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*Full Length Research Paper*

# Gender differences and challenges in cassava production and processing in Abia State, Nigeria

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**The study investigated the level of involvement of male and female farmers in small scale production and processing of cassava in Abia State, Nigeria. The objectives of the study were to determine the socioeconomic profile of the cassava farmers, identify the constraints in cassava production and suggest the possible remedies to improve cassava production in the study area. The result revealed that despite the fact that both male and female farmers were actively involved in cassava production and processing, but in terms of labour, the women dominated in most of the activities like planting, weeding and harvesting of cassava. There is also a significant difference in the labour involvement both in production and processing. It also revealed that land ownership was one of the major problems in the area. Women do not own land according to the tradition, and this discourages agricultural production among women. Other constraint like lack of input, lack of fund and high cost of labour were militating against cassava output in Abia State, Nigeria. Based on the constraints, the farmers suggested ways to improve cassava production in the study area. The major suggestions were provision of input, provision of agricultural subsidy/labour. It was recommended that state government should address the problem of farmers by assisting them with improved inputs. Loans should be given to farmers. Land ownership in the rural areas should be addressed by government so that everybody will have equal right to land ownership.**

**Key words:** Gender differences, challenges, cassava production, cassava processing and food security.

## INTRODUCTION

Cassava (*Manihot esculenta* Cranz) originated from South America and is grown in over ninety countries of the world mostly in the tropics where it constitutes one of the most important sources of energy in the diet and provides livelihood for over 500 million people (Okogbenin et al., 2008). Cassava is an important root crop that is widely grown throughout the tropical areas. It ranks fourth in terms of production and output after wheat, rice and maize (International Institute of Tropical Agriculture, 2000). Cassava is particularly important

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because it has the ability to tolerate drought condition and poor soil, and has relatively low requirement for labour which makes it attractive for nations in search of food securities for their people (Anuebuwa and Iloka, 1998).

Cassava is a major crop consumed in various processed forms in Nigeria (Anuebuwa and Iloka, 1998). It is a major source of energy with high food security value similar to most cereal crops (Achnewhu and Owuamanam, 2001). Every part of cassava is useful. The roots of some varieties are eaten boiled or roasted. The roots are processed into flour and garri. Cassava flour is mixed with maize flour and steamed to make fufu which is a major food in many homes in Nigeria (Ugwu, 1996). The flour is increasingly being partly substituted for wheat flour in bakery and fast food industries. Cassava leaves are a significant source of protein, minerals and vitamins (Philips et al., 2004; FAO, 2004). It is also found to be important for feeding livestock and as an industrial raw material e. g. flour and starch for food industry (Anuebuwa et al., 1998). The peels are used as livestock feed (Unanma, 2003). Cassava is gaining more recognition as a cash crop for international trade.

Cassava is a major cash crop for most of the farmers in Nigeria. Reports have further revealed that a higher proportion of cassava farmers, in Nigeria get a higher income from its production than they get from most other major staples (Ugwu, 1996). Hence, it has great potentials and plays a crucial role in contributing to food security, income generation, poverty alleviation and socioeconomic growth of Nigeria. World food monitoring reports indicated that Nigeria has consistently maintained the leading position as world largest producer of cassava in the recent years (Philips et al., 2004). The annual production record of cassava in the country stands at about 38.17 million metric tones (FAO, 2005). Yet most of the starches for the food industries are being imported while Nigeria has the potential of producing all the starch required by the food industries.

Cassava based farming communities coped better in hunger stressed times and uncomfortable situations (Moses et al, 2007). Cassava can be processed into a number of important food products including ‘gari’ that can store for over a year without any loss in food quality. Cassava chips, when properly fried, can store a year or more without any loss in quality. Cassava roots (traditional varieties) can be stored in the ground for up to 24 months, and some varieties for up to 36 months. Harvest may be delayed until market, processing, or other conditions are favourable (Onyeka et al., 2005). These attributes make cassava an important food security crop in Nigeria and several other sub-Saharan African countries that produce the crop.

In South eastern Nigeria, before the Nigeria civil war in 1967-1970, cassava was regarded as a women’s crop (Unamma, 2003). Then it was only women that cultivated cassava. They earned their money from growing cassava either as intercrops within their husbands’ yam field or intercropped it with other minor crops. The trend has change in the recent times as men are going into cassava production and processing even though their level of involvement and contributions along side with their female counterparts are not equal. However, it is a known fact that men and women perform different functions, have unequal decision power and differences in access to production resources in agricultural production (Ironkwe et al., 2007). Because of these differences, their views, needs and priorities to improve their productive potentials also differ. This could strongly affect their various outputs even in cassava production and processing. Hence, the understanding of gender contributions to food output in rural households in Nigeria is important in view of the recent global food crisis and the need to increase and sustain agricultural production in the country. This will ensure effective allocation of production resources for increased and sustainable production within the rural household. In effect, the increase in agricultural production arising from this will increase the farm output of the households, and improve their standard of living (Onyemauwa et al., 2007).

## Objective of the study

The general objective of the study was to determine the differences in the level of involvement of both gender in production and processing of cassava in Abia State, Nigeria. Specific objectives include:

(1) Determine the socio-economic characteristic of the male and female farmers.
(2) Determine the level of involvement of male and female farmers in cassava production and processing in the study area.
(3) Identify and analyze the constraints militating against cassava production and processing by male and female farmers.
(4) Suggest remedies to improve production by the male and female farmers.

## Research questions

In view of the objective of this study, these research questions were asked:

(1) Is there any difference between the level of involvement of male and female farmers in cassava production and processing in the study area?
(2) What are the constraints militating against cassava production and processing in Abia state.

## Hypothesis

(1) There is no significant difference in the level of involvement of male and female farmers in cassava

production
(2) There is no significant difference in the level of involvement of male and female farmers in cassava processing
(3) There is no significant difference in the constraints militating against the male and female farmers in the area.

## METHODOLOGY

The survey research design was adopted in this study. The approach was used because it provided the researchers the opportunity of sampling the opinions of large representative sample. According to Isangedighi et al. (2004), survey approach enables a researcher to study large and small populations by selecting and studying samples from the population in order to discover the relative incidence, distribution, interrelation of sociological and psychological variables.

The study was conducted in Abia State in 2012. Out of the seventeen local government areas in the State, five local government areas were randomly selected. They were Umunneochi, Bende, Umuahia South, Isiala Ngwa North and Ukwa East Local Government Areas. Two communities were randomly selected from each Local Government Area making it a total of 10 communities. The population comprised of all cassava farmers in the ten selected communities. From each community, a stratified random sampling technique was used to select five female and five male cassava farmers making 50 male and 50 female cassava farmers. A total of 100 cassava farmers were used for the study.

A well structured questionnaire was used as an instrumental guide to interview the respondents. Information sought from respondents included their personal characteristics, activities carried out on production and processing of cassava, cost of production and income from cassava per hectare, production resources used, constraints faced etc. The questionnaire was initially validated by two test experts in Department of Agricultural Economics, University of Nigeria, Nsukka. Thereafter, it was trial tested on 10 cassava farmers in Umuahia urban. Cronbach Alpha Analysis was used to ascertain the internal consistency of the items. Result gave overall Reliability Coefficient value of 90.

Data were analyzed using descriptive statistics such as mean, frequency and percentages. The t-test was used to analyze the extent of involvement of male and female cassava farmers in production and processing of cassava. A three point likert continuum of (3) serious, (2) less serious and (1) not serious constraints were used to determine the level of constraints in cassava production and processing in the area. The mean calculated as follows: 3+2+1=6/3=2. Anything less than 2 is not a constraint while 2.0 and above is a constraint (Alfred, 2006).

## RESULTS AND DISCUSSION

The socio-economic characteristics of the cassava farmers are presented in Table 1. Table 1 revealed that more of the younger female farmers than male were in cassava production in the State. This shows that cassava farmers in Abia State are more of female youths than male youths who are between 20 to 40years. This agrees with Pur et al. (2007) that the level of male youth involvement in agriculture has reduced due to urban migration, schooling and part-time farming. Majority of the respondents were married though greater population of the female (84%) where married than the male (70%) farmers. Majority 42% of both of the male and female farmers had the same household size (6-10). This indicates that they are involved in farming in order to take care of their large household. This is in agreement with Imoudu (2005).

Greater proportion of the women (36%) had no formal education while more than half of the male (76%) had both primary and secondary education; that is why (Heidi and Uday, 2001) emphasized on girl-child education for economic development, poverty alleviation and food security. However, greater proportion of the male (40%) had more years of farming experience (21-30 years) then the female folks. On the other hand, a greater percentage (64%) of female than male (24%) is full time farmers. The male 56% had larger farmer size (4 to 6 ha) than the female 44% (less than 1 ha). This shows that women are small scale farmers and this agrees with Ironkwe et al. (2007) who observed that women farm sizes are small and scattered. According to them this affects their production. The table also revealed that farm land was mostly owned by men as indicated by majority (88%) of male and (6%) of female respondents. This result agrees with Ironkwe and Asumugha (2007) that women do not own land due to culture and tradition in Africa.

In order to determine the level of involvement of male and female cassava farmers in cassava production and processing, the activities carried out in cassava production and major processed products were examined as shown in Tables 2 and 4 respectively. Table 2 reveals the distribution of respondents according to the activities carried out in cassava production. From the table, the total female farmers (married and single) dominated mostly in five major production activities out of eight. These include cutting of planting materials 85%, planting 75%, weeding 82%, harvesting 88% and fertilizer application 70%. The male dominated in two major production activities which include land clearing 83% and ridging 56%. Both genders participated equally in haulage and transportation 60% each. Also the table shows the female gender level involvement to be 62.5% that is $\frac{5}{8} x \frac{100}{1} = 62.5\%$ while the male involvement is $\frac{2}{8} x \frac{100}{1} = 25\%$. This indicates that females are more involved in the activities carried out in cassava production. This result also shows that women, both married and single are involved in less tedious farm activities than men. The tedious farm works were done by the men. For those whose husbands are sick or weak and single women, they hire the men to do the tedious work for them. This is in conformity with Achinowhu and Owuamanam (2001) that men are more involved in tedious farm activities than women.

The t-test on Table 3 showed that there was a significant difference between the male and female farmers involvement in cassava production. The t-

**Table 1.** Socio-economic Characteristics of the Respondents (Cassava Farmers).

| S/N | Variables | Male | | Female | | Total | |
|---|---|---|---|---|---|---|---|
| | | F | % | F | % | F | % |
| | **Age range (years)** | | | | | | |
| | 21-30 | 0 | 0 | 3 | 6 | 3 | 3 |
| 1 | 31-40 | 10 | 20 | 30 | 60 | 40 | 40 |
| | 41-50 | 28 | 56 | 10 | 20 | 38 | 38 |
| | > 50 | 12 | 24 | 7 | 14 | 19 | 19 |
| | **Marital status** | | | | | | |
| 2 | Single | 15 | 30 | 8 | 16 | 23 | 23 |
| | Married | 35 | 70 | 42 | 84 | 77 | 77 |
| | **Household size** | | | | | | |
| | 1-5 | 6 | 12 | 13 | 26 | 19 | 19 |
| 3 | 6-10 | 21 | 42 | 21 | 42 | 42 | 42 |
| | 11-15 | 14 | 28 | 9 | 18 | 23 | 23 |
| | >15 | 9 | 18 | 7 | 14 | 16 | 16 |
| | **Educational status** | | | | | | |
| | No formal education | 4 | 8 | 18 | 36 | 22 | 22 |
| **4** | Primary education | 20 | 40 | 16 | 32 | 36 | 36 |
| | Secondary education | 18 | 36 | 12 | 29 | 30 | 30 |
| | Tertiary education | 8 | 16 | 4 | 8 | 12 | 12 |
| | **Farming experience (yrs)** | | | | | | |
| | <1-10 | 5 | 10 | 7 | 14 | 12 | 12 |
| **5** | 11- 20 | 15 | 30 | 22 | 44 | 37 | 37 |
| | 21-30 | 20 | 40 | 17 | 22 | 31 | 31 |
| | > 30 | 10 | 20 | 10 | 20 | 20 | 20 |
| | **Farm involvement** | | | | | | |
| **6** | Fill time | 12 | 24 | 32 | 64 | 14 | 14 |
| | Part time | 38 | 76 | 18 | 36 | 56 | 56 |
| | **Farm size (ha)** | | | | | | |
| | <1 | 0 | | 22 | 44 | 22 | 22 |
| **7** | 1-3 | 10 | 20 | 15 | 30 | 25 | 25 |
| | 4-6 | 28 | 56 | 8 | 16 | 36 | 36 |
| | >6 | 12 | 24 | 5 | 10 | 17 | 17 |
| | **Land ownership** | | | | | | |
| **8** | Yes | 44 | 88 | 3 | 6 | 47 | 47 |
| | No | 6 | 12 | 47 | 94 | 53 | 53 |

Sources: Field survey 2012.

calculation of 2.44 exceeded the t-critical of 1.96 thereby the hypothesis which states that there is no significant difference in the involvement of male and female farmers in cassava production is rejected. Table 4 shows the major processed products by the respondents.

Table 4 revealed that both gender processed their cassava into the same products. Such products were garri, fufu, tapioca, alibor, starch etc. The result shows that both gender equally produced alibor and starch. However it was observed that more of females than the

**Table 2.** Distribution of respondents according to activities carried out in cassava production.

| Activities | Male | | | | | | Female | | | | | | Dominant gender |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Married | | Single | | Total | | Married | | Single | | Total | | |
| | F | X | F | X | F | X | F | X | F | X | F | X | |
| Land clearing | 70 | 2.1 | 13 | 0.39 | 83 | 2.49 | 12 | 0.36 | 5 | 0.15 | 17 | 0.5 | Male |
| Ridging | 46 | 1.38 | 10 | 0.30 | 56 | 1.68 | 40 | 1.2 | 4 | 0.12 | 44 | 1.32 | Male |
| Cutting of planting materials | 10 | 0.3 | 5 | 0.15 | 15 | 0.45 | 77 | 2.3 | 8 | 0.24 | 85 | 2.55 | Female |
| Planting | 20 | 0.6 | 5 | 0.15 | 25 | 0.75 | 68 | 2.04 | 7 | 0.21 | 75 | 2.25 | Female |
| Weeding | 10 | 0.3 | 8 | 0.24 | 18 | 0.54 | 64 | 1.93 | 8 | 0.24 | 82 | 2.46 | Female |
| Fertilizer application | 20 | 0.6 | 10 | 0.30 | 30 | 0.90 | 62 | 1.86 | 8 | 0.24 | 70 | 2.1 | Female |
| Harvesting | 18 | 0.54 | 3 | 0.09 | 22 | 0.66 | 80 | 2.4 | 8 | 0.24 | 88 | 2.64 | Female |
| Haulage and transportation | 40 | 1.2 | 10 | 0.30 | 50 | 1.5 | 43 | 1.29 | 7 | 0.21 | 50 | 1.5 | Both gender |
| Overall | | 0.88 | | 0.24 | | 1.12 | | 1.67 | | 0.21 | | 1.9 | |

Source: Field survey 2012, Multiple responses were recorded, The number of farmers is 100 which means the frequencies are the same with percentage.

males were involved in processing of cassava into various food forms. The male gender level of involvement is $\frac{1}{8} x \frac{100}{1} = 12.5\%$ while female gender level of involvement is $\frac{5}{8} x \frac{100}{1} = 62.5\%$ . The results agree with Unamma (2003) who recorded that cassava production and processing is dominated by women and cassava being regarded as women's crop.

The t-test on Table 5 showed that there was a significant difference between the male and female farmers involvement in cassava processing. The t-calculation of 2.56 exceeded the t-critical of 1.96 thereby the hypothesis that there is no significance difference in the involvement of the male and female in cassava processing is rejected.

Table 6A and B shows the constraints militating against cassava production and processing in the study area. Table 6 shows the constraints militating against male farmers in cassava production and processing in the study area. Majority ($\bar{x}$ 2.40), ($\bar{x}$ 2.28), (($\bar{x}$2.16),($\bar{x}$2.10), ($\bar{x}$ 2.10), ($\bar{x}$ 2.04) saw lack of fund, lack of improved planting materials, use of crude implements, lack of inputs (that is fertilizer and herbicides), climate change and lack of processing machines respectively as major constraints militating against male cassava farmers.

Table 6B shows the constraints militating against female cassava farmers in the study area. Majority ( ($\bar{x}$ 3.0), ($\bar{x}$ 2.7),($\bar{x}$2.53),($\bar{x}$2.4), ($\bar{x}$2.3),($\bar{x}$2.1),($\bar{x}$2.1),($\bar{x}$2.0)) saw land tenure system, lack of fund, lack of improved planting materials, high cost of labour, crude implements, low storability, lack of processing machine and labour intensive respectively as major constraints militating against women farmers in the area.

Land tenure problem was one of the most constraints facing the women in cassava production. The way land is owned often discourages land utilization. Land is owned by inheritance hence land is fragmented over generations. Women, according to tradition do not own land. So, their farm land is the one given by their husbands or fathers which they also use for their yam cultivation. Most of the times, it is when the men permits that the women can plant on the land. Another major constraint faced by the women is labour intensiveness. The women experience labour shortages because of unending migration of able bodied youth from the rural to the urban areas creating labour shortage, especially at the peak period when labour is required for land preparation, planting, weeding, harvesting etc. Also, hired labour shortage has driven up the cost of labour making such labour unprofitable to the farmer. The women are faced with the challenges of doing most of the labour themselves.

Lack of fund affected both genders because they are poor, they cannot secure the necessary

**Table 3.** The t-test statistical analysis of the significance difference between mean ratings of male and female farmers' involvement in cassava production.

| Respondents | N | X | SD | df | Cal-t | Crit-t | SL | Decision |
|---|---|---|---|---|---|---|---|---|
| Male | 50 | 1.12 | 0.62 | 98 | 2.44 | 1.96 | 0.05 | Rejected |
| Female | 50 | 1.92 | 0.89 | | | | | |

Source: Calculated from survey data 2912.

**Table 4.** Distribution according to major processed products.

| Processed products | Male | | | Female | | | Total | | Dominant gender |
|---|---|---|---|---|---|---|---|---|---|
| | F | % | X | F | % | X | F | % | |
| Garri | 40 | 80 | 2.40 | 50 | 100 | 3.00 | 90 | 90 | Female |
| Fufu | 24 | 48 | 1.44 | 44 | 88 | 2.64 | 68 | 68 | Female |
| Tapioca | 25 | 50 | 1.25 | 45 | 90 | 2.70 | 70 | 70 | Female |
| Alibor | 15 | 30 | 0.90 | 15 | 30 | 0.90 | 30 | 30 | Both gender |
| Abacha | 20 | 40 | 1.20 | 42 | 84 | 2.52 | 62 | 62 | Female |
| Chips | 42 | 84 | 2.52 | 22 | 44 | 1.32 | 64 | 64 | Male |
| Cassava flour | 34 | 68 | 2.04 | 45 | 90 | 2.70 | 79 | 79 | Female |
| Starch | 8 | 16 | 0.48 | 8 | 16 | 0.48 | 16 | 16 | Both gender |
| Overall | | | 1.53 | | | 2.03 | | | |

Source: Field survey 2012, Multiple responses were recorded.

**Table 5.** The t-test statistical analysis of the significance difference between mean ratings of male and female farmers' involvement in cassava processing.

| Respondents | N | X | SD | df | Cal-t | Crit-t | SL | Decision |
|---|---|---|---|---|---|---|---|---|
| Male | 50 | 1.53 | | 98 | 2.56 | 1.96 | 0.05 | Rejected |
| Female | 50 | 2.03 | | | | | | |

Source: Calculated from survey data 2012.

collateral for loans, cannot have access to credit facilities and cannot procure the sophisticated machines for processing. Climate change was another constraint for both genders, they experience drought or long period without rain leading to poor harvest, excessive sunshine leading to increase in temperature and unfavourable climate that reduces farm activities. Lack of improved planting materials, lack of processing machines and crude implements also affected both genders. Farmers still rely on the use of tools like hoes, cutlass, rake, shovel etc for their activities instead of ploughs, cultivators, etc. and this leads to drudgery of farmers, time wasting, low yield and low farm income. Improved planting materials were not readily available and the processing machines are limited. Tables 6A and B revealed that both male and female cassava farmers are faced with many constraints. The results are in agreement with Ironkwe et al. (2007) that farmers are faced with constraints that hinder the productivities. It can also be ascertained that the female farmers has more constraints example land tenure system, labour intensiveness etc than their male counterparts. Therefore the hypothesis which states that there is no significant difference in the constraints between male and female cassava farmers in the area is thereby rejected. Table 7 reveals the suggested remedies to improve cassava production in Abia State, Nigeria. Table 7 shows the suggested remedies by the male and female farmers to improve cassava production in Abia State, Nigeria. Majority 80% suggested provision of loan 78% provision of inputs, 68% suggested reduced cost of herbicides and fertilizer and 60% suggested provision of land while 40% suggested development of variety that resist goat attack.

## CONCLUSION AND RECOMMENDATIONS

The study revealed that both male and female farmers were actively involved in cassava production and processing, but female farmers dominated in most

**Table 6A.** Distribution of male farmers according to constraints in cassava production and processing.

| S/N | Constraints | Serious | Less serious | Not serious | Mean |
|---|---|---|---|---|---|
| 1 | Lack of fund | 40 | 5 | 5 | 2.40 |
| 2 | Lack of improved planting material | 38 | 10 | 2 | 2.28 |
| 3 | Crude implements | 34 | 10 | 6 | 2.16 |
| 4 | Lack of inputs | 35 | 10 | 5 | 2.10 |
| 5 | Climate change | 35 | 15 | 0 | 2.10 |
| 6 | Lack of processing Machines | 34 | 8 | 8 | 2.04 |
| 7 | High cost of labour | 30 | 13 | 7 | 1.80 |
| 8 | Land tenure system | 18 | 22 | 10 | 1.08 |
| 9 | Low storability | 10 | 30 | 10 | 0.60 |
| 10 | Labour intensive | 10 | 28 | 12 | 0.60 |
| 11 | Lack of extension contact | 5 | 18 | 27 | 0.30 |
| 12 | Poor marketing | 5 | 15 | 30 | 0.30 |
| 13 | Poor yield | 5 | 10 | 35 | 0.30 |
| 14 | Low adaptability | 5 | 10 | 35 | 0.30 |
| 15 | High mortality | 2 | 9 | 39 | 0.12 |
| 16 | Pest/ disease attack | 2 | 10 | 38 | 0.12 |
| 17 | Poor branding | 0 | 10 | 40 | 0 |
| 18 | Poor products Quality | 0 | 5 | 45 | 0 |

Source: Field survey 2012, Multiple responses are recorded.

**Table 6B.** Distribution of female farmers according to constraints in cassava production and processing.

| S/N | Constraints | Serious | Less serious | Not serious | Mean |
|---|---|---|---|---|---|
| 1 | Land tenure system | 50 | 0 | 0 | 3.0 |
| 2 | Lack of fund | 45 | 5 | 0 | 2.70 |
| 3 | Lack of planting material | 42 | 7 | 1 | 2.53 |
| 4 | High cost of labour | 40 | 7 | 3 | 2.40 |
| 5 | Crude implements | 38 | 12 | 0 | 2.30 |
| 6 | Climate change | 35 | 15 | 0 | 2.10 |
| 7 | Lack of processing Machines | 35 | 10 | 5 | 2.10 |
| 8 | Labour intensive | 34 | 10 | 6 | 2.0 |
| 9 | Lack of inputs | 30 | 15 | 5 | 1.80 |
| 10 | Low storability | 25 | 15 | 10 | 1.50 |
| 11 | Poor marketing | 25 | 15 | 10 | 1.50 |
| 12 | Poor yield | 10 | 15 | 25 | 0.60 |
| 13 | Lack of extension contact | 7 | 8 | 35 | 0.40 |
| 14 | Poor products Quality | 5 | 7 | 38 | 0.30 |
| 15 | Low adaptability | 5 | 10 | 35 | 0.30 |
| 16 | Pest/ disease attack | 5 | 10 | 35 | 0.30 |
| 17 | High mortality | 3 | 7 | 40 | 0.18 |
| 18 | Poor branding | 0 | 10 | 40 | 0 |

Source: Field survey 2012, Multiple responses are recorded.

activities in terms of labour in production and processing. They had some similar constraints though the women had more constraints like labour intensiveness, land tenure problem and marketing problems. Therefore policies geared towards achievement of cassava production in the state should be gender based. Such policies should critically examine the farmers' production environment, constant priorities needs and goals on gender basis. This is necessary if cassava production and processing must be increased and sustained to

prevent hunger in the face of the global food crisis, increase farmers' income and improve standard of living of the rural households. It was recommended that state government should address the problem of farmers by assisting them with improved inputs. Adopting of modern farming and husbandry procedures such as planting of improved seedlings, application of agricultural chemical for pest and disease control, and enhanced yield should be facilitated by assisting farmers in sourcing improved technologies. Small scale irrigation should be promoted and strengthened. Government should increase funding of the agricultural sector so as to improve efficiency of institutional agencies for agricultural development. Loan should be given to farmers. Land ownership in the rural areas should be addressed by government so that everybody will have equal right to land ownership.

## Conflict of Interest

The authors have not declared any conflict of interest.

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*Review*

# Solar photovoltaic water pumping system for irrigation: A review

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**Irrigation is a well established procedure on many farms and is practiced on various levels around the world. It allows diversification of crops, while increasing crop yields. However, typical irrigation systems consume a great amount of conventional energy through the use of electric motors and generators powered by fuel. Photovoltaic energy can find many applications in agriculture, providing electrical energy in various cases, particularly in areas without an electric grid. In this paper the description of reviews on a photovoltaic irrigation system, is presented. Photovoltaic water pumping system is one of the best alternative methods for irrigation. The variation of spatial and temporal distribution of available water for irrigation makes significant demand on water conservation techniques. Hence solar powered Automated Irrigation System provides a sustainable solution to enhance water use efficiency in the agricultural fields using renewable energy system removes workmanship that is needed for flooding irrigation. The use of this photo-irrigation system will be able to contribute to the socio-economic development. It is the proposed solution for the energy crisis for the Indian farmers. This system conserves electricity by reducing the usage of grid power and easy to implement and environment friendly solution for irrigating fields.**

**Key words:** Solar photovoltaics, water pumping system, irrigation, photovoltaic (PV) pumping system

## INTRODUCTION

Solar energy is the most abundant source of energy in the world. Solar power is not only an answer to today's energy crisis but also an environmental friendly form of energy. Photovoltaic (PV) generation is an efficient approach for using the solar energy. Solar panels (an array of photovoltaic cells) are now extensively used for running street lights, for powering water heaters and to meet domestic loads. The cost of solar panels has been constantly decreasing which encourages its usage in various sectors. One of the applications of this technology is used in irrigation systems for farming. Solar powered irrigation system can be a suitable alternative for farmers in the present state of energy crisis in India. This is green way for energy production which provides free energy once an initial investment is made (Harishankar et al., 2014).

Today the generation is heading towards ultra-technologies. Water pumping has a long history; so many methods have been developed to pump water. People have used a variety of power sources, namely human

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energy, animal power, hydro power, wind, solar and fuels such a diesel for small generators.

The most common pumps used in remote communities are:

i) Hand pumps
ii) Direct drive diesel driven borehole pumps
iii) Electric submersible pumps with diesel generator
iv) Solar submersible pumps

**Photovoltaic cells**

Photovoltaic cells are devices which 'collect the light and convert it into electricity. The cells are wired in series, sealed between sheets of glass or plastic, and supported inside a metal frame. These frames are called solar modules or panels. They are used to power a variety of applications ranging from calculators and wrist-watches to complete home systems and large power plants. PV cells are made of thin silicon wafers; a semi-conducting material similar to that used in computer chips. When sunlight is absorbed by these materials, the solar energy knocks electrons loose from their atoms, allowing the electrons to flow through the material to produce electricity. This process of converting light (photons) to electricity (voltage) is called the "photovoltaic effect".

**PV applications**

Solar panels are used in a variety of applications. The applications vary from small simple lanterns to large elaborate power plants.

i) Rural and urban households for domestic purposes like lighting.
ii) Communities, small industries and institutions like schools, for lighting as well as for powering television sets, computers, etc.
iii) Water pumping systems.
iv) Telecommunications, as these systems are often installed in isolated places with no other access to power.
v) Refrigeration of vaccines at health center in rural areas. Such solar refrigerators are also utilized to store blood plasma. WHO supports programmers that install solar power for medical purposes.

**System components**

The whole system of solar pumping includes the panels, support structure with tracking mechanism, electronic parts for regulation, cables, pipes and the pump itself.

