



# African Journal of Agricultural Research

Volume 9 Number 29 17 July 2014

ISSN 1991-637X



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*Full Length Research Paper*

## Productivity of improved plantain technologies in Anambra State, Nigeria

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Received 2 June, 2014; Accepted 26 June, 2014

The poor plantain output problem in Anambra centers on the efficiency with which farmers use resources on their plantain farm. It also borders on how the various factors that affect plantain production can be examined, so as to improve plantain production in the country. The inefficiency problem is attributed to factors such as use of low input technologies, lack of knowledge of high input technologies, amongst others. The study therefore examined farmers' productivity of improved plantain technologies in Anambra State, Nigeria. Data collected were analyzed using descriptive statistics, productivity indices, multiple regression and gross margin analysis. Analysis of productivity indices based on resource use efficiency shows that the farmers are highly efficient in the use of planting materials and labor. The results of the multiple regression analysis revealed that farmers' age, farm size, household size, educational status, planting materials, extension contact and labor are the main determinants of plantain productivity in the state. Gross margin per hectare of plantain averaged ₦988750 while net farm income averaged ₦980250. Rate of returns on capital invested is ₦2.3 implying that plantain production is a profitable and viable venture. The study therefore recommends the organization of field days and farmers' training on the use of high yielding planting materials. Procurement and distribution of improved varieties to farmers by Anambra State Agricultural Development Programme at the right time and at affordable prices are effective strategies for stimulating plantain productivity in the area.

**Key words:** Gross margin, plantain production technologies, productivity, resource-use.

### INTRODUCTION

Plantain (*Musa* spp., ABB genome) is a giant herb that is cultivated in humid forest and mid- latitude zone of sub-Saharan Africa. Its origin is believed to be the South East Asia. However, a remarkable diversity of plantain exists in sub-Saharan Africa. The food crop is generally triploid, sterile and develops fruits by parthenocopy. Total world production is estimated to be over 76 million metric tons. Twelve million metric tons are produced in Africa annually (INIBAP in Fakayode et al., 2011).

Nigeria is one of the largest plantain producing countries in the world. It is the largest producer in West Africa with annual production of about 2.4 million metric tons mostly obtained from the southern states (FAO, 2006). Despite its prominence, Nigeria does not feature among plantain exporting nations. It produces more for local consumption than for export (Fortaleza, 2012). To harness the export potential of plantain, the current level of its production must be improved. This implies that the limited resources

available to plantain farmers have to be optimized. The poor plantain output problem in Nigeria therefore centers on the efficiency with which farmers use resources on their plantain farm. It also borders on how the various factors that affect plantain production can be examined, so as to improve plantain production in the country. This quest therefore raises research questions as to how could farmers be enhanced to produce a basic staple crop like plantain more efficiently? How productive is the plantain enterprise? How viable is it? This study thus examined the productivity of plantain farms in Nigeria using Anambra State as a case study.

However, Kebede (2001) predicated the growth on productivity gained through greater technical and allocative efficiencies of the farmers in response to the changing technological and production environment. He also stated that despite all human and material resources devoted to Nigerian agriculture, the productive efficiency of farmers for most crops still fall below 60%. The inefficiency problem is attributed to factors such as use of low input technologies, lack of knowledge of high input technologies and poor farm management skills, poor extension services, unavailability and high cost of inputs (Obasi, 2005; Anyanwu and Obasi, 2010a, b). Previous studies on efficiency of resource utilization and productivity (Ike and Ogba, 2004; Oluwatosin, 2006; Moses and Adebayo, 2006) showed that there are wide variations in the levels of productivity and productive efficiency for the major food crops and the levels are far from the optimum. This indicates therefore that ample opportunities exist for the farmers to increase their productivity and productive efficiency. However, the importance of plantain in National economy has caused several researches to be carried out on plantain production. International Institute of Tropical Agriculture (IITA) concluded a five year US\$ 4 million project that improved plant breeding techniques and developed new cultivars to increase yields of Musa crops (banana and plantain). This is for application in poverty reduction and income generation efforts throughout sub-Saharan Africa (SSA).

The project also developed new methods for deploying the varieties in a way that preserves traditional varieties while offering additional value-adding processing options (IITA, 2009). An underlying factor behind much of these works is that if farmers are not making efficient use of existing technology, introducing new technologies as a means of increasing agricultural output would be defeated, thus efforts designed to improve efficiency would be more cost-effective (Shapiro in Fakayode et al., 2011). In an economy where resources are scarce and opportunities for new technologies are lacking, efficiency studies can show the possibility of raising productivity by improving efficiency without expanding the resource base. Plantain farmers can thereby maximize profit and produce more, leading to food security and competitiveness in plantain production. This study will therefore serve as a guide to agricultural key players on plantain production

investment decisions.

## Objectives of the study

Specifically, this study was designed to examine the socio-economic characteristics of plantain farmers in the study area, identify the major plantain cultivars and techniques used by farmers, analyze the productivity, cost and returns of plantain production in relation to technologies used and determine the factors affecting plantain productivity of the farmers in the study area.

## METHODOLOGY

### Area of the study

The study was conducted in Anambra State, which is located in the south-east geopolitical zone of Nigeria. It consist of twenty-one Local Government Areas grouped under four agricultural zones of Anambra State Agricultural Development Programme, these are Ogbaru, Ayamelum, Anambra west, Aguata and Awka North. The study area has an approximated land area of 4,416 km<sup>2</sup> and lies between longitude 6°20'N and 7°00'E and latitudes of 7°16'N and 7°00'E. Its boundaries are formed by Delta State to the West, Imo State to the South, Enugu State to the East and Kogi State to the North (NBS, 2007).

The State Agricultural Development Programme currently provide extension services, imparts new technologies and financial assistance to apex farmers. The apex farmers are involved in all types of food production including research and often collaborate with the institute of tropical Agriculture and university of agriculture Umudike.

### Method of data collection

Data used for the study were sourced mainly from primary and secondary sources. Primary data were collected using structured questionnaire administered to the plantain farmers in the study area. The secondary data were sourced from journals, articles and relevant extension agencies in the area. A multi-stage random sampling technique was used for the study.

The first stage involved the random selection of four Local Government Areas. In each selected Local Government Area, five communities/villages were randomly selected. Lastly, ten farmers involved in plantain production were randomly selected from each community from a list obtained from the Local Government Area. This gave a total of two hundred respondents. However, due to incomplete information in some questionnaires, only one hundred and eighty six of the respondents constituted the sample size for the study.

### Method of data analysis

Descriptive statistical tools such as frequency counts, percentages and means were used to describe the data collected, while inferential statistical tools such as Multiple Regression Model, Gross Margin Model and Productivity Indices were also employed to analyze the data for the study.

### Multiple regression model

The regression equation estimated is stated as Equation (1)



$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 + b_6X_6 + b_7X_7 + b_8X_8 + b_9X_9 + b_{10}X_{10} + e_i \quad (1)$$

Where; Y = Gross value of plantain productivity {Output (₦)}, X<sub>1</sub> = Age of farmers {Years}, X<sub>2</sub> = Farm size {Hectares}, X<sub>3</sub> = Household size {Number}, X<sub>4</sub> = level of education {years}, X<sub>5</sub> = Farm income {Naira}, X<sub>6</sub> = Farming experience {Years}, X<sub>7</sub> = Farmers contact with extension agents {Monthly}, X<sub>8</sub> = Expenditure on planting materials (₦), X<sub>9</sub> = Expenditure on chemical fertilizer (₦), X<sub>10</sub> = Labour input (man-days), e<sub>i</sub> = Stochastic error term, b<sub>0</sub> = intercept, b<sub>1</sub> – b<sub>10</sub> = regression coefficients of the explanatory variables, X<sub>1</sub> – X<sub>10</sub> = explanatory variables (socio-economic characteristics)

### The gross margin analysis

The gross margin analysis was employed to determine the overall gross margin per hectare and net farm income (NFI) per hectare. The gross margin and net farm income were estimated as Equations (2) and (3)

$$GM = TR - TVC \quad (2)$$

$$NFI = GM - TFC \quad (3)$$

Where; GM = Gross Margin (₦), TR= Total Revenue (₦), TVC = Total Variable Cost (₦), NFI = Net Farm Income (₦), TFC = Total Fixed Cost (₦)

Other estimations from the gross margin were profit margin and return per naira outlay. The profit margin (%) is the ratio of profit/net farm income to total revenue. The Rate of Return on Capital Invested (RORCI) is the ratio of the profit / net farm income to the total cost of production. It indicates what is earned by the business per naira outlay. The profit margin (%) and the rate of return on capital invested were estimated as Equations (4) and (5).

$$\text{Profit margin (\%)} = \text{profit} / \text{total revenue} \quad (4)$$

$$\text{Rate of return on capital invested (\%)} = \text{Profit} / \text{total cost of production} \quad (5)$$

### Productivity indices

This shows the output earning per naira expenditure on the resources used. The resource productivity for labour and material used were estimated as Equations (6) and (7).

#### Resource productivity

$$\text{Labour productivity (\%)} = \text{total revenue} / \text{Labour input} \quad (6)$$

$$\text{Material productivity (\%)} = \text{total revenue} / \text{material input} \quad (7)$$

## RESULTS

### Socio-economic characteristics of farmers

Table 1 shows the summary statistics of some socioeconomic variables. The mean age of the respondent is 45 years. This is because younger farmers have the tendency to operate more efficiently than older farmers and the more experienced they ought to be in terms of their knowledge of improved plantain

technologies and good varieties of plantains. About 67% are male while 33% are female. This could be due to the fact that men are stronger, more active and have the potential to work for longer hours on the farm than their women. By implication, the level of productivity of the farmers in term of the application of labor is expected to be higher than that of the female.

The mean household size is 6 persons. According to Onu (2005), large family size could be as a result of polygamous nature of the rural farmers. He further opined that this could be linked to the fact that most rural farmers look at large household size as a good and economical way of maximizing farm returns by using free family labor. There is high level of literacy in the study area, 85% has a formal education while only 15% has no formal education. Access to education as well as exposure to agriculture workshops better the farmers' skill and his or her overall productivity (Apata et al., 2010). In addition, education is reported to have a significant impact on farmers' efficiency in production (Arene, 1996; Maurice, 2004). 84.4% had a plantain farm between 0.5 to 1.0 ha. While only 1.6% of the respondents had above 2 ha. This implies that the respondents are mainly smallholder farmers. However, plantain production may not be greatly influenced by farm size since farmers with fragmented farm land often try to make maximum use of their plots. Also majority (32.3%) of the farmers had farming experience of over 12 years in plantain production an indication that many of the farmers are quite knowledgeable about plantain production in the study area. It was observed that majority of the respondents were low income earners, with farming being their major occupation. Majority (60.8%) of the respondents had no visits. It could be that, lack of assistance from national extension systems is often major reasons why farmers do not adopt farming innovations which might lead to low productivity (Agwu and Afieroho, 2007).

### Major plantain varieties and technologies used by respondents

#### Plantain varieties cultivated by respondents

Table 2 shows that 57.5% of the respondents cultivated only local varieties of plantain and 22.6% cultivated improved varieties only. However, 19.9% cultivated both (local and improved) varieties. This reveals that the improved varieties were poorly used and may be as result of limited availability in the area and the poor extension practice on plantain technologies.

The local varieties cultivated by the farmers in the study area include; Agbagba, Une ukam, Aka nkita, Aka Agboha, Ovudaa, Une Ogbanu, Anumuyoho, Mkpalliki and Mkpuene. The improved varieties cultivated are Pita 3 and 7, Pita 14, Obino l' ewai, Ogba Ibugo, Ojoko Osukwu, among others. Most of the farmers could not identify the improved varieties by their current scientific

**Table 1.** Distribution of respondents socioeconomic characteristics (n = 186).

<b>Gender</b>	<b>Frequency</b>	<b>Percentage</b>
Male	124	66.7
Female	62	33.3
<b>Age of the farmers (years)</b>		
Less than 20	5	2.7
20-30	11	5.9
31-40	47	25.3
41-50	66	35.5
Above 51	57	30.6
<b>Marital status</b>		
Singled	11	5.9
Married	140	75.3
Divorced	11	5.9
Widowed	24	12.9
<b>Household size</b>		
1-3	37	19.9
4-6	95	51.1
7-9	40	21.5
10-13	9	4.8
13 and above	5	2.7
<b>Educational status</b>		
No formal Education	28	15.1
Primary Education	74	39.8
Secondary Education	45	24.2
Tertiary Education	39	21.0
<b>Occupational status</b>		
Farming	98	52.7
Civil service	45	24.2
Trading	40	21.5
Others	3	1.6
<b>Farm size (Ha)</b>		
Less than 0.5	20	10.8
0.5-1.0	157	84.4
1.5-2.0	6	3.2
Above 2.0	3	1.6
<b>Farmers income (₦)</b>		
Less than 100000	74	39.8
100,001 to 200,000	36	19.4
200,001 to 300,000	31	16.7
300,001 to 400,000	12	6.5
400,001 to 500,000	6	3.2
Above 500,000	27	14.5
<b>Farming experience (years)</b>		
Less than 1	3	1.6

**Table 1.** Contd.

1-3	26	14.0
4-6	50	26.9
7-9	36	19.4
10-12	11	5.9
Above 12	60	32.3
<b>Extension visits</b>		
No visit	113	60.8
Once in a month	35	18.8
Twice in a month	21	11.3
Thrice in a month	8	4.3
Four times in a month	9	4.8
<b>Total</b>	<b>186</b>	<b>100.0</b>

Source: Field survey, 2014.

**Table 2.** Distribution of respondents according to plantain varieties cultivated.

Varieties cultivated	Frequency	Percentage
Local varieties	107	57.5
Improved varieties	42	22.6
Both varieties	37	19.9
<b>Total</b>	<b>186</b>	<b>100.0</b>

Source: Field survey, 2014.

**Table 3.** Distribution of respondents according to reasons for cultivating variety chosen.

Reason (s)	Variety	Frequency	Percentage
Resistance to disease/pest attack	Improved	147	79.0
High yield	Improved	140	75.3
Early maturation	Improved	131	70.4
Taste of the plantain fruit	Improved	119	64.0
Height of the plantain tree	Improved	107	57.5
Shape of the plantain fruit	Improved	97	52.2
Availability of the cultivar	Local	75	40.3

Source: Field survey, 2014, multiple responses.

names. However local names were given to the cultivars by the farmers.

#### **Reasons for cultivating variety chosen by respondents**

Table 3 shows that majority (147 respondents representing 79%), opined that they cultivated the variety they chose because of its resistance to disease attacks and 140 respondents because they believed the chosen variety was high yielding. Only 40.3% of the respondents

cultivated the cultivar chosen because of such reason as its availability.

#### **Techniques practiced by respondents**

Analysis in Table 4 shows that propping (staking) is the most practiced plantain technique. Majority 69.9% of the respondents practiced propping, followed by fertilizer / manure application, plant spacing, mulching and pruning having 59.7, 59.7, 55.9, and 54.8%, respectively. The least practiced plantain production technique was

**Table 4.** Percentage distribution of respondents according to production techniques practiced.

Techniques practiced	Frequency	Percentage
Propping (staking)	130	69.9
Fertilizer / manure application	111	59.7
Plant spacing	111	59.7
Mulching	104	55.9
Pruning	102	54.8
Sucker multiplication	37	19.9
Sucker cleaning	24	12.9
Debudding	14	7.5
Hot water treatment	1	0.54

Source: Field survey, 2014, multiple responses.

**Table 5.** Result of productivity analysis.

Resource	Value (₦)
Total revenue	1713750
Labour input	82900
Material input	451500
<b>Resource productivity</b>	
Labour productivity	$\frac{1713750}{82900} = 20.67$
Material (capital) productivity	$\frac{1713750}{451500} = 3.8$

Source: Field survey, 2014.

hot water treatment with only 0.54% of the respondents practicing it. The reasons for most of the respondents practicing propping may not be unconnected with the height of most local varieties they cultivated and manuring was basically organic compost. Tall plantain plants are more susceptible to lodging by wind than short ones. Propping, manuring, plant spacing, mulching and pruning may not be new to the farmers since these techniques were traditionally practiced. Other improved technologies and techniques which plantain farmers were expected to adopt include; desuckering, debudding, hot water treatment, inorganic fertilizer application, among others. These were technologies promoted by Agricultural Development Programme and IITA in the area.

#### **Plantain productivity of the farmers in the study area**

Table 5 shows that the Labor productivity of the farmers was 20.67. This shows that output earning per ₦1 expenditure on labor was ₦20.67, implying that labor was well utilized. Material productivity of the farmers was 3.8. This also shows that output earning per ₦1 expenditure on material was ₦3.8, implying that material was

well utilized.

#### **Costs and return of plantain production in relation to technologies used in the study area**

Table 6 shows the estimated costs and return of plantain farmers cultivating 1 hectare on the average were ₦733500 and ₦1713750 per annum, respectively. Among the cost components, cost of material input had the largest share of the total cost (61.55%), followed by labor inputs (11.30%). The gross margin and net farm income on the average for plantain farmers was ₦988750 and ₦980250 respectively. The profit margin percentage was 57.2%, while return per naira outlay was ₦2.3 implies that for every ₦1 invested in plantain production enterprise there is a return of ₦2.3 to the enterprise. These measures of performances indicate that plantain production in the study area is viable and profitable.

#### **Determinants of plantain productivity in the study area**

Table 7 shows the result of the multiple regression analysis on the determinants of productivity of improved

**Table 6.** Cost and returns of plantain production in relation to technologies used in the study area.

Variable	Amount in Naira (₦)
Total value of production (revenue)	1713750
Total variable cost	725000
Gross margin	988750
Total fixed cost	8500
Net farm income	980250
Profit margin %	57.2%
Rate of return on investment (ROR) %	₦ 2.3

Source: Field survey, 2014.

**Table 7.** Multiple regression result of the determinants of plantain productivity.

Variable	Coefficient	Standard error	t- statistics	Sig T
Farmers' Age (X <sub>1</sub> )	0.097*	0.058	1.667	0.107
Farm Size (X <sub>2</sub> )	0.555***	0.162	3.423	0.001
Household Size (X <sub>3</sub> )	0.013*	0.019	1.487	0.139
Educational Status (X <sub>4</sub> )	0.152**	0.060	2.526	0.019
Farmers' Income (X <sub>5</sub> )	0.022	0.237	0.093	0.926
Farmers' Years of Experience (X <sub>6</sub> )	0.015	0.201	0.073	0.942
Frequency of extension visit (X <sub>7</sub> )	-0.072*	0.042	-1.714	0.101
Planting materials (X <sub>8</sub> )	1.093***	0.252	4.343	0.000
Chemical fertilizer (X <sub>9</sub> )	-0.102	0.155	-0.659	0.513
Labour input (X <sub>10</sub> )	0.113**	0.052	2.173	0.04
(Constant)	56.698	11.628	4.876	0.000
R <sup>2</sup>	0.794			
$\bar{R}^2$	0.736			
F- Ratio	23.624***			

Source: Data analysis, 2014. \*\*\*Significant at 1%,\*\*Significant at 5%, \*Significant at 10%.

plantain technologies in study area. Based on the magnitude of the models' R<sup>2</sup>, the number of independent variables that were statistically significant, and the number of independent variable's co-efficient signs that conform to a *priori* expectation, the double log function was chosen as the lead equation and used for further analysis of the data.

The coefficient of multiple determination (R<sup>2</sup>) of 0.79 implies that 79% of the variations in productivity are explained by the joint action of the independent variables while the remaining 21% is due to error term. This is high and seems to show that the variables may be responsible for the productivity of the technologies in the study area. The analysis shows that farmers' age (X<sub>1</sub>), farm size (X<sub>2</sub>), household size (X<sub>3</sub>), educational level (X<sub>4</sub>), planting materials (X<sub>8</sub>) and labor (X<sub>10</sub>) had positive and significant relationships with productivity.

This suggests that total factor productivity will increase significantly if these factors are increased above their present levels of use. It is expected that productivity will increase if more educated farmers with

large household size as family labor and also hired labor with adequate planting materials, cultivate greater hectares of farm land. However extension contact (X<sub>7</sub>) is significant at 10% but inversely related to productivity. It negates a *prior* expectation. The negative coefficient implies that a unit increase in this variable will reduce the productivity of plantain. It could be due to the fact that extension support for the productivity of the improved plantain technologies under study was almost non-existent with 60.8% respondents recorded a no visit by extension agents.

## DISCUSSION

Out of the 186 respondents interviewed in the study area, majority (67%) of farmers are men while 33% are women. This implies that higher proportion of the plantain being produced in the study area is carried out by men. The mean age of the farmers in the study area is about 45 years and majority is within the age group of



41 to 50 years. This shows majority of the farmers are in their productive age. Further analysis on the socio-economic characteristics shows that 75.3% are married.

The average household size in the study area is 6 persons with the majority (51.1%) having between 4 and 6 persons, an indication of large family size in the study area. Households with greater numbers of members are more likely to have available labor compared to households with fewer household members for timely execution of important farm activities. The majority of the respondents (85%) had formal education while only 15% had no formal education. This shows a high level of literacy in the study area, this will enable the farmers to be fast adopters of innovation. Majority (52.7%) of the respondents in the study area are engaged in farming activities as their primary source of income while the rest engaged in civil service (24.2%), trading (21.5%) and others (1.6%). This shows that agriculture is main occupation in the study area. The result also shows that majority (39.8%) earned between ₦100001 and ₦200000 per cropping season, an indication that the general income per cropping season from plantain production seems low and may be attributed to the low productivity of cultivars used.

Majority (84.4%) had plantain farm between 0.5 to 1.0 ha. This implies that the respondents are mainly smallholder farmers which might not really be favorable for adoption of plantain technologies. The result also shows that majority (32.3%) of the farmers had farming experience of over 12 years. This reveals that many of the farmers are quite knowledgeable about plantain production in the study area. Majority 69.9% of the respondents practiced propping, followed by fertilizer or manure application, plant spacing, mulching and pruning having 59.7, 59.7, 55.9 and 54.8%, respectively. The least practiced plantain production technique was hot water treatment with only 0.54% of the respondents practicing it. It can be because propping, manure application, plant spacing, mulching and pruning may not be new to the farmers since these techniques were traditionally practiced. Also, 57.5% of the respondents cultivated local varieties, 26.6% cultivated improved plantain varieties while 19.9% cultivated both. The reasons for cultivating the chosen varieties are because of; its resistance to pest and disease attack, high yield, early maturation, the height, based on its availability and among others.

The analysis of the regression shows that farmers' age, farm size, household size, educational level, planting materials and labor had positive and significant relationships with productivity. Among the included independent variables, extension contacts deviated from the *a priori* expectation in terms of the sign. This study further determined the viability of plantain production in the area using the gross margin analysis. The total variable cost (TVC) was found to be ₦725000 per hectare while the total revenue (TR) per hectare basis

was found to be ₦1713750 thus giving a gross margin (GM) of ₦988750, which indicated a positive gross margin, proving plantain production in the area to be viable.

The low production of plantain in Anambra State is largely attributed to several factors one of which was farmers' use of local varieties and production practices. Improved production technologies were developed by IITA Ibadan. The production technologies have potentials of boosting the productivity of plantain. Based on the results of the analysis, we conclude that plantain productivity will increase in the area if the level used of factors such as farm size, labor input, household size and planting materials is increased. Similarly, productivity will also increase if farmers with higher educational level and greater years of farming experience engage in agriculture.

## Conclusion

From the overwhelming findings conveyed by the data in this study, it can be concluded that, provided the innovation is profitable, compatible, simple, triable, and accessible to the farmers, and the disincentive that can inhibit farmers from its usage are removed or weakened through the visible and or feasible motivations, farmers will not hesitate to make a positive decision. As such, a thorough examination of the felt need or constraints through baseline survey should be accorded importance before technology development and transfer is undertaken.

The importance of agricultural sector in the country cannot be overemphasized, particularly in the area of promoting the economic growth. Increased use of improved plantain technologies through increase in agricultural productivity would generate multiplier effect that goes beyond the farm to the wider rural economy helping to improve standard of living. The measures of performances from the gross margin analysis result, indicates that plantain production in the study area is viable and profitable. Finally, technology delivery should target peers' support rather than relying on external influence of research and extension that is not sustainable and lacks proximity to the end users.

## RECOMMENDATIONS

Based on the findings of this study, the following below are the suggested recommendations:

- (i) The state government through the Agricultural Development Programme (ADPs) should partner with research institutions such as IITA to encourage increased sucker multiplication, procurement and distribution of improved varieties at the right time and at affordable prices are effective strategies for stimulating plantain

productivity. This recommendation flows from the significance of the planting materials. Also the ADPs should be intensified to sensitize and motivate farmers towards enlisting in farmers' co-operative societies with the aim of encouraging them to use improved plantain technologies which are capable of improving their productivity.

(ii) Efforts should be made at making lands available to enhance plantain production. This recommendation flows from the significance of farm size. Land which is a very scarce commodity, especially in the study should be made available readily to the plantain farmers. In the light of this, Government and other stake holders should sought ways by which some of the degraded soils in the study area could be reclaimed for agricultural uses.

(iii) Farmers' adult education / enlightenment workshops are needed to provide information on the technologies. This recommendation flows from the significance of the age which rules them out of regular schools. Farmers also need to play a lead role in the development and testing of improved plantain technology, assessing on-station trials, conducting researcher designed and farmer-designed trials, and providing feedback to researchers on their experiences.

(iv) Also, from the findings, it was observed that the techniques used were not new to the farmers and were practiced traditionally. Therefore it is expected that improved plantain technologies developed should be appropriate for farmers. This requires enhancing the partnership between researchers and farmers. Researchers and farmers together need to understand the circumstances, problems, and preferences of rural households and how these vary among different types of farmers. Participatory techniques are available to ensure that farmers take the lead in this diagnostic process (Chambers et al in Harrison and Okoedo-Okojie, 2013).

### Conflict of interests

The author has not declared any conflict of interests.

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*Full Length Research Paper*

# Markov chain analysis of dry, wet weeks and statistical analysis of weekly rainfall for agricultural planning at Dhera, Central Rift Valley Region of Ethiopia

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Received 5 March, 2014; Accepted 18 June, 2014

Knowledge of rainfall pattern is very important for making decision on crop planning and water management. In the present study, Markov Chain probability model was performed to explain the long term frequency behavior of wet or dry weather spells during the main rainy season at Dhera, Central Rift Valley Region of Ethiopia. The study used 24 years (1984-2010) of rainfall data and weekly rainfall data was considered as standard to study the probabilities of occurrences of dry and wet weeks. Some reasonable and significant conclusions regarding specific time for land preparation, supplementary irrigation and soil conservation measures were obtained. The main rainy season starts on the 26<sup>th</sup> week (25<sup>th</sup> June - 1<sup>st</sup> July) and remains active upto the 40<sup>th</sup> week (1<sup>st</sup> - 7<sup>th</sup> October) this shows a total of 105 days of main rainy season that could occur. The coefficients of variation at the onset and withdrawal week are 69.4 and 99.2% respectively for the study site during the main rainy season. The probability of occurrences of initial and conditional probability is more than 50% on the 26<sup>th</sup> week at 10 mm per week threshold limit and 28<sup>th</sup> week at 20 mm per week threshold limit, therefore land preparation for planting could be undertaken in 26<sup>th</sup> and 28<sup>th</sup> weeks respectively for the main rainy season crop cultivation. Initial and conditional probabilities at 20 mm threshold limit per week showed that, supplementary irrigation and moisture conservation practice need to be practiced between 38<sup>th</sup> and 40<sup>th</sup> week for short duration crops and if the crop duration extend after 40<sup>th</sup> week it's evident that supplementary irrigation is needed. In addition, harvesting runoff water for supplementary irrigation and construction of soil erosion measures need to be practiced between 28<sup>th</sup> and 33<sup>rd</sup> weeks for better rain water management.

**Key words:** Markov chain model, onset week, withdrawal week, agricultural planning.

## INTRODUCTION

Ethiopian farming is mainly dependant on rainfed smallholder agricultural system as a means of food and income for its population (Hordofa et al., 2008). Although agricultural production from rainfed agricultural system

depends on all climatic parameters, rainfall is the most important and sensitive one. Both shortage and surplus of rainfall during the length of the crop growing period can lead to full or partial crop failure. However, agricultural

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production can be increased and risk minimized by selecting the right time for the onset of rainfall based on informed decision by analyzing the long term rainfall pattern and variability as well as harvesting excess water and utilizing it in periods of shortage as supplementary irrigation.

The rift valley region of Ethiopia has an average of 725 mm rainfall with a maximum of 1990 mm and minimum of 90 mm (FAO, 1997). Despite its high average amount, the magnitude and distribution of rainfall are highly variable across time and space (Menker and Erkosa, 2006). In addition, rainfall variability increases as rainfall quantity decreases (Menker and Erkosa, 2006). Thus, rainfall variability is greater in rift valley region where this study has been undertaken because of low agricultural production due to absence of sufficient rainfall. Rainfall variability has major implications on crop production and productivity. Hence, rainfall pattern needs to be studied for the purpose of crop planning and water management as it is the main factor for yield of a crop under rainfed condition (Singh et al., 2013). During occurrences of good rainfall years, agricultural production increased and improved farmer's livelihood. Conversely, during inadequate and variable rainfall years, partial or complete crop failures could occur (Gautam and Rao, 2007).

In the study area, farmers usually made traditional crop planning decision based on the onset of rainfall for Belg season (small amount of rain before the main rainy season). If the rain comes early, then farmers plant medium duration crops. Yet, if the rains are delayed, then farmer's plant short duration crops (Walker et al., 2010). According to Yemenu and Chemedda (2010), irregularity of onset, temporal and spatial distribution of rainfall caused low crop yield to the rainfed agricultural system. Therefore, analyzed data on the onset and withdrawal of the main rainy season can be very important for planning of agricultural activities, especially to determine time of land preparation before sowing. In the case of Bisoftu District, planning of agricultural activities is fairly simple and less risky because of stability of onset dates of the main rainy season which is at the second decade of June and the withdrawal of rainy season is at the last decade of September (Yemenu et al., 2010).

Knowledge on sequences of dry and wet spells can assist to acquire specific information for agricultural planning (Reddy et al., 2008). Understanding the events of occurrences of dry spells and intensive rainfall are crucial to decrease the adverse effects of dry spells at sensitive crop development stage and occurrences of runoff in the middle of the season from damaging the crops in the field respectively (Yemenu et al., 2013). Many studies on agricultural planning were performed by Markov Chain probability model (Pandharinath, 1991; Reddy, 1991; Dash and Senapati, 1992; Babuan and Lakshminarayana, 1997; Reddy et al., 2008; Shahraki et al., 2012; Panigrahi et al., 2002; Senthilvelan et al., 2012; Mangaraj et al., 2013; Mandal et al., 2013; Singh et al.,

2013) used weekly rainfall values as a standard to determine initial, conditional and consecutive dry and wet spells analysis. Determination of dry and wet spells can prove to farmers its usefulness regarding improving crop productivity and intensity (Mandal et al., 2013).

This study has been carried out to determine the onset and withdrawal of rainy season, probabilities of occurrences of onset and withdrawal of rainy seasons and the initial and conditional probabilities of dry and wet weeks using threshold limits of 10 mm and 20 mm of rainfall during the main rainy season at Dodota area, Central Region of Ethiopia.

## MATERIALS AND METHODS

### Study area

The study area is located at Dhera, Dodota District under the Central Rift Valley region of Ethiopia (Figure 1). Dhera has an average annual maximum temperature of 27°C, average annual minimum temperature of 8.36°C and 656.91 mm of average annual rainfall. Daily rainfall data recorded at Dhera Meteorological Station (08° 19' 10" N, 39° 19' 13" and height of 1650 m above sea level) for a period of 24 years (1984-2010) were used for the present study. Rainfall data records on 1992, 1998 and 2008 season were not included in this study due to missing of recorded data. The weekly rainfall values have been computed from daily values and were used for the present study.

### Methods of computation for onset and withdrawal of main rainy season

Time of onset, withdrawal and length of rainy season were selected as key parameters to characterize the rainfall season for crop production (Punyawardena, 2002). Determination of onset, withdrawal and length of rainy season is important for crop planning, choice of suitable crop varieties and to plan compressive strategies for efficient rain water management (Mandal et al., 2013). Dash and Senapati (1992) suggested a procedure to compute the onset and withdrawal of rainy season from weekly rainfall data by forward and backward accumulation method. The present study uses the suggested procedure to compute onset and withdrawal of rainy season from weekly rainfall data. Each year is divided into 52 meteorological weeks, starting the first week from 1<sup>st</sup> to 7<sup>th</sup> January upto the last week 24<sup>th</sup> to 31<sup>st</sup> December. According to Panigrahi and Panda (2002), accumulation of 75 mm of rainfall was considered as the onset time for sowing of rainfed crops. The onset time of rainy season was determined by forward summation of weekly rainfall starting from 20<sup>th</sup> week until 75 mm of rainfall accumulated (Dash and Senapati, 1992). Results from Babuan and Lakshminarayana (1997), had chosen a 20 mm of rainfall accumulation for the withdrawal of the rainy season, which is sufficient for ploughing of fields after harvesting of the crops. The withdrawal of rainy season was determined by backward summation of weekly rainfall starting from 48<sup>th</sup> week until 20 mm of rainfall is accumulated.

The probabilities of onset and withdrawal of the main rainy season was calculated by using Weibull's formula. The percent probability (P) of each rank was calculated by arranging them in ascending order and by selecting the highest rank allotted for a particular week. The following Weibull's formula has been used for calculating percent probability of onset and withdrawal (Mandal et al., 2013). The mean, maximum, minimum, standard deviation and

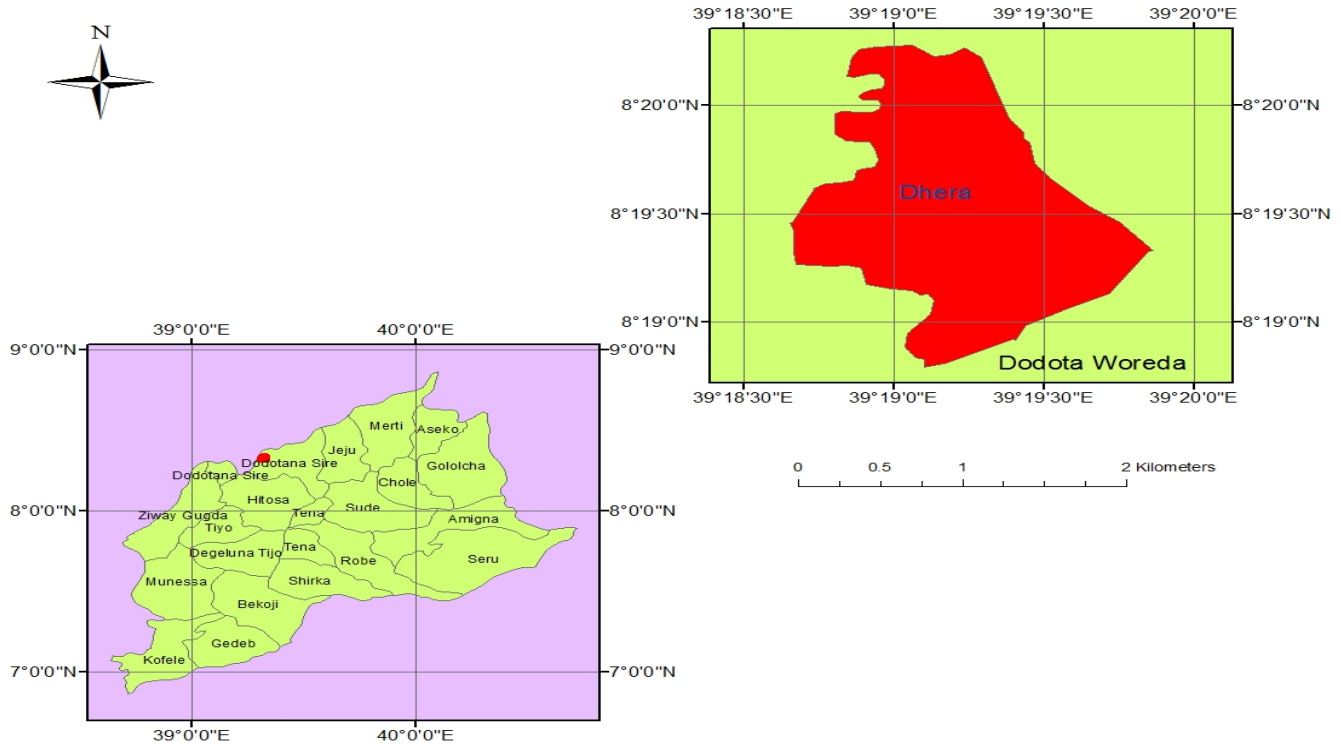


Figure 1. Study area map (Source: EMA 2012).

coefficient of variation of weekly rainfall were calculated manually.

$$P = \frac{m}{N+1} \times 100 \quad (1)$$

Where,  $m$  is the rank number and  $N$  is the number of years of data used.

#### Methods of computation for initial and conditional probabilities of main rainy season

The information on initial and conditional probability were given based on weekly level using First Markov chain model (Equation 2 to 7). Probability of a week being dry or wet is under initial probability while in case of conditional probability, if a given period  $i$  is wet or dry, then the chance of  $(i+k)^{\text{th}}$  period is wet and given as wet/wet or wet/dry are estimated. A threshold limit of 10 mm and 20 mm of rainfall were selected as critical for different purpose of agricultural planning purpose. The weekly analysis of rainfall is very important concerning agricultural planning and week period has been considered as the optimum length of time (Reddy, 1991; Reddy et al., 2008). The dry and wet spell analysis was carried out using weekly rainfall based on Markov Chain Model considering less than 20 mm rainfall in a week as a dry week and vice versa (Pandharinath, 1991). The different formulas used for this analysis are shown below:

#### Initial probabilities

$$Pd = Fd/N \quad (2)$$

$$Pw = Fw/N \quad (3)$$

#### Conditional probabilities

$$Pww = Fww/N \quad (4)$$

$$Pwd = 1 - Pdd \quad (5)$$

$$Pdw = 1 - Pww \quad (6)$$

$$Pdd = Fdd/N \quad (7)$$

Where,  $Pd$  = probability of the week being dry,  $Pw$  = probability of week being wet,  $Fd$  = number of dry weeks,  $Fw$  = number of wet weeks,  $N$  = number of years of data,  $Pdd$  = probability of a dry week preceded by a dry week,  $Pww$  = probability of a wet week preceded by a wet week,  $Pwd$  = probability of a wet week preceded by a dry week,  $Pdw$  = probability of a dry week preceded by a wet week,  $Fdd$  = number of dry weeks preceded by another dry week,  $Fww$  = number of a wet week preceded by a wet week,

The calculation of initial and conditional probability for 10 mm and 20 mm threshold limit follow the same procedure, the initial probability (probability of a week being dry or wet) calculated by dividing the counted number of dry week or wet week to the number of years of the data, which is 24 years. The conditional probability (probability of a dry week preceded by dry week) calculated by dividing the counted number of dry week preceded by dry week to the counted number of dry week, the same goes to the calculation of probability of a wet week preceded by a wet week. Finally the probability of wet week preceded by dry week and dry week



**Table 1.** Characterization of the main rainy season at Dhera (1984-2010)

Particulars	Week No	Date
Mean week of onset of the main rainy season	26	25 June - 1 July
Earliest week of onset of the main rainy season	21	21 May - 27 May
Delayed week of onset of the main rainy season	29	16 July - 22 July
Mean week of withdrawal of the main rainy season	40	1 October - 7 October
Earliest week of withdrawal of the main rainy season	35	27 August - 2 September
Delayed week of withdrawal of the main rainy season	47	19 November - 25 November
Mean length of the main rainy season		15 weeks (105 days)
Duration of the main rainy season		
Longest		23 weeks (161 days)
Shortest		6 weeks (42 days)

**Table 2.** Probability of onset and withdrawal of the main rainy season at Dhera.

		Onset												
Week		21	22	23	24	25	26	27	28	29				
P (%)		4.00	8.00	16.00	26.00	34.00	48.00	64.50	84.00	96.00				
		Withdrawal												
Week		35	36	37	38	39	40	41	42	43	44	45	46	47
P (%)		4.00	8.00	12.00	32.00	56.00	68.00	78.00		84.00	88.00	92.00		96.00

preceded by wet week is calculated by subtracting probability of a dry week preceded by dry week and probability of a wet week preceded by a wet week from 100.

