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**African Journal of Agricultural Research**

**Table of Contents: Volume 12 Number 20, 18 May, 2017**

## ARTICLES

African Journal of Agricultural Research

Table of Contents: Volume 12 Number 20, 18 May, 2017

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*Full Length Research Paper*

# Neonicotinoid insecticide treatment improves physiological performance of melon and watermelon seeds

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**Thiamethoxam is a neonicotinoid insecticide, which is believed to induce the expression of enzymes and proteins that activate plant defense mechanisms, thus improving the plant resistance to stress factors. The aim of this study was to evaluate the physiological performance of melon and watermelon seeds treated with thiamethoxam. The seed treatment consisted of five doses of thiamethoxam, 0, 2, 4, 6 and 8 mL per kilogram of seeds. The parameters evaluated were measured by a standard germination test, first count of germination test, laboratory vigor tests (accelerated aging and cold tests), and a field vigor test (seedling emergence). Thiamethoxam treatment resulted in increase in the normal seedling percentage in the standard germination, first count of germination, accelerated aging, cold and final seedling emergence tests. The doses of 4.0 to 5.0 mL of thiamethoxam per kilogram of seeds were most efficient in improving the physiological performance of melon and watermelon seeds.**

**Key words:** Thiamethoxam, seedling vigor, *Cucumis melo*, *Citrullus lanatus*.

## INTRODUCTION

Watermelon (*Citrullus lanatus*) belongs to the family Cucurbitaceae and is thought to have originated from Southern Africa (Paris, 2015). Today, watermelon is cultivated all over the world; the plant prefers warm seasons and is resistant to drought. The total world production of watermelon is about 110 million tonnes on approximately 3.5 million ha. The main producers at the global level are China (about 70 million tonnes), Turkey (3.8 million tonnes on 157,585 ha), Iran (3.75 million tonnes on 146,630 ha), Brazil (about 2.1 million tonnes on 92,000 ha) and the U.S.A. (about 1.8 million tonnes on 49,910 ha) (FAOSTAT, 2016).

Melon (*Cucumis melo*, Cucurbitaceae) is one of the most widely grown vegetable crops throughout the

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warmer regions of the world. The production levels for the five top producers are estimated to be 15 million tonnes in China (423,000 ha harvest area), 1.67 million tonnes in Turkey (100,578 ha harvest area), 1.4 million in Iran (80,211 ha harvest area), 1.03 million tonnes in Egypt (39,683 ha harvest area) and 990,890 tonnes in the U.S.A. (33,508 ha harvest area) (FAOSTAT, 2016).

Watermelon and melon have low germination and growth rates at both low (below 13°C) and high (above 40°C) temperatures, which creates problems for melon cultivation in northwestern and southern Brazil (Kozik and Wehner, 2014; Lira et al., 2015). These temperature effects can affect germination, seedling emergence and vigor in the cucurbits species. High seedling vigor is required to achieve good initial development of melon and watermelon so that production levels sustain the demand for these products.

Seed treatment is a term that describes the use of both fungicide and insecticide products and processes. The use of specific products can improve the growth environment for the seeds, seedlings and young plants. Thiamethoxam is a broad-spectrum neonicotinoid insecticide used to control a wide variety of commercially important crop pests. Neonicotinoids are the most important chemical class of insecticides introduced to the global market after synthetic pyrethroids (Jeschke et al., 2011). New horizons of crop protection have been opened by the development of seed treatment with neonicotinoids (Elbert et al., 2008).

Thiamethoxam is available as a seed treatment and is widely used in cropping systems (Jeschke et al., 2012). Its application to seeds has been observed to increase seed germination, root growth, seedling height and biomass accumulation in various crops, such as rice (Clavijo, 2008), oat (Almeida et al., 2012), cotton (Lauxen et al., 2010), soybean (Tavares et al., 2008) and maize (Afifi et al., 2015). Until now, the mechanism is not well known. A number of studies have demonstrated that the use of thiamethoxam as a seed treatment enhances agricultural production (Castro et al., 2009; Pynenburg et al., 2011; Sirchio and Sutton, 2007). It has been reported in the literature that this molecule fosters potential gains in seedling vigor (Macedo and Castro, 2011).

This study aimed to investigate the effects of increasing doses of the neonicotinoid insecticide thiamethoxam on seedling vigor of melon and watermelon under the climate conditions of southern Brazil, a subtropical region that may present adverse conditions for the initial establishment of melons and watermelons.

## MATERIALS AND METHODS

### Experimental location

This study was conducted at the Department of Crop Science Seed Laboratory and at the experimental area of Faculdade de Agronomia Eliseu Maciel, Capão do Leão, Rio Grande do Sul State, Brazil. The experimental area was located at a latitude of 31°52'00'' S, longitude of 52°21'24'' W, and altitude of 30 m above the sea level.

### Seed treatment

Five different doses (0, 2, 4, 6 and 8 mL of the product per kilogram of seeds) of the neonicotinoid insecticide thiamethoxam (35 g a.i./L) were tested. Seed treatment was carried out according to the method proposed by Nunes (2005). The solution volume used (product + water) was 1.6 L per 100 kg of seeds. The product slurry was distributed over the walls of a plastic bag. Seeds were placed in the container, which was sealed and shaken for 3 min to coat seed uniformly with the insecticide slurry. The seeds (3,000 for each treatment) were then placed on paper towels and allowed to air-dry in the laboratory. This procedure was performed for each species (melon and watermelon) separately.

### Standard germination test and first count of germination

The standard germination test (ISTA, 2015) was conducted using 200 seeds for each treatment, which were incubated at 25°C for 14 days for watermelon and eight days for melon on moistened paper towels. The first count of germination test was conducted along with the standard germination test, and seedlings were counted at five days for watermelon and four days for melon. The tests were replicated four times, and only normal seedlings were counted.

### Vigor tests

#### *Accelerated aging test*

The accelerated aging test was performed by aging seeds at 41°C for 48 h using a wire-mesh tray method (McDonald and Phaneendranath, 1978). A single layer of 200 seeds from each treatment was placed on a 10 × 10 × 3 cm copper wire mesh tray inside an 11 × 11 × 3.5 cm plastic box containing about 40 mL of water at the bottom. Following the incubation, the seeds were germinated at 25°C for four and eight days for melon and watermelon, respectively, as described above. The percentage of normal seedlings was recorded.

#### *Cold test*

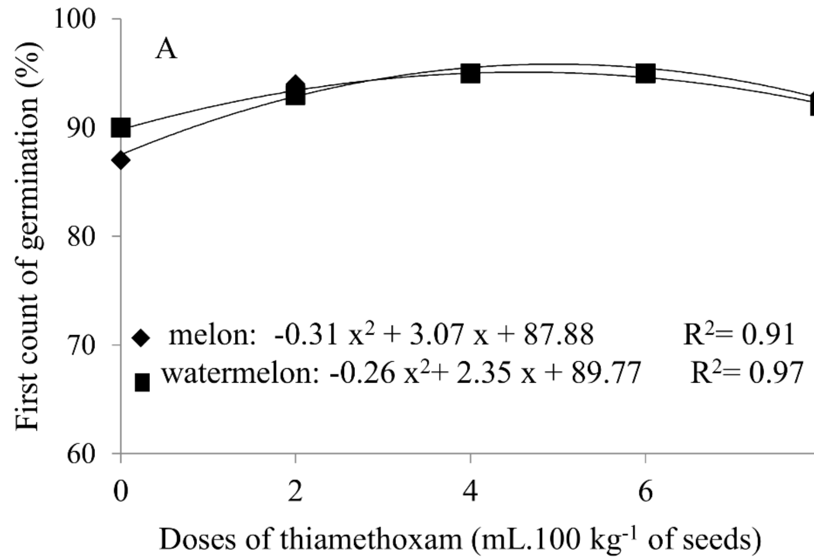
The cold test was performed on four 200-seed replications of each treatment by exposing the samples to 10°C for five days on moistened blotter paper and then transferring to 25°C for five and four days for melon and watermelon, respectively, for germination.