**i) Solar panels or modules:** Solar panels are the main components used for driving the solar pump. Several solar panels connected together in arrays produce DC electricity, interconnections are made using series or parallel combinations to achieve desired voltage and power for the pump.
**ii) Solar pump:** Centrifugal or submersible pumps are connected directly to the solar array using DC power produced by the solar panels. Solar pumps are available in several capacities depending upon the requirement of water.
**iii) Support structure and tracking mechanism:** Support structure provides stability to the mounted solar panels and protects them from theft or natural calamities. To obtain maximum output of water, a manual tracking device is fixed to the support structure. Tracking increases the output of water by allowing the panels to face the sun as it moves across the sky.
**iv) Foundations (array and pump):** Foundations are provided for support structures and pump.
**v) Electrical interconnections:** A set of cables of appropriate size, junction boxes, connectors and switches are provided along with the installation.
**vi) Earthing kit:** Earthing kit is provided for safety in case of lightning or short circuit.
**vii) Plumbing:** Pipes and fittings required to connect the pump come as part of the installation.

**How the solar pump system works**

A 50-watt photovoltaic solar panel can power a 12-volt pump, which can move 1,300 to 2,600 L/h. Standard plastic fittings and half-inch piping connect these elements to a water saving tank of 500 to 1,000 L. A sturdy stand should be built for the water tank to provide gravity flow, and a frame should also be constructed to provide the best angle for the solar panels. Multiple filters are needed to protect the life of the pump and minimize clogging in sprinkler emitters and tubes. A solar pump combined with affordable drip irrigation kits can be used with a wide variety of high-value crops to increase water efficiency, minimize fertilizer loss, and irrigate hilly terrains.

**Aspects**

In general, the investment required for a PV pumping system is Rs 250-300/Wp (where Rs is the Indian rupee and Wp is watts peak). For example, the cost of a 900 Wp unit would be Rs 225,000-270,000, but with subsidies, this will be reduced to Rs 50,000. To make the best use of solar energy, the PV system, the groundwater pump and the water distribution system have to be well matched. The PV power provided must cover the power demand of the pump adequately. This is determined by the relationship between the required discharge flow, the total head and the pump efficiency. This depends on the

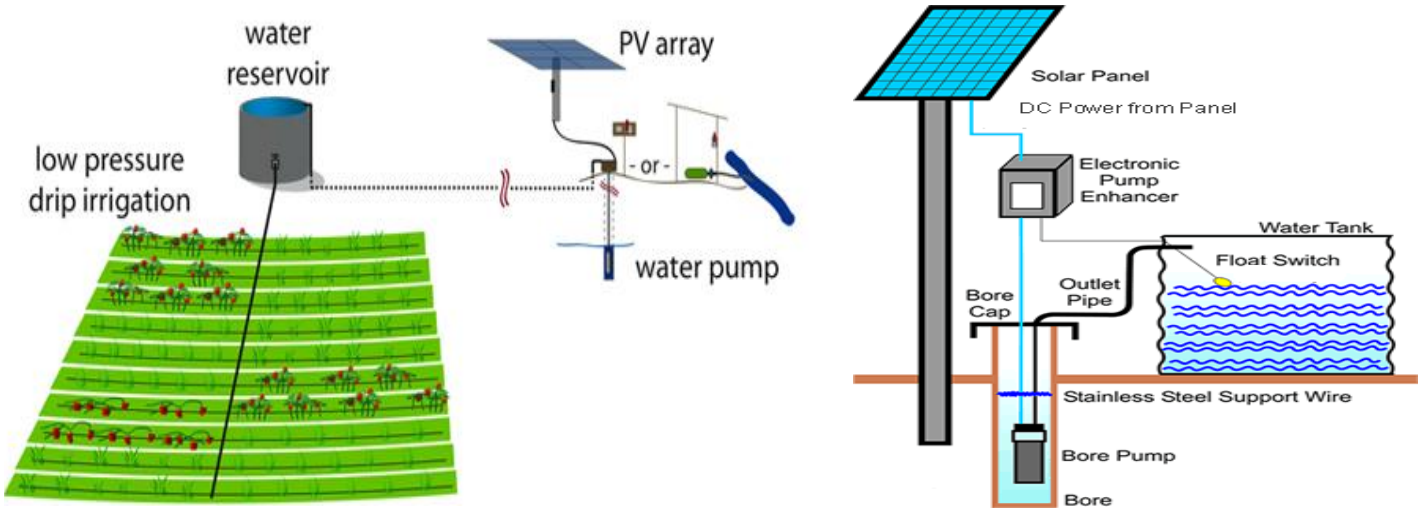


**Figure 1.** Components of solar PV irrigation system.

type of pump, which in turn depends on the depth of the available water source. Although positive displacement pumps are preferred for large heads, centrifugal pumps are most commonly used for this as shown in Figure 1.

Photovoltaic (PV) panel electrical outputs are rated according to industry Standard Test Conditions (STC) of 1000 W $m^{-2}$ incident solar radiation at an operating cell temperature of 25°C and under an absolute air mass of 1.5. Environmental conditions met outside the laboratory will cause a decrease in PV performance from the STC rating, the magnitude of which depends on the module technology. Many additional losses are incurred due to the inefficiencies in transferring energy from the PV panels to a load, such as a pump or battery bank, thus resulting in a secondary decline of performance. Though there have been studies measuring outdoor performance of PV modules, there is a great need for further field studies of complete PV systems.

Another important aspect would be the ability to model the potential solar radiation, PV power output, and subsequent water output for the purpose of irrigation scheduling. Photovoltaic powered water pumping systems (photo-irrigation) have been studied by researchers for many years. Studies mostly concentrated on DC motors because energy obtained from solar panel is DC (Lawrance et al., 1995; Dursun and Saygin., 2005). These are shown that better results were obtained for performance analysis (Kolhe et al., 2004; Kolhe et al., 2000). Photo-irrigation system has advantages over flood irrigation, for bringing efficient utilization of water sources, preventing erosion and growing of weeds (Cuadros et al., 2004), decreasing moisture stress (Pande et al., 2003), no operation cost, providing opportunity for local energy sources and exhibiting a parallel point of view with water requirement (Ghoneim, 2006). In terms of automation, developed wireless technologies, researches focused on automatic irrigation with sensors in agricultural systems (Kim and Evans, 2009; Stone et al., 1985).

The advantages of using wireless sensor is to reduce wiring and piping costs, and easier to install and maintain especially over large areas (Dursun and Ozden, 2010). Energy of pumps used for the agricultural irrigation is generally provided from electrical energy or fossil fuels. Solar energy that is sensitive to environment, clean and requiring no maintenance is an alternative renewable energy source especially for countries like Turkey having a high amount of annual solar irradiation rate. Means for requirement for irrigation PV pumping systems has advantage of water demand (Anis and Metwally, 1994). In summer months obtained solar energy increases and also naturally water requirement of trees increases.

The cost of solar PV has come down and cost of diesel has been regularly increasing. At present the cost of solar PV is very much less than diesel, solar PV cost shall be half of diesel within three to four years, since approaching towards grid parity. 400,000 telecom towers are associated with diesel generating sets having capacity 3 to 5 kW. 60% Telecom towers located in urban and semi urban areas and 100% located in the villages are run by diesel generating sets. In fact, off-grid potential is unlimited in India and is about 20 to 25% potential of the world (Arora, 2014). Solar water pumps are often thought of as being an expensive technology, which is not able to pump enough water and which is not durable. However, solar water pumps have come a long way in 25 years and today there are solar pumps on the market which have improved on previous technology, e.g.: Submersible pumps which can pump up to 200 m heads; pumps that are able to pump larger volumes of water, e.g.: At 100 m, about 10,000 L/day; At 50 m, about 20,000 L/day. Above performance can be doubled through dual systems (if the borehole allows this).

i) Low maintenance requirements (3 to 5 years);
ii) Good performance which means fewer solar panels to pump the same amount of water;
iii) Some of the pump models can be backed-up by a genset to pump additional water with the same pump during the night or during overcast days;
iv) Good quality and reliability
v) Simple to install.

**Furthermore, solar pumps are well known for having the following features:**

i) Require minimal attention as they are self-starting;
ii) Solar pumps are “good” for boreholes as they pump over the whole day;
iii) Weak boreholes can be used effectively with a low volume pump due to pumping 8 to 10 h a day;
iv) In most cases, a solar pump offers an ideal solution to the diesel option which requires operating funds (with uncertainty about future diesel prices), time investment for operating pump (manual starting etc.) and logistics for fuel, maintenance, installation and de-installation;
v) Tracking arrays can be used to increase daily water pumping rates;
vi) Solar pumps offer clean solutions with no danger of borehole contamination.

Photovoltaic (PV) technology is used for generating electricity from the incoming solar radiation. Several attempts have been made to evaluate, monitor and improve the performance of different components of a PV system: a PV module (Abdallah, 2004; Vick and Clark, 2004; Huang and Sun, 2007; Hansen et al., 2000; Lorenzo, 1994), a controller (Hohm and Ropp, 2003), a battery (Copetti et al., 1993; Gergaud et al., 2003; Achaibou et al., 2012), a pump (Vick and Clark, 2011), and a pump motor (Bhat et al., 1987). These, and similar studies have been effective for improving the efficiency of the PV system components. However, several factors need to be considered for an optimal PV system design to achieve the desired reliability of the system in a given environment. This involves a detailed investigation of all interacting physical (plant and soil type, irrigation system specifications, PV system sizing, site attributes), meteorological (solar radiation, air temperature, relative humidity, wind speed, precipitation) and managerial (irrigation scheduling) variables with the aim of achieving the desired reliability of the PV system. Ultimately, a technique that combines the center pivot irrigation system characteristics, daily crop water requirements, soil moisture status, irrigation applications, PV array output, load demands, and energy storage is required for evaluating a solar-powered center pivot irrigation system in terms of its reliability. This sort of holistic approach could be very beneficial for effective sizing of the system.

Environmental conditions met outside the laboratory will cause a decrease in PV performance. Important environmental conditions to consider are the insolation, ambient temperature, and wind speed (Van Dyk et al., 2005).

The setup of a PV system is also very flexible. The most efficient use of solar energy is when the panels are directly connected to the load. In fact, the success of water pumping lies partly with the elimination of the intermediate phase, namely the battery bank, for energy storage. With a direct connection between the PV array and the pump, water can be pumped during sunlight hours. The most efficient form of direct-connect systems is when the water is being pumped to an elevated storage tank, thus the electrical energy from the panels is converted to potential energy of the elevated water, to be used on demand, often by gravity (Hamidat et al., 2003). The overall efficiency, from sunlight to water flow, has been recorded to exceed 3% (Daud and Mahmoud, 2005).

This system is easy to implement and environment friendly solution for irrigating fields. The system was found to be successful when implemented for bore holes as they pump over the whole day. Solar pumps also offer clean solutions with no danger of borehole contamination. The system requires minimal maintenance and attention as they are self-starting. To further enhance the daily pumping rates tracking arrays can be implemented. This system demonstrates the feasibility and application of using solar PV to provide energy for the pumping requirements for sprinkler irrigation. Even though there is a high capital investment required for this system to be implemented, the overall benefits are high and in long run this system is economical (Harishankar et al., 2014).

After economic analyzing, it is shown that photovoltaic pumping system for irrigation in Bangladesh is more feasible than diesel engine pumping system. In economic view point, PV pumping system for only one season irrigation is a little bit higher than the diesel engine pumping system due to high cost of PV module and its components (Haque, 2001). The automation of an irrigation system will largely reduce the gap between requirement and consumed energy and further conserves the resources thereby reducing the wastage of resource. The main advantage of this project is optimizing the power usage through water resource management and also saving government’s free subsidiary electricity. This proves an efficient and economy way of irrigation and this will automate the agriculture sector (Yalla et al., 2013).

India, developing a grid system is often too expensive because rural villages are frequently located too far away from existing grid lines. Even if fuel is available within the country, transporting that fuel to remote, rural villages can be difficult. There are no roads or supporting infrastructure in many remote villages. The use of renewable energy is attractive for water pumping applications in remote areas of many developing countries. Transportation of renewable energy systems,

such as photovoltaic (PV) pumps, is much easier than the other types because they can be transported in pieces and reassembled on site (Khatib, 2010). The life cycle cost analysis done that covered both systems proves that the PV water pumping system is the more economical choice over the diesel water pumping system (Narale et al., 2013).

According to Cuadros et al. (2004), this method was suitable for determining the size and thus viability of these solar powered irrigation systems since the cost of photovoltaic (PV) systems is fairly high. Not only is the viability looked at in terms of the cost of PV systems but also the land area required for implementation. Glasnovic and Margeta (2009) investigated the maximum areas which could be economically irrigated. Similar work done by Kelley et al. (2010) suggested that PV irrigation was technically and economically feasible, provided that there was enough land available for the solar array. One of the concerns regarding the use of solar panels for producing power is the amount of panels required and the area they would occupy. In the case of agriculture this is especially important since it directly impacts the area that would be left for planting This work showed that only a small percentage would be required on the two-acre plot for the panels. This demonstrates the feasibility and application of using solar PV to provide energy for the pumping requirements for drip irrigation.

Some of the factors were taken into consideration to calculate the pumping requirement and thus the solar panel area included the crop chosen, the size of the planting region, the number of peak sun hours, the efficiency of the solar array and its electronics, the pumping elevation and the pump efficiency. These factors would thus affect the feasibility of such systems. This study showed encouraging results for the use of solar panels in terms of the area required to house them to be used to generate power for the pumping requirement for drip irrigation of hot peppers on a two acre plot (Persad et al., 2011). Specific studies have looked at using PV systems on small farms (Roul, 2007) and previous feasibility studies evaluated either the economic feasibility or the technical feasibility of PV irrigation. Most of the studies were system size-specific and location-specific. Studies focusing on systems with power requirements on the order of 1 kW have been conducted for sites in Namibia, Jordan and India (Mahmoud, 1990; NAMREP, 2006; Meah et al., 2008). Most of the literature concluded that PV irrigation is both technically feasible for very small systems in the order of one acre (Kelley et al., 2010). Solar pumps have been talked about in India for some time. According to one 2005 estimate (Purohit and Michaelowa, 2005), some 7000 were already in operation in the field. However, solar-powered tube wells in actual use by farmers are not easy to find. With the cost of photo voltaic (PV) cells following the More's Law and falling steadily and the price of diesel soaring, solar- powered pumping has emerged as an economically feasible idea.

Water pumping has long been the most reliable and economic application of solar-electric (photovoltaic, or PV) systems. Most PV systems rely on battery storage for powering lights and other appliances at night or when the sun is not shining. Most PV pumping systems do not use batteries – the PV modules power the pump directly. Without batteries, the PV pumping system is very simple. It consists of just three components: the solar array, a pump controller and the pump. The only moving part is the pump. The solar modules are warranted to produce for 20 to 25 years. The expected life of most controllers is 5 to 10 years. Pump life can vary from 5 to 10+ years (and many are designed to be repaired in the field). Unless the pump or controller fails, the only maintenance normally required is cleaning the solar modules every 2 to 4 weeks. This task obviously can be done cheaply by non-skilled local labor (Aligah, 2011). Recently, Hammad (1999), presented a study related to the usage of photovoltaic generated electricity for pumping water from 13 wells spread across the east and south east desert which is far from the national grid, as well as in the southern parts of the Jordan which has a complicated topographical situation. These pumps are capable of pumping 40–100 $m^3$ of water per day individually to meet the daily demands of individuals living in those areas. A fully automated irrigation system is designed, built and tested using solar PV cells and a digital controller. The system is economical, reliable, portable, and compact. Savings in electricity bills and water bills can justify the initial cost, which may be a bit more than the conventional system, over a period of time. It causes less damage to the environment and releases the public utility from an extra load. It can be used in small or big farms, gardens, parks and lawns. Also, it can be used as a universal solar-based-controller to control building doors, water heaters, and air-conditioning control systems (Ali, et al., 2001).

The solar water-pumping technology is commercially available, has-proven record of reliability, require, minimal skilled manpower once in operation, and operation and maintenance cost is also very minimal and affordable. The photovoltaic pumps have many advantages including they operate on freely available sunlight and therefore incur no fuel or electrical costs. They are also environmentally friendly, reliable and have a long working life (Yingdong et al., 2011). The advantage of using solar energy for pumping the water is that major quantities of water are required during day time and that too during time when the sun is on top of our head, and during these times the PV panels produce maximum energy and hence the water quantity. These solar pumps can be installed in locations which are not connected to national electric grid (Ahmet, 2012).

PV systems for the pumping of groundwater are also used in Upp er Egypt, proving that the cost of the water unit pumped by PV systems is significantly lesser than

that pumped by diesel systems (Yingdong, 2011). 9 million pump sets for irrigation run by diesel out 21 million pump sets in India (3.73 kW (5 HP)). Out of these 9 million diesel pump sets 75% are assumed to be in solar resource region; total number of diesel pump sets in solar resource region comes to 6.75 million. Out of 6.75 million diesel pumps, 70% have land for installation of PV System; total numbers of pump sets in solar resource region and have land for installation of solar PV comes to 4.725 million, that is, 16,785 mW (just half of diesel pumps). The replacement of 4.5 million diesel pumps saves 223,800 million liter of diesel and 469.98 billion kg carbon dioxide per annum (Arora, 2013). The procedures reported above have shown that the optimal nominal electric power of the PV generator, for reference parameters in the Arilje region, with decade average daily water requirements of 12.8 $m^3$ $ha^{-1}$ $day^{-1}$, that would satisfy the raspberry demands throughout the entire irrigation observed period (Gajic et al., 2013). At annual operation of 2000 h, Claro Energy's 8.5 kW solar pumps costing Rs. 1 million will save some 17000 kWh of electricity each per year valued at Rs. 85000/year (Mukherji, 2007).

## Advantages

i) Low operating cost: One of the important advantages is the negligible operating cost of the pump. Since there is no fuel required for the pump like electricity or diesel, the operating cost is minimal.
ii) Low maintenance: A well-designed solar system requires little maintenance beyond cleaning of the panels once a week.
iii) Harmonious with nature: Another important advantage is that it gives maximum water output when it is most needed, that is, in hot and dry months.
iv) Flexibility: The panels need not be right beside the well. They can be anywhere up to 20 m away from the well, or anywhere you need the water. These pumps can also be turned on and off as per the requirement, provided the period between two operations is more than 30 s.

## Limitations

i) Low yield: Solar pumping is not suitable where the requirement is very high. The maximum capacity available with solar is very low. However, the output of the solar DC pump is more than a normal pump.
ii) Variable yield: The water yield of the solar pump changes according to the sunlight. It is highest around noon and least in the early morning and evening. So it should be operate during noon time.
iii) Theft: Theft of solar panels can be a problem in some areas. So the farmers need to take necessary precautions. Ideally, the solar system should insured against theft as well as natural hazards like lightning. It should be avoided by keeping fencing around it.

## Conclusions

Photovoltaic systems are especially designed to supply water and irrigation in areas where there is no mains electricity supply. Their main advantages over hand pumps or internal combustion engine pumps are their practically zero maintenance, their long useful life, that they do not require fuel, that they do not contaminate, and finally that they are straightforward to install. Another important characteristic is that, as they use the sun as their energy source, the periods of maximum demand for water coincide with the periods of maximum solar radiation. When compared to diesel powered pumping systems, the cost of solar PV water pumping system without any subsidy works out to be 64.2% of the cost of the diesel pump, over a life cycle of ten years. Solar pumps are available to pump from anywhere in the range of up to 200 m head and with outputs of up to 250 m³/day. In general photovoltaic pumps are economic compared to diesel pumps up to approximately 3 kWp for village water supply and to around 1 kWp for irrigation. Solar photovoltaic (SPV) sets represent an environment-friendly, low-maintenance and cost effective alternative to irrigation pump sets which run on grid electricity or diesel. It is estimated that India's potential for Solar PV water pumping for irrigation to is 9 to 70 million solar PV pump sets, that is, at least 255 billion litres/year of diesel savings.

A solar irrigation pump system methods needs to take account of the fact that demand for irrigation system water will vary throughout the year. Peak demand during the irrigation system seasons is often more than twice the average demand. This means that solar pumps for irrigation are under-utilized for most of the year. Attention should be paid to the system of irrigation water distribution and application to the crops. The irrigation pump system should minimize water losses, without imposing significant additional head on the irrigation pumping system and be of low cost.

## Conflict of Interest

The authors have not declared any conflict of interest.

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# Cloning and gene expression analysis of ascorbic acid biosynthesis enzymes in *Moringa oleifera*

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**Moringa (*Moringa oleifera*), which is a semi-tropical plant, is used as food and for the production of medicines and oil products, because of a large amount of various nutrients including ascorbic acid (AsA). Although Moringa leaf has a high AsA content, the molecular mechanisms of AsA accumulation in Moringa have received little attention. In this study, we isolated Moringa cDNAs for enzymes, belonging to the major AsA biosynthesis pathway (Smirnoff-Wheeler pathway) in higher plants. The predicted amino acid sequences showed 70% or more similarity to those of *Arabidopsis*. Quantitative RT-PCR indicated that Moringa GDP-L-galactose phosphorylase (*GGP*) is most highly expressed in Moringa during leaf development and light exposure. A significant high promoter activity of the Moringa *GGP* gene was detected by promoter assay in *Arabidopsis* protoplast.**

**Key words:** *Moringa oleifera*, ascorbic acid, biosynthesis enzymes, gene expression.

## INTRODUCTION

Ascorbic acid (AsA), vitamin C is reported as the sum of AsA and dehydroascorbic acid (DHA), is an essential human nutrient. Vitamin C, however, cannot be endogenously synthesized in the human body due to the absence of the last enzyme in the AsA biosynthesis pathway (Chatterjee, 1973), and must instead be obtained from fruit and vegetables in the diet. AsA has a variety of physiological roles (Smirnoff and Wheeler, 2000). For example, through its antioxidant properties, AsA scavenges reactive oxygen species (ROS) that are produced by abiotic stresses such as light (Asada, 2006), high and low temperature (Suzuki and Mittler, 2006), and drought (Helena and Carvalho, 2008). AsA also plays major roles in cell growth, photosynthesis, and control of anthesis (Barth et al., 2006; Mano et al., 2004). Scrutiny of AsA biosynthesis has led to several proposed synthesis pathways in plants, and one major pathway is the Smirnoff-Wheeler pathway, in which AsA is synthesized via D-mannnose and L-galactose (Kanter et al., 2005; Radzio et al., 2003; Wheeler et al., 1998; Wolucka et al., 2001; Zhang et al., 2008). The Smirnoff-Wheeler pathway has been characterized, and involves eight AsA biosynthesis enzymes, namely, phosphomannose isomerase, phosphomannomutase,

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GDP-D-mannose pyrophosphorylase (*GMP*), GDP-D-mannose-3',5'-epimerase (*GME*), GDP-L-galactose phosphorylase (*GGP*), L-galactose-1-phosphate phosphatase (*GPP*), L-galactose dehydrogenase (*GDH*), and L-galactono-1,4-lactone dehydrogenase (*GalLDH*) (Wheeler et al., 1998). The *vtc1* mutant of *Arabidopsis*, which is deficient in *GMP* gene, has 25% of wild-type AsA, and the *vtc2 Arabidopsis* mutant, which is deficient in *GGP* gene, has 10 to 20% of wild-type AsA (Conklin et al., 1999; Dowdle et al., 2007). Although other AsA biosynthesis pathways, such as the galacturonate and *myo*-inositol pathways, have been found to function in plant AsA biosynthesis (Agius et al., 2003; Lorence et al., 2004), the Smirnoff-Wheeler pathway appears to be predominant in higher plants.

Moringa (*Moringa oleifera* Lam), which is native to northwest India, is an important multi-purpose tree that is used as food and for the production of medicines and oil products (Morton, 1991). The many potential uses of Moringa have led to the recent publication of a number of reports discussing the use of seed- and leaf-powders and extracts for, among others, water purification, nutrition, and medicine (Anselme et al., 1995; Anwar and Bhanger, 2003; Bhuptawat et al., 2007). Moringa can grow rapidly in tropical areas as well as in soils with relatively low nutrients and low humidity (Morton 1991), and has a high leaf AsA content (Sreelatha and Padma, 2009), suggesting that it could serve as a valuable dietary source of vitamin C for populations in less-developed countries. However, an increase in AsA content is required to optimally utilize the already high production capacity in Moringa. To date, the molecular mechanisms through which AsA accumulates in Moringa have not been determined, and elucidation of these molecular mechanisms is essential for the future improvement of AsA content in Moringa leaf. In this study, Moringa cDNAs for AsA biosynthesis enzymes were identified and used to evaluate gene expression. Quantitative RT-PCR analysis indicated that *MoGGP* was most highly expressed in Moringa among biosynthesis gene of the six investigated. The 5'-upstream region of the *MoGGP* gene was determined, allowing investigation of *cis*-element(s) enhanced promoter activity using *Arabidopsis* protoplasts.

## MATERIALS AND METHODS

### Plant materials

Moringa plants were grown at 25°C in 16 h of light (light intensity; 55.6 µmol/s/$m^2$) and 8 h of dark in a greenhouse. To compare the AsA contents and mRNA expression levels of AsA biosynthesis enzymes in the leaves of Moringa and *Arabidopsis*, the small (leaf length; <10 mm), medium (leaf length; 10 to 15 mm) and large leaves (leaf length; >15 mm) were prepared from Moringa plant. To test the effects of light on mRNA expression levels of AsA biosynthesis enzymes, leaf discs (8 mm diameter) were prepared from Moringa small leaves using a biopsy punch (8.0 mm, Kai industries). Leaf discs were floated on water in a petri dish, incubated in the dark overnight, and were then exposed to continuous light treatment (100 µmol/s/$m^2$), or were left to continuous darkness (0 µmol/s/$m^2$) at 25°C for 24 h. After light and dark treatment, discs were assayed for AsA content and mRNA expression levels of AsA biosynthesis gene.

*Arabidopsis thaliana* cv. Columbia seeds were placed on soil in a pot (8 cm wide by 7.5 cm high). The seedlings were soil-cultivated in a plant growth incubator at 25°C in 16 h of light (66.7 µmol/s/$m^2$) and 8 h of dark for 3 weeks. To measure AsA contents and mRNA expression levels AsA biosynthesis enzymes, rosette leaves were used.

### Determination of ascorbic acid content

Total vitamin C content, as ascorbic acid content, was determined using an ascorbate oxidase method. Moringa and *Arabidopsis* leaves were harvested and ground in liquid nitrogen. The powdered tissues (100 mg) were homogenized in 1.0 ml of cold 6.0% (v/v) perchloric acid and centrifuged at 12,000 × g for 10 min at 4°C. To determine total vitamin C content, supernatants (350 µl) were combined with 110 µl of 1.25 M $K_2CO_3$ and the volume brought to 525 µl with distilled water. The extract (420 µl) was combined with 4 µl of 1 M dithiothreitol, incubated in the dark at 30°C for 30 min, and then centrifuged at 12,000 × g for 10 min. The supernatant (50 µl) was then combined with 446 µl of 200 mM succinate buffer and the absorbance was immediately measured at 265 nm. The absorbance at 265 nm was remeasured 30 s after the addition of 2.5 U of ascorbate oxidase. Total vitamin C content was calculated using a standard curve.

### Reverse transcription-polymerase chain reaction

Total RNA was isolated from Moringa leaves using an RNeasy Plant Mini Kit with DNase I (Qiagen) according to the manufacturer's protocol. First-strand cDNA was synthesized from total RNA using a ReverTra Ace kit (Toyobo) and an oligo$(dT)_{20}$ primer. The cDNA was used as a template for polymerase chain reaction (PCR) using KOD Dash (Toyobo). Primers for AsA biosynthesis genes were designed based on the conserved amino acid sequences of plant AsA biosynthesis enzymes. The sequences for primers were used from the cDNA sequences of *Arabidopsis thaliana* AsA biosynthesis enzymes, and are listed in Supplementary Table 1. After initial denaturation for 2 min at 94°C, 40 cycles of amplification were carried out with 10 s denaturation at 98°C, 30 s annealing at 64°C for GalLDH, 56°C for GDH, GPP, GGP and GME or 55°C for GMP, and 60 s extension at 72°C. The primary PCR products were used as templates for nested PCR with additional gene-specific primers. Nested PCR was performed using KOD Dash, with the following thermocycler conditions: initial denaturation for 2 min at 94°C, followed by 40 cycles of 10 s denaturation at 98°C, 30 s annealing at 58°C, and 60 s extension at 72°C. PCR products were subcloned into the pGEM-T Easy vector (Promega, Madison, WI) and sequenced using an Applied Biosystems 3130xl Genetic Analyzer (Applied Biosystems) with a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), according to the manufacturer's protocol.

