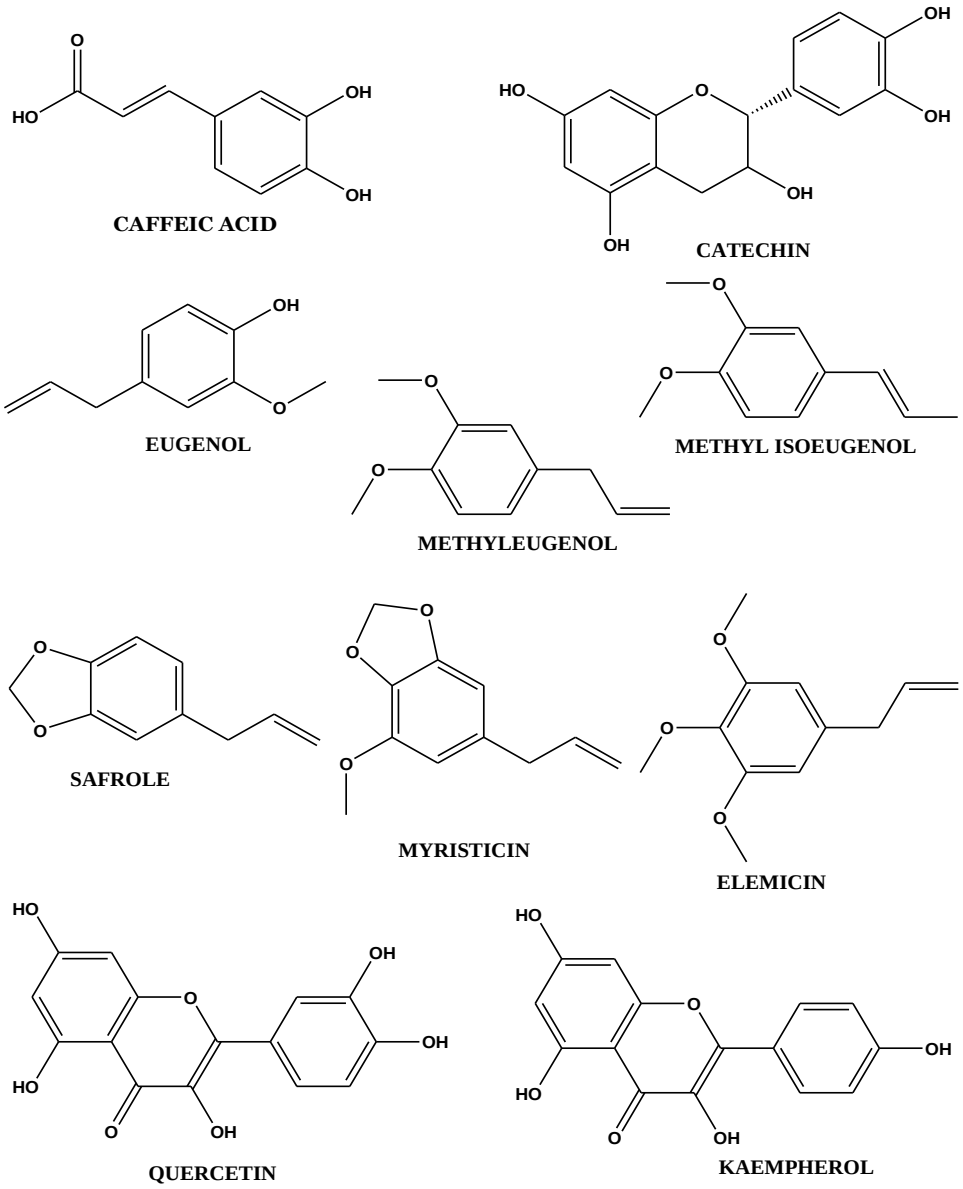

Figure 2. Structures of major phenolics identified in the seeds of *Monodora myristica* and *Monodora tenuifolia*

Table 3. Major phenolics in the seeds of *Monodora myristica* and *Monodora tenuifolia* as identified by GC-FID and their percentage composition.

S/N	Identified phenolics	% composition	
		M. myristica	*M. tenuifolia*
1.	Myristicin	42.60	41.87
2.	Caffeic acid	23.39	22.48
3.	Safrole	11.19	11.86
4.	Methyl Eugenol	5.40	6.28
5.	Catechin	4.88	5.27
6.	Elemicin	3.82	3.37
7.	Quercetin	2.71	2.97
8.	Kaempherol	2.23	2.27
9.	Methyl Isoeugenol	1.39	1.45
10.	Eugenol	1.02	0.95

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Review

Future research trends of forest landscape degradation: The effect of changing status and use of rural road networks

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The degradation of forest landscape was mainly driven by anthropogenic disturbance at short temporal and small spatial scale. However, the execution (intensity or frequency) of anthropogenic disturbance was determined by the accessibility of road network connection. The passing effect of rural road network must be considered, when understanding on the degradation of forest landscape and selecting restoration measures for degraded forest landscape. The existing literatures deeply understood the influences of the natural passing effect on forest landscape degradation. And they, to a certain extent, also identified the function of huge road network to forest landscape degradation. But, rural road network more widely distributed, and its passing effect on forest landscape degradation involved less. Moreover, the evolution of the rural road network (e.g., change of use, renovation and expansion, new build) was greatly accelerated, due to land consolidation favoring to agricultural industrialization and new village construction helping to urbanization. Certainly, under such environment, the degradation of forest landscape will be greatly intensified. Therefore, the passing effect of rural road network was considered, when the study of forest landscape degradation carried out. And future priority field should focus on: the corresponding relationship between forest landscape degradation and the passing effect of rural road network was firstly identified. Secondly, underlying driving factors must be understood, as they determined the evolution direction of the above relationship. Finally, preferred recovery block and recovery model of degraded forest landscape were arranged through consultation with local residents together. The understanding of forest landscape degradation, under the function of the passing effect of rural road network, can provide the best path for the identification of degradation process and the selection of recovery strategy.

Key words: Forest landscape, degradation, passing effect, rural road network.