## RESULTS AND DISCUSSION

### Onset and withdrawal of main rainy season at Dhera

The onset and withdrawal of the main rainy season at the study site is 25<sup>th</sup> June and 7<sup>th</sup> October respectively. According to Table 1 the main rainy season starts on the 26<sup>th</sup> week (25<sup>th</sup> June to 1<sup>st</sup> July) and remains active upto the 40<sup>th</sup> week (1<sup>st</sup> to 7<sup>th</sup> October). This implies the mean length of the main rainy season for the study site is 15 weeks or 105 days. The earliest and delayed weeks of onset of the main rainy season are 21<sup>st</sup> week (21<sup>st</sup> to 27<sup>th</sup> May) and 29<sup>th</sup> week (16<sup>th</sup> to 22<sup>nd</sup> July), respectively. Similarly, the earliest and delayed week of withdrawal of the main rainy season are 35<sup>th</sup> week (27<sup>th</sup> August to 2<sup>nd</sup> September) and 47<sup>th</sup> week (19<sup>th</sup> to 25<sup>th</sup> November) respectively.

### Probabilities of main rainy season at Dhera

The results of probabilities of onset and withdrawal of the main rainy season are presented in Table 2 and the results reveal that there are a 96% chance that both the onset and withdrawal of the main rainy season will occur

during at 29<sup>th</sup> and 47<sup>th</sup> week. There are no withdrawal probability occurrences of main rainy season at 42<sup>th</sup> and 46<sup>th</sup> weeks; therefore these two weeks are not included in the probability calculation.

The weekly rainfall for mean, maximum, minimum, standard deviation and coefficient of variation of Dhera area rainfall were calculated and the result is shown in Table 3, there are 12 weeks (26<sup>th</sup> to 37<sup>th</sup> week) where the rainfall exceeds more than 20 mm and 3 weeks (38<sup>th</sup> to 40<sup>th</sup> week) where rainfall is less than 20 mm during the main rainy season. The coefficient of variation during the main rainy season varies from maximum of 99.2% at 40<sup>th</sup> week to minimum of 68.4% at the 30<sup>th</sup> and 31<sup>st</sup> week. The coefficients of variation at the onset and withdrawal week are 69.4% and 99.2% respectively for the study site during the main rainy season. The threshold limit for coefficient of variation for weekly rainfall should be less than 150% (Senthilvelan et al., 2012). During the rainy season at the study site, the coefficient of variation is less than 150%, the lesser the variability of rainfall during rainy season showed the higher dependability of rainfall, therefore agricultural operation and growing rainfed crops is possible during the rainy season. This conclusion is similar to the result of Senthilvelan et al. (2012).

### Initial and conditional probabilities of main rainy season at Dhera area

The initial and conditional probability of 10 and 20 mm

**Table 3.** Descriptive statistics on weekly rainfall data for Dhera.

Week	Mean (mm)	Max (mm)	Min (mm)	SD	CV (%)
1	2.1	19.2	0.0	3.6	169.8
2	0.0	0.3	0.0	0.0	295.4
3	3.9	16.5	0.0	4.0	101.5
4	7.4	27.5	0.0	7.6	102.4
5	1.9	21.0	0.0	3.3	178.1
6	6.8	33.7	0.0	7.1	103.9
7	2.8	31.2	0.0	4.5	163.0
8	6.4	55.9	0.0	8.6	135.6
9	7.7	41.0	0.0	9.2	119.2
10	7.2	37.5	0.0	8.0	112.0
11	11.8	66.6	0.0	12.2	102.8
12	13.7	32.1	0.0	12.0	87.6
13	13.2	35.5	0.0	11.7	88.5
14	12.4	41.2	0.0	11.2	90.5
15	10.8	40.3	0.0	9.9	91.1
16	13.2	58.5	0.0	13.3	100.8
17	13.3	42.5	0.0	11.4	86.0
18	14.7	52.5	0.0	14.2	96.6
19	11.6	40.0	0.0	10.9	94.3
20	15.6	54.8	0.0	13.6	87.1
21	9.2	45.0	0.0	8.8	95.9
22	9.2	24.2	0.0	7.8	84.1
23	12.3	33.5	0.0	10.6	86.8
24	9.2	20.0	0.0	6.9	74.6
25	14.6	67.6	0.0	13.7	93.6
26	26.4	39.5	0.0	18.3	69.4
27	20.3	31.2	0.0	14.7	72.4
28	40.2	52.0	0.0	27.3	67.9
29	32.9	49.5	0.0	23.0	70.1
30	31.9	53.2	0.0	21.8	68.4
31	25.9	39.5	0.0	17.7	68.4
32	36.7	48.2	0.0	26.8	73.1
33	39.1	72.4	0.0	28.6	73.2
34	27.7	38.8	0.0	19.6	70.6
35	27.7	39.0	0.0	19.2	69.4
36	25.9	33.3	0.0	18.2	70.2
37	22.2	36.3	0.0	15.7	70.8
38	17.8	36.7	0.0	13.4	75.2
39	9.6	28.0	0.0	7.6	78.7
40	6.7	28.0	0.0	6.6	99.2
41	5.7	25.5	0.0	6.1	107.0
42	3.8	30.0	0.0	4.7	125.0
43	3.1	20.0	0.0	3.9	125.1
44	1.8	22.0	0.0	3.1	173.4
45	2.2	24.6	0.0	3.3	148.3
46	0.3	4.8	0.0	0.7	196.5
47	1.9	12.2	0.0	2.2	116.5
48	0.8	17.0	0.0	2.1	259.7
49	3.7	37.6	0.0	6.1	162.4
50	1.9	42.0	0.0	5.2	279.6
51	1.7	28.0	0.0	3.7	217.4
52	0.1	1.4	0.0	0.2	170.2

threshold limit rainfall were summarized in Tables 4 and 5 for all 52 weeks respectively. However, this paper

**Table 4.** Initial and conditional probabilities at 10mm threshold limit of rainfall for Dhera.

Week	Pd	Pw	Week	Pdd	Pww	Pwd	Pdw
Initial probabilities (%)			Conditional probabilities (%)				
1	88.0	8.0	1	86.4	0.0	13.6	100.0
2	96.0	0.0	2	95.8	0.0	4.2	100.0
3	80.0	16.0	3	80.0	25.0	20.0	75.0
4	72.0	24.0	4	77.8	33.3	22.2	66.7
5	92.0	4.0	5	91.3	0.0	8.7	100.0
6	72.0	24.0	6	83.3	50.0	16.7	50.0
7	84.0	12.0	7	85.7	0.0	14.3	100.0
8	76.0	20.0	8	73.7	20.0	26.3	80.0
9	80.0	16.0	9	80.0	0.0	20.0	100.0
10	72.0	24.0	10	66.7	16.7	33.3	83.3
11	60.0	36.0	11	60.0	33.3	40.0	66.7
12	64.0	32.0	12	62.5	25.0	37.5	75.0
13	64.0	32.0	13	68.8	37.5	31.3	62.5
14	52.0	44.0	14	30.8	27.3	69.2	72.7
15	60.0	36.0	15	80.0	55.6	20.0	44.4
16	60.0	36.0	16	46.7	22.2	53.3	77.8
17	64.0	32.0	17	56.3	12.5	43.8	87.5
18	64.0	32.0	18	68.8	37.5	31.3	62.5
19	68.0	28.0	19	76.5	28.6	23.5	71.4
20	52.0	44.0	20	46.2	27.3	53.8	72.7
21	72.0	24.0	21	72.2	16.7	27.8	83.3
22	60.0	36.0	22	73.3	55.6	26.7	44.4
23	56.0	40.0	23	64.3	50.0	35.7	50.0
24	64.0	32.0	24	56.3	25.0	43.8	75.0
25	60.0	36.0	25	66.7	44.4	33.3	55.6
26	24.0	72.0	26	16.7	66.7	83.3	33.3
27	36.0	60.0	27	11.1	46.7	88.9	53.3
28	8.0	88.0	28	0.0	90.9	100.0	9.1
29	12.0	84.0	29	0.0	81.0	100.0	19.0
30	16.0	80.0	30	25.0	85.0	75.0	15.0
31	24.0	72.0	31	0.0	66.7	100.0	33.3
32	16.0	80.0	32	25.0	85.0	75.0	15.0
33	12.0	84.0	33	0.0	81.0	100.0	19.0
34	12.0	84.0	34	0.0	81.0	100.0	19.0
35	24.0	72.0	35	16.7	66.7	83.3	33.3
36	16.0	80.0	36	25.0	80.0	75.0	20.0
37	24.0	72.0	37	33.3	83.3	66.7	16.7
38	28.0	68.0	38	14.3	58.8	85.7	41.2
39	64.0	32.0	39	62.5	37.5	37.5	62.5
40	72.0	24.0	40	66.7	16.7	33.3	83.3
41	84.0	12.0	41	85.7	33.3	14.3	66.7
42	88.0	8.0	42	86.4	0.0	13.6	100.0
43	80.0	16.0	43	80.0	25.0	20.0	75.0
44	92.0	4.0	44	91.3	0.0	8.7	100.0
45	88.0	8.0	45	86.4	0.0	13.6	100.0
46	96.0	0.0	46	95.8	0.0	4.2	100.0
47	88.0	8.0	47	86.4	0.0	13.6	100.0
48	92.0	4.0	48	91.3	0.0	8.7	100.0
49	88.0	8.0	49	86.4	0.0	13.6	100.0
50	92.0	4.0	50	91.3	0.0	8.7	100.0
51	88.0	8.0	51	86.4	0.0	13.6	100.0
52	96.0	0.0	52	95.8	0.0	4.2	100.0

**Table 5.** Initial and conditional probabilities at 20mm threshold limit of rainfall for Dhera.

Week	Pd	Pw	Week	Pdd	Pww	Pwd	Pdw
Initial probabilities (%)			Conditional probabilities (%)				
1	92.0	4.0	1	91.3	0.0	8.7	100.0
2	96.0	0.0	2	95.8	0.0	4.2	100.0
3	92.0	4.0	3	91.3	0.0	8.7	100.0
4	72.0	24.0	4	77.8	33.3	22.2	66.7
5	92.0	4.0	5	91.3	0.0	8.7	100.0
6	84.0	12.0	6	85.7	0.0	14.3	100.0
7	92.0	4.0	7	91.3	0.0	8.7	100.0
8	84.0	12.0	8	81.0	0.0	19.0	100.0
9	84.0	12.0	9	85.7	0.0	14.3	100.0
10	80.0	16.0	10	80.0	25.0	20.0	75.0
11	68.0	28.0	11	64.7	14.3	35.3	85.7
12	72.0	24.0	12	72.2	16.7	27.8	83.3
13	72.0	24.0	13	77.8	33.3	22.2	66.7
14	64.0	32.0	14	56.3	25.0	43.8	75.0
15	76.0	20.0	15	84.2	40.0	15.8	60.0
16	68.0	28.0	16	64.7	28.6	35.3	71.4
17	76.0	20.0	17	73.7	20.0	26.3	80.0
18	76.0	20.0	18	78.9	40.0	21.1	60.0
19	72.0	24.0	19	72.2	16.7	27.8	83.3
20	64.0	32.0	20	68.8	37.5	31.3	62.5
21	84.0	12.0	21	85.7	0.0	14.3	100.0
22	80.0	16.0	22	80.0	25.0	20.0	75.0
23	76.0	20.0	23	78.9	40.0	21.1	60.0
24	88.0	8.0	24	86.4	0.0	13.6	100.0
25	76.0	20.0	25	73.7	20.0	26.3	80.0
26	36.0	60.0	26	22.2	46.7	77.8	53.3
27	56.0	40.0	27	42.9	20.0	57.1	80.0
28	24.0	72.0	28	33.3	77.8	66.7	22.2
29	28.0	68.0	29	28.6	64.7	71.4	35.3
30	24.0	72.0	30	16.7	72.2	83.3	27.8
31	40.0	56.0	31	40.0	57.1	60.0	42.9
32	20.0	76.0	32	40.0	84.2	60.0	15.8
33	24.0	72.0	33	33.3	72.2	66.7	27.8
34	44.0	52.0	34	18.2	30.8	81.8	69.2
35	52.0	44.0	35	53.8	36.4	46.2	63.6
36	36.0	60.0	36	22.2	53.3	77.8	46.7
37	56.0	40.0	37	42.9	30.0	57.1	70.0
38	60.0	36.0	38	66.7	44.4	33.3	55.6
39	80.0	16.0	39	75.0	0.0	25.0	100.0
40	80.0	16.0	40	75.0	0.0	25.0	100.0
41	84.0	12.0	41	85.7	33.3	14.3	66.7
42	92.0	4.0	42	91.3	0.0	8.7	100.0
43	96.0	0.0	43	95.8	0.0	4.2	100.0
44	92.0	4.0	44	91.3	0.0	8.7	100.0
45	92.0	4.0	45	91.3	0.0	8.7	100.0
46	96.0	0.0	46	95.8	0.0	4.2	100.0
47	96.0	0.0	47	95.8	0.0	4.2	100.0
48	96.0	0.0	48	95.8	0.0	4.2	100.0
49	92.0	4.0	49	91.3	0.0	8.7	100.0
50	92.0	4.0	50	91.3	0.0	8.7	100.0
51	92.0	4.0	51	91.3	0.0	8.7	100.0
52	96.0	0.0	52	95.8	0.0	4.2	100.0

discusses only initial and conditional probability of dry and wet week during the main rainy season (26<sup>th</sup> to 40<sup>th</sup> week). Considering 20 mm threshold limit, the initial and conditional probability of dry weeks ranges from 20 to 80% and 16.7 to 75% respectively.

At the first week of the main rainy season, the chance of occurrence of dry week (Pd) and dry week preceded by dry week (Pdd) are 36 and 22.2% respectively, similarly at the end of the main rainy season, dry week (Pd) and dry week preceded by dry week (Pdd) has a chance of 80 and 75% occurrences respectively. During the main rainy season, the initial and conditional probability of wet weeks ranges from 16 to 76% and 0 to 84.2% respectively. At the first week of the main rainy season chance of occurrence of wet week (Pw) and wet week preceded by wet week (Pww) is 60 and 46.7% respectively, similarly at the end of the main rainy season, wet week (Pw) and wet week preceded by wet week (Pww) has a chance of 16 and 0% occurrences respectively.

The detailed characterization and statistical analysis of Dhera rainfall can be useful for agricultural planning and water management. According to Senthilvelan et al. (2012) and Reddy (1991), based on threshold limit of 10 mm per week at more than 50% for initial and conditional probability during the main rainy season is adequate for crop activities like land preparation and the conditional probability of occurrences of rainfall at 20 mm per week above 50% is the right week for planting.

At 10 mm per week threshold limit, the probability of occurrences of initial and conditional probability is 72 and 66.7% respectively for 26<sup>th</sup> week at the start of the main rainy season, therefore the land preparation could be undertaken in 26<sup>th</sup> week. At 20 mm per week threshold limit, the conditional probability of wet week (Pww) during the main rainy season is more than 50% with a value of 77.8% for 28<sup>th</sup> week; therefore 28<sup>th</sup> week is the right week for planting at the study site. On 28<sup>th</sup> week a mean rainfall amount of 40.2 mm was contributed and this amount of rainfall is good for germination and there will be no moisture stress during the germination period. At rainfall amount of more than 25 mm, there won't be moisture stress and it is good for germination period (Reddy et al., 2008). Since the planting is undertaken in 28<sup>th</sup> week and the total rainy season is 84 days counting from the planting weeks, short growing duration of wheat, lowland pulses and lowland barley need to be considered by the breeder when releasing new varieties.

At 20 mm threshold, the probability of dry week (Pd) being more than 50% is during 27<sup>th</sup>, 35<sup>th</sup> and 37<sup>th</sup> to 40<sup>th</sup> week and also the chance of dry week preceded by dry week (Pdd) is more than 50% during 35<sup>th</sup> and 38<sup>th</sup> to 40<sup>th</sup> week for the main rainy season, therefore during those dry weeks specifically at the end of the main rainy season, supplementary irrigation and moisture conservation practice need to be undertaken. The probability of a wet week (Pw) being more than 75% is

during 32<sup>nd</sup> week and the probability of wet week preceded by wet week (Pww) being more than 75% is during 28<sup>th</sup> and 32<sup>nd</sup> week, in addition 28<sup>th</sup>, 32<sup>nd</sup> and 33<sup>rd</sup> week has weekly rainfall more than 35 mm. Therefore, during this week's harvesting of excess amount of runoff water for supplemental irrigation and soil erosion control measures need to be practiced.

## CONCLUSION AND RECOMMENDATION

This study finds out the chance of weeks for appropriate crop planning and water management. A mean of 105 days of rainy season could occur with taking 26<sup>th</sup> week as onset and 40<sup>th</sup> week as withdrawal of the main rainy season at Dhera area. Land preparation for planting and planting could be undertaken between 26<sup>th</sup> and 28<sup>th</sup> weeks for the main rainy season crop cultivation. Supplementary irrigation and moisture conservation practice need to be practiced during 38<sup>th</sup> to 40<sup>th</sup> week for short duration crops and supplementary irrigation and moisture conservation practice could be extended if the crop is long duration crop. Harvesting runoff water and construction of soil erosion measures need to be practiced during 28<sup>th</sup> to 33<sup>rd</sup> weeks for better water management.

## Conflict of Interests

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENT

The author would like to acknowledge the Department of Soil and Water Research Process, Kulumsa Agricultural Research Center for offering all the facilities.

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## Full Length Research Paper

# Increasing efficiency of seed inoculation with bio-fertilizers through application of micronutrients in irrigated chickpea

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Received 12 January, 2014; Accepted 16 June, 2014

The present study was aimed to determine the efficiency of different combinations of seed inoculation with micronutrients [Ammonium Molybdate:  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ ] and cobalt sulphate:  $\text{CoSO}_4$ ] on chickpea plant height, nodule count and seed yield. The experiment was carried out for three consecutive winter seasons from 2010 - 2011 to 2012 - 2013 in Gujarat State of India. The inoculation treatments influenced significantly the chickpea plant height, nodules/plant and therefore, the seed yield. Application of recommended dose of fertilizers (RDF) + ammonium molybdate (2.0 g/kg seed) + Rhizobium (*Mesorhizobium ciceri*) + phosphate solubilizing bacteria (PSB species *Bacillus subtilis*) recorded significantly highest chickpea plant height (40.9 cm) and nodules/plant (26.5) and remained equivalent with application of RDF + ammonium molybdate (2.0 g/kg seed) +  $\text{CoSO}_4$  (2.0 g/kg seed) + Rhizobium + PSB. In contrast, RDF without inoculation (control) had the lowest chickpea plant height (37.4 cm) and nodules/plant (21.0). Significantly, the highest chickpea seed yield (1882 kg/ha) was produced with combination of RDF + ammonium molybdate (1.0 g/kg seed) + Rhizobium + PSB over control (1538 kg/ha) and remained equal with RDF + ammonium molybdate (2.0 g/kg seed) + Rhizobium + PSB (1832 kg/ha) and RDF + Rhizobium + PSB (1805 kg/ha) and produced 22.4, 19.1, and 17.4% more seed yield over control, respectively.

**Key words:** Chickpea (*Cicer arietinum*), cobalt, molybdenum, *Mesorhizobium ciceri*, *Bacillus subtilis*, yield.

## INTRODUCTION

Chickpea or Bengal gram or gram (*Cicer arietinum* L.) is one of the important annual grain legumes of the world which is used extensively as the primary source of protein for human beings as well as nitrogen for many cropping systems and is widely grown in all Indian States. India is the largest producer of chickpea accounting to

75% of the world production. Madhya Pradesh, Uttar Pradesh, Rajasthan, Maharashtra, Gujarat, Andhra Pradesh and Karnataka are the major chickpea producing States sharing over 95% area (Anonymous, 2011 - 2012). Being a leguminous and hardy crop chickpea does very well under dry tracts, which receive

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an annual rainfall of 60 to 100 cm. It prefers fairly cold weather but frost is deadly harmful, especially at flowering and grain formation stages. Hailstorm at maturity causes great damage to the crop. The best type of soil is clay loam with an efficient drainage and free from soluble salts preferably having neutral Ph (Vittal et al., 2005).

It is used in many forms as *dal*, *chhole*, in sweets and in some attractive dishes (Poonia and Pithia, 2011). Its leaves contain malic and citric acids, which are useful for stomach ailments, and it is best blood purifier. Its seed contains about 17 to 21% protein, 62% carbohydrates and good amount of fat; besides, being rich source of Ca, Fe, vitamin C, and B<sub>1</sub> (Singh, 1988). Its feed and straw are highly rich in nutrients. Chickpea plays a significant role in improving soil fertility by fixing the atmospheric nitrogen. Chickpea meets 80% of its nitrogen (N) requirement from symbiotic nitrogen fixation and can fix up to 140 kg N ha<sup>-1</sup> from air (Gaur et al., 2010). Currently in Gujarat State, chickpea is grown on 2399 thousand ha area, 2733 thousand metric tons production and productivity of 1139 kg/ha. During 2000 - 2001, the productivity was far below (517 kg/ha) than the national level (744 kg/ha). This is not because of availability of improved chickpea varieties seed but proper fertilizer to the crop, soil moisture and nutrient deficiencies leads to poor chickpea seed production.

Mineral nutrient deficiencies are a major constraint limiting legume nitrogen fixation and yield. Rhizobial growth and survival in soils is not usually limited by nutrient availability. Multiplication of Rhizobia in the legume rhizosphere is limited by low calcium availability. Nodule initiation is affected by severe cobalt deficiency through effects on Rhizobia. Nodule development is limited by severe boron deficiency via an effect on plant cell growth. Nodule function requires more molybdenum than does the host plant, and in some symbioses nitrogen fixation may be specifically limited by low availability of Ca, Co, Cu and Fe (Graham et al., 1988).

Chickpea crop do not need much nutrition and usually can be grown on the marginal lands. Gupta et al. (1991) and Hale et al. (2001) stated that the molybdenum is a component of some bacterial nitrogenase and, therefore, is especially important for plants that live in symbiosis with nitrogen-fixing bacteria. In another research, it was found that molybdenum is also essential for nitrate reductase and nitrogenase enzyme activity (Westermann, 2005) and *Rhizobium* bacteria fixing nitrogen needs molybdenum during the fixation process (Vieira et al., 1998).

Molybdenum is a trace element found in the soil and is required for growth of most biological organisms including plants (Agarwala et al., 1978). Deficiency of molybdenum cause poor assimilation of atmospheric nitrogen fixation in chickpea. Molybdenum is responsible for the formation of nodule tissue and increase in nitrogen fixation and without it microbial activity depressed with poor nitrogen

fixation. Sharma et al. (1989) reported that inoculation with *Rhizobium* strains resulted in a significant increase in chickpea seed yield. In contrast, a competitive and persistent rhizobial strain is not expected to express its full capacity for nitrogen fixation if limiting factors (e.g., salinity, unfavorable soil pH, nutrient deficiency, mineral toxicity, temperature extremes, insufficient or excessive soil moisture, inadequate photosynthesis, plant diseases, and grazing) impose limitations on the vigor of the host legume (Peoples et al., 1995). Availability of soil moisture and mineral nutrients are the most factors that limit nitrogen fixation.

The chickpea production practices adopted by most Indian farmers are ancient and traditional with no or few input application. Inadequate agronomic practices like insufficient fertilizer use and seed inoculation, late planting, inadequate seed rate, poor weed control and no use of micronutrients significantly cause poor productivity of chickpea. Application of recommended dose of fertilizers (RDF) and seed inoculation are recommended as the most agronomic research practice to achieve higher chickpea seed yield. The inoculation of chickpea seed is not adopted by majority of the farmer's due to insufficient knowledge about inoculation and doubts. Chickpea responds positively to inoculation when grown in soils that contain native chickpea rhizobia (Sharma et al., 1983). Many researchers studied to determine effect of soil moisture and micronutrients on nitrogen fixation. Dudhade et al. (2009) reported 9.89% increased yield due to application of *Rhizobium* to chickpea seed.

The seed inoculation must be emphasized not only for the benefit of chickpea inoculation, but also for the combination of that with micronutrients in order to obtain higher yields. Jain et al. (2007) reported that *Rhizobium* (*Rhizobium leguminosarum*) inoculation along with application of phosphorus (40 kg/ha), Zn (4 kg/ha), Mo (0.6 kg/ha), and Boron (0.1 kg/ha) resulted in the highest plant height, nodulation, and dry matter production of green gram cv. Pusa Vishal at Uttar Pradesh district of India. Togay et al. (2008) found that seed treatment with ammonium molybdenum has a positive effect on growth, yield parameters and yield in legume crops.

The study was conducted in order to analyze the effect of different micronutrients [Ammonium Molybdate:(NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>] and cobalt sulphate:CoSO<sub>4</sub>] and seed inoculation [*Rhizobium* through *Mesorhizobium ciceri* and phosphate solubilizing bacteria (PSB) through *Bacillus subtilis*] on chickpea seed yield.

The main objective of the study is to determine the efficiency of seed inoculation in combination with bio-fertilizers through application of micronutrients and their effects on chickpea seed yield.

## MATERIALS AND METHODS

Field trials were carried out in Junagadh district in South Saurashtra region of Gujarat State (India) and receive an average rainfall of

**Table 1.** Mean monthly air temperature and total monthly precipitation during 2010 - 2011 to 2012 - 2013 and long-term (1965 to 2010).

Month	Long-term average (1965 - 2010)				2010 - 2011				2011 - 2012				2012 - 2013			
	Maximum temp. (°C)	Minimum temp. (°C)	RH (%)	Rainfall (mm)	Maximum temp. (°C)	Minimum temp. (°C)	RH (%)	Rainfall (mm)	Maximum temp. (°C)	Minimum temp. (°C)	RH (%)	Rainfall (mm)	Maximum temp. (°C)	Minimum temp. (°C)	RH (%)	Rainfall (mm)
October	36.3	20.9	64	7.9	35.0	23.0	44	0.0	35.4	23.0	53	0.0	37.0	21.5	30	0.0
November	33.9	17.0	57	3.5	32.0	20.0	49	0.0	34.7	20.	49	0.0	33.8	15.3	23	0.0
December	31.3	11.5	56	0.5	28.8	11.9	32	0.0	31.1	13.7	47	0.0	32.1	15.7	25	0.0
January	30.1	10.8	54	0.1	31.0	14.0	23	0.0	28.6	11.4	50	0.0	28.1	11.6	45	0.0
February	32.6	14.0	53	0.3	33.0	15.0	19	0.0	30.7	14.6	52	0.0	31.2	13.3	36	0.0
March	36.6	18.7	54	0.3	38.0	22.0	16	0.0	36.6	18.5	40	0.0	36.0	17.7	37	0.0
Total	-	-	-	12.6	-	-	-	0.0	-	-	-	0.0	-	-	-	0.0
Mean	33.5	15.5	-	-	33.0	17.7	-	-	32.9	16.9	-	-	33.0	15.9	-	-

\* RH, Relative humidity.

680 mm with a variability of 61% on average. But in chickpea growing season the rainfall is negligible during the experimentation. The mean monthly maximum and minimum temperature are 33.5 and 15.5°C and 12.6 mm mean seasonal rainfall, based on long-term average (Table 1). The soil structure has a medium black clay soil with alluvial characteristics. It contains 204 kg/ha available nitrogen, 99.5 kg/ha available potassium, 226 kg/ha available phosphorus, 0.08 ppm/kg soil weight molybdenum, 11.61 mg/kg Fe, 1.13 mg/kg Zn, 14.15 mg/kg Mn, 1.93 mg/kg Cu and 0.82% organic carbon. The soils were saline in reactions (pH = 7.8 - 8.1). The average monthly temperature during the growing period in the experimental years (respectively 33.0, 32.9, and 33.0°C) was lower than climatic data of long-term period (33.5°C). Total amount of precipitation during the growing period in all the 3 years were nil (0.0 mm) which were lower than average of long-term period (12.6 mm).

The experiments were designed in a randomized block design with six replications in each year. Plantings were made on 30 November, 2010, 18 November, 2011 and 23 November, 2012. The plot size was 5.0 × 2.7 m. Each plot had six rows. Inter and intra spacing was kept 45 and 10 cm, respectively. Plot size was 4.0 × 1.8 m (7.2 m<sup>2</sup>) at harvest time. Each plot had two rows at the beginning and at the end of the plot for protection which were removed before harvest. A popular local released cultivar named

GG 1 with a seed rate of 60 kg/ha was tested in all the 3

years containing 36 total plots.

The study contains the following treatments:

T<sub>1</sub> = RDF (20-40-00 NPK kg/ha = Control)

T<sub>2</sub> = RDF + *Rhizobium* (through *M. ciceri*) + PSB (through *B. subtilis*)

T<sub>3</sub> = RDF + 1.0 g ammonium molybdate/kg seed + *Rhizobium* (through *M. ciceri*) + PSB (through *B. subtilis*)

T<sub>4</sub> = RDF + 2.0 g ammonium molybdate/kg seed + *Rhizobium* (through *M. ciceri*) + PSB (through *B. subtilis*)

T<sub>5</sub> = RDF + 2.0 g CoSO<sub>4</sub>/kg seed + *Rhizobium* (through *M. ciceri*) + PSB (through *B. subtilis*)

T<sub>6</sub> = RDF + 2.0 g ammonium molybdate/kg seed + 2.0 g CoSO<sub>4</sub>/kg seed + *Rhizobium* (through *M. ciceri*) + PSB (through *B. subtilis*)

In this experiment, RDF were applied to all treatments as per recommended package of practices. The basal dose of fertilizers ((20-40-00 NPK kg/ha) were applied with a hand and placed into the top 5 to 10 cm depth of soil at sowing time. All plots were fertilized with 20 kg/ha nitrogen and 40 kg/ha phosphorus. Nitrogen source was urea (46% nitrogen) and phosphorus source was di-ammonium phosphate (16% nitrogen and 46% phosphorus) in this experiment. Seed inoculation was performed with and without application of ammonium molybdate

[(NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>] and cobalt sulphate (CoSO<sub>4</sub>) as described in the treatments. The *Rhizobium* (*M. ciceri*) and PSB that is, PSB (*B. subtilis*) culture were supplied from Senior Microbiologist, Department of Microbiology, Choudhary Charan Singh Haryana Agricultural University, Hisar, Haryana (India).

Seed inoculation was performed just before sowing. No pesticide was used at sowing to seed and soil. The first irrigation was done after seed sown in plots. The second irrigation was at vegetative growth stage, third was done at flowering stage, and the fourth one was done at pod filling stage. These growth stages were selected as the reports of Silim and Saxena (1986) and Zaman and Malik (1988). Weeds were controlled by hand hoeing done twice (at vegetative stage and the beginning of the flowering) by hand hoe every year. Plots were harvested manually in March to determine seed yield and yield components. Plant height (cm) and nodules per plant were measured on five randomly selected plants from all plots at each year. Nodules were counted by excavating the roots of five random selected plants from the central rows of each plot at the flowering stage. A spade was used to uproot an undisturbed soil sample (approximately 20-cm depth) containing the roots. The nodules were counted separately from each plot. The seed yield was measured after mature plants harvested manually from four rows each 4.0 m in length and threshed. The data were analyzed as a randomized block design. All the data were subjected to

**Table 2.** Effect of seed inoculation through bio-fertilizers and molybdenum / cobalt on chickpea plant height and root nodulation.

Treatment	Plant height (cm)				Nodules/plant			
	2010 - 2011	2011 - 2012	2012 - 2013	Pooled	2010 - 2011	2011 - 2012	2012 - 2013	Pooled
RDF (20-40-00 NPK kg/ha = Control)	37.5	39.0	35.7	37.4	20.3	21.0	21.8	21.0
RDF + <i>Rhizobium</i> (through <i>M. ciceri</i> ) +PSB (through <i>B. subtilis</i> )	37.2	38.3	37.3	37.6	24.2	23.3	23.8	23.8
RDF + 1.0 g ammonium molybdate/kg seed + <i>Rhizobium</i> (through <i>M. ciceri</i> ) + PSB (through <i>B. subtilis</i> )	37.3	39.5	38.3	38.3	25.0	23.4	23.4	23.9
RDF + 2.0 g ammonium molybdate/kg seed + <i>Rhizobium</i> (through <i>M. ciceri</i> ) +PSB (through <i>B. subtilis</i> )	37.7	42.7	42.4	40.9	27.2	26.8	25.5	26.5
RDF + 2.0 g CoSO <sub>4</sub> /kg seed + <i>Rhizobium</i> (through <i>M. ciceri</i> ) + PSB (through <i>B. subtilis</i> )	38.8	39.9	35.7	38.1	27.2	24.3	23.4	24.9
RDF + 2.0 g ammonium molybdate/kg seed + 2.0 g CoSO <sub>4</sub> /kg seed + <i>Rhizobium</i> (through <i>M. ciceri</i> ) + PSB (through <i>B. subtilis</i> )	37.2	43.1	36.9	39.1	27.5	25.6	25.1	26.1
S.Em±	0.69	1.53	1.60	0.80	0.52	1.04	0.78	0.48
CD at 5%	NS	NS	NS	2.3	1.1	3.0	2.3	1.4
LSD (= 0.05)	4.5	9.3	10.4	8.5	5.0	10.5	8.0	8.1
Interaction (Y × T)								
S. Em ±	-	-	-	1.35	-	-	-	0.81
LSD (= 0.05)	-	-	-	NS	-	-	-	NS

analyses of variance (ANOVA) for all treatments using Statistical Analysis System (version 9.3). Results of ANOVA for single experiments were combined over years. The significance of the treatments and years were determined at the 0.05 and 0.01 probability levels using appropriate F-values. The F-protected least significant difference (LSD = 0.05) and standard error of the mean (S.Em±) were calculated according to Cochran and Cox (1967).

## RESULTS AND DISCUSSION

### Plant height (cm)

The applications of *Rhizobium* and PSB seed

inoculation with ammonium molybdate/cobalt sulphate had statistically significant effect on the plant height. Gupta and Gangwar (2012) in a similar study found the same result in chickpea plant height. Sharma et al. (1983) reported that the effect of fertilizer application was positive on plant height. The plant height was not affected significantly across the years (Table 2) whereas in pooled analysis plant height had a significant effect. Significantly, highest plant height (40.9 cm) was recorded with application of RDF + 2.0 g ammonium molybdate/kg seed + *Rhizobium* + PSB over rest of the treatments. The same treatment remained equivalent with application of RDF + 2.0 g ammonium molybdate/kg seed + 2.0

g CoSO<sub>4</sub>/kg seed + *Rhizobium* + PSB which produced a plant height of 39.1 cm. The lowest plant height (37.4 cm) was obtained from control plot (RDF alone).

The plant height in chickpea tended to increase in *Rhizobium*, PSB, and micronutrients inoculated treatments compared to uninoculated plots. This was attributed due to quick release of available nitrogen synthesized by root rhizobia to the plant at the time of vegetative growth. The high soil nitrogen fixation and its quick availability in rhizosphere derivation from better nodulation cause excessive vegetative growth. All of these beneficial attributes might be effective on plant vegetative growth. Yagmur and Kaydan (2011)



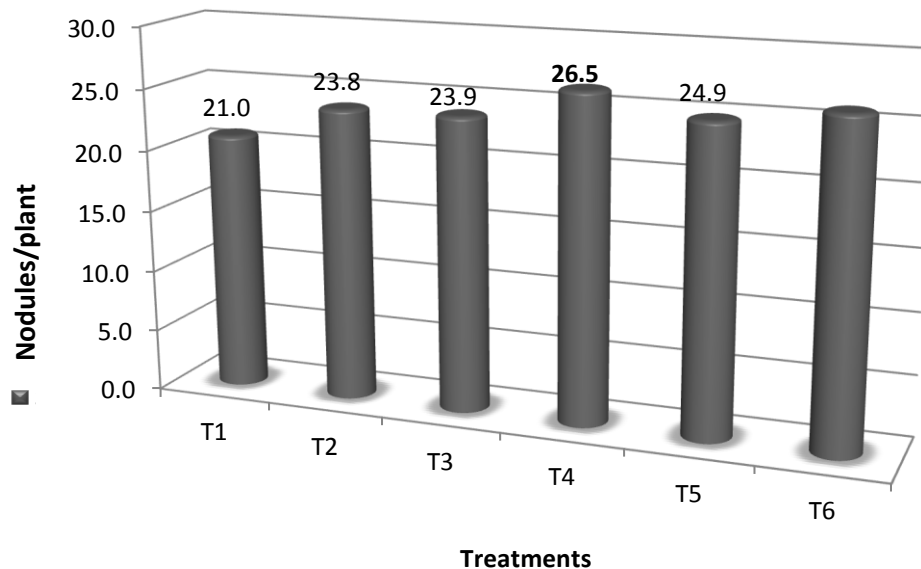


Figure 1. Significant effects of seed inoculation treatments on nodules/plant.

reported that seed inoculation and nitrogen fertilizers were increased the plant height in chickpea. Gupta and Gangwar (2012) reported that application of ammonium molybdate either soil application or through seed treatment in combination with *Rhizobium* + PSB + RDF significantly increased plant height and nitrogen uptake in chickpea over control (RDF alone).

#### Number of nodules per plant

It was found that chickpea seed inoculation with *Rhizobium* and PSB through application of ammonium molybdate and cobalt sulphate significantly influenced the number of nodules on the root system. Therefore, inoculated seed with *Rhizobium*, PSB, and ammonium molybdate/cobalt sulphate had statistically significant effect on the nodule count per plant. The number of nodules per plant was affected significantly in all the years by application of ammonium molybdate/cobalt sulphate as seed inoculation to chickpea. According to pooled data in Table 2, the highest number of nodules (26.5 nodules/plant) were recorded from the application of RDF + 2.0 g ammonium molybdate/kg seed + *Rhizobium* + PSB and remained equivalent to T6 (RDF + 2.0 g ammonium molybdate/kg seed + 2.0 g  $\text{CoSO}_4$ /kg seed + *Rhizobium* + PSB). The lowest nodules (21.0 nodules/plant) one was obtained from the application of RDF alone. Chickpea plots inoculated with *Rhizobium*, PSB, and 2.0 g ammonium molybdate/cobalt sulphate gave higher nodules per plant compared to without or lower dose micronutrients amended plots. Groups that are significantly different in nodule count per plant are also graphically depicted in Figure 1.

Moreover, inoculation with 2.0 g ammonium molybdate/cobalt sulphate was found significantly superior. There was a constructive increase in number of nodules per plant with the combination of ammonium and cobalt as seed inoculation because of the synergistic effect of both in nodule formation as mentioned by Gupta et al. (1991) and Hale et al. (2001). The increased nodulation in inoculated seeds with higher doses of molybdenum/cobalt could be attributed to better plant development through efficient utilization of molybdenum/cobalt for nodule formation by the plant grown from inoculated seeds.

#### Seed yield (kg/ha)

Six treatments of this study on seed yield of irrigated chickpea (*C. arietinum* L.) were significantly different ( $p < 0.05$ ) in years 2010 - 2011, 2011 - 2012, and pooled data (Table 3). Groups that are significantly different in chickpea seed yield are also graphically depicted in Figure 2. In pooled data the best effects were obtained on seed yield from the treatment of RDF + 1.0 g ammonium molybdate/kg seed + *Rhizobium* + PSB and remained equivalent to the treatments of RDF + 2.0 g ammonium molybdate/kg seed + *Rhizobium* + PSB and RDF + *Rhizobium* + PSB as 1882, 1832, 1805 kg/ha, respectively. The lowest seed yield was determined from control (T1) plots as 1538 kg/ha.