### 5'- and 3'- rapid amplification of cDNA ends

Gene-specific primers (Supplementary Table 1) were designed and used for 5'- and 3'-rapid amplification of cDNA ends (RACE) to determine the nucleotide sequences of partial cDNA fragments. For 3'-end amplification, single-stranded cDNA was synthesized from total RNA (500 ng) using an oligo $(dT)_{17}$ adaptor primer and

ReverTra Ace. PCR for 3'-RACE was performed using KOD FX (Toyobo) with an adaptor primer and gene-specific primers.

After initial denaturation for 2 min at 94°C, 35 cycles of amplification were carried out with 10 s denaturation at 98°C, 30 s annealing at 55°C and 60 s extension at 68°C. The primary PCR product was used as templates for nested PCR, using an adaptor primer and a gene-specific primer and the same thermocycler conditions for the primary PCR. The resultant PCR products were subcloned and sequenced as described above. For 5'-RACE, circularized cDNA was synthesized from total RNA. Total RNA (5 µg) was reverse-transcribed using ReverTra Ace and a 5'-end phosphorylated oligo$(dT)_{17}$ primer. After hydrolysis of total RNA with RNase H (TaKaRa) at 30°C for 60 min, cDNA was circularized by ligation with T4 RNA Ligase (TaKaRa) at 15°C overnight. PCR was performed with KOD Dash and primers (GPP, oKT066 and oKT087; GGP, oKT064 and oKT065), using the circularized cDNA as templates. PCR conditions were as follows: 40 cycles of 30 s denaturation at 94°C, 10 s annealing at 55°C for GPP or 60°C for GGP, and 60 s at 72°C. PCR products were subcloned and sequenced as described above.

### Cloning and sequencing of the Moringa GGP genomic sequence

The genome sequence of MoGGP was determined using gene-specific primers (Supplementary Table 1) designed against the MoGGP cDNA sequence. Genomic DNA was isolated from Moringa leaves using a DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's protocol. PCR was performed using KOD Dash with gene-specific primers, with 40 cycles of 30 s denaturation at 94°C, 10 s annealing at 60°C, and 60 s extension at 72°C. PCR products were subcloned and sequenced as described above. The 5'-upstream region of the MoGGP gene was isolated using the cassette-ligation mediated PCR method with an LA PCR *in vitro* Cloning Kit (TaKaRa). After digestion of genomic DNA with EcoRI or XbaI, the cleaved genomic DNA fragments were ligated to the appropriate double-stranded DNA cassette. Primary PCR was performed with a cassette-specific primer (C1) and a gene-specific primer, using KOD FX Neo reagents (Toyobo). PCR conditions were as follows: initial denaturation for 2 min at 94°C, 40 cycles of 10 s denaturation at 98°C, 30 s annealing at 64°C, 60 s extension at 68°C. Subsequent nested PCR used the primary PCR product as templates, alongside a cassette-specific primer (C2) and a gene-specific primer, and used the same thermocycler conditions as the primary PCR. PCR products were subcloned and sequenced as described above.

### Real-time PCR

For internal reference of quantitative RT-PCR, partial cDNA fragment of Moringa rRNA was cloned by RT-PCR as described above. Primers (Supplementary Table 1) for Moringa rRNA were designed from the conserved sequences of plant rRNA; *Arabidopsis* (Acc. No. X52320), *Brassica napus* (Acc. No. D10840), *Cercidiphyllum japonicum* (Acc. No. AF274639) and *Disanthus cercidifolius* (Acc. No. AF274645). Reaction conditions were as follows: initial denaturation 30 s for 95°C, followed by 30 cycles of 5 s at 95°C and 10 s at 60°C. The resultant PCR products were subcloned and sequenced. The cDNA sequences are deposited in GeneBank under an accession number LC005430.

Total RNA was isolated from Moringa leaves using an RNeasy Plant Mini Kit with DNase I (Qiagen). Single-stranded cDNA was synthesized from RNA (500 ng) using the ReverTra Ace qPCR RT Kit (Toyobo), according to the manufacturer's protocol. Real-time PCR quantitation of transcripts of AsA biosynthesis enzymes was performed using Chrome4 (BioRad) and SsoFast EvaGreen Supermix (BioRad), according to the manufacturer's protocol. Reaction conditions were as follows: initial denaturation 30 s for 95°C, followed by 30 cycles of 5 s at 95°C and 10 s at 60°C with respective gene-specific primers (Supplementary Table 1). From the sequences of Moringa (Acc. No. LC005430) and *Arabidopsis* (Acc. No. X52320) rRNA, gene-specific primers were designed and used as respective internal reference. Transcript levels were determined using standard curves generated with DNA samples of known concentration. Normalization was performed by dividing Moringa AsA biosynthesis gene transcript levels with those of rRNA. PCR specificity was assured by examining the melting curve between 65°C and 95°C every 2 s with increments of 0.2°C, and by use of agarose gel electrophoresis to check for a single amplifying band. Real-time PCR experiments were performed in triplicate.

### Promoter assay

The 5'-upstream region of the *MoGGP* gene was amplified by PCR using primers designed for In-Fusion PCR cloning, according to the manufacturer's instructions. For the *Arabidopsis GGP* promoter, 878 bp upstream of the *AtGGP* initiation codon was used. Amplified fragments were subcloned upstream of the *Renilla* luciferase coding sequence using In-Fusion PCR cloning. Preparation and transformation were performed using *Arabidopsis* protoplasts. Briefly, *Arabidopsis* protoplasts were prepared from rosette leaves of approximately 3-week-old *Arabidopsis thaliana* (ecotype Columbia) plants using cellulase "onozuka" R10 (Yakult Pharmaceutical Industry Co., Ltd.) and macerozyme R10 (Yakult Pharmaceutical Industry Co., Ltd.). Isolated protoplasts were suspended in MMg (0.4 M mannitol, 15 mM $MgCl_2$, and 4 mM MES pH 5.7) and used for protoplast transformation, which was performed in a 96-well plate (96-well U-bottom plate, Thermo SCIENTIFIC) using polyethylene glycol. Plasmid (1 pmol) expressing full-length *Renilla* luciferase under the control of the *MoGGP* promoter was used to transfect *Arabidopsis* protoplasts ($3.0 \times 10^5$ cells). Plasmid (0.5 pmol) expressing full-length click beetle red luciferase under the control of the cauliflower mosaic virus 35S promoter was co-transformed for use as an internal reference. Luciferase luminescence was measured using a microplate luminometer (ARVOx4 2030 Multilabel Reader, Perkin Elmer, MA). The luminescence signals in each well were integrated for 3 s, 20 min after adding ViviRen (Promega) in each well. Luciferase luminescence was normalized for transformation efficiency by dividing the relative light units (RLU) of *Renilla* luciferase by the RLU of beetle red luciferase. Beetle luciferase activity was assessed by measuring luminescence through a red filter (effect filter 106, Always CO., LTD), with a 3 s integration period after the addition of the substrate.

## RESULTS AND DISCUSSION

### Isolation of cDNA clones encoding Moringa ascorbic acid biosynthesis enzymes

Reverse transcription (RT)-PCR was performed using primers designed from the cDNA sequences of *Arabidopsis* AsA biosynthesis enzymes. Partial cDNA fragments were isolated, and their nucleotide sequences were highly similar to those of the *Arabidopsis* AsA biosynthesis genes (data not shown). Rapid amplification of cDNA ends (RACE) methods were subsequently used to determine full-length cDNA sequences for Moringa

*GGP* and *GPP*. The cDNA of Moringa *GGP* (*MoGGP*) has a 1,320 bp open reading frame (ORF) that is predicted to encode a protein of 440 amino acids with a calculated molecular mass of 48,963 Da (Supplementary Figure 1). Primary structure analysis using the Conserved Domain Database (CDD) (Marchler-Bauer et al., 2013) suggested that *MoGGP* contains a histidine triad motif found in *Arabidopsis GGP* (*AtGGP*). The cDNA of Moringa *GPP* (*MoGPP*) has an 804 bp ORF that is predicted to encode a protein of 268 amino acids with a calculated molecular mass of 28,954 Da (Supplementary Figure 2). Primary structure analysis using CDD suggested that *MoGPP* has related domains of inositol monophosphatase family. *MoGGP* and *MoGPP* have 75 and 81% identities, respectively, to the *Arabidopsis* proteins, as indicated in Supplementary Table 2. We successfully identified the 3'-downstream sequences of Moringa *GMP*, *GME*, *GDH* and *GalLDH* cDNAs using 3'-RACE, but in this study we were unable to determine the 5'-upstream sequences of cDNAs using 5'-RACE. Comparison of the sequences between the *Arabidopsis* AsA biosynthesis enzymes and the partial predicted Moringa proteins indicated that high levels of similarity exist at the amino acid sequence level (Supplementary Table 2). We therefore concluded that the partial cDNAs isolated in this study are those encoding the Smirnoff-Wheeler pathway AsA biosynthesis enzymes in Moringa. The cDNA sequences are deposited in GeneBank under the following accession numbers: AB924374 for *MoGalLDH*, AB924375 for *MoGDH*, AB924376 for *MoGPP*, AB924377 for *MoGGP*, AB924378 for *MoGME*, and AB924379 for *MoGMP*.

## Transcriptional levels of AsA biosynthesis enzymes in Moringa leaves

Genetic and biochemical studies indicate that the Smirnoff-Wheeler pathway is both ubiquitously expressed and is the dominant AsA biosynthesis pathway in higher plants (Conklin, 2001; Conklin et al., 2000; Wheeler et al., 1998; Wolucka and Van Montagu, 2007). Figure 1A shows that Moringa small (< 10 mm), medium (10 to 15 mm) and large (> 15 mm) leaves contained 10.9, 5.2 and 4.1 µmol/gFW (gram fresh weight) AsA, respectively. AsA content in *Arabidopsis* rosette leaf was 3.6 µmol/gFW. Sreelatha and Padma reported that Moringa leaf contained about 30.5 µmol/gFW AsA (Sreelatha and Padma, 2009). The Moringa plants used in this study were grown under fluorescent at 25°C in a green house. The comparative-low AsA contents in Moringa leaves used in this study, may be due to growth condition, such as light intensity, humidity or temperature, because AsA content in plant is subject to ambient growth condition (Davey et al., 2000). To evaluate AsA biosynthesis at transcriptional levels in Moringa leaves, mRNA levels of AsA biosynthesis enzymes isolated in this study were measured in small, medium and large leaves using quantitative RT-PCR (Figure 1B). The mRNAs encoding all six Moringa AsA biosynthesis enzymes were expressed during Moringa leaf development, indicating that the Smirnoff-Wheeler pathway is likely functional during Moringa leaf development. As indicated in Figure 1B and 1C, the transcriptional patterns of the Moringa and *Arabidopsis* genes tested in this study were different from each other. Of the biosynthesis genes tested, the *MoGGP* mRNA was expressed at the highest levels at all developmental stages. The *Arabidopsis vtc2* mutant is defective in the production of *GGP*, and contains only 10 to 25% of the AsA of a wild-type plant (Conklin et al., 1999; Dowdle et al., 2007). On the other hand, estrogen inducible transiently overexpression of *AtGGP* resulted in an increase in AsA contents (Yoshimura et al., 2014). This indicates that *AtGGP* is a key enzyme in *Arabidopsis* AsA biosynthesis. In acerola, which contains extremely abundant AsA, expression levels of *GGP* mRNA were the highest of the AsA biosynthesis mRNAs tested (Badejo et al., 2009). The acerola data, alongside our findings here, suggest that *MoGGP* may be the predominant enzymes contributing to AsA accumulation in Moringa leaves.

## Effects of light exposure on mRNA levels of AsA biosynthesis enzymes in Moringa leaf

Exposure of plants to light causes an increase in AsA levels (Cruz-Rus et al., 2010; Fukunaga et al., 2010; Li et al., 2009; Yabuta et al., 2007). Multiple AsA biosynthesis enzymes are thought to participate in light-triggered AsA biosynthesis in plants. We evaluated the effects of light on AsA production in Moringa by measuring mRNA levels in leaf discs treated with continuous light exposure. As indicated in Figure 2A, AsA content was significantly higher in leaf discs illuminated at 100 µmol/s/$m^2$ light than in those incubated in the dark (0 µmol/s/$m^2$). Quantitative RT-PCR analysis revealed that *MoGGP* was the only AsA biosynthesis gene to display significantly increased expression on exposure to light treatment (Figure 2B). In *Arabidopsis* transiently overexpressing *AtGGP*, the increase in AsA levels was enhanced under continuous light (Yoshimura et al., 2014). The finding that only the *MoGGP* mRNA levels increase with light exposure suggests that this is the dominant enzyme involved in light-triggered AsA biosynthesis in Moringa.

## Sequence and activity of the Moringa GGP promoter

The *MoGGP* gene sequence (Acc. No. AB924665) was determined through amplification of genomic DNA using primers designed from the *MoGGP* cDNA. The *MoGGP* gene structure was determined by comparing the genomic and cDNA sequences, and was found to comprise seven exons (Figure 3A).

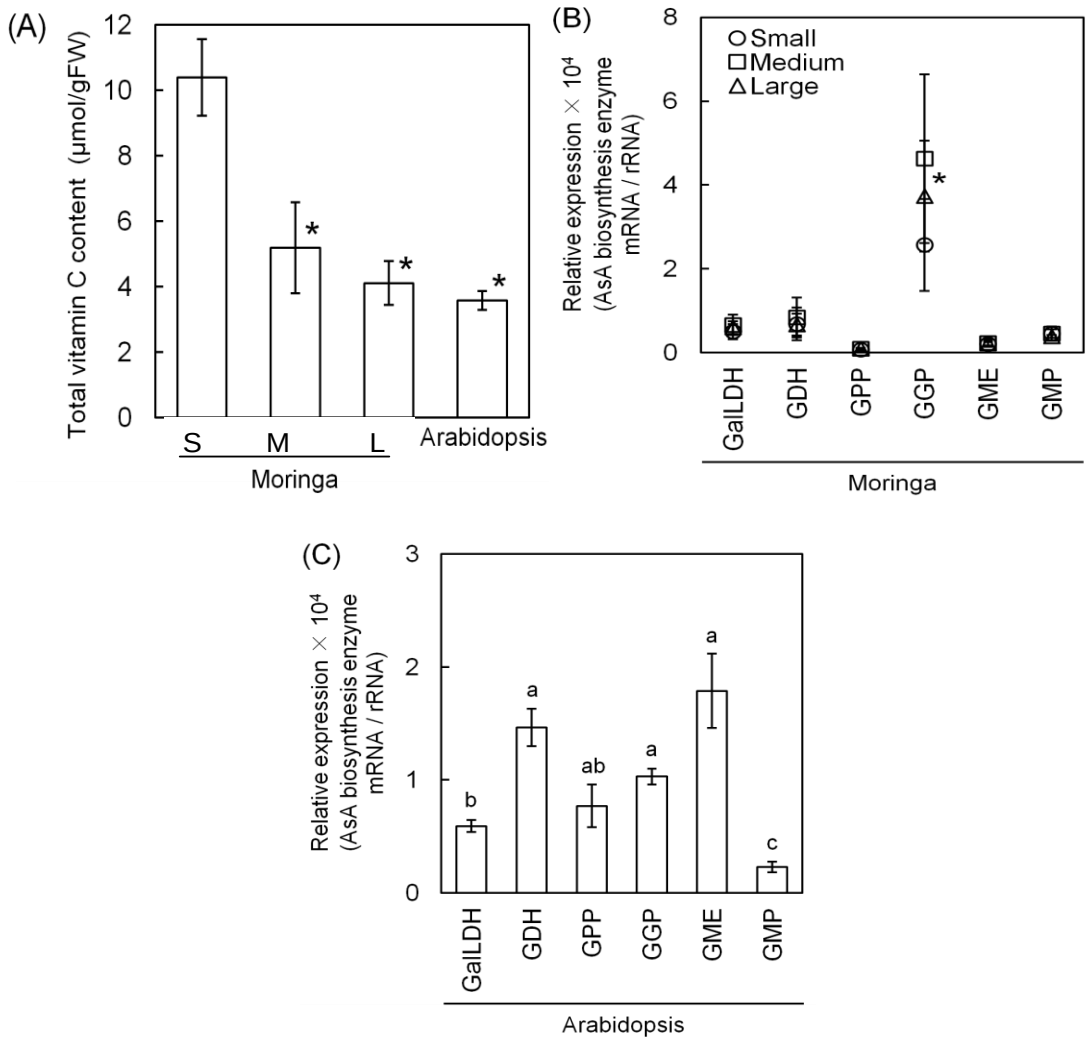


**Figure 1.** The AsA contents and mRNA expression levels of AsA biosynthesis enzymes in Moringa leaves. (A) The extracts were prepared from the small (S, < 10 mm), medium (M, 10 to 15 mm) and large (L, > 15 mm) Moringa leaves, and the rosette leaves of *Arabidopsis*. The total vitamin C contents, as the AsA contents, were determined in the extracts using ascorbate oxidase as described in materials and methods section. Bars represent means ± SE (n = 3). Asterisks indicate significant difference as compared to total vitamin C content in small Moringa leaves ($p < 0.05$). (B) Total RNA (500 ng) was extracted from the Moringa leaves. The mRNA levels of AsA biosynthesis enzymes in the Moringa leaves were measured using total RNA by quantitative RT-PCR as described in materials and methods section. The mRNA expression levels were normalized with rRNA levels. Bars represent means ± SE (n = 3). (C) The mRNA levels of AsA biosynthesis enzymes in the *Arabidopsis* leaves were measured by quantitative RT-PCR. The mRNA expression levels were normalized with rRNA levels. Bars represent means ± SE (n = 3). Values followed by the same letter are not significantly different ($p <0.05$).

The genomic and cDNA sequences were compared between *MoGGP* and *AtGGP*, which indicated that both exon number and length were well conserved. This suggests that the *GGP* gene likely arose prior to the divergence of *Arabidopsis* and Moringa. We also sequenced ~0.8 kb of the 5'-region upstream of the *MoGGP* initiation codon (Figure 3B). The 215 bp directly upstream of the initiation codon was identical to the 5'-UTR of the *MoGGP* cDNA, indicating that the transcription start site (TSS) of the *MoGGP* gene is more than 200 bp upstream of the initial ATG. The 5'-upstream region was searched for transcription factor recognition sites using the PLACE program (Higo et al., 1999). Several consensus elements were found, including two SORLIP1AT (GCCAC; Hudson and Quail, 2003) and IBOX (GATAAG; Giuliano et al., 1988) motifs within a 600 bp promoter region. Expression of *MoGGP* was induced by light exposure, suggesting that these sequences are likely to function as light responsible *cis*-elements. We could not, however, find TATA or CAAT boxes upstream

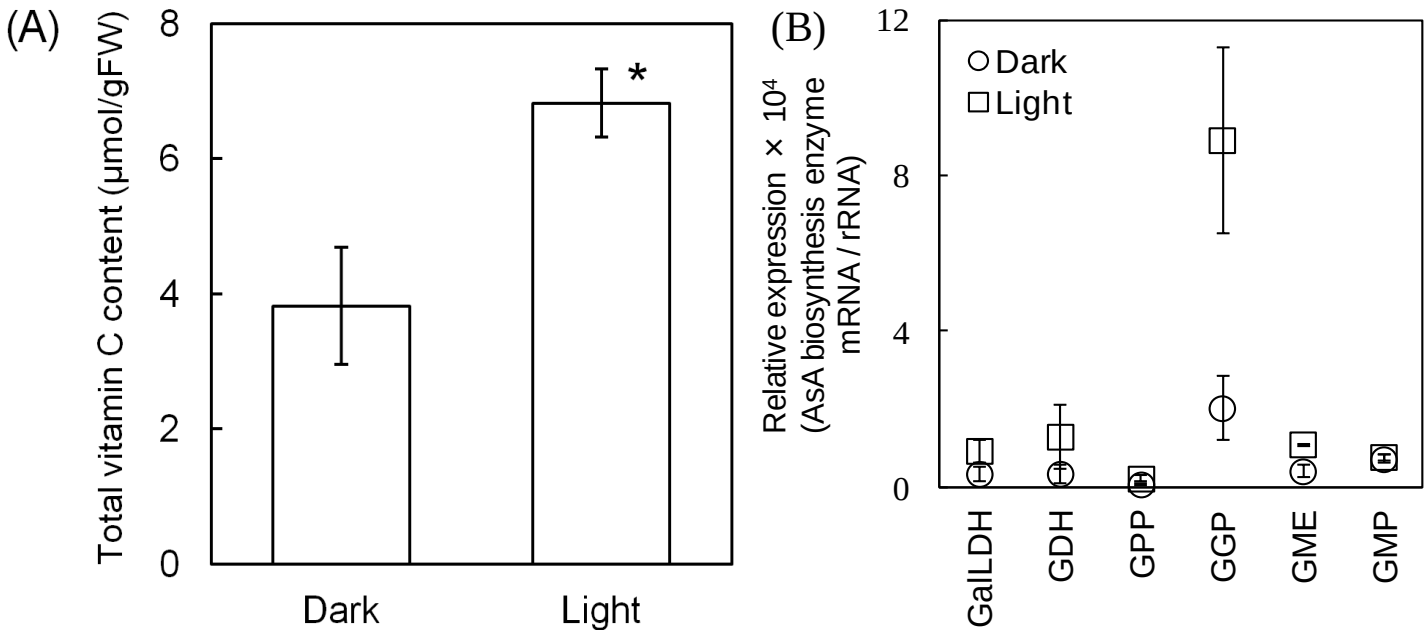


**Figure 2.** The AsA contents and mRNA expression levels of AsA biosynthesis enzymes in response to light exposure. (A) Before light treatment, leaf discs were floated on water in the dark overnight. The total vitamin C contents of Moringa leaf discs were measured at 24 h after continuous light (100 µmol/s/m$^2$) or darkness (0 µmol/s/m$^2$). An asterisk indicates significant difference as compared to the total vitamin C content in the leaf discs treated with darkness. (B) The mRNA levels of AsA biosynthesis enzymes in the Moringa leaf discs treated with light were measured by quantitative RT-PCR. The mRNA expression levels were normalized with rRNA levels. Bars represent means ± SE (n = 3). Asterisks indicate significant difference as compared to the mRNA expression level in the leaf discs treated with darkness ($p < 0.05$).


**Figure 3.** The sequence and promoter activity of Moringa GDP-L-galactose phosphorylase. (A) The length of each exon and intron. The exons and the introns are showed by white boxes and black lines respectively. The respective lengths are indicated in bp. The diagram is not to scale. ATG and asterisks indicate the initiation codon and the stop codon respectively. (B) The nucleotide sequence of the 5'-upstream region of *MoGGP* gene. The position of the initiation codon (ATG) is represented by +1 at the adenine nucleotide. Underlining indicates sequences that are similar to *cis*-element reported previously. (C) Luciferase activities driven by the Moringa *GGP* promoters. The *MoGGP* promoter was subcloned into the upstream of the *Renilla* luciferase. In promoter assays, after transfection with 1 pmol of the plasmid and 0.5 pmol of a plasmid containing the click beetle red luciferase gene driven by the cauliflower mosaic virus 35S promoter to *Arabidopsis* protoplasts, luciferase activities in the protoplasts were measured using a microplate luminometer described in the materials and methods section. For each transfection, *Renilla* luciferase activity was normalized with click beetle red luciferase activity. Bars represent means ± SE (n = 3). Asterisks indicate significant difference as compared to control ($p < 0.05$).

of the *MoGGP* gene, and the core promoter was a minimum promoter region capable of initiation basal transcription. By contrast, the PLACE program located two TATA boxes at 292 and 344 bp upstream of the *Arabidopsis GGP* TSS (data not shown), suggesting that different transcription factors are involved in *GPP* promoter activation in the two species. We tested the *cis*-element promoter activity of *MoGGP* with a transient expression assay using *Arabidopsis* protoplasts. Protoplasts were isolated from *Arabidopsis* leaves and transiently transfected with *MoGGP*-LUC reporter construct. Protoplasts transfected with *MoGGP*-LUC exhibited 2-fold higher luminescence intensities than mock-treated protoplasts (Figure 3C). Although TATA boxes do not appear to be present in the *MoGGP* promoter region determined in this study, an unknown *MoGGP* promoter element may be driving expression. Further investigation of the promoter regions upstream of *MoGGP* is necessary to address this question.

In this study, we wished to examine the biosynthesis pathways underlying the high levels of AsA found in Moringa leaves. We identified six novel Moringa genes putatively encoding AsA biosynthesis enzymes in the Smirnoff-Wheeler pathway, and suggest that these genes play a major role in AsA biosynthesis during leaf development and under light conditions. Unlike acerola, in which several enzymes are important for AsA synthesis, *MoGGP* seems to be the major important enzyme regulating AsA content in Moringa. No TATA boxes were found in the 5'-region upstream of *MoGGP*. We found that the putative *cis*-elements may be involved in the light response, but we were unable to provide direct evidence of *cis*-element(s) enhanced gene expression in this study. Further investigation of more distal 5'-regions is necessary to determine the elements through which *MoGGP* expression is regulated, providing valuable information concerning the mechanisms underlying *GGP* transcription in Moringa.

**Abbreviations: AsA,** Ascorbic acid; ***GalLDH*,** L-galactono-1,4-lactone dehydrogenase; ***GDH,*** L-galactose dehydrogenase; ***GGP*,** GDP-L-galactose phosphorylase; ***GME*,** GDP-D-mannose-3',5'-epimerase; ***GMP*,** GDP-D-mannose pyrophosphorylase; ***GPP*,** L-galactose-1-phosphate phosphatase; **RACE,** rapid amplification of cDNA ends; **RLU,** relative light units; **ROS,** reactive oxygen species; **TSS,** transcription start site.