INTRODUCTION

Forest landscape plays an important role in sustaining the balance of global atmospheric carbon and oxygen and climate stability (Hellmann and Pineda-Krch, 2007). However, forest landscape, with the high natural formation, was commonly controlled by the integration of natural succession and interference pattern, due to the

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high intensity of continuous disturbance of human activities (Lugo and Gucinski, 2000; Narayanaraj and Wimberly, 2012). This gradually led to the transition towards a simple stand structure, with the reverse development trajectory from natural forest, degraded natural forest, secondary forest, degraded forest, artificial forest to farmland (Quine and Watts, 2009). Consequently, the basement of forest landscape was usually damaged, and the high sensitivity of the response of forest landscape to anthropogenic disturbance mostly occurred (Nitschke and Innes, 2008; Albers and Robinson, 2013). Except for natural protected areas, natural forest landscape can only be found in high mountains and steep slope, where local residents had difficulties in carried out agricultural activities (Grainger, 2012).

Anthropogenic disturbance has been the main driving force of the evolution and conversion of forest landscape at short temporal and small spatial scale and the expansion or diffusion of interference, and its frequency of occurrence were often constrained by the accessibility of road network reflecting obvious post construction effect. Unfortunately, the existing literature is too much focused on the agglomeration and radiation of logistics and information exchange along large road networks in huge geomorphic units (Trombulak and Frissell, 2000; Wijewardana, 2008; Liu et al., 2008). Especially, the road network of county or above level possessing post-construction effect, clearly driving the occurrence of the heterogeneity and diversity of forest landscape, presented greater interest (Pfaff et al., 2013).

The patch use transformation of forest landscape driven by the rural road network (e.g., tractor road and production road) less caused researcher's interest and attention, due to the effect of rural road network on forest landscape possessing the following characteristics: local disturbance, occurrence concealment and effect hysteresis (Mon et al., 2012). The objectives of this study was to consider future research directions of forest landscape degradation, following the development of rural road networks, through refining existing literature on forest landscape degradation and post-construction passing effect, and summing up research progress made so far. The implication of this paper was to help people understand the mechanism of the forest landscape degradation, and to provide ideas for the restoration of degraded forest landscape in the future.

EXISTING PROGRESS OF PASSING EFFECT

Post construction effect resulted from the control drive of traffic accessibility to landscape evolution trajectory. Understanding forest landscape degradation must consider the evolution of traffic accessibility. People often say that forest landscape is being "cut in pieces", which was the most concentrated expression of above effect, concluding selective logging and usage alteration (Pfaff et al., 2013). However, the study on post construction effect can be traced back to the significant function of huge valley and mountain on the passing of water vapor and runoff transmission under the background of large geomorphic units. Further, this effect is transferred to forest landscape, and will influence its health evolution and spatial difference distribution (Narayanaraj and Wimberly, 2013). High mountains presented significant obstacles to water vapor transport and human activity expansion (Li et al., 2007). But, only adaptive strategies could be taken to deal with this effect, due to mountain shape being not remodeled or changed in a short time.

Large valley possessed clearly transfer effect to the transmission of water vapor and runoff, and the extension of human activities (Wijewardana, 2008). The agglomeration of town and rural settlements, and the occurrence of various land-use activities, often focused on the valley area, or gathered in the interchange of main traffic routes, and usually extended along valley trend or traffic network development direction. Finally, the transfer effect of large valley had great disturbance to forest landscape, which distributed around valley area and main traffic routes (Li and Zhang, 2010). Moreover, for reducing the influence of transfer effect on forest landscape degradation, the way environmental impact assessment was often adopted to evaluate the destruction scene of forest landscape induced by human activities. At the same time, response or adaptive measures could be provided to recover degraded forest landscape.

The roads are the channels of artificial landscape and the transmission belt of economic development. They were mostly built for economic purposes (that is, economic development, traffic). Moreover, they widely distributed, were rapidly built, and deeply impacted their surrounding landscape. Zong et al. (2003) considered that the roads affected 15 to 20% area of global terrestrial ecosystem, depending on five functions (e.g., channel, barrier, filtration, habitat, source and sink). The effects of roads on forest landscape degradation presented the following two aspects: transient interference in the process of construction and potential, long-term and slow interference in the process of operation (Liu et al., 2008; Freitas et al., 2012). The former promoted forest landscape degradation being "point" or "strip" patterns, while the latter resulted in the occurrence of forest "island" or the reduction of forest ecological integrity.

China's road construction has been rapidly speeded since 1990s. Especially, backbone road network basically formed after the adjustment of the important development strategy at the national level (e.g., Western development, Northeastern old industrial base reconstruction and Midlands rise). With this correspondence, the study of the impact of the transfer effect of roads on surrounding forest landscape was mainly focused on county or higher level road network, while rural road network was seldom involved.

However, a large number of rural road network that directly helped agricultural production, farmer's trip and rural life obtained rapid development, associating with the promulgation of the policies aiming at the issues of "agriculture, rural areas and farmers", e.g., rural-urban integrated development, new rural construction and land consolidation. Although the impact of rural road network on the degradation of forest landscape also depended on the road grade and the distance from the roads, and the connecting or adjoining degree of rural road network with the main roads, more emphasis on the transfer of tractor road and production road to natural landscape availability (Tang et al., 2010; Sonwa et al., 2012). That is, the effect of low grade roads on the degradation of forest landscape was the most direct and most significant. As we know, rural road network connecting the radius of residence and production, itself with narrow pavement, was only suitable for small agricultural vehicles or pedestrians. But, the influence of the transfer effect induced by low grade roads on the degradation of forest landscape generally strengthened, along with the upgrade of road level (Agbenyega et al., 2009; Mon et al., 2012).

The more important, public welfare forest was protected by ecological compensation measures at the national or regional level (Yin and Xu, 2002). The direct effects of large traffic network on forest landscape was relatively weak, the trend of forest landscape degradation has been basically controlled. However, forest landscape scattering in the farmland was faced with the threat of further development or transformation under the impact of rural road network (Ali et al., 2005; Avon et al., 2013). Because this part of forest landscape was non-public welfare forest. Certainly, not only lack of adaptable protection policies limiting the development behavior were arranged, but also no appropriate compensatory measures encouraging participation protection were formulated. The rural road network not only was carrying the direct function of residents' disturbance behavior to scattered forest landscape, but also was a carrier of the implementation of forest conservation policy at the national and regional scale (Freitas et al., 2010; Sonwa et al., 2012). Hence, it was necessary to understand the impact of the road status and use effect on forest landscape degradation through selecting the rural road network as the object.