#### *Seedling emergence*

The seedling emergence test was conducted on four 100-seed replications in soil (Planosol Haplic Eutrophic Solodic) at the experimental area of the Crop Science Department. Seed samples from each treatment were manually distributed in 1.5-m long and 0.25-m apart rows at a 2 to 3 cm depth. Emerged seedlings were counted on 14th day.

### Experimental design and statistical analysis

The experimental design was completely randomized with four replications for each treatment and species. Data were subjected to regression analysis using the Statistical Analysis System for



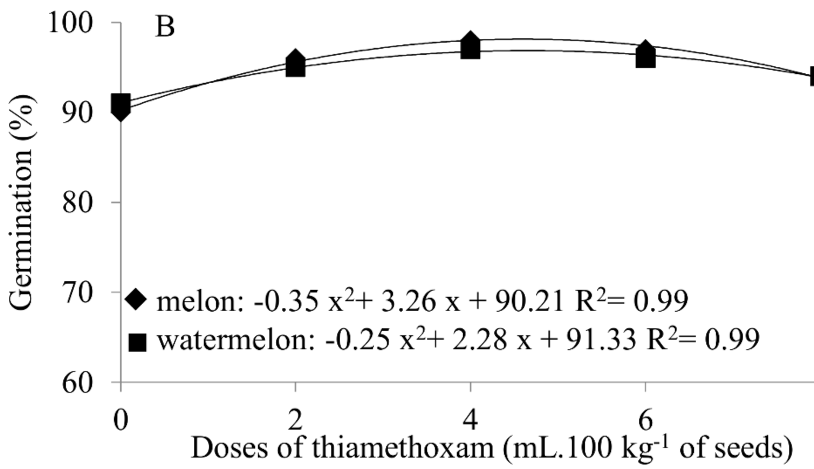




**Figure 1.** Percentage of germination (A); first count of germination (B) of melon and watermelon seeds treated with doses of thiamethoxam.

Windows software (WinStat, v. 1.0) by choosing the most suitable model based on the F-test at 5% probability and the highest coefficient of determination ($R^2$). Results were fitted by an orthogonal polynomial, second-degree regression equation based on the significance of the F-test at 5% probability. Results were fitted to the second-degree regression equation according to the doses used in this study.

## RESULTS AND DISCUSSION

The standard germination test, first count of germination, accelerated aging, cold test and seedling emergence results showed an increase with the doses, from zero up to the maximum dose point located between 4 and 5 mL of thiamethoxam per kilogram of seeds for both species, melon and watermelon. From this point, the results decreased as the dose of thiamethoxam increased.

### Germination rate and first count of germination

According to Figure 1, the seed treatment with the bio-activator increased the germination rate for both evaluated species, watermelon and melon, with the increases of 8 and 6% for melon and watermelon, respectively. The maximum effective dose was between 4.6 and 4.7 mL per kilogram of seeds for both species, watermelon and melon. Similar results were obtained by

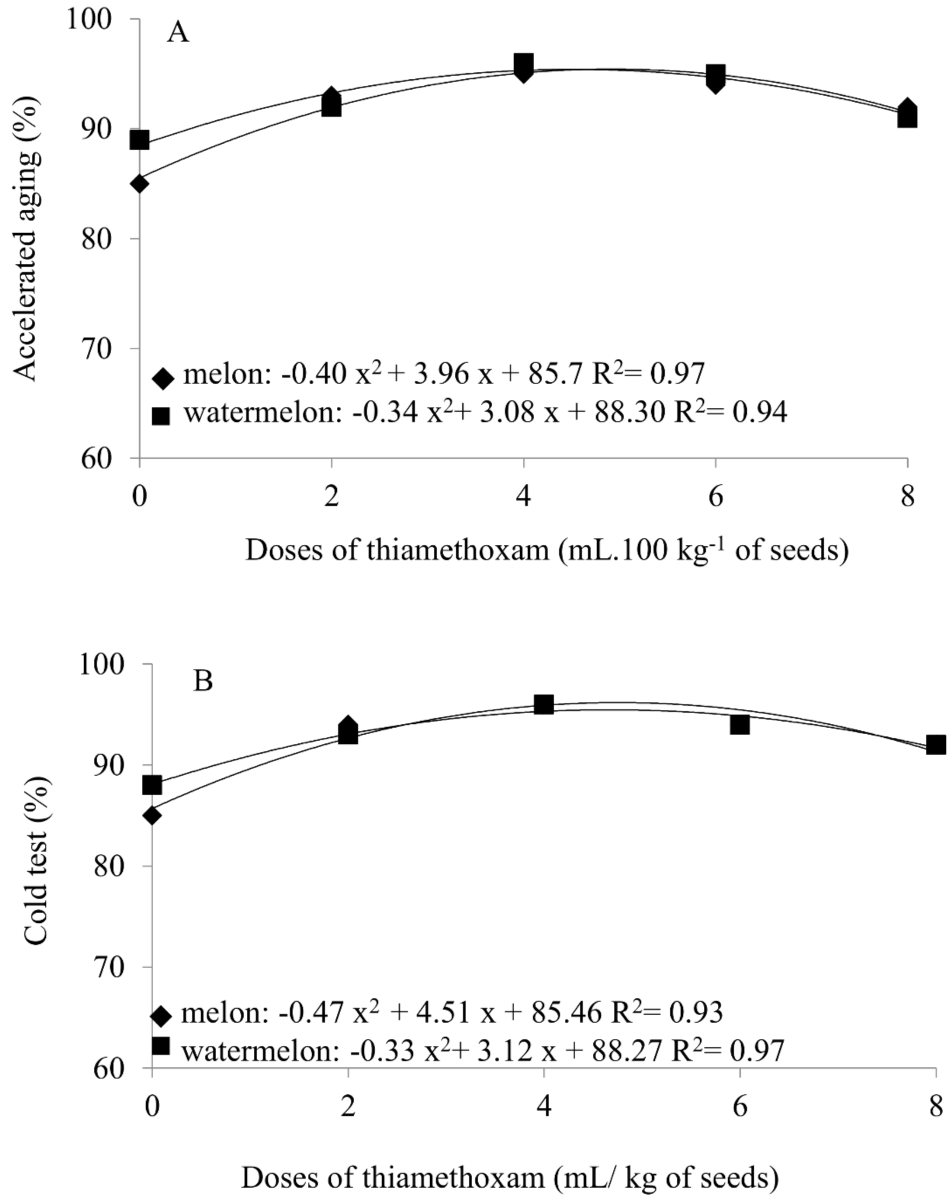



**Figure 2.** Accelerated aging (%)(A); cold test (%) (B) of melon and watermelon seeds treated with doses of thiamethoxam.