## Conflict of Interest

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

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## SUPPLEMENTARY

### Supplementary Figure

```
cggtagcccttccgatctcctcttccttgctggcggcggcggcagcgccagcagtttctctcctctatat 70
tagtactagttttagggagtagatttatctttatacaaactagttgtttaagaatttagcggcaagaagt 140
cttggtttttctgggttttatttgaaattaaaaaaaaaagaaaaaaaagagagaagaagaacaagATGTT 210
                                                                  M  L

GAGGATCAAGAGGGTTCCCACTGTTGTCTCGAATTACCAGAAGGAAGAGGGGGAAGAAGGTGCTCGCCGA 280
  R  I  K  R  V  P  T  V  V  S  N  Y  Q  K  E  E  G  E  E  G  A  R  R

GAAGGCGGCTGTGGCCGGAATTGCCTCAACAAGTGTTGCATACAAGGGGCGAAGATTCCATTATATGTTT 350
 E  G  G  C  G  R  N  C  L  N  K  C  C  I  Q  G  A  K  I  P  L  Y  V

TCAAAGGGCTGAACAAGACTGGTGGCAGCAAGGGTGTGCTTGGGCATGAGAACGGGGAGCCTCCTGTTGC 420
F  K  G  L  N  K  T  G  G  S  K  G  V  L  G  H  E  N  G  E  P  P  V  A

CTTTCTTGAGTCACTCCTTCTTGGGGAGTGGGAGGATCGCTCGGAAAGAGGGCTTTTTCGGTATGATGTC 490
  F  L  E  S  L  L  L  G  E  W  E  D  R  S  E  R  G  L  F  R  Y  D  V

ACTGCCTGTGAAACCAAGGTGATCCCGGGTGACTATGGCTTCATAGCCCAGCTGAACGAGGGCCGCCATC 560
 T  A  C  E  T  K  V  I  P  G  D  Y  G  F  I  A  Q  L  N  E  G  R  H

TCAAGAAGAGGCCAACTGAATTCCGTGTTGATAAGGTCCTGCAGCCTTTTGATGGGAACAAATTCAACTT 630
L  K  K  R  P  T  E  F  R  V  D  K  V  L  Q  P  F  D  G  N  K  F  N  F

CACCAAAGTTGGGCAGGAAGAGGTACTCTTCCAGTTTGAAGCAAGTGAAGATGGTGAAGTTCAGTTCTTT 700
  T  K  V  G  Q  E  E  V  L  F  Q  F  E  A  S  E  D  G  E  V  Q  F  F

CCAAGTGCACCCATTGATGTTGAGAATTCTCCTAGTGTTGTTGCTATAAATGTTAGTCCTATTGAGTATG 770
 P  S  A  P  I  D  V  E  N  S  P  S  V  V  A  I  N  V  S  P  I  E  Y

GGCATGTGCTGTTGATCCCTCGTGTTCTTGAGTGCTTGCCACAGAGGATTGACCGTGACAGCTTCTTGCT 840
G  H  V  L  L  I  P  R  V  L  E  C  L  P  Q  R  I  D  R  D  S  F  L  L

TGCCCTTCACATGGCAGCTGAAGCTGGAAATCCATATTTCCGGTTGGGTTACAACAGTTTAGGTGCATTT 910
  A  L  H  M  A  A  E  A  G  N  P  Y  F  R  L  G  Y  N  S  L  G  A  F

GCTACTATCAATCATCTGCACTTCCAGGCTTATTACCTGGCTATGCCCTTTCCAGTTGAGAAGGCTCCCA 980
 A  T  I  N  H  L  H  F  Q  A  Y  Y  L  A  M  P  F  P  V  E  K  A  P

CCAAGAAGATTACCACCACTGATGGTGGGGTGAGGATCTCAGAACTTTTGAATTACCCAGTTAGAAGTCT 1050
T  K  K  I  T  T  T  D  G  G  V  R  I  S  E  L  L  N  Y  P  V  R  S  L

TGTCTTCGAGGACGGTGAAACTGTGCAAGACTTATCCAACACGGTATCGGATGCCTGCATTTGCCTCCAA 1120
  V  F  E  D  G  E  T  V  Q  D  L  S  N  T  V  S  D  A  C  I  C  L  Q

AACAGCAACATTCCTTACAATGTCCTCATTGCTGATTGTGGAAACCGGGTCTTTGTCTTCCCGCAGTGCT 1190
 N  S  N  I  P  Y  N  V  L  I  A  D  C  G  N  R  V  F  V  F  P  Q  C

ATGCTGAGAAACAAGCTCTTGGGGAAGTGAGTCCTGAGCTTCTGGATACCCAAGTTAACCCTGCTGTTTG 1260
Y  A  E  K  Q  A  L  G  E  V  S  P  E  L  L  D  T  Q  V  N  P  A  V  W

GGAAATCAGTGGGCATATGGTATTGAAGAGGAAGAAGGATTATGAAGAGGCATCCGAGGAAAATGCTTGG 1330
  E  I  S  G  H  M  V  L  K  R  K  K  D  Y  E  E  A  S  E  E  N  A  W

AGGCTCCTTGCTGAGGTGTCTCTTTCTGAAGAGAGGTTCAGGGAAGTGAGTGCTCTGATCTTTGAGGCCA 1400
 R  L  L  A  E  V  S  L  S  E  E  R  F  R  E  V  S  A  L  I  F  E  A

TTGCTTGCAGTGAAAATGGTGAGGCAAGCAATGAACAGAGCTCGGTGAACAAAAATGTGCATGCCATAAA 1470
I  A  C  S  E  N  G  E  A  S  N  E  Q  S  S  V  N  K  N  V  H  A  I  K

GAAGAGCTCTCACTCTGCTATAGTGACTGGGACACAAGAATGCCTGGTTCTCCAGTAGaatcgggtgttt 1540
  K  S  S  H  S  A  I  V  T  G  T  Q  E  C  L  V  L  Q  *

ggttatcggacttgctgtgtttgtgattctgaagtatgagactttgcacatgagatcatatgggtttgtg 1610
gcactagtggcctaaataagcaaacctggtttgcactggttgtggtagtcctttgtgtttatgtgttgtg 1680
cttgttagcctggtgatgtactgtgtggagccttgttcaagtactttgttgtataatgtttgctgctata 1750
agctaatgaatttgtcttattctgtaaaaaaaaaaaaaaa                               1791
```

**Figure 1.** Nucleotide and deduced amino acid sequences of *MoGGP*. An asterisk shows the termination codon.

```
cgatatttttttcatgtgatacgtgaaaacctccaacacagagcacaacctatcacccatcagcttcctcc 70
ccccctggcgtatcactgatcaacccagtgaagaaaaaaATGGCTGAAAATTTTTCGCTGGAAGAATTCT 140
                                        M  A  E  N  F  S  L  E  E  F

TGGCATCTGCAGTTGATGCAGCTAAGAAAGCTGGCGAGATAATCCGTAAAGGATTTTACCAAACCAAACA 210
L  A  S  A  V  D  A  A  K  K  A  G  E  I  I  R  K  G  F  Y  Q  T  K  H

TGTGGAACATAAAGGCCAGGTAGATTTGGTCACAGAGACTGATAAAGCATGTGAGGATCTTGTATTTAAT 280
  V  E  H  K  G  Q  V  D  L  V  T  E  T  D  K  A  C  E  D  L  V  F  N

CATCTCAAGCAGCATTACCCCAATCATAAGTTCATTGGGGAAGAAACTACTGCTGCTTATGGTGCTACAG 350
 H  L  K  Q  H  Y  P  N  H  K  F  I  G  E  E  T  T  A  A  Y  G  A  T

AGCTGACTGATGAACCCACTTGGATAGTTGATCCTCTTGATGGAACGACTAACTTTGTCCATGGATTCCC 420
E  L  T  D  E  P  T  W  I  V  D  P  L  D  G  T  T  N  F  V  H  G  F  P

CTTTGTGTGCGTCTCTATTGGTCTCACGATCAAGAAGGTTCCTACAGTTGGTGTTGTGTACAATCCGATT 490
  F  V  C  V  S  I  G  L  T  I  K  K  V  P  T  V  G  V  V  Y  N  P  I

ATGGAAATTCGATTTACTGGCATCCACGGGAAAGGTGCTTTCTTGAATGGAAACCCTATAAAAGTATCAT 560
 M  E  I  R  F  T  G  I  H  G  K  G  A  F  L  N  G  N  P  I  K  V  S

CTAAAACTGAACTTGTCAAATCCCTTCTTGCAACTGAGGCTGGAACAAAACGGGATAAGGCAACTCTAGA 630
S  K  T  E  L  V  K  S  L  L  A  T  E  A  G  T  K  R  D  K  A  T  L  D

TGATTCGACGAACAGAATCAATAATCTGCTTTCCAAGGTGAGATCTCTTCGAATGAGTGGCTCGTGTGCA 700
  D  S  T  N  R  I  N  N  L  L  S  K  V  R  S  L  R  M  S  G  S  C  A

TTGAACCTCTGCGGAATTGCCTGTGGAAGGCTTGATCTGTTTTACGAACTTGGATTTGGTGGCCCATGGG 770
 L  N  L  C  G  I  A  C  G  R  L  D  L  F  Y  E  L  G  F  G  G  P  W

ATGTTGCTGGAGGTGCTGTTATTGTTAGAGAAGCTGGAGGACTTGTGTATGATCCATCTGGTAAAGATTT 840
D  V  A  G  G  A  V  I  V  R  E  A  G  G  L  V  Y  D  P  S  G  K  D  F

TGATATCACATCTCAGCGAGTTGCAGCTTCAAACCCTTCCCTGAAGGATTTGTTCGTGGAGGCTTTGCAG 910
  D  I  T  S  Q  R  V  A  A  S  N  P  S  L  K  D  L  F  V  E  A  L  Q

CTTTAAgaacgacagtgcacctaaagcctatgaagactagatgtagccgttatttaaatataatttaatc 980
 L  *

aagttctctcatttttccagcaatttctattcttaccatgaggattcaaaatgtatcatctctgagagag 1050
ttgatgacaacaattcttccaattggcatgtggccttattcctgtgccagattgctagtagaacctaatg 1120
gttaatatgtatttgtatcactttcaaattatcgcattgttgagctctaaaaaaaaaaaaaaaaaa     1186
```

**Figure 2.** Nucleotide and deduced amino acid sequences of *MoGPP*. An asterisk shows the termination codon.

**Table 1.** Primers used for cDNA cloning, quantitative RT-PCR and promoter assay.

| Primer name | Oligonucleotide sequence (5'-3') | Purpose |
|---|---|---|
| oYF089 | CATCTAGCTCGATGTGGCCT | GalLDH RT-PCR forward |
| oYF090 | GCCCAATGTTCATACGCAGA | GalLDH RT-PCR reverse |
| oYF067 | GTTGGTTTTGGTGCCTCTCCG | GDH RT-PCR 1st forward |
| oYF070 | TCTTTACAGGCTCGAGAATAGCTTCAAC | GDH RT-PCR 1st reverse |
| oYF071 | CACAAAGGCCAGGTGGATTTGG | GPP RT-PCR 1st forward |
| oYF074 | GTCCCATGGACCACCGAAACC | GPP RT-PCR 1st reverse |
| oYF075 | GGCTGTGGACGGAATTGCCTC | GGP RT-PCR 1st forward |
| oYF078 | CCTCTTCAGTACCATGTGACCACT | GGP RT-PCR 1st reverse |
| oYF079 | GCTGCTGATATGGGTGGTATGGG | GME RT-PCR, 3'-RACE 1st forward |
| oYF082 | AGGAGCCCAACCAAGCTTTTC | GME RT-PCR, 3'-RACE 1st reverse |
| oYF083 | GGAGGTTTTGGCACTCGCTTG | GMP RT-PCR 1st forward |
| oYF086 | GTTATGCAACACAACCCCTCCATT | GMP RT-PCR 1st reverse |
| oYF068 | CTCGGTATCAACTTCTTCGACACCT | GDH RT-PCR 2nd forward |

**Table 1.** Contd.

| | | |
|---|---|---|
| oYF069 | AGCAGGGTGCCATTCAGGAGG | GDH RT-PCR 2nd reverse |
| oYF072 | TTCATAGGAGAAGAAACTACAGCTGCA | GPP RT-PCR 2nd forward |
| oYF073 | TCCATTCAAGAATGCTCCTTTCCC | GPP RT-PCR 2nd reverse |
| oYF076 | GCTCAGCTTAACGAGGGTCGT | GGP RT-PCR 2nd forward |
| oYF077 | ACCTAGAGCCTGTTTCTCTGCGTA | GGP RT-PCR 2nd reverse |
| oYF080 | TCGAGTGCTTGTATCTATCCAGAG | GME RT-PCR 2nd forward |
| oYF081 | CAACCTGAGTACACCTTCAACACA | GME RT-PCR 2nd reverse |
| oYF084 | GAAGTCGTCTTGGCCATTAACTACGAG | GMP RT-PCR 2nd forward |
| oYF085 | CCGACCGTCGAGTGCCA | GMP RT-PCR 2nd reverse |
| oKT003 | TCAAGCCGAAGCTGAGTTTT | GalLDH 3'-RACE 1st forward |
| oKT004 | GGGATACAGAGTGGGATGGA | GalLDH 3'-RACE 2nd forward |
| oKT005 | GGGTGTTGGTGTAATCAGTGC | GDH 3'-RACE 1st forward |
| oKT006 | CACTTGCAATGGGACTCCTT | GDH 3'-RACE 2nd forward |
| oKT007 | GGTTCCTACAGTTGGTGTTGTG | GPP 3'-RACE 1st forward |
| oKT008 | TACTGGCATCCACGGGAAA | GPP 3'-RACE 2nd forward |
| oKT009 | CCTCATTGCTGATTGTGGAA | GGP 3'-RACE 1st forward |
| oKT010 | GTCTTTGTCTTCCCGCAGTG | GGP 3'-RACE 2nd forward |
| oKT027 | TCAAAATATGGGGTGGTGGT | GMP 3'-RACE 1st forward |
| oKT028 | CCACAGGGAAAGTTGAAAGG | GMP 3'-RACE 2nd forward |
| oKT066 | ATGTGGCCTTATTCCTGTGC | GPP 5'-RACE forward |
| oKT087 | TGCCTTATCCCGTTTTGTTC | GPP 5'-RACE reverse |
| oKT064 | ACTGTGTGGAGCCTTGTTCA | GGP 5'-RACE forward |
| oKT065 | TCAGTTGGCCTCTTCTGGAG | GGP 5'-RACE reverse |
| oKT200 | TAGCCCTTCCGATCTCCTCT | Moringa GGP genomic DNA forward |
| oKT201 | TACTTGAACAAGGCTCCACACA | Moringa GGP genomic DNA reverse |
| oKT118 | AAAAGCTGTTCCCCCTGTCT | Moringa Real-time PCR GalLDH forward |
| oKT046 | ACAAAGACGTCCACCTCCAC | Moringa Real-time PCR GalLDH reverse |
| oKT106 | AATGTTGCTGCTGCCTCAGA | Moringa Real-time PCR GDH forward |
| oKT107 | GATGTCAGCCCTGCTGGATT | Moringa Real-time PCR GDH reverse |
| oKT176 | TTAGAGAAGCTGGAGGACTTGTG | Moringa Real-time PCR GPP forward |
| oKT177 | CTTCATAGGCTTTAGGTGCACTG | Moringa Real-time PCR GPP reverse |
| oKT156 | GGACACAAGAATGCCTGGTT | Moringa Real-time PCR GGP forward |
| oKT157 | AGGCCACTAGTGCCACAAAC | Moringa Real-time PCR GGP reverse |
| oKT212 | AGTGAGGAGCATAAGAGCTACCC | Moringa RealTime-PCR GME forward |
| oKT213 | GGACAGGTATCAGGCCAATTAGT | Moringa RealTime-PCR GME reverse |
| oKT158 | CGCATGTATATCCAGCAGCA | Moringa Real-time PCR GMP forward |
| oKT125 | TCTCTTTGTGGGGCAGAACT | Moringa Real-time PCR GMP reverse |
| oKT240 | GTAAACGGCGGGAGTAACTATG | Moringa, Arabidopsis RT-PCR Real-time PCR rRNA forward |
| oKT241 | AGTCGGACTAGAGTCAAGCTCAA | Moringa, Arabidopsis RT-PCR Real-time PCR rRNA reverse |
| oKT159 | CAACAACATGGTGGAAAAGC | Arabidopsis Real-time PCR GalLDH forward |
| oKT127 | TGGATTCACTCATTCCTCACC | Arabidopsis Real-time PCR GalLDH reverse |
| oKT128 | CAAGGAGATTTCGTCGGTGT | Arabidopsis Real-time PCR GDH forward |
| oKT129 | TTCTTGATCCATCCCCAGAC | Arabidopsis Real-time PCR GDH reverse |
| oKT130 | CCATCCGGTAAAGATTTGGA | Arabidopsis Real-time PCR GPP forward |
| oKT131 | GACTTCATGCCCCTGTAAGC | Arabidopsis Real-time PCR GPP reverse |
| oKT132 | CGAGTGCCTTGTCCTTCAGT | Arabidopsis Real-time PCR GGP forward |
| oKT133 | ATGCCCACAACCATCATACA | Arabidopsis Real-time PCR GGP reverse |
| oKT134 | AGCTTGGTTGGGCTCCTAAT | Arabidopsis Real-time PCR GME forward |
| oKT135 | ACCACCTTTGATGACCCGTA | Arabidopsis Real-time PCR GME reverse |
| oKT160 | CACTCAACGGTTGGTCAATG | Arabidopsis Real-time PCR GMP forward |
| oKT161 | TGATCTCCTTGTGTGGCAAA | Arabidopsis Real-time PCR GMP reverse |
| oKT249 | CCAAGAAGGAGTGACTCAAGAAAG | Moringa GGP promoter cloning EcoRI cassette 1st reverse |
| oKT252 | CGTGTATAGCCGTGATGTCTACAACT | Moringa GGP promoter cloning XbaI cassette 1st, 2nd reverse |
| oKT243 | GTAATTCGAGACAACAGTGGGAAC | Moringa GGP promoter cloning EcoRI cassette 2nd reverse |

**Table 2.** Identity (%) of amino acid sequences among Moringa and *Arabidopsis* AsA biosynthesis enzymes.

| Parameter | GalLDH | GDH | GPP | GGP | GME | GMP |
|---|---|---|---|---|---|---|
| Identity (%) | 77 | 81 | 81 | 75 | 92 | 86 |

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*Full Length Research Paper*

# Sensitive parameters for EPIC model evaluation and validity under soil water and nutrients limited conditions with NERICA cropping in West Africa

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**Crop models known to be based on the theory of crop physiology for describing the dynamic process of crop growth are recently explored for their uncertainties in model application under resource limited conditions. The aim of this study was to test Environmental Policy Integrated Climate (EPIC), on upland land rice production by taking into account seasonal variability in Guinean and Guinean-Sudanian zones in Benin and Nigeria (West Africa). A range of data available under farmer or experimental conditions in rainfed agriculture were measured or used from literature. The results show the accuracy of the model to simulate LAI, total above ground biomass and grain yield of upland rice for 2 NERICA rice cultivars. After calibration, the model showed average mean relative error between 0.06 and 0.15 with the model efficiency up to 0.98% in the case of LAI. The assessment of the model performances about sensitivity to N or P fertilizer application is also discussed under Ultisols. Large root mean square (RMSE) in calibration and the validation (>100) process suggested that robustness of the model became restrictive under severe environmental conditions such as in drought or flooding condition. Performance of the model at large scale should be executed of with land marginality classification.**

**Key words:** Environmental Policy Integrated Climate (EPIC), modeling, upland rice, West Africa.

## INTRODUCTION

The operation of crop growth models is of interest for filling gap between information needed and that created by traditional experimental trials in soil and agronomic research or for extrapolating results gained on experimental stations leading to better integration of knowledge.

Beside, simulation modeling represents a research tool for assessing climatic change patterns and their impacts on crop growth and yield. Modeling a cropping system requires to understand the complex crop-water-soil interaction and to suggest some empirical parameters which can be applicable to diverse conditions and environments. However, the attempt to use crop growth models under extremely unfavourable growth conditions that is, water scarcity combined with low soil fertility or with indigenous management practices remains a

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challenge in tropical cropping systems such as in Africa or in Latin America (de Barros et al., 2004; Gaiser et al., 2010).

Indeed, for the single crop rice that has a relatively long history in modeling, model development is now geared to the issue of resources limitation due to expansion of rainfed rice systems. For instance the water and nitrogen modules in the latest version of ORYZA2000 formerly developed for estimating potential rice production suggest repeated model simulations with real-world data in order to increase the confidence in the suitability of the model for a certain purpose (Bouman and Van Laar, 2006). Even the agroecological system models such as Environmental Policy Integrated Climate (EPIC), which addresses crop simulation in response to weather, and nutrient cycling, is still not widely used to explore management strategies (Probert, 2004). As result, in rainfed low-input systems such as smallholder farms in West Africa, models developed for optimal management conditions fail to meet the needs of researchers and extension workers. That can be a key issue in Africa where about 80% of the rice production depends on rainfed conditions.

Although the basic use of crop models was to calculate crop growth and development for a single field, there is increasing interest in studies that concern multiple fields (Leenhardt et al., 2007; Hartkamp et al., 1999). This depends on the assumption that field scale model can be useful for evaluating management strategies at a broader scale. In rainfed upland systems in West Africa, rice yield is seldom above 2 $Mgha^{-1}$. Constraints are shown to be in addition to rainfall uncertainty in West Africa, weeds and soil nitrogen availability. Indeed soil nutrient availability for upland rice culture has also been described to be related to land use and ecology (Becker and Johnson, 2001). It is then important for crop modeling targeted on upland rice to be tested on various environmental and management conditions to provide more confidence for further upscaling exercises. The adopted upland rice is NERICA cultivars that have been showed relatively high yieldsyield which vary in a controlled environment from 4.0 to 7.0 $Mgha^{-1}$ (Akintayo et al., 2008). NERICAs are developed from interspecific crosses and tested for their ability to overcome drought (Asch et al., 2005) or to tolerate temporary inundation via flash flooding (Kawano et al., 2009) or for low nitrogen environment (Saito and Futakuchi, 2009; Oikeh et al., 2008). New cultivars including NERICA 1 and NERICA 4 are assessed through the participatory varietal selection (PVS) in order to identify genotypes that perform well across or within a specific target environment.

The objective of this study was a multisite calibration and validation of the EPIC model for NERICA cultivars in contrasting ecological condition and the identification of site-specific model sensitive parameters. For this purpose we analyzed the sensitivity of the crop model to fertilizer and water input with data from experimental and on farm fields in Guinean and Sudanian zones in Benin and Nigeria (West Africa). The EPIC model was chosen due to its capacity to consider the effect of limiting water stress and of nutrients such as nitrogen and phosphorus on rice production (Gaiser et al., 2010).

## MATERIALS AND METHODS

### Study area

The model evaluation followed a calibration and validation process. Experimental data were collected from 8 experiments carried out in 2004-2010 in Benin and in Nigeria, West Africa (Table 1). The locations are listed from South to North: (The International Institute for Tropical Agriculture (IITA), Cotonou) (4), Tohoué (8), Ikenne (1, Nigeria), Niaouli (3), Bohicon (2), Kpakpazoumé (6), Tchankpéhoun (7) and Pingou (5). The calibration dataset is obtained from sites 1, 4, 6, 7 and 8. Validation plots concerned sites 2, 3 and 5.

The experiments ranged from the Guinean to the Sudanian Zone (Table 2) toward the North. The Guinean zone has two rainy seasons and the Sudanian Zone has a monomodal rainfall distribution. The annual precipitation is about 1100 mm with slightly declining rainfall in northward direction; there is regionally higher rainfall close to the Atacora mountain range for the case of Pingou and Tchankpéhoun (Röhrig, 2008). The length of growing season decreases also from South to North, from 250 to 130 days. In general, the rainfall distribution allows cultivation of two crops per year in the southern areas (Igué, 2000).

### Model data input and source

Crop management dates are summarized in Table 3. For the experiments in site 1 through 4 experimental layouts and number of replications are described and published in previous works (Oikeh et al., 2008; Saito and Futakuchi, 2009; Sokei et al., 2010; Koné et al., 2008). For 5 to 8 sites, the experimental design varies according to the area. Plots of 3 m x 15 m were used in Kpakpazoumé and Pingou. The farmland in Tohoué occupied 1250 $m^2$ and 5000 $m^2$ in Tchankpéhoun. In all experiments cultivar 'NERICA1' or 'NERICA4' were used.

Soil information was provided from soil profiles dug during the fallow period in 2009 for sites 8, 3, 2 and 4. Atchade (2006) reported chemical and physical characteristics of soil profiles in IITA, Niaouli and Bohicon (Cana Sud). The top soil properties (0 to 15 cm) were adapted according to Saito and Futakuchi (2009) at IITA. Two fields were used at IITA: one with low soil fertility ($IITA_{low}$) and the other with high soil fertility ($IITA_{high}$). Soil data in Ikenne were obtained from Heuberger (1998). The profile was described during the fallow period in Kpakpazoumé, Pingou and Tchankpéhoun in 2009. Topsoil sample were randomly collected from the fields at 5 points of 0 to 20 cm depth along a profile down to the root-table. The samples were sieved to pass through a 2 mm mesh before analysis. The pH was determined using a soil-water ratio of 1:2. The organic carbon and organic N were analysed using the elemental analysis for Kpakpazoumé and Pingou (Fujine, 2014). The dichromate oxidation method of Walkley and Black (1934) was used for Kobli and Tanguiéta. Exchangeable bases (Mg, K, Ca, and Na) were extracted with 1 mol $L^{-1}$ NH4 Acetate; Ca and Mg in the extract were measured using the atomic absorption spectrophotometer (AAS) while Na and K were determined by flame photometry. The potential cation exchangeable capacity was determined by extraction with 1 mol $L^{-1}$ $BaCl_2$. Ikenne, Niaouli have sandy textured topsoil. However, except Tohoué, all the sites have loamy to clayey subsoils (Alfisols and Ultisols).

**Table 1.** Dataset for calibration and validation of crop growth simulation. GY: Grain Yield, TAB: Total Aboveground Biomass, LAI: Leaf Area Index, C refers to data used for Calibration and V for Validation.

| Site No | Location | Latitude Longitude | Elevation (m) | Year | Variables for simulation | Activity | References |
|---|---|---|---|---|---|---|---|
| 1 | Ikenne | 6°54´N 3°42´E | 71 | 2004 | GY | C | Oikeh et al. (2008) |
| 2 | Bohicon | 7°11´N, 2°04´E | 77 | 2006, 2007 | GY | V | Sokei et al. (2010) |
| 3 | Niaouli | 6° 44´N 2° 07´E | 81 | 2005, 2006 | GY | V | Koné et al. (2008) |
| 4 | IITA | 6° 20´N 2° 20´E | 457 | 2006, 2007 | LAI, TAB, GY | C | Saito and Futakuchi, 2009) |
| 5 | Pingou | 10° 45´N 0° 59´E | 100 | 2009, 2010 | GY | V | |
| 6 | Kpakpazoumé | 7° 55´N 2° 15´E | 174 | 2009, 2010 | GY; TAB | C | |
| 7 | Tchankpéhoun | 10° 45´N 0° 59´E | 187 | 2009, 2010 | GY; TAB | C | |
| 8 | Tohoué | 6° 25´N 2° 40´E | 14 | 2009 | GY; TAB | C | |

The soils were usually acid with low nitrogen content except in Bohicon and $IITA_{high}$. The soil type was sandy to sandy loam (Table 2). Down to 15 cm, soil organic carbon level was classified in the order :

Bohicon>$IITA_{high}$>Kpakpazoumé>Pingou>Ikenne>Tchankpéhoun>Niaouli>$IITA_{low}$> Tohoué.

Daily meteorological data (maximum and minimum air temperature and global solar radiation) were collected from the synoptic weather station nearest to the fields (Table 2). For synoptic data in Ikenne, the model weather generator was used from FAO climate database LocClim for monthly mean for temperature. Solar radiation at Ikenne was derived from Apkadio and Etuk (2002) and the Hargreaves (Hargreaves and Samani, 1985) method was used for ETP estimation. For all other sites, Penman Monteith Method was applied. All these methods have been successfully tested for ETo estimation in (Rahimi et al., 2015; Valipour, 2014a, b, c;d; e; f;g;h, Valipour, 2015). Daily rainfall was retrieved from the closest rainfall gauge.

**Modeling with EPIC**

The version 3060 of the EPIC model (Williams et al, 1990) was used to simulate rice productivity. EPIC is a field-based model designed to simulate crop production based on information about soil, crop rotation and management system. A full description is presented in the model documentation by Izaurralde et al (2006). Among various subroutines, the model considers N and P cycling by flows between inorganic and organic stocks. For N mineralization, EPIC couples C and N cycling in the soil. Simulated C and N compounds in EPIC are stored in either biomass, slow, or passive soil organic matter pools. Direct interaction is simulated between these pools as the function of soil moisture, temperature, nutrient content and clay content functions (Izaurralde et al., 2006; Gaiser et al., 2010). For P mineralization, the model uses the approach given in Jones et al. (1984). Two sources of mineralization are considered: the fresh organic P pool, associated with crop residue and microbial biomass, and the stable organic P pool, associated with the soil humus. The mineral P is then transferred among three pools: labile (which comprises fertilizer), active mineral, and stable mineral. Flow between the labile and active mineral pools is governed by the equilibrium equation that implies the mineral P flow, the amount in the active mineral P pool and P sorption coefficient defined as the fraction of fertilizer P remaining in the labile pool after the initial rapid phase of P sorption is completed.

***Model evaluation***

The evaluation of the model was firstly done by graphical presentation of the agreement between measured and calculated values for crop model by producing linear regressions between measured and simulated variables and calculating the coefficient of determination ($R^2$) derived from 1:1 regression line where data are considered to meet independence assumption. The different comparison methods in Table 4 that highlight the feature of data and the model response were also used. The mean error (ME), the mean relative error (MRE), the mean absolute error (MAE) and the root mean square were presented where n is the sample number, x is the observed, y is the simulated value. The MRE is positive when the model in total overestimates compared to observation. The negative sign is related to underestimation. The root mean square error (RMSE) estimates the precision and reliability of the prediction for single yield estimation points. Model efficiency is used to assess the predictive power of the model taking into account the variability inside the observation data set. All these calculations were done in Microsoft Excel sheet.

## RESULTS AND DISCUSSION

### Calibration of crop parameters

The calibration and validation runs started with a warm

**Table 2.** Pedoclimatic conditions of test sites used for model calibration and validation.

| Sites | Climate zone | Rainfall[1] (mm) | Synoptic station | Station | Soil type FAO/US classification | Texture[2] | Soil organic carbon (%)[2] | References for soil profile |
|---|---|---|---|---|---|---|---|---|
| 1 | Guinean | 1287 | FAO | Ibeju-Ode | Typic Haplustult/Ultisol | S | 0.86 | Heuberger (1998) |
| 2 | Guinean | 1208 | Bohicon | Bohicon | Haplic Alisol/Alfisol | SL | 2.38 | Atchade (2006); CENAP |
| 3 | Guinean | 1065 | Cotonou | Niaouli | Acrisol/Alfisol | S | 1.89 | Atchade (2006); Koné et al. (2008) |
| 4 | Guinean | 1352 | Cotonou | IITA | Haplic Alisol/ Alfisol | S /SC | 1.96/0.7 | Atchade (2006); Saito and Futakuchi (2009) |
| 5 | Soudan-Guinean | 1103 | Natitingou | Matéri | Dystric Plinthisol /Alfisol | SL | 0.84 | Our results |
| 6 | Soudan-Guinean | 1209 | Savé | Kpakpazoumé | Dystric Plinthisol /Alfisol | SL | 0.91 | Our results |
| 7 | Soudan-Guinean | 1103 | Natitingou | Matéri | Luvisol/Alfisol | LS | 0.82 | Our results |
| 8 | Guinean | 1082 | Cotonou | Porto Novo | Dystric Cambisol / Inceptisol | S | 0.65 | Our results |

1. Rainfall in site 1, in 2005, in site 2 is average 2007 and 2008, in site 3 is average 2005 and 2006, in site 4 is average 2006, and 2007, in site 5 and 7 are average 2009 and 2010, in site 6, average 2009 and 2010, site 8 refers to 2009,
2. Texture ans soil organic carbon in 0-20cm or 15 cm depth.

up period of 8-9 years in order to stabilize the soil organic carbon pools in the model. The approach used for the calibration was to modify some initial values of the model parameters in order to iteratively fit simulation values as close as possible to the observed yield values. Therefore, we adjusted the default crop parameters for rice to the NERICA cultivars. However no cultivar distinction was taken into account in the crop file. The NERICA 1 and 4 passport data published by the Africa Rice Centre represented no feature for distinguishing the two cultivars in the crop file of the EPIC model such as the number of days to maturity which determine the Potential Heat Unit (PHU) or flowering age.