The impact of rural road network on forest landscape was the explanation or decomposition of the function of the use effect of large road network on forest landscape degradation at the national or regional level. However, the influence of large road network on forest landscape at the national or regional level was the concentration or accumulation of the stress of the use effect of rural road network on forest landscape degradation. Namely, the disturbance scenarios of forest landscape around large road network at the national or regional level could rely on the accumulation of the use effect of a number of rural road networks connecting with large roads by the way of coupling interaction. Identifying the above effects of rural road network can serve macro decision-making for protecting forest landscape at the national or regional level.

The study of the impact of the use effect of road network on forest landscape was being transferred from large road network at the national or regional level to rural road network. It is often easier to develop or disturb local area where the density of rural road network was higher, and this development or disturbance usually extended along roads (Hickey and Nitschke, 2007). Where the density of rural road network was higher, the accessibility was better. In this region, the degree of influence of anthropogenic disturbance for pursuing maximum economic yield was stronger. Thus, forest landscape near rural road network was easily developed as farmland or woodland. Even then, some hidden intrusion or implicit damage was carried by farmers who replanted local precious species into natural forest landscape. The consequence of development and invasion resulted in the loss or damage of the ecological functions of forest landscape (Nagendra, 2012). Moreover, the effect of this loss or damage did not appear in a short time, while it presented regional effect with the help of local accumulation magnification.

FUTURE DIRECTIONS OF ROAD STATUS AND USE EFFECT

Forest landscape reflected the clear vulnerability characteristics, resulting from the overlap of ecology and economy, and the frequent disturbance of human activities in the key forest areas (Nitschke and Innes, 2008). However, most of them were controlled by the morphology of rural road network induced by the disturbance of human activities (Freitas et al., 2012). Identifying road use effect associated with the accessibility media of rural road network not only contributed to the understanding of the degradation process of forest landscape, but also favored the recovery scheme of degraded forest landscape.

Most of the influence of rural road network on forest landscape presented was corresponding to "channel", and no way was equal to "barrier". Namely, the impact of rural road network on forest landscape played only a "channel" for local residents whose perturbation behavior could be touched or not (Van de Walle, 2002). But, the extent of the influence only expanded outward along roads with the characteristics of local sporadic "point" shape, and performed for the spatial jump of interference. Rural road network altered or modified the effect of large road network on forest landscape, and it determined the possibility of forest landscape conversion. The conversion of forest landscape that was much closer to the road was earlier, due to the demand of farmers' traffic, crop's

harvest and agricultural goods' transportation. Moreover, the comprehensive effect of forest landscape fragmentation or correction was more obvious. Forest landscape that occurred around rural road network was used first or concerted with the patterns of "point" or "strip" shape along the both sides of road, when no major road network occurred in this region. There was a significant positive correlation between the reduction of forest landscape and the distribution of rural road network. This correlation expanded outward along the two sides of rural road network accessibility corridor. The consequence of expansion resulted in the decrease of forest landscape. Even then, shrub was very rare in this region. Further, it induced the damage of the function of soil and water conservation of forest landscape along rural road network (Neary et al., 2009). Under such environments, a high risk of ecological signal was released: parts of roads were seasonal being used in the dry season only. In turn, the passing effect of rural road network was greatly reduced.

The degradation of forest landscape presented the patterns of sporadic and isolated "point" shape, with the single, stability and chronic characteristics, when the rural road network was sparse. However, local residents often developed the larger range along the two sides of roads through the stronger disturbance action, along with the development of rural road network. Thus, the degradation of forest landscape gradually evolved towards "strip" shape, and the degraded patches of "point" and "strip" shape obviously appeared. Finally, the degradation of forest landscape showed strong diffusion convergence effect.

The disturbance of "strip" shape of local farmers to forest landscape would move with the feature of "skirt strip" shape along the foot of the mountain upward, resulted from the grid development of rural road network, when meeting with undulating micro topography. Then, the consequence of "skirt strip" shape disturbance drove the formation of a large number of forest landscape "islands" (Rotherham, 2007). Below the mountain where is easy to close, with the following characteristics, e.g., good accessibility, fertile and thick soil, good matching between water and soil resources, and low risk for production investment. Under such environment, part of forest landscape was firstly used for farmland and artificial woodland.

Moreover, accessibility determined by rural road network, relying on the point, corridor and superposition effect of point-corridor-network, induced that "isolated island" gradually atrophied, and "strip" gradually widened and lengthened connecting "isolated islands" (Sonwa et al., 2012). Consequently, the ecological integrity of local forest landscape was damaged. Certainly, once the signs of forest landscape degradation or ecological integrity damage occurred at the regional scale. At this time, in the location, long-term human disturbance must occur terribly, and evoked amazing patch fragile of forest landscape that was difficult to restore in the short time. Thus, the important role of forest landscape played in the balance of global atmospheric carbon and oxygen, and climate stability, was influenced by the accumulation of forest landscape degradation through the local to regional approach.

The obstructing effect of micro topography was very obvious in which rural road network was less. The spatial distribution of forest landscape presented significant difference, associating with the distance from the roads. However, the obstructing effect of original micro topography was broken, and forest landscape was heavily changed, along with the increase of the density of rural road network. Along the accessibility of rural road network radiating outwards, the strengthen degree that forest landscape was disturbed was generally slowed. Thus, for forest landscape, large patches controlled the dominant position, its patches' area increased, and its diversity index decreased. Accordingly, in the reachability scope, traffic was the more convenient, and the use of original forest landscape transformed. The process of forest landscape transformation was more complicated, and showed the number of patches was much more, the middle trajectory being difficult to identify. Certainly, the difficulty was much greater, if this part of the forest landscape was restored to near natural condition (Basu and Nayak, 2011).