These results indicated that seed inoculation affects on chickpea significantly when plots were treated with molybdenum/cobalt at 1.0 or 2.0 g/kg seed. The seed inoculated with *Rhizobium* and PSB with molybdenum/cobalt at 2.0 g/kg seed has small increases

**Table 3.** Effect of seed inoculation through bio-fertilizers and molybdenum / cobalt on chickpea seed yield (kg/ha).

Treatment	Chickpea seed yield (kg/ha)				Chickpea fodder yield (kg/ha)			
	2010 - 2011	2011 - 2012	2012 - 2013	Pooled	2010 - 2011	2011 - 2012	2012 - 2013	Pooled
RDF (20-40-00 NPK kg/ha = Control)	1486	1948	1181	1538	1895	1940	1301	1712
RDF + <i>Rhizobium</i> (through <i>M. ciceri</i> ) + PSB (through <i>B. subtilis</i> )	1796	2267	1352	1805	2027	2880	1407	2105
RDF + 1.0 g ammonium molybdate/kg seed + <i>Rhizobium</i> (through <i>M. ciceri</i> ) + PSB (through <i>B. subtilis</i> )	1817	2421	1403	1882	1919	3049	1392	2120
RDF + 2.0 g ammonium molybdate/kg seed + <i>Rhizobium</i> (through <i>M. ciceri</i> ) + PSB (through <i>B. subtilis</i> )	1778	2343	1376	1832	1842	2901	1325	2022
RDF + 2.0 g CoSO <sub>4</sub> /kg seed + <i>Rhizobium</i> (through <i>M. ciceri</i> ) + PSB (through <i>B. subtilis</i> )	1667	2248	1339	1752	1738	2737	1415	1963
RDF + 2.0 g ammonium molybdate/kg seed + 2.0 g CoSO <sub>4</sub> /kg seed + <i>Rhizobium</i> (through <i>M. ciceri</i> ) + PSB (through <i>B. subtilis</i> )	1647	2114	1321	1727	1778	2726	1408	1971
S.Em±	61.7	99.8	51.0	41.2	68.3	116.4	41.2	130.7
CD at 5%	180	291	NS	116	NS	339	NS	NS
LSD (= 0.05)	8.9	10.9	9.4	10.3	9.0	10.5	7.4	10.1
Interaction ( Y × T)								
SEm ±	-	-	-	73.9	-	-	-	81.5
LSD (= 0.05)	-	-	-	NS	-	-	-	230.0

in seed yield. It was concluded that application of RDF + 1.0 g ammonium molybdate/kg seed + *Rhizobium* + PSB had 22.4% increases in seed yield over control treatment (RDF alone). Though, treatment T4 (RDF + 2.0 g ammonium molybdate/kg seed + *Rhizobium* + PSB) determined 3.3% lower yield but statistically remained at par with treatment T3.

Seed yield had highly depended on plant height and nodulation count under irrigated conditions. Higher yield was determined from treatment T3 and T4 was a reflection of high nitrogen supply due to good nodulation when inoculated with molybdenum/cobalt fertilizers, resulting in high

nitrogen fixation and better plant growth and yield contributing parameters. Deo and Kothari (2002) stated that seed treatment with 3.5 g sodium molybdate/kg seed enhance the seed yield in chickpea.

#### Fodder yield (kg/ha)

The effect of seed inoculation with *Rhizobium* and PSB through application of molybdenum/cobalt and years was found not significant (Table 3) except in 2011 - 2012 year alone. In the year 2011 -2012, highest fodder yield (3049 kg/ha) was

obtained from treatment RDF + *Rhizobium* + PSB inoculated with 2.0 g ammonium molybdate/kg seed. Moreover, the treatment statistically remained at par with all treatments except control (RDF alone). The lowest fodder yield (1940 kg/ha) was determined from plots of RDF alone without inoculation.

#### Economics

Economic analysis (Table 4) indicated that maximum net income rupees 48146/ha was obtained in the treatment T3 where chickpea

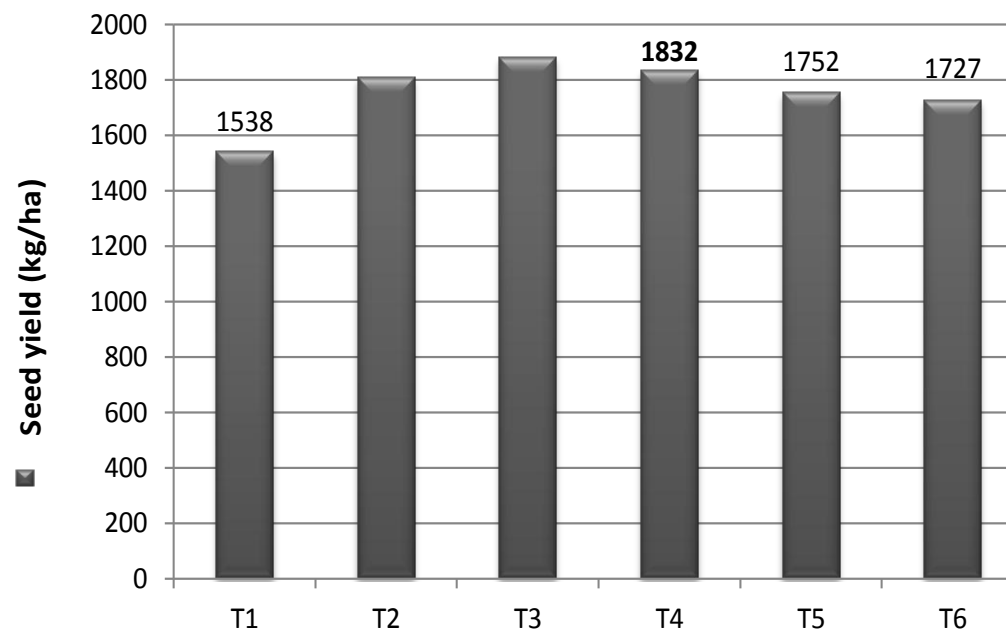


Figure 2. Significant effects of seed inoculation treatments on chickpea seed yield.

Table 4. Effect of different seed inoculation treatments on economics of chickpea production.

Treatment	Pooled chickpea seed yield (kg/ha)		Gross return (Rupees/ha)	Cost of cultivation (Rupees/ha)	Net return (Rupees/ha)	Benefit : Cost ratio
	Seed	Straw				
RDF (20-40-00 NPK kg/ha = Control)	1538	1712	54857	18126	36731	2.03
RDF + <i>Rhizobium</i> (through <i>M. ciceri</i> ) +PSB (through <i>B. subtilis</i> )	1805	2105	64438	18277	46161	2.52
RDF + 1.0 g ammonium molybdate/kg seed + <i>Rhizobium</i> (through <i>M. ciceri</i> ) + PSB (through <i>B. subtilis</i> )	1882	2120	67142	18996	48146	2.53
RDF + 2.0 g ammonium molybdate/kg seed + <i>Rhizobium</i> (through <i>M. ciceri</i> ) + PSB (through <i>B. subtilis</i> )	1832	2022	65333	19716	45617	2.30
RDF + 2.0 g CoSO <sub>4</sub> /kg seed + <i>Rhizobium</i> (through <i>M. ciceri</i> ) +PSB (through <i>B. subtilis</i> )	1752	1963	62498	19476	43022	2.20
RDF + 2.0 g ammonium molybdate/kg seed + 2.0 g CoSO <sub>4</sub> /kg seed + <i>Rhizobium</i> (through <i>M. ciceri</i> ) + PSB (through <i>B. subtilis</i> )	1727	1971	61628	20916	40712	1.95

\*Selling price (Rupees/kg): Chickpea seed: 35.0, Chickpea fodder: 0.60.

seed is treated with 1.0 g ammonium molybdate/kg seed with *Rhizobium* and PSB. The same treatment determined a maximum benefit cost ratio of 2.53 followed by treatment T2 plots. Moreover, application of ammonium molybdate and cobalt sulphate above 1.0 g dosage/kg seed had not showed significant improvement on the economics of chickpea seed production but improved the nodulation count in chickpea to some extent which benefits in soil nitrogen economy.

## Conclusion

According to the results, inoculation practices were dwelled upon at farmer's field through application of molybdenum as micronutrient for inoculation to save nitrogen fertilizing cost and contaminating nitrate to clean water. Therefore, the greater economic benefits can be obtained when the chickpea seed is treated with *Rhizobium* (through *M. ciceri*) + PSB (through *B. subtilis*) microbial fertilizers should be inoculated with ammonium molybdate at 1.0 g/kg seed under assured irrigated conditions in South Saurashtra region of Gujarat State (India).

## Conflict of Interests

The authors have not declared any conflict of interests.

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## Full Length Research Paper

# Mechanization, fertilization and staking options for environmentally sound yam production

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Received 9 January, 2014; Accepted 26 June, 2014

**An on-station study at Fumesua and Ejura in Ghana with two yam varieties (Pona and Dente), seedbed option (ridge and mound) and NPK fertilizer rates (0, 45-45-60, 60-60-60 and 60-60-80 kg ha<sup>-1</sup> N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O) revealed significant ( $p < 0.05$ ) increases in soil carbon and phosphorus with fertilizer application to yam. Fertilized yam had acceptable culinary qualities. Another study on staking options using a promising non-staked yam line TDR95/19177 showed that non-staked yam resulted in a high (32.5%) yield reduction, suggesting the need for further plant breeding work. On-farm studies showed significant ( $P \leq 0.05$ ) interaction between fertilizer rate and seedbed preparation method on continuously cropped lands, with mechanised and manual ridging having significantly ( $p < 0.05$ ) higher yam plant population (5,503-7,483 plants ha<sup>-1</sup>) than farmers' mounding (4,219-4,579 plants ha<sup>-1</sup>), and a greater yield response to fertilizer. Benefit Cost Ratio (BCR) was highest (2.7:1) when 45-45-60 kg ha<sup>-1</sup> N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O was applied to yam on ridges. On newly cleared fields, yam planted on ridges with trellis/minimum staking gave highest tuber yields and BCR of 3.8:1. Mechanised ridging for yam seedbed preparation fertilizer application and the significant reduction in the use of stakes will reduce drudgery and the contribution of yam production to deforestation and climate change.**

**Key words:** Continuously cropped fields, ridging, staking, yam.

## INTRODUCTION

Yam is one of the two major root crops produced and consumed in Ghana and West Africa. It is currently the major non-traditional export crop of Ghana. For the past decade, Ghana since 2008 has ranked second in world yam production and contributes about 16% to the National Agricultural Gross Domestic Product (FAOSTAT, 2014). Ghana is also Africa's leading exporter of yam, exporting 94% of yam from West Africa (FAO, 2013). However, yam production is characterized by clearing of new areas on yearly basis in search for fertile lands leading to deforestation and soil degradation.

Yam is a heavy soil nutrient feeder; a ton of yam is reported to extract 3.8 to 4.0 kg/ha, 0.39 to 1.1 kg/ha, 4.2 to 5.9 kg/ha of N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O, respectively (Ferguson and Haynes 1970; Le Buanec, 1972). The struggle for fertile lands coupled with the increasing human population has led to pressure on cropland and forestlands in the yam growing communities (Akwaag et al., 2000; Asante, 1996). There is therefore, reduced length and quality of fallowed land and available fertile lands, and distances to yam fields are increasing and becoming more difficult to access. Where pressure on

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land is high, farmers are increasingly compelled to grow yam on non-fallowed land. For example in Sekyere-West and Ejura-Sekyedumasi districts of Ghana, a major yam growing area, the forested land before 1983 was 782 km<sup>2</sup>, it was predicted that by 2010 it would reduce to 78.2 km<sup>2</sup> while the grasslands would increase from 1337 to 2247 km<sup>2</sup> in the same period (Akwaag et al., 2000).

Gradually, the transitional zones are giving way to grasslands resulting in shortage of staking material for the cultivation of yam especially in the Guinea savannah zones. Farmers have therefore reported declining yields of yam (Asante, 1996; Sagoe and Sally, 2006). Moreover, staking which is an integral part of yam production has been identified as a major contributor to deforestation and a major constraint for yam production especially in the Guinea savannah where there is unavailability or scarcity of stakes (Akwaag et al., 2000; Asante, 1996; Ndegwe et al., 1990). The search for stakes is laborious and forms about 20% of the labour requirement and major cost of yam production (Asante, 1996; Koli, 1973; Wholey and Haynes, 1971). There is therefore the need to focus research activities on breeding, agronomy, soil management and other management practices to provide farmers with technology to produce yam without stakes or with minimum number of stakes without a corresponding reduction in yields. This will increase yam production and enhance the potential of the crop to reduce household food insecurity and reduce poverty among producers, processors and traders (IITA, 2010) and mitigate This paper reports on-station and on-farm studies from 2009 to 2011 with the goal of investigating the contribution of chemical fertilizer, mechanized seedbed preparation and staking options to sedenterization of yam production and reduce the associated drudgery. The specific objectives were:

- i) To evaluate the effect of staking options on the growth and yields of a promising non-staked yam line TDR95/19177,
- ii) To evaluate effect of fertilizer and seedbed preparation options on yam varieties on-station,
- iii) To evaluate the effect of seedbed preparation options and staking options on performance of yam on newly cleared farmers' fields,
- iv) To evaluate the effect of fertilizer and seedbed options on the performance of yam on continuously cropped farmers' fields.

## MATERIALS AND METHODS

### Seedbed preparation and fertilizer study

The study was conducted at Fumesua in the Forest, and also in Ejura in the Forest savannah Transition where there is a greater chance for mechanization. It was on-station researcher managed in years 1 (2009) and 2 (2010) and then moved on-farm in year 3 (2011) in Hiawoanwu, Aframsso/TeacherKrom, and Atebubu communities in the Forest-transition zone. The mean annual rainfall during the study period in Fumesua and Ejura were similar with

1,356 and 1,360 mm, respectively (Table 1). The on-station was conducted in a split-split plot design with three replications. The main plot treatments were yam varieties with two levels (*Dioscorea rotundata*) and subplot treatments with two levels of seedbed preparation (Ridges and Mounds) while the sub-subplot treatments were fertilizer rates (0, 45-45-60, 60-60-60 and 60-60-80 kg ha<sup>-1</sup> N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O). Ridges were constructed mechanically (Ejura) and manually (Fumesua). Fertilizer was 50% split applied at 4 and 12 to 16 weeks after planting. Yam was planted at a spacing of 1.2 × 1.2m with target population of 6,944 plants ha<sup>-1</sup>. Planting was done with first rains in March and April. Data were collected on soil physico-chemical properties (0 to 30 cm depth) at planting and at harvest, stand establishment, rate of sprouting, shoot growth (leaves and vine), yields, yield components and tuber shape.

At on tuber, a randomized complete block experiment with each farmer as replication was established in 2011 for 4 farmers on continuously cropped fields, traditionally not used for yam production. A 2 × 2 Factorial Randomized Complete Block Design with each farmer as a replicate was used. The factors were fertilizer rate (0,45-45-60 kg ha<sup>-1</sup> N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O) and seedbed preparation (farmers' mound, mechanized/manual ridge of 40 to 45 cm height). Farmers' variety *Dioscorea alata* and *Dioscorea rotunda* data were used in Hiawoanwu and Aframsso/TeacherKrom communities, respectively near Ejura. Fertilizer was 50% split and applied at 4 and 12 to 16 weeks after the planting). Data was collected on soil physico-chemical properties at 0 to 30 cm depth at planting and at harvest, fresh and dry weight of tuber and yield components.

### Seedbed preparation and staking options study

The study was conducted in Fumesua in 2011 with TDR 95/19177, a yam line with potential for high yield under no-staking (Otoo et al., 2008). The experimental design was a Randomized Complete Block Design (RCBD) with three treatments (Vertical staking, Trellis/minimum staking (80% the number of stakes in vertical staking) and no-staking) and three replications. There were two plants within a row to one stake in the vertical staking. Stakes used were 1.5 m high for Trellis/minimum staking and 2 m for the vertical stakes. Yam was planted at a spacing of 1.2 × 1.2 m with target population of 6,944 plants ha<sup>-1</sup>. Data were collected on number of stands at 31st day after planting till time of harvest, above ground biomass (leaf and vine weight), yield and its components. Following the on-station study, farmer managed on-farm verification and demonstrations were conducted on typical newly cleared yam fields in Hiawoanwu and Aframsso/TeacherKrom farming communities in the Ejura/Sekyedumase district in 2011. The study was conducted in a 2 × 2 factorial Randomized Complete Block Design with 4 farmers. Each farmer was considered as a replicate the number of stakes in the trellis were further reduced to 50% of vertical staking. The two factors therefor were staking (trellis/minimum staking 50% of vertical, and farmers' staking) and seedbed preparation (mechanized/manual ridge and farmers' mound). Stakes ranged between 1.5 to 3 m high for trellis/minimum staking 1.5 to > 5 m (trees) for farmers's staking. Planting on ridges was at 1.2 × 1.2 m, set size ranged between 170 to 400 g and no fertilizer was applied. The total annual rainfall during the period of the study on-farm was 1186.7 mm (Table 1). Data in all the studies were subjected to analysis of variance at 5% significant level using the Statistical Analysis Software (SAS, 2007).

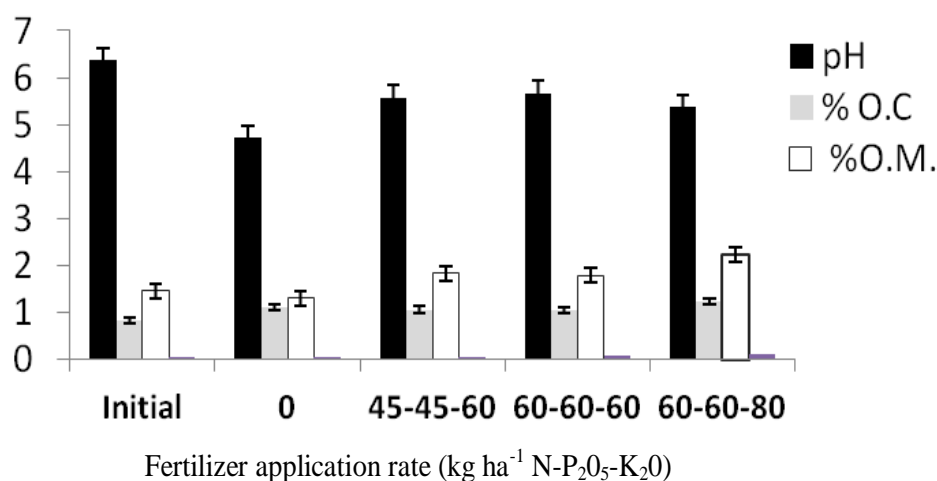
## RESULTS

### Changes in soil chemical properties

There were significant ( $p \leq 0.05$ ) increases in soil carbon,

**Table 1.** Locations and total monthly rainfall data for the period used in the study (2009 -2011).

Location	Year/Months	Total monthly Rainfall (mm)												Totals
		Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	
Fumesua	2009	-	-	36	142	145	479	215	32	46	106	42	-	1244
	2010	-	77	69	121	79	208	111	136	145	249	92	35	1322
	2011	43	134	181	73	138	283	147	41	122	297	44	0.2	1144
Means (mm)		14	70	95	112	121	324	158	70	104	217	59	12	1356
Ejura	2009	-	49	102	163	103	289	236	61	79	129	97	13	1319
	2010	-	38	62	128	224	166	161	248	198	221	127	0.2	1574
	2011	-	31	54	77	116	321	86	104	181	218	0.2	-	1187
Means (mm)		-	39	72.6	123	148	259	161	138	153	189	75	4.4	1360

**Figure 1.** Changes in soil pH, percentage organic carbon and percentage organic matter after 2 years of continuous cropping (2009 and 2010).

organic matter and phosphorus with fertilizer application to yam. However, there was no effect of seedbed preparation method on the soil chemical properties (Figures 1 and 2).

### Yields

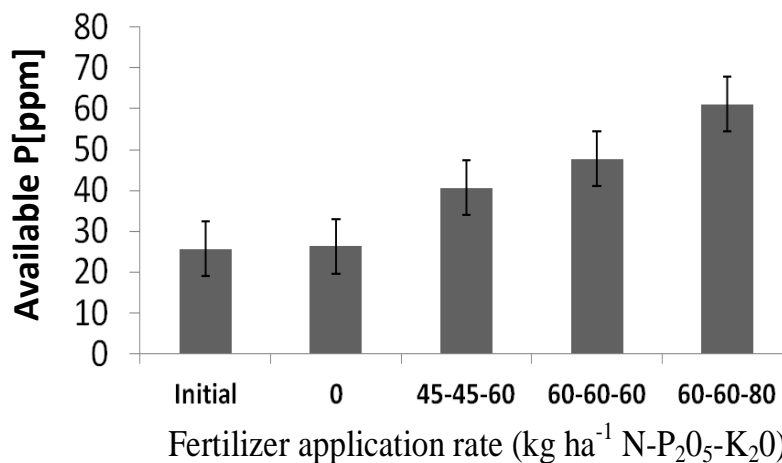
There were no significant difference between yam planted on ridges and mounds (Table 2). The similarity of yields on ridges and mounds and of fertilizer application (Table 3) at the station can be attributed mainly to similarity in plant population and poor rainfall pattern. At the on-farm, higher plant population density on ridges compared to farmers' mounds may have been a major contributor to the higher tuber yields on ridges (Figure 3). In farmers' fields, there was a significant ( $p \leq 0.05$ )

interaction between seedbed preparation method and fertilizer application with similar yields obtained for yam on ridges without fertilizer application and yam on mounds with 45-45-60 kg ha<sup>-1</sup> N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O. Yield increase due to fertilizer application ranged from 22% on mound to 28% on ridges.

Contrary to the perception by farmers and consumers, that fertilizer application to yam reduces its culinary features, sensory evaluation by farmers and consumers in the study found fertilized yam to have acceptable culinary qualities (Table 4).

### Seedbed preparation and staking study

The on-station study showed significant ( $p \leq 0.05$ ) differences in the fresh and dry weight of the treatments



**Figure 2.** Changes in soil phosphorus after 2 years of continuous cropping (2009 and 2010).

**Table 2.** Effect of seedbed preparation after 2 years continuous cropping: on-station.

Location	Seed bed option	Number of tubers harvested	Yam tuber yield (t ha <sup>-1</sup> )			
			Marketable (M)	Seeds (S)	Total (M+S)	Unmarketable
Ejura	Ridge	6351	13.8	0.9	14.7	0.3
	Mound	6684	14.6	1.0	15.6	0.3
	P value (0.05)	0.43 <sup>ns</sup>	0.50 <sup>ns</sup>	0.80 <sup>ns</sup>	0.50 <sup>ns</sup>	0.90 <sup>ns</sup>
Fumesua	Ridge	6467	4.7	1.3	6.0	0.4
	Mound	6033	4.3	1.0	5.3	0.3
	P value (0.05)	0.40 <sup>ns</sup>	0.70 <sup>ns</sup>	0.06 <sup>ns</sup>	0.50 <sup>ns</sup>	0.003*

ns- Means are not significantly different from each other at 5% significant level, \* means are significant different from each other at 5% significant level.

**Table 3.** Effect of fertilizer application on yam after 2 years continuous cropping: on-station.

Location	Fertilizer rate (Kgha <sup>-1</sup> N-P <sub>2</sub> O <sub>5</sub> -K <sub>2</sub> O)	Number of tubers harvested	Yam tuber yield (t ha <sup>-1</sup> )			
			Marketable (M)	Seeds (S)	Total (M+S)	Unmarketable
Ejura	0	6568	14.8	0.8	15.6	0.3
	45-45-60	6829	15.3	0.9	16.2	0.3
	60-60-60	6452	13.2	0.9	14.1	0.3
	60-60-80	6221	13.4	1.0	14.4	0.3
	P value (0.05)	0.28 <sup>ns</sup>	0.20 <sup>ns</sup>	0.80 <sup>ns</sup>	0.24 <sup>ns</sup>	0.57 <sup>ns</sup>
Fumesua	0	6308	4.7	1.2	5.9	0.3
	45-45-60	6337	4.4	1.1	5.6	0.4
	60-60-60	5874	4.2	1.1	5.3	5.3
	60-60-60	6481	4.6	1.3	5.9	5.9
	P value (0.05)	0.27 <sup>ns</sup>	0.87 <sup>ns</sup>	0.59 <sup>ns</sup>	0.76 <sup>ns</sup>	0.73 <sup>ns</sup>

ns- Means are not significantly different from each other at 5% significant level.

(Table 5). Vertical staking option had highest fresh and dry leaf and vine weight which translated into highest fresh and shoot weights, tuber yields and numbers (Table 6). No significant ( $p \leq 0.05$ ) differences were found in the shoot growth, tuber yield and tuber numbers between the

trellis/minimum and no-staking options. No-staking led to 32.5% reduction in yield as compared to vertical staking option. At on farm, a significant interaction ( $p \leq 0.05$ ) was observed between seed bed preparation method and staking option in tuber yield and its components.



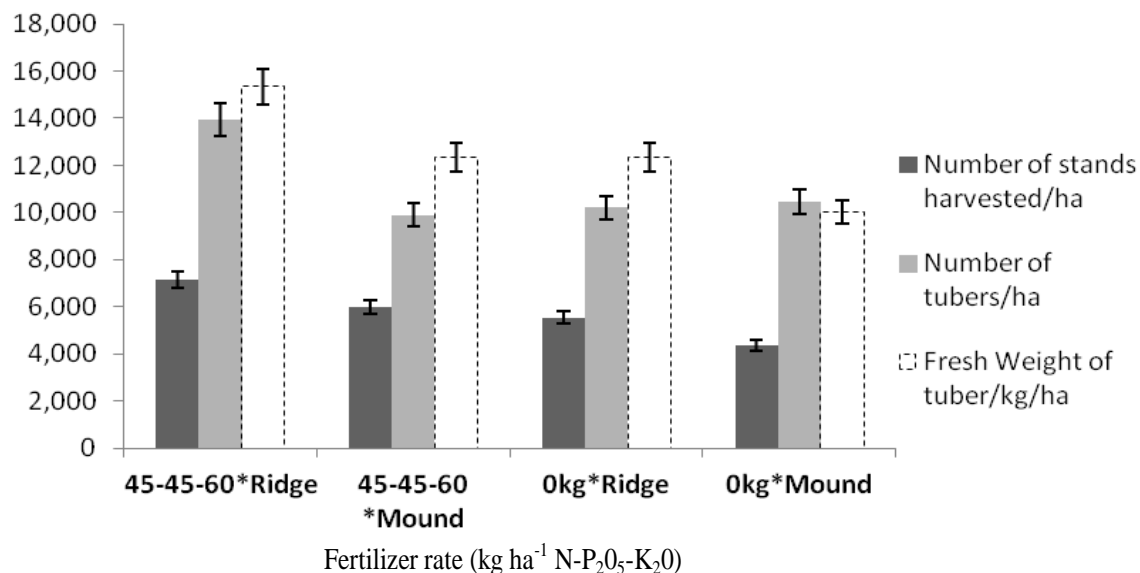


Figure 3. Effect of seedbed preparation and fertilizer application on yam plant stand and yields on-farm 2011.

Table 4. Sensory evaluation of boiled Dente yam variety. Ejura, on-station, 2010.

Fertilizer rate kg ha <sup>-1</sup> N-P <sub>2</sub> O <sub>5</sub> -K <sub>2</sub> O	Taste	Texture	Aroma	Overall acceptability	STD (acceptability)
0	2.7	3.3	3.1	3.1	0.97
45-45-60	2.9	2.9	2.8	2.8	0.91
60-60-60	2.7	2.9	3.0	2.7	0.75
60-60-80	2.0	2.2	2.4	2.1	0.80

N = 24, Scale 1 - 5: 1 = best, 5 = worst.

Table 5. Fresh and dry shoot weight of TDR95/19177 yam line, on-station 2011.

Treatment	Fresh leaf weight	Dry leaf weight	Fresh vine weight	Dry vine weight	Fresh shoot weight	Dry shoot weight
	Kg ha <sup>-1</sup>					
No-staking	1,223 <sup>b</sup>	246 <sup>b</sup>	1,157	434 <sup>b</sup>	2,381 <sup>a</sup>	680 <sup>b</sup>
Vertical staking	1,826 <sup>a</sup>	657 <sup>a</sup>	1,678	656 <sup>a</sup>	3,504 <sup>a</sup>	1,313 <sup>a</sup>
Trellis/Minimum staking	997 <sup>b</sup>	203 <sup>b</sup>	1,039	382 <sup>b</sup>	2,036 <sup>b</sup>	585 <sup>b</sup>
S E	276	145	405 <sup>ns</sup>	105	568	133

Means with the same letter are not significantly different; ns- Means are not significantly different from each other at 5% significant level.

Irrespective of the staking option, yam planted on mechanized and manual ridges had significantly ( $p \leq 0.05$ ) more number of stands at harvest which translated into significantly ( $p \leq 0.05$ ) more number of tubers and total tuber fresh weight than yam on mounds (Figure 4). However, ridges with Trellis/minimum staking had significantly ( $p \leq 0.05$ ) higher number of stands at harvest, number of tubers and total fresh weight of yields as compared to the other treatments.

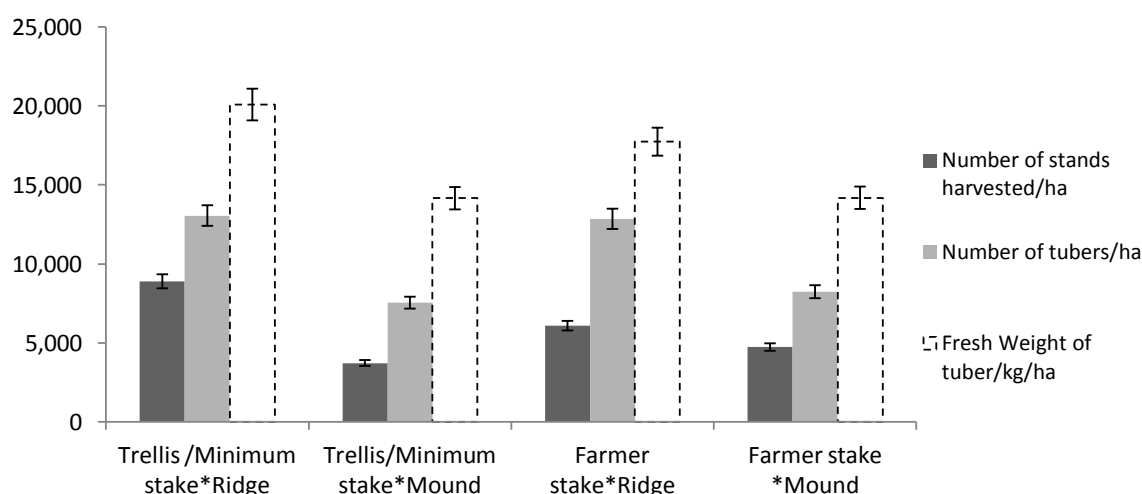
#### Partial budget and benefit cost ratio analysis of on-farm studies

Partial budget and benefit cost ratio for yam onfields which had been continuously cropped for 5 to 20 years showed that net benefit value for yam planted on ridges and fertilized gave the highest value of 4,815.5 per hectare (Table 7). It was even more profitable to plant yam on ridges without applying fertilizer than on mounds

**Table 6.** Yield and components of Non-staking, vertical staking and trellis/minimum staking of TDR95/19177 Yam line, on-station 2011.

Treatment	Number of stands harvested/ha	Number of tubers harvested/ha	Weight per tuber (Kg)	Total fresh tuber yield (Kg/ha)
No-staking	7, 176	10, 417 <sup>b</sup>	1,40 <sup>a</sup>	14, 688 <sup>b</sup>
Vertical staking	7, 407	13, 310 <sup>a</sup>	1,60 <sup>a</sup>	21, 748 <sup>a</sup>
Trellis/minimum staking	7, 292	11, 111 <sup>b</sup>	1,11 <sup>b</sup>	12, 338 <sup>b</sup>
SED	222 <sup>ns</sup>	771	0,18	2, 712

Means with the same letter are not significantly different; ns - Means are not significantly different from each other at 5% significant level.

**Figure 4.** Effect of staking options and seedbed preparation on yam plant stand and yields on-farm, 2011.

with fertilizer application. The benefit cost ratio of 2.7:1 was highest for this treatment. This implies that a farmer who invests ₦1.00 would gain an additional ₦1.70 when yam is planted on mechanized or manual ridging and fertilizer is applied. Partial budget and benefit cost ratio for yam on newly cleared lands with trellis/minimum staking and farmer staking options also revealed that the ridge option still gave the highest net benefit values compared to mounds with trellis/minimum staking resulting in the highest benefits (Table 8), with benefit cost ratio was 3.8:1. A farmer would gain an additional ₦2.8 for every ₦1.00 that is invested in planting yam on ridges with Trellis/minimum stakes than farmers' current practice.

## DISCUSSION

The significant ( $p \leq 0.05$ ) high shoot growth of the vertical staking treatment (Table 5) suggests vertical staking enhanced more shoot development as compared to trellis/minimum stake and no staking. This translated into significantly higher tuber numbers and yields (Table 6). However, no staking treatment option resulted in a 32.5%

yield reduction of TDR95/19177 yam. This reduction is high, and consistent with an earlier report by Otoo et al. (2008) which however in addition reported a significantly ( $p \leq 0.05$ ) higher yield reduction (46.5 to 53.5%) of no-staked *D. rotundata* and *D. alata* varieties compared to TDR95/19177. The use of stakes to support yam vines has been identified as one of the reasons why farmers clear new lands and major contributor to deforestation (MTADP, 1990). There is the need to breed either through varietal screening or hybridization for a yam variety which can produce higher yields with minimum or no staking than TDR95/19177. This variety may have among other characteristics, high biomass production and high resistance to diseases and pests and high weed suppression. This would reduce the pressure on forest and the labour associated with the search for stakes for staking. The insignificant ( $p > 0.05$ ) differences in the yields of the yam under ridges and mounds (Table 3) at the on-station might be due to the similar number of stands. However, in the verification trials on the farmers fields (Figure 4), there was a significant ( $p \leq 0.05$ ) interaction between seedbed preparation method and fertilizer application with similar yields obtained for yam on ridges without fertilizer application and yam on

**Table 7.** Partial budgeting of continuously cropped farmers' fields with and without fertilizer application 2011.

Parameter	Ridge + fertilizer	Mound + fertilizer	Ridge, no fertilizer	Mound, no fertilizer
Average yield (kg/ha)	17000	13000	13000	11000
Adjusted yield (kg/ha)	15300	11700	11700	9900
Farm gate price in Dec 2010 (C/kg)	0.5	0.5	0.5	0.5
Gross benefit (C/ha)	7650	5850	5850	4950
Cost of land clearing and stumping (C/ha)	100	50	100	50
Cost for ploughing (C/ha)	63	63	63	63
Construction of seedbed (C/ha)	63	150	63	150
Planting material(seed yam) cost (C)	1125	1125	1125	1125
Cost of planting (C/ha)	50	50	50	50
Cost of stakes (C/ha)	250	250	250	250
Labour cost of staking (C/ha)	280	280	280	280
Cost of weeding and reshaping (C/ha)	450	600	450	600
Cost of fertilizer (C/ha)	253.5	253.5	0	0
Cost of fertilizer application (C)	100	100	0	0
Cost of harvesting (C/ha)	100	100	100	100
Total costs that vary (C)	2834.5	3021.5	2481	2668
Net Benefit (C)	4815.5	2828.5	3369	2282
benefit cost ratio (BCR)	2.7	1.9	2.4	1.9

**Table 8.** Partial budget with trellis/minimum and farmer staking on newly cleared yam fields, 2011.

Parameter	T+R	T+M	F+R	F+M
Average yield (kg/ha)	20000	15000	18000	15000
adjusted yield (kg/ha)	18000	13500	16200	13500
Farm gate price in Dec 2010 (C/kg)	0.5	0.5	0.5	0.5
Gross benefit (C/ha)	9000	6750	8100	6750
cost of land clearing and stumping (C/ha)	100	50	100	50
cost for Ploughing (C/ha)	63	63	63	63
Construction of seedbed (C/ha)	63	150	63	150
Planting material(seed yam) cost (C)	1125	1125	1125	1125
cost of planting (C/ha)	50	50	50	50
cost of stakes (C/ha)	125	125	250	250
labour cost of staking (C/ha)	280	280	280	280
cost of Weeding and reshaping (C/ha)	450	600	450	600
cost of Harvesting (C/ha)	100	100	100	100
Total costs that vary (C)	2356	2543	2481	2668
Net benefit (C)	6644	4207	5619	4082
Benefit cost ratio (BCR)	3.8	2.7	3.3	2.5

NB: T = Trellis/minimum staking; M = Mound; R = Ridge.

mounds with 45-45-60 kg ha<sup>-1</sup> N-P2O5-K2O. Ridges appears to increase the efficiency of fertilizer use possibly through increased plant population over farmers practice of mounding (Figure 4), and also increased moisture retention (Ennin et al., 2009; Tetteh and Saakwa, 1994) Yield increase due to fertilizer application ranged from 22% on mound to 28% on ridges. It is recommended that emphasis be put on ensuring that

ridgers are accessible to farmers in the major yam producing areas in Ghana and the sub region. In Ghana, the Government's policy of establishment of Agriculture Mechanization centres must be capitalized on, to promote access to ridgers. This would reduce the drudgery associated with yam production and expand the scale of yam production.

The significant increases in soil carbon when yam was

fertilized could be attributed to greater vegetative growth, and subsequent litter fall and decay in the soil (Figure 1). The increase in soil phosphorus availability to yam increased with increasing rate of fertilizer application (Figure 2) and could be attributed to reported Mycorrhiza association with root and tuber crops (Onwueme and Charles, 1994; Zaag et al., 1980). The response of yam to fertilizer application could be improved with supplementary irrigation due to intermittent drought spells that occur during the growing period of yam.

Fallowed fields/virgin lands which have soil fertility restored are what farmers would normally use for yam production (Akwaag et al., 2000; Asante, 1996). However, due to population increases and pressure on croplands, these lands are not available or farmers have to travel longer distances to grow yams compared to fields of other food crops (Young, 1997; Nair, 1984). Figures from the Ministry of Food and Agriculture indicate that the yam production in Ghana increased over 51.6% between 1998 and 2007 with a corresponding increase in area of cultivation of about 53.6% in the same period. It is therefore projected that growth rate for yam in Ghana would be 2.71% per annum (PPMED, 2007). This suggests that, a 1% increase in area under yam cultivation would lead to a corresponding increase in yam yields of about 1%. However, with the increased pressure on cropping land as a result of population increase and the concomitant shortening of fallow periods, it would not be able to support this trend to ensure food security (Quansah et al., 2001; Garrity, 2004).

The result of this study has demonstrated fertilizer application can be used as a technique to increase productivity on continuously cropped lands and still achieve appreciable yields, thereby reducing the rate of deforestation due to yam cultivation. Also, contrary to the view that the use of fertilizer in yam production affects the quality of yam, sensory evaluation showed that the culinary qualities of fertilized yam is good and could even be better than unfertilized yam (Table 4). Reducing the number of stakes from optimum (2 plants per stake) by 50% (trellis/minimum staking) did not reduce the yields. Therefore, it appears trellis/minimum staking with stakes with heights of 1.5 m to 3 m high would favour shoot growth and better display of leaves for capture of photosynthetic active radiation for plant growth and higher tuber yields. Quantity of stakes can be further reduced by the use of ropes and this is being verified/demonstrated in 2010 in farmers' fields. It is recommended that farmers reduce the number of stakes used in staking by 20 to 50% while breeding effort continues in search of a yam variety, which would give high yields without staking.

## Conclusion

Ridging as a method of land preparation for yam production is agronomically feasible and has a positive

interaction with fertilizer application, resulting in higher yields compared to farmers' practice of manual mound construction. It has the potential to remove drudgery and increase scale of production through mechanization, and improve fertilizer use efficiency. It is recommended that, emphasis be put on ensuring that ridgers are accessible for purchase or ridging provided as a hired service by farmers. Trellis/minimum staking with stakes taller than 1.5 m high of yam planted on ridges has the potential for higher yam yields and profits than farmers' current staking practices and planting on mounds. No staking of TDR95/19177 a line with promise for high yields under no-staking resulted in 32% yield reduction, and further breeding work is also required to develop varieties which will have minimum yield reduction under no-staking.

Continuously cropped fields hitherto not used for yam production can support yam crop with minimum fertilizer application and is profitable. Further studies are needed in water management and time of planting to increase the fertilizer use efficiency. This coupled with mechanised ridging for yam seedbed preparation and the significant reduction or elimination of the use of stakes will greatly reduce the contribution of yam production to deforestation and climate change in the face of the increasing importance of yam as a food security and export crop in the West African sub-region.

## ACKNOWLEDGEMENTS

The authors wish to express their sincere gratitude to the West Africa Agricultural Productivity Program (WAAPP) for providing financial support for the study. The technical support from Messer S. Obeng Antwi of the CSIR-Crops Research Institute, Kumasi is duly acknowledged.

## Conflict of interests

The authors have not declared any conflict of interests.