Clavijo (2008) and Almeida et al. (2009, 2012) for rice, oat and carrot seeds and by Tavares et al. (2008) for soybean seeds. These authors observed that thiamethoxam accelerated the seed germination of the investigated species.

The seed vigor, evaluated by the first count of germination (Figure 1B), was higher for the seeds treated with the neonicotinoid insecticide, which showed increases of 8 and 5% for melon and watermelon, respectively. An increase in the first count of germination could be observed from the zero dose, reaching the point of maximum effectiveness at a dose of 4.99 mL per kilogram of seeds for melon and 4.53 mL per kilogram of seeds for watermelon. According to Castro and Pereira (2008), thiamethoxam stimulates the enzyme activity, thus providing better initial development and uniform seedling emergence for soybean seeds treated with this product.

### Accelerated aging

The same tendency was observed in the accelerated aging test (Figure 2A). Thiamethoxam treatment resulted in a higher percentage of seed germination after a period of stress induced by the high temperature and humidity.

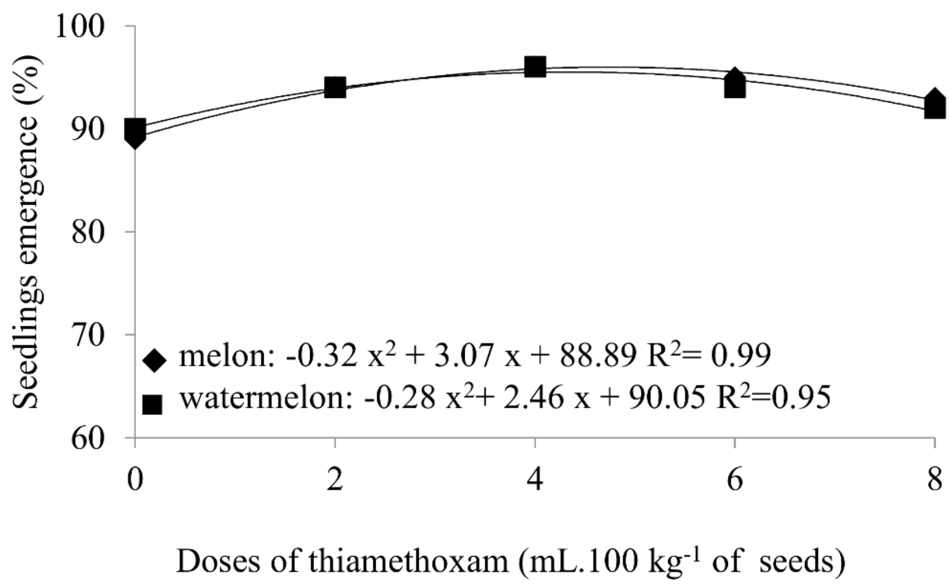



**Figure 3.** Seedlings emergence (%) of melon and watermelon seeds treated with doses of thiamethoxam.

The germination of the melon seeds treated with thiamethoxam increased by 10%, and that of the watermelon seeds treated with thiamethoxam increased by 7%. The response in the accelerated aging test increased from the zero dose to a dose of 4.88 mL per kilogram of seeds for melon and 4.48 mL per kilogram of seeds for watermelon. Lauxen et al. (2010) reported similar results for cotton seeds treated with thiamethoxam, which were subjected to the accelerated aging test. Tavares and Castro (2005) have suggested that this response occurs because thiamethoxam stimulates the protection mechanisms in plants exposed to various types of stress. The neonicotinoid insecticide penetrates plant cells and activates various physiological reactions, such as the expression of functional proteins involved in plant defense mechanisms.

### Cold test

Figure 2B confirms that thiamethoxam conferred increased resistance to seeds during the cold test and increased the percentage of normal seedlings by up to 11% for melon seeds and 8% for watermelon seeds. This resistance may be observed as an increasing curve from zero to the maximum, which corresponds to doses of 4.76 and 4.64 mL per kilogram of seeds for melon and watermelon, respectively. From the maximum point, the curve decreases, that is, the increasing dose of thiamethoxam decreases the percentage of normal seedlings. Based on these results, the effect of the insecticide is the improvement of seed resistance to various stress factors.

### Seedling emergence

As shown in Figure 3, thiamethoxam promoted the seedlings emergence of the species evaluated. The increase in seedling emergence was 7 and 6% for melon and watermelon, respectively. This increase is seen from zero up to a maximum, which was observed at a dose of 4.7 mL per kilogram of melon seeds and 4.38 mL per kilogram of watermelon seeds. Similar results were obtained for carrot (Almeida et al., 2009), rice (Clavijo, 2008), oat (Almeida et al., 2012), cotton (Lauxen et al., 2010) and soybean seeds (Tavares et al., 2008).

The results showed that thiamethoxam fostered potential gains in vigor of melon and watermelon seedlings. Thus, this neonicotinoid insecticide has the potential for use with these species, which exhibit slow, low and irregular growth characteristics of their low-vigor seeds.

Thiamethoxam stimulates the physiological performance of melon and watermelon seeds. Based on the regression analysis, the dose of 4 to 5 mL of the neonicotinoid insecticide thiamethoxam per kilogram of seeds improves the physiological performance of melon and watermelon seeds. This neonicotinoid insecticide was shown to increase the seed vigor of melon and watermelon and improve the initial establishment of these species. Further investigations are necessary to verify whether this effect may last through the life cycle of these crops.

### CONFLICT OF INTEREST

The authors have not declared any conflict of interest.

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*Full Length Research Paper*

# Evaluation of cowpea root system and yield traits in humid tropical agro-ecology

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**Roots are essential to plants and can be the focus for yield improvement in the environment the plant grows. The objectives of this study were to evaluate some yield components and root traits in cowpea (*Vigna unguiculata* (L) Walp) and establish the linear association between the yield and root traits to explore improvement potentials of the crop in humid ecosystem. Ten cowpea accessions were tested in Calabar, Nigeria with humid tropical rainforest ecology. The accessions were grown in perforated polyethylene pipes arranged in completely randomized design, in five replications and on the field arranged in a randomized complete block design, in three replications. TVu-215 had longest roots, heaviest root biomass and largest root volume. TVu-10983, TVu-1515 and TVu-7906 also had long roots. TVu-7857, TVu-1130, TVu-1131, TVu-215 and TVu-1515 had the greatest root density. With respect to yield traits, TVu-1131 and TVu-16196 had the highest yield per hectare; TVu-7857 also had high yield per hectare. Root traits did not significantly correlate with most of the yield traits, but there was significant negative moderate correlation (-0.51) between root volume and number of pods per plant. Root of cowpea does not require much robustness in humid ecology to absorb water and nutrient, which accounts for the non-significant correlation between root system and yield traits in the environment. TVu-16196, TVu-1130, TVu-1131, TVu-215, TVu-7857 and TVu-1515 are selected for the improvement of the root and yield traits.**

**Key words:** Cowpea yield traits, root traits, humid tropical ecology, Calabar, Nigeria.

## INTRODUCTION

The root system is an essential part of plants; it is responsible for anchorage of the plant to the surface on which it grows, it also aids the process of water and nutrient uptake from the soil and involved in the biotic interactions between the plant roots and the microorganisms within the soil region, such as creating barrier that inhibits pathogen and pests penetration into the plant (Ma et al., 2014; Radhakrishnan et al., 2014). In some species, the root functions as reservoir of nutrients in addition, for example, cassava roots store starch. The

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root system also contributes to soil health, which refers to the capacity of the soil to function as a living ecosystem that sustains microorganisms, plants and animals (Xue et al., 2014; Saikia et al., 2015). The root system is also explored for soil erosion control (Ola et al., 2015). Some plants can absorb heavy metals and other toxic compounds from the soil and accumulate the substances in aerial parts which can then be harvested and disposed off in a process known as soil phyto-remediation (Glick, 2003).