In future scenario, the trend of the function of the accessibility induced by use effect on the further fragmentation of forest landscape was very clear. The area of large patches continued to shrink, and the number of small patches continued to increase. At the same time, the shape of large patches tended to be broader and the diversity tended to be simple (Bahadur, 2011). The ecological effects of forest landscape fragmentation caused habitat loss, the edge effect of fragmentation also seriously impaired the ability of maintaining the quality and population of forest landscape.

Human disturbance was blocked, and the ecological integrity of forest landscape was maintained relatively well, due the obstructing of micro relief where rural road network was absent. However, the construction of rural road network made the accessibility better and anthropogenic disturbances spread. The damage of forest landscape ecological integrity often occurred. But, the particularity of forest landscape site resulted in human disturbance was very frequent along both sides of rural road network. The consequence of perturbation led to the loss of a large amount of forest ecological information, and economic development into a vicious spiral circle. That is, the maintenance of forest landscape ecological integrity was facing double choice of economic development and ecological protection. Their balance became the most important problem at present, adapting to and coping with global climate change.

The World Bank considered that international or

interregional developed areas should include the protection of the forest landscape of poor areas. The framework of the international climate convention: "The Kyoto Protocol" and IPCC's "fourth report on climate change" also analyzed economic development, CO_2 emissions, afforestation or reforestation. Carbon trading was promoted by the rapid development of economy under the background of global climate change. But, ultimate measures for solving global climate change were the restoration of degraded forest landscapes. However, the current framework of carbon trading still consider afforestation and reforestation as a basic way of forest carbon sink, and ignores the huge potential value of the increase in carbon sink from degraded forest landscape restoration and need for a simple carbon sequestration accounting. In fact, afforestation, reforestation, and restoration of the ecological function of existing degraded forest landscape, possess the absorptive effect of atmospheric CO_2. Moreover, the restoration of degraded forest landscape had obvious advantages, either the investment of capital or management, or the occurrence of landscape effect in time, compared with the afforestation and reforestation. Currently, the main problem lies in the lack of accurate understanding to the complex process and core mechanism of degraded forest landscape. And, it was just the basic premise for efficient, accurate accounting the carbon effect of forest landscape restoration. Under such environment, the explanation of the use effect for forest landscape degradation and restoration was in line with the future demand of national economic strategy and the interest of present international research.

In addition, the central government work report, the central document and the new government's policy in 2013 also took agricultural industrialization, industrial new village and new village urbanization as strategic task for the next 10 years. The key issues of these documents were to implement the appropriate concentration of farmers contracted land and rural residential land, and to strengthen the infrastructure facilities. Especially, the density and level of rural road network was one of the key considerations. The density and level of the existing rural road network could not meet the demand of the development of new agricultural industrialization and new rural construction. Rural road system needed a new layout, and upgrading of rural road design standards. Moreover, the original transit road lost the previous meaning, due to the emergence of a large number of hollow, abandoned or unused villages. And it had gradually evolved into a village road and production road, and some where even abandoned. Certainly, the construction of new village residents gave rise to the emergence of a new road type. Under the process of contracted land and rural residential land focused on, the number of patches greatly reduced, and the area of single patch increased. Thus, the original production roads aimed at helping to local residents living or farming would no longer exist, after the number of patches reduced. Originally, the region where the trunk road was rarely planned, and road density increased for the efficient transport of agricultural goods.

Certainly, the road was wasted, and its use was changed. The change of the changing use effect of road must occur, and the degree of degradation of forest landscape resulting from this changed (Rotherham, 2007). The road use effect was terminated, associated with road abandonment. Thus, the degradation of forest landscape also stopped. The use effect greatly reduced due to the conversion from tractor to production road, and the degradation degree of forest landscape also obviously weakened. But, the change of use effect, resulted from improving road construction standards or new roads, also induced the change of the degree of degradation of forest landscape. The changed status and use effect when road construction standards were promoted from production to tractor road would greatly enhance, and the response of forest landscape degradation on it was more obvious. The use effect suddenly occurred due to new road building and the degradation of forest landscape also appeared.

Hence, the study of degraded forest landscape in the future must consider the impact of the passing effect of rural road network on it, meeting new rural construction, modern agricultural development and protection of forest landscape embedding in farmland. This meets the requirement of restoring forest landscape, dealing with global climate change and transforming economic development mode.

FUTURE RESEARCH IDEA OF CHANGING STATUS AND USE EFFECT

The study of forest landscape degradation considering the effect of use of rural road network was to understand the response of forest landscape degradation on it, and plan the recovery strategy of forest landscape sustainable utilization in the future. Therefore, the key scientific problem to be solved included: How to find out the dynamics of forest landscape patch and the changes of use effect, and understand the relationships between them? How to identify the driving intensity of the response of the conflicts of different interests groups on the use effect? How to arrange the landscape restoration scenarios, to set the priority recovery block, and to construct sustainable utilization mode?

The research objectives need to aim at four aspects: 1. Understanding the spatial differences of the trajectory of forest landscape degradation induced by the use effect of rural road network, and analyzing the impact and function process of the evolution of use effect on these differences; 2. Identifying the conflicts of different interest groups caused by the use effect of rural roads, and finding out the specific driving process and future

possible trend of these conflicts to forest landscape degradation; 3. Simulating the future scenarios of forest landscape restoration under the constraints of ecological integrity, considering the use effect, and determining priority block of landscape restoration; 4.The content arrangement needs to integrate the framework of pattern, process, driving forces and measures, and answer the relations between forest landscape degradation and use effect of rural road network. Specifically, the future research must identify the use effect of forest landscape degradation and restoration by means of rural road network transferring or diffusing human disturbance. At the same time, the trajectory and characteristics of forest landscape degradation should be analyzed. And the relationship between this trajectory and the use effect of rural road network should be assessed. On the basis of above researches, the underlying potential driving factors can be understood. In addition, the future trend of the function and response of different interests groups on the use effect of rural road network should be investigated. Finally, the appropriate scenario of the restoration of degraded forest landscape needs to be simulated.

The analysis methods need to integrate high resolution remote sensing data (e.g., aerial photos, SPOT, Quickbird, etc.) and non- remote sensing data (e.g., household interviews, historical statistics, field survey, etc.). Quantitative mathematical method (e.g., CLUE-S, strength of interference effect, ecological time limit, logistic regression, multi-agent, ecological fragmentation degree, etc.), and patch dynamics atlas featured the spatial distribution of forest landscape, are used to calculate and simulate the response of forest landscape patch atlas on the change of the passing effect of rural road network.