In the process of LAI calibration, the parameters DLAP1 and the DLAP2 were used to control the crop growth. The DLAP1 was changed from 30.01 to 30.20 and the DLAP2 from 70.95 to 60.95 for the two cultivar cultivars. The plant population density has been also modified from 12600 to 50.600 in PPC1 and 250.600 to 250.900 in PPC2. Félix (2006) considered that the sub-model of EPIC for LAI development is based on a strong amount of empiricism, as the mechanism that controls the rate of development of LAI is not yet well understood.

As result, the model outputs and the observations with regard to the LAI before and after the calibration were graphically compared. Figure 1 shows that the model first underestimated the values of LAI with a negative mean error of -0.22 (Table 6). After calibration the average relative difference between the observed values and simulated LAI was approximately 6% with a model efficiency of 98%. The LAI development was rather satisfactory calibrated similar to Yoshida et al (2007) using a complex and detailed phenological model as a function of relative crop growth rate, leaf nitrogen content and air temperature. The LAI was estimated under full irrigation at relatively high soil fertility level compared to farmers field as described in Table 2 (Corg =19.6 $gkg^{-1}$ and total nitrogen up to 2.2 $gkg^{-1}$). The observed value is average of 5 cultivars including NERICA1 grown under high soil fertility conditions (Saito and Futakuchi, 2009). The authors detected no difference in rice cultivars in LAI at 42 and 56 DAS and no traits from the early vegetative stage were observed related to grain yield. The relative increase of measured LAI at mid stage (DLAP1) compared to the default value in the model is in line with the high weed competitiveness feature demonstrated for

**Table 3.** Description of the experiments with field operation. N1 and N4 refer to Nerica1 and Nerica4 respectively.

| Site | Year | Cultivar | Planting density (cm x cm) | Amount of inorganic fertilizer ($kgha^{-1}$) | | | Sowing date | Irrigation application |
|---|---|---|---|---|---|---|---|---|
| | | | | N | P | K | | |
| **Research station** | | | | | | | | |
| 1. Ikenne (Oikeh et al., 2008) | 2004 | N1 | 20x20 | 0 | 0 | 25 | 16 Jun | no |
| | | | | 30 | 0 | 25 | | |
| | | | | 60 | 0 | 25 | | |
| | | | | 120 | 0 | 25 | | |
| | | | | 0 | 26 | 25 | | |
| | | | | 30 | 26 | 25 | | |
| | | | | 60 | 26 | 25 | | |
| | | | | 120 | 26 | 25 | | |
| 2. Bohicon (Sokei et al., 2010) | 2007 | N1 | 20x20 | 60 | 13 | 25 | 29 May | no |
| | 2008 | | | 0 | 0 | 0 | 31May | |
| | | | | 0 | 0 | 0 | | |
| 3. Niaouli (Koné et al., 2007) | 2004 | N4 | 20x20 | 100 | 100 | 100 | 3 Jun | no |
| | 2005 | | | 0 | 100 | 100 | 5 May | |
| | | | | 100 | 0 | 100 | | |
| 4. IITA (Saito and Futakuchi, 2010) (Sone et al., 2009) | 2006 | N1 | 20x20 | 50 | 13 | 25 | 19 Sep | yes |
| | 2007 | | | 50 | 13 | 25 | 27 Feb | |
| **On farm –research** | | | | | | | | |
| Pingou | 2009 | N4 | 30x10 | 66 | 14 | 27 | 4 Aug | no |
| | 2010 | | | 34 | - | - | 13 Jul | no |
| 6. Kpakpazoumé | 2009 | N1 | 30x10 | 63 | 14 | 27 | 14 Jul | no |
| | 2010 | | | 66 | 17 | 33 | 15 Jul | no |
| **Farmland** | | | | | | | | |
| 7.Tchankpéhoun | 2009 | N1 | 30x10 | 39 | 14 | 27 | 28 Jul | no |
| | 2010 | | | 35 | 7 | 13 | 14 Jul | no |
| 8. Tohoué | 2009 | N1 | 30x10 | 44 | 16 | 25 | 27 May | yes |

**Table 4.** Measure of agreement between a model and observed data.

| Name | Equation | Optimum value |
|---|---|---|
| Mean error | $\frac{1}{n}\sum_{i=1}^{n}(y_i - x_i)$ | 0 |
| Mean relative error | $\mathrm{MRE} = \frac{1}{n}\sum_{i=1}^{n}\frac{(y_i - x_i)}{x_i}$ | 0 |
| Mean absolute error | $\mathrm{MAE} = \frac{1}{n}\sum_{i=1}^{n}(\lvert y_i - x_i\rvert)$ | 0 |
| Model efficiency | $\mathrm{EF} = \frac{\left[\sum_{i=1}^{n}(x_i - \bar{x})^2 - \sum_{i=1}^{n}(y_i - x_i)^2\right]}{\sum_{i=1}^{n}(x_i - \bar{x})^2}$ | 1 |
| Root mean square | $\mathrm{RMSE} = \left[\frac{1}{n}\sum_{i=1}^{n}(y_i - x_i)^2\right]^{0.5} \times \frac{100}{\bar{x}}$ | 0 |

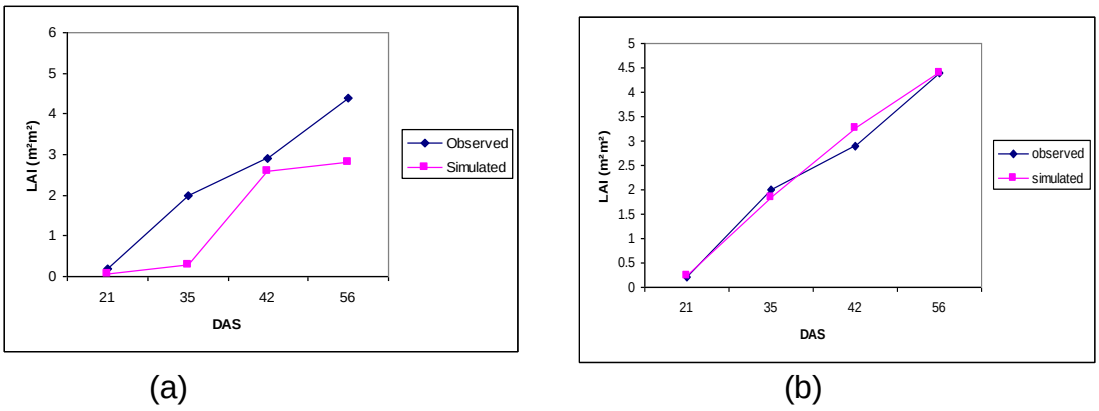



**Figure 1.** Comparison between simulated and observed leaf are index (LAI), (a) situation before and (b) after calibration.

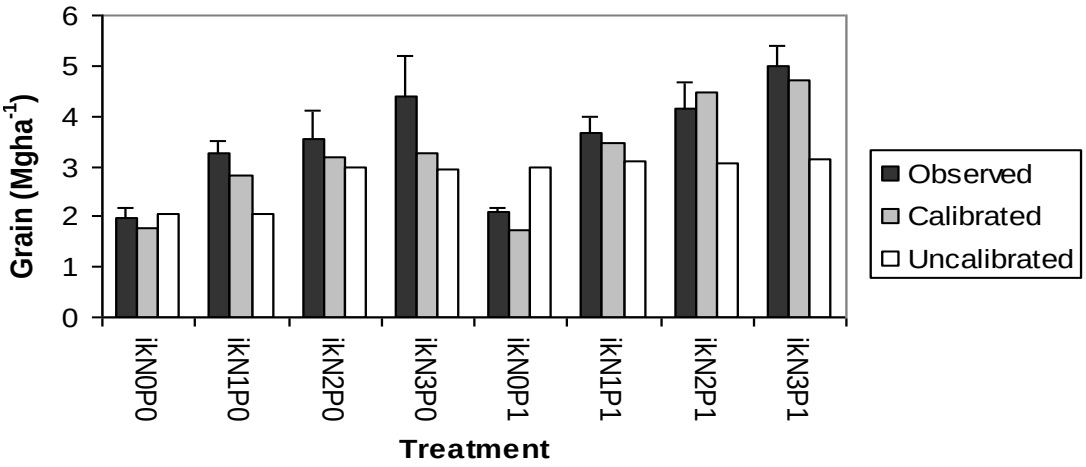



**Figure 2.** Model sensitivity to supply of N and P before and after calibration for Ikenne site in 2004 (N0, N1, N2, N3 is 0, 30, 60 and 120 kgNha$^{-1}$, P0 and P1 is 0 and 26 kgPha$^{-1}$ respectively).

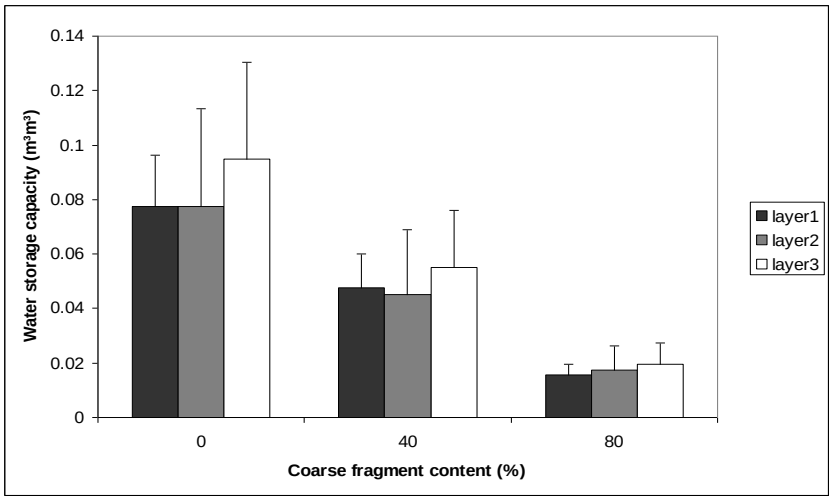
NERICA lines (Ekeleme et al., 2009).

## Model sensitivity for soil parameter

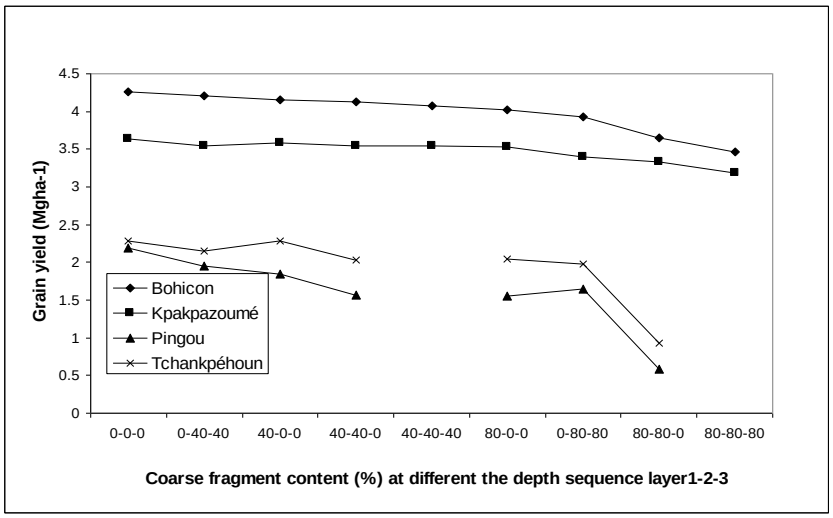
Before calibration, the model showed low sensitivity to the supply of inorganic N and P on a highly weathered and strongly acid low-activity clay soils at Ikenne (Figure 2), as the experimental layout was made to test the effect of fertilizer application in the humid forest zone on Ultisols (Table 3). Leenhardt et al. (2006) suggested the use of pedotransfer functions to estimate soil properties during the simulation process as solution for unavailable data. However, Gaiser et al. (2010) using sensitivity analysis estimated the fraction of microbial biomass across some different soil types under cropland in West Africa. The fraction of biomass in the soil organic matter pool (FBM) triggers the mineralisation of soil nitrogen, which is the main growth constraint in low-input small-holder systems in West Africa. The authors set a value of FBM to 0.01 instead of 0.04 that is more representative for soils with high organic matter content (Niu et al., 2009). The recommended value of 0.01 was then used for all sites.

In addition, the fraction of humus in the passive pool expresses the proportion of carbon (and nitrogen) in the soil organic matter pool that has a low turnover rate. It was set to 0.99 making less nitrogen available to the plant, thus generating more response of the crop to additional nitrogen supply.

More sensitivity of yield to fertilizer P application in the model was found when initial labile phosphorus concentration in the first layer for the acid Ultisol was set to a value of 0.05 ppm. Labile phosphorus (CSP) is considered to have contributions to be correlated to P uptake (Sharpley, 1985). The labile P concentration factor allows optimum uptake rates when CSP is above




(a)




(b)

**Figure 3.** Sensitivity analysis of coarse fragment content on: (a) mean water storage capacity of Bohicon, Pingou, Tchankpéhoun and Kpakpazoumé (b) grain yield with the variations of coarse fragment content value at different soil layers.

20 ppm which was the default value used as critical labile P concentrations for a range of crops and soils. The soils in Ikenne are classified by USDA as Ultisols: They are considered to be low in CEC and bases due to the translocation of the clay in the subsoil and high leaching. They present a high P sorption to Fe and Al-hydroxides in the subsoil (Mokwunye, 1979) or kaolinite in the clay fraction (Wisawapipat et al., 2009). Daroub et al. (2004) in developing a soil-plant P model for highly weathered soils recorded for maize an overestimation of the P uptake by the model. Apparently, their model was not able to reproduce P fixation which is much higher than in less-acid soils developing in temperate climates.

The analysis of rainfed upland system refers also to evaluation of the water availability with depends on soil texture. The coarse fragments influence soil physical hydraulic properties. In EPIC, the role of this parameter addresses directly to the water erosion engine but it has soil functioning oriented for estimation of water storage capacity at the same stand as the bulk density. In fact Chow et al. (1997) observed by incorporating 10 to 30% coarse fragments into the plow layer of the Northen American Podsol, it increased significantly the soil bulk density and this increase reduced the porosity and soil water-holding capacity. The sensitivity analysis of coarse fragments content was done in 4 sites where substantial coarse fragments were identified in soil profile to show influence of this parameter the grain yield.

Figure 3 shows at 2 to 3 soil layers over 4 sites (Bohicon, Kpakpazoumé, Pingou and Tchankpéhoun) variation in coarse fragments content from 0 to 80%. It appeared that strong influence of coarse fragment

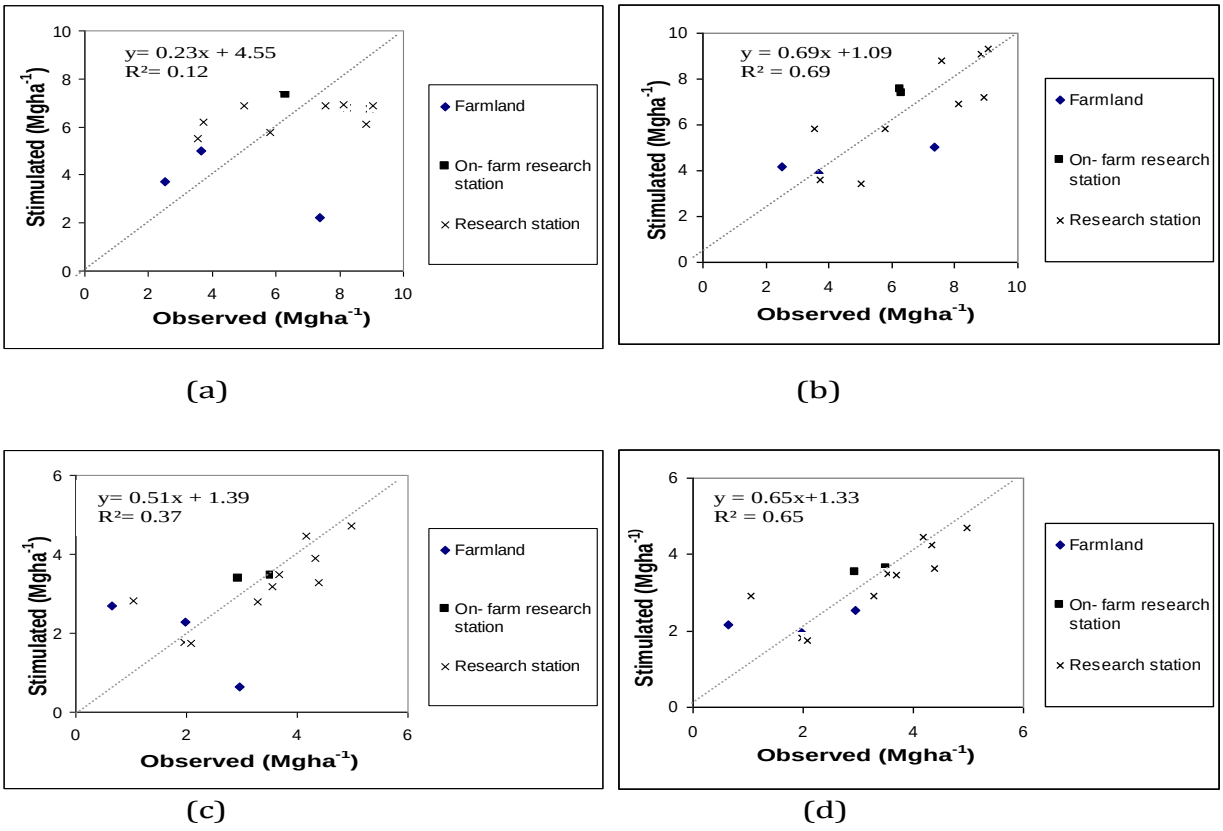



**Figure 4.** Scatter plot between observed and simulated total above ground biomass before (a) and after the calibration (b), grain yield before (c) and after calibration (d).

content was obtained when all the layers are concerned by the limitation and the upper layers showed the higher sensitivity for grain yield.

## Simulation of total aboveground biomass and grain yield

Figure 2 shows that the model reflects after calibration the effect of N and P application on NERICA yield on Ultisols when P and N are limiting. This is in accordance with Nigerian forest agroecosystems where high split application of 90 to 120 kg N $ha^{-1}$ has been recommended for the rice cultivars to optimize yields (Enwezor et al., 1989). The model results in 7 over 8 treatments were not significantly different from the observed yield of NERICA. The P stress has been simulated adequately to allow the expression of nitrogen stress among the treatments with application and without application of P. By simulating adequately the processes in P deficient soils, the model agrees with the results of Sahrawat et al. (1995) suggesting that P fertilization of acid-tolerant upland rice can significantly improve its productivity under Ultisols. Before the calibration and over all five sites, grain yield and total aboveground plant biomass had a RMSE of more than 30% (Table 6). Farmland fields contributed the most to overestimation of model by 88% for 2 years on average (Figure 4). For the calibration in Tchankpéhoun, the plant density was reduced from the theoretical plant population to the measured plant density at maturity. During the two seasons, heterogeneous planting density translated by the missing of plant hills led to the reduction of the total yield observed. Affholder (2001) pointed out that a model developed in high input environment such as the US where the planting density is very homogeneous need numerous modifications to be applied under the conditions of West Africa where the variability of the densities of sowing is a big factor of the variability of the productivities. Oikeh et al. (2009) did not show relationship between the grain yield and NERICA density whereas density effects appeared only for tiller and panicle densities. This study was undertaken with seasonal differences in rainfall distribution and moisture availability that may reduce simple effects of N and spacing (plant density). Tchankpéhoun got adequate monomodal rainfall supply for the two years. After calibration, the goodness of fit of the model was improved for both total aboveground biomass and the grain yield (Figure 4). Lower RMSE after calibration indicated that a

**Table 5.** Parameter setting for rice in the EPIC crop file: original defaults and values after calibration (WA, biomass-energy conversion factor; HI, potential harvest index; WSYF, minimum harvest index; LAImax, maximum leaf area index; PPC1/PPC2, plant density, LAI parameters DLAP1, DLAP2 ).

| Parameter | Explanation | Original | Used in the parameterization |
|---|---|---|---|
| WA | Radiation use efficiency (kg $ha^{-1}$/MJ$m^{-2}$) | 25 | 25 |
| HI | Harvest index (decimal fraction) | 0.50 | 0.55 |
| PHU | Potential heat unit (degree days) | 1500 | 1500 |
| WSYF | Minimum harvest index under water stress condition (decimal fraction) | 0.25 | 0.01 |
| LAI max | Potential maximum leaf area index ($m^2$ $m^2$) | 6 | 6 |
| DLAP1 | First point on optimal leaf area curve .Percentage of heat unit | 30.01 | 30.20 |
| DLAP2 | Second point on optimal leaf area curve .Percentage of heat unit | 70.95 | 60.95 |
| PPC1 | 1st point of plant population density for crops (plants $m^2$)/Fraction of potential leaf area index at 1st point (decimal fraction) | 125/600 | 50/600 |
| PPC2 | 2nd point of plant population density (plants $m^2$)/PPT2 Fraction of potential leaf area index at 2nd point (decimal fraction) | 250/900 | 250/600 |

higher fraction of the measured variations were accounted by the model (Table 6).

## Model validation

The calibration of the EPIC model for upland rice was focused on nitrogen and phosphorus as main constraints to crop growth. The validation was carried out on three sites (Niaouli, Bohicon and Pingou) over two seasons. At Niaouli the experiment tested different levels of N and P input, at Bohicon NPK application was tested and Pingou was an on-farm field experiment (reference in Table 3).

The validation of the model showed that a relatively high gap between averages simulated and observed yield (Table 7). The mean error was 1.2 Mg $ha^{-1}$ whereas the mean relative error was 3.0 Mg $ha^{-1}$, which showed a very large overestimation of the simulated yields at plot level. The variation of the individual plots was also quite high resulting in root mean square error of more than 100%. The observed grain mean grain yield was lower than the average in the calibration suggesting various stress effects. Indeed some causes of rice failure attributed to floods and drought were reported b for NERICA evaluation on 5 locations with similar pedoclimatic conditions to these experiments in Benin republic (JAICAF, 2007). Therefore, before the use of the model in the assessment of impacts of and adaptations to climate variability and climate change in spatial studies, there is still a need for improvement in the amount and quality of available data collection.

Figure 5 showed a scatter plot of the observed and estimated value of sites used for model validation. The average yield in plots used for validation was relatively low, this is due to crop failure in 2006 in Niaouli where the average yield was below 1 Mg$ha^{-1}$ leading to the model overestimation. In fact, the experimental design was originally set up to evaluate the tolerance to drought with nutrients application for NERICA cultivar. Niaouli is located in the sub humid zone with bimodal rainfall pattern. The mid-season rainfall pattern associated with the sandy topsoil texture induced severe drought stress. The soil type “terre de barre” was described by Azontonde (1991) as soil with good physical hydraulic characteristics but with low water storage and their structure can be rapidly destroyed when there is no proper technique for maintaining organic matter.

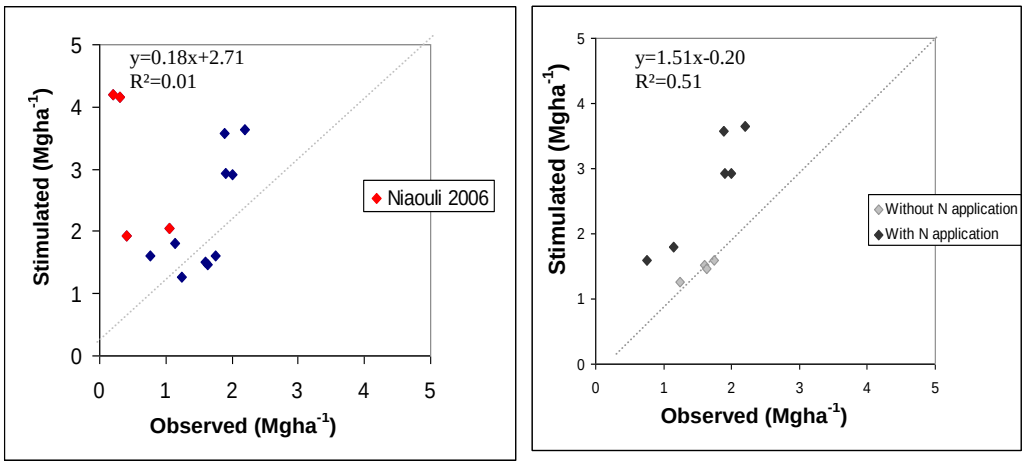
The sensitivity of NERICA cultivar to water stress is well documented. Akinbile et al. (2007) showed that with NERICAs yield decreased under optimal satisfactory conditions almost linearly with evapotranspiration thus indicating that water application remained dominant factor at all the stages of production. In EPIC model, the potential harvest index is adjusted daily according to water stress suffered by the crop (Williams, 1995). During the calibration, the sensitivity of model was increased by setting the water stress impact (WSYF parameter), which allowed harvest index to drop to 0.01 in case of severe drought. The effect of water stress could be in fact limited to HI reduction. Fuji et al. (2004) reported that some

**Table 6.** Mean simulated and observed rice LAI ($m^2m^2$), total above ground biomass (TAB), grain yields in Mg $ha^{-1}$ as well as mean error (ME in Mg $ha^{-1}$), mean relative error (MRE), mean absolute error (MAE), model efficiency (EF) and mean root square error (RSME) before and after model calibration over 6 sites.

| Sites | n | Obs. | Sim. | ME | MRE | MAE | EF | RMSE |
|---|---|---|---|---|---|---|---|---|
| **Before calibration** | | | | | | | | |
| LAI ($m^2m^2$) | 4 | 2.38 | 2.16 | -0.22 | 0.07 | 1.55 | 0.00 | 32.84 |
| TAB(Mg $ha^{-1}$) | 15 | 6.33 | 6.04 | -0.30 | 0.04 | 1.55 | 0.09 | 30.15 |
| GY(Mg $ha^{-1}$) | 15 | 3.03 | 2.97 | -0.06 | 0.23 | 0.71 | 0.32 | 33.13 |
| **After calibration** | | | | | | | | |
| LAI ($m^2m^2$) | 4 | 2.38 | 2.44 | 0.06 | 0.06 | 0.14 | 0.98 | 8.39 |
| TAB(Mg $ha^{-1}$) | 15 | 6.33 | 6.33 | 0.00 | 0.05 | 1.55 | 0.61 | 21.10 |
| GY(Mg $ha^{-1}$) | 15 | 3.03 | 3.15 | 0.11 | 0.24 | 0.47 | 0.67 | 23.01 |

**Table 1**. Validation of the EPIC model with respect to yield of rice in Mg $ha^{-1}$. Obs. is observed and sim. is simulated value, n is the number of pair of observed and simulated grain yield, a is the regression slope. The mean error (ME in Mg $ha^{-1}$), mean relative error (MRE), mean absolute error (MAE) and mean root square error (RSME) are calculated over 3 sites.

| Grain yield (Mg $ha^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|
| N | Obs. | Sim. | ME | MRE | MAE | RMSE (%) |
| 14 | 1.3 | 2.5 | 1.2 | 3.0 | 1.2 | 134.74 |




(a) (b)

**Figure 5.** Scatter plots for NERICA validation, (a) represents model validation for all plots, (b) refers to plot without the particular year Niaouli 2006.

Nerica lines showed high dry matter production under drought condition among other rice cultivars, and this have been correlated with stomata conductance (r=0.63**). However, intensive rains of short duration followed by long dry spells that occurred during the flowering period which lead to increased sterility and decrease in grain weight (O´Toole and Moya, 1981). De Barros et al. (2005) observed slight overestimation of grain yield by the EPIC simulations was attributed to high rates of floral abortion caused by dry spells during the flowering periods since this factor is not considered in the model.