Roots are capable of actively foraging resources from their environment. The root system is the interface between nutrient in the soil and the ability to achieve yield in plants. The availability of nutrients in the soil solution determines root growth and proliferation (Lynch, 1995; Akpan and Mbah, 2016). Nitrogen and phosphorus are the most important inorganic nutrient for plant growth, and they often limit the primary productivity in natural systems as well as cropping systems, unless when they are supplied as fertilizer (Treder et al., 2014). The length of the root is related to the amount of nutrient the plant can take in, while the limit to which the root grows affect the plant productivity and yield (Lynch, 1995; Hodge et al., 2009). Quantifying the morphology and spatial distribution of the rooting system (root architecture) is important as a means of determining the availability and accessibility of water and nutrients and hence improving crops and managing soils for higher crop yield (Gregory, 2009). The root architecture includes the entire root system or a large part of the rooting system of an individual plant. This character has not been measured in most crops in the humid rainforest zone of Nigeria, most especially; no study has focused on the relationship between cowpea yield and its root traits. Yield in crops is quantitatively inherited, improvement of yield trait requires an approach of selecting plant characteristics that have high heritability and which correlate with yield.

The root system arises from the coordinated control of an endogenous genetic system and the action of environmental stimuli such as the pH, temperature, water level, microbial and chemical constituent. The nutrient level, health and type of substrate strongly influence the rooting characteristics, that is, the root growth, structure and architecture of the root system, as such, a healthy root system play vital role in the crop productivity. Most plant breeding efforts fail to focus on the relationship between root system and crop yield, probably because of the underground location of the root system. Paez-Gracia et al. (2015) submitted that plant breeding efforts centred on the modification of root traits to increase crop yield can result in the development of more stress-tolerant crops and enhance the capacity of the plant to explore the soil for better absorption of water and nutrient. The ability to improve root traits to translate into higher yields would be remarkable accomplishment for the breeder, such parental lines with the desirable root characters can be selected as breeding lines to be advanced through the crop improvement process.

Cowpea (*Vigna unguiculata* (L) Walp.) is an important legume crop grown in the West African cropping systems; it is a very useful source of plant protein. Cowpea has many beneficial qualities, such as (1) it has early maturation characteristic; many cultivars flowers in 48 days and reach maturity in 60 days, (2) it is highly digestibility, (3) it fixes atmospheric nitrogen (N) in the soil, (4) it has high phosphorus (P) use efficiency, (5) it can reproduce itself by seed dispersal, (6) it is a desired forage and cover crop, (7) it can improve soil structure with its deep roots, (8) it reduces erosion and weeds through its rapid growth and soil coverage (Singh et al., 1997; Ola et al., 2015). Cowpea is nutritionally very useful; it has high digestible protein, 15 to 26%; carbohydrate 55 to 66%; several minerals and vitamins, such as iron, zinc, B-vitamins (Umoren, 1997), the leaves and green pods are also consumed as a leafy vegetable and as animal feed. The average area under cowpea cultivation is about 11.3 million hectare and the world production is 3.67 million tonnes (Singh et al., 2002), yet the production is grossly insufficient based on the demand for the crop as dependable protein source. Cowpea is a severely neglected crop because of its African origin, it deserves greater attention especially for people living in sub-Saharan Africa.

The objectives of this study were to evaluate some yield components and root morphology traits in cowpea, and establish the relationship between the yield component and root traits. The result of this work can serve as novel resource for plant breeders and others interested in elucidating the valuable factors affecting root architecture for practical crop improvement.

## MATERIALS AND METHODS

The experiment was conducted in the Teaching and Research Farm of the Department of Crop Science, Faculty of Agriculture, Forestry and Wildlife Resources Management, University of Calabar, Calabar, Nigeria. Calabar (latitude 4.5 °N; longitude 8.0 °E) is in the rainforest ecology; it is rain-fed with rainfall ranging between 2000 and 3500 mm, temperature ranging from 27 to 35°C and relative humidity of 70 to 85% annually. The rainy season spans from March to October annually, it is bimodal with peaks in July and September and has a short dry spell in August, although, there is really no month without rain which results in persistent flooding. Udoimuk et al. (2014) deduced that the rainfall pattern in Calabar has enhanced wind erosion and desertification, soil erosion and coastal flooding.

Ten accessions of cowpea were obtained from the Genetic Resource Centre, International Institute of Tropical Agriculture, Ibadan, Nigeria; the accessions were TVu-215, TVu-1130, TVu-1131, TVu-1515, TVu-7838, TVu-7857, TVu-1358, TVu-7906, TVu-10983 and TVu-16196. The accessions were grown in polyethylene pipes; 60 cm long, 15 cm in diameter, perforated along their length. Three seeds were sown per polyethylene pipe, placed in a completely randomized design, in five replications; the number of seedlings per pipe was thinned down to one on the 15$^{th}$ day after

**Table 1.** Root traits of the cowpea accessions grown in humid tropical ecology.

| Accession | Root length (cm) | Root biomass (g) | Root volume ($cm^3$) | Root density (g per $m^3$) | Lateral root number | Root dry matter | Root moisture content |
|---|---|---|---|---|---|---|---|
| TVu-10983 | 105.67[b] | 13.12[d] | 12.00[c] | 1.125[ab] | 17.67[ab] | 1.89[c] | 11.23[cd] |
| TVu-1130 | 100.00[bc] | 15.85[c] | 10.33[c] | 1.538[a] | 17.00[abc] | 3.41[b] | 12.44[c] |
| TVu-1131 | 81.00[de] | 15.69[b] | 10.67[c] | 1.483[a] | 11.67[c] | 3.59[b] | 12.10[cd] |
| TVu-1358 | 77.00[e] | 7.47[e] | 10.33[c] | 0.728[c] | 16.67[abc] | 1.79[cd] | 5.70[ef] |
| TVu-1515 | 100.67[bc] | 13.09[d] | 10.33[c] | 1.325[a] | 13.00[abc] | 1.79[cd] | 11.30[cd] |
| TVu-16196 | 99.67[bc] | 18.76[b] | 23.00[b] | 0.821[bc] | 18.33[a] | 3.68[b] | 15.07[b] |
| TVu-215 | 137.13[a] | 39.78[a] | 28.00[a] | 1.435[a] | 13.67[abc] | 6.76[a] | 33.02[a] |
| TVu-7833 | 88.00[d] | 7.03[ef] | 11.67[c] | 0.605[c] | 12.00[bc] | 0.99[e] | 6.04[e] |
| TVu-7857 | 95.67[c] | 12.79[d] | 11.67[c] | 1.67[a] | 12.67[abc] | 1.72[cd] | 11.07[d] |
| TVu-7906 | 100.00[bc] | 6.02[f] | 9.00[c] | 0.673[c] | 15.0[abc] | 1.42[d] | 4.60[f] |
| CV% | 4.3 | 4.5 | 16.0 | 20.2 | 9.05 | 8.9 | 5.9 |
| SE | 3.49 | 0.547 | 1.789 | 0.1797 | 0.872 | 0.196 | 0.586 |

Mean with the same letters in the same column are not significantly different at 5% probability level of Duncan's new multiple range test. CV = coefficient of variation in per cent; SE = standard error

sowing. The pipes were filled with top soil to two-third their length before seed sowing; the pipes were placed outside the screen house and irrigated manually as needed. At maturity, the pipes were slit open and the soil soaked in water to loosen the roots for measurement. The second experiment was on 338 $m^2$ field; three seeds were sown on 30 plots measuring 3 x 2 m each, laid out in a randomized complete block design, in three replications; the planting distance of 50 cm intra-row, 60 cm inter-row and 100 cm inter-block.