Completing of the above ideas, the key technology that must be solved is: 1.Quantitative technique by which the relationship between the degradation of forest landscape and the use effect of rural road network should be analyzed; 2. Recognition technique by which driving factors impacting forest landscape degradation are understood, considering the influence of the use effect of rural road network. The technical difficulty that must be resolved: 3.Distinguishing what extent the influence of the use effect of rural road network on the degradation of forest landscape will appear? Otherwise, the relationships between the degradation of forest landscape and the passing effect of rural road network is very difficult to find; 4.How to use the use effect of rural road network to restore degraded forest landscape. Otherwise, the sustainable utilization mode of degraded forest landscape may not achieve.

CONCLUSIONS

The study of the impact of the use effect of road network on forest landscape degradation is shifting from large road at the national or regional level to rural road network. The spatial distribution of forest landscape degradation presented strong heterogeneity, resulting from the difference of the status and use effect of rural road network. However, understanding the impact of the use effect of rural road network on forest landscape degradation must first be determined using the relationships between them, and find out the reason of forest landscape degradation, under the influence of the changing use of rural road network. Moreover, the future evolution trend of rural road network, and the future scenarios of forest landscape degradation induced by the changing use passing effect of rural road network should be quantitatively simulated. Finally, the future restoration scheme of degraded forest landscape should be determined by consulting with the local inhabitants, considering the use effect of rural road network. However, completing this research idea and direction, the most critical technical problems cannot be resolved without two aspects: what is the influence threshold value of the passing effect of rural road network on forest landscape degradation? How to use the passing effect of rural road network to restore degraded forest landscape?

Conflict of Interests

The authors have not declared any conflict of interests.

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Review

Biology and management of ber fruit fly, *Carpomyia vesuviana* Costa (Diptera: Tephritidae): A review

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Ber fruit fly, *Carpomyia vesuviana* Costa (Tephritidae: Diptera) is one of the notorious monophagous pests of ber in India, Pakistan and Middle East countries. The fly infest most of the *Ziziphus* species grown in the world and cause the damage internally and in serious case it causes severe yield loss up to 80% or even upto 100% damage. The external temperature, relative humidity and rainfall and soil moisture, soil temperature and soil depth are found to be critical factors for the activity and the adult fly emergence from soil. The favourable temperature for pupal development and adult emergence is 30°C, pupation at 3 to 6 cm depth of soil was ideal for adult emergence. Alternating rainfall ranging from 20 to 40 mm and 62 to 85% relative humidity also promotes fly activity. Prophylactic measures are the essential components for the successful management of *C. vesuviana*. Field sanitation, destruction of wild bushes, collection of infested fruits and summer ploughing to expose the overwintering pupa to hot summer breaks the reproduction cycle of fly. Growing of resistant cultivars like Tikidi, Umran, Mundia, Banarasi, Sanaur-1, Safeda selection, Illaiciihi, Mirchia, Zg-3 and Chhuhara would give better yield and also reduces the protection cost. However, synthetic chemicals are presently employed as major tools against fruit fly, organophosphate and synthetic pyrethroid insecticides are in extensive use. Availability of potential biocontrol agents and botanic pesticides are very limited. Therefore, it is necessary to incorporate the all available tactics in integrated manner and incorporation of neem based formulations and biological pesticide, spinosad, bait application, male annihilation technique are essential to manage the *C. vesuviana* in a successful manner in the scenario of organic cultivation.

Key words: *Ziziphus* sp., *Carpomyia vesuviana*, bio-ecology, integrated pest management (IPM).

INTRODUCTION

Fruit fly, *Carpomyia vesuviana* Costa (Diptera: Tephritidae) is the most destructive pest of ber, a multipurpose tree that supplies fruit, timber, fuel wood and also fodder (Joshi and Shinde, 1971; Lakra, 1998; Muhammad, 2006; Kavitha and Savithri, 2002; Zavitha et al., 2002; Balikai et al., 2013). It is the monophagous pest that infesting only on *Zizyphus* species growing under arid and semi arid region in Oriental Asia India also in,

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Table 1. Host range.

Species	References
Z. zizyphus	Sohi et al. (1990)
Z. mauritiana	Ahmed et al. (2005), Farrer et al. (2004), Singh (1984), Lakra (1984), Bagle (1992)
Z. spina-cheresti ; Z. numularia; Z. lotus	(Lakra (1984), Farrer et al. (2004)
Z. jujube	(Joshi and Shinde (1971), Berdyeva (1978)
Z. sativa	(Bagdavadze (1971), Giray (1979)
Z. rotuntifolia	Lakra and Singh (1984)

Middle East, Temperate Asia, China and South Europe (Kapoor, 2005; Kirichenko, 1940; Stonehouse et al., 2002; Hu et al., 2010; Farrar et al., 2004). The pest contributes towards low yield and poor quality of fruits and it causing loss up to 80% under severe infestation (Batra, 1952; Cherian and Sundaram, 1941; Karuppaiah et al., 2010). The incidence of *C. vesuviana* reduces the yield from 13 to 20% per plant (Bagle, 1992), 90 to 100% (Joshi and Shinde, 1971). In Georgia the *Carpomyia* attacking *Z. sativa* causes more than 70% losses (Bagdavadze, 1977). The ber species *Z. jujube* was infested severely by *C. vesuviana* and it causes up to 60 to 70% fruit damage in Turkmenia (Berdyeva, 1978). In Iran the intensity of damage was 30 to 100% (Farrar et al., 2003).

The brownish yellow adult flies are emerges from the soil and starts to infest on ber fruits at pea stage. Adults inserts the eggs on the young developing fruits by making puncture with small cavity using protrusive ovipositor just below the epidermis of the immature fruits (Lakra and Singh, 1983; Dashad et al., 1999). Thus, newly emerged maggots' starts to feed on the pulp and making the galleries with accumulated excreta. In severe case infestation may cause the fruit deformation and finally leads into fruit drop (Lakra and Singh, 1989). Since the maggots causing internal damage, the curative measures using chemical insecticides are showing unsatisfied results. Therefore, it is essential to explore prophylactic and curative measures together to manage this pest successfully. Besides the synthetic insecticides, incorporation host plant resistance along with bio-control agents and botanical pesticides would give satisfactory control than single approach. Hence, the documented information about *C. vesuviana* has been reviewed in this paper to explore the available technologies for the successful management.