**Table 8.** Validation data results without Niaouli 2006 , with reference to fertilizer treatment, year and observed grain yield, + symbol refers to presence and - the absence of fertilizer input.

| Year | Site | Treatment (fertilizer) | | Grain yield (Mgha$^{-1}$) | |
|---|---|---|---|---|---|
| | | N | P | Observed | Simulated |
| 2007 | Bohicon | - | - | 1.64 | 1.49 |
| 2008 | Bohicon | - | - | 1.24 | 1.36 |
| 2007 | Bohicon | + | + | 2.20 | 3.74 |
| 2008 | Bohicon | + | + | 1.88 | 3.67 |
| 2009 | Pingou | + | + | 1.14 | 1.80 |
| 2010 | Pingou | + | + | 0.73 | 1.60 |
| 2005 | Niaouli | - | - | 1.60 | 1.51 |
| 2005 | Niaouli | - | + | 1.75 | 1.60 |
| 2005 | Niaouli | + | - | 1.90 | 2.62 |

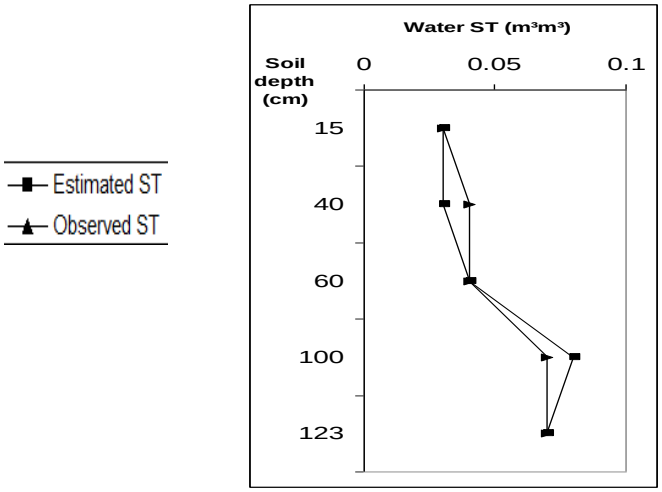



**Figure 6.** Water storage capacity simulation after content in the layers for drought experiment in Niaouli in the rooting zone.

After removing the plots with crop failure induced by drought in 2006 in Niaouli, the goodness of the fit of the model was improved from 0.01 to 0.51. Table 8 lists the simulation results of the remaining plots. Pingou siteo held in 2010 the lowest yield below 1 Mgha$^{-1}$. In this year it was observed a shallow groundwater during the wet season at sowing (end of July) and was followed by transplanting. Therefore the first possibility for the model overestimation is that the model could not consider transplanting shock that caused a delay in phenological development resulting in reduced vegetation period in the field. No reported analyses on negative impact of flooding on upland NERICA were available. In controversy Fofana (2008) highlighted the recovery and the improvement possibility of NERICA1 production after short and intense moisture stress at the seedling emergence stage. The presence of ferric cuirasses in Pingou may result in low saturated conductivity at the middle soil depth, thus increasing the submergence and runoff risk. The relatively high soil moisture should have been the explanation of low yield due to direct seeding in 2009. Indeed Ogunremi et al. (1986) demonstrated direct-seeded rice was adversely affected by the transient flooding conditions during the seedling stage on Ultilisol in Southern Nigeria. The obtained grain yield decreased indeed with increasing penetrometer resistance.

The tendancy of overestimation remained of yield response to fertilizer was observed in Bohicon and Niaouli. Even at Niaouli in 2005, where experiment also applied relatively high amount of NP (100 kg ha$^{-1}$) that were less efficient in observation than in modeling. Under limiting water conditions there could be less capacity of crop to continue water uptake that can probably reduce transport to the roots through mass flow. Indeed some traits of upland rice (japonica type) related to less adventious roots per hill result in relatively week ability in N uptake (Zhang, 2008). In addition, a severe drought that occurred just after the application of the first split of N could have induced urea volatilization resulting from the lack of N dissolution Oikeh et al. (2008).

From data drawn from the upland experiments, the EPIC model was calibrated and parameterized for a multisite evaluation, which is particularly important for rice production because of its high dependency to nutrients and water. In the model validation the variations of individual plots also were higher than the observed. The model overestimated the yield under drought condition in the site of Niaouli. The model indeed estimated effective lower water retention in the first soil depths by the use of texture. Figure 6 shows the relatively good agreement of the model estimation for water storage capacity in the Niaouli site that allowed condition for water stress experiment. However, it has been also reported in the sensitivity analysis of the model that coarse fragment content had more or less high influence on water storage capacity in the soil layers. In four sites out of eight sites, the model was parameterized with coarse fragment content limiting water storage capacity in different layers.

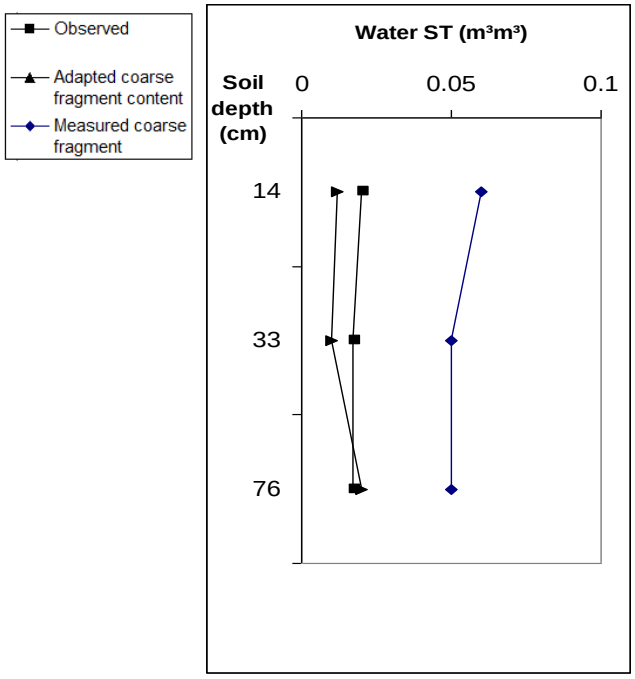



**Figure 7.** Comparison between water storage model estimation after adjustment of water storage capacity in Bohicon.

Adapted value was needed in case of Bohicon while the model still overestimated the water retention capacity with the measured variables. The results of final calibration are shown in Figure 7. Indeed, the model estimation of water storage required modification in Bohicon and reduced the yield gap between observed and simulated value from 3 to 2 $Mgha^{-1}$ on average. As consequence, when simulating rainfed rice it appear to be a prerequisite to provide detailed site-specific soil input parameters including water retention among soil physical characteristics.

At the level of discussion of plant nutrition causes on the overestimation of the model, if the model was adequately tested to respond to NP fertilizer from calibration there is still issue of the simulation of micronutrients. Several experiments conducted on highly weathered soils in Africa showed that as sufficient N and P is applied in maize, micro nutrient deficiencies may appear (Gaiser et al., 1999). Voortman et al. (2000) estimated micronutrients deficiencies on about 60% of the cropland in sub-Saharan Africa. This confirms the requirement to improve micronutrient effects in crop models. Furthermore, Koné et al. (2009) proved that there was also a significant (P = 0.004) decreasing effect of Zn (28%), N (34%) and K (36%) exclusion on the mean grain yield in the Ferralsol soils Benin. These results attested the existence of Zn and K deficiencies which may reduce the sustainability of upland rice production.

In the study through the modeling tool, interspecific genotypes were evaluated under with local farmer's conditions which include in addition to low inherent soil fertility, the occurrence of drought or flood. This multisite evaluation did not show any type of interaction between variety and environment that should be seen in the simulation outputs. Several kind of stress may limit varietal selection progress under unfavourable environments (Banziger and Cooper, 2001). In fact, Mandel et al. (2010) found smaller genetic variance for grain yield under low-input conditions in India.

The higher effect of soil texture input on grain yield confirmed the importance of water capacity retention estimation as a key soil parameter indicating that management of water supply (bunds building or reduction drainage) are likely to be useful for improving rice production

## Conclusion

The EPIC model was tested with mix of collected and secondary data from an experimental station, on farm research field and farmland in the tropical humid with P fixing soils to semi-humid zones. The study showed the relevant crop and soil parameters for calibration in upland rice cropping conditions, in particular different pools of nitrogen and phosphorus in the soil. In calibration, the model presents a good response to the application of N and P mineral fertilizers. A multiple year calibration for multiple variables such as plant biomass, leaf area index and yield improve our confidence in the model calibration. Although the EPIC shows the sensitivity of rice to seasonal rainfall, its robustness under severe water stress become limited. With a multiple year calibration for multiple variables such as plant biomass, leaf area index and yield, the uncertainty in the model prediction in validation is related at first place, to the lack or quality on input data (estimation of impact drought spells on grain yield) and secondly on the model weakness (reduction of HI linked to crop phenology and fertilizer responsiveness under low input environments).

## Conflict of Interest

The authors have not declared any conflict of interest.

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# Response of lettuce crop to magnetically treated irrigation water and different irrigation depths

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**The effects of irrigation with magnetically treated water (MTW) and common water (CW) on lettuce plantations at different irrigation depths were analyzed. Current assay was conducted in a greenhouse and featured an experimental 2 x 5 design with randomized blocks and ten replications, comprising two water sources (MTW and CW) and five irrigation depths (replacement of 25, 50, 75, 100 and 125% of evapotranspiration of the crops - ETc). Two crop cycles were undertaken to observe the repeated effects of the treatments. The number of leaves and aerial green weight were underscored among the several effects. An increase in the number of leaves was reported due to irrigation depths in types of water, with a positive effect of MTW at 100% and 125% depths of ET in the two cycles. Increase in the green phytomass of the aerial section was reported when replacement depth of 100% of ET in the two cycles was employed.**

**Key words:** Magnetism, productivity, efficiency, evapotranspiration.

## INTRODUCTION

Planet Earth has 98% of sea water and 2% of fresh water for human consumption. Further, 87% of fresh water lie on the poles and in glaciers (Moraes and Jordao, 2002), with a mere 0.26% of total fresh water available, in a non-frozen form, for consumption.

Agricultural activities consume 70% of the water available since water is a determining factor in plants´ physiology, nutrition and growth. The absorption of nutrients by plants mainly occurs through the root system by mass flow, diffusion and interception, which are practically and entirely dependent on water. The relevance of irrigation for plant development triggers a search for the best use of water in every possible way (Silva, 2008). This is why several researchers study magnetically treated water (MTW) and its applications in agriculture.

Several studies point to evidence that the water exposed to the magnetic field has different properties from the untreated water. The major changes were observed in the water adsorption of water on surfaces (Ozeki et al., 1996), crystallization and precipitation of salts (Katsuki et al., 1996; Kronenberg, 1985), the solubility of some minerals (Hasson and Bramson, 1985; Herzog et al., 1989; Bogatin et al., 1999; Gehr et al., 1995) and surface tension (Joshi and Kamat, 1966; Bogatin et al., 1999) when subjected to the magnetic field water observed that degassing occurs, thus increasing

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**Table 1.** Climatic parameters during the experiment.

| Parameters | | Cycle-1 | Cycle-2 |
|---|---|---|---|
| Temperature (°C) | Minimum | 16.29±3.80 | 16.08±3.25 |
| | Maximum | 34.40±3.99 | 31.15±6.38 |
| | Means | 23.63±2.04 | 21.60±3.96 |
| Humidity (%) | Minimum | 43.22±9.85 | 41.96±12.84 |
| | Maximum | 92.60±3.28 | 89.88±14.70 |
| | Means | 75.48±6.22 | 72.88±12.88 |
| Evaporation (mm) | | 105.4 | 102.6 |

Legend: means ± standard deviation.

the permeability in soil, which consequently increases the efficiency of irrigation.

Maheshwari and Grewal (2009), Mohamed (2013) and Grewal and Maheshwari (2011) studied the use of salt water and MTW for the irrigation of several types of crops. These authors employed a magnetizer in water treatment and observed different crop reactions on the increase in productivity and quality when magnetized saline water was applied. In fact, when Lin and Yotvat (1990) used MTW in the production of several cash crops, they reported that crops with MTW showed considerable difference from those treated with common water (CW). Further, Yaofu et al. (2007) employed from growth to harvest magnetic water in the irrigation of tobacco plants and reported higher production and quality when compared to results from plants treated with CW. Research assays in which MTW was used for plant irrigation showed that the soil's mineral salts are taken to deeper regions and that the sediment is not formed at the surface.

The plant is thus impaired in nutrient absorption and forms additional roots to further absorb the required elements for its growth and development. The above process may cause anatomical and physiological abnormalities that are demonstrated in the process of the plant´s formation and production. Consequently, energy waste occurs for its root formation (Nimm and Madhu, 2009; Nasher, 2008).

It has been demonstrated that MTW contributes towards an increase in farmers´ income and that production increases in quantity and quality. In fact, reports from Russia and China underscore the efficiency of MTW for many types of crops (Silva, 2008). The need for new technologies that optimize the use of irrigation water without losing quality rates in production and increase in food production has helped establish the hypothesis that there is a positive influence in productivity when irrigation water is induced into a magnetic field (Silva et al., 2011; Putti et al., 2013). However, few researches with MTW have been conducted. Current assay tries to give support the scientific basis of procedures and to confirm the occurrence of changes in the production of food and other possible advantages when MTW is used for irrigation.

## MATERIALS AND METHODS

The assay was performed in March and April 2012 in a protected area in the Department of Rural Engineering of UNESP at the Faculty of Agronomy Sciences on the Lageado Experimental Farm, Botucatu SP Brazil, 22° 51' S and 48° 26' W, altitude 786 m. According to classification by Köppen (Koppen and Geiger, 1928), the region has a Cfa climate (Subtropical Humid Climate). Climate parameters were recorded by an automatic meteorological station. Table 1 shows the climatic details during the experiment.

The soil of the greenhouse is Red Nitosol Dystrophic soil with a moderate clayey structure, according to Carvalho et al. (1983). The chemical characteristics of the soil comprised pH ($CaCl_2$) = 5.9; M.O. = 24 g $dm^{-3}$; P (resin) = 191 mg $dm^{-3}$; K = 4.8 mmolc $dm^{-3}$; Ca = 68 mmolc $dm^{-3}$; Mg = 25 mmolc $dm^{-3}$; H+Al = 17 mmolc $dm^{-3}$; SB = 67 mmolc $dm^{-3}$; B = 0.51 mmolc $dm^{-3}$; Cu = 4.8 mmolc $dm^{-3}$; Fe = 20 mmolc $dm^{-3}$; Mn = 10.10 mmolc $dm^{-3}$; Zn = 8 mmolc $dm^{-3}$ CTC = 114 mmolc $dm^{-3}$; V = 85%.

The soil was prepared by a tractor with a rotation plough that revolved an approximately 30 cm surface layer and limited the plots. Weeds were manually uprooted when required.

Seeds were sown in expanded polystyrene trays with three seeds per cell and thinning occurred after 14 days of sowing, leaving only one plant per cell. Seedlings were transferred to the plots in 25 cm x 25 cm spacing when the former had four or five permanent leaves. Experimental plots were 1.2 m wide by 3 m long, totaling 3.6 m², with four rows. Plants on the side rows were discarded and only the plants within the central rows were taken into consideration.

The experimental design comprised 5 x 2 randomized blocks with 10 treatments and ten replications; repetition comprised a lettuce plant. Treatments consisted of irrigation depths to replace 25, 50, 75, 100 and 125% of crop evaporation (ETc) and two water sources, common water (CW) and magnetically treated water (MTW). Sylocymol Rural equipment, manufactured by Timol, was employed for the magnetization of water. The experiment had two independent systems of drip irrigation made up of a main line with the direct insertion of Amandani-type drips manufactured by Petroísa Irrigações Ltda. Spacing between drips was 0.30 m, with a mean discharge of 1.47 $L.h^{-1}$, at a pressure of 10 m.c.a.

Irrigation and reading of Class A tank were undertaken daily at 8 am and irrigation time was determined as follows:

**Table 2.** Mean number of leaves according to type of water and irrigation depths.

| Irrigation depths (%) | 1st Cycle | | 2nd Cycle | |
|---|---|---|---|---|
| | MTW | CW | MTW | CW |
| 25 | 32.8±2.17$^{Ba}$ | 30.0±2.92$^{ABa}$ | 19.2±0.84$^{Ba}$ | 19.2±2.39$^{Aa}$ |
| 50 | 31.6±2.40$^{Ba}$ | 26.00±2.45$^{Bb}$ | 18.6±1.14$^{Bb}$ | 20.8±1.30$^{Aa}$ |
| 75 | 31.4±3.20$^{Ba}$ | 28.2±1.92$^{ABb}$ | 20.2±1.48$^{Ba}$ | 19.6±1.52$^{Aa}$ |
| 100 | 39.0±1.58$^{Aa}$ | 26.4±1.34$^{ABb}$ | 24.6±1.14$^{Aa}$ | 17.8±1.92$^{Ab}$ |
| 125 | 35.0±3.67$^{Aba}$ | 30.20±1.79$^{Ab}$ | 22.8±1.30$^{Aa}$ | 20.4±2.07$^{Ab}$ |
| s($\hat{m}$) | 1.09 | | 0.70 | |

Means followed by the same small letter in the line and by the same capital letter in the column, for each cycle, do not differ at a 5% probability, by test *t*.

$$Ti = 6.000 . \frac{Kc . Kp . Eca . Sl . Sg . TR}{Ei . Vg} \quad (1)$$

where, $Ti$ is irrigation time; $Kc$ is the coefficient of the crop; $Kp$ is the coefficient of the tank; $Eca$ is the evaporation of Class A tank (mm day$^{-1}$); $Sl$ is the spacing between the sides (m); $Sg$ is the spacing between drippers (m); $Ei$ is the efficiency of irrigation (%); $Vg$ is the discharge of drippers (L h$^{-1}$).

Total irrigation depth applied was calculated according to Snyder (1992) in which evaporation (Kp) was obtained by the equation below:

$$kp = 00482 + 0.024 \ln(B) - 0.00376.V + 0.0045.R \quad (2)$$

where $Kp$ is the coefficient of the tank; $B$ is the edge of the vegetation area surrounding the tank (m); $V$ is the speed of the wind at a height of 2 m (m/s); $UR$ is mean relative humidity (%). $Kc$ rates followed instructions by FAO 56 (1998) in which 0.7 are used at the beginning; 1 in the middle; 0.95 at finish.

Due to the limited weather data, it was not possible to use the FAO Peanman-Monteith model to estimate reference crop evapotranspiration. However, empirical methods including mass transfer-, radiation-, temperature-, and pan evaporation-based methods have been developed for estimation of the reference crop evapotranspiration using limited data and some studies shows the successful applications of this method (Valipour, 2014a, b, c, d, e, f, 2015a, b).

Number of leaves, green and dry phytomass of the aerial section, green and dry phytomass of the root, length of root, rates of Chlorophyll *a* and *b* and total chlorophyll were assessed.

Data were processed by Anderson-Darling normality tests and by Bartlett´s variance homogeneity tests, followed by analysis of variance (F test) at 5% significance level.

## RESULTS AND DISCUSSION

### Number of leaves

After checking normal and homegeinidade data, analysis of variance (ANOVA) revealed eststísticas differences between treatments and also interaction siginificativa at the level of 5%. Therefore, the unfolding was carried out in the levels of water and irrigation factors for each variable in each cycle analyzed.

Water depths with MTW in the analysis of NL for the first cycle had the highest responses, with no significant difference for 25% ETc depths (Table 2). Irrigation depths with 100% and 125% of ETc with MTW had the highest number (Table 2).

As perceived for the number of leaves of the first cycle, CW-irrigated treatment and irrigation depth of 125% of ETc showed significant difference when compared to the others, since it had the highest number of leaves. On the other hand, the lowest number of leaves was observed at irrigation depth of 50% of ETc, with a 16.15% difference between the numbers of leaves.

Depths of water irrigated with MTW had the highest number of leaves with 100% of ETc and showed significant difference, whereas a smaller amount of leaves was obtained in depths irrigated with 75% of ETc. However, a 24.20% increase in the number of leaves revealed no statistical difference. Depth at 25% of ETc did not show any significant difference when the behavior of water type was analyzed according to irrigation depths.

Irrigation depths of 50, 75, 100 and 125% of ETc showed a significant difference, with 21.53, 11.34, 47.72 and 15.89% increase in the number of leaves, respectively. There was no significant difference for irrigation depths with CW in the second cycle.

The number of leaves in the irrigation depths varied when MTW-irrigated treatment was analyzed. Highest production occurred with irrigation at 100% ETc and the lowest at a depth of 50% ETc, or rather, a 32.25% increase in the number of leaves.

When the depth between the two types of water was assessed, there was a significant difference in depths of 100% and 125% of ETc irrigated with MTW, respectively, with a 38.20 and 11.76% increase. However, in the case of depth of 50% of ETc, the highest number of leaves occurred when irrigated with CW, featuring an 11.82% increase.

An analysis of the two cycles showed that the highest number of leaves occurred for depth of 100% ETc irrigated with MTW. Nevertheless, in the case of MTW,

**Table 3.** Mean green phytomass of the aerial segment (GPAS) (g) according to the type of water and depth.

| Depths (%) | 1st Cycle | | 2nd Cycle | |
|---|---|---|---|---|
| | MTW | CW | MTW | CW |
| 25 | 271.6±39.9$^{BCa}$ | 199.6±39.7$^{Ab}$ | 174±23.3$^{Ca}$ | 140.3±57.7$^{Ba}$ |
| 50 | 238.4±14.1$^{Ca}$ | 205.1±21.4$^{Aa}$ | 123.4±28.5$^{Ca}$ | 148.3±39$^{Ba}$ |
| 75 | 308.3±26.3$^{ABa}$ | 226.9±31.5$^{Ab}$ | 169.1±43.4$^{Ca}$ | 215±42.6$^{ABa}$ |
| 100 | 355.54±14.9$^{Aa}$ | 217.8±27.2$^{Ab}$ | 397.5±42.4$^{Aa}$ | 243.2±36.3$^{Ab}$ |
| 125 | 290.8±37.3$^{BCa}$ | 256.1±37.6$^{Aa}$ | 272.1±27.3$^{Ba}$ | 238.1±51.4$^{Aa}$ |
| s($\hat{m}$) | 6.09 | | 8.10 | |

Means followed by the same small letter in the line and by the same capital letter in the column, for each cycle, do not differ at a 5% probability, by test *t*.

the amount of leaves for depth of 75% of ETc had better results than those with conventional depths. The above, verified in the two cycles, reduced irrigation water by 33.33%.

In their studies on the variation of water depths for lettuce plantations irrigated with CW, Hamada and Testez (1995) obtained their greatest amount of leaves for the depth of 120% of ETc. Villas Boas et al. (2007) also harvested the greatest number of leaves at a depth of 120% of ETC, with an average rate of 23.06 leaves per lettuce plant. According to Lima Junior (2012), the greatest amount of leaves per plant occurred at a depth of 100% of ETc.

In the case of depths irrigated with MTW, Selim and El-Nady (2011) reported that the production of tomato at different tensions rated 40, 60, 80 and 100% of ETc. They were actually significant differences when compared to control where water was not affected by the magnetic field.

## Green phytomass of the aerial segment

Analysis of the green phytomass of the aerial segment for the first cycle revealed that the highest phytomass rate occurred at depths of 25, 100 and 125% of ETc in irrigation depth with MTW (Table 3). On the other hand, in the second cycle, irrigation depths of 100 and 125% produced the highest green aerial phytomass rate, with a significant difference (Table 3).

There was no significant difference between irrigation depths in conventional treatment in the first cycle. The above may be due to the fact that irrigation occurred daily and water tension remained within the crop development bracket.

Highest production of lettuce with MTW occurred at irrigation depth of 100% of ETc and the lowest production occurred at irrigation depth of 50% of ETc, with a 49.11% increase.

When the types of water in each irrigation depth were compared, there was significant difference only for irrigation depths of 50 and 125% of ETc. However, irrigation depths of 25, 75 and 100% of ETc showed the positive effect of magnetism due to a respective 37.44, 35.87 and 63.66% increase in the green phytomass of the aerial segment.

In the second cycle, there were significant differences in irrigation depths with CW. Highest production occurred at the irrigation depth of 100% of ETc, whereas the lowest occurred at the irrigation depth of 25% of ETc, with a 73% increase.

There was significant difference for irrigation depths with MTW. Highest phytomass production occurred with irrigation depth of 100% of ETc and the lowest was obtained with irrigation depth of 50% of ETc, even though it was not statistically different from irrigations depths of 25 and 75% of ETc, with a 122.13% production difference between the irrigation depths.

Only the 100% irrigation depth differed when the effect of water type in the irrigation depths was analyzed, with a 63.44% increase for MTW treatment.

Results showed that the effects for green phytomass of the aerial segment in the two cycles were very close, with highest production at the irrigation depth of 100% of ETc. However, in the first cycle, the production with irrigation depth of 75 of ETc was higher than the other irrigation depths with CW. Consequently, there was a 33.33% save in water, a fact not reported in the second cycle.

With regard to variation in irrigation depth with CW, several researches in the literature showed that increase of water for lettuce crops failed to provide a higher production.

Andrade Junior and Klar (1997) reported maximum production at irrigation depth of 75% of ETc, with an average of 818.72 g plant$^{-1}$ of the variety American-type Mesa 659 lettuce. Maximum production for the variety crisp head lettuce occurred at irrigation depth of 118.8%, with average per plant reaching 296.43 g planta$^{-1}$ (Villas Boas et al., 2007). Araújo et al. (2007) registered the best productivity for cultivar Veronica with irrigation depth of 100% of ETc.

MTW-irrigated chickpea crops had an increase in the green phytomass of the aerial segment in the two cycles.

**Table 4.** Mean dry phytomass of the aerial segment according to water type and irrigation depths.

| Irrigation depths (%) | 1st Cycle | | 2nd Cycle | |
|---|---|---|---|---|
| | MTW | CW | MTW | CA |
| 25 | 9.56±2.15$^{BCa}$ | 8.19±1.11 $^{Ba}$ | 9.73±1.12$^{Ba}$ | 7.94±2.67$^{Aa}$ |
| 50 | 7.8±1.33$^{Ca}$ | 6.88±1.75$^{Ba}$ | 7.43±1.17$^{Bb}$ | 11.42±1.9$^{Aa}$ |
| 75 | 9.21±1.23$^{BCa}$ | 8.27±1.07$^{ABa}$ | 9.70±1.79$^{Ba}$ | 9.89±1.59$^{Aa}$ |
| 100 | 12.74±1.43$^{Aa}$ | 8.03±0.74$^{Bb}$ | 16.07±1.79$^{Aa}$ | 8.45±1.48$^{Ab}$ |
| 125 | 11.34±1.33$^{ABa}$ | 11.11±2.42$^{Aa}$ | 13.76±1.00$^{Aa}$ | 10.51±1.99$^{Ab}$ |
| s($\hat{m}$) | 0.30 | | 0.34 | |

Means followed by the same small letter in the line and by the same capital letter in the column, for each cycle, do not differ at a 5% probability, by test *t*.

There were 12.51 and 11.16% increases respectively in the first and second cycle (Hazayn and Quados, 2010a). No significant difference was reported in celery (*Apium graveolens*) when compared to that irrigated with CW (Hazayn and Quados, 2010a). Selem and El-Nady (2011) reported that an increase in green phytomass of the aerial segment occurred in tomato cultivated with four reposition irrigation depths (40, 60, 80, 100%), which respectively caused a 108.07, 50.147, 37.38 and 36.17% increase when compared to the same irrigation depth with CW. Increase in the production of green phytomass of the aerial segment in tomato was corroborated by Souza et al. (2005).

## Dry phytomass of the aerial segment

In the analysis of dry phytomass of the aerial segment in the first cycle, MTW irrigation provided greater production with all irrigation depths, even though only the irrigation depth of 100% was statistically different (Table 4).

In the second cycle, irrigation depth 50% of ETc with CW differed statistically from that with MTW. Irrigation depths of 100 and 125% of ETc provided greater production and thus they were underscored when irrigated with MTW.

The highest accumulation rate occurred with irrigation depth of 125% of ETc for dry matter within the first cycle irrigated with CW, with significant difference from the others. The lowest production rate occurred with irrigation depth of 50% of ETc, with a 61.48% decrease.

MTW irrigation had the best performance with irrigation depth of 100% of ETc. The lowest production of dry matter occurred with irrigation depth of 50% of ETc, with a 63.33% decrease.

A difference was reported only for irrigation depth of 100% of ETc when irrigation depths were analyzed according to type of water. In this case, MTW-treatment had a 58.06% higher production. However, no significant difference was reported for CW irrigation depth in the second cycle.