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## Full Length Research Paper

# Association of statistical methods used to explore genotype × environment interaction (GEI) and cultivar stability

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Received 11 December, 2013; Accepted 26 June, 2014

This study aims to acquaint breeders of the need to use statistical tools that will help resolve the identification of consistently better performing genotypes across various environmental conditions. It also aim to reveal the relationship among the various statistical methods used to describe genotype × environment interaction (GEI) and cultivar stability. A mixed model with fixed genotypes and random environments were used for the analysis of variance (ANOVA). In the present study, twenty released bread wheat cultivars were evaluated during the 2009 main cropping season using a randomized complete block design (RCBD) with three replications at seven different environments. The combined ANOVA revealed the presence of a highly significant GEI ( $p < 0.01$ ) for grain yield indicating its influence on cultivar selection and recommendation. Spearman's rank correlation coefficient revealed a perfect correspondence between Wricke's ecovalence ( $W_i$ ) and Shukla's stability variance ( $\sigma^2$ ). These stability measures also showed a highly significant positive rank correlation with deviation from regression ( $S_{di}^2$ ), coefficient of determination ( $r_i^2$ ), AMMI stability value (ASV), variance of ranks ( $S_i^{(2)}$ ), rank sum (R-sum), and mean absolute rank difference ( $S_i^{(1)}$ ) indicating their similarity in cultivar ranking. The principal component analysis (PCA) clearly showed three groupings of the statistical methods as the static concept of stability, the dynamic concept of stability and yield performance measures. Therefore, it is imperative to consider one stability measure from the dynamic concept and one from the yield performance measures for efficient cultivar recommendation.

**Key words:** Bread wheat, genotype × environment interaction (GEI), principal component analysis (PCA), rank correlation, stability measures.

## INTRODUCTION

It is true that agricultural production has become increased during the past decades mainly because of the innovative ideas and efforts of agricultural researchers. However, 870 million people in the world are still suffering

from food shortage and malnutrition. The problem is so serious in sub-Saharan Africa being the home of about 26.5% of the world's hungry people (FAO, 2012). This indicates that the increased production and productivity

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could not keep pace with the world's population growth especially in the developing countries. The world population, currently 6.78 billion, is expected to be 10 billion by the middle of the 21<sup>st</sup> century (IDB, 2008). To feed such huge ever increasing population an assignment has remained for the agricultural scientist and other concerned bodies, at least to double the current food crops production. The key to doubling agricultural production is therefore, increasing efficiency in the utilization of resources (increased productivity per hectare and per dollar) and this includes a better understanding of the impacts of genotype  $\times$  environment interaction (GEI) on cultivar recommendation and ways of exploiting it (Kang, 2002).

There are three ways of cultivar recommendation and exploiting the GEI in a crop breeding program (Eiseman et al., 1990). (i) Ignoring them, that is, using genotypic means across environments as a criterion for cultivar recommendation even when GEI exists. Interaction, however, should not be ignored when they are significant and of crossover type (Crossa, 1990). (ii) Avoiding them - involves reducing the influence of significant cross over interaction by grouping similar environments (forming mega environments) and the main goal of crop breeding program in this case will be cultivar recommendation for each mega environment or specific adaptation (Annicchiarico, 2002; Basford and Cooper, 1998). By clustering environments, however, potentially useful information such as the aim of international and national breeders to develop cultivars with broad adaptation may be lost (Kang, 2002) and the number of cultivars recommended will be so large creating difficulties in the seed system of countries with diverse agro-ecologies like Ethiopia. (iii) Exploiting them - where the breeding program focuses on assessing the stability of genotypic performance across diverse environments by analyzing and interpreting the impact of GEI for broad adaptation. Therefore, breeders have to exploit the potential embedded in genotypes towards minimal GEI and enable breeders to identify cultivars performing well under different growing conditions.

To analyze and determine the extent of GEI under varying growing conditions, a number of univariate parametric and nonparametric as well as multivariate statistical methods have been developed by different researchers. The most commonly used are the parametric methods that require the fulfillment of some statistical assumptions such as normal distribution, independence, homogeneity of error variance and absence of outliers (Sabaghnia et al., 2006). Eberhart and Russell regression coefficient ( $b_i$ ) and deviation from regression ( $S^2_{di}$ ), Coefficient of determination ( $r_i^2$ ), Wricke's ecovalence ( $W_i$ ), Shukla stability variance ( $\sigma^2$ ), cultivar superiority measure ( $P_i$ ), coefficient of variation (CV) and environmental variance ( $S^2_{xi}$ ) are under this category.

AMMI stability value (ASV) is among the multivariate group. However, if the aforementioned assumptions are violated the nonparametric stability measures might be a

good option (Huehn, 1990). Mean absolute rank differences ( $S_i^{(1)}$ ), and variance of ranks ( $S_i^{(2)}$ ) are among the commonly applied nonparametric methods. The stratified ranking (TOP and LOW), where genotypes are ranked at each environment separately and the number of sites at which the cultivar occurred in the top and bottom third of the ranks computed (Fox et al., 1990) and rank sum (R-sum) where both yield and Shukla's stability variance are used as a criterion for cultivar ranking (Kang, 1988) are also among the nonparametric methods.

However, different opinions still exist among the leading scientist and the users of the different statistical methods in identifying the best and most suitable procedures to be used for multi location and year data set or production environments. For example, Fox et al. (1990) criticized the Lin and Binns cultivar superiority measure, noting that it may be influenced by scale of measurement; Freeman and Perkins (1971) noted that joint linear regression approach has a number of statistical and biological limitations; and the parametric stability measures might not be good when some assumptions are violated (Huehn, 1990). All these indicate the need of assessing the relationship among the stability measures developed for the analysis and interpretation of multi-environment data. Therefore, this study was carried out with the following objective: to see the relationship among the various statistical methods used to describe GEI and stability analysis.

## MATERIALS AND METHODS

The study was conducted at seven environments during the 2009 main cropping season. The environments are different in soil type, altitude, mean maximum and minimum temperatures, amount of rainfall and relative humidity (Table 1). Twenty cultivars were planted at each environment in a randomized complete block design (RCBD) with three replications. Each experimental unit had six rows of 2.5 m length with 0.2 m spacing between rows (3 m<sup>2</sup>). A 1.5 m alley was left between blocks. A seeding rate of 150 kg/ha was used. The recommended fertilizer doses of each environment (92-46 kg N-P<sub>2</sub>O<sub>5</sub>/ha for Adet cambisol and nitosol; 138 - 46 kg N-P<sub>2</sub>O<sub>5</sub>/ha for Motta, Injibara, Debark and Debre Tabore; 64 - 46 kg N-P<sub>2</sub>O<sub>5</sub>/ha for Finote Selam) was applied in the form of urea and diammoniumphosphate (DAP). The whole DAP was applied at planting but Urea was split into one third at planting and the remaining two third at tillering stage. Other management practices were performed following the recommendation.

Combined analysis of variance (ANOVA) was carried out using the PROC MIXED model of SAS program (SAS, 2002). Genotypes were assumed fixed and environmental effects as random. Significance levels of the ANOVA procedure for mixed model were determined as suggested by McIntosh (1983) and Romagosa and Fox (1993). Variance components were estimated following the PROC VARCOMP of SAS program. Fourteen stability measures were computed in accordance with Wricke's (1962) ecovalence ( $W_i$ ) as cited in Becker and Leon (1988), Eberhart and Russell's (1966) coefficient of regression ( $b_i$ ) and deviation from regression ( $S^2_{di}$ ), Shukla's (1972a) stability variance ( $\sigma^2$ ), Pinthus's (1973) coefficient of determination ( $r_i^2$ ), Francis and Kannenberg's (1978) CV and environmental variance ( $S^2_{xi}$ ), Lin and Binns (1988) cultivar superiority measure ( $P_i$ ), Nassar and Huehn's (1987) mean

**Table 1.** Description of the test environments.

Test environment	Soil type	Altitude	Latitude	Longitude	Temperature		Rain fall	Relative humidity
					Max	Min		
Adet	Nitosol	2240	11° 16'N	37° 29'E	25.8	11.8	869	68.3
Adet	Cambisol	2164	11° 16'N	37° 29'E	25.8	11.8	869	68.3
Motta	Nitosol	2470	11° 20'N	37° 88'E	23.3	12.6	783	66.1
Debre Tabore	Luvisol	2630	11° 89'N	37° 29'E	21.0	10.2	1079	68.7
Finote Selam	Nitosol	1935	10° 84'N	37° 36'E	28.4	12.6	884	na
Injibara	Acrisol	2610	10° 85'N	36° 8'E	na	na	2024	na
Debark	Cambisol	2900	13° 9'N	17° 53'E	18.7	9.6	804	66.4

Note: na = data not available.

absolute rank difference ( $S_i^{(1)}$ ) and variance of ranks ( $S_i^{(2)}$ ), Kang's (1988) rank sum, Fox et al. (1990) TOP and LOW parameters and Purchase's (1997) ASV. Most of these stability measures were computed using AGROBASE20 computer program (Agrobase, 2000). Whereas, Fox et al. (1990) TOP and LOW stability measures were computed using a SAS program called SASG × ESTAB (Hussein et al., 2000).

To see the association among the stability measures, Spearman's rank correlation coefficient were computed between all possible pairs of stability measures including grain yield using AGROBASE20 computer program and principal component analysis (PCA) using Genstat program. In order to determine Spearman's rank correlation coefficient as outlined by Steel and Torrie (1980) between the different procedures, all the genotypes evaluated was respectively assigned stability values and ranked according to the procedure and definitions used. Ranking numbers are whole numbers and when two or more equal ranking numbers occur, the average of ranking numbers that they otherwise would have received are ascribed to each genotype. Consider  $n$  genotypes are arranged in the same following order for the two stability measures;  $X_i$  indicates the ranking order or number of the  $i^{\text{th}}$  genotype for the first stability measure,  $Y_i$  indicates the ranking order of the  $i^{\text{th}}$  genotype of the second stability measure, then  $d_i = X_i - Y_i$  ( $i = 1, 2, 3, \dots, n$ ) and Spearman's rank correlation coefficient ( $r_s$ ) can be described as:

$$r_s = 1 - \frac{6 \sum d_i^2}{n(n^2 - 1)}$$

The significance of rank correlation coefficient between any two stability measures was tested by means of student's t test as described by Steel and Torrie (1980) with  $n-2$  degrees of freedom:

$$t = \frac{r_s \sqrt{n-2}}{\sqrt{1-r_s^2}}$$

## RESULTS AND DISCUSSION

### Cultivar performance and genotype × environment interaction

The combined ANOVA for grain yield indicated that there were a highly significant difference between genotypes,

environments and GEI. The significant GEI indicated that genotypes under different environments behave differently for the expression of their performance. It means a particular genotype may not exhibit the same phenotypic performance under different environmental conditions or different genotypes may respond differently to a specific environment. The grain yield performance of cultivars were ranged from 3.78 to 4.49 ton/ha (Table 2). The variance component estimation for grain yield also indicated that environments, genotypes and GEI contributed about 72.25, 5.35 and 10.87% of the total variation. This indicates that the test environments were highly variable and had the highest influence on the yielding potential of bread wheat cultivars. The variance components due to GEI is higher than the genotypes variance indicating one could not ignore the influence of GEI on cultivar recommendation for a specified growing condition.

### Association among stability measures

Spearman's rank correlation coefficients were computed for the various parametric and nonparametric stability measures including mean grain yield and presented in Table 3. Mean grain yield had statistically highly significant positive rank correlation with the cultivar superiority measure, TOP, LOW and with the R-sum. It had also a significant rank correlation with the CV. Flores et al. (1998) reported a significant rank correlation of grain yield with  $P_i$  and CV and they suggested that yield has an important influence on the ranking of genotypes by these stability measures. The highly significant rank correlation of mean grain yield with  $P_i$ , LOW, R-sum and TOP indicates that selection for increased grain yield in bread wheat would change yield stability by decreasing  $P_i$ , LOW and R-sum, but by increasing the TOP value. This further indicates the need to develop genotypes that are specifically adapted to environments with optimal growing condition. Similarly, a significant positive rank correlation of grain yield with TOP and R-sum was reported by Sabaghnia et al. (2006) and Solomon et al.



**Table 2.** Mean grain yield and genotype ranking based on the various stability parameters.

Cultivar	Yield	P <sub>i</sub>	$\sigma^2_{di}$	W <sub>i</sub>	b <sub>i</sub>	S <sub>di</sub> <sup>2</sup>	r <sub>i</sub> <sup>2</sup>	S <sub>i</sub> <sup>(1)</sup>	S <sub>i</sub> <sup>(2)</sup>	TOP	LOW	R-Sum	ASV	CV	S <sub>xi</sub> <sup>2</sup>
Gassay	4.49	1	5	5	12	6	6	5	5	1	1	1.5	7	6	11
Tay	4.39	2	4	4	15	5	4.5	7	7	5.5	3	1.5	6	4	14
Senkegna	4.30	4	19	19	13	19	17.5	16	13.5	2	2	11.5	17	11	19
Paven 76	4.24	3	7	7	5	7	7.5	1.5	4	9.5	8	3	4	1	2
Tossa	4.18	5	11	11	10	11	12	14	12	5.5	5	8.5	15	2	10
Shina	4.14	6	8	8	19	8	7.5	8	8	9.5	8	6.5	10	14	20
Dinknesh	4.10	8	9	9	11	10	9	17	15	14	4	8.5	3	8	9
Kubsa	4.07	10	14	14	18	14	11	14	11	5.5	8	11.5	18	15	17
Menze	4.03	7	3	3	20	3	1	6	6	14	11.5	4	1	13	18
Densa	4.00	11	20	20	3	20	20	12	20	3	8	15.5	20	10	12
Galema	3.96	9	2	2	16	1	2.5	3.5	2	9.5	16	5	2	9	13
KBG-01	3.81	13	6	6	17	4	4.5	3.5	3	14	13	10	8	18	15
Guna	3.80	12	1	1	7	2	2.5	1.5	1	14	16	6.5	5	5	3
Warkaye	3.79	15	18	18	1	18	19	19	18	5.5	8	17.5	19	7	4
Digalu	3.78	14	12	12	9	12	13.5	9	9	14	11.5	13	11	12	8
Hawi	3.69	17	16	16	2	13	16	10	10	18.5	16	17.5	12	3	1
Jiru	3.67	16	13	13	8	15	15	11.5	17	9.5	16	15.5	16	16	7
ET-13	3.47	18	17	17	4	17	17.5	18	19	18.5	16	19.5	13	17	6
Sirbo	3.18	19	10	10	6	9	10	11.5	13.5	18.5	19	14	9	19	5
Mellinium	3.11	20	15	15	14	16	13.5	14	16	18.5	20	19.5	14	20	16

(2007) on durum wheat and lentil, respectively. On the contrary, Mohammadi et al. (2007) reported a negative but non-significant rank correlation between grain yield and the TOP value on wheat genotypes. Even though the correlation coefficient is not significant and strong, grain yield had negative relationship with the coefficient of regression ( $r = -0.38$ ). This result disagrees with the previous results of Piepho and Lotito (1992), Mekbib (2003) and Akcura et al. (2006) who reported a positive and significant rank correlation between grain yield and coefficient of regression. Mean grain yield had also a non-significant negative rank correlation with the Francis and Kannenberg's environmental variance. Conversely, mean grain yield had weak positive correlation with the other stability measure.

The Eberhart and Russell's regression coefficient ( $b_i$ ) shows a highly significant positive rank correlation with the Francis and Kannenberg's environmental variance. This indicates that the two stability measures are equivalent in genotype ranking. This result supports the findings of Akcura et al. (2006) and Ferney et al. (2006). Except with CV, however, the regression coefficient had negative rank correlation with most of the stability measures. For example, it had a significant negative rank correlation with  $\sigma^2$ ,  $W_i$ ,  $S_{di}^2$ ,  $S_i^{(2)}$ ,  $r_i^2$  and with R-sum. This result supports the findings of Piepho and Lotito (1992) who reported a negative rank correlation of  $b_i$  with most of the stability measures on sugar beet. However, it disagrees with the results of Mekbib (2003). The significant negative rank correlation between regression coefficient ( $b_i$ ) and coefficient of determination ( $r_i^2$ )

indicated that the genotypes that were highly responsive to high yielding environments were less responsive to low yielding environments and vices versa.

The Eberhart and Russell's deviation from regression showed a highly significant correspondence with Shukla's stability variance, Wricke ecovalence,  $S_i^{(1)}$ ,  $S_i^{(2)}$ , ASV,  $r_i^2$  and with R-sum; but non-significant positive rank correlation with mean grain yield and CV. In line with this, Mekbib (2003) reported a significant positive correlation between  $S_{di}^2$ ,  $\sigma^2$  and  $W_i$ . It also had negative but negligible rank correlation with the TOP, LOW and with the environmental variance. This negligible rank correlation suggested that it is imperative to include the deviation from regression, while using the TOP, LOW and environmental variance as a tool for cultivar stability assessment and recommendation.

Shukla's stability variance had a highly significant rank correlation with most of the stability measures (such as deviation from regression, mean absolute rank difference, variance of ranks, ASVs, coefficient of determination and R-sum). This indicates that either of these stability measures could be used for bread wheat genotype recommendation. A perfect rank correlation between Shukla's stability variance and Wricke's ecovalence ( $r = 1.00$ ) indicates that these two stability measures were equivalent for genotype ranking purposes. This may be due to their biometrical relationship that Shukla's stability variance is the linear combination of the ecovalence. In line with this result, Solomon (2006) on maize reported a perfect correspondence between them.

The Lin and Binns cultivar superiority measure ( $P_i$ )

**Table 3.** Spearman's rank correlation coefficients for all the stability parameters including mean grain yield of cultivars.

Variable	Yield	CV	P <sub>i</sub>	σ <sup>2</sup>	W <sub>i</sub>	b <sub>i</sub>	S <sup>2</sup> <sub>di</sub>	S <sub>i</sub> <sup>(1)</sup>	S <sub>i</sub> <sup>(2)</sup>	ASV	r <sub>i</sub> <sup>2</sup>	TOP	LOW	R-sum
CV	0.55*	1												
P <sub>i</sub>	0.98**	0.57**	1											
σ <sup>2</sup>	0.32	0.21	0.43	1										
W <sub>i</sub>	0.32	0.21	0.43	1.00**	1									
b <sub>i</sub>	-0.38	0.32	-0.41	-0.49*	-0.49*	1								
S <sup>2</sup> <sub>di</sub>	0.28	0.22	0.38	0.98**	0.98**	-0.48*	1							
S <sub>i</sub> <sup>(1)</sup>	0.25	0.27	0.35	0.79**	0.79**	-0.27	0.82**	1						
S <sub>i</sub> <sup>(2)</sup>	0.38	0.33	0.46*	0.86**	0.86**	-0.46*	0.90**	0.89**	1					
ASV	0.23	0.22	0.34	0.88**	0.88**	-0.36	0.88**	0.67**	0.73**	1				
r <sub>i</sub> <sup>2</sup>	0.34	0.12	0.44	0.98**	0.98**	-0.62**	0.97**	0.76**	0.86**	0.87**	1			
TOP	0.75**	0.40	0.71**	-0.06	-0.06	-0.11	-0.12	0.01	0.02	-0.28	-0.07	1		
LOW	0.90**	0.51*	0.84**	0.02	0.02	-0.16	-0.03	-0.08	0.06	-0.03	0.03	0.80**	1	
R-sum	0.78**	0.47*	0.86**	0.82**	0.82**	-0.51*	0.79**	0.68**	0.77**	0.71**	0.82**	0.41	0.53*	1
S <sup>2</sup> <sub>xi</sub>	-0.38	0.41	-0.36	-0.08	-0.08	0.86**	-0.05	0.03	-0.08	0.04	-0.22	-0.30	-0.28	-0.24

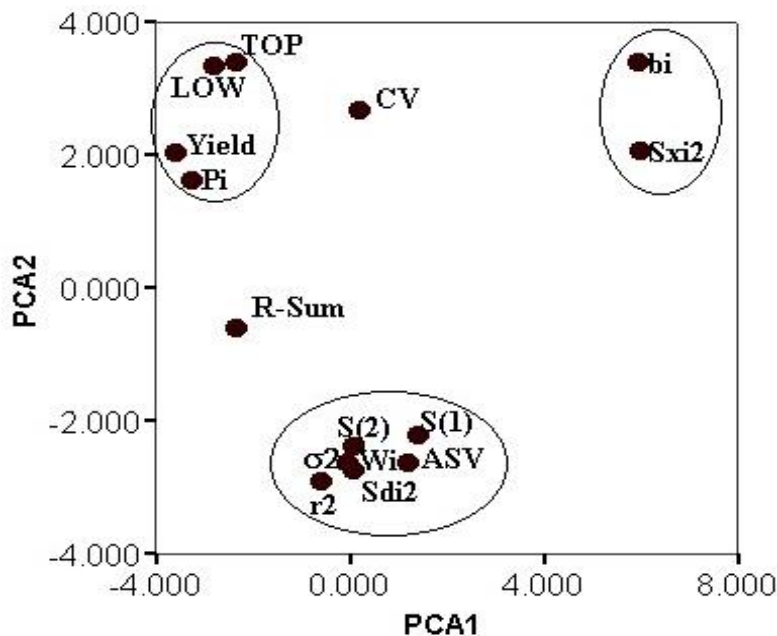
shows a highly significant positive rank correlation with the TOP, LOW and R-sum. This indicated that either of these stability measures could be sufficient for cultivar stability assessment and recommendation. In this case, however, care should have to be taken. Because Lin and Binns (1988) defined cultivar superiority measure (P<sub>i</sub>) as the mean square distance between a cultivar's yield and highest yield achieved, it may be therefore influenced by scale of observations which will be more important when ranges of site mean yields are large as commonly seen in multi-environment trials. There was no significant relationship between P<sub>i</sub> and most of the parametric stability measure depicting P<sub>i</sub> is not normally a stability measure rather a performance indicator. Similar result was reported by Purchase et al. (2000).

The Wricke's ecovalence shows a highly significant positive rank correlation with S<sup>2</sup><sub>di</sub>, r<sub>i</sub><sup>2</sup>, ASV, S<sub>i</sub><sup>(2)</sup>, R-sum, and S<sub>i</sub><sup>(1)</sup>. Similarly, a positive

correlation between W<sub>i</sub> and S<sup>2</sup><sub>di</sub> were reported by Duarte and Zimmermann (1995) and Mekbib (2003). A positive but negligible rank correlation was also observed between ecovalence and the LOW parameter. On the other hand, ecovalence had negligible negative rank correlation with the parameters TOP and S<sub>xi</sub><sup>2</sup>. Because of their biometrical relationship, the observed high correspondence between W<sub>i</sub>, (S<sub>i</sub><sup>(1)</sup>) and (S<sub>i</sub><sup>(2)</sup>) is highly expected. The nonparametric stability measures (S<sub>i</sub><sup>(1)</sup>) and (S<sub>i</sub><sup>(2)</sup>) are based on the ranks of values  $X_{ij} - (\bar{X}_{i.} - \bar{X}_{..})$  within each environments, where  $X_{ij}$ ,  $\bar{X}_{i.}$ ,  $\bar{X}_{..}$  denotes the observed values of genotype i in environment j, the mean of genotype i in all environments and the overall mean respectively. Subtracting the environmental mean ( $\bar{X}_{.j}$ ) from the above term will not affect the ranking within environments. So ranking  $X_{ij} - (\bar{X}_{i.} - \bar{X}_{..})$  values is equivalent to ranking

of  $(X_{ij} - \bar{X}_{i.} - \bar{X}_{.j} + \bar{X}_{..})$  values (Piepho and Lotito, 1992). It is known that the Wricke's ecovalence (W<sub>i</sub>) is the sum of squares of the term  $X_{ij} - \bar{X}_{i.} - \bar{X}_{.j} + \bar{X}_{..}$ . This relationship clearly justifies that these stability measures are almost similar for genotype ranking.

Similarly, because ecovalence may be partitioned into two components: the covariance between GEI effects and environmental effects  $[(b_i - 1)^2 \sum_j (\bar{X}_{.j} - \bar{X}_{..})^2]$  and sum of squared deviations from regression which is just another expression of S<sup>2</sup><sub>di</sub>  $[\sum_j d_{ij}^2]$  (Becker and Leon, 1988), the higher rank correlation between W<sub>i</sub> and S<sup>2</sup><sub>di</sub> indicates that the covariance component explains only a small portion of the ecovalence values. In other words, since the regression coefficient (b<sub>i</sub>) in this study was non-significantly different from unity and the sum of squares of



**Figure 1.** Principal component analysis (PCA1 and PCA2) plot of the various stability parameters based on the rank correlation matrix.

environmental effects  $\left[ \sum_j (\bar{x}_{.j} - \bar{x}_{..})^2 \right]$  is constant for all genotypes, most of the ecovalence value was contributed by the deviation from regression.

Nassar and Huehn's mean absolute rank difference ( $S_i^{(1)}$ ) and variance of ranks ( $S_i^{(2)}$ ) showed a highly significant positive rank correlation with each other. These two nonparametric stability measures also had a highly significant positive rank correlation with the ASV, coefficient of determination, deviation from regression and with the R-sum. This result suggests their similarity and consequently, only one of these stability measures would be enough to identify stable genotypes in a breeding program. Kang's rank-sum showed a significant positive rank correlation with most of the stability measures except with  $b_i$  being negatively correlated. The ASV had a significant rank correlation with the coefficient of determination. In addition, the percentage of sites for which each genotype occurred in the top (TOP) and bottom (LOW) third of entries in each trial showed a significant positive correspondence with each other indicating their similarity for genotype ranking purposes. Similar finding was reported by Solomon et al. (2007).

### Principal component analysis (PCA)

To understand the relationships among the various stability measures, PCA based on the rank correlation matrix was performed. The first two PCA's explained 77.5% (41.9 and 35.6% by PCA1 and PCA2, respectively) of the total variance of the original variables.

The relationships among the different stability measures are graphically displayed as a two dimensional scatter plot of PCA1 and PCA2 (Figure 1). This scatter plot clearly reveals three different groups of the stability measures. The mean grain yield, cultivar superiority measure ( $P_i$ ), the TOP and LOW parameters scattered together in one group indicating their being performance measures rather than stability. The second group consists of  $S_i^{(1)}$ ,  $S_i^{(2)}$ ,  $W_i$ ,  $\sigma^2$ ,  $S_{di}^2$ , and  $r_i^2$  together (representing the dynamic concept of stability); and the regression coefficient ( $b_i$ ) and the environmental variance ( $S_{xi}^2$ ) in the third group which represents the static concept of stability. Whereas the CV as well as the R-sum was not grouped in any of the three classes. They were clustered separately indicating they are different from the other stability measures in genotype ranking like CV or associated with most of the stability measures like R-sum. This biplot clustering has indicated the similarity and dissimilarity of the various stability measures in cultivar ranking.

### Conclusion

The observed strong positive association among ecovalence ( $W_i$ ), stability variance ( $\sigma^2$ ), deviation from regression ( $S_{di}^2$ ), ASV and coefficient of determination ( $r_i^2$ ) indicate their similarity in cultivar ranking and therefore a breeder can use only one of them depending on their simplicity and the nature of data set. In addition, mean absolute rank difference ( $S_i^{(1)}$ ), variance of ranks ( $S_i^{(2)}$ ) and R-sum that showed a strong association with

the aforementioned measures can be a good alternative for cultivar stability assessment and recommendation. This holds more important especially in cases where the data set exhibited a problem of outliers, violation of assumptions such as normal distribution, independence and homogeneity of error variance. Besides these stability measures, the genotypes' grain yield performance measures should always be considered together with the stability measures.

## Conflict of interests

The authors have not declared any conflict of interests.

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## Full Length Research Paper

# Genetic improvement of *Gossypium arboreum* L. using molecular markers: Status and development needs

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Received 12 March, 2014; Accepted 16 June, 2014

The genus *Gossypium* has 45-50 species, of which only four (two allotetraploids and two diploids) enjoy the status of cultivated cotton. Among the four cultivated ones, *Gossypium arboreum* holds a special place because of the inherent ability to withstand drought, salinity and remarkable resistance to sucking pests and leaf curl virus. However, the species suffer with poor fiber quality traits, low yield and has certain undesirable plant and boll features. Improvement of fiber quality traits of *G. arboreum*, without disturbing its unique characteristics, has been a long sought after dream of scientists world over. For conservation, evaluation and documentation of existing accessions, gene banks of diploid cultivated cotton has been established by different countries, of which India holds the highest number of *G. arboreum* collections. Traditional breeding efforts made to improve fiber quality of *G. arboreum* have met with limited success due to paucity of polymorphic phenotypic markers and polygenic nature of the desired traits. Genetic engineering approach is highly genotype dependent and much success has not been achieved by this way also. Molecular breeding approach, based on the strength of breeding with high polymorphic nature of molecular markers, has yielded significant results in the improvement of *G. arboreum*. A number of molecular markers have been developed and used in various cotton improvement programs but dominantly these efforts have been made for the allotetraploid species *G. hirsutum*. Some efforts have been made for generating fingerprint database of *G. arboreum* germplasm, though much more efforts are needed in this direction. Linkage maps of *G. arboreum* have been generated using different markers systems which has enabled the mapping of gene/QTLs of desired traits on the chromosome of *G. arboreum*. More research inputs need to be devoted to produce consensus and saturated genomic maps, if the aim of 'breeding by design', by retaining suitable features and improving the undesirable ones, is to be realized for *G. arboreum*.

**Key words:** Asiatic cotton, molecular breeding, cotton fiber quality, molecular breeding, genetic diversity.

## INTRODUCTION

Cotton is the world leading natural fibre crop on which the textile industries worldwide are largely based on. It is also an important oilseed crop contributing significantly to bio-energy production. Cotton occupies a pivotal position in

the world economy, and very often, it is known as 'white gold'. The term cotton is used to describe cultivated species of the genus '*Gossypium*' (family Malvaceae) (Wendel et al., 1992; Wendel and Brubaker, 1993;

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Dongre and Kharbikar, 2004; Esmail et al., 2008), which has 45-50 species, 40-45 being diploid ( $2n=2x=26$ ) and 5 being allotetraploid ( $2n=4x=52$ ). Spinnable fibers are obtained from two allotetraploid (*Gossypium hirsutum* and *Gossypium barbedense*) and two diploid (*Gossypium herbaceum* and *Gossypium arboreum*) species and hence only these four species enjoy the status of cultivated cotton. The diploid species are popularly called as old world, or desi cotton (in India and Pakistan), while the allotetraploids species are commonly known as new world cotton (Wendel et al., 1992). The present paper is an attempt to highlight the strengths and weaknesses of *G. arboreum* germplasm and to discuss present status and development need of various improvement programs.

### Domestication of *G. arboreum*

There is evidence of its cultivation as old as 2000 BC by the Harapan civilization of the Indus Valley for the cotton textiles. With time, *G. arboreum* germplasm was dispersed and domesticated in different regions leading to the development of different races viz. indicum, burmanicum, cernuum, sinense, bengalense, soudanase. Over the years of cultivation, various ecotypes of each race have been identified in different parts of Indian subcontinent. Bengal Desi, of race 'bengalense', is popularly grown in North India and Pakistan and is found to be highly productive but yielding short (12-20 mm length) and very coarse fibers (>5.5 micronaire). Various ecotypes of race 'indicum' have developed to grow successfully in the diverse harsh environmental conditions of Tamil Nadu, Gujrat, Karnatka and Andhra Pradesh. One ecotype (Gharo cotton) of race 'cernuum' is found to yield very big balls and is popularly cultivated in North-Eastern regions of India and adjoining parts of Bangladesh (Kulkarni et al., 2009).

### Characteristics features of *G. arboreum*

*G. arboreum*, also called true cotton, is a species native to Indian sub-continent. *G. arboreum* has certain inherent qualities like the ability to withstand drought and salinity (Maqbool et al., 2010; Tahir et al., 2011) making it suitable for low input conditions. Many studies demonstrated that the asiatic cotton is tolerant and shows remarkable resistance to several pests and disease, including bollworms (Dhawan et al., 1991), aphids and leafhoppers (Nibouche et al., 2008), rust, fungal (Wheeler et al., 1999) and viral (Mehetre et al., 2004; Akhtar et al., 2010) diseases. The reasons for resistance to sucking pests are presence of lower palisade layer, relatively higher distance between lower epidermis of midrib and phloem, and densely arranged midrib cortex cells in *G. arboreum* plants. In fact, presently *G. arboreum* is being

used as donor species in introgressive breeding to improve tetraploid cotton, especially for disease resistance and insect tolerance (Ansingkar et al., 2004; Kulkarni, 2002). Natural *G. arboreum* fibers display various colours (e.g. white, off-white and tan) also and some of the accessions produce fibers with high strength (Mehetre et al., 2003).

### Present cultivation scenario of *G. arboreum*

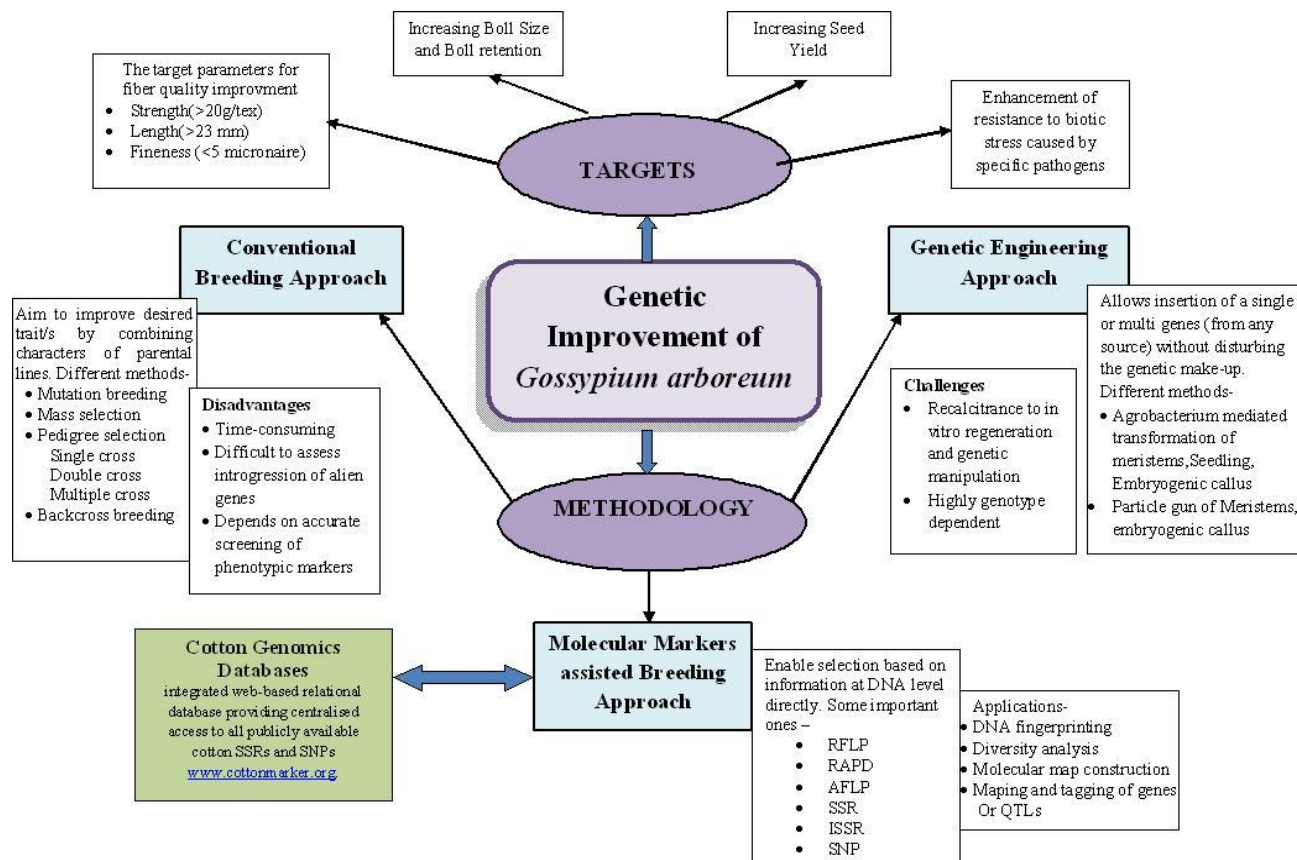
Till the 1950s, a major part of cotton area of Old World (Asia and Africa) was occupied by diploid cultivated cotton (Kulkarni et al., 2009). After that with increase in global trade, American upland cotton and Egyptian/sea-island cotton viz. *G. hirsutum* and *G. barbadense* respectively, replaced the diploid cotton. At present, the area of diploid cotton cultivation in India is around 25% (17% *G. arboreum* and 11% *G. herbaceum*), while for rest of old world countries it has come to a very low scale (Kulkarni et al., 2009). The major cause for this change was due to fibre, boll and plant features of diploid cotton. The primary fibre properties affecting textile manufacturing and end product quality include fibre length and uniformity, strength, elongation, fineness and maturity (Chee and Campbell, 2009). Diploid cultivated cotton are characterized by short (<23 mm fibre length), coarse (>5.0 micronaire) and weak (<20 g/tex at 3.2 mm gauge) fibres, which are not suitable for spinning by mechanized textile technology (Kulkarni et al., 2009) (Figure 1). The lanky plant structure with indeterminate growth and small bolls with less locule retention are major hurdles in cotton harvesting, impairing the economics of cultivation (Figure 1). Still, poor and marginal farmers prefer *G. arboreum* because of the characteristics features discussed above.

Genetic improvement of *G. arboreum* for better fiber quality traits, without disturbing its unique characters, is needed to promote its cultivation. Such efforts will boom the fiber industry and go a long way for strengthening the world economy. In the following section, an effort is being made to explore the various on-going efforts and approaches for genetic improvement of *G. arboreum* so as to analyze the strengths, achievements made so far as well as to identify the challenges being faced by these methods. Further, current status of cotton molecular marker technology and the scope, potential and future perspective of this technology for the genetic improvement of *G. arboreum* are discussed in light of suitable examples.

### Improvement of *G. arboreum*: Status and challenges

The success of genetic improvement program of any crop is based on the identification of well defined targets and adoption of reliable methodology. Although, diploid





**Figure 1.** A diagrammatic description of various targets for genetic improvement of *G. arboreum* along with various methodologies adopted for this, with characteristics features of each.

cultivated cottons are grown in nearly eleven countries (India, Pakistan, China, Burma, Nepal, Cambodia, Malaysia, Indonesia, Namibia, Vietnam, Laos), organized genetic improvement programs are dominantly made in India. Diploid cotton improvement projects are a part of All India Coordinated Cotton Improvement Project (AICCIP) and supported by Technology Mission on Cotton (TMC), Mini Mission I and Rainfed Cotton Agroecosystem (RCPS) programs of the National Agriculture Technology Project ([www.cicr.nic.in](http://www.cicr.nic.in)). The research projects designed for improvement of *G. arboreum* are generally targeted at: (i) Improvement of fiber quality traits, like length and fineness (ii) Increase in the boll size (iii) Increase in yield (iv) enhancement of resistance to various biotic stress- though *G. arboreum* is resistant to sucking pests and immune to leaf curl viral disease, certain other disease and pests affect it. Grey mildew (*Ramularia areola* Atk), Leaf spot (*Alternaria macrospora*) and Fusarium wilt are important disease while bollworms (*Helicoverpa armigera*, *Earias vitella* and *Pectinophora gossypiella*) are insect pests causing economic damage to *G. arboreum* (Kulkarni et al., 2009). A significant level of variability, for the desired characters, exists in the germplasm of *G. arboreum*, indicating good

potential for genetic improvement. Appreciable genetic variability has been observed in the *G. arboreum* germplasm for yield and yield components (Singh and Singh, 1984; Kumar and Rajmani, 1994), fiber properties (Singh, 1986), boll size (Kulkarni et al., 2009) and locule retention (Singh and Nandeshwar, 1983). Various improvement efforts have been made to exploit this genetic variability using traditional plant breeding methods based on morphological markers. Traditional approaches to increase the seed yield have been quite successful; varieties released during 1970s and 1980s had less seed cotton yield potential (1600 to 1800 kg/ha) compared to varieties released in 1990s and 2000s (e.g. LD 327, HD 107 and RG8 (2500-2600 kg/ha), a hybrid AAH1 (3900 kg/ha) in north Indian irrigated conditions (Singh, 1998; Lather et al., 2001). Efficient enhancement of fiber properties and boll feature of *G. arboreum* has also been reported through *G. hirsutum* gene introgression program (Kulkarni et al., 2003). Some successful breeding attempts were also made for improving the locule retention of *G. arboreum* bolls; CINA36 is an improved line which has been registered for good locule retention (Kulkarni et al., 2009).