HOST RANGE AND DISTRIBUTION

The infestation of *C. vesuviana* has been observed in all wild and cultivated species *viz*., *Z. zizyphus, Z. mauritiana, Z. spina-cheresti, Z. numularia, Z. lotus, Z. jujube, Z. sativa* and *Z. rotuntifolia* (Table 1). The distribution is found in all the ber growing region of India and world. The occurrence of this pest is reported in many countries like India, Pakistan, Iran, Georgia, Bangladesh, Turkey, Turkumenia, Mauritius, Indian Océan Island, Uzbekistan, Temperate Asia, China and South Europe and Oman (Table 2).

NATURE AND EXTENT OF DAMAGE

The infestation starts at onset of fruiting, adult fly lays eggs singly in the young developing fruits after 2 to 5 days. Upon hatching, the maggots feed on the pulp and create galleries with accumulated excreta and results in rotting of fruits. The infested fruits are become deformed and their growth gets retarded in severe cases such fruits are drop off (Singh, 2008). The fruit fly damages flowers and fruits and the adults puncture the ripening fruits and lay their eggs inside the epidermis the young maggots feed on the fleshy and juicy pulp of fruits (Gupta and Sharma, 2006; Lakra and Singh, 1989). The maggots burrow in to the flesh around the centre leaving excreta that give fruits a bitter taste (Bagdavadze, 1977). The full grown larvae come out by making hole in the fruit skin and drop to the ground for pupation.

Variations in the damage intensity found to be associated with external factors like, rainfall, relative humidity and temperature (Lakra and Singh, 1985). However, the physical factors like soil moisture, soil temperature and soil depth also found to play a crucial role in the adult fly emergence from soil. The optimum temperature for pupal development was 30°C leading to high adult emergence (74%) and short pupal duration (15.65 days) at 10, 16 and 40°C no adult emergence up to 50 days and 3 to 6 cm pupation depth was ideal for adult emergence (Sangwan and Lakra, 1992). High temperature >40ºC and low relative humidity <20 to 30% was unfavorable and prolonged immature stage occurs at temperature beyond 5°C. The intermittent light rainfall ranging from 20 to 40 mm also promotes fly activity and moderate and heavy rainfall, 50 to 120 mm per week curtails the activity (Lakra and Singh, 1985). The incidence of fruit fly was high when the relative humidity was 62 to 85% and temperature ranging between 17 and

Table 2. Geographical distribution of *C. vesuviana.*

Country	References
India	Batra (1953), Cherian and Sundaram (1941), Basha (1952), Saen (1986), Jothi and Tandon (1995), Patil et al. (1997), Balikai (1999), Kavitha and Savithri, (2002), Balikai et al. (2013)
Pakistan	Abhasi et al. (1994), Stonehouse et al. (2002), Ahmed et al. (2005), Sarwar (2006)
Iran	Farrar et al. (2004), Farrar and Chou (2000)
Georgia	Bagdavadze (1977)
Turkey	Giray (1979)
Turkmenia	Berdyeva (1978)
Mauritius	(Sookar et al. (1998), White et al. (2000)
Indian ocean islands (Rodrigues, Seychelles)	White et al. (2000)
Uzbekistan	Kimsanboev et al. (2000)
Oman	Azam et al. (2004)

25.5°C and minimum at 2.3 to 4.8°C (Dashad et al., 1999). The relationship between the pest incidence and temperature was positive and it was negative with relative humidity, wind speed and cloud cover (Nandihalli, 1996). The intensity of damage is influenced by the surviving pupa of preceding years (Dashad, 1999). The early maturing varieties had higher infestation than late maturing verities (Singh, 2002). The larvae bore down into the soil up to a depth of 2 to 12 cm where it pupates (Batra, 1953).

STATUS OF *C. VESUVIANA* IN INDIA

In India, the time of activity and number of generations found to be varies with season from region to region (Table 3). In northern states of India, the infestations occur from November to April and activity was very high during fruit maturity. There may be a 2 to 3 generation per year (Batra, 1953) and incidence was most abundant in December and least in March (Lakra and Singh, 1983) and it was 6 to 9 overlapping generation per year (Larka and Singh, 1986). The pupa hibernates in soil during April to August lead to the unusual activity of fly during off season fruits of *Z. zizyphus* at Punjab (Sohi et al., 1990). The shortest generation time 23 days was recorded in the eggs laid on September (Larka and Singh, 1986). In the central part of India (Gujarat) the fruit fly infestation starts around mid October and increased suddenly in mid November, continuing till December (Bagle, 1992). In the Southern state, Karnataka*, in Z. mauritiana* the activity of *C. vesuviana* is prevalent from fortnight of December to fortnight of February (Nandihalli, 1996).

BIOLOGY

Life-cycle of the fruit flies varied with environmental factors. Adults are small yellowish brown fly little smaller than the common housefly having brownish bands on hyaline wings and black spots on the thorax. The pre oviposition, oviposistion and post oviposition periods lasted after 2 to 12, 3 to 44 and 0 to 14 days, respectively and about 80% of the females deposited eggs after 3 to 7 days and laid an average of 22.99 eggs. The egg stage was 1 to 4 days with the viability of 70.21 to 94.44%, and the larval period found to be 7 to 24 days and pre-pupal stage was 3 to 8 h (short 5 to 42 days) long (43 to 122 days) cycle pupation occurred in 80 and 20% of pupa, respectively. The pupal duration was more in November, December and April (Lakra and Singh, 1986). The incubation period was 2 to 5 days and of larval and pupal stage was 9 to12 and 2 weeks, respectively (Batra, 1953). The fly larvae enter soil and pupate after 3 to 4 h in puparia that over winter in the soil around the tree trunks and occasionally pupation takes place with infested fruits (Bagdavadze, 1977). Adult emergence from pupa between 9 and 14 h, pairing and oviposition occur during day light hours at night the flies usually rest in the tree canopy and its complete two generation per year at Turkmenia (Berdyeva, 1978). The adult longevity was 3 to 48 days in lab and it varied respect with month. The sex ratio was 1:1 and pre-oviposition, oviposition and post oviposition periods lasted 2 to 8, 3 to 35 and 0 to 12 days. Females laid averagely 19.1 ± 5 eggs, generally 1 to 4 ovipunctures per fruit. About 72% of the egg laying was occurs between 3 to 7 days and higher fecundity during November, February and lower in March. The incubation period was 1 to 4 days and 70.4 to 91.9% of eggs were viable. The larval and pupal period was long during December and short during March and the average was 6 to 22 days and 8 to 320 days. The maggots took 1.8 to 5 h to pupate. The shortest pupal duration 8 days was observed in March to April and longest 320 days in September. The eggs laid during march April and January had shortest life cycle and eggs laid in September October had the life cycle duration of320 days. The fly completes about 8 to 10 overlapping