Data were taken on the following traits at harvest: root length (cm); root volume ($cm^3$) determined by measuring volume of displaced water after gently dipping root system into water in Eureka can; root biomass (g) measured with Havana weighing balance model SP 20; root density (g per $m^3$) equals biomass divided by volume; number of lateral roots; weight of root dry matter (g) and root moisture content (g) equals difference between root biomass and root dry matter. The yield component traits were number of days to pod maturity, number of pods per plant, pod length per plant (cm), number of seeds per pod, weight of pods per plant (g), weight of seeds per plant (g), shelling percentages and 100-seed weight. Standard agronomic practices regarding ridge making, weeding and pest control were carried out. The data collected were subjected to analysis of variance (ANOVA) using GENSTAT (2005) software and significant means were separated with Duncan new multiple range test (DNMRT).

Linear correlation coefficient (r) was computed between the cowpea root and yield traits:

$$r_{xy} = \frac{cov_{(x,y)}}{\sqrt{v_x v_y}}$$

Where; $r_{xy}$ = correlation coefficient between trait x and trait y; $cov_{(x,y)}$ = covariance between x and y; $v_x$ = variance of x and $v_y$ = variance of y.

## RESULTS

Means of the root traits of the cowpea accessions grown in the humid tropical rainforest agro-ecology are presented in Table 1. TVu-215 had significantly the longest roots (137.1 cm), TVu-1358 had the shortest roots (77 cm). TVu-215 had the heaviest root biomass (39.8 g), TVu-7906 had the least biomass (7.47 g). TVu-215 had the largest root volume (28 $cm^3$), followed by TVu-16196 (23 $cm^3$). TVu-1130, TVu-1131, TVu-215 and TVu-1515 had the largest root densities. The number of lateral roots per plant ranged from 11.7 in TVu-1131 to 18.3 in TVu-16196; TVu-10983, TVu-1130, TVu-1358, TVu-215 and TVu-1515 also had large number of lateral roots. The root dry matter of the cowpea accessions ranged from 0.99 g in TVu-7833 to 6.67 g in TVu-215. TVu-215 also had the most moisture content of 33.0 g, while TVu-7906 had least root moisture content (4.6 g).

Yield characteristics in the ten cowpea accessions are presented in Table 2. The number of days from planting to pod maturity ranged from 64.3 days in TVu-16196 to 75.67 days in TVu-10983. TVu-16196 had the highest number of pods per plant (32.3), while TVu-215 had the least (16 pods). TVu-16196 had significantly the longest pods (26.3 cm) and TVu-1358 had the shortest pods (13.3 cm). TVu-16196 also had the most seeds in a pod (19.67), while TVu-1358 had the fewest seeds per pod (11 seeds). The weight of pods per plant ranged from 22.5 g in TVu-1515 to 140.6 g in TVu-16196; the weight of pods per plant in TVu-16196 was significantly the heaviest among the accessions. TVu-16196 also had significantly the heaviest seeds per plant (118.3 g), TVu-1515 had the lightest seeds (19.2 g). TVu-16196 with 19.9 g had significantly the heaviest 100-seed weight and TVu-10983 had the lightest (10 g). TVu-7906 had the least shelling percentage of 69.3%, while TVu-1130 had the highest (89.7%). TVu-1131 and TVu-16196 had significantly the highest yield per hectare; 1583.5 and 1286.4, kg/ha respectively, TVu-7857 produced 986.2

**Table 2.** Some yield characteristics in the cowpea accessions grown in the humid ecology.

| Accession | Days to pod maturity | Pods per plant | Pod length per plant (cm) | Number of seeds per pod | Wt of pods per plant (g) | Wt of seeds per plant (g) | 100 seeds weight | Shelling percentage | Seed wt per hectare (Kg/ha) |
|---|---|---|---|---|---|---|---|---|---|
| TVU-10983 | 75.67$^{a}$ | 16.33$^{de}$ | 18.33$^{cd}$ | 13.67$^{cd}$ | 29.50$^{cd}$ | 21.11$^{d}$ | 10.0$^{b}$ | 70.90$^{c}$ | 58.6$^{e}$ |
| TVU-1130 | 64.67$^{ef}$ | 21.33$^{bc}$ | 21.00$^{b}$ | 17.33$^{ab}$ | 50.10$^{b}$ | 44.50$^{d}$ | 13.0$^{ab}$ | 89.74$^{a}$ | 123.6$^{d}$ |
| TVU-1131 | 67.00$^{de}$ | 20.33$^{bcd}$ | 18.67$^{cd}$ | 17.67$^{ab}$ | 69.80$^{ab}$ | 57.00$^{ab}$ | 16.1$^{ab}$ | 81.94$^{ab}$ | 1583.5$^{a}$ |
| TVU-1358 | 64.67$^{ef}$ | 22.67$^{b}$ | 13.33$^{e}$ | 11.00$^{e}$ | 36.00$^{bcd}$ | 30.00$^{cd}$ | 13.0$^{ab}$ | 83.37$^{ab}$ | 833.4$^{b}$ |
| TVU-1515 | 72.00$^{b}$ | 6.67$^{g}$ | 21.33$^{b}$ | 15.67$^{bc}$ | 22.51$^{d}$ | 19.20$^{d}$ | 18.7$^{ab}$ | 77.55$^{bc}$ | 533.4$^{c}$ |
| TVU-16196 | 64.33$^{f}$ | 32.33$^{a}$ | 26.33$^{a}$ | 19.67$^{a}$ | 140.60$^{a}$ | 118.30$^{a}$ | 19.9$^{a}$ | 86.16$^{ab}$ | 1286.4$^{a}$ |
| TVU-215 | 70.33$^{bc}$ | 16.00$^{f}$ | 18.33$^{cd}$ | 13.33$^{cd}$ | 36.20$^{bcd}$ | 27.80$^{cd}$ | 14.5$^{ab}$ | 76.61$^{bc}$ | 772.3$^{bc}$ |
| TVU-7833 | 67.33$^{d}$ | 16.33$^{de}$ | 17.00$^{d}$ | 13.67$^{cd}$ | 25.90$^{d}$ | 20.00$^{d}$ | 10.3$^{b}$ | 77.21$^{bc}$ | 555.6$^{c}$ |
| TVU-7857 | 70.00$^{bc}$ | 18.67$^{cde}$ | 19.000$^{c}$ | 19.00$^{a}$ | 44.91$^{bc}$ | 35.5$^{bc}$ | 10.6$^{b}$ | 79.05$^{bc}$ | 986.2$^{b}$ |
| TVU-7906 | 68.33$^{cd}$ | 18.33$^{cde}$ | 21.00$^{b}$ | 16.00$^{bc}$ | 37.9$^{bcd}$ | 26.30$^{cd}$ | 10.1$^{b}$ | 69.34$^{c}$ | 730.6$^{bc}$ |
| CV% | 0.90 | 2.90 | 3.30 | 2.30 | 3.60 | 4.20 | 39.9 | 3.20 | 4.22 |
| SE | 1.11 | 1.78 | 0.85 | 1.19 | 7.40 | 5.9 | 19.0 | 4.28 | 5.9 |

Mean with the same letters in the same column are not significantly different at 5% probability level of Duncan's new multiple range test.