The various conventional breeding approaches are

generally very time consuming (Figure 1); generally takes five to six generations for transfer of a trait, within a species, into the high yielding locally adapted cultivars and a large number of progenies have to be planted to select the plants with appropriate combinations of traits. After that, the improved lines so developed have to go through a number of multi-location trials before a variety could be identified for cultivation by the farmers. In way, a total of 7-10 years or more are involved from the beginning to the end of the project. Further, sometimes it is difficult to combine more than one trait in a single breeding experiment, and improvement in one aspect is many-a-times found to be associated with undesirable characteristics of another aspect e.g. 'Fiber improvement' breeding programs which used 'indicum' as source for long and fine fibers, suffered with small boll size problem and when 'cernuum' was used to improve boll size it led to undesirable fiber properties (Kulkarni et al., 2009). Breeding efforts in fiber quality have been very effective, which used mostly 'indicum' source for long and fine fiber. Mass selection generated a wide range of exploitable genetic variation for fiber properties but boll size remained small (Rao et al., 2004).

Genetic transformation is one of the powerful techniques offered by biotechnology for genetic improvement of crop plants with desired traits. The most important application of this has been the development of Bt cotton which also happens to be first genetically modified crop marketed successfully and still very popular. *In vitro* regeneration of all the four cultivated species of cotton has been a difficult goal to achieve because morphogenic response is highly genotype dependent (Trolinder and Xhixian, 1989; Cousins et al., 1992; Rajasekaran et al., 1996) (Figure 1). Much of the genetic transformation work in cotton has been done for tetraploid species and for this also, foreign gene to be introduced is generally put into Coker (highly responsive to *in vitro* regeneration) followed by transferring into elite cotton cultivars through conventional breeding method like back cross breeding which take longer time. Very few studies have been carried out for genetic transformation in *G. arboreum*. Generally *G. arboreum* genotypes are more tolerant to insects than *G. hirsutum*, but in case of severe outbreaks economic losses are observed. Further there are certain genotypes which have desired fiber traits but suffer with high susceptibility to insects attack e.g. DLSa-17 which is long linted but susceptible to all lepidopteran pests. As there is no germplasm accession that provides durable resistance to bollworms, the transgenic approach offers the solution. An effort has been made to transfer cry 1F gene into DLSa-17 to make it insect resistant; transformants were obtained at low frequency (Sangannavar, 2008). Various factors affecting transformation efficiency of shoot apices of *G. arboreum* cultivar LD 694, using *Agrobacterium* strain GV3101 carrying plasmid pPZP200 vector with cry1AC gene, have also been reported (Sanghera et al., 2011). In

addition to developing bioengineered insect resistant desi cotton, other traits like lint yield, fiber quality, seed oil content, resistance to fungal and viral infection also need to be addressed by genetic transformation. The prerequisite for this is the development of protocols which overcome the recalcitrance of cotton tissues to genetic manipulation and *in vitro* regeneration, which seems to be a far dream though. So if genetic transformation is compared with breeding approach, latter seems to be more reliable and stable approach for cotton with wider applicability.

The only drawback of breeding approach is dependence on less polymorphic morphological markers. With the advent of molecular marker technology, a new era of breeding has emerged. DNA marker technology enables the plant breeders to select desirable plants directly on the basis of genotype instead of phenotype. DNA marker technology has enabled the development of a sufficiently large number of genetic markers to accommodate the needs of modern plant breeding, also termed as 'Molecular breeding'. With the use of molecular marker technology, breeding efforts have become more specific, less time consuming and more yielding in terms of the target level achieved. In the coming section a brief discussion has been made about different types of molecular markers developed and applied in cotton followed by different applications these molecular markers offer for genetic improvement of *G. arboreum*.

### Cotton molecular markers: Types and database

Since the last three decades, a lot of efforts have been put in for developing various types of DNA markers, each having a differential set of advantages for any particular applications. Application of this technology towards cotton improvement programs started in the 1990s, Meredith (1992) in a study of heterosis and varietal origins reported the first restriction fragment length polymorphism (RFLP) evaluation in upland cotton. First detailed RFLP map of cotton with 41 linkage groups was developed by Reinisch et al. (1994). Since then, numerous types of marker systems have been developed and applied to cotton improvement programs. In the coming section, a brief overview is made about various types of molecular markers being developed and used for cotton genomics followed by a discussion about cotton marker database.

### Types of molecular markers

The cotton genome is large and complex with 26 chromosomes, which require identification of a large number of evenly spaced DNA markers for significant applications (Park et al., 2005; Qureshi et al., 2004). A number of molecular markers have been developed and used for cotton (Figure 1).



### Restriction fragment length polymorphism (RFLP)

It is hybridisation based technique in which organisms are differentiated by analysis of patterns derived from cleavage of their DNA by restriction enzymes. The main steps involve isolation of DNA, digestion with restriction enzymes, separation of restriction fragments by agarose gel electrophoresis, transfer of fragments to nylon membrane, hybridization with probes, and scoring of polymorphism by autoradiography. RFLP markers are reliable, reproducible and co-dominant making them ideal tool for genome mapping and gene tagging experiments, though the technique is time-consuming, cumbersome and not amenable to automation (Table 1). RFLPs have been widely used in gene mapping studies because of their high genomic abundance, ample availability of different restriction enzymes and random distribution throughout the genome (Neale and Williams, 1991). RFLPs have played a strategic role in initiating and pushing the research work in cotton genomics (Rahman et al., 2009) and a number of researchers have applied this technique to various *Gossypium* spp. for different purposes (Reinisch et al., 1994; Jiang et al., 2000; Mei et al., 2004; Ulloa et al., 2005). However, at present, RFLPs are not popular in cotton genome studies because of low ability to detect polymorphism in cotton compared to other plant taxa (Brubaker and Wendel, 2000).

### Random amplified polymorphic DNA (RAPD)

It is a polymerase chain reaction (PCR) based technique, based on enzymatic amplification of target or random DNA segments with arbitrary primers. Polymorphism is obtained because of sequence variation in the genome for primer binding sites, making RAPDs as dominant marker. RAPD marker system is easy to carry out, needs no prior sequence information, requires very less amount of DNA and is amenable to automation, however, the technique suffers with low reproducibility (Rafalski, 1997) and some more disadvantages mentioned in Table 1. The poor reproducibility of RAPD profiles within and between laboratories can be attributed to various factors like quality of DNA, composition of PCR reaction mixture, nature of DNA polymerase and the skills of the working person, to quote a few. RAPDs have been used for diversity, genome mapping and phylogenetic studies in cotton (Rahman et al., 2002b; Zhang et al., 2002; He et al., 2007; Rahman et al., 2008b; Rana and Bhat 2004). RAPDs are not popular for genetic mapping and gene tagging studies in cotton because of their low informativeness and non-locus specific nature.

### Amplified fragment length polymorphic (AFLP)

It is a technique which combines reliability of RFLP with the ease of RAPD (Vos et al., 1995). The process

involves three simple steps: (i) restriction of genomic DNA and ligation of oligonucleotide adaptors, (ii) pre and selective amplification of restriction fragments, and (iii) gel analysis of amplified fragments. The polymorphic fragments are detected as present or absent making it a dominant marker system. The technique can be automated and allows the simultaneous analysis of many genetic loci per experiment (Table 1). AFLP produces more polymorphic loci per primer than RFLPs, SSRs or RAPDs (Maughan et al., 1996). The availability of many different restriction enzymes and corresponding primer combinations provide a great deal of flexibility, enabling the direct manipulation of AFLP fragment generation for defined applications. The AFLP technique has been extensively used in various cotton improvement programs like phylogenetic studies (Iqbal et al., 2001), linkage and quantitative trait loci (QTL) mapping (Jixiang et al., 2007; Mei et al., 2004; Hawkins et al., 2005; Altaf et al., 1997), genetic diversity studies (Abdalla et al., 2001; Rana et al., 2004a; Zhang et al., 2005) and map saturation studies (Lacape et al., 2003; Zhang et al., 2005).

### Simple sequence repeats (SSRs)

These are also commonly called as microsatellites and were first described in humans (Litt and Luty, 1989). These are short tandem repeats of 2-8 nucleotide motifs. The repeat number of core nucleotide sequence varies from a few to hundreds of times at many independent loci. The SSRs are flanked by unique sequences which have remained conserved between the members of a gene pool during the course of evolution. PCR amplification of SSRs is done by using primers complementary to the flanking regions. The length of the amplified product varies according to the number of repeated motif (Ellegien, 1993). SSRs are found highly polymorphic (Powell et al., 1996) and the polymorphism is due to either slippage of DNA polymerase during replication or unequal crossing over, resulting in differences in copy number of the core nucleotide sequences (Rahman et al., 2002a). Because of their abundance in the genome and codominant nature, microsatellites are considered ideal markers in gene mapping studies (Han et al., 2006). SSRs are highly reliable, allow exchange of data among laboratories and are more robust than RAPD and AFLP (Table 1). Expansion and contraction of SSR repeats in genes of known function can be tested for association with phenotypic variation or, more desirably, biological function (Ayers et al., 1997).

In cotton genome, one microsatellite on an average per 170 kb of genomic DNA has been reported (Zhao et al., 1994), making them a good tool for genome mapping. Out of 10000 SSRs containing genomic fragments isolated from *G. hirsutum*, 588 were sequenced to identify SSRs and primers have been designed for 307 of these (Reddy et al., 2001). SSRs have been used in

**Table 1.** A summarized description of some important molecular markers used commonly in cotton breeding programs.

S/No	Features	RFLP	RAPD	AFLP	SSRs	ISSR	SNPs
1.	DNA Required ( $\mu$ g)	10	0.02	0.5 to 1.0	0.05	0.02	0.05
2.	PCR based	No	Yes	Yes	Yes	Yes	Yes
3.	No. of polymorphic loci analysed	1-3	1.5-50	20-100	1-3	1.5-50	1
4.	Ease of use	Not easy	Easy	Easy	Easy	Easy	Easy
5.	Amenable to automation	Low	Moderate	Moderate	High	Moderate	High
6.	Reproducibility	High	Unreliable	High	High	Moderate	High
7.	Need of sequence data	Not required	Not required	Not required	Required	Not required	
8.	Level of polymorphism	Low	Low-Moderate	Low-Moderate	High	Low-Moderate	High
9.	Dominance	Co-dominant	Dominant	Dominant	Co-dominant	Dominant	Co-dominant
10.	Interspecific transferability	Moderate-high	Low-Moderate	Low-Moderate	Low-Moderate	Low-Moderate	Low-Moderate
11.	Utility in marker assisted selection	Moderate	Low-Moderate	Low-Moderate	High	Low-Moderate	High
12.	Cost and labour involved in generation	High	Low-Moderate	Low-Moderate	High	Low-Moderate	High
13.	Advantages	-High genomic abundance -Can be used in plants reliably -Needed for map based cloning	-High genomic abundance -No radioactive labeling -Relatively faster	-High genomic abundance -Can be used across species -Useful in preparing contig maps	-High genomic abundance -No radioactive labeling -Multiple alleles	-High genomic abundance -Require low quantity of DNA	-High throughput automation
14.	Disadvantages	-Need large amount of good quality DNA -Need radioactive labeling -Cloning and characterization of probe are required	-Cannot be used across species	-Very tricky due to changes in patterns with respect to materials used -Need to have very good primers	-Cannot be used across species	-Non-homology of similar size fragments	-High costs

cotton genomics for studies like phylogenetics and diversity analysis (He et al., 2007; Lacape et al., 2007), linkage mapping (Yu et al., 2005), gene tagging and QTL mapping (Karaca et al., 2002; Zhang et al., 2003), association mapping (Kantartzi and Stewart, 2008).

### Inter simple sequence repeats (ISSRs)

ISSRs were developed by modifying the SSR marker technology. The modification involved the

use of primers complementary to SSR motif itself instead of the flanking sequence thus ruling out any prior sequence information (Wolfe et al., 1998). The procedure was first applied to fungi (Meyer et al., 1993) and was found to work successfully with plants (Gupta et al., 1994) as well as animals (Perring et al., 1993).

Amplification with ISSR primers result in DNA fragments of about 100-3000 bp located between adjacent, oppositely oriented microsatellite regions. ISSR analysis is reported to be

technically simple, highly reproducible and capable of revealing high polymorphism (Rahman et al., 2009). ISSRs have been reported as quite useful markers for revealing polymorphism in cotton genotypes (Liu and Wendel, 2001).

### Single nucleotide polymorphism (SNPs)

SNPs are a novel class of DNA markers that has emerged recently and have become highly

preferable in genomic studies. The SNPs arise due to change in a single nucleotide position (point mutation) by various genetic phenomena. SNPs are highly abundant making them suitable markers for generating high density genetic maps. SNP markers are co-dominant, distributed normally and sometimes associated with morphological changes (Lindblad-Toh et al., 2000). SNP analysis is useful for cultivar discrimination in crops where it is difficult to find polymorphism. These DNA markers have also been used in cotton for fingerprinting (Rahman et al., 2002b, 2008b), linkage map construction (Reinisch et al., 1994; Zhang et al., 2002; Lacape et al., 2003; Mei et al., 2004; Rong et al., 2004), gene mapping (Shappley et al., 1998; Ulloa and Meredith, 2000) and genetic diversity studies (Rahman et al., 2002b, 2008b).

### Cotton markers database

The cotton marker database (CMD) is an integrated web-based relational database providing centralised access to all publicly available cotton microsatellites and single nucleotide polymorphisms ([www.cottonmarker.org](http://www.cottonmarker.org)). CMD is initiated and funded by Cotton Incorporated. This database also displays data for various microsatellite projects that have been screened against a standardised panel of core germplasm including 12 diverse genotypes selected from cultivated and exotic cottons. Various data mining tools like SSR server, BLAST server, FASTA server, CAP3 server and CMap are accessible at CMD. Hosting of web and database for the CMD is provided by Bioinformatics and Chemical Informatics (BCI) group at School of Computing, Clemson University and Computational resources are provided by Clemson Computing and Information Technology (CCIT). This collection of all publicly available cotton SSR and SNP markers into a centralised, readily accessible web-enabled database provides a more efficient utilization of molecular marker resources and will help to accelerate basic and applied research in molecular breeding and genetic mapping in *Gossypium* species.

### Applications of molecular markers in *G. arboreum* improvement programs: Generating a DNA fingerprint database of the germplasm, existing worldwide

Different countries have established Gene Banks of diploid cultivated cotton for conservation, evaluation and documentation of existing accessions. In India, Central Institute of Cotton Research (CICR), Nagpur, is maintaining a global germplasm collection of diploid cultivated cotton; it holds the highest number of collections belonging to *G. arboreum* (1870) (Anonymous, 2005). The French cotton germplasm collection, preserved in Centre de Cooperation Internationale en Recherche Agronomique pour le

Development (CIRAD), Montpellier, maintains 69 accessions of *G. arboreum* (Dessauw and Hau, 2006). The United States of America (USA) maintains 1730 accessions of *G. arboreum* at Southern Plains Agricultural Research Center (SPARC), Crop Germplasm Research Unit, College Station, Texas (Anonymous, 2005). Likewise, nearly 369 accessions are maintained at Chinese Academy of Agriculture Science, Nanjiang, China (Liu et al., 2006). Systematic race-wise characterization of *G. arboreum* germplasm, existing worldwide, has been difficult due to overlapping morphological traits and non-availability of race specific phenotypic markers. The DNA fingerprint database will help in right documentation of the germplasm.

Further, every country has some policy to carry out various germplasm expeditions of different crops/plants for sampling the existing variability. Such programs are also being conducted for exploring the germplasm of *G. arboreum* and variability is recorded based on morphological characters only, which is unable to give the real picture due to poor polymorphic index. Generation of DNA fingerprint of a newly explored local cultivar and matching it with the existing fingerprint database would help to make a right entry for it.

Liu et al. (2006) used SSR markers to create DNA fingerprint database of 39 *G. arboreum* L. genotypes of China. Of the 358 microsatellite markers analyzed, 74 primer pairs detected 165 polymorphic DNA fragments and 12 genotypes could be fingerprinted with one or more SSR markers.

### Genetic diversity analysis

Genetic diversity is the basis for genetic improvement of any crop. Genetic diversity is usually thought of as the amount of genetic variability among individuals of a variety, or population or species (Brown, 1983). The knowledge of genetic variation between and within populations of *G. arboreum* is desirable not only to establish a theoretical basis for conserving the Asiatic cotton germplasm resources but also to target and improve certain ideal characteristics such as fibre quality and various plant features for exploiting these germplasm in modern cotton productions. There exist only a few studies for genetic diversity analysis of *G. arboreum* using molecular markers. Abdalla et al. (2001) used 16 AFLP primer combinations on 3 diploid species and 26 allotetraploid species of genus *Gossypium* and observed genetic similarities, among all taxa, ranging from 0.21 (between the diploid species *G. arboreum* and *G. raimondii*) to 0.89 (within *G. barbadense*). Rana and Bhat (2004) used RAPD markers to assess the genetic relationship among 30 genotypes of *G. arboreum*; of 45 primers surveyed, 63% were found polymorphic which revealed genetic similarity in the range of 47.05 to 98.73%. The results indicated a close genetic relationship

among the cultivated genotypes indicating a narrow genetic base.

Guo et al. (2006) studied the genetic diversity of selected *G. arboreum* accessions collected from different regions of China using SSR markers and observed that largest number of alleles and maximum number of polymorphic loci was present in the A03 linkage group. The polymorphism information content for the 22 polymorphic microsatellite loci varied from 0.52 to 0.98, with an average of 0.89. Genetic diversity of 19 elite genotypes of diploid and tetraploid cotton has been carried out by Dongre et al. (2007) using SSR and ISSR markers, the similarity coefficient obtained ranged from 0.59 to 0.90 and 0.59 to 0.93, respectively, thus suggesting considerable genetic variation between the selected genotypes.

Kantartzi et al. (2009) studied genetic diversity in 96 accessions of *G. arboreum* L. using genomic and EST-derived microsatellite markers. They selected a set of 25 SSR primers based upon high level of informativeness and production of clear PCR bands on agarose gel. The 25 SSR loci were found to reveal 75 allelic variants (polymorphism) ranging from 2-4 alleles per locus. RAPD markers have also been used to study genetic diversity in 20 selected genotypes of *G. arboreum*; of the 40 primers screened, 20 failed to amplify any genotype, 11 showed good band profile in few genotypes while remaining nine gave amplification in all the 20 genotypes (Deosarkar et al., 2010). Out of the total amplified products, 52 bands showed 70.58% polymorphism, while remaining products were monomorphic across the genotypes. Dongre et al. (2011) studied genetic diversity of 16 cultivars of *G. arboreum* using 20 RAPD primers and 24 SSR primers. Fifteen selected polymorphic RAPD primers produced a total of 178 fragments, of which 112 fragments were found to be polymorphic, resulted in 65.14% polymorphism. Twenty selected polymorphic SSR primers produced a total of 64 alleles, of which 41 were found to be polymorphic, resulting in 69.50% polymorphism. More such studies are needed to investigate genetic diversity among the existing germplasm of *G. arboreum*.

### Construction of molecular maps via linkage or association mapping techniques

Linkage maps shows the relative positions of genetic markers along a chromosome that is determined by the recombination frequency during crossover of homologous chromosomes. The mapping of functional genes plays an important role in studies of genome structure, function, and evolution, as well as allowing gene cloning and marker-assisted selection to improve agriculturally important traits. Many linkage maps of tetraploid cotton have been constructed using different molecular markers and mapping populations (Reinisch et al., 1994; Ulloa et

al., 2002; Lacape et al., 2003; Rong et al., 2004). Unfortunately, molecular marker technology has not been applied extensively to map the genome of *G. arboreum*. Only a few reports are present for genetic mapping in *G. arboreum*. To increase the numbers of microsatellites available for use in constructing a genetic map, and facilitate the use of functional genomics to elucidate fiber development and breeding in cotton, sampling of microsatellite sequences from expressed sequence tags (ESTs) transcribed during fiber elongation in the A-genome species *G. arboreum* was done to evaluate their frequency of occurrence, level of polymorphism and distribution in the At and Dt subgenomes of tetraploid cotton (Han et al., 2004). There are only a few reports available for linkage mapping of *G. arboreum* genome. Han et al. (2004) published a genetic map ((TM1 X Hai7124) X TM1) based on 99 SSR markers developed from fiber ESTs of *G. arboreum*. A total of 111 loci detected with these 99 EST-SSRs integrated with 511 SSR loci and two morphological marker loci assorted to 34 linkage groups containing 2 to 41 markers each, covering 5644.3 cm with an average intermarker distance of 9.0 cm. Intraspecific genetic linkage map of the A-genome diploid cotton was constructed with newly developed SSR markers using 189 F<sub>2</sub> plants derived from the cross of two Asiatic cotton cultivars (*G. arboreum* L.) 'Jianglingzhongmian' × 'Zhejiangxiaoshanl'ushu' (Ma et al., 2008). For this study, 268 pairs of SSR primer with better polymorphism were picked out of 6092 pairs which generated a total of 320 polymorphic bands. Linkage map was generated with Join Map 3.0. The total length of the map was 2508.71 cm and average distance between adjacent markers was 9.40 cm. Comparisons among the 13 suites of orthologous linkage groups revealed that the A-genome chromosomes are largely collinear with the At and Dt subgenome chromosomes. Construction of intraspecific genetic linkage map of *G. arboreum* with SSR and RAPD markers and using 180 F<sub>2</sub> plants derived from cross of two cultivars- Ravi X Entry-17 of *G. arboreum*, was presented by Shaheen et al. (2013). Another step after construction of linkage map is QTL analysis for identifying/mapping gene of desired traits. Shaheen et al. (2013) mapped 7 QTLs, including 5 for productivity traits and 2 for fiber traits, in the intraspecific *G. arboreum* linkage map prepared by them. However, identification of map position of a QTL is only the first step for planning a MAS (marker assisted selection) program. After a QTL has been identified, several other things need to be addressed like-(i) Will the QTL work in other genetic background, (ii) Will the QTL have same phenotypic effects in other genetic backgrounds, (iii) is the QTL linked to other undesirable traits, (iv) Will the QTL-marker linkage relationship hold in different generations, (iv) what is the cost and efficiency of applying MAS compared to phenotypic selection alone? (Chee and Campbell, 2009).

Association mapping, based on linkage disequilibrium

(LD) where collection of cultivars, lines, or landraces are genotyped with densely spaced markers, is emerging out as a better technique of mapping (Sorkheh et al., 2008; Mackay and Powell, 2007). There are number of reasons for this, firstly, in the population a broader genetic variation in a more representative background is available. It means that one is not limited to marker and trait loci that happen to differ between two parents (Kraakman et al., 2006). Secondly, LD mapping may attain a higher resolution, because of the use of all the meiosis accumulated in the breeding history. Thirdly, historic phenotypic data on cultivars can be used to link markers to the traits, without the need for new trials with special mapping populations (Sorkheh et al., 2008).

In fact, association mapping was first successfully used for identification of alleles at loci contributing to susceptibility to human diseases (Goldstein et al., 2003). Later on this approach was applied to different plant species as well such as wheat (Bresseghele and Sorrells, 2006), rice (Agrama et al., 2007), barley (Kraakman et al., 2006), maize (Stich et al., 2005) etc. In cotton, association studies are very limited; LD has been measured only in a collection of tetraploid *G. hirsutum* lines (Abdurakhmonov et al., 2007). There is a single report on association analysis of fiber traits in *G. arboreum* accessions (Kantartzi and Stewart, 2008), analysing 56 *G. arboreum* germplasm accessions introduced from nine regions of Africa, Asia and Europe for 8 fiber characters. Genotyping was carried out with 98 SSR markers. Population structure analysis identified six main clusters for the accessions which corresponded to different geographic regions, indicating agreement between genetic and predefined populations. The general linear model method was used to disclose marker trait association and these associations were investigated by fitting single marker regression models for phenotypic traits on marker band intensities with correction for population structure. More studies are needed in *G. arboreum* germplasm and to carry out association analysis between quantitative traits and molecular markers which will also account for the effect of population structure.

### Future perspective

*G. arboreum* is a poor man crop with low input conditions. The genetic improvement in desirable traits of *G. arboreum* will definitely gear up the economy of poor farmer as well as the whole cotton industry. Mapping of genes/QTLs of economically important traits in *G. arboreum* (diploid with AA genome) will also be a great help for cracking similar things in comparatively complex genomes of *G. hirsutum* and *G. barbadense* (tetraploid, AADD genome). Much of the initial years of molecular genetics research into cotton has been devoted to developing core infrastructure facility like polymorphic markers, discrete mapping populations and extensive

linkage maps. This has given an insight about the number and locations of QTL associated with desirable traits. With the advancement made so far and continuous advancement of molecular technology, the coming years will definitely give a clearer picture of the genetic basis of various desirable traits like cotton fiber quality, drought resistance, biotic resistance etc. Molecular breeding is definitely having a bright future in cotton and MAS is going to make an effective complement to phenotypic screening in the future genetic gain for desirable traits.

### CONCLUSION

The Asiatic cotton grows easily in dry land as well as in saline conditions and has inherent resistance to several abiotic and biotic stresses, making it a low input crop. However, the fibers produced by *G. arboreum* are in low yield and of poor quality (in terms of mechanised spinning). A huge germplasm of *G. arboreum* exists worldwide. Well defined and well planned efforts are needed to explore the existing resources for the improvement of fiber quality traits. Different approaches are being carried out for this, amongst which molecular markers assisted breeding seems to be far reaching. Various molecular markers technology has been applied to cotton improvement program in general as well as to *G. arboreum* in specific. Continuous and intensive efforts are needed to create saturated maps as well as to identify of markers linked to fiber quality genes/QTLs. The improvement of *G. arboreum* fibers will be very fundamental to the gearing up of fiber industry as well as to the world economy.

### Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

# Productivity of transplanted rice as influenced by weed control methods

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Received 15 April, 2013; Accepted 26 June, 2014

Rigorous research efforts are being made by scientists around the world to evolve different strategies for improving rice yield. Most of the improved crop management practices in rice cultivation failed due to poor and improper practices for containing weeds. At present, no single approach, that is, uses of herbicides or manual or mechanical weeding is effective in containing the weed menace. Hence, the present investigation was aimed to study the influence of integrated weed control (chemical + hand weeding) on the productivity of transplanted rice. Ten weed control treatments like application of herbicides alone and their integration with one-hand weeding, two-hand weeding and unweeded check were tested in randomised block design with three replications. The highest weed control efficiency (90 and 93%) and maximum grain yield (5831 and 8783 kg ha<sup>-1</sup>) were recorded under two-hand weeding during both years respectively which was at par with post emergence application of bispyribac sodium 25 g ai ha<sup>-1</sup> supplemented with hand weeding at 45 DAT. Uncontrolled weed growth reduced grain yield to the tune of 47.02 and 53.79% during 2011 to 2012 and 2012 to 2013, respectively.

**Key words:** Transplanted rice, integrated weed control, herbicides, hand weeding, weed control efficiency, yield.

## INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food for more than 60% of the world population. It is the most important cereal crop and is extensively grown in tropical and subtropical regions of the world. Rigorous efforts are being made under several research programmes by scientists around the world to evolve different strategies for improving rice yield. Most of the improved crop management practices in rice cultivation failed due to poor and improper practices for containing weeds.

In India, rice is cultivated in an area of 44.07 million hectares annually with a production of 103.4 million tonnes, with an average productivity of 2.3 t ha<sup>-1</sup> (FAO, 2012).

There are several reasons for low productivity and the one due to weeds is the most important. Weeds compete with rice for moisture, nutrients, light, temperature and space. Uncontrolled weeds have caused yield reduction of 28 to 45% in transplanted rice (Singh et al., 2007; Manhas et al., 2012). Furthermore, any delay in weeding will lead to increased weed biomass which has a negative correlation with yield.

Butachlor, anilofos, oxadiargyl and pretilachlor are herbicides presently used for weed control in transplanted rice. These herbicides provide effective control of annual grasses, but not annual sedges and broad leaved weeds.

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For example, it has been reported that whenever there is effective control of grasses due to these herbicides, annual sedges and broad leaved weeds emerge in high density competing with crop and resulting into heavy yield losses (Singh et al., 2004). At present, no single approach of either use of herbicides or manual/mechanical weeding is effective in containing weed menace. Therefore, there is a necessity that these herbicides are supplemented with hand weeding to widen weed control spectrum. Hence, the present investigation was to study the influence of integrated weed management package on weed control efficiency and productivity of transplanted rice.

## MATERIALS AND METHODS

Field experiment was conducted at Tamil Nadu Rice Research Institute, Aduthurai (11° N latitude, 79° E longitude and at an altitude of 19.5 m above mean sea level) in Cauvery Delta Zone of Tamil Nadu during wet seasons of 2011 to 2012 and 2012 to 2013 to study the effect of integrated weed management packages on weed control, growth and yield of transplanted rice. The soil of the experimental field was clay with slightly alkaline pH (8.2), medium in organic carbon (0.52%), low total nitrogen (161 kg ha<sup>-1</sup>), high available phosphorus (54.5 kg ha<sup>-1</sup>) and medium available potassium (206 kg ha<sup>-1</sup>). The experiment was laid out in randomised block design with three replications. Treatments consisted of application of pre emergence (PE) herbicides viz., clomazone 500 g ai ha<sup>-1</sup>, clomazone + 2, 4-DEE (ready mix) 500 g ai ha<sup>-1</sup> alone and their integration with one-hand weeding (HW) at 45 DAT; butachlor 1250 g ai ha<sup>-1</sup> + HW at 45 DAT; pretilachlor 750 g ai ha<sup>-1</sup> + HW on 45 DAT; post emergence (POE) herbicide bispyribac sodium 25 g ai ha<sup>-1</sup> + HW at 45 DAT and pre plant incorporation of glyphosate at 15 days before transplanting followed by PE application of bensulfuron methyl plus pretilachlor 660 g ai ha<sup>-1</sup> tested with two-HW at 25 and 45 DAT and unweeded control for weed control efficiency and productivity of transplanted rice. Hand weeding were carried out as per the treatment schedule.

Long duration (155 days) high yielding paddy variety CR 1009 was transplanted two seedlings per hill 7 and 17 September, 2011 and 2012, respectively with a spacing at 20 × 15 cm. The crop was fertilized with recommended dose of 150: 50: 50 kg N,P<sub>2</sub>O<sub>5</sub>,K<sub>2</sub>O ha<sup>-1</sup>. The entire dose of phosphorus was applied as basal in addition to zinc sulphate 25 kg ha<sup>-1</sup> and gypsum 500 kg ha<sup>-1</sup> while nitrogen and potassium were applied in four equal splits at basal, active tillering (4 Weeks After Transplanting-WAT), panicle initiation (8 WAT) and heading stages (12 WAT). Pre emergence herbicides were mixed with sand at 50 kg/ha and applied uniformly to the field on 3 DAT. The post emergence herbicide was sprayed at 2 to 3 leaf stage of weeds (15 DAT) by using knapsack sprayer fitted with flat fan nozzle. A thin film of water was maintained at the time of herbicide application. All other agronomic and plant protection measures were adopted as per the standard recommendations.

Weed species present in the experimental plot were identified at flowering stage of crop from weedy check plot and grouped as grasses, sedges and broad leaved weeds. The data on weed density and weed dry weight (60 DAT) were recorded with the help of a quadrat (0.25 m<sup>2</sup>) at four places randomly chosen and then expressed in number per square metre and kilogram per hectare. The weed control efficiency was worked out on the basis of weed dry matter recorded in each treatment at 30, 60 DAS/DAT and at harvest by using the formula suggested by Mani et al. (1973).

$$\text{WCE (\%)} = \frac{\text{Total weed dry weight in unweeded control (kg ha}^{-1}\text{)} - \text{Total weed dry weight in treated plot (kg ha}^{-1}\text{)}}{\text{Total weed dry weight in unweeded control (kg ha}^{-1}\text{)}} \times 100$$

Values were subjected to square root transformation ( $\sqrt{x+0.5}$ ) prior to statistical analysis to normalize their distribution. Observations on yield attributes like panicles per meter square (m<sup>2</sup>), grains per panicle, 1000 grain weight and grain yield of paddy were recorded at harvest.

The data recorded were analysed statistically in Randomized Block Design (RBD) as per the method suggested by Gomez and Gomez (1984). Wherever the treatment means were significant, critical differences were calculated at 5% probability level for comparisons of mean values. Non significant differences among treatment means were denoted as NS.

## RESULTS AND DISCUSSION

### Weed growth

The dominant weed flora of experimental fields consisted of *Echinochloa crusgalli*, *Echinochloa colonum* and *Leptochloa chinensis* among grasses, *Cyperus difformis*, *Cyperus iria* and *Fimbristylis miliacea* among sedges and *Marselia quadrifolia*, *Eclipta alba*, *Ammania baccifera*, *Bergia capensis* and *Ludwigia parviflora* among broad leaved weeds.

### Effect on weeds

All the weed control treatments significantly reduced total density and dry weight of weeds during both years (Table 1). The lowest density (5.67 and 4.33 No./m<sup>2</sup>) and dry weight (2.04 and 1.47 g/m<sup>2</sup>) of weeds were recorded under two-hand weeding on 25 and 45 DAT followed by post emergence application of bispyribac sodium 25 g ai ha<sup>-1</sup> supplemented with HW at 45 DAT during both years. The reduced density and dry weight of weeds might be attributed to broad spectrum and season long weed control by the application of post-emergence herbicides followed by HW as observed in the plots in which two-hand weeding were given. These results are in line with findings of Singh et al. (2012) who reported that density and dry weight of weeds were greatly reduced under two-hand weeding in transplanted rice. The highest weed density and dry matter was recorded with unweeded check during both years. It means that if weeds were not controlled properly within critical period of crop weed competition, their density continuously remained increasing and crop growth badly affected. The highest weed control efficiency (90 and 93%) was observed under two-hand weeding in both years (Table 1) which was at par with post emergence application of bispyribac sodium 25 g ai/ha supplemented with HW at 45 DAT (87 and 92%) during both years. Yadav et al. (2009) and Upendra Rao et al. (2009) also reported that results of post emergence herbicide bispyribac sodium were found comparable with two-hand weeding. No significant differences were observed on weed control efficiency among pre emergence application of pretilachlor, butachlor and clomazone + 2,4-DEE ready mix followed by one-hand weeding at 45 DAT and pre plant post emergence application of glyphosate 2.5 L/ha at 15 days before

**Table 1.** Influence of weed management practices on weed density, weed dry weight, and weed control efficiency (WCE) (60 DAT) in transplanted rice.

Treatment	2011 to 2012			2012 to 2013		
	Weed density (No./m <sup>2</sup> )	Weed dry weight (g/m <sup>2</sup> )	WCE (%)	Weed density (No./m <sup>2</sup> )	Weed dry weight (g/m <sup>2</sup> )	WCE (%)
Clomazone 500 g ai/ha	7.65(58.00)	3.28(10.29)	51.68	7.56(56.67)	3.24(9.99)	53.03
Clomazone + 2, 4 DEE 500 g ai/ha	6.89(47.00)	3.16(9.50)	55.39	6.62(43.33)	3.04(8.74)	58.91
Clomazone 500 g ai/ha + HW 45 DAT	3.54(12.00)	2.53(5.92)	72.22	3.34(10.67)	2.29(4.73)	77.74
Clomazone + 2, 4 DEE 500 g ai/ha + HW 45 DAT	3.39(11.00)	2.39(5.20)	75.58	2.80(7.33)	2.22(4.47)	78.99
Butachlor 250 g ai/ha + HW 45 DAT	2.92(8.00)	2.27(4.67)	78.09	2.74(7.00)	2.18(4.37)	79.46
Pretilachlor 500 g ai/ha + HW 45 DAT	2.80(7.33)	2.29(4.77)	77.62	2.80(7.33)	2.22(4.50)	78.84
Bispyribac Sodium 25 g ai/ha + HW 45 DAT	2.55(6.00)	1.82(2.83)	86.70	2.27(4.67)	1.45(1.62)	92.40
PPI Glyphosate 2.5 L/ha. 15 DBT + Bensulfuron methyl + Pretilachlor 660 g ai/ha	2.92(8.00)	2.31(4.85)	77.21	2.86(7.67)	2.25(4.56)	78.57
Two HW at 25 and 45 DAT	2.48(5.67)	1.57(2.04)	90.41	2.20(4.33)	1.40(1.47)	93.10
Unweeded control	10.06(100.67)	4.67(21.30)	0.00	10.01(99.67)	4.65(21.26)	0.00
SEd	0.21	0.12	-	0.20	0.20	-
CD (P = 0.05)	0.44	0.26	NA	0.42	0.42	NA

Figures in parentheses are original values, which were subjected to square root transformation ( $\sqrt{x + 0.5}$ ) before statistical analysis, NA - Not Analysed, DBT - Days Before Transplanting, DAT - Days After Transplanting and HW – hand Weeding.

transplanting followed by post plant pre emergence application of bensulfuron methyl + pretilachlor 660 g ai/ha at 3 DAT. These results are in conformity with the findings of Ramachandra et al. (2010).

#### Yield and yield attributes of rice

From the research findings, it was found clearly that yield attributes like panicles per square metre, grains per panicle and grain yield of transplanted rice were significantly influenced by weed control treatments in both years (Table 2). Maximum number of panicles (319 and 362 m<sup>-2</sup>) was recorded in two-hand weeding at 25 and 45 DAT followed by post emergence application of bispyribac sodium 25 g ai ha<sup>-1</sup> supplemented with HW at 45 DAT (315 and 355 m<sup>-2</sup>) and unweeded check recorded minimum

number of panicles (222 and 263 m<sup>-2</sup>) during both years. Increase in panicles per meter square, grains per panicle might be due to better environment with increased uptake of both macro and micro nutrients and ultimate development of large sink created out of reduced crop weed competition.

The highest grain yield (5831 and 8783 kg ha<sup>-1</sup>) were recorded in two-hand weeding over application of herbicides alone, herbicides followed by one-hand weeding and unweeded check during both years of study. Similar results have been reported by Deepthi Kiran and Subramanyam (2010). Superiority of two-hand weeding could be attributed to reduced competition by weeds due to frequent elimination of weeds from the field and hence better crop growth.

Weeds in weedy plot caused 47.02 and 53.79% reduction in grain yield of paddy as compared to two-hand weeding. This reduction in yield was

mainly due to high density of weeds (100.67 and 99.67 m<sup>-2</sup>) in both years. Grain yield (5613 and 8653 kg/ha) recorded under post emergence application of bispyribac sodium 25 g ai ha<sup>-1</sup> followed by HW at 45 DAT was comparable with that of two-hand weeding. Higher grain yield under these treatments might be due to increased panicles/m<sup>2</sup> and grains/panicle. Similar findings were also obtained by Veeraputhiran and Balasubramanian (2010) and Nalini et al. (2012). Grain yield under pre plant application of glyphosate at 15 days before transplanting followed by post plant application of bensulfuron methyl + pretilachlor at 3 DAT were comparable with application of butachlor, pretilachlor, clomazone + 2,4-DEE followed by HW at 45 DAT. These results are in agreement with the findings of AICRIP (2011) and Kishor Jalindar et al. (2012). This might be attributed to better growth of

**Table 2.** Influence of weed management practices on yield and yield attributes of transplanted rice.

Treatment	2011 to 2012			2012 to 2013		
	Panicles/m <sup>2</sup>	Grains/panicle	Grain yield (kg/ha)	Panicles/m <sup>2</sup>	Grains/panicle	Grain yield (kg/ha)
Clomazone 500 g ai/ha	238	115	4141	286	117	6162
Clomazone + 2, 4 DEE 500 g ai/ha	249	118	4330	291	128	6643
Clomazone 500 g ai/ha + HW 45 DAT	272	126	4701	308	131	7826
Clomazone + 2, 4 DEE 500 g ai/ha + HW 45 DAT	293	132	4934	331	142	8013
Butachlor 250 g ai/ha + HW 45 DAT	309	135	5180	347	147	8277
Pretilachlor 500 g ai/ha + HW 45 DAT	300	133	5062	341	148	8067
Bispyribac Sodium 25 g ai/ha + HW 45 DAT	315	141	5613	355	155	8653
PPI Glyphosate 2.5 L/ha. + Bensulfuron methyl + Pretilachlor 660 g ai/ha	284	136	4928	326	150	7973
Two HW at 25 and 45 DAT	319	142	5831	362	163	8783
Unweeded control	222	112	3089	263	114	4059
SEd	14	8	230	13	8	297
CD (P = 0.05)	29	17	483	27	17	624

plants on account of reduced crop - weed competition resulting in increased availability of nutrients, water and light.