However, the highest production occurred with MTW irrigation depth of 100% of ETc which did not significantly differ from that of irrigation depth of 125%. On the other hand, the others had lower and different rates.

Irrigation depths of 100 and 125% of ETc with MTW had a higher accumulation of dry matter, respectively with 90.17 and 30.92% increase. It must be underscored that, in the two cycles, irrigation depth of 100% of ETc with MTW provided higher productions. The lowest were treatments irrigated with CW, with irrigation depths of 50% of ETc for the first cycle and 25% of ETc for the second cycle.

Decrease of dry phytomass of the aerial segment was not reported for irrigation with CW by Hamada and Testez (1995). In fact, the greatest phytomass was registered in the irrigation depth of 100% of ETc. The others had lower rates. In fact, water deficit provided a lower number of leaves and, consequently, a lower green phytomass of the aerial segment.

Villas Boas et al. (2007) underscored that an increase in irrigation depth above 100% of ETc reduced the production of dry matter, also corroborated by Andrade Junior and Klar (1994).

Hazan and Qados (2010a) reported that in MTW irrigation, the treatment with irrigation depth of 100% of ETc indicated a 5.76% difference in the accumulation of dry matter during the first cycle of chick peas. A high accumulation of dry matter, featuring 2.70%, occurred during the second cycle. A 2.04% increase in the dry matter of peas and an 8.05% increase in the production of celery irrigated with MTW were reported (Maheshwari and Grewal, 2009).

Selem and El-Nady (2011) submitted tomato crop to different irrigation depths with MTW and obtained the highest production rate in total dry phytomass at irrigation depth of 80% of ETc. An accumulation of 61.37% in irrigation depths 100, 60 and 40% of ETc provided respective increases of 44.19, 73.15 and 84.61%.

MTW effects on the variables average number of leaves, green phytomass of the aerial segment and dry phytomass of the aerial segment showed the same

**Table 5.** Mean green phytomass of root (g) according to type of water and irrigation depths.

| Irrigation depths (%) | 1st Cycle | | 2nd Cycle | |
|---|---|---|---|---|
| | MTW | CW | MTW | CW |
| 25 | 14.20±3.18$^{Aa}$ | 7.98±1.46$^{Bb}$ | 9.03±0.42$^{Ca}$ | 6.83±1.36$^{Bb}$ |
| 50 | 11.36±1.80$^{Aa}$ | 7.64±0.80$^{Bb}$ | 8.91±1.18$^{Ca}$ | 10.44±2.02$^{Aa}$ |
| 75 | 13.88±2.59$^{Aa}$ | 9.07±2.05$^{Bb}$ | 13.47±2.31$^{ABa}$ | 7.75±2.06$^{ABb}$ |
| 100 | 14.52±2.31$^{Aa}$ | 7.66±1.45$^{Bb}$ | 14.28±1.17$^{Aa}$ | 6.89±1.18$^{Bb}$ |
| 125 | 11.51±1.32$^{Aa}$ | 13.11±2.85$^{Aa}$ | 11.31±0.80$^{BCa}$ | 9.63±1.31$^{ABa}$ |
| s($\hat{m}$) | 0.29 | | 0.41 | |

Means followed by the same small letter in the line and by the same capital letter in the column, for each cycle, do not differ at a 5% probability, by test *t*.

amount of the variable or an increase. The above is due to changes that occur when water passes through a magnetic field and used in irrigation.

The soil was saturated at the start of the cycle. Since irrigation occurred daily, soil tension did not reveal great differences till mid-cycle and crops failed to provide great differences in the variables for variation in irrigation depths.

According to Zhou et al. (2000), MTW has a low pH rate, with more acid soil throughout the crop cycle, with benefits for the development of some crops such as the lettuce. Since a decrease in the strength of water molecules occurs, the water is removed by the plants from the soil. The soil´s matrix potential probably becomes weaker and the soil´s tension decreases (Khoshraves et al., 2011).

## Green phytomass of the root

The green phytomass of the root was influenced by the type of water and by the irrigation depths. In the first cycle, treatments irrigated with MTW provided higher rates of green phytomass of the root in irrigation depths of 25, 50, 75 and 100% of ETc, with significant differences (Table 5). Treatments irrigated with MTW had a significant effect in depths of 25, 75 and 100% of ETc for the second cycle (Table 5).

Green phytomass rate of the root irrigated with CW in the first cycle was higher in the irrigation depth of 125% of ETc, with significant difference. The lowest phytomass of the root was reported in the irrigation depth of 50% which was not statistically different from the others. Increase in phytomass reached 71.59%. On the other hand, irrigation with MTW did not show any significant difference between the irrigation depths under analysis.

When irrigation depths are analyzed according to the type of water; only irrigation depth of 125% failed to be different from the others. There was a 77.94, 48.69, 53.03 and 89.55% increase in phytomass, respectively, for the irrigation depths of 25, 50, 75 and 100%.

In the second cycle, there was significant difference for irrigation depths with CW. Highest rate of green phytomass of root developed in the irrigation depth of 50% of ETc and the lowest phytomass rate occurred in the irrigation depth of 25%, with a difference of 52.85%.

A significant difference occurred in irrigation depths with MTW. The highest rate of green phytomass of root was reported in the irrigation depth of 100% of ETc and the lowest in the irrigation depth of 50% of ETc, with a 51.17% reduction.

It must be underscored that, in the first cycle, the rates of the green phytomass of the root did not differ among the magnetically treated irrigation depths and the same phytomass may be obtained by using irrigation depth of 25% of ETc. Irrigation depth of 100% of ETc in the second cycle, featuring the highest phytomass, was affected by the magnetism on the green phytomass of the root, with a positive effect for the variable.

## Dry phytomass of the root

The type of water in irrigation and the irrigation depths affected the dry phytomass of the root. In the first cycle, the treatments irrigated with MTW had a significant effect on the irrigation depths of 25, 50 and 100% of ETc, as Table 6 shows. In the second cycle, treatments irrigated with MTW had a significant effect on the irrigation depths of 100 and 125% of ETc (Table 6).

In fact, the dry phytomass of the root for the first cycle did not show any significant differences between irrigation depths for the types of water.

However, the effect of magnetism was positive when the effect of the type of water on irrigation depth is taken into account. Irrigation depths 25%, 50% and 100% of ETc had a respective 60.16, 80.48 and 95.86% increase.

In the second cycle, the variation of irrigation depths with CW showed a significant difference and the treatment irrigated with 75% of ETc had the highest rate of dry phytomass of the root. The lowest occurred in the irrigation depth of 25% of ETc. There were no significant

**Table 6.** Means of dry phytomass of the root (g) according to the type of water and irrigations depths.

| Irrigation depths (%) | 1st Cycle | | 2nd Cycle | |
|---|---|---|---|---|
| | ATM | AC | ATM | AC |
| 25 | 1.97±0.70$^{Aa}$ | 1.23±0.35$^{Ab}$ | 0.95±0.22$^{Aa}$ | 0.70±0.15$^{Ba}$ |
| 50 | 2.22±0.37$^{Aa}$ | 1.23±0.06$^{Ab}$ | 1.03±0.12$^{Aa}$ | 0.96±0.25$^{ABa}$ |
| 75 | 1.62±0.39$^{Aa}$ | 1.69±0.31$^{Aa}$ | 0.90±0.32$^{Ab}$ | 1.18±0.08$^{Aa}$ |
| 100 | 2.37±0.36$^{Aa}$ | 1.21±0.36$^{Ab}$ | 1.29±0.21$^{Aa}$ | 0.72±0.13$^{Bb}$ |
| 125 | 1.53±0.34$^{Aa}$ | 1.68±0.30$^{Aa}$ | 1.01±0.11$^{Aa}$ | 0.85±0.21$^{Bb}$ |
| s($\hat{m}$) | 0.07 | | 0.04 | |

Means followed by the same small letter in the line and by the same capital letter in the column, for each cycle, do not differ at a 5% probability, by test *t*.

**Table 7.** Mean length of root (cm) according to type of water and irrigations depths.

| Irrigation depths (%) | 2nd Cycle | |
|---|---|---|
| | MTW | CW |
| 25 | 17.9±2.75$^{Ba}$ | 15.2±1.61$^{Ba}$ |
| 50 | 16.9±1.52$^{Ba}$ | 19.3±1.53$^{ABa}$ |
| 75 | 21.8±1.44$^{Aa}$ | 15.5±2.62$^{Bb}$ |
| 100 | 18.2±2.56$^{ABa}$ | 20.1±2.84$^{Aa}$ |
| 125 | 18.0±0.94$^{Ba}$ | 19.2±2.54$^{ABa}$ |
| s($\hat{m}$) | 0.95 | |

Means followed by the same small letter in the line and by the same capital letter in the column, for each cycle, do not differ at a 5% probability, by test *t*.

differences for irrigation depths with MTW.

When the effect of the type of water in the irrigation depths is analyzed, it must be underscored that irrigation depth of 75% of ETc with CW had the highest phytomass rate, with a 31.11% increase. Irrigation depths of 100 and 125% with MTW had a positive effect, respectively with a 79.16 and 18.82% increase of phytomass.

A positive effect of MTW for the dry phytomass of the root has been registered when the two cycles are analyzed. In the first cycle the effect occurred only between the two types of water in the irrigation depth. However, in the case of CW, there was a difference for the dry phytomass of the root.

## Length of the root

Water types in irrigation and irrigation depths affected the length of the root (cm) for the second cycle only. In the case of the second cycle, treatments irrigated with MTW had a significant effect on the irrigation depth of 75% of ETc, as shown in Table 7. In the case of irrigation depths with CW, a significant difference occurred where irrigation depth of 100% provided the longest root, whereas irrigation depth of 25% of ETc provided the smallest growth, with a 32.23% reduction. There were significant differences for irrigation depths with MTW. The irrigation depth with 75% of ETc provided the longest root whilst irrigation depth 50% of ETc provided the shortest, with a 28.99% decrease.

According to Filgueira (2000), the lettuce has an axial root system, although the primary root is not highly developed. The equilibrium of energy is directed towards the development of the aerial segment, just where the perpetuation of the species occurs (Santos, 2004).

Farias et al. (2010) show that water deficit for sugar cane plantations favors a surface root zone with a reduction of the root´s length and interferes in its volume which is related to the green phytomass of the root. Soares (2012) reports that the root of the tomato develops in great length and volume in irrigation depth of 120% of ETc.

Variations in irrigation depths directly affect the development of the root, according to Zotarelli et al. (2009), Jensen et al. (2010), Bai and Li (2010) and Yan et al. (2012). However, irrigation management must also be taken into account. According to Bandeira et al. (2011), when the lettuce is daily irrigated, it does not

**Table 8**. Analysis of variance for the variable Chlorophyll *a* (Cl *a*), Chlorophyll *b* (Cl *b*) and total Chlorophyll (Cl total).

| Causes of variation | DF | MS | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1st Cycle | | | 2nd Cycle | | |
| | | Cl *a*[1] | Cl *b*[1] | Total Cl [1] | Cl *a*[1] | Cl *b*[1] | Cl total[1] |
| Water | 1 | $3.12^{ns}$ | $0.20^{ns}$ | $3.67^{ns}$ | $0.21^{ns}$ | $0.22^{ns}$ | $5.10^{ns}$ |
| Irrigation depth | 4 | $5.76^{ns}$ | $0.16^{ns}$ | $7.69^{ns}$ | $4.45^{ns}$ | $0.36^{ns}$ | $6.54^{ns}$ |
| Water x Irrigation depth | 4 | $6.70^{ns}$ | $0.28^{ns}$ | $9.69^{ns}$ | $4.97^{ns}$ | $0.19^{ns}$ | $5.12^{ns}$ |
| CV (%) | | 17.10 | 17.74 | 16.48 | 16.03 | 25.63 | 16.37 |

(1) Data transformed by Box-Cox´s method.

reveal any difference between the green and dry phytomass of the root when compared with the lettuce irrigated by tensiometry. In spite of the above, there was a difference for the root´s diameter.

MTW in irrigation had a positive effect for green and dry phytomass and for the root´s length (Selem and El-Nady, 2011; Souza et al., 2005).

Selem and El-Nady (2011) reported that tomato with different magnetic irrigation and conventional depths had greater length and greater green and dry phytomass of the root. The best development, however, occurred in treatments irrigated with MTW and in the treatment of 80% of ETc.

The positive effect of MTW also occurred in the variables under analysis, with a length greater than or equal to the roots or green and dry phytomass. The above probably occurs due to the acidification of the water and, subsequently, the acidification of the soil which is highly beneficent to lettuce. The greatest rate of green phytomass of the root is directly related to an increase in the root´s volume which increases on the surface in contact with the soil and, consequently, obtains a greater absorption of water and nutrients.

The root´s greater volume (the green phytomass of the root) provided a greater growth of the plant, with a greater rate of aerial green phytomass and number of leaves.

## Chlorophyll

Analysis of variance for chlorophyll *a*, chlorophyll *b* and total chlorophyll did not have significant differences for any assessment between irrigation depths and type of water. Effects proved to be equal for the cycles (Table 8). There was no significant difference with regard to the effect of the variation of chlorophyll rate according to irrigation depth of CW. Effect is due to the fact that no great variations occurred in the soil´s water tension. However, water deficit in plants, derived from the lowest irrigation depths, trigger a low photosynthesis rate (Buchanan et al., 2000), with the lowest pigmentation rate and leaf number, directly affecting production (Engel and Poggiani, 1991).

Treatments irrigated with MTW did not show any difference in chlorophyll rates. According to Hazayn and Qados (2010a), chlorophyll rates in chick pea crops increased 26.57, 21.18 and 24.91% respectively for chlorophyll *a*, chlorophyll *b* and total chlorophyll.

Selim and El-Nady (2011) reported that tomato plants with different water tension in the soil and irrigated with MTW had different rates of chlorophyll. Plantation irrigated with MTW had a positive effect when compared to that irrigated with CW. On the other hand, MTW-irrigated wheat exhibited mean rates of chlorophyll *a* and total chlorophyll with higher significant differences. Chlorophyll *a* did not vary (Hazan and Qados, 2010b).

## Conclusion

The variables of MTW-irrigated lettuce crops showed more positive results when compared with those irrigated with CW.

The lettuces aerial green weight irrigated with MTW reveals a production higher than or equal to when compared to that irrigated with CW, for the two cycles, with an approximate 63% increase.

The technology of water magnetization for irrigation produces new possibilities for production increase and water volume decrease.

Current research looks forward to further studies, preferentially with multi-disciplinary teams, for a better understanding of the phenomenon which is brought about when water passes through a magnetic field, especially in its application to irrigation.

## Conflict of Interest

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Characterization of *Erwiniachrysanthemi* isolates inciting stalk rot disease of sorghum

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**Stalk rot of sorghum plants caused by *Erwiniachrysanthemi* is one of the most destructive diseases of sorghum crop. Twenty one of bacterial isolates were isolated from stalk of sorghum plants from different location of U.S. Nagar district of Uttarakhand, India. Biochemical, physiological and morphological characterization of *E.chrysanthemi* was done for confirmation of the bacterium specie. Bacterial suspension [0.7% Tween-40 (v/v) + 2 × $10^8$ cell/ml] of each isolate was inject-inoculated with a 21G hypodermic needle into the stalk of plants for pathogenicity testing of the test bacterium. Reactions of biochemical and physiological testing are clearly evident enough to support the confirmation of test bacterium to taxonomic assignation of *E. chrysanthemi*causing soft roton sorghum plants. Out of 31 diseased samples of different locations examined, in 21 samples, the pathogen was detected as *E.chrysanthemi*by usingset of biochemical and physiological testing. As all the bacterium produced typical stalk rot disease symptoms on sorghum plant and water-insoluble blue pigment (indigoidine) on nutrient glycerol $MnCl_2.4H_2O$ (2 mM) agar medium it was confirmed as *chrysanthemi* species.**

**Key words:**Blue pigment, characterization,*Erwiniachrysanthemi,* Stalk rot, sorghum.

## INTRODUCTION

Soft rot erwinias are very important primary pathogens of both growing plants and the harvested crop (Pérombelon and Kelman, 1980). However, strains from different host plants differ in their specific host range as well as in the pathogenic and phenotypic properties (Dickey, 1979; Janse and Ruissen, 1988). The genus *Erwinia*is named after one of the founder of phytobacteriology, Erwin Frink Smith, and was established by Winslow et al. (1917) to include the plant pathogenic entereobacteria. Like other entereobacteria, the *Erwinia*are motile by means of several to many peritrichous flagella usually 8-11 (Burkholder et al., 1953; Dickey, 1981), gram-negative, non-spore forming, straight rod with rounded ends, and occurs singly or in pairs. Its size varies from 0.8-3.2 × 0.5-0.8 µm (average 1.8 × 0.6 µm) depending on carbon source present in the medium and growth conditions (Grula, 1970). Stalk rot of sorghum caused by *Erwiniachrysanthemi*Burkholder, McFadden, and Dimock is oneof the most destructive diseases of sorghum crop. Saxenaet al. (1991) reported this bacterium causing

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stalk and top rot of sorghum under natural conditions in India during 1987-1988 crop season in sorghum field at Pantnagar, Uttarakhand. The disease was wide spread and affected 60 to 80% of plants in different sorghum genotypes. Recently, the occurrence of disease incidence ranging from 7.50 to 46.85 in Tarai region of Uttarakhandhas been also shown by Kharayat and Singh (2013).

Early and accurate diagnoses of plant disease are necessary to predict outbreaks and allow time for development and application of mitigation strategies. A number of different biochemical methods are presently being employed for microbial identification and taxonomic classification. Moreover, each method has its advantages and disadvantages; with regard to ease of application, reproducibility, requirement for equipment and level of resolution. Present investigation was aimed to devise the set of biochemical testing to characterize the isolates of destructive soft rot bacterium, *E. chrysanthemi*.

## MATERIALS AND METHODS

On the basis of visual observation of suspected stalk with typical soft rot symptoms, sampling was done from 31 different locations in the growing season 2011-2012. Samples were brought to the laboratory and kept in refrigerator at 4°C. Isolations were made from samples and the pathogen was identified by using set of biochemical and physiological testing.

### Isolations and purifications of *E. chrysanthemi*

Isolation of bacterium was done as per the method described by Janse (2005). Pieces of tissue taken from the margin of healthy and diseased tissues were disinfected with 70% alcohol and placed in a sealed tube with sterile water and tissues were left for 30 min in suspension so that the bacteria could diffuse out of the tissues. Subsequently 100 µl of the suspension was plated onto crystal violet sodium polypectate (CVP) medium. Characteristically deep-pit forming colony on CVP medium purified on yeast dextrose calcium carbonate medium by streaking using freshly growing single colony and these plates were incubated at 28°C for five days.

### Pathogenicity tests

To confirm the pathogenicity of isolatesfrom various locations [Figure 1, 1-Pantnagar-1, 2-Pantnagar-2, 3-Pantnagar UTMC-535, 4-Majra Farm, 5-Banjari farm, 6-Chhinki Farm, 7-Sailanigot,8-Tanakpur,9-Khetalsanda,10-Kashipur,11-Haldhwani-1(HLD1),12-Kisanpur (Haldhwani), 13-Bajpur,14-Bajpur (Doraha),15-Rudrapur,16-Kashipur (Sultanpur),17-Tanda Kajal (Kashipur), 18-Gadarpur, 19-Barhani (Bajpur), 20-Sitarganj and 21-Nagina (Sitarganj)], stem inoculation was done on of 21 days old susceptible sweet sorghum plants variety SPSSV 6 under controlled glasshouse conditions. Isolates were grown on Luria Broth for 24 h at 28°C. The bacterial cells were suspended in sterile distilled water and the cell density adjusted to $2\times10^8$cfu/ml. Bacterial suspension [0.7 % Tween-40 (v/v) + $2 \times 10^8$ cell/ml] of each isolate was inject-inoculated with a 21G hypodermic needle into the stalk of plants. Control plants were inject-inoculated with sterilized water. Experiment was conducted twice to confirm the result.

### Biochemical, physiological and morphological characterization of *E. chrysanthemi*

Test pathogen was screened for characterization upto species level by using a set of biochemical and physiological testing (Table 1) to detect the presumptive *E. chrysanthemi*which were selected according to keys ofSchaad et al. (2001).

### Scanning electron microscopy

SEM preparation for *E.chrysanthemi*Pantnagar isolate was done using procedure described by Kaláb et al. (2008). Twenty-four hours old actively growing bacterial cells on Luria broth medium were harvested by centrifuge at 6000 rpm. Then, the bacterial cells were fixed with 2.5% gluteraldehyde in 0.05 M sodium phosphate, pH 6.8, for 24 h at 4ºC, washed with sodium cacodylate buffer three times (10 min each wash). They were finally fixed in 1% osmium tetraoxide for 1 h and washed with 0.1 M sodium cacodylate buffer as before. Further, the cells were dehydrated through a series of graded acetone (10, 20, 30, 40, 50, 70, 80, 90 and 100%). These cells were soaked for 15 min at each concentration. The drying was completed by placing the sample in a flow of $CO_2$ in critical point dryer. The cells were mounted on aluminum stubs and coated with gold using Hummer V sputter coater, and viewed and photographed under a scanning electron microscope.

## RESULTSANDDISCUSSION

Reactions of biochemical and physiological testing presented in Table 1 are clearly evident enough to support the confirmation of test bacterium to taxonomic assignation of *E. chrysanthemi*causing soft roton sorghum plants. Out of 31 diseased samples of different locations (Figure 1) examined, in 21 samples, the pathogen was detected as *E.chrysanthemi*by usingset of biochemical and physiological testing. These set of test also has been used by several other investigators to detect the presumptive *E. chrysanthemi,* viz.oxidative/fermentative test (Hugh and Leifson, 1953), oxidase test, deep pit formation on crystal violet sodium polypectate, CVP medium + 0.4% tetrazolium chloride solution (Cuppels and Kelman, 1974; Tomlinson and Cox, 1987;Perombelon and Burnett, 1991;Bdliyaet al., 2004;Kaneshiroet al., 2008; Zhu et al., 2010),indole production,sensitivity to erythromycin at 15 µg/ml (Jensen et al., 1986; Olabiyi, 2010), colony morphology on yeast dextrose calcium carbonate agar medium (YDC) (Dye, 1968; Goto, 1979; Kaneshiroet al., 2008), and Growth at 37°C (Pérombelon and Kelman, 1980; Lelliott and Dickey, 1984; Pérombelon and Hyman, 1986; Hyman et al., 1998). As the bacterium produced water-insoluble blue pigment (indigoidine) on NGM (Nutrient Glycerol $MnCl_2.4H_2O$ (2 mM) agar medium itwas confirmed as *chrysanthemi* species. As it has been already reported that *chrysanthemi* is only species under the genus which produced water-insoluble blue pigment

**Table 1.** Biochemical, physiological and morphological characterization of isolates of *Erwiniachrysanthemi* isolated from sorghum plants.

| ***E. chrysanthemi* isolates** / **Test** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Indole production | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Methyl red | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Citrate utilization | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Glucose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adonitol | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Arabinose | + | + | + | + | + | - | + | + | + | + | - | + | + | - | + | + | - | + | + | + | + |
| Lactose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Sorbitol | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Mannitol | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Sucrose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Gram reaction | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3% KOH test | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Oxidase | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Deep pit on CPV medium[Ψ] | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Blue pigment on NGM[*] | + | + | + | + | + | + | + | + | + | + | - | - | + | + | - | + | + | - | + | + | + |
| Sensitivity to erythromycin (15 µg/ml) | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Growth at 37°C | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Undulate margin on NYDA[Φ] | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pathogenicity test | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |

[Ψ]Size of deep pit was found variable it may be due to variability; [Φ]Allformed **c**olony with undulate or lobate margin after 4 days, later becomes feathery on NYDA; [□]Mostly all isolates produced blue pigment but some were lack.

(Starr et al., 1966; Lee and Yu, 2006;Olabiyi, 2010). SEM analysis showed that the shape and size of bacterium, straight rod with rounded ends (Figure 2) and 1.50 × 0.50 µm respectively. In pathogenicity test, the bacterium found potential pathogen as it produced typical symptom on stalk of sorghum plant as naturally occurred in field conditions after 4 days of inoculation (Figure 3). The symptom mainly affect sorghum stem showing water-soaked symptoms that later turned reddish dark brown color. The infected stem pith disintegrated and showed slimy soft-rot symptoms after 7 days of inoculation. Several other workers also reported same symptoms (Zummo, 1969; Hepperly and Davila, 1987; Saxenaet al., 1991; Hseuet al., 2008).

## Conclusion

Biochemical and physiological methods are easy to use, reproducible and less costly than molecular and serological methods and can be readily used for identification of *E. chrysanthemi*.

## Conflict of Interest

The authors have not declared any conflict of interest.

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**Figure 1.** The number in map showing various locations of *Erwiniachrysanthemi* isolates.

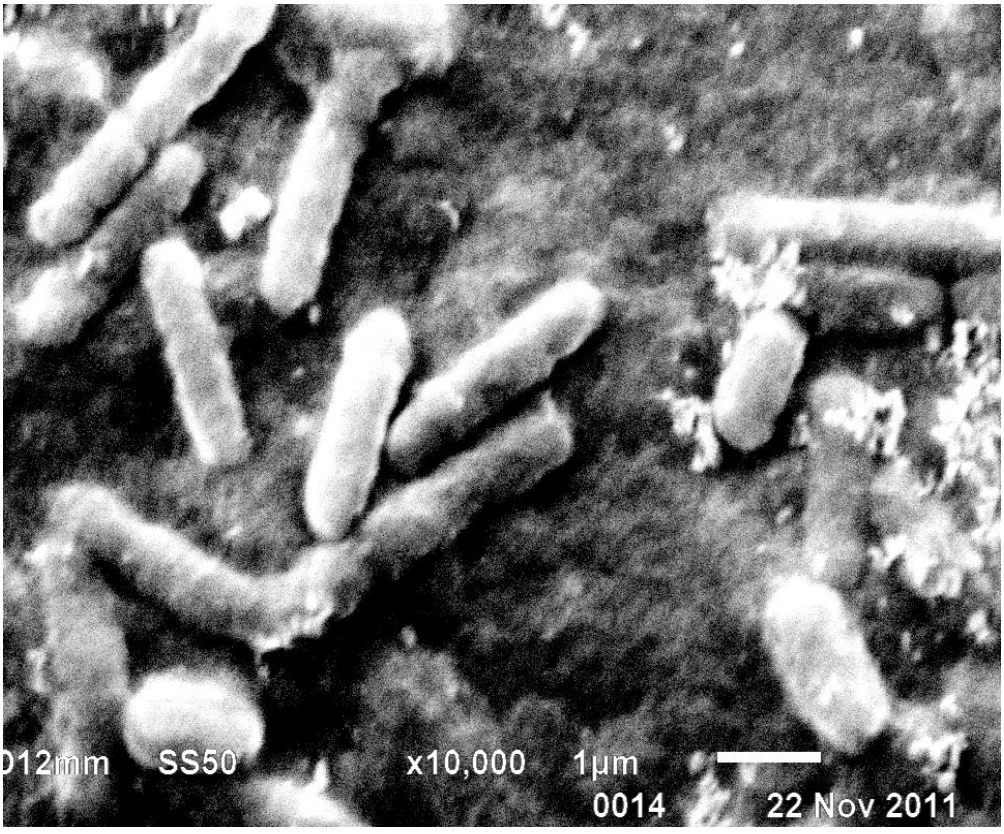


**Figure 2.** Scanning electron micrograph showing *E. chrysanthemi* cells with straight rod.

**Figure 3.** Typical stalk rot symptom produced by *E. chrysanthemi* on 21 days old susptible sorghum plant.

glasshouse facilities during the course of investigation.