**Table 3.** Linear correlation coefficient of root and yield characters in cowpea accessions grown in humid tropical ecology.

| | DPM | NLR | RBM | RDM | RMM | RLM | RMC | RVM | NPP | PLP | SPP | SHP | WPP | WSP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 100SD | 0.24 | 0.09 | 0.27 | 0.13 | 0.34 | -0.03 | 0.25 | 0.34* | -0.09 | 0.43 | 0.01 | 0.35 | 0.58 | 0.58 |
| DPM | | -0.47 | 0.34 | 0.80* | 0.23 | 0.31 | 0.36 | -0.06 | -0.85 | -0.21 | -0.04 | -0.42 | -0.25 | -0.28 |
| NLR | | | -0.04 | -0.27 | 0.05 | 0.08 | -0.04 | 0.16 | 0.11 | 0.09 | -0.09 | -0.04 | 0.22 | 0.23 |
| RBM | | | | 0.46 | 0.95* | 0.82* | 0.99* | 0.88* | -0.35* | 0.20 | -0.04 | 0.14 | 0.06 | 0.08 |
| RDM | | | | | 0.43 | 0.32 | 0.49 | 0.08 | 0.54* | 0.16 | 0.31* | 0.05 | 0.04 | 0.02 |
| RMM | | | | | | 0.68* | 0.93* | 0.82* | -0.20 | 0.21 | 0.08 | 0.25 | 0.21 | 0.22 |
| RLM | | | | | | | 0.84* | 0.72* | -0.47 | 0.35 | -0.02 | -0.28 | -0.17 | -0.16 |
| RMC | | | | | | | | 0.87* | -0.38 | 0.20 | -0.02 | 0.12 | 0.03 | 0.05 |
| RVM | | | | | | | | | -0.52 | 0.30 | 0.05 | 0.38* | 0.27 | 0.31* |
| NPP | | | | | | | | | | 0.09 | 0.52 | 0.70* | 0.65* | 0.66* |
| PLP | | | | | | | | | | | 0.71* | 0.12 | 0.63* | 0.59 |
| SPP | | | | | | | | | | | | 0.49 | 0.76* | 0.73* |
| SHP | | | | | | | | | | | | | 0.73* | 0.77* |
| WPP | | | | | | | | | | | | | | 0.99* |

*Significant correlation at 0.05% probability; 100SD = 100 seed weight; DPM = number of days to pod maturity; NLR = number of lateral roots; RBM = root biomass; RDM = root density; RMM = root dry matter; RLM =root length; RMC = root moisture content; RVM = root volume; SHP = shelling percentage; NPP = number of pods per plant; PLP = pod length per plant; SPP = number of seeds pet plant; WPP = weight of pod per plant; WSP = weight of seed per plant.

kg/ha, TVu-1358 produced 833.4 kg/ha, while TVu-10983 had the least seed yield per hectare.

The linear correlation coefficient expressing the relationship between the cowpea root and yield characters are presented in Table 3. There was poor correlation ($p > 0.05$) between 100-seeds weight and root volume (0.34). Number of days from seed sowing to pod maturity correlated ($p > 0.05$) positively and highly (0.8) with the root density. High but negative correlation (-0.85) was observed between number of days to pod maturity and number of pods per plant. Root density correlated ($p > 0.05$) moderately with the number of pods per plant (0.54) but poorly with the number of seeds per pod (0.31). Negative moderate correlation was observed between root volume and number of pods per plant (-0.52), and positive but low correlation was found between root volume and the shelling percentage (0.38) and weight of seeds per pod (0.31).

## DISCUSSION

Most studies on crop yield improvement focus on shoot biomass, branching, other shoot traits, diseases and pests resistance, and hardly on the root traits. The root system is underground in most plant species, including cowpea; it is a very important character in plants because of its roles in water and nutrient absorption from the soil, in anchorage, in interfacing with the soil microorganisms and as food storage structure. According to Akpan and Mbah (2016), the root system is the link between the soil nutrient and plant productivity. Paez-Garcia et al. (2015) noted that different root traits enable plants to respond, adapt and thrive in different environments, which implies that root traits can be the focus of crop breeding effort. Watanabe et al. (1997), Singh et al. (1999) and Comas et al. (2013) identified large root diameters, long roots and heavy root biomass and density as traits associated with the development of plant productivity in drought conditions. Some traits in this study were at variance with results of Watanabe et al. (1997), Singh et al. (1999) and Comas et al. (2013), for example, root biomass related inversely with the number of pods per plant. Most of the root traits had poor correlation with the yield traits. Therefore, root system traits in the humid ecology do not need robustness to capture water and nutrients from the soil.

Secondly, some root traits such as root volume, root biomass and root length are controlled by multiple genes, indicating that non-genetic factors would play significant role in the inheritance of the traits, whereas the yield traits such as number of seeds per plant, pod length and number of pods per plant are controlled by single genes (Sangwan and Lodhi, 1995). Cowpea is a self pollinated crop, therefore adoption of mass selection as breeding method to improve root and yield traits at the same time would be slow.

TVu-16196, TVu-1130, TVu-1131, TVu-215, TVu-7857 and TVu-1515 were the best in yield performance, these accessions are therefore selected for improvement of the root and yield traits and recommended for the humid tropical agro-ecology.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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# Traditional sheep production systems and breeding practice in Wolayita Zone of Southern Ethiopia

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**The study was conducted in Wolayita zone of Southern, Nations, Nationalities and People Regional State with the objectives to explain its production systems, breeding practice with major constraints of sheep productivity. Purposive sampling techniques were employed to select target farmers. Structured questionnaire, focused group discussions, secondary data sources and field observations were used to generate the required data. A total of 184 households were selected from four woredas (8 rural kebele) in both weyna-dega and dega agro-ecologies. The survey results revealed that the overall total family size and land holding were 6.4 and 1.1 ha, respectively and the overall mean sheep holding was 5.2 sheep per households. The purpose of keeping sheep was as source of income followed by manure and meat production. The key feed resources in both agro-ecologies were communal grazing and private pastures. Most important causes of sheep mortality in the study were disease and parasite, water and nutritional deficiency, drought and absence of animal clinic. The overall mean value of age at first lambing, lambing interval and twining rate are 13.5, 7.9 months and 1.5 lambs, respectively. The constraints that delay sheep production in the study area was feed and grazing land shortages disease, drought, labor shortage, water shortage and loss of sheep by predators with index value of 0.30, 0.24, 0.16, 0.15, 0.09 and 0.05, respectively. It was concluded that indigenous sheep had a potential for multipurpose role to generate income for smallholders. Therefore, genetic improvement program should aim at farmers need to cope with trait preference and existing traditional herding and breeding practice.**

**Key words:** Sheep production system, breeding practice, selection criteria, trait preferences.

## INTRODUCTION

Sheep are the second most important species of livestock in Ethiopia. The estimated sheep population is about 27.3 million head (CSA, 2015) out of which, 99.9% of the total sheep population is indigenous breeds (Gizaw et al., 2007). There are 14 traditional populations (Gizaw et al., 2007; Gizaw, 2008); Ethiopia has highly diversified

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indigenous sheep types which are parallel to the diversity in ecology (Galal, 1983), ethnic communities and production systems in the country (Solomon, 2008). Sheep can survive under harsh environments such as feed scarcity, disease challenges and are highly adapted to low-input systems (Markos et al., 2006). They are also considered as living banks for their owners and serve as a means of ready cash income to meet immediate needs such as acquiring agricultural inputs, paying school fees or tuition, taxes, medical bills and purchasing large animals and a reserve against economic and agricultural production hardship or monetary saving and investment in addition to many of other socio-economic and cultural functions (Markos, 2006)

The productivity of sheep as in case of most of the ruminants is markedly low due to several genetic and environmental factors besides the institutional, environmental and infrastructure constraints (Markos, 2006; Kosgey et al., 2007). Therefore, genetic improvement of the indigenous livestock through appropriate techniques or selection and breeding programme is the need of the day especially under such constraints (Yakubu, 2010).