## Conclusion

Application of post emergence herbicide bispyribac sodium 25 g ai/ha at 15 DAT followed by hand weeding at 45 DAT produced higher grain yield and this was at par with two-hand weeding due to lower crop-weed competition. Sequential application of herbicides viz., glyphosate 2.5 L/ha at 15 DBT followed by bensulfuron methyl + pretilachlor 660 g ai/ha at 3 DAT was also found promising and it can also be recommended for weed control in transplanted rice during the peak period of labour scarcity. No doubt, the results of two-hand weeding are significantly better in terms of weed control and rice grain yield, but as it is time consuming,

laborious and expensive, it can not be recommended for large scale rice production. From the research findings, it can be concluded that application of post emergence herbicide bispyribac sodium 25 g ai/ha followed by HW at 45 DAT can be recommended for effective weed control and higher productivity in transplanted rice.

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## Full Length Research Paper

## Parasitism of plum tree (*Dacryodes edulis*, Burseraceae) by *Loranthaceae* in the locality of Fotetsa- Dschang (West-Cameroon)

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Received 16 February, 2014; Accepted 16 June, 2014

Plum tree (*Dacryodes edulis*) in Cameroon is a fruiting and shading tree in agro ecosystems. It is of nutritional, medicinal and economical values to local population. Its productivity is reduced partially in some localities by the *Loranthaceae* parasites. These parasites were censused on plum trees in all the nine villages of the locality Fotetsa (West Region of Cameroon) where they are mainly shading plants in coffee farms. In each village, all fruits bearing plum trees located in a perimeter of 1850 × 60 m were observed and characterized using the stem diameter, number of *Loranthaceae* present and the taxon of the parasite. At Fotetsa, four *Loranthaceae* species attack plum trees: these are *Tapinanthus apodanthus*, *Phragmanthera capitata*, *Tapinanthus oleifolius* and *Viscum congolense*. The rate of parasitism varied significantly (from 41.46 to 62.74%) between the nine villages of the locality of Fotetsa in function of the different classes of plum tree stem circumference. Also, the number of *Loranthaceae* per plum tree varied in function of localities with maxima within 17 and 29 *Loranthaceae* per tree. In each village, average mean number of *Loranthaceae* per tree and average mean stem circumference were correlated. Considering the negative impact of *Loranthaceae* parasite on fruiting plant production, it is imperative to envisage preventive methods (limitation of species propagation) and curative methods (mechanical destruction of species fixed on the host plant) for fight against these hemiparasite phanerogames.

**Key words:** Cameroon, plum tree, *Loranthaceae*, inventory, identification.

### INTRODUCTION

Plum tree (*Dacryodes edulis*) is an oleiferous fruiting plant that originated from humid inter-tropical regions of Africa (Aubreville, 1962; Okafor, 1983). The species is

much diversified, but only two varieties are well identified up to now: var. *edsulis* and var. *parvicarpa* (Verheij, 2002). As many other non-timber products, plum trees

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are not yet domesticated. However, it is frequently used as a shading plant in coffee and cocoa farms. The fruits called plums are drupes with diameter varying between 3 and 18 cm (Kengue, 2002; Giacomo, 1982). These fruits are used by the local consumers as food and as exporting. In fact, the pulp of these fruits contains considerable amounts of carbohydrates, lipids, proteins, fibres and mineral salts: especially calcium, magnesium, potassium and sodium (Aiyelaegbe et al., 1998; Kiakouma and Silou, 1990). In addition, they contain non-saturated fats like linoleic acid (18.3%) and oleic acid (29.5%) and many amino-acids which give the plum the protective and energetic values (Umoti and Oky, 1987). The oil yield of plum is estimated at 7 tons per hectare (Kiakouma and Silou, 1990). Leaves, stems and stem backs are used in ethno-botany in the treatment of otitis, amoebic dysentery, leprosy, anaemia and yellow fever (Anon, 1991). With its non-neglected yearly production, plum tree occupied the 8<sup>th</sup> position amongst tropical fruit trees behind banana tree, coco tree, mango tree, pineapple, pawpaw tree, pear tree and orange tree (Anon, 1991).

In Cameroon, plum tree is mainly cultivated in the West, Centre and Littoral Regions. The yearly production is about 500 kg of fruit per tree (Kengue, 2001). However, its production is reduced by the attack of several pests. These pests are generally bacteria, viruses, fungi, insects and small animals which attack all plant organs and in particular flowers and fruits, provoking their falling before maturity (Silou, 1996; Onana, 1998). In the West Region of Cameroon, plum trees are mostly found in the area of Fouban and Fotetsa where coffee (*Coffea arabica*) is intensively grown and where it serves as shading tree in the coffee farms. In the locality of Fotetsa (05°20' latitude North and 10°03' longitude East), where the population (about 25310 inhabitants) is essentially made up of farmers who make a good part of their incomes through plum harvesting and marketing (Nkuekeng, 2006). But in addition to devastating effect of microorganisms, insects and small animals, plum production is limited due to parasitism by *Loranthaceae* on plum trees in farms (Dibong et al., 2009a). The *Loranthaceae* are hemiparasite phanerogames who carry out photosynthesis using water and mineral salts from the host plant. No work has yet been done on the identification of this parasite and the control of their devastating action in the locality of Fotetsa. Hence, an inventory and identification of *Loranthaceae* found on plum trees was done in the nine villages that constitute the locality of Fotetsa. The rate of parasitism on plum trees and the mean number of *Loranthaceae* per tree were determined in each village.

## MATERIALS AND METHODS

### Collection and identification of *Loranthaceae*

Prospections were carried out in the agro ecosystems of the nine villages of the locality of Fotetsa: Melah, Megang, Makong, Zimlah,

Tsekoug, Ngonlah, Toulah, Zimtetsa and Tdissang. These prospections were done between the months of April and July 2011 and 2012 which corresponded to the flowering and fructification periods of the *Loranthaceae*. In each locality, all the plum trees above 19 cm of stem circumference (Dibong et al., 2009a) located within a perimeter of 1850 x 60 m were observed. The area of study was divided into 111 quadrats of 1000 m<sup>2</sup> and the total numbers of 504 plants were prospected with a single replication. The pictures of *Loranthaceae* present within the prospected area and some of their organs (flowers, fruits, hostorium) that could enable their identification were made using a numerical camera (model Nikon). The identification of the collected samples of *Loranthaceae* was done by comparing them to the specimen at the national herbarium of Cameroon and by using the identification key for *Loranthaceae* of Cameroon (Balle, 1982). The species of *Loranthaceae* found without flowers and fruits to enable their identification were not taking into consideration.

### Parameters measured and analysis

For each plum tree considered:

- i. The stem circumference was assessed at 1.20 m above ground level to establish a relation between the parasitism by *Loranthaceae* and the host;
- ii. The number of *Loranthaceae* carried by each plum tree was counted;
- iii. The percentages of infected plum trees in the villages prospected were calculated. The variation of this parameter from one village to another was compared by analysis of variance (ANOVA) using Duncan test at  $P \leq 0.05$ .

A clustering of data into classes of circumferences was done according to Sturges (1926) which aided to determine the minimal number K of classes in function of the number N of a series studied trees:

$$K = 1 + [10 \log_{10} N / 3]$$

The size of the class was obtained by dividing the size of the series by K-1.

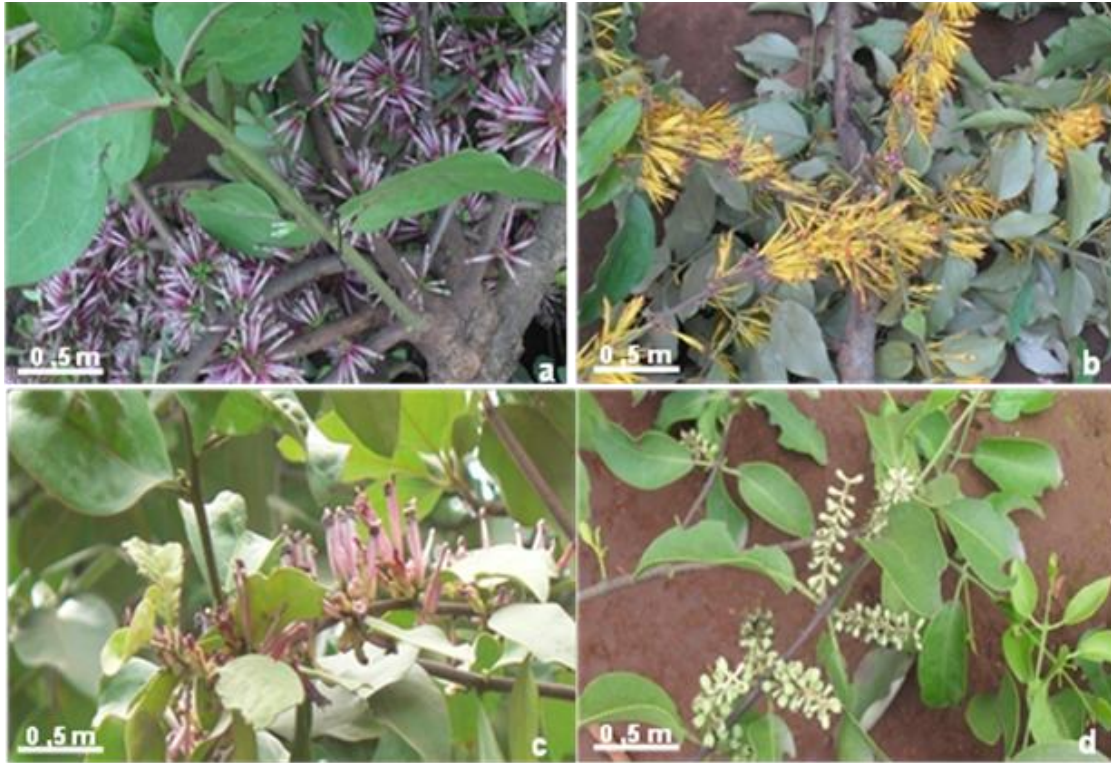
The analysis of the regression was done to verify the existence of a relation between the number of *Loranthaceae* and the circumferences of the plum tree observed.

## RESULTS

### Species of *Loranthaceae* found on plum tree in the different villages

Four species of *Loranthaceae* attack plum trees in the study area: *Tapinanthus apodanthus* (Sprague) characterized by violet inflorescences (Figure 1a), *Phragmanthera capitata* (Sprengel) S. Balle characterized by dark-yellow inflorescences (Figure 1b), *Tapinanthus oleifolius* (S. Moore and Sprague) characterized by pinkish inflorescences (Figure 1c) and *Viscum congolense* (De Wild) characterized by green-whitish inflorescences (Figure 1d). Some of these species can be found alone or in association with others on a plum tree individual. These species can be found on other host trees like pear, mango, cacao, guava, and some citrus.





**Figure 1.** Different species of Loranthaceae parasite of plum trees in the nine villages of the locality Fotetsa-Dschang (West-Cameroon): a-*Tapinanthus apodanthus*; b-*Phragmenthera capitata*; c-*Tapinanthus oleifolius*; d-*Viscum congolense*.

### Rate of parasitism on plum tree

The percentages of plum trees attacked are relatively high and varied from 41.46% in the village of Meganh to 66.67% in the village of Ngonlah (Table 1). The percentage of infected tree was high in the villages of Ngonlah, Toulah and Makong; and lower in the villages of Meganh, Zimtetsa and Tsekoug. The circumference of prospected plum trees varied from 19 to 272 cm. Plum trees in the class 1 (19 to 47 cm) which were the youngest and individuals of classes 6 (157 to 187 cm), 7 (187-215 cm), 8 (215 to 243 cm) and 9 (243 to 271 cm) which were the oldest were slightly attacked compared to young individuals of classes 2 (47 to 75 cm), 3 (75 to 103 cm), 4 (103 to 131 cm) and 5 (131 to 159 cm) which were strongly attacked in almost all the villages of the locality Fotetsa (Figure 2).

### Average number of *Loranthaceae* per attacked plum tree

The analysis of the average number of *Loranthaceae* per tree and the circumference of the stem of the host plant in the nine villages showed significant differences. The highest average number of *Loranthaceae* per attacked

tree was significantly higher ( $S = 0.013$ ) in the localities Meganh and Tsegoug ( $5.24 \pm 6.73$  and  $5.18 \pm 6.00$ ) (Table 2) compared to the one in the localities of Toulah ( $4.94 \pm 7.72$ ) and Zimtetsa ( $4.79 \pm 7.48$ ). Lowest number of *Loranthaceae* was obtained in plants from the localities of Zimlah ( $4.23 \pm 4.23$ ); Melah ( $4.19 \pm 7.31$ ); Ngonlah ( $3.61 \pm 7.48$ ); Makon ( $2.62 \pm 5.30$ ) and Tdissang ( $2.04 \pm 2.83$ ); and these means were not significantly different (Table 2). The mean plant stem circumference was significantly the same in all localities except that of Tsekoug which was higher ( $83.11 \pm 37.33$ ). These numbers compared between the different villages aided in the distribution of attacked trees into three different groups: Highly attacked (Ngonlah, Toulah and Makong), averagely attacked (Tdissang, Zimlah and Melah), lowly attacked (Tsekoug, Zimtetsa and Meganh) (Table 1). The linear regression analysis between the stem circumference and the mean number of *Loranthaceae* per plum tree in all the villages showed a relation between these two parameters (Figures 3a, b, c, d, e, f, g, h and i).

### DISCUSSION

The results showed that in the locality of Fotetsa-



**Table 1.** Distribution of investigated plum tree in the village Fotetsa per locality and per class of stem circumference in function of their attack by Loranthaceae parasite species.

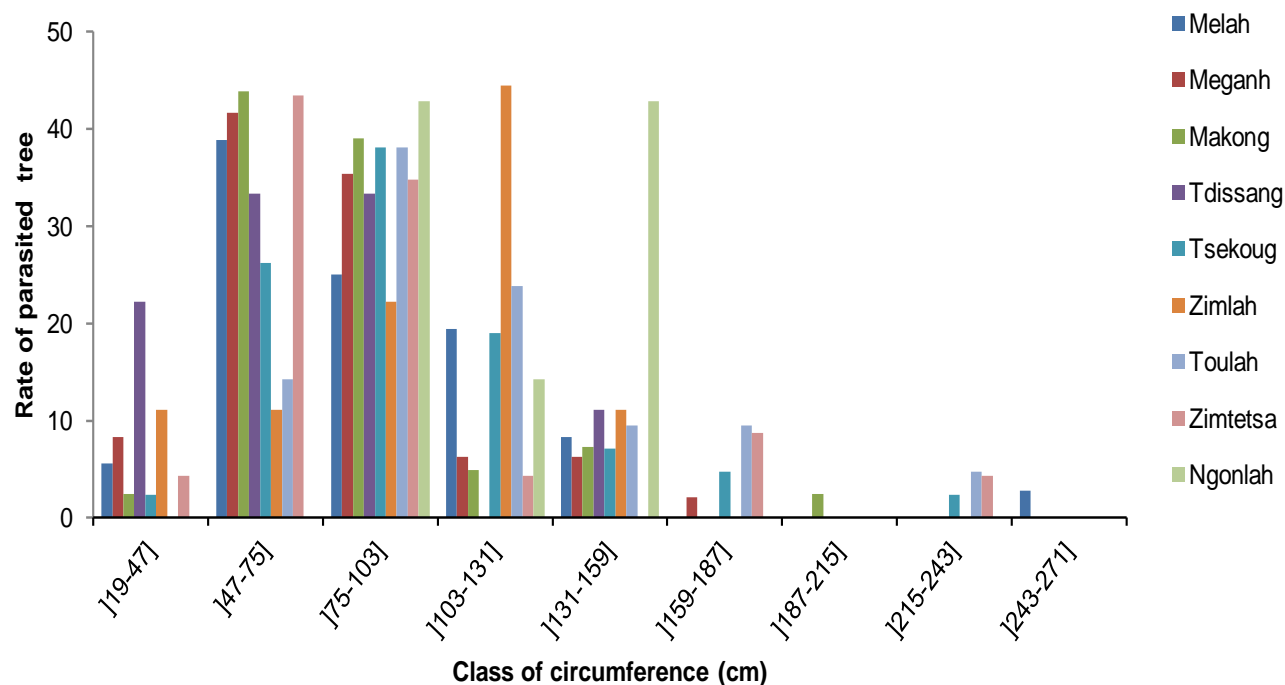
Localities	Attacked plum tree	Classe de circumference (cm)							Total	Infected tree (%)
		19-47	48-75	76-103	104-131	132-159	160-187	>188		
Melah	No	2	14	9	7	3	3	1	39	-
	Yes	25	11	9	0	1	0	0	46	43.90
Meganh	No	4	20	17	3	3	1	0	48	-
	Yes	13	13	7	1	0	0	0	34	58.53
Makong	No	1	18	16	2	3	9	1	41	-
	Yes	28	22	13	2	0	0	0	65	36.60
Tdissang	No	2	3	3	0	1	0	0	9	-
	Yes	6	3	2	1	0	0	0	12	42.85
Tsekoug	No	1	11	16	8	3	2	1	42	-
	Yes	10	13	4	1	0	0	0	28	60
Zimlah	No	1	1	2	4	1	0	0	11	-
	Yes	3	3	3	3	0	0	0	14	42.85
Toulah	No	0	3	8	5	3	2	1	19	-
	Yes	15	13	4	0	0	0	0	32	38.88
Zimtetsa	No	1	10	8	1	0	2	1	23	-
	Yes	9	7	4	0	0	0	0	20	53.50
Ngonlah	No	0	0	3	1	3	0	0	7	-
	Yes	9	4	1	0	0	0	0	14	33.33

Dschang (West –Cameroon), plum trees were attacked by four species of *Loranthaceae* family. This number of species is comparable to the one observed by Sonke et al. (2000) on pear tree in the locality of Yaoundé (Centre Region Cameroon). Among these four species of

*Loranthaceae* observed on plum trees in the study area, one of them (*P. Capitata*) was also observed on pear trees in the Centre Region of Cameroon by Sonke et al. (2000). Others of these species were found on other plant trees like *Mangifera indica* and *Psidium guajava* in the locality of

Tubah (North West-Region, Cameroon) by Feguem (2011). Therefore, it can be seen that *Loranthaceae* parasites are not specific to a host plant, or to a locality.

In fact, Balle (1982) has shown that the area of distribution of *Loranthaceae* parasite in Africa



**Figure 2.** Variation of the rate (%) of plum trees attacked by Loranthaceae in the nine localities of the village Fotetsa- Dschang (West-Cameroon) in function of the plant stem circumference classes.

**Table 2.** Average number of tufts of Loranthaceae and prospected mean plum stem circumferences in the nine prospected localities of the village Fotetsa-Dschang (West-Cameroon).

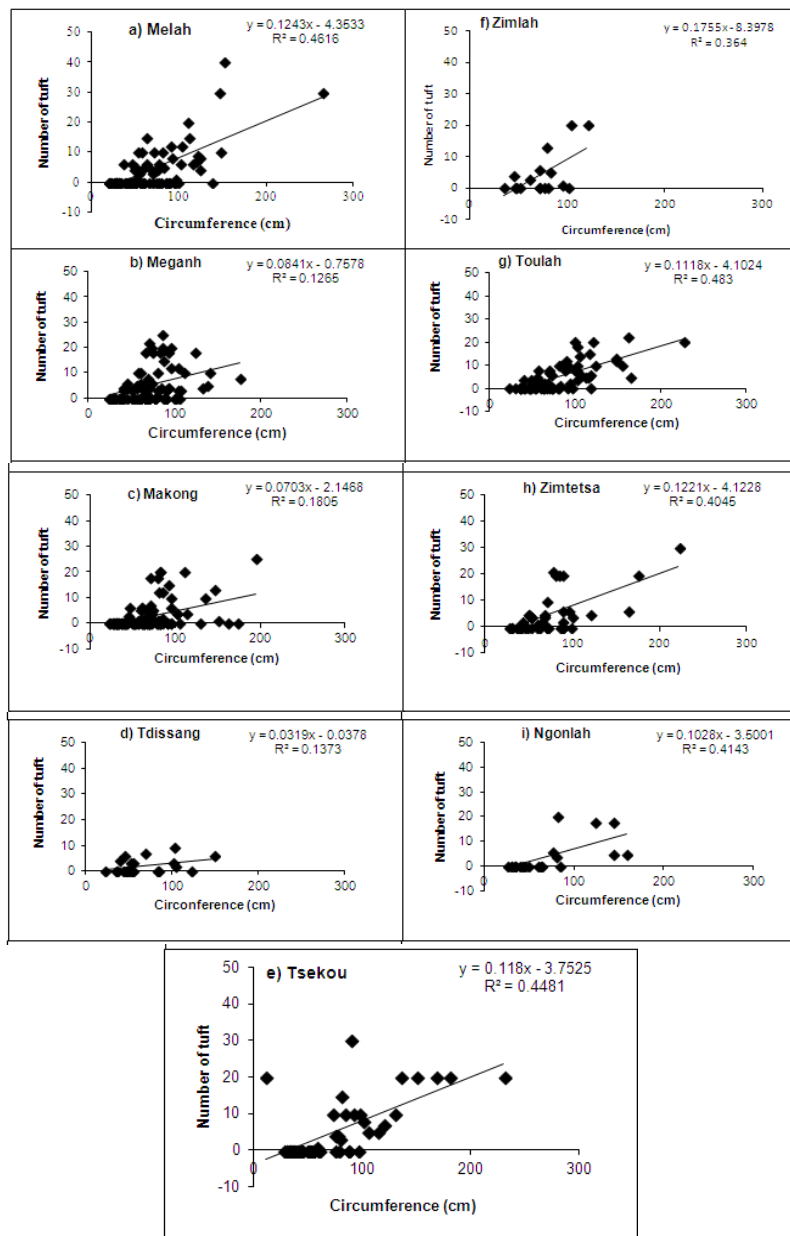
Localities	Mean tuft number $\pm$ Sd	Mean plant stem circumference $\pm$ Sd	Number of prospected plum trees
Melah	4.19 $\pm$ 7.31 <sup>a</sup>	68.36 $\pm$ 39.82 <sup>a</sup>	85
Meganh	5.24 $\pm$ 6.73 <sup>c</sup>	71.36 $\pm$ 28.47 <sup>a</sup>	82
Makong	2.62 $\pm$ 5.30 <sup>a</sup>	68.29 $\pm$ 32.17 <sup>a</sup>	106
Tdissang	2.04 $\pm$ 2.83 <sup>a</sup>	65.29 $\pm$ 32.98 <sup>a</sup>	21
Tsekoug	5.18 $\pm$ 6.00 <sup>bc</sup>	83.11 $\pm$ 37.33 <sup>c</sup>	70
Zimlah	4.23 $\pm$ 4.23 <sup>ab</sup>	71.97 $\pm$ 23.55 <sup>ab</sup>	25
Toulah	4.94 $\pm$ 7.72 <sup>bc</sup>	73.66 $\pm$ 43.82 <sup>ab</sup>	51
Zimtetsa	4.79 $\pm$ 7.48 <sup>bc</sup>	73.02 $\pm$ 39.018 <sup>ab</sup>	43
Ngonlah	3.61 $\pm$ 7.48 <sup>a</sup>	69.23 $\pm$ 41.40 <sup>a</sup>	21
F- value	2.549	12.806	
Significance	0.013	0.049	

Means with the same letter in the same column are not significantly different at  $P < 0.05$  (Duncan test).

in general and in Cameroon in particular is very wide. It extends from the south of the 7<sup>th</sup> North parallel, passing through Atlantic costal forest and continues to the transition area between the Biafrean forest and the Congolese forest, prolonging into the mountain forest and then the Guinean savannah. The association between *Loranthaceae* species and host plant species as well as the density of parasite that were observed from one host plant to the other might be conditioned by many factors such as: the climate type (Raynal-Roques and Paré, 1998), the variety and sensitivity of the host (Balle, 1982;

Sallé and Aber, 1986), the geographical positioning and floristic parameters (Dibong et al., 2009a; b).

In the locality of Fotetsa, the percentage of attacked plum trees varied significantly from one village to another with a maximum of 66.67% in Ngonlah and a minimum of 41.46% in Meganh. Similar results were obtained by Dabou (2009) for the same species in the locality of Manjo (Littoral Region, Cameroon) but, only two of the four species recorded in Fotetsa were found in this locality (*T. Apodanthus* and *P. Capitata*). The variation of the parasitism rate from one village to another could be



**Figure 3.** Regression lines between mean tufts number of Loranthaceae on prospected plum tree and mean plant stem circumference in the nine prospected localities of the village Fotetsa-Dschang (West-Cameroon).

explained at least partly by a change in the microclimate when passing from one village to another (Balle, 1982).

Therefore, the fact that Meganh and Zimlah presented the same parasitism rate (41%) could imply that these two localities may have the same microclimate and the same flora diversity of the host plants (Dabou, 2009; Boussin et al., 1993a). The results of this study showed differences in the prevalence of parasitism in the different villages. These differences could be due to some factors such as the variety of the plum tree, the history and age

of the tree, the sensitivity and degree of infestation of the host plant (Dabou, 2009; Boussin et al., 1993b) as well as the availability of avian species responsible for the dissemination of the *Loranthaceae* seeds (Jiofack, 2005). In fact, plum trees observed in the study area could belong to different varieties, therefore presenting different degree of sensitivity towards the parasites as reported by Sonké et al. (2000). In addition, the pruning of attacked branches as a curative practice in many of these villages, the flora composition of the agro ecosystems and the

vicinity between the hosts plant could also explained the difference in the prevalence between the different villages (Raynal-Roques and Paré, 1998; Dabou, 2009; Dibong et al., 2009c). The maxima number of *Loranthaceae* (17; Melah, Zimla, Toulah, Zimtetsa and 29; Meganh, Tsekoug) observed in some trees in the study area are in the same range with the results of Feguem (2011) on *Psidium guajava* in the locality of Tubah (North-West Region, Cameroon) and Dibong et al. (2009a; c) on fruiting trees in the Littoral Region of Cameroon. Such a high parasite density ( $\geq 20$  *Loranthaceae* per tree) can lead to the total death of the host plant (Boussin et al., 2009a). A variation and significant correlation between the mean stem circumference and the number of *Loranthaceae* on attacked plum trees was also noticed.

Similar observations were made by Sonké et al. (2000) and Tatchou (2009) on pear tree in the locality of Yaoundé (Centre Region, Cameroon) and Banganté (West-Region, Cameroon) respectively. Dongmo (1998) also reported similar facts on *Hevea brasiliensis* in the Fako Division (South-West Region, Cameroon). Such variation and correlation could at least partially be explained by the genetic and microclimatic factors associated to the growth and development of the host and parasite (Okafor, 1983; Sallé and Aber, 1986; Sallé, 1994), the degree of sensitivity and resistance of the host plant tissues to the fixation by the parasite (Dibong et al., 2009a) and the efficacy of protection method carried out in the study area.

The fight against *Loranthaceae* parasite on fruiting plants in general and plum tree in particular can be envisaged preventively or curatively. The preventive measure will consist into limiting considerably the propagation of the parasite seeds in the agro ecosystems (Boussin et al., 1993a). Genetic and biotechnological methods of fight can be used to produce improved host plant individuals which can resist the parasites. Curative measure will consist to fight against these parasites by pruning or cutting down the attacked branches. This cultural method which seems for now very effective however presents certain limits when considering the high density of parasites on certain trees in the investigated area. In fact, cutting down the attacked branches may lead to the destruction of all the branches of the tree therefore leading to the death of the plant and subsequently decrease in the productivity of plum fruits in the study area. The physical elimination of only parasite at the level of the contact point (haustorium) seems to be the best fighting method against parasites while maintaining the host plant alive and avoiding a drastic reduction in plum productivity.

## Conclusion

This work shows that plum trees in the locality of Fotetsa (West-Cameroon) are attacked by four species of the *Loranthaceae* family. These *Loranthaceae* which can be

found alone or in association on the same plum tree were not specific to this host plant. In fact, they are found on other fruiting plant trees in the study area. The rate of attacked plants and the parasite density varied with villages and host plant stem circumference.

## Conflict of Interests

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

Authors acknowledge the team of the General Biology Laboratory of the Higher Teacher Training College (HTTC) of the University of Bamenda for the perfect collaboration and all the referees for their comments and suggestions.

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## Full Length Research Paper

# Role of trap colors and exposure time of pheromone on trapping efficacy of males of the tomato leafminer, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera:Gelechiidae)

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Received 22 May, 2014; Accepted 16 June, 2014

Field studies were conducted in two sites (Saheline and Chott-Mariem) in the Centre-East of Tunisia in 2012 and 2013 to evaluate the influence of different colors of pan water traps and age of pheromone dispenser on capture of males of the tomato leafminer, *Tuta absoluta*. Three experiments were setup using colored traps (white, yellow, orange, red and green) in a randomized block design with four replicates. The first trial was conducted during the period from November, 2012 to February, 2013, the second between December, 2012 and April, 2013 and the third during April to May, 2013. Results indicate, in all experiments, that there is no significant difference in male capture according to trap color. However, in the third trial, green colored traps captured, respectively, more than 5-fold, 4-fold, 4-fold and 2-fold for red, orange, yellow and white colored traps. The experiments aimed to determine the effect of pheromone aging on male trapping, results revealed a significant difference between aged lures and control. Fresh lures captured from 2 to 13-more moths than weathered pheromone capsules.

**Key words:** Aging lure, color, fresh lure, pan water trap, pheromone, Tunisia, *Tuta absoluta*.

## INTRODUCTION

The tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae) is the most important constraint to tomato production in Tunisia (Photo 1) where this crop plays an important role in farming production both in greenhouses and open fields. In spite of health hazards, occurrence of resistant biotypes of the pest, high cost and destruction of population of natural enemies, insecticides are routinely applied as sole control strategy against this pest (Braham et al., 2012).

Lepidoptera pheromones have been successfully used

for insect monitoring and mating disruption of insects (Wyatt, 1998). Virgin female of tomato leafminer releases a sex pheromone that strongly attracts males (Quiroz, 1978) which is identified by Attygalle et al. (1996) as (3E, 8Z, 11Z)-3,8,11-tetradecatrien-1-yl acetate. Pheromones are used to monitor population of insects to determine the correct timing to apply control strategies such as introduction of natural enemies, intensification of mass trapping and applying insecticides. Complementary strategies of pest management through an efficient

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monitoring of pest population need to be determined, including the use of monitoring pheromones to correct timing of pesticide application and other means of control. To our knowledge, in Tunisia, the only study relating to the use of *T. absoluta* pheromone lure is made by Abbes and Chermiti (2011) in which two marks of pheromone capsules were compared for their efficiency in trapping males under tomato greenhouse.

The objectives of this study are: (1) to test the attractiveness of pheromone traps differently colored to *T. absoluta* males since farmers use these kind of traps usually self made recyclable buckets, (2) to test the effectiveness of capsule pheromone traps following field aging. Farmers are advised to replace pheromone capsules every 5 to 6 weeks, but little information is available on factors influencing the longevity of field-aged capsules. These parameters are important to define in order to improve trapping technique either for monitoring or for mass trapping.

## MATERIALS AND METHODS

### Effect of Trap colour on attractiveness of males of *Tuta absoluta*

For all experiments, tomato plants, *Lycopersicon esculentum* Mill (cv Rio Grande) were sown in climatic chamber. Seeds were deposited in cells in a rectangular polyester tray of 60 × 40 × 5 cm filled with peat (Potgrond, Germany).

In Chott-Mariem locality, tomato seedlings were planted in a sample row (1 m between rows and 0.4 m on the row) on November 26, 2012, while in Saheline locality, two crops were established one in autumn, the other in spring. The autumn crop, seeds were sown on 16 August 2012 and seedlings were transplanted on 12 September 2012 in a plot of about 2400 m<sup>2</sup> (60 × 40 m). Plants were spaced as mentioned above to get a density of about 25000 plants/ha. In the spring crop, sowing was done on 17 February 2013 and plantation on 22 March 2013. The plot of about 4000 m<sup>2</sup> (65 × 62 m) was planted with tomato in 1600 m<sup>2</sup> (28 rows, 14 in each side) and 36 rows of potato in the middle to obtain large area and increase the inter-traps distance. Traps were setup in a randomized block (Figure 1). In all experiments, to avoid trap interference, the distances between traps and between blocks were more than 15 m (Bacca et al., 2006). Pan traps made of plastic were purchased from a local store, originally colored in light red (height from bottom to top = 14 cm, diameter = 35 cm).

Each trap was perforated with two holes parallel on the edge of the trap. Each plug of pheromone was placed inside a plastic cylindrical container (7 cm length and 3 cm diameter) perforated by many holes. The pheromone container was fixed on the top middle of the trap using metal wire tied to the body of the trap. The container is used to provide shelter and avoiding direct exposure of pheromone to sunlight. The inside and outside of the bucket trap were painted with the commercially available paints using a brush. The colours were selected based on reported evaluations of trap colours influencing the attraction of various Lepidoptera species (Hendrix and Showers, 1990; Bloem et al., 2005) and on our observations on what is used by farmers. Each trap was filled with approximately 4 L of tap water then traps were setup in a randomized block (Figure 1) and the water renewed at every inspection date.

In all experiments, to avoid trap interference, the distances between traps and between blocks were more than 15 m (Bacca et

al., 2006). Caught insects were removed with a piece of wood and moths of *T. absoluta* were counted. Captured insects were removed with a piece of wood and *T. absoluta* moths counted. In Chott-Mariem, traps were setup in the field on 27 November 2012, while in Saheline on 9 September 2012 and on 25 April 2013, respectively for autumn and spring tomato.

### Effect of age of pheromone on attraction males of *Tuta absoluta*

The objective of the study is to determine the attractive period of pheromone lure in the field. Two sets of 24 pheromone capsules were setup in pan traps without water in the field in 2012 and one set in 2013. The first set was put on 7 March 2012 in the edge of uncultivated plot in Saheline region and 4 pheromones capsules were removed at weekly interval (on 14 March, 22 March, 29 March, 13 April and 20 April 2012) wrapped in aluminum foil and put in the freezer at 0°C until use. Aged capsules (from 1 to 6 weeks) and fresh ones were deployed in traps in tomato crop on 27 April 2012.

The second set was put on in the same place on 20 April 2012 and 4 pheromone capsules were collected at weekly interval (on 27 April, 4 May, 11 May, 17 May, 24 May and on 31 May) and stocked in the freezer. Aged capsule and 4 fresh were setup in a tomato field on 4 June 2012. The second trial lasted from 4 June 2012 to 11 July 2012. Tomato field planted with Rio Grande cultivar having approximately 2000 m<sup>2</sup> (50 × 40 m in length). Traps were set up in a randomized block design at the edge of the plots and spaced at about 10 to 12 m.

The third set was put in the field on 17 October 2012; four pheromone capsules were collected at weekly interval then put in a freezer (on 24 October, 01 November, 9 November, 15 November and 23 November 2012). Traps with aged capsules (from 1 to 5 weeks) were deployed in the tomato field as described above on 1 March 2013. Data on the minimum, maximum and average monthly temperatures for the Saheline were reported from meteorological station at about 12 km from the site. Meteorological data of Chott-Mariem were not very different from Saheline locality, both located at the border of Mediterranean Sea (about 25 km from each other).

### Statistical analysis

The mean number of captured *T. absoluta* males was analyzed statistically using one way analysis of variance (ANOVA). When ANOVA indicates that significant differences were found, ( $P < 0.05$ ) means were separated by least significant difference test (LSD). Tests were run with Minitab software (13.0).

## RESULTS

### Effect of Trap colour on attractiveness of males of *Tuta absoluta*

#### Chott-Mariem trial

The result revealed that, the mean number of male captured varied from 3 adults in late February 2013 to 17 in December 2012 (Figure 2). In the region, January and February are the coldest months of the year (Figure 3). The ANOVA of the average number of captured males according to inspection dates, shows no significant difference between different tested colored traps on





Photo 1. Symptoms of infestation by *Tuta absoluta* on tomato leaves.

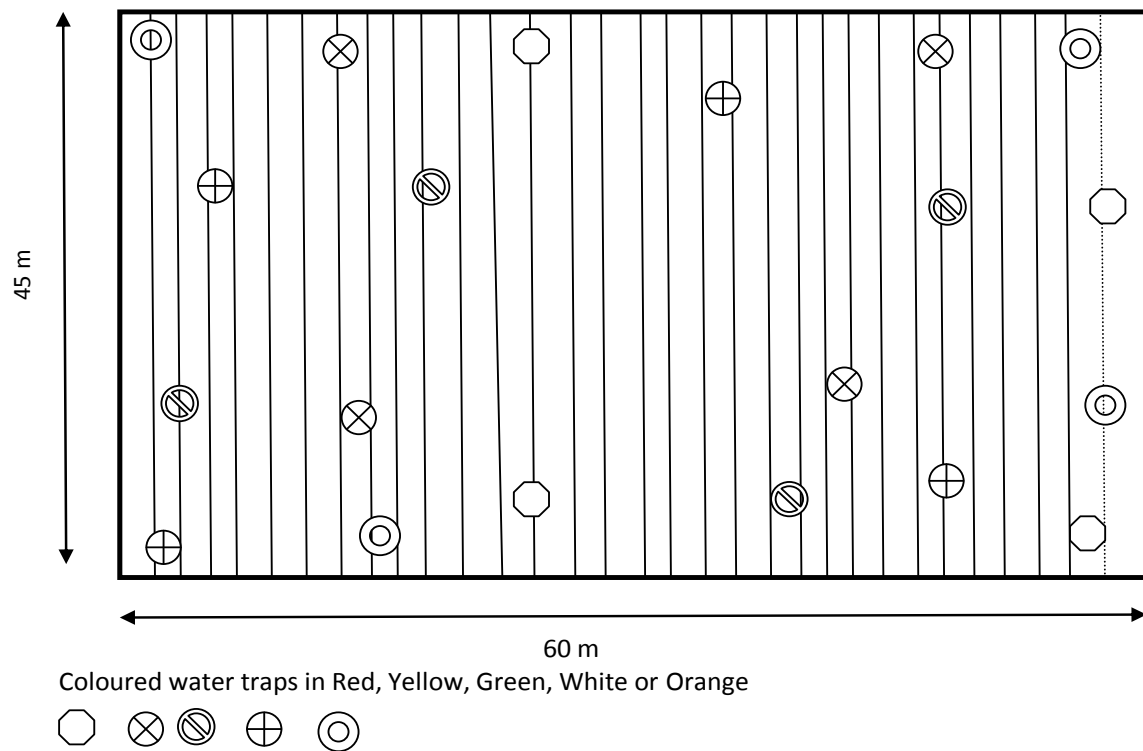
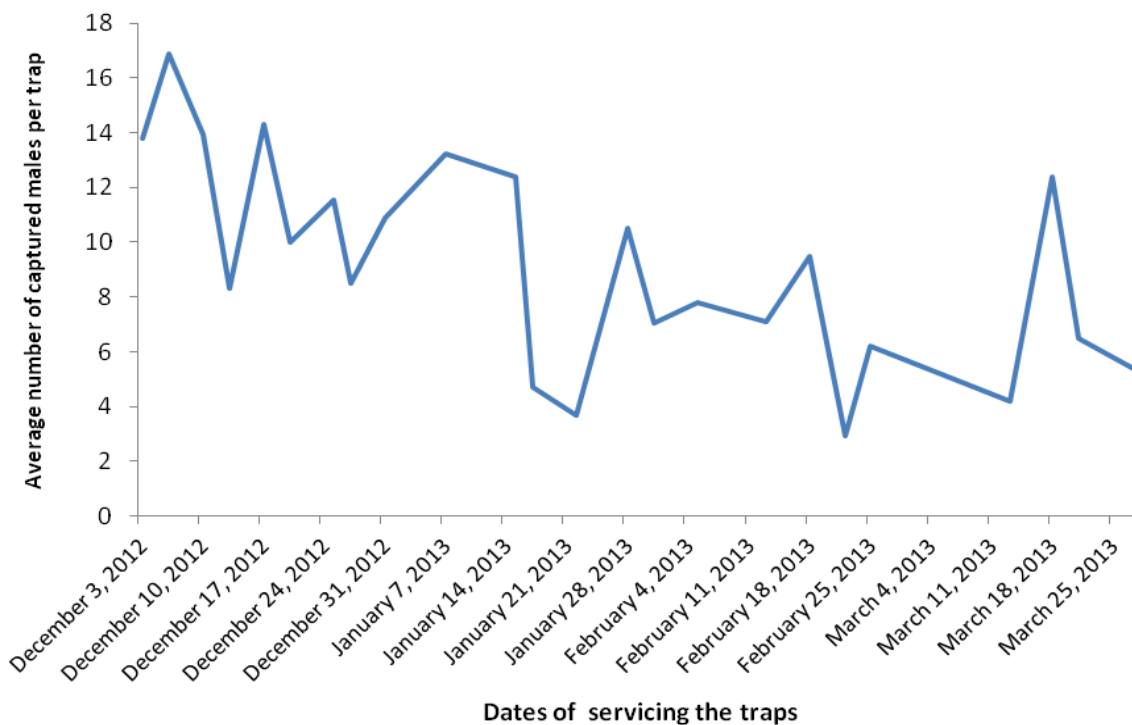


Figure 1. Layout of the experimental plot used for trap comparison colour (Saheline, 2012).