Table 3. Season of activity of *C. vesuviana* in India.

Month of activity	State	Reference
August-October	Uttar Pradesh	Gupta and Sharma (2006)
October-December	Gujarat	Bagle (1992)
December-February	Karnataka	Nandihalli (1996)
August-September (on *Z. numularia*) 'July - April (on *Z. mauritiana*)	Haryana	Lakra and Singh (1985)
July - February	Andhra Pradesh	Kavitha and Savithri (2002)

Table 4. Resistance cultivars to *C. vesuviana.*

Cultivar	Remarks	Reference
Sanaur-1, Safeda selection,Illacihi, Chinese, Mirchia, Zg-3,Umran	Resistant	Mann and Bindara (1976)
Tikidi	Resistant	Singh (1984)
Gola, Illaichi	Moderately resistant	
Tikidi, Illaichi	Resistant	Sharma et al. (1998)
Umran,Tas bataso, Deshi Alwar, Kismis	Moderately resistant	
Cv. Illaichi, Chhuhara	Resistant	Nandihalli et al. (1996)
Tikidi, Mundia	Moderately susceptible	Pareek et al. (2003)
Tikidi	Highly resistant	Pramanick et al. (2005)

generation in a year (Farrar et al., 2003).

INTEGRATED MANAGEMENT STRATEGIES

Fruit fly, *C. vesuviana* cause damage internally and it is very difficult to manage this pest without insecticides. The egg laying can reduce by prophylactic spraying. The maggot has the low possibilities to expose against key mortality factors like harsh environment, pathogen predators and parasites. The lack of early detection techniques also boosts the pest population built-up. Apart from this, the fruits are harvested with short intervals for consumption and it is consumed directly by consumers. In the consumption point of view it will not be recommended to rely on pesticides as a major component to mange this pest. Hence it is necessary to include eco-friendly soft pesticides with low residual toxicity and short waiting period (Table 4).

PROPHYLACTIC MEASURES

Clean cultivation

Field sanitation is an effective preventive measure in fruit fly management and need to be done systematically as a primary component to break the reproduction cycle and minimize the population built up and infestation (Singh, 2008). The residual pupae are the major source of infestation, which is present in the surrounding of the tree trunk through the physiological adaptation like aestivation and hibernation (Singh et al., 1973). The collection of all fallen, bird damaged and infested fruit at regular interval that is twice in a week from fruit setting to harvest and proper destruction and feed such fruits to sheep goats, camels or other farm animals or bury them at least on one meter deep in compact soil can avoid the fly's emergence (Srivastava and Nanda, 1983; Lakra, 1998). Birds attack on unripe and semi ripped fruits results in a built up of initial population of tephritids causing heavy losses at later stage of crop (Grewal and Kapoor, 1986). Harvesting of matured fruits before the colour change (green to yellow), deep and through raking up of soil and ploughing the orchards during hot summer and winter months expose the pupae to drastic environment and natural enemies. Cultural operation also been destroy the over wintering pupae through mechanical injury during the operations (Lakra, 1998). Clean cultivation surrounding the areas of orchard by destroying burning and destroying the pruned parts of cultivated as well as the wild bushes (*Z. numularia, Z. mauritiana* var. *rotundifolia*) which serve as a good source of multiplication and help in carrying over of fruit fly to cultivated ber species (Chauhan and Yadav, 2000). The early fruit setting and off season fruit bearing of wild bushes provide link to breed and switch over to main crop. Early harvesting of fruits at colour change stage,

avoiding the over ripening of fruits on trees also promote less survival of fruit fly (Lakra, 2004). Cultivation of orchards soil during spring (Singh et al., 1973a), summer (Chundawat and Srivastava, 1978) and rainy season (Bakhshi and Singh, 1974) destroy the hibernating pupae by exposing them to bright sunlight and birds making the considerable reduction in the infestation. Heating of soil by burning grass and irrigation during summer also kill the pupae (Singh, 2008).

Host plant resistance

Host plant resistance promotes cumulative protection against fruit fly without any environmental hazards with least management cost (Singh, 1984; Singh and Vashistha, 1985; Sharma et al., 1998). The success of developing varieties which is resistance against the fruit fly has the limitations like crossable barriers, heritability of quality characters and less availability of resistance source etc. Use of biotechnological tools to transfer the resistance gene from the source genotypes to cultivated genotypes could be a better option. However, in India some successful work has been reported in the resistant breeding programme (Table 4). Faroda (1996) attempted cross between Seb x Tikidi and obtained F_1 generation showed 90% resistant with poor fruit quality and backcrossed BC_1 line showed 87 to 90% resistance and desirable fruit characters. None of the *Z. mauritiana* cultivars were immune to the fruit fly but it has wide range of susceptibility from 10 to 50% damage (Sharma et al., 1998). The expression of resistance also depends up on the bio physical and bio chemical characters of fruits. The cultivar with moderate sugar content and hard texture of fruit coupled with resistant to fruit fly (Pramanick et al., 2005). The adult flies avoid egg laying in the fruits which are less than 9 × 4.5 mm size and lay more than 50% of the eggs in 20 × 9 mm size fruits (Lakra and Singh, 1983). The mechanism of resistance such as antibiosis and non preference in ber genotypes against fruit fly has been studied by and certain cultivar for egg laying and no significant variation among them respect to egg laying, same time significance variation was observed in the larvae hatching indicate the antibiosis (Singh, 1984). The infestation of fruit fly was positively correlated with fruit weight, pulp stone ratio, total soluble solids and total sugars and negatively correlated to acidity, vitamin C and total phenol (Arora et al., 1999). Fruit size, fruit weight and pulp ratio showed positive correlation with fly infestation and cultivar with round fruits and early varieties contributing higher infestation (Singh and Vashishtha, 2002). The early maturing cultivar with moderate to bigger fruit size, sweet soft and thin skinned, more juicy and attractive flavoured pulp are more susceptible to fruit fly (Saxena and Rawat, 1968). Varieties such as Kakara, Umran, Mundia, Banarasi and Chhuhara exhibited fewer incidences of *C. vesuviana* and Gola, Kaithali and Ajmeri showed higher incidence.