Therefore, assessing the production system, indigenous knowledge of selection, management, identification of breeding goals, describing morphological characters and productivity level of the breed/type in their habitat are prerequisites to set up a genetic improvement program at the smallholder and pastoral levels (Kosgey et al., 2006b).

Wolayita zone is geographically located in Southern, Nations, Nationalities and Peoples Regional State (SNNPRs). Even though the study area is rich in livestock resource very little has been done to characterize, identify and document the existing production systems of the zone. The overall objective of this study is, therefore, to describe the performance characteristic of indigenous sheep types and to assess the production system in the study areas.

The objectives of this study were to evaluate reproductive and production performance of Wolayita sheep under traditional management and describe their production system for the establishment of community-based sheep breeding strategy.

## MATERIALS AND METHODS

### Description of the study areas

The study was conducted in Wolayita zone of Southern Nations, Nationalities and Peoples Regional State (SNNPRS) of Ethiopia which is divided into 12 woredas and 302 rural and 22 urban kebeles. Wolaita zone possesses agroecological zones of 11% of wet highlands, 57% of intermediate wet highlands and 32% of semi-dry lowlands. Altitude in the zone ranges between 1500 and 2500 m.a.s.l. except for some parts where it falls below 1500 m. Mean annual rainfall in the area varies between 800 and 1400 mm. Average temperature varies between 17 to 31°C in the zone (CSA, 2009). According to South agricultural office report (2009), the study area the livestock population in the study area encompassed 905523 cattle, 220,150 sheep, 112,550 goat, 524.281 poultry, 4,011Horse, 37,527 Donkey and 3,001 Mules. Out of which Sheep account for about 12.18% of the total livestock population.

### Sampling techniques

A multi-stage purposive sampling technique was employed for selection of districts and peasant associations for the study. In the first stage, districts known for their sheep populations were identified and followed by identification of potential peasant association and villages. Potentials for sheep production and road accessibility were used as criteria in selecting the sites. Thus, four districts were purposively selected based on sheep population potential and road accessibility. From each districts two peasant associations was selected purposively based on the same criteria. A total of 184 households (23 from each PA and 46 from each district) were purposefully selected based on possession of sheep for interview.

### Methods of data collection

Data were generated by administrating a structured questionnaire, organizing group discussion and from secondary data sources. The questionnaires were pre-tested before administration and some re-arrangement, reframing and correcting in accordance with respondent perception were done to get information on:

1. Socio economic characters like sex, age, education level, household size, and livestock possession, economic benefit of sheep and major production constraints;
2. Reproductive performances like age at first lambing interval, litter size (number of lambs born per ewe per lambing) and lambing pattern.
3. Breeding practices like sheep production objectives, selection criteria and castration practices.
4. Feed situation, like major feed sources, supplementation, grazing method and water source
5. Major diseases of sheep in the area
6. Routine husbandry practices like access for sheep extension, housing and so on;

Focused group discussions were held with elderly farmers in the study areas. The group was composed of youngsters, women sheep owners, village leaders and socially respected individuals who are known to have better knowledge on the present and past social and economic status of the area and incorporate local knowledge on sheep breed.

General information of the area, vegetation cover, topography, climate data and population size were obtained from secondary data and district office of agricultural and rural development of wolayita zone.

### Data management and analysis

Data collected through questionnaire were entered into Statistical Package for Social Sciences (SPSS for window, 16.0, 2006). Chi-square was employed when required to test the independence of categories or to assess the statistical significance. Indices were calculated to provide ranking of selection criteria and the reasons of keeping sheep; and calculated as Index = Sum of (3 for rank 1 + 2 for rank 2 + 1 for rank 3) given for an individual reason divided by the sum of (3 for rank 1 + 2 for rank 2 + 1 for rank 3) for overall reasons. Similar indexes were calculated for ranking selection criteria for breeding females and males, constraints for sheep

**Table 1.** Demographic characteristics of the sample households in Wolayita zone Ethiopia (Mean ±SD).

| Descriptor | High-land N(92) | Mid-land N(92) | Over all mean N(184) |
|---|---|---|---|
| Family size | 5.5± 2.4 [b] | 7.3±2.3 [a] | 6.4±2.5 |
| Sex of household head | Percent | Percent | Over all mean |
| Male | 79.4 | 94.6 | 87 |
| Female | 20.6 | 5.4 | 13 |
| Educational level | Percent | Percent | Over all |
| Illiterate | 35.9 | 28.3 | 32.1 |
| Read and write | 28.3 | 28.3 | 28.3 |
| From 5th-8th Grade | 22.8 | 27.2 | 25 |
| Secondary school | 13 | 15 | 14.1 |

*P≤0.01,* [a,b] values with different super scripts differ significantly across rows.

production and sheep diseases.

## RESULTS AND DISCUSSION

### General household characteristics

The study indicated that the majority (87%) of the families were headed by the males where as 16.7% of the proportion was headed by females (Table 1). Educational status of the respondents did not differ significantly across the studied sites. In contrast to the results of the present study higher proportion of illiterate and those with lower level of education were reported from Southern Ethiopia (Seduce, 2007). Larger family size as was observed, in the mid altitude areas which can be attributed to polygamy practiced in the area. The family size as observed in the study is comparable to the values reported by Seduce and Entries (2011) (Wolayita zone and Dawuro district) as well as Kebebe et al., 2006 (Umbulo-wacha watershed of Sidama). On the other hand, it is lower than the values reported by Endeshaw (2007) (from Dale district of Sidama zone).

The large numbers of breeding ewes in a flock is attributing to higher lamb numbers (as obtained in the present study) across both the agro-ecologies (Table 2). The results obtained in this study are similar to the observations of Zelalem and Fletcher (1991). However, the results further illustrate that there was no significant association between agro ecologies when it came to the numbers of lambs and rams. The results of the present findings were in agreement with the results obtained by Berhanu (1995) in South Western Ethiopia, and higher than the proportion of breeding ewes reported by Solomon et al. (2005) in East Wollega and West Shoa Zones as well as Tsedeke and Endrias (2011) for Wolayita and Dawuro zones.