**Figure 2.** Average number of captured males per trap (mean of 20 colored traps) during the study period in Chott-Mariem.

**Table 1.** Mean numbers ( $\pm$ SD) of captured *Tuta absoluta* males in Chott-Mariem region.

Trap colors	Mean $\pm$ SD*	N
White	9.90 $\pm$ 3.73 <sup>a</sup>	24
Yellow	8.57 $\pm$ 3.74 <sup>a</sup>	24
Orange	10.77 $\pm$ 5.67 <sup>a</sup>	24
Red	9.36 $\pm$ 6.71 <sup>a</sup>	24
Green	7.57 $\pm$ 4.41 <sup>a</sup>	24

\*Means with the same letter are not statistically different at  $P = 0.05$  ( $N =$  number of dates of trap servicing).

attraction males of *T. absoluta* ( $F_{4.115} = 1.45$ ;  $P = 0.22$ ) (Table 1).

### Saheline trial

**Autumn crop:** As presented in Figure 4, for autumn tomato cultivation, statistical analysis shows no significant difference between different colored traps tested on attracting males of *T. absoluta* ( $F_{4.54} = 0.53$ ;  $P = 0.71$ ) (Table 2).

**Spring crop:** For spring cultivation, the ANOVA of the average number of captured males according to

inspection dates, shows no significant difference between colored traps ( $F_{4.29} = 2.69$ ;  $P = 0.054$ ; Table 3). The mean number of captured males varies between 3 and 17 (Figure 5).

### Effect of age of pheromone on attraction males of *Tuta absoluta*

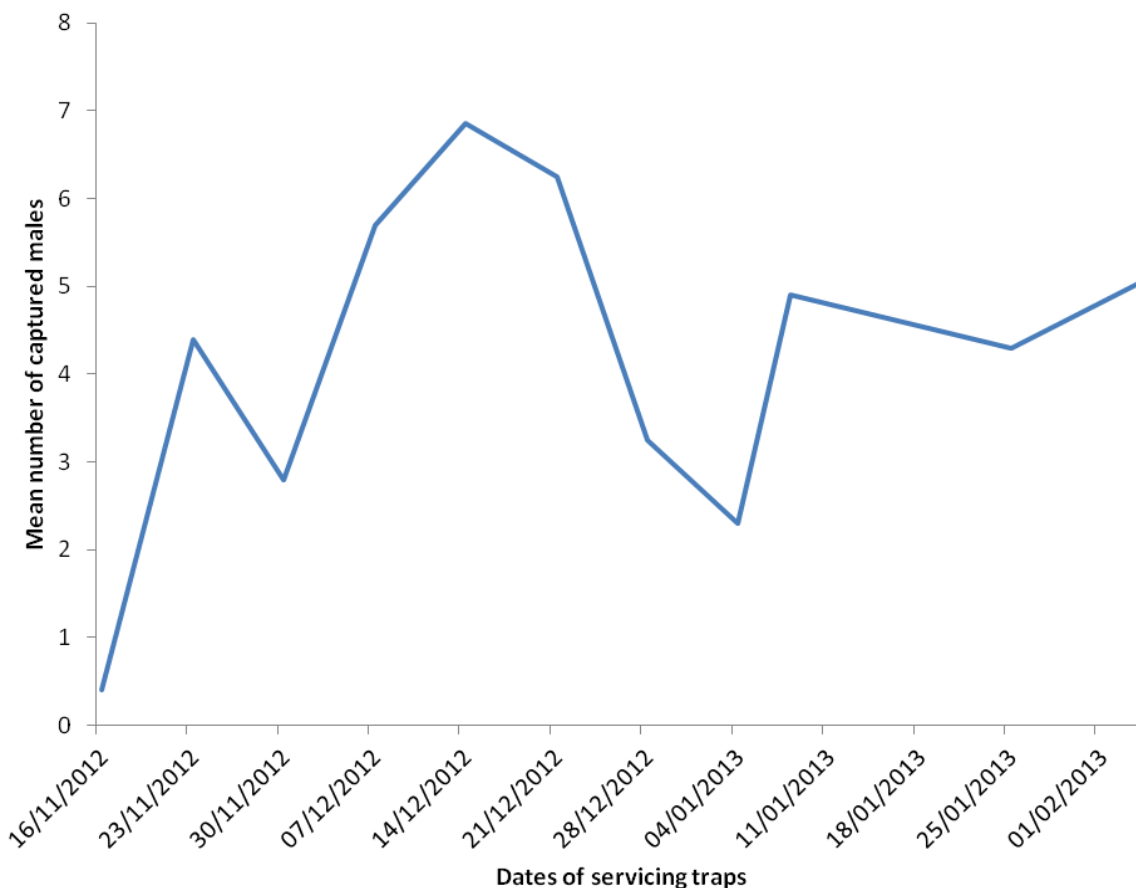
For the first trial (from April 2012 to June 2012), significant difference was observed between aged and fresh lures (ANOVA, one factor  $F_{6.63} = 6.06$ ;  $P = 0.001$ ). Fresh lures captured from 2-fold to 4-fold *T. absoluta* males (Table 4). Surprisingly, lures aged 5 weeks captured numerically more moths than capsules aged 1 week.

*T. absoluta* (ANOVA, one factor  $F_{6.63} = 8.30$ ;  $P = 0.002$ , Table 5). Aged lures did not show significant differences between them. Fresh lure captured 5 to 13-fold males of *T. absoluta* (Table 5).

Concerning the third trial, there is no significant difference regarding d capsules and fresh ones probably due to low adult population (ANOVA, 1 factor  $F_{5.30} = 1.12$ ;  $P = 0.73$ ) (Table 6 and Figure 8).

## DISCUSSION

The tomato Tomato leafminer, *T. absoluta* can be



**Figure 4.** Average number of captured males per trap (mean of 20 colored traps) during the study period in tomato autumn crop.

**Table 2.** Mean numbers ( $\pm$ SD) of captured *Tuta absoluta* males according to color trap (autumn tomato cultivation, 16 November 2012 to 4 February 2013).

Trap colors	Mean $\pm$ SD*	N
White	4.29 $\pm$ 2.19 <sup>a</sup>	11
Yellow	4.29 $\pm$ 2.49 <sup>a</sup>	11
Orange	3.40 $\pm$ 1.46 <sup>a</sup>	11
Red	4.93 $\pm$ 3.16 <sup>a</sup>	11
Green	4.06 $\pm$ 2.80 <sup>a</sup>	11

\*Means with the same letter are not statistically different at P = 0.05. N = number of inspection dates.

considered as a potential pest owing to its presence a year round in the coastal region of Tunisia (Centre-East) in an open tomato fields. Although originates from hot regions of South American countries (Miranda et al., 1998), the insect is able to survive winter under Mediterranean climate and causes serious damage to winter tomato crop which indicated by male captures in January-February, the coldest months of the year.

**Table 3.** Mean numbers ( $\pm$ SD) of captured *Tuta absoluta* males according to color trap (spring tomato cultivation).

Trap colors	Mean $\pm$ SD*	N
White	7.45 $\pm$ 4.81 <sup>a</sup>	6
Yellow	4.50 $\pm$ 4.32 <sup>a</sup>	6
Orange	4.16 $\pm$ 2.51 <sup>a</sup>	6
Red	2.41 $\pm$ 2.52 <sup>a</sup>	6
Green	17.5 $\pm$ 18.08 <sup>a</sup>	6

\*Means with the same letter are not statistically different at P = 0.05. N = number of inspection dates.

The author did not evaluate level of infestation on tomato leaves and fruits, but low damage was noticed.

Trap color may be important to increase the effectiveness of a pheromone monitoring or mass trapping system. The parameters that make one color more effective than another are poorly understood. Trap color has been shown to improve trapping efficiency in other economically important Lepidoptera (Knight and Miliczky, 2003). Similar numbers of male tomato leafminer were captured in bucket traps at different colors demonstrating



**Figure 5.** Mean number of captured males according to trap inspection in spring tomato cultivation (Saheline, 2013).

**Table 4.** Mean numbers ( $\pm$ SD) of captured males in different aged lure traps (first trial, 27 April 2012).

Capsule age	Mean $\pm$ SD*	N
1 Week	2.62 $\pm$ 2.32 <sup>a</sup>	10
2 Weeks	4.65 $\pm$ 3.03 <sup>a</sup>	10
3 Weeks	2.35 $\pm$ 1.82 <sup>a</sup>	10
4 Weeks	3.25 $\pm$ 1.56 <sup>a</sup>	10
5 Weeks	4.75 $\pm$ 2.88 <sup>a</sup>	10
6 Weeks	3.02 $\pm$ 2.61 <sup>a</sup>	10
Control (fresh)	8.55 $\pm$ 4.20 <sup>b</sup>	10

\*Means with the same letter are not statistically different at P = 0.05. N = number of inspection dates.

**Table 5.** Mean numbers ( $\pm$ SD) of captured males in different aged lure traps (second trial, 4 June 2012).

Capsule age	Mean $\pm$ SD*	N
1 Week	2.40 $\pm$ 1.21 <sup>a</sup>	10
2 Weeks	2.79 $\pm$ 2.43 <sup>a</sup>	10
3 Weeks	3.99 $\pm$ 4.04 <sup>a</sup>	10
4 Weeks	1.59 $\pm$ 2.20 <sup>a</sup>	10
5 Weeks	3.80 $\pm$ 2.28 <sup>a</sup>	10
6 Weeks	2.74 $\pm$ 1.96 <sup>a</sup>	10
Control (fresh)	20.00 $\pm$ 17.43 <sup>b</sup>	10

\*Means with the same letter are not statistically different at P=0.05. N = number of inspection dates.

the superiority of pheromone attraction over color. Two hypothesis may explain this: (1) pheromone plumes are

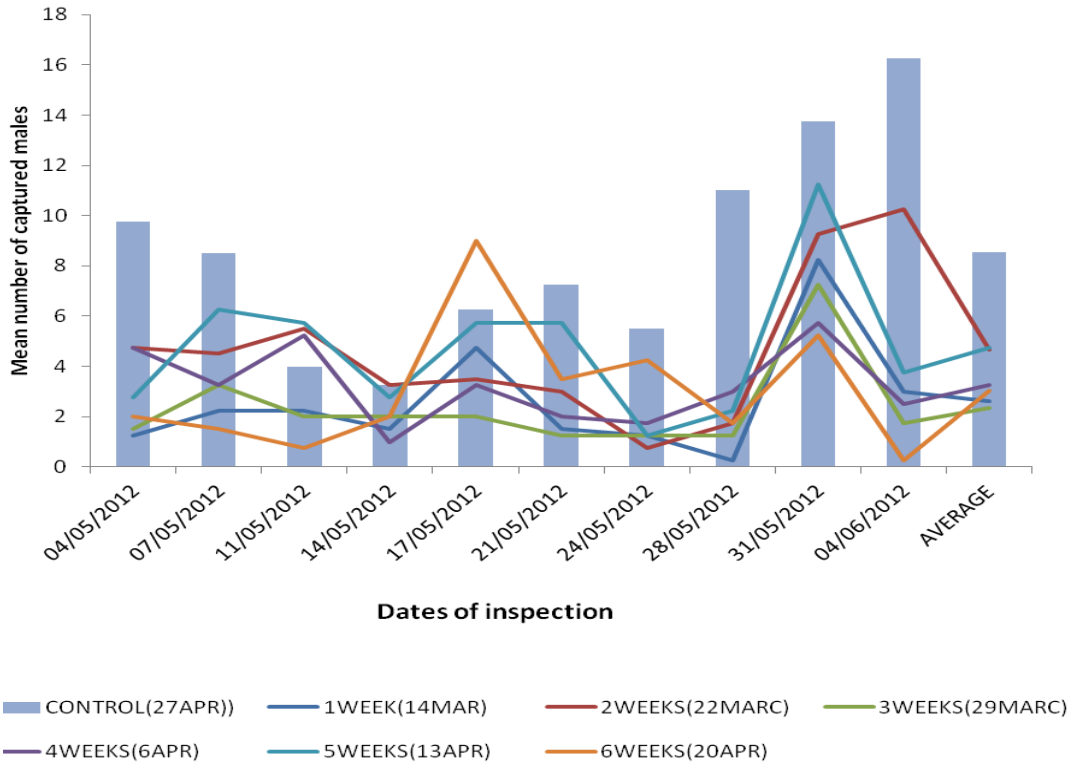
**Table 6.** Mean numbers ( $\pm$ SD) of captured males in different aged lure traps (third trial, June 2013)

Capsule age	Mean $\pm$ SD*	N
1 Week	3.75 $\pm$ 1.83 <sup>a</sup>	6
2 Weeks	2.75 $\pm$ 0.90 <sup>a</sup>	6
3 Weeks	2.45 $\pm$ 1.37 <sup>a</sup>	6
4 Weeks	2.20 $\pm$ 1.10 <sup>a</sup>	6
5 Weeks	2.02 $\pm$ 0.55 <sup>a</sup>	6
Control (fresh)	3.50 $\pm$ 2.85 <sup>a</sup>	6

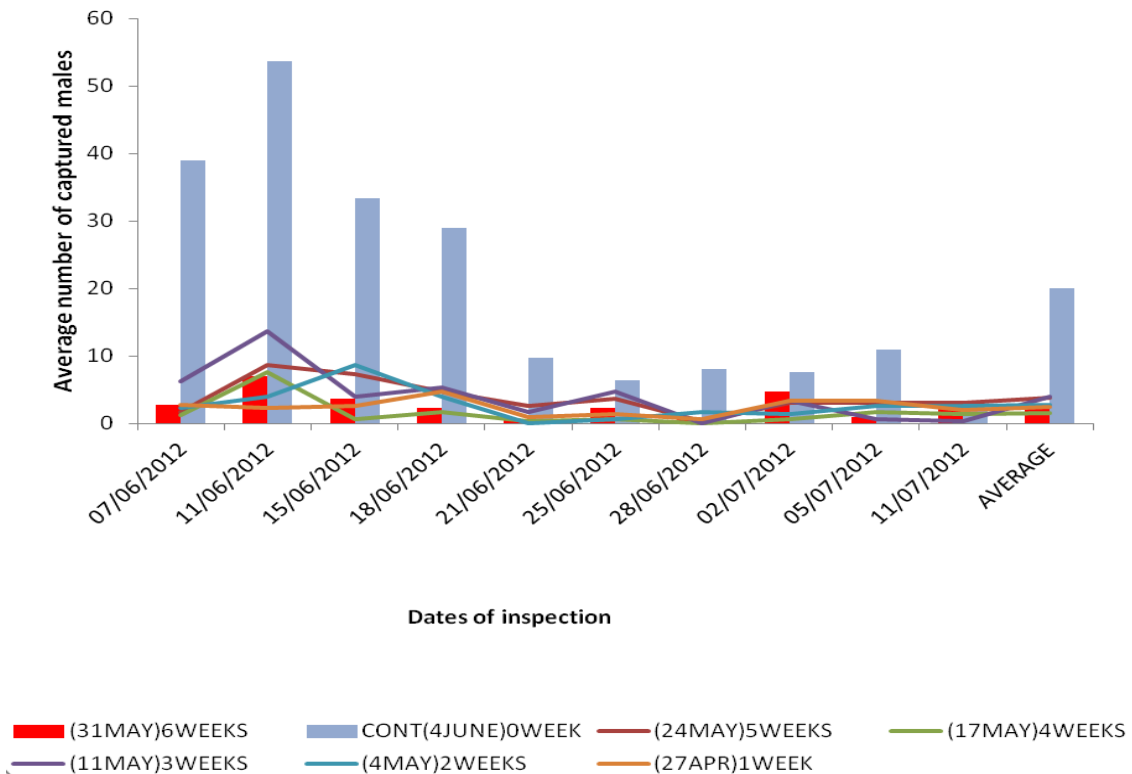
\*Means with the same letter are not statistically different at P = 0.05; N = number of inspection dates.

perceived over large distances hiding the trap color, (2) the distance between traps and blocks (15 m) may be relatively short resulting in maximum interference and competition among the pheromone plumes of neighboring traps (Elkinton and Cardé, 2008) and (3) color may be hidden by plant canopies; bucket trap are deployed on the ground.

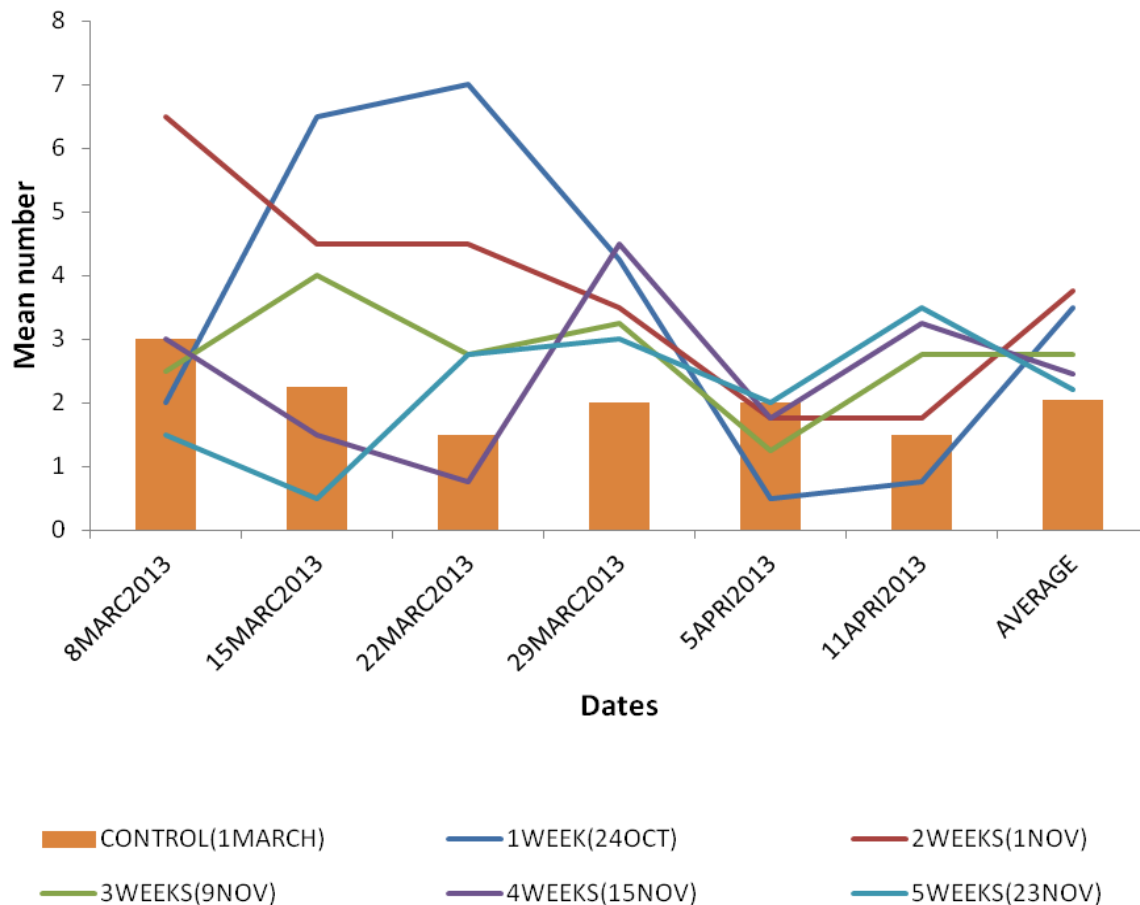
In spring tomato trial, though not shown statistically, green traps performed better. It may be due to the presence of tomato and potato in the same plot. It is known that *T. absoluta* attacks potato (*Solanum tuberosum*) leaves but no studies relating to the preference of the insect when potato and tomato cultivations are mixed or planted side by side which is common in the Centre-East region of Tunisia. Accordingly, Knight and Miliczky (2003) evaluated different trap colors for attraction of males of codling moth *Cydia pomonella* (Lepidoptera: Tortricidae), honeybees



**Figure 6.** Average number of captured *Tuta absoluta* males (Effect of weather factor on attractiveness of pheromones, first trial).



**Figure 7.** Mean number of males captured in traps in weathering capsule tests (second trial).



**Figure 8.** Mean number of *Tuta absoluta* captured in bucket traps according to weathering capsules (third trial).

and non-target insects reported a significantly high number of males of codling moth were caught in green delta traps versus the unpainted white ones.

To our knowledge, this is the first study aiming to demonstrate the effect of trap color on the attraction of *T. absoluta* males; we hypothesize that trap color may enhance the effectiveness of pheromone baited-trap. Roubos and Liburd (2008) reported a significant difference regarding the effect of bucket trap color on the male capture of the grape root borer, *Vitacea polistiformis* (Harris) (Lepidoptera:Sesiidae) using synthetic sex pheromone. Green and yellow colored traps caught more grape root borer males than white or blue. It was suggested that males do not distinguish between green and yellow colors which have similar reflectance.

Concerning the effect of weather factors on the decrease of pheromone attraction, the tests of the two experiments of 2012 (April and June), fresh lures captured from 2 to 13-fold more *T. absoluta* than aged lures (from 1 to 6 weeks). In spring, fresh lures can be attractive for 4 weeks (Figure 6) and in summer, this period is about 2 weeks (Figure 7). Indeed, in Tunisia, Abbas and Chermiti (2011) evaluated the efficiency

of two marks of sex pheromone dispensers used for trapping *T. absoluta* males under greenhouse showed that the number of trapped males peaked the second week following lure installation to decrease afterwards until its renewal.

In a study relating the effect of aging lures on the attractiveness of male codling moths *C. pomonella*, Kehat et al. (1994) demonstrated that captures in traps were negatively correlated with the aging of lure.

## Conclusion

Among five different colors used in bucket pheromone traps to capture *T. absoluta* males, no preference was noticed. However, green-colored traps colored are more suitable. Capsule pheromone lures for the monitoring and/or mass trapping of *T. absoluta* males should be replaced after 4 weeks in spring and 2 weeks in the summer at the most. Because of high temperature, the effect of aging of dispenser was more marked during summer than during spring. More research on the design of the tube used to host pheromone capsule providing a

shelter is needed.

### Conflict of Interests

The authors have not declared any conflict of interests.

### ACKNOWLEDGEMENTS

The author would like to thank Miss Bendhiefi A. and Mr Chtiwi L. for technical assistance in the field and laboratory work. This research was funded by Institution de la Recherche et de l'Enseignement Supérieur Agricoles (IRESA) of the Tunisian Ministry of Agriculture. Many thanks to the former and current IRESA Presidents Professors: Amamou H. and Darghouth A.

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## Full Length Research Paper

# Effects of limiting frequency of free access to milk on growth and intake of holstein calves during pre- and early post-weaning period

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Received 26 November, 2013; Accepted 16 June, 2014

New born (male and female) calves (n = 26) weighing  $33.3 \pm 0.71$  kg were used to evaluate effects of restricting frequency of free access to milk before and 2 weeks after weaning. Calves were randomly assigned at birth to 1 of 2 treatments being free access to whole milk twice daily (2X) or free access to whole milk once a day (1X). Milk and starter intakes were recorded daily, body weight (BW) was recorded weekly and metabolisable energy intake estimated. Before weaning, 2X calves had greater milk intake (8.76 L/d) and lower starter intake dry matter intake (DMI); 0.11 kg DM/d compared to 1X calves (7.11 L/d and 0.21 kg DM/d, respectively;  $P < 0.01$ ). Pre-weaning body weight (54.21 kg) and BW gain average daily gain (ADG) 0.74 kg/d were greater ( $P < 0.01$ ) for 2X calves compared to 1X (45.30 kg and 0.58 kg/d, respectively). Feed and energy conversion ratio did not differ ( $P > 0.05$ ) between treatments before weaning, but were greater ( $P < 0.03$ ) in 1X calves compared to 2X calves after weaning. Calves fed 2X lost weight a week after weaning, while calves fed 1X maintained growth. Limiting frequency of free access to milk improved solid feed intake and helped calves maintaining apparent growth after weaning.

**Key words:** dairy calf, milk intake, energy intake, weaning.

## INTRODUCTION

The amount of milk consumed by young dairy calves influences gut development (Anderson et al., 1982) and determines intake of starter feed as well as their health and growth (Appleby et al., 2001). In conventional calf-rearing systems, the amount of milk or milk replacer is limited to 8 to 10% of body weight (BW) during the first few weeks of life (Drackley, 2005) in order to encourage solid dry matter (DM) intake and allow early weaning. Studies to improve milk feeding systems for dairy calves through *ad libitum* milk feeding have shown higher milk

consumption and BW gain, with reduced starter intake, compared to restricted milk feeding (Appleby et al., 2001; Hammon et al., 2002; Jasper and Weary, 2002). Delayed solid feed intake because of *ad libitum* milk consumption during the pre-weaning period (Appleby et al., 2001; Hammon et al., 2002) results in delayed ruminal development which is associated with poor post-weaning performance (Baldwin et al., 2004). Restricted milk feeding as well as reduced starter intake due to *ad libitum* milk feeding, lead to poor growth and welfare (Khan et

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al., 2007). Alternative milk feeding approaches for better performance and welfare of dairy calves are needed. Khan et al. (2007) reported that step-down milk feeding may prevent the problems of depressed solid feed intake associated with *ad libitum* milk feeding and of low BW gain with conventional milk feeding of dairy calves. A review of feeding systems (Khan et al., 2011) revealed that low milk feeding regimens (4 L/d) leaves calves hungry thereby compromising growth, health, welfare and future milk yield, but that higher milk allocation reduces feed consumption thereby increasing growth check in the days after weaning, suggesting that the optimal milk feeding strategy occurs between conventional and *ad libitum* intake. Khan et al. (2011) suggested that effects of solid feed level should be evaluated on calves fed higher amounts of milk. The hypothesis of the current study was that limiting free access to milk would keep high level of milk intake and increase intake of solid feed. Therefore the objective was to compare effects of free access to milk fed twice daily and free access to milk fed once daily on solid feed consumption, metabolisable energy (ME) intake, BW gain, and feed efficiency in Holstein calves during the pre-weaning and early post-weaning periods.

## MATERIALS AND METHODS

The experimental protocol and procedures were approved by the Animal Ethics Committee (APIEC11/028) of the Agricultural Research Council at Irene (Pretoria) South Africa.

### Animals and treatments

Twenty six holstein female ( $n = 16$ ) and male ( $n = 10$ ) calves averaging  $33.3 \pm 0.71$  kg of BW at birth were used. Calves were born between September 2012 and December 2013, at the Agricultural Research Council Institute (S: 28013' 0" and E: 250 55' 0", altitude 1523 m) in South Africa. Calves were raised in individual calf pens until 70 d of age in an open front shed housing unit, with an exercise yard and proper floor rubber mats. Calves were blocked by birth date and randomly allocated to one of 2 experimental groups being: Free access to whole milk twice a day (2X) or free access to whole milk once a day (1X). All calves were fed colostrum for 3 d after birth. From day 4, calves fed 2X had free access to whole milk at 0800 and 1400 h, while calves fed 1X had free access to milk only at 0800 h. Milk was offered in 5 L buckets. The bucket was immediately refilled when empty until the calves stop drinking, and removed 30 min after to ensure cessation of drinking. From day 52, all calves were fed 4 L once a day at 0800 h until weaning at 56 days, when it was stopped completely. Fresh water and starter pellet (88.5 % dry matter (DM), 18.0 % crude protein (CP)) were available *ad libitum* from day 4 to 70. Average DM content of whole milk was 12.3 %, which contained 26.7 % fat, 28.2 % protein, and 34.2 % lactose.

### Intake and measurements

Daily individual calf starter intakes were measured throughout the experiment, milk and water consumption by each calf was measured daily. Calf starter was sampled and analyzed for DM by oven drying at 60°C for 48 h and CP according to AOAC (2000)

procedure 968.06. Composite daily milk samples from milk collected in the morning and evening were collected monthly to determine fat, CP, and lactose at lacto lab (Pty) (Irene) using a system 4000 infrared analyser (Foss Electric, Hillerod, Denmark). Calves were weighed at birth and at 7 day intervals throughout the experiment. Average body weight (BW) gain, total dry matter intake (DMI), feed conversion ratio (kg BW gain/kg of total DMI) and metabolisable energy (ME) conversion ratio (kg BW gain/kg of total ME intake) were calculated. Metabolisable energy concentration of whole milk was calculated according to NRC (2001). Faecal scoring to determine faecal fluidity and consistency occurred daily at 0800 h with the score ranging from 1 to 4, with '1' indicating formed stools, '2' when soft or of moderate consistency, '3' when runny or mild diarrhoea, and '4' when watery and profuse diarrhoea. At weaning, calves in both groups continued on the starter feed and remained in individual pens until 70 d of age when the experiment ended.

### Statistical analysis

Data were analyzed for two periods (pre-and post-weaning) using a mixed-effects analysis of variance in SAS (2009) with frequency of access to milk and its interaction with time as fixed effects, and calf as random effect. Data were analyzed as average and repeated measures. Initial BW for the pre-weaning data, and BW at weaning for the post-weaning data, was both included in the model as covariates. Tukey's test (Samuels, 1989) was used for testing differences in Least Squares Means. Significance was declared at  $P < 0.05$  and tendency to differ were accepted if  $P < 0.10$  but  $> 0.05$ .

## RESULTS

Milk intake (L/d, % BW and kg of DM/d) was greater ( $P < 0.05$ ) and starter DMI (kg/d and % BW) was lower ( $P < 0.05$ ) in calves fed 2X compared to calves fed 1X (Table 1). Total DMI and ME intake did not differ between treatments averaging 1.13 kg/d and 23.5 MJ/d, respectively. As expected, there was a week effect ( $P < 0.01$ ) on intake. As calves aged, starter and milk intake increased. Average BW, BW gain and average daily gain (ADG) were greater ( $P < 0.01$ ) for the 2X treatment compared to 1X, but BW gain as % of initial BW did not differ. Starter DMI as % BW was greater ( $P < 0.05$ ) in calves fed 1X. There was a week effect ( $P < 0.001$ ) on BW and DM intake as % BW. Feed conversion ratio and energy conversion ratio (ECR) were similar in both treatments. Post-weaning starter DMI (kg/d and % BW) and ME intake (MJ/d) did not differ between treatments and averaged 1.47 kg/d and 19.5 MJ/d respectively (Table 2). Body weight tended ( $P < 0.10$ ) to be greater for 2X calves, while BW gain (kg), ADG, feed conversion ratio (FCR) and energy conversion ratio (ECR) were lower ( $P < 0.05$ ) in the same group of calves.

The patterns of starter DMI and Milk intake are presented in Figures 1 and 2 respectively, and after 70 d, the general trend was still for the DMI to increase. In both treatments, starter DMI spiked from day 49 to 70. The spike was related to milk withdrawal. Starter DMI, g/kg BW remained higher ( $P < 0.05$ ) for 1X calves before



**Table 1.** Pre-weaning (56 days) effects of frequency of free access to milk on intake, growth and efficiency (Least square means).

Item	Treatments		SEM	P-value	
	2X	1X		Treatment	Week
<b>Intake</b>					
Milk, L/d	8.76	7.11	0.45	0.008	<0.0001
Milk, % of BW	15.4	13.9	0.83	0.009	<0.0001
Milk, kg of DM/d	1.02	0.86	0.05	0.008	<0.0001
Starter, kg of DMI/d	0.10	0.20	0.03	0.006	<0.0001
Total DM, kg/d	1.12	1.06	0.07	0.378	<0.0001
ME, MJ/d	22.71	20.62	0.71	0.143	<0.001
Starter DMI, % BW	0.18	0.44	0.66	0.001	<0.001
Total CP kg/d	0.30	0.28	0.01	0.170	<0.001
<b>Growth</b>					
BW initial, kg	34.3	32.3	0.71	0.308	-
BW average, kg	54.2	45.3	2.95	0.001	<0.001
BW gain, kg	41.2	32.7	2.80	0.005	<0.001
ADG, kg/d	0.74	0.58	0.05	0.005	<0.001
<b>Gain: feed efficiencies</b>					
FCR, kg/kg	0.69	0.59	0.15	0.081	-
ECR, kg/kg	0.03	0.03	0.001	0.072	-

Means in the same row differ if  $P < 0.05$ ; 2X: free access to milk twice (0800 and 1400 h) (56 days); 1X: free access to milk once a day (0800 h) (56 days); FCR: Feed conversion ratio; ECR: Energy conversion ratio.

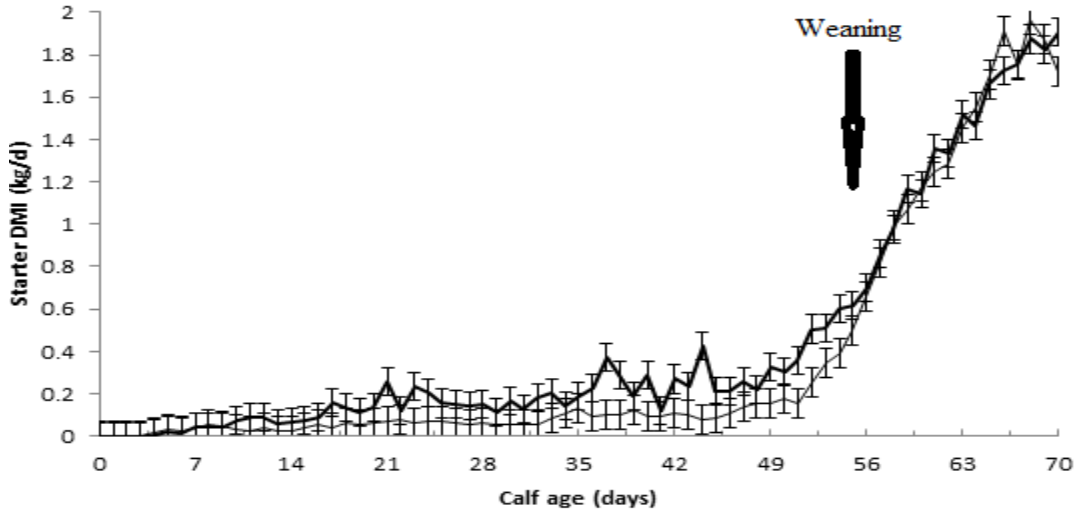
**Table 2.** Post-weaning (14 days) effects of frequency of free access to milk on intake, growth and efficiency (Least squares means).

Item	Treatments		SEM	P-value	
	2X	1X		Treatment	Week
<b>Intake</b>					
DM, kg/d	1.46	1.47	0.12	0.981	<0.0001
ME, Mcal/d	19.50	19.54	0.80	0.981	<0.0001
Starter DMI, % BW	1.93	2.09	1.78	0.378	<0.0001
Total CP kg/d	0.26	0.26	0.1	0.978	<0.0001
<b>Growth</b>					
BW, kg	76.70	70.8	2.95	0.052	-
BW gain, kg	5.10	12.40	2.60	0.015	<0.001
ADG, kg/d	0.41	0.89	0.19	0.015	<0.001
<b>Gain: feed efficiencies</b>					
FCR, kg/kg	0.40	0.78	0.15	0.030	-
ECR, kg/kg	0.03	0.06	0.01	0.030	-

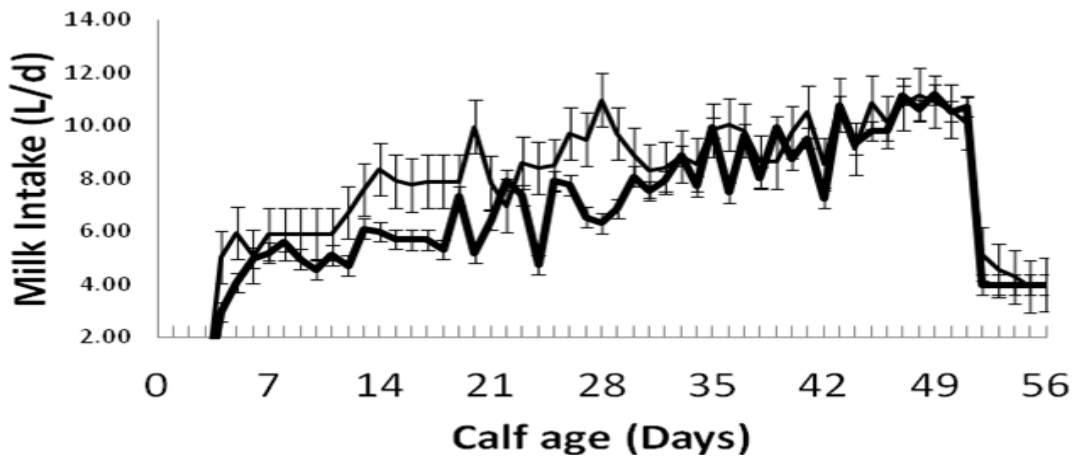
Means in the same row differ if  $P < 0.05$ ; 2X: free access to milk twice (0800 and 1400 h) (14 days); 1X: free access to milk once a day (0800 h) (14 days); FCR: Feed conversion ratio; ECR: Energy conversion ratio.

weaning (Table 1) and only numerically greater after weaning (Table 2). Figure 3 illustrates the growth curves of calves on 2 and 1X through 70 days of age. Higher

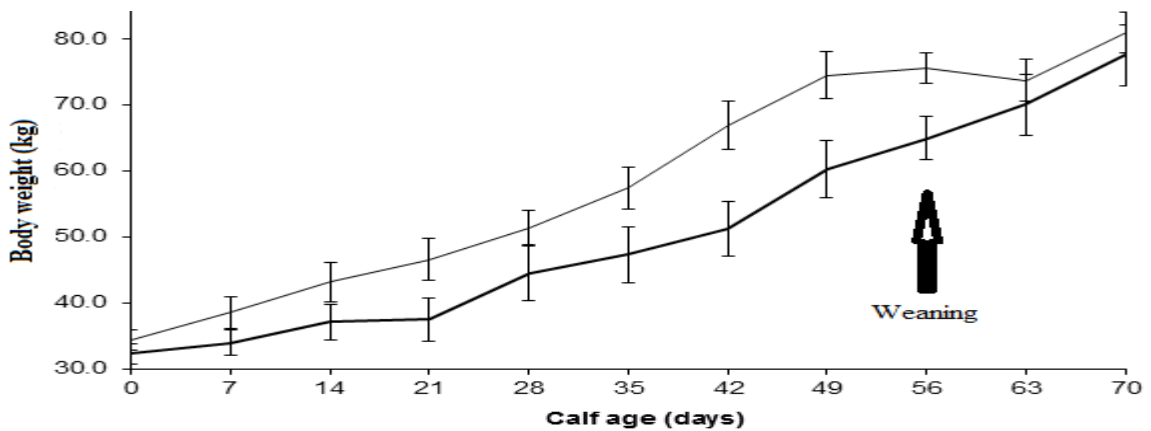
BW's occurred for 2X calves until weaning (75.5 vs 64.9 kg for 2 and 1X; respectively). Calves in treatment 2X had a decline on BW during the first week after weaning



**Figure 1.** Starter DMI of calves with free access to milk twice daily (08:00 and 14:00 h) (—) or once daily (08:00 h) (---).



**Figure 2.** Milk intake of calves having free access to milk twice daily (0800 and 1400 h) (—) or once daily (0800 h) (---).



**Figure 3.** Body weight of calves having free access to milk twice daily (08:00 and 14:00 h) (—) or once daily (08:00 h) (---).

**Table 3.** Least squares mean for faecal score ( $\pm$ SD) over period for calves having free access to milk twice (8:00 and 14:00) (2X) or once a day (8:00) (1X).