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*Full Length Research Paper*

# Growth and efficiency of water use of papaya cultivars (*Carica papaya* L.) under doses of bovine biofertilizer in hydroponics cultivation

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**Among the fruit plants cultivated in Brazil, papaya (*Carica papaya* L.) stands out by having high productivity of fruit quality. The seedling production system of this culture needs a technology that promotes the production of plants with high physiological and sanitary quality. Thus, we aimed to evaluate the growth, dry matter accumulation and the efficiency of water use of papaya cultivars under doses of bovine biofertilizer in hydroponic culture. We used a completely randomized design with eight treatments in a factorial scheme 4 x 2, with six replications, and a useful plant per repetition totalizing 48 useful plants. Four doses of biofertilizers (D = 10, 20, 30 and 40% v/v) were tested and applied in two varieties of papaya (Sunrise Solo (C1) and Tainung-01 (C2)). During the first 60 days after sowing, the papaya cultivars were evaluated for growth, dry matter accumulation and water use efficiency in accordance to their doses of biofertilizers. The cultivar Tainung-01 has a higher potential for growth, biomass accumulation and efficient use of water in comparison with the Sunrise Solo cultivar. The doses estimated of 25 and 35% (v/v) of bovine biofertilizer promoted the greater growth and dry matter accumulation for the cultivars Sunrise Solo and Tainung-01, respectively.**

**Key words:** *Carica papaya* L., organic fertilization, hydroponics, seedling production.

## INTRODUCTION

Among the fruit crops in Brazil, the papaya tree (*Carica papaya* L.) stands out for presenting high productivity of fruit quality. In the year of 2012, there was a national production of 1,517,696 tons, being the world's largest producer and the third largest exporter of papaya, with the Northeast Region (902,000 tons) being the largest producer of this fruit, followed by the Southeast (549,000 tons), North (42,000 tons), Midwest (6,000 tons) and the

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**Table 1.** Chemical composition of biofertilizer solution enriched with bovine manure 60 days after the start of anaerobic fermentation.

| pH | ECw (dS $m^{-1}$ e) | $Ca^{+2}$ | $Mg^{+2}$ | $Na^{+}$ | $K^{+}$ | $Cl^{-}$ | $CO_3^{2-}$ | $HCO_3^{-}$ | $SO_4^{2-}$ | P (mg $dm^{-3}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | (cmolc $L^{-1}$) | | | | | | | | |
| 6.34 | 8.08 | 3.71 | 2.40 | 3.27 | 1.69 | 4.59 | 0.43 | 2.03 | 1.02 | 56.00 |

South (4,000 tons), respectively (IBGE, 2014).

The culture has shown great economic and social expression, mainly in the states of Bahia, Espírito Santo, Rio Grande do Norte and Ceará. Regarding the exports, the state of Espírito Santo accounts for 50% of the total (Serrano and Catteano, 2010). Due to the expanded cultivated areas and the need to increase productivity and final product quality, efforts are made to always improve productivity levels and reduce production costs (Guimarães et al., 2012). Thus, new technologies have been introduced in the papaya culture aiming to raise productivity levels. As such, the use of biofertilizers and seedling production systems through hydroponics emerge as a promising alternative, considering that the phase of seedlings and their initial development interfere directly in the orchard productivity (Trinidad et al., 2000). Among the papaya cultivars most commonly grown in Brazil are those of Solo and Formosa groups. Cultivars from the 'Solo' group are intended mainly for the export market, for having smaller fruits. The main cultivars of the 'Formosa' group are imported hybrids that produce larger fruits that are intended mainly for the domestic market, being used in these conventional, integrated and organic crop practices (Hafle et al, 2009; Serrano and Cattenao, 2010).

The cultivation of papaya seedlings in a protected environment favors the production of high quality physiological and sanitary plants. According Fochesato et al. (2007), this needs to be done in containers where the seedlings produced alter their development complying with culture medium, when compared to the process in the field, with limited space for root growth. A good alternative for this is the optimization of propagation methods in hydroponics, which targets the time reduction to obtain seedlings, as well as a greater control of nutrition and phytosanitary conditions (Souza et al., 2013).

In most cases of hydroponic cultivation, the nutrient solutions are produced from a mixture of different fertilizer salts of high solubility in water (Resh, 1997), but they can also be produced from organic biofertilizers, a system known as “organoponics”, or as part of the solution, as it occurs in organic-inorganic hydroponics (Martins, 2000).

Several studies have been reported in the literature with promising results of the use of biofertilizers in the seedling production from different cultures: Medeiros et al. (2008) with lettuce, Probst et al. (2008) in forage, Cocco et al. (2008) with tobacco and Dantas et al. (2014) with acerola. However, there are too few studies that enable the production of papaya seedlings using biofertilizers, especially when they are related to hydroponic production.

Based on the above considerations, this study aimed to evaluate the growth, dry matter accumulation and efficiency of water use of papaya cultivars under doses of bovine biofertilizer in hydroponic cultivation.

## MATERIALS AND METHODS

The experiment was carried out from February 3rd to April 3rd, 2012 in a seedling nursery at the Universidade Estadual of Paraíba (UEPB), Campus IV, Catolé do Rocha - PB, covered with a nylon shading screen for 50% brightness inside.

We used a completely randomized design with 8 treatments in a factorial 4 x 2, with six replications, and a useful plant per repetition, totalizing 48 useful plants. Four doses of biofertilizers (D = 10, 20, 30 and 40% v/v) were tested and applied in two varieties of papaya (Sunrise Solo (C1) and Tainung-01 (C2)).

The plants were grown in a hydroponic system using modified Leonard jars, made with pet bottles according to the methodology of Santos et al. (2009). The bottles were cut 14 to 15 cm from the base and together with the caps they underwent a sterilization process at a 250 L water tank with sodium hypochlorite (10%) for one hour. After this period, all parts of the bottles were rinsed in tap water to remove excess sodium. To each vessel, it was added one liter of washed sand, which was sterilized by autoclaving at a temperature of 121°C for two consecutive days number of hours. After being filled, the pots were seeded (three seeds per pot) and covered with paper bags, in order to prevent algae growth in the solution.

The bovine biofertilizer was obtained by anaerobic fermentation, mixing equal parts of fresh cattle manure and slightly water win electrical conductivity - ECw = 0.8 dS $m^{-1}$, adding 2 kg of leaves and branches of the leguminous plant cowpea (*Vigna unguiculata* L.) (Table 1). For the preparation of the biofertilizer, plastics biodigesters with a capacity for 200 L were used, kept hermetically sealed for 45 days. To release the methane gas produced during fermentation, a thin hose was connected at the upper base and the other end was submerged in a water container to prevent the entrance of air and loss of quality of the organic feedstock (Santos, 1992). For being applied in liquid form, it was analyzed as if it were water for irrigation, as the data in Table 1, as a suggestion of Dantas et al. (2014). The total volume of the solution was 0.7 L, being replaced weekly based on culture evapotranspiration (ETc), as shown in Table 2.

According to the methodology proposed by Benincasa (2003), relative growth rates in height (RGRH) were determined by equation 1 and in stem diameter (RGRSD) by equation 2. Based on the growth in stem diameter, and height the papaya seedlings reached in the end of the total emergency 15 after sowing in relation to the analyses performed at 30, 45 and 60 days after sowing (DAS).

$$RGRH = \frac{lnH2 - lnH1}{t2 - t1} \quad (1)$$

In which: RGRH = Relative growth rate in height of plants (cm $cm^{-1}$

**Table 2.** Water and biofertilizer consumption for papaya (*Carica papaya* L.) seedlings during 60 days in organic hydroponic cultivation.

| Biofertilizer doses (%) | Water volume | Biofertilizer volume | Total volume |
|---|---|---|---|
| | | ml | |
| 10 | 1485 | 165 | 1650 |
| 20 | 1320 | 330 | 1650 |
| 30 | 1015 | 435 | 1450 |
| 40 | 870 | 580 | 1450 |

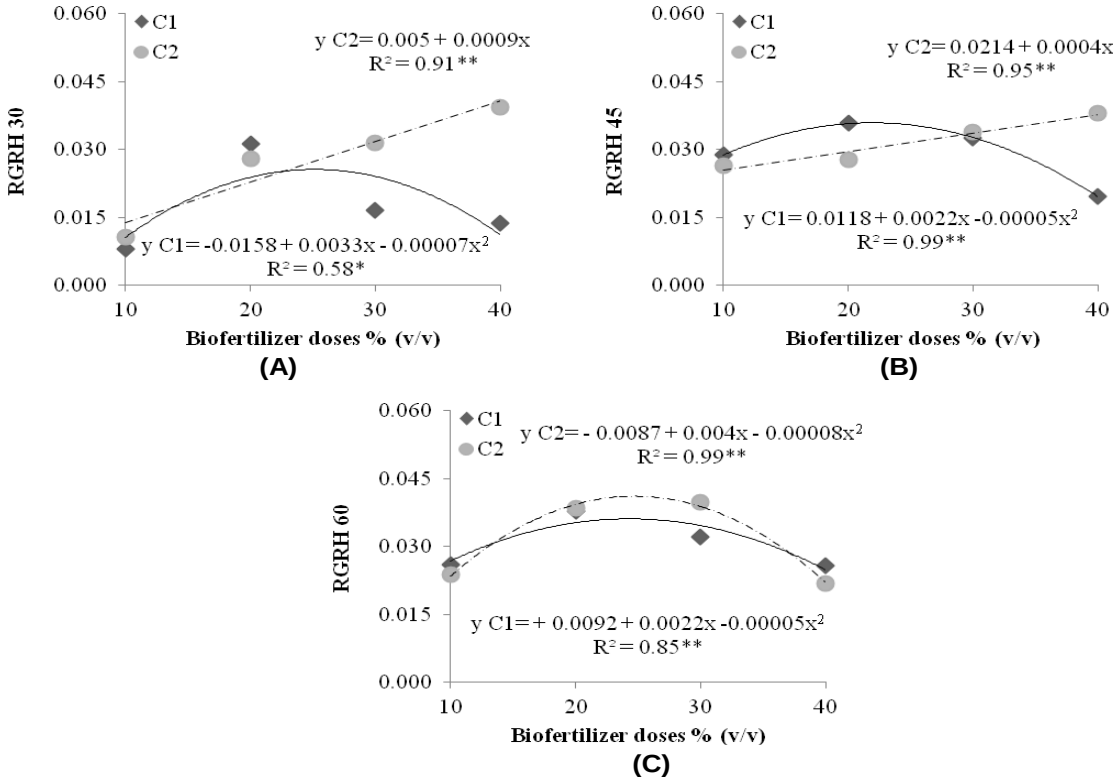


**Figure 1.** Relative growth rates in height (cm cm$^{-1}$ day$^{-1}$) of papaya cultivars (*Carica papaya* L.) (C1 = 'Sunrise Solo' and C2 = 'Tainung-01') at 30 (A), 45 (B) and 60 (C) days after sowing, in function of biofertilizer.

day$^{-1}$); H1 = plant height in time t1 (cm); H2 = plant height in time t2 (cm), and ln = logaritmo natural.

$$RGRSD = \frac{lnSD2 - lnSD1}{t2 - t1} \quad (2)$$

In which: RGRSD = Relative growth rate stem diameter (mm mm$^{-1}$ day$^{-1}$); SD1 = plant stem diameter in the time t1 (mm); SD2 = plant stem diameter in the time t2 (mm), and ln = logaritmo natural.

Also at 60 (DAS) the plants were collected to obtain the leaf dry matter (LDM) (g), stem dry matter (g) (SDM) (g) and root dry matter (RDM) (g), from the biomass partition of the collected material and packaging in an air circulating oven (DL-AF Dellta) at 65°C to dry the material for 72 h. After this period, the plants were weighed on an analytical balance (ABT 120-5DM Polimate**)**. With the data of dry matter and water consumption by papaya, we determined the efficiency of water use (EWU) by the relationship between the produced dry matter and water consumed by the plant expressed in g L$^{-1}$.

The results were submitted to analysis of variance (F test) and, when the parameters were significant, we used the Tukey mean comparison test (5%), for the cultivar factor and regression analysis, for the doses of biofertilizers with help from the SISVAR Software (Ferreira, 2011).

## RESULTS AND DISCUSSION

To the relative growth in height of the cultivar C1 (Sunrise Solo), we verified a quadratic behavior at 30, 45 and 60 days after sowing, so that it reached the growth peak when cultivated under the doses of 24, 22 and 22% (v/v) of biofertilizer, respectively (Figure 1). Teixeira et al. (2009) also investigated reductions in height growth of papaya trees due to increasing doses of Lithothamnium.

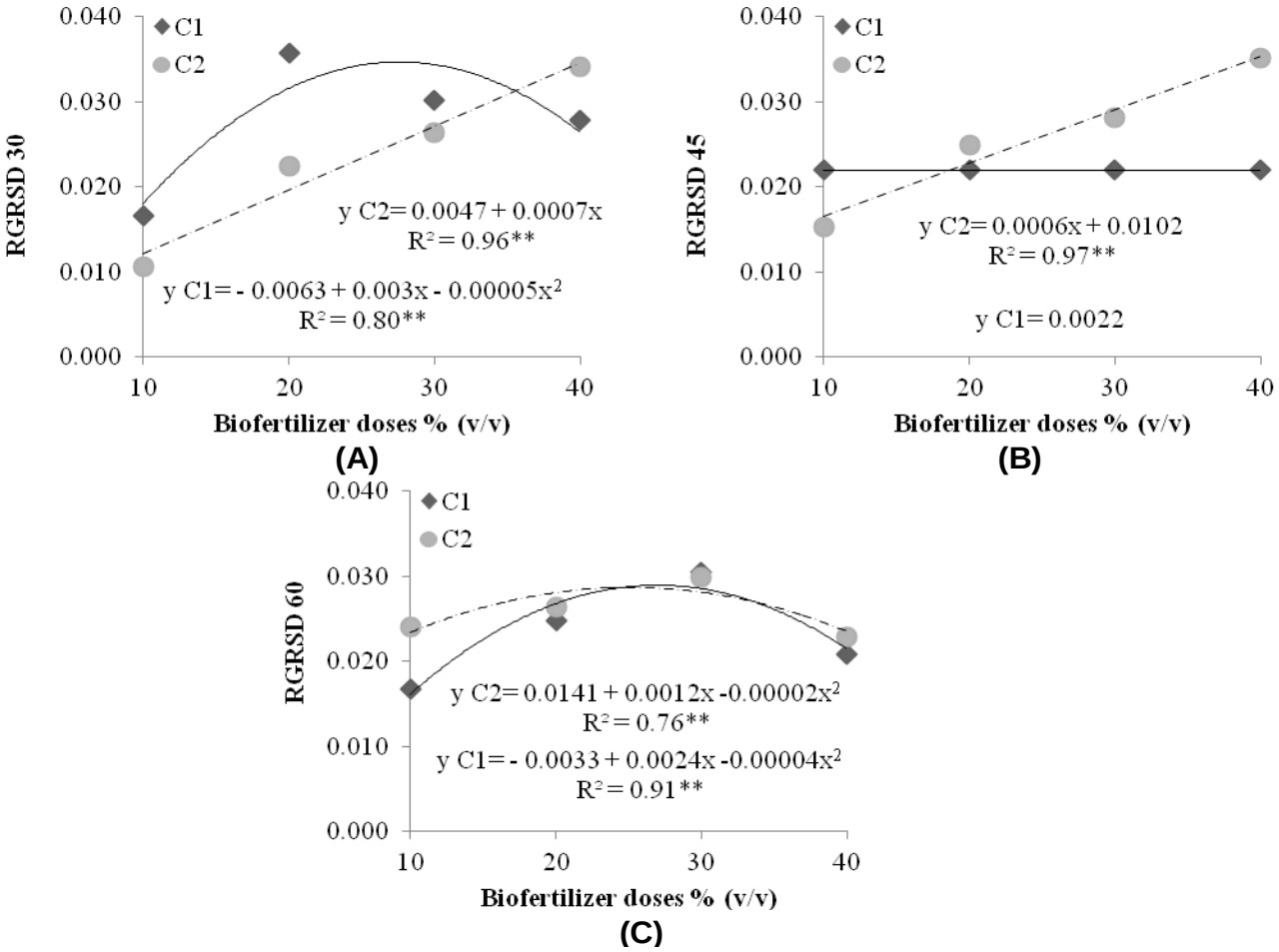


**Figure 2.** Relative growth rates stem diameter (RGRSD) (mm mm-1 day$^{-1}$) of the stem thickness of papaya cultivars (*C. papaya* L) (C1 = Sunrise Solo and C2 = Tainung-01) at 30 (A), 45 (B) and 60 (C) days after sowing, in function of biofertilizer.

A fact confirmed by Dantas et al. (2014) in acerola seedlings, where the height of the seedlings responded in a quadratic way to bovine biofertilizer doses. The authors believe that these results were influenced by the increase in substrate fertility providing toxic effects.

In order to cultivate C2 ('Tainung-01') a linear increase behavior was observed for the relative growth in height during the first 30 and 45 days after sowing due to the increase of biofertilizer doses up to the maximum level studied (40% v/v). It was also verified that at 60 days after sowing this behavior became quadratic, so that the higher relative growth rates in height were achieved at a 25% (v/v) dose of biofertilizer (Figure 1). Guimarães et al. (2012) also observed linear response of height growth in seedlings of Carica papaya. Tainung-01 in function of biofertilizer doses during the first 40 days after sowing. One can conclude from this that the need for larger doses of biofertilizer during the first 45 days after sowing may be related to lower efficiency of the plants' root system in this growth phase, so that at 60 days after sowing, when the seedlings had a more developed root system, they were able to meet their nutritional needs in hydroponic solution containing lower biofertilizer doses.

For the growth in height, divergent behavior can be observed between the papaya (*C. papaya)* cultivars studied under biofertilizer doses at 30, 45 and 60 days after sowing (Figure 1). It was ascertained that the cultivar C1 ('Sunrise Solo') has lower nutritional requirements in relation to cultivar C2 ('Tainung-01'), thus demanding lower doses of biofertilizer to maximize its growth index. It was also observed that the cultivar C2 ('Tainung-01') holds the greatest potential for growth, especially under favorable nutritional conditions.

For the growth of stem diameter, it is found that C2 ('Tainung-01') was similar to that observed in height, so that the papaya plants obtained linear relative growth rates of stem diameter according to biofertilizer doses during the first 30 and 45 days after sowing, denoting the initial growth potential of the cultivar and biofertilizer efficiency in papaya plant nutrition (Figure 2).

However, at 60 days after sowing it was observed a quadratic behavior of the relative growth in stem diameter of C2 (Tainung-01), tending to reduce when cultivated in biofertilizer doses greater than 30% (v/v). Possibly after 45 days of sowing the papaya plants tend to reduce the growth in stem diameter due to the limitations of the container and a lower incidence of light in the nursery, reflecting the need for transplanting the seedlings. Thus, the reduction of growth limits nutrient and water absorption, such that larger doses of biofertilizer may have exerted a toxic effect on the papaya plants after this time.

Sunrise Solo (C1) showed a quadratic behavior to the relative stem diameter growth at 30 days after sowing, reaching the maximum growth under 30% (v/v) dose of biofertilizer (Figure 2A and B). It was also observed that at 45 days after sowing, there was no significant influence of the doses in the relative growth in stem diameter of papaya plants (Figure 2C and D). Such a fact may be related to the reduction of secondary growth activity of Sunrise Solo papaya plants, since at 45 days after sowing there was an increase in growth rates in height relative to the first 30 days after sowing, denoting the greater investment in primary growth.

However, at 60 days after sowing, a quadratic behavior was once again verified in the relative growth of papaya cultivars, so that the seedlings produced at doses of 30% (v/v) of biofertilizer obtained the highest growth rates of 0.04 (mm $day^{-1}$), similar to results obtained by cultivar Tainung-01, which also reached maximum growth at the respective dose. Lima et al. (2007) also found no differences in the relative growth of papaya plants Tainuing-01 and Golden due to the evaluation period.

It is noteworthy that at 30 and 60 days after the sowing, both cultivars had similar growth rates, differing only in response to biofertilizer doses at 30 days, where cultivar Tainung-01 responds linearly to the doses, a fact that follows due to higher nutritional requirements of this cultivar in the first days after emergence, possibly due to having lower reserves from the seeds (cotyledons) compared to the cultivar Sunrise Solo who responded in a quadratic way to biofertilizer doses.

For the leaf dry matter, a quadratic response was observed in both cultivars in relation to doses of biofertilizer (Figure 3A), noting that the cultivar Tainung-01 had the highest leaf dry matter accumulation (0.71 g) under the dose of 34% (v/v) of biofertilizer. This value was 43.7% greater than the maximum leaf dry matter accumulation observed in cultivar Sunrise Solo (0.40 g), achieved at a dose of 21% (v/v) of biofertilizer.

Based on these results, it is possible to explain the greater growth potential of cultivar Tainung-01 in relation to cultivar Sunrise Solo, given that the leaves are the organs responsible for the plant's photosynthetic activity and with it, the greater accumulation of leaf dry matter denotes the largest investment in active photosynthetic area, favoring the higher photosynthetic potential, encouraging further growth. This fact was observed in cultivar Tainung-01, that got high relative growth rates in stem diameter and height during the first 45 and 60 days after sowing respectively, in relation to cultivar Sunrise Solo (Figures 1 and 2).

Similar behavior was ascertained by Diniz et al. (2011) in passion fruit plants, on which the supply of more than 50% (v/v) of biofertilizer caused decline in leaves dry matter accumulation.

For stem dry matter, differing responses were verified between papaya cultivars depending on the increase of biofertilizer doses, and a quadratic response was found for cultivar Sunrise Solo with maximum accumulation of stem dry matter (0.27 g) in 26% (v/v) dose of biofertilizer (Figure 3B). For that, a linear and increasing behavior of cultivar Tainung-01 was examined based on the biofertilizer doses reaching the maximum accumulation of 0.49 g in a dose of 40% (v/v) of biofertilizer, being this accumulation 45% higher than the cultivar Sunrise Solo (Figure 3C).

These results are possibly related to higher growth rates in height and stem diameter observed in cultivar Tainung-01 in relation to Sunrise Solo. In addition to that, the behavior observed for dry matter accumulation in papaya plants with biofertilizer doses was similar to that seen in the growth, so that the best responses from cultivar Sunrise Solo were at doses estimated close to 25% (v/v) of soil biofertilizer, while the best performance of cultivar Tainung-01 occurred at levels close to 40% (v/v) of biofertilizer. This denotes the genetic variability among papaya cultivars belonging to the Solo and Formosa groups regarding nutritional needs.

As for the root dry matter, positive linear correlation was ascertained of cultivar Sunrise Solo to biofertilizer doses, obtaining increments of 0.007 g for each unit increase in the biofertilizer dose, reaching a maximum of 0.51 g in a dose of 40% (v/v) of biofertilizer (Figure 3C). The stimulation of root growth may be related to the need for greater selectivity of nutrient in cultivation solution by the plant, promoting with this the exclusion of ions at high concentrations, considering a reduction of growth and leaf biomass accumulation of cultivar Sunrise Solo under higher doses of biofertilizer (Figures 1, 2 and 3A).

For cultivar Tainung-01, a quadratic behavior was verified, a fact that confirms their stem and leaf dry matter accumulation. It was observed that in this cultivar the peak accumulation of root dry matter is reached in the dose of 34% (v/v) of biofertilizer, noticing a decrease thereafter (Figure 3C). This behavior can be related to the toxic effect of some nutrients with an increasing dose of biofertilizer, making the plant reduce its root system due to the increase of nutrient concentration (salts) in solution.

The divergence of the root system behavior of these cultivars due to the increase of biofertilizer doses may be related to its tolerance capacity to salt content in the solution. Sá et al. (2013) points out that the cultivar

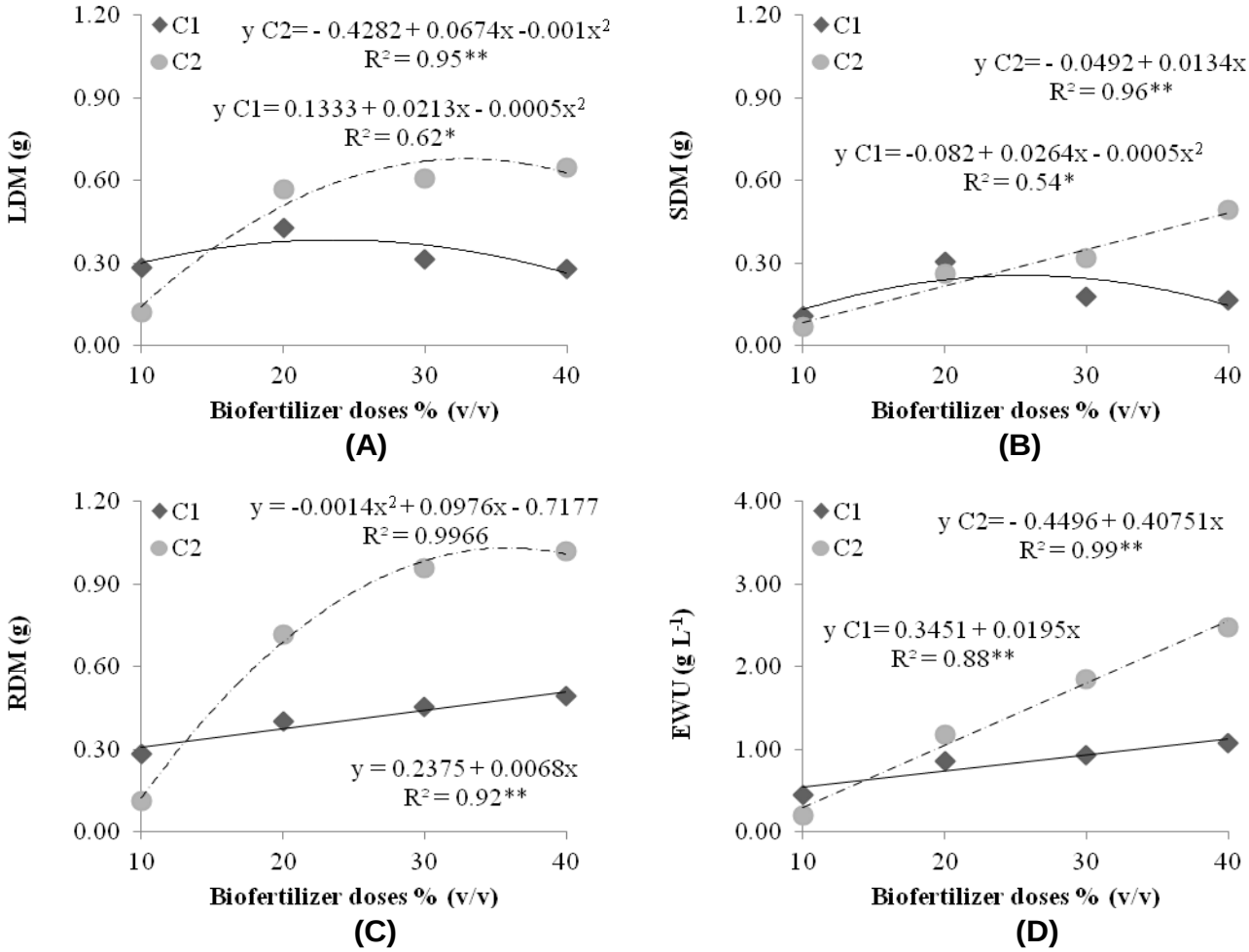


**Figure 3.** Leaf dry matter (LDM) (A), stem dry matter (SDM) (B), root dry matter (RDM) (C) and efficiency of water use (EWU) (D) of papaya cultivars (*Carica papaya* L) (C1 = Sunrise Solo and C2 = Tainung-01) at 60 days after sowing, in function of biofertilizer.

Sunrise Solo shows a higher potential of salt tolerance in relation to cultivar Tainung-01. This explains the root system growth capacity of cultivar Sunrise Solo even under the higher doses of biofertilizer, where there is a greater concentration of salts and nutrients in hydroponic solution.

It was observed that efficiency water use of both papaya cultivars increased linearly with the increase of bovine biofertilizer doses in the cultivation solution (Figure 3D). The efficiency water use is expressed by the relation between the biomass accumulation ($CO_2$ fixed during photosynthesis) and water consumption of the plant (sweating), so that the values denote the amount of carbon fixed by the plant by each unit of water lost (Taiz and Zeiger, 2013). It is believed that the higher doses of biofertilizer promoted an increase in the availability of nutrients in hydroponic solution, favoring root absorption, making it more efficient, and thereby promoting a reduction in the need of water uptake by the plants, since along with it, the essential nutrients for their growth are absorbed, favoring the increase in water use efficiency.

It is noteworthy that cultivar Tainung-01 had the highest efficiency of water use in relation to cultivar Sunrise from doses greater than 15% (v/v) of biofertilizer, when compared under the same culture condition (Figure 3D). It was also verified that the cultivar Taining-01 obtained unitary increments 75% higher than those observed for the cultivar Sunrise Solo with the increasing dose of biofertilizer. Thus, the greatest growth potential of cultivar Tainung-01 in relation to cultivar Sunrise Solo may be related to the first's higher efficiency in water use, denoting its greatest photosynthetic potential ($CO_2$ fixation) under increased nutrient availability.

## Conclusions

The cultivar Tainung-01 has a higher growth potential,

biomass accumulation and efficient use of water in comparison to the cultivar Sunrise Solo. The doses estimated of 25 and 35% (v/v) of bovine biofertilizer promoted the greater growth and dry matter accumulation for the cultivars Sunrise Solo and Tainung-01, respectively. The cultivar Sunrise Solo has lower nutritional requirements to achieve its maximum growth in relation to cultivar Taining-01.

## Conflict of Interest

The authors declared that they have no conflict of interest.

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