Biological control

There is no successful record of parasitoids, predators and pathogens against the *C. vesuviana.* Singh (1989) reported that the braconid *Biostres vandenboschi* Fullaway as a parasitoid of *Carpomyia* from India but the proportion was very negligible. The parasitoids *Bracon fletcheri, Opius carpomyia* and *Omphalia* sp. were also noticed (Kavitha and Savithri, 2002). However, the population reduction of pest was not insignificant (Saxena and Rawat, 1968). The wasp parasitoid *Fopus carpomyia* was found at larval stage of fruit fly and the ovipositor is very suitable to parasitize the hidden host in fruits. The rate of parasitization was 21 to 26.7% (Farrar et al., 2004). The successful suppression of *C. vesuviana* with parasitoid can made through the augmentative release. The lack of mass culturing and efficacy testing techniques should be developed to overcome this bottleneck by generating new ideas for the practical application in the integrated management programme.

Chemical control

Management of ber fruit fly is mostly depending upon the chemical insecticides along with botanicals. Though it is unsatisfactory, the lack of alternative best approaches like attractive baiting and male annihilation with lures and management with biological pesticide has not yet developed against this pest. Moreover, pest can be managed below the economic threshold through the proper insecticide schedule. Spraying of Malathion (0.05 %) during January showed better control of fruit fly (Joshi and Shinde, 1971) and fenthion found to be most effective when it was applied 3 times in a season (Patel et al., 1989).

Two application of 0.2% dichlorvos at the pea stage of the fruits and 15 days later gave the better control than the monocrotophos 0.036%, malathion 0.05% and phasalone 0.07% (Ragumoorthi and Arumugam, 1992). Dimethoate, fenthion, phosphomidon and deltamethrin were most effective and endosulfan was the least effective against *C. vesuviana* (Patel, 1990).

The syntheytic pyrethroids fenvelarate 0.005% and decamethrin 0.0015% showed consistent action against the fruit fly activity (Bagle, 1992). Diptrex at onset of fruiting showed significant reduction in larval infestation (Abbasi et al., 1994). Soil application of fenitrothion or quinalphos dust at 20 kg/ha under the canopy showed 80 to 95% reduction in adult emergence (Lakra et al., 1991). The schedule comprising 0.03% dimethoate in late October and early November and second spray after 45 days followed by 0.075% endosulfan followed 0.1% carbaryl later 0.05% malathion with 1% sugar solution at

10 days interval proved effective against this pest (Lakra et al., 1991). Insecticides schedule consist of monocrotophos 0.03%, fenthion 0.05% and carbaryl 0.01% at 15 days interval were most effective (Dashad et al., 1999). Spraying of dimethoate followed by eco-neem was showed good reduction in infestation (Singh et al., 2008) and lowest incidence of *C. vesuviana* was observed with lambda cyhalothrin 0.0025% followed by 0.0018% beta-cyfluthrin (Gyi et al., 2003). Application of fenthion 0.1% at pea stage and second spray 30 days later showed lowest fruits damage. The extract of azadiractin 1% and *Ocimum sanctum* 1% were effective up to 10 days after spraying (Rajaram and Siddeswaran, 2006). Dipterex, imidacloprid, triazophos and neem products are notable insecticides against ber fruit fly (Abbasi et al., 1992; Singh et al., 2000). The integrated management with dipterex 100g/ acre+ 5% molasses baiting and hoeing and collection of fallen fruits throughout the season proved better than single treatment applied trees (Ahmed et al., 2005). Application of neem powder and tobacco extracts significantly reduced the infestation of *C. vesuviana* and they are the potential candidates for organic cultivation of ber (Mari et al., 2013).

Post harvest management of *C. vesuviana*

Transfer of infected fruits and planting materials from the area of infested to other non infested area for domestic consumption is one mode of spread of insects pests. This can be checked through tight domestic quarantine and post harvest disinfestations of fruits or planting material. The post harvest treatment of ber fruits with 0.45 kg ethylene dibromide per 28 m^3 for 6 h with 0.25 kg for 12 h in a sealed earth ware or air tight space would be effective in causing mortality of eggs and larvae of fruit flies but because of small size it does not seem convenience method (Lakra, 2004).

Conclusion

The fruit fly *C. vesuviana* is a major pest of ber. The present management strategies are solely relies on the insecticides and organophosphates and synthetic pyrethroids are the major insecticides groups. In India, use of newer compounds, neo- nicotinoids, microbial origin compounds like spinosad are very less in fruit fly management programme. The spinosad is good alternative for malathion, which is widely used in fruit fly management programme as a poison in baits. This could be exploited, besides it is essential to develop the augmentative release technique for notified bio control agent in ber ecosystem. The pest can be suppressed below the level of economic injury with the other integrated pest management (IPM) tools like growing fruit fly resistant varieties, proper field sanitation measures with scheduled application of soft insecticides native botanicals. So far no reports on attractive compound for *Carpomyia,* like methyl euginol for *Bactrocera*. The incorporation of bait application techniques (BAT) and insect transgenic, embryo specific lethality and sterile insect release techniques in wide area management can be exploited. However the economics cost and returns should be considered before to initiate any advanced technology.

Conflict of Interests

The authors have not declared any conflict of interests

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