Item	Treatments		SEM	P
	2X	1X		
	<b>Faecal score</b>			
Week1	1.9	2.0	0.067	0.231
Week2	2.6	2.6	0.159	0.780
Week3	2.8	2.4	0.214	0.045
Week4	1.9	2.4	0.162	0.003
Week5	1.9	2.3	0.140	0.009
Week6	2.0	2.0	0.110	0.838
Week7	2.0	2.0	0.057	0.324
Week8	2.0	2.0	0.071	0.348
Total period	2.1	2.2	0.123	0.322

Means in the same row differ if  $P < 0.05$ ; 2X: free access to milk twice (8:00 and 14:00) (56 days); 1X: free access to milk once a day (8:00) (56 days); Score 1: formed stools; 2: soft or of moderate consistency; 3: runny or mild diarrhoea and 4: watery and profuse diarrhoea.

but recovered during the following week. Body weight gain for 1X calves was not affected during the transition to a solid diet. Results on faecal scores during the preweaning period are presented in Table 3. Faecal score did not differ ( $P > 0.05$ ) between 2 and 1X averaging 2.16. Differences in faecal scores were observed only during weeks 3, 4 and 5, when it was higher for 1X calves compared to calves 2X calves.

## DISCUSSION

Consistent with previous reports (Borderas et al., 2009; Appleby et al., 2001; Jasper and Weary, 2002; Khan et al., 2007), all calves increased milk consumption during the first 2 weeks, and consumed high amounts without noticeable increase in diarrhoea as observed previously. Calves fed 2X consumed more milk, with intake level comparable to previously reported levels (Jasper and Weary, 2002; Moallem et al., 2010; Borderas et al., 2009) for calves fed milk *ad libitum*. Calves fed 1X consumed 18.8% less milk and doubled starter than 2X calves. This greater starter intake can be attributed to the hyperphagic response resulting from the reduction in milk availability by limiting free access (Khan et al., (2007). Early studies indicated that limiting milk intake encourages intake of calf starter (Maynard and Norris, 1923), and that rumen papillae growth is stimulated by VFA production, in particular butyrate, which is high in calves consuming large amount of starter (Tamate et al., 1962). Hence calves are often fed reduced volumes of milk with the aim of increasing starter DM intake, promoting rumen development and reducing the age of weaning. However, restricting milk affects calf welfare since calves naturally drink milk several times during the day. However, in our study calves fed 1X consumed much greater volume of

milk (7.11 L/d) than conventional milk feeding (Jasper and Weary, 2002; Borderas et al., 2009), respectively 4.6 and 3.8 L/d) and improved starter DMI throughout the pre-weaning period. The total amount of starter DMI of 1X-fed calves (0.20 kg/d) was also much greater than the 0.17 kg/d (as fed) for calves fed milk conventionally (Jasper and Weary, 2002). In the latter study, milk weight totalled 0.1 g/kg of the calf's BW and was distributed evenly between two feedings.

The difference on starter and milk intake between the two groups of calves did not entail differences in total DM and ME intake. Greater amounts of milk by 2X calves resulted in increased ADG during the milk feeding period, and despite higher pre-weaning started DMI, calves fed milk 1X gained 20% less BW than those fed 2X, probably due to a decrease in milk intake and associated decrease in nutrients availability. Calves fed 2X had BW (75.5 kg) at weaning (56 d) being comparable to Moallem et al. (2010) at weaning (60 d) for calves fed *ad libitum* whole milk (82.7 kg) and milk replacer (85.6 kg). Because total DM intake and ME intake before weaning did not differ between treatments, it is not surprising that there was no difference in FCR and ECR.

There was no differences in starter intake after weaning, suggesting that the difference in milk intake prior to weaning did not affect starter intake 2 weeks after weaning. Factors regulating intake and feeding frequency should be evaluated to help understanding the interaction of the amount and composition of nutrients from milk on solid feed intake. In contrast, Terre et al. (2007) and Weary et al. (2008) reported that high milk intakes before weaning can depress solid feed intakes after weaning if milk feeding is ended abruptly. In our study, the amount of milk was reduced to 4 L in the morning from d 52 to 56.

Calves fed 2X did not maintain their growth advantage after weaning. Their low post-weaning ADG may be

attributed to reduced DM digestibility related to low DM intake of starter before weaning (Terre et al., 2007 and Hill et al., 2010). It is also possible that increased DMI in 2X-calves after weaning has resulted in part of increased nutrient intake filling the gut rather than improving growth.

The earlier adaptation to high feed intake cushioned calves on 1X free access to milk from transitional effects of milk removal. Khan et al. (2007) indicated that earlier initiation of solid feed consumption and, possible, related ruminal activity can mitigate negative effects of *ad libitum* milk intake on pre-weaning and post-weaning feed intake, growth and performance of dairy calves. Although DMI per kg BW was higher for 1X calves through the entire experimental period, its effects on increasing BW gain only occurred after weaning.

## Conclusion

Results suggest that high level of milk intake, similar to *ad libitum* milk feeding, and starter intake, similar to conventional milk feeding; can be attained when offering calves free access to milk only in the morning with *ad libitum* starter feed available all day. Body weight and feed efficiency post-weaning are comparable to unrestricted calves. However, further research is warranted to document long term effects of higher milk feeding frequency on performance of heifers, and in their first lactation.

## Conflict of Interest

The authors have not declared any conflict of interest.

## ACKNOWLEDGMENTS

This research was supported by the Agricultural Research Council, Animal Production Institute, South Africa, which is gratefully acknowledged.

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## Full Length Research Paper

**Genetic variability, correlation and path analysis among different traits in *desi* cotton (*Gossypium arboreum* L.)****C. S. Erande<sup>1</sup>, H. V. Kalpande<sup>1\*</sup>, D. B. Deosarkar<sup>1</sup>, S. K. Chavan<sup>1</sup>, V. S. Patil<sup>1</sup>, J. D. Deshmukh<sup>1</sup>, V. N. Chinchane<sup>1</sup>, Anil Kumar<sup>1</sup>, Utpal Dey<sup>2</sup> and M. R. Puttawar<sup>1</sup>**<sup>1</sup>Department of Agricultural Botany, Marathwada Agricultural University, Parbhani, Maharashtra, India.<sup>2</sup>Department of Plant Pathology, Marathwada Agricultural University, Parbhani, Maharashtra, India.

Received 28 May, 2013; Accepted 16 June, 2014

Genetic variability, correlations and path coefficients were studied in *desi* (Indian) cotton (*Gossypium arboreum* L.) taking 20 phenotypically diverse genotypes along with two viz., PA- 402 and PA- 255, one NH-615 and one hybrid check NHH-44. A wide range of variation was found for almost all the characters. All the characters showed very small difference between genotypic coefficient of variation (GCV) and respective phenotypic coefficient of variation (PCV), indicated that all the characters were least affected by environment. The high heritability estimates coupled with high expected genetic advance were observed for number of monopodia plant<sup>-1</sup>, number of sympodia plant<sup>-1</sup>, plant height, number of bolls plant<sup>-1</sup>, average boll weight, lint yield plant<sup>-1</sup>, harvest index, oil content, seed cotton yield plant<sup>-1</sup>, 2.5% span length and micronaire value indicating the presence of additive gene action and phenotypic selection may be more fruitful. The correlation studies revolved positive and significant genotypic and phenotypic correlation for most of the characters. Considering the association and path analysis, lint yield plant<sup>-1</sup>, bolls plant<sup>-1</sup>, ginning outturn, lint index, total biomass, number of sympodia plant<sup>-1</sup> and plant height had high positive effect on seed cotton yield plant<sup>-1</sup>.

**Key words:** Genetic variability, correlation, path analysis, *Gossypium arboreum*.

**INTRODUCTION**

Cotton (*Gossypium* spp.) is an important fibre and cash crop of the country which provides lint as raw material to the textile industry (Perchival and Kohel, 1990). It plays a key role in Indian economy by earning more than 30% of foreign exchange (Patel et al., 2007). It is also known as "White Gold". It generates employment at various stages during cultivation, ginning, spinning and garment making. Out of four cultivated species of genus *Gossypium*, only two species, that is, *Gossypium hirsutum* and *Gossypium arboreum* are being mostly cultivated in Maharashtra,

India. In the last few years there has been a significant reduction in area of *G. arboreum* cotton across the country and particularly in Maharashtra because of lower productivity and inferior fibre properties as compared to tetraploid cotton in rain fed eco-system and non availability of Bt variety / hybrid.

Genetic cotton could be gained either through combination or exploitation of hybrid vigour. Therefore, more emphasis should be given to increase the seed cotton yield unit area<sup>-1</sup>, by developing varieties with short

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structure, big boll size and medium to longer staple length with sustained yield in multiple environments. To achieve such desirable characteristics in a new variety, proper breeding strategies should be followed. The progress in breeding programme depends on magnitude of genetic variability present in breeding material. The existence of variability is essential for resistance of biotic and abiotic factor as well as for varietal adaptability. Selection is also effective when there is high degree of genetic variability among the individuals in a population. Correlation analysis measures the mutual relationship between various plant characters and determines the component character on which selection can be based for genetic improvement in yield.

Direction and magnitude of correlation between yield and yield contributing character must be considered for selecting the superior genotypes from diverse genetic population, but correlation does not provide information about direct and indirect effects of independent variables on dependent one, for this path coefficient analysis is essential. Path analysis splits correlation coefficient into measures of a direct and in direct effects. The present study on genetic variability, correlation and path analysis among different traits in *desi* (Indian) cotton was undertaken with the following objective viz., to study the performance of quality *arboreum* derived from breeding material and germplasm, the pattern of genetic variability, to estimate direct and indirect effects on seed cotton yield.

## MATERIALS AND METHODS

Field trial was conducted during *Kharif* (monsoon) season of 2008 with twenty elite genotypes of *G. arboreum*, two *G. arboreum* checks and one *G. hirsutum* hybrid check were sown in Randomized Block Design in two replication along with a spacing of 60 x 30 cm between rows and plants, respectively at Cotton Research Station, Mehboob Baugh, Marathwada Agricultural University, Parbhani, Maharashtra, India. Recommend package practices for the region were followed.

Five competitive plants were randomly selected for recording data on days to 50% flowering, days to 50% boll bursting, monopodia plant<sup>-1</sup>, sympodia plant<sup>-1</sup>, bolls plant<sup>-1</sup>, plant height (cm), boll weight (g boll<sup>-1</sup>), seed cotton yield (g plant<sup>-1</sup>), lint yield (g plant<sup>-1</sup>), seed index, lint index, ginning outturn, harvest index, oil content, total biomass, 2.5% span length, micronaire value, fibre strength, uniformity ratio, maturity ratio, fibre elongation and short fibre index.

The analysis of genotypic and phenotypic coefficient of variance was calculated according to Burton (1952). Heritability (Broad sense) was done by the methods of Allard (1960). The correlation coefficients were calculated according to Johnson et al (1955) whereas path coefficient analysis as described by Dewey and Lu (1959).

## RESULTS AND DISCUSSION

Analysis of variance revealed highly significant differences among genotypes for all the characters

indicating considerable amount of genetic variation present in the material. High magnitude of variation in the experimental material was reflected by high values of mean and range for almost all the characters.

Range of variation on the basis of mean was more for the characteristics viz., seed cotton yield plant<sup>-1</sup>, days to 50% flowering, days to 50% boll bursting, number of sympodia, plant height, number of bolls plant<sup>-1</sup>, lint yield plant<sup>-1</sup>, seed index and lint index, ginning outturn oil content, 2.5% span length, fibre strength and uniformity ratio. Similar results were reported by Nageshwara et al (2001), Neelam and potdukhe (2002), Phundan et al. (2004), Ghumber et al. (2005) and Kumari and Subbramma (2006).

## Genetic variability

The estimates of genetic parameters like genotypic variance ( $\sigma^2_p$ ), phenotypic variance ( $\sigma^2_g$ ), genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (broad sense), genetic advance (GA) and expected genetic advance (EGA) are presented in Table 1.

## Variance components and coefficient of variation

The values of phenotypic variance were more than the genotypic variance for all the characters. High genotypic and phenotypic variances were observed for the characters seed yield plant<sup>-1</sup>, number of bolls plant<sup>-1</sup>, plant height and days to 50% flowering. Low genotypic and phenotypic variances were observed for the characters of number of monopodia (0.01 and 0.02), Earliness index (0.001 and 0.002), average boll weight (0.051 and 0.068), Harvest index 0.012 and 0.014), maturity ratio (0.0004 and 0.0005) as well as fibre elongation (0.011 and 0.021), fibre strength (0.535, 0.670), micronaire value (0.280, 0.399), lint index (0.249, 0.350) and seed index (0.353, 0.53) and short fibre index.

In present investigation through the phenotypic coefficient of variations were greater than genotypic coefficient of variations. The differences between them were of lower magnitude, that is, they were more or less close to each other. This indicates that there is small effect of environment on characters and selection may be effective. Sambamurty et al. (2004) and Gumber et al. (2005) also reported greater PCV values than GCV values for all the characters.

High estimates of genotypic and phenotypic coefficient of variation were observed for number of sympodia, number of monopodia, number of bolls plant<sup>-1</sup>, plant height, average boll weight, seed cotton yield plant<sup>-1</sup>, lint yield plant<sup>-1</sup>, harvest index, total biomass, maturity ratio, short fibre index and 2.5% span length and fibre elongation. Kumari and Subbramma (2006) reported

**Table 1.** Parameters of genetic variability for yield and yield contributing characters of cotton.

S/N	Characters	Range	General mean	GV (%)	PV (%)	GCV (%)	PCV (%)	Heritability (%)	Genetic advance	EGA (%)
1	Days to 50% flowering	69.00-78.25	72.92	6.73	7.59	3.56	3.78	88.70	5.03	6.90
2	Days to 50% boll bursting	107.60-113.75	110.71	2.79	3.07	1.51	1.58	90.80	3.28	2.96
3	Number of monopodia	1.10-1.60	1.29	0.01	0.02	9.45	10.91	75.00	0.25	19.38
4	Number of sympodia	9.80-21.60	15.88	15.19	16.02	24.54	25.20	94.80	7.81	49.24
5	Earliness index	0.77-0.88	0.83	0.001	0.002	3.02	4.61	42.90	0.03	4.08
6	Plant height	120.40-202.40	163.50	200.0	250.70	8.63	9.67	80.00	29.89	18.26
7	Number of bolls plant <sup>-1</sup>	8.50-20.00	14.76	13.95	14.96	25.30	26.20	93.20	7.43	50.34
8	Average boll weight	2.01-2.63	2.30	0.05	0.07	9.86	11.37	75.00	0.40	17.62
9	Seed cotton yield	16.88-35.66	29.69	75.64	131.20	29.67	38.57	60.70	13.49	45.03
10	Lint yield plant <sup>-1</sup>	7.14-12.97	10.90	8.51	9.00	26.84	27.51	95.10	5.88	53.94
11	Seed index	5.80-8.34	6.93	0.35	0.53	8.56	10.47	66.80	1.00	14.42
12	Lint index	3.45-4.47	4.16	0.25	0.35	11.99	14.22	22.43	0.93	22.44
13	Ginning outturn	33.61-39.87	37.71	1.66	2.50	3.41	4.19	66.50	20.16	5.74
14	Harvest index	0.16-0.55	0.36	0.01	0.01	30.96	32.25	92.10	0.22	61.02
15	Oil content	14.90-19.50	17.39	1.60	1.69	7.27	7.49	94.19	2.52	14.54
16	Total biomass	23.80-120.40	57.45	830.49	852.80	50.16	50.49	97.30	5.47	10.00
17	2.5% span length	26.20-27.70	27.24	4.17	4.97	7.49	9.19	80.04	3.22	11.83
18	Micronaire value	4.40-6.00	5.22	0.28	0.39	10.15	12.11	70.20	0.91	17.52
19	Fibre strength	17.50-20.50	19.27	0.54	0.67	3.80	4.25	79.00	1.34	6.99
20	Uniformity ratio	48.00-53.00	50.66	1.97	3.00	2.79	3.42	65.70	2.34	4.63
21	Maturity ratio	0.81-0.86	0.84	0.001	0.002	2.05	2.35	79.40	0.03	3.69
22	Fibre elongation	5.80-6.20	5.90	0.01	0.02	1.78	2.47	51.90	0.15	2.64
23	Short fibre index	7.60-11.80	9.55	1.59	1.65	13.10	13.44	94.90	2.61	26.29

GV = Genotypic variance, PV = Phenotypic variance, GCV = Genotypic coefficient of variation, PCV = Phenotypic coefficient of variation, EGA = Expected genetic advance.

that the estimates of GCV and PCV were high for number of boll, seed cotton yield plant<sup>-1</sup> (g), fibre properties viz., 2.5% span length and micronaire values.

The lowest genotypic and phenotypic coefficients of variation were observed for days to 50% flowering and boll bursting, uniformity ratio, fibre elongation ginning outturn and oil content. Similar finding were reported by Sumathi and Nadarajan (1996) for days to 50% flowering and

ginning percentage and Gumber et al. (2005) for ginning percentage.

#### Heritability and genetic advance

Heritability estimate for characters under study is given in Table 1. Heritability values are useful in predicting the expected progress to be achieved through the process of selection. Genetic

coefficient of variation along with heritability estimate provides a reliable estimate of the amount of genetic advance to be expected through phenotypic selection (Wright, 1921).

Heritability ranged from 42.90% for Earliness index to 97.30% for total biomass. According to Singh (2001) heritability values greater than 80% are very high, values from 60 to 79% are moderately high and values from 40 to 59% are low. Accordingly, characters like days to 50%

**Table 2a.** Genotypic correlation between yield and its components in cotton.

Character	Days to 50% flowering	Days to 50% boll bursting	Number of monopodia	Number of sympodia	Earliness index	Plant height (c.m)	Number of bolls plant <sup>-1</sup>	Average boll weight (g)	Lint yield plant <sup>-1</sup> (g)	Seed index (g)	Lint index (g)	Ginning outturn (%)
Days to 50% flowering	1.00	0.199	-0.257	0.312	0.333	0.943**	0.459*	0.044	0.024	0.760**	-0.199	0.045
Days to 50% boll bursting		1.000	-0.089	0.061	0.559**	0.380	0.400*	0.286	0.117	0.475*	0.630**	0.088
Number of monopodia			1.000	0.731**	0.241	0.190	0.402*	0.521**	0.859**	0.529**	-0.417*	0.277
Number of sympodia				1.000	0.336	0.089	0.375	0.374	0.471*	0.014	-0.469	0.430*
Earliness index					1.000	0.852**	0.390	-0.161	-0.022	0.476*	-0.124	0.169
Plant height						1.000	0.638*	-0.015	0.314	0.966**	0.201	0.035
Number of bolls plant <sup>-1</sup>							1.000	0.422*	0.096	0.501**	0.049	0.366
Average boll weight								1.000	0.619**	0.098	0.360	0.008
Lint yield plant <sup>-1</sup>									1.000	0.389*	0.010	0.044
Seed index										1.000	0.224	0.472*
Lint index											1.00	0.431*
Ginning outturn												1.000

\*, \*\* significant at 5 and 1% probability levels respectively.

flowering (88.70%), number of monopodia (75.00%), number of sympodia (94.80%), plant height (80.00%), number of bolls plant<sup>-1</sup> (93.20%), average boll weight (75.20%), lint yield plant<sup>-1</sup> (95.10%), harvest index (92.10%) and seed cotton yield (60.70%) had very high and moderately high heritability. This indicates that selection will be the best step for selecting genotypes having traits with very high and moderately high heritability. This is because there would be a close correspondence between the accessions and the phenotype due to relative small contribution of the environment to the total variability. These results are in conformity with the results reported by Deshmukh et al. (1999), Rao and Raddy (2001) and Kapoor and Kaushik (2003).

The range of genetic advance as percent of mean was from 2.64% for fibre elongation to 61.02% for harvest index. High heritability estimates coupled with high expected genetic

advance were observed for the characters number of sympodia, plant height, number of monopodia, number of boll plant<sup>-1</sup> average boll weight, harvest index, oil content, total biomass, fibre strength, short fibre index, seed cotton yield plant<sup>-1</sup>, lint yield plant<sup>-1</sup>, lint index, harvest index, 2.5% span length and micronaire value indicating additive gene action. Kapoor and Kasushik (2003) reported highest heritability coupled with highest expected genetic advance for number of bolls plant<sup>-1</sup>, seed cotton yield plant<sup>-1</sup> and boll weight. High heritability estimates coupled with low expected genetic advance were observed for days to 50% flowering, days to 50% boll bursting, ginning percentage, maturity ratio indicating non additive gene action. Similar result were reported by Rao and Raddy (2001) for days to 50% flowering (Neelam and Potdukhe, 2002; Sambamurthy et al., 2004; Kumari and Subramamma, 2006). Low heritability estimates

coupled with low expected genetic advance were observed for the characters like earliness index and fibre elongation.

#### Association among characters

Estimates of correlation coefficient measures the degree of relationship between pairs of characters are presented in Tables 2a, b and 3a, b. Computation of correlation between yield and yield contributing characters of considerable importance in plant selection. In the present study, the genotypic and phenotypic correlations of seed cotton yield with number of monopodia, number of sympodia, earliness index, plant height, number of bolls plant<sup>-1</sup>, average boll weight and lint yield plant<sup>-1</sup> were positive and significant indicating the increase in seed cotton yield is mainly because of increase in one or more of the above characters.



**Table 2b.** Genotypic correlation between yield and its components in cotton.

Character	Harvest index	Oil content	Total biomass	2.5% Span length	Micronaire value 10 <sup>-6</sup> /inch	Fibre strength	Uniformity ratio	Maturity ratio	Fibre elongation	Short fibre index	Seed cotton yield
Days to 50% flowering	0.284	0.114	-0.184	-0.164	0.124	-0.226	0.144	0.154	-0.188	0.087	0.296
Days to 50% bollbursting	0.407*	-0.147	-0.393*	-0.248	-0.173	-0.227	-0.032	-0.020	-0.169	0.089	0.309
Number of monopodia	-0.325	-0.209	0.780**	0.343	-0.849**	-0.236	-0.680	-0.801**	-0.679**	0.477*	0.499**
Number of sympodia	-0.123	-0.134	0.480	-0.290	-0.318	0.023	0.219	-0.295	-0.535**	0.605**	0.425*
Earliness index	-0.565**	-0.284	-0.392	0.150	0.236	-0.032	0.023	0.220	-0.278	0.301	0.461*
Plant height	0.227	0.126	0.044	0.233	0.045	-0.352	0.162	0.079	0.056	0.173	0.480*
Number of bolls plant <sup>-1</sup>	0.379	0.062	0.048	0.118	-0.311	-0.359	-0.165	-0.310	-0.269	0.320	0.783**
Average boll weight	0.009	0.387*	0.353	0.368	-0.436*	-0.203	-0.295	-0.454*	-0.253	0.170	0.631**
Lint yield plant <sup>-1</sup>	0.056	0.134	0.276	0.275	-0.579**	-0.018	-0.519**	-0.674**	-0.298	0.169	0.419*
Seed index	-0.371**	0.029	-0.491**	-0.230	0.220	-0.537**	0.078	0.130	-0.398*	0.167	0.333
Lint index	-0.224	0.620**	-0.259	0.885**	0.129	-0.365	-0.130	-0.028	-0.042	0.219	0.189
Ginning outturn	0.776**	-0.168	0.162	-0.105	0.697**	0.217	0.270	-0.436*	-0.413	0.396*	0.703**
Harvest index	1.000	0.204	-0.200	0.026	-0.077	-0.329	0.350	0.059	-0.229	-0.071	0.379
Oil content		1.000	-0.185	0.347	0.128	-0.213	-0.007	-0.097	-0.081	-0.194	0.342
Total biomass			1.000	-0.093	-0.514**	0.288	-0.327	-0.464*	-0.160	0.334	0.149
2.5% span length				1.000	-0.559**	0.576**	-0.935**	-0.358	0.283	-0.699**	0.447*
Micronaire value					1.000	0.239	0.729**	0.929**	0.679**	-0.712**	-0.932**
Fibre strength						1.000	0.497**	0.368	0.786**	-0.286	-0.547**
Uniformity ratio							1.000	0.726**	0.658**	-0.693**	-0.643**
Maturity ratio								1.000	0.774**	-0.572**	-0.912**
Fibre elongation									1.000	-0.937**	-0.761**
Short fibre index										1.000	0.622**

\*, \*\* significant at 5 and 1% probability levels respectively.

Similar results were reported by Rajarathinam et al. (1993), Mandloi et al. (1998) and Gumber et al. (2005) for numbers of bolls plant and boll weight and Ahuja and Kumar (2001) for number of bolls plant<sup>-1</sup> and plant height. Rao and Mary (1996) reported positive and significant correlation for seed cotton yield plant<sup>-1</sup> with seed index, lint index, harvest index and ginning outturn (%), Patel et al. (2003) reported positive correlation of seed index seed cotton yield with seed oil percent. Plant height exhibited positive and significant

association with number of bolls plant<sup>-1</sup>, lint yield and seed cotton yield plant<sup>-1</sup>. The significant and positive correlation of number of sympodia plant<sup>-1</sup> with number of bolls plant<sup>-1</sup> and boll weight indicates the possibility of increase in seed cotton yield through the simultaneous improvement of these characters. The number of bolls plant<sup>-1</sup> showed significant positive correlation with boll weight. The results indicate that the direct selection procedure to increase number of bolls plant<sup>-1</sup> would help full for increasing boll weight.

The character average boll weight showed significant positive correlation with seed cotton yield.

The Earliness index (Bartlett's index) exhibited negative non significant correlation with average boll weight at both genotypic and phenotypic levels. The results are in accordance with the studies conducted by Carvalho et al. (1994). The fibre quality parameters viz., micronaire value, fibre strength, uniformity ratio exhibited negative correlation with seed cotton yield plant<sup>-1</sup>, whereas

**Table 3a.** Phenotypic correlation between yield and its components in cotton.

Character	Days to 50% flowering	Days to 50% boll bursting	Number of monopodia	Number of sympodia	Earliness index	Plant height (c.m)	Number of bolls plant <sup>-1</sup>	Average boll weight (g)	Lint yield plant <sup>-1</sup> (g)	Seed index (g)	Lint index (g)	Ginning outturn (%)
Days to 50% flowering	1.000	0.189	-0.177	0.271	0.236	0.471*	0.407*	0.007	0.012	0.545**	-0.108	0.042
Days to 50% boll bursting		1.000	-0.076	-0.046	0.349	0.252	0.379	0.247	0.110	0.358	0.369	0.059
Number of monopodia			1.000	0.441*	-0.048	-0.176	0.397*	0.400*	0.507**	-0.285	-0.265	0.402*
Number of sympodia				1.000	0.194	0.410*	0.391*	0.308	0.460*	0.034	-0.252	0.390*
Earliness index					1.000	0.423*	0.160	-0.033	0.565**	0.395*	-0.086	0.125
Plant height						1.000	0.456*	0.037	0.145	0.482*	-0.134	0.028
Number of bolls plant <sup>-1</sup>							1.000	0.449*	0.080	0.432*	0.016	0.241
Average boll weight								1.000	0.492**	0.085	0.233	0.036
Lint yield plant <sup>-1</sup>									1.000	0.315	0.105	0.071
Seed index										1.000	0.205	0.021
Lint index											1.000	0.027
Ginning outturn												1.000

\*, \*\* significant at 5 and 1% probability levels respectively.

the trait 2.5% span length showed positive correlation with seed cotton yield plant<sup>-1</sup>. Similar results were obtained by Rajarathinam et al. (1993), Rao et al. (2001) and Sharma (2005).

In present study, significant and positive genotypic and phenotypic correlation was observed between numbers of bolls plant<sup>-1</sup>, number of monopodia and sympodia plant<sup>-1</sup>, boll weight and plant height with seed cotton yield plant<sup>-1</sup>, which is considerable significant to breeder because component breeding would be very effective under such situation. Selection for all these traits might be helpful in evolving high yielding varieties of *desi* (Indian) cotton.

### Path analysis

In order to know the specific forces in building up of the total correlation, it is essential to resort to path coefficient analysis (Table 4a, b). It was

observed that genotypic level of analysis was more important as a plant breeder viewpoint so more emphasis was placed on path coefficient analysis at genetic level. At genotypic level, lint yield plant<sup>-1</sup> (0.877), number of bolls plant<sup>-1</sup> (0.767). Similar findings were reported by Gururajan (2000), and Alther and Singh (2003). Seed index (0.747), ginning outturn (0.513), plant height (0.371), lint index (0.364) and total biomass exerted highest direct effect on seed cotton yield. Whereas, weak positive indirect effects were observed for the characters of days to 50% boll bursting (0.182), number of sympodia plant<sup>-1</sup> (0.153), average boll weight (0.118), 2.5% span length (0.257), uniformity ratio (0.148), maturity ratio (0.152), fibre elongation (0.130) and negative indirect effects were showed by the traits days to 50% flowering, number of monopodia, harvest index, fibre strength, earliness index and oil content with seed cotton yield. The fibre quality characteristics *viz.*, short fibre index, 2.5% span

length, micronaire value, maturity ratio and uniformity ratio exhibited direct effect on seed cotton yield plant<sup>-1</sup>, while fibre strength showed negative in direct effects. Similar results were reported by Rajarathinam et al. (1993), Rao et al. (2001) and Ekinici et al. (2010).

The path analysis studies revealed that, the traits which had positive direct effects on seed cotton yield should be given due to emphasis for making selection for high yielding genotypes in *desi* (Indian) cotton.

Irum et al. (2011) noticed that the genotypes exhibited a wide range of variability for all the characters except shoot length. Moderate to high heritability estimates were found for all characters. All the seedling traits showed positive and significant correlation with cotton yield both at genotypic and phenotypic level. Path coefficient analysis showed that root length had the highest and positive direct effect on cotton yield.

Positive direct effects were produced by shoot

**Table 3b.** Phenotypic correlation between yield and its components in cotton.

Character	Harvest index	Oil content	Total biomass	2.5% span length	Micronaire value 10 <sup>-6</sup> /inch	Fibre strength	Uniformity ratio	Maturity ratio	Fibre elongation	Short fibre index	Seed cotton yield
Days to 50% flowering	0.262	0.092	-0.174	-0.096	0.057	-0.185	0.033	0.154	-0.134	0.057	0.239
Days to 50% boll bursting	0.379	-0.174	-0.368	-0.01	-0.143	-0.186	0.009	0.016	-0.157	0.068	0.232
Number of monopodia	-0.239	-0.171	0.521**	0.03	-0.534	-0.164	-0.361	-0.534**	-0.162	0.336	0.222
Number of sympodia	0.133	-0.14	0.461*	-0.424*	-0.433	-0.043	0.176	-0.345	-0.426*	0.562**	0.387*
Earliness index	-0.372	-0.152	-0.26	0.399	0.05	-0.122	0.019	0.064	0.017	0.168	0.456*
Plant height	0.085	0.005	0.008	0.124	0.025	-0.202	0.014	0.021	0.277	0.125	0.469*
Number of bolls plant <sup>-1</sup>	0.369	0.038	0.041	0.044	-0.385	-0.317	-0.109	-0.256	-0.396*	0.317	0.570**
Average boll weight	0.034	0.389*	0.324	0.099	-0.460*	-0.189	-0.255	-0.364	-0.181	0.145	0.419*
Lint yield plant <sup>-1</sup>	0.043	0.139	0.258	0.124	-0.479*	-0.113	-0.385	-0.551**	-0.244	0.156	0.393*
Seed index	-0.463*	0.022	-0.411*	-0.176	0.142	-0.435*	0.02	0.142	-0.511**	0.146	0.203
Lint index	-0.396	0.388*	-0.405	0.025	0.062	-0.144	0.029	-0.002	0.02	0.128	0.24
Ginning outturn	0.142	-0.073	-0.107	-0.065	0.454	0.078	0.083	-0.461*	-0.278	0.345	0.588**
Harvest index	1	-0.188	-0.799**	0.038	-0.037	-0.468*	0.095	0.126	-0.123	-0.076	0.273
Oil content		1	-0.181	0.153	0.133	-0.185	-0.018	-0.112	-0.016	-0.172	0.223
Total biomass			1	-0.056	-0.403	0.253	-0.276	-0.434*	-0.123	0.331	0.123
2.5% span length				1	-0.498*	0.125	-0.075	-0.032	0.285	-0.263	0.438*
Micronaire value					1	0.17	0.611**	0.672**	0.445**	-0.542**	-0.588**
Fibre strength						1	0.466*	0.271	0.402*	-0.226	-0.465
Uniformity ratio							1	0.610*	0.451*	-0.538**	-0.414*
Maturity ratio								1	0.543*	-0.530**	-0.510**
Fibre elongation									1	-0.677**	-0.436*
Short fibre index										1	0.467*

\*, \*\* significant at 5 and 1% probability levels respectively.

**Table 4a.** Direct and indirect (Genotypic) effects of 22 causal variables on seed cotton yield.

Character	Days to 50% flowering	Days to 50% boll bursting	Number of monopodia	Number of sympodia	Earliness index	Plant height (cm)	Number of bolls plant <sup>-1</sup>	Average boll weight (g)	Fibre elongation	Short fibre index (g)	Lint index	Ginning outturn
Days to 50% flowering	-0.101	0.036	-0.038	0.359	-0.148	0.350	0.352	-0.005	-0.020	-0.568	0.072	0.036
Days to 50% boll bursting	0.020	0.182	0.013	0.070	0.249	-0.141	-0.306	-0.033	0.103	0.355	0.229	0.071
Number of monopodia	0.026	0.016	-0.150	0.843	-0.107	-0.070	0.308	0.061	0.784	-0.395	0.151	0.225
Number of sympodia	-0.032	0.011	0.110	0.153	-0.149	-0.032	-0.287	-0.044	0.413	0.010	0.170	-0.349
Earliness index	-0.034	0.101	0.036	0.387	-0.445	0.316	-0.222	-0.019	0.018	-0.355	0.045	0.137
Plant height	0.096	0.069	0.028	0.102	-0.379	0.371	-0.489	-0.001	0.275	0.722	0.218	-0.028
Number of bolls plant <sup>-1</sup>	0.047	0.072	0.060	0.432	-0.129	-0.236	0.767	-0.050	0.084	-0.374	0.017	-0.297
Average boll weight	0.004	-0.052	-0.078	-0.431	0.071	0.005	0.324	0.118	-0.542	0.073	-0.130	-0.006
Lint yield plant <sup>-1</sup>	0.002	0.021	0.135	0.543	-0.009	-0.116	-0.073	-0.073	0.877	0.291	-0.003	-0.035
Seed index	0.077	0.086	-0.079	0.016	0.212	-0.358	0.384	0.011	0.341	0.747	81.000	0.139
Lint index	-0.020	0.144	0.062	-0.200	0.055	0.223	0.037	0.042	0.008	0.167	0.364	0.025
Ginning outturn	-0.005	-0.016	-0.042	-0.495	-0.075	0.001	0.280	-0.001	-0.038	0.128	-0.011	0.513

**Table 4a.** Contd.

Harvest index	0.029	0.074	0.049	-0.141	-0.251	-0.084	-0.290	-0.001	0.049	-0.426	0.277	0.143
Oil content	0.001	0.026	-0.031	-0.154	0.126	-0.046	-0.047	0.046	0.117	0.021	-0.189	-0.136
Total biomass	-0.019	-0.710	-0.117	-0.553	0.174	0.016	0.036	0.042	0.241	-0.367	0.240	0.131
2.5% span length	-0.017	0.045	-0.051	-0.911	-0.512	0.860	0.090	0.043	0.241	0.172	-0.322	0.041
Micronaire value	-0.013	0.031	-0.031	-0.597	0.105	-0.016	-0.392	-0.051	-0.507	-0.164	0.047	0.567

**Table 4b.** Direct and indirect (Genotypic) effects of 22 causal variables on seed cotton yield.

Character	Harvest index	Oil content	Total biomass	2.5% span length	Micronaire value 10 <sup>-6</sup> /inch	Fibre strength	Uniformity ratio	Maturity ratio	Fibre elongation	Short fibre index	Seed cotton yield
Days to 50% flowering	0.311	0.043	-0.164	0.043	0.111	0.263	0.150	-0.023	0.025	0.029	0.239
Days to 50% boll bursting	-0.446	-0.056	-0.351	0.064	-0.155	-0.263	0.033	0.003	0.021	0.030	0.232
Number of monopodia	-0.357	-0.080	0.697	0.088	0.765	0.274	0.713	0.122	0.088	0.016	0.222
Number of sympodia	0.135	0.051	0.429	-0.203	-0.467	0.026	0.229	0.060	0.069	0.208	0.387*
Earliness index	-0.620	-0.108	-0.351	0.296	-0.213	0.037	0.023	-0.033	0.036	0.103	0.456*
Plant height	-0.249	0.048	-0.039	0.060	-0.040	0.409	0.169	-0.012	-0.007	0.059	0.469*
Number of bolls plant <sup>-1</sup>	-0.415	0.023	0.042	0.030	-0.460	-0.417	0.172	0.047	0.061	0.111	0.570**
Average boll weight	0.009	0.147	0.316	0.094	-0.393	0.235	-0.309	0.069	0.033	0.058	0.419*
Lint yield plant <sup>-1</sup>	0.062	0.051	0.246	0.070	-0.521	0.208	-0.543	0.103	0.038	0.058	0.419*
Seed index	0.628	0.011	-0.436	-0.059	0.198	0.624	-0.081	-0.019	0.051	0.057	0.203
Lint index	0.685	0.198	-0.590	-0.228	0.116	0.424	-0.136	0.004	-0.005	-0.075	0.24
Ginning outturn	-0.193	0.064	0.144	-0.130	0.628	-0.252	0.283	0.066	0.053	0.129	0.588**
Harvest index	-0.098	0.078	-0.715	0.006	0.069	0.615	0.068	-0.008	0.029	-0.024	0.273
Oil content	0.224	-0.382	-0.165	-0.089	0.115	0.247	-0.006	0.014	0.010	-0.066	0.223
Total biomass	-0.878	-0.070	0.494	0.023	-0.463	0.334	0.343	0.070	0.020	0.118	0.213
2.5% span length	-0.029	0.132	-0.083	0.257	0.503	-0.321	-0.980	0.054	-0.036	-0.240	0.438*
Micronaire value	0.084	-0.048	0.460	-0.144	0.301	0.278	0.764	-0.142	-0.088	-0.245	-0.588**
Fibre strength	-0.581	0.081	0.257	0.071	-0.251	-0.162	-0.521	-0.056	-0.102	-0.098	-0.465
Uniformity ratio	-0.071	0.003	-0.292	-0.240	0.657	0.578	0.148	-0.111	-0.085	-0.238	-0.414*
Maturity ratio	0.064	-0.360	-0.414	0.092	0.837	0.428	-0.761	0.152	-0.100	-0.196	-0.510**
Fibre elongation	-0.251	-0.300	-0.143	-0.073	0.612	0.914	-0.690	-0.118	0.130	-0.322	-0.436*
Short fibre index	-0.077	-0.070	0.307	0.180	-0.642	-0.332	0.726	0.087	0.122	0.344	0.467*

\*, \*\* significant at 5 and 1% probability levels respectively.

length, root length, shoot/root length ratio, shoot weight and root weight, while shoot/root weight ratio had negative direct effects. Salahuddin et al.

(2010) recorded that highly significant positive correlation ( $r = 0.567$ ) was displayed by sympodial cotton yield was greatly influenced by sympodial

branches. The coefficient of determination ( $r^2 = 0.321$ ) revealed 32.1% variation in the seed cotton yield plant<sup>-1</sup> due to its relationship with sympodial

branches plant<sup>-1</sup>. Regression coefficient (b = 5.66) showed that a unit increase in sympodial branches plant<sup>-1</sup> resulted into a proportional increase of 5.66 gm in seed cotton yield plant<sup>-1</sup>, whereas bolls plant<sup>-1</sup> exhibited strong positive association with seed cotton yield (r = 0.959). The coefficient of determination (r<sup>2</sup> = 0.92) revealed 92% of the total variation in seed cotton yield attributable to the variation in number of bolls plant<sup>-1</sup>. The regression coefficient (b = 3.37) indicated that for a unit increase in bolls plant<sup>-1</sup>, there would be a proportional increase of 3.37 gm in seed cotton yield plant<sup>-1</sup>. Boll weight displayed a highly significant positive correlation (r = 0.597) with seed cotton yield plant<sup>-1</sup>.

The result of genetic variability, character association and path coefficient analysis confirmed that the characters number of monopodia and sympodia plant<sup>-1</sup>, plant height, number of bolls plant<sup>-1</sup>, average boll weight, lint yield plant<sup>-1</sup> and span length were important in respect of genetic variability, correlation and path coefficient analysis. The greater variability in these characters would give a prime scope for the development of high yielding through selection in the segregating generation.

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