### Purpose of keeping sheep

The reasons for which sheep are reared by the agrarian society in the studied area are presented in Table 3. The primary reason for rearing sheep in the study area is for generating additional sources of income this is closely followed by rearing them for obtaining manure and as a source of meat, while some of them reported that they rear sheep to meet the cultural and social obligations and only a few reported that sheep are reared for sacrificial rituals. The findings are similar to the observations of Markos (2006), Sisay (2006), Endeshaw (2007), Tsedeke (2007), Getahun (2008) and Belete (2009) from different parts of the country. In contrast to these findings, Kosgey (2004) reported low ranking of small ruminants for breeding purpose among the smallholders and pastoralists in Kenya. Tesfay (2008) also illustrated that the primary reason of keeping Afar sheep breed was for the purpose of milk yield. Different studies addressed the importance of multiple values of indigenous livestock breeds in developing countries under low input system (Kosgey, 2004; Mwacharo and Drucker, 2005; Wurzinger et al., 2006; Zewdu et al., 2008).

### Labor division in sheep husbandry and decision making

The results from Table 4 indicated that the various management tasks which were undertaken mainly by the family members and involvement of individuals outside the family is exceptional across the study sites. The results indicate that marketing and breeding is the domain of the senior male family members, the results are similar with the observations of Verbeek et al. (2007) from Kenya. The results also indicated that a majority of the male respondents were involved in the treatment of the sick animals along with providing feed supplements to the flock. Most of the women were engaged in taking care of the sick animals and providing feed supplements to the animals, this may be because most of the women were home makers and stayed at home while the male members had other activities to perform. The younger members of the family were mostly engaged in herding

**Table 2.** Flock size and structures in the study areas.

| Class of sheep | High-land | | Mid -land | | Over all | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| Lambs | 158 | 34.4 | 187 | 37.9 | 314 | 34.4 |
| Breeding ewes | 236 | 51.4[a] | 238 | 48.3[b] | 469 | 51.4 |
| Breeding rams | 65 | 14.2 | 68 | 13.8 | 129 | 14.1 |

$\chi^2 \leq 0.05$, [a,b] values with different subscripts differ significantly across rows.

**Table 3.** Ranked purpose of keeping sheep as indicated by respondents (%).

| Production objectives | 1st | 2nd | 3rd | 4th | 5th | Index |
|---|---|---|---|---|---|---|
| Income | 180 | 2 | 0 | 0 | 0 | 0.36 |
| Manure | 0 | 30 | 75 | 6 | 1 | 0.27 |
| Meat | 3 | 107 | 40 | 0 | 0 | 0.22 |
| Social and cultural function | 0 | 20 | 17 | 74 | 2 | 0.11 |
| Sacrifices | 0 | 1 | 0 | 26 | 42 | 0.04 |

Index = sum of [5 for rank 1 + 4 for rank 2 + 3 for rank 3+2 for rank 4+1 for rank 5] for particular trait divided by sum of [5 for rank 1 + 4 for rank 2 + 3 for rank 3+2 for rank 4+1 for rank 5] for all traits.

**Table 4.** Daily activities of the respondents on sheep managements (%).

| Responsible bodies | Herding | Marketing | Breeding | Sick animas care | Feeding | $\chi^2$ cal |
|---|---|---|---|---|---|---|
| Males ≥ 15 years | 75.2 | 94.8 | 92.5 | 88.7 | 85.7 | 8.9 * |
| Female ≥ 15 years | 58.1 | 67.7 | 47.3 | 90.6 | 89.5 | 4.5 |
| Male <15 years | 66.7 | 2.35 | 11.8 | 37.7 | 45.7 | 9.9 |
| Female<15 years | 48.6 | - | 6.5 | 30.2 | 34.3 | 9.7 * |
| Hired labor | 3.8 | - | 3.2 | - | - | - |

* Chi-square significant at, P<0.05.

the animals besides assisting their parents in the day to day livestock husbandry activities. The contribution of the hired labour is negligible in all the activities mentioned. There are both positive and negative aspects of the same, while in one hand engaging children can benefit the family by reducing the burden of hiring an external hand thus helping the resource challenged family but while on the other hand it also leads to higher school dropouts and thus spiraling into the vicious cycle of poverty.

## Feed resources and grazing management

The results indicating the feed resources are presented in Figure 1. The results of the study indicate that there were hardly any differences between the two studied agro ecologies. According to the group discussions with the key informants, grazing on, crop residues (maize and sorghum stovers and straws from barley, tef, and wheat), parts of root and tuber crops (cassava, sweet potato), sugar cane, grains, parts of enset and banana plants, weeds and tillers from crop fields and leaves and browses from local trees are major feed resources in different seasons of the year. Similar results were also reported by Tsedeke and Endris (2011) under livestock rearing system in smallholder crop-livestock mixed farming system of Wolayita and Dawuro districts. Reports by Tsedeke (2007) and Yeshitila (2007) indicated that farmers use crop fillers and tillers during the wet season in Alaba area of SNNPRS. Feed leftovers, local minerals (bole) and other agro industrial by-products especially from the local beverages are supplemented to improve utilization of crop residues and roughages. After crops are harvested, livestock freely graze on grazing and crop lands and afterwards either they graze (tethered) or under the supervision of herdsmen. The results also indicate that most of the respondents, in high-land (44.5, 30.5 and 22.7%) and in mid altitude (44, 32.8 and 21.1%) depend on the grazing

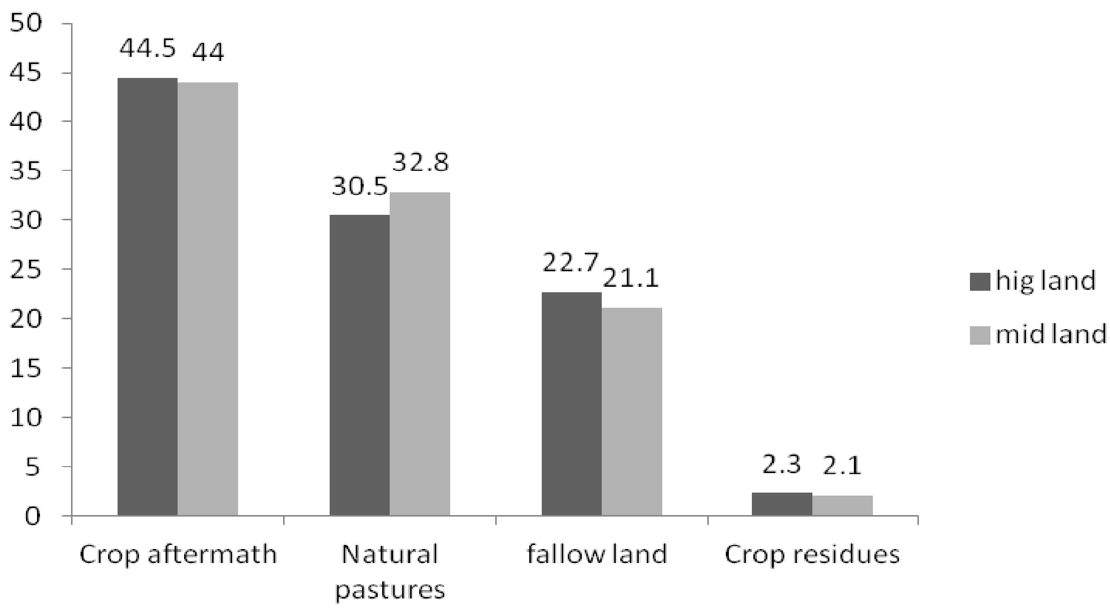


**Figure 1.** Common feed resources used in the study areas in High-land and mid-land agro-ecologies (%).

**Table 5.** Average days per week and hours in grazing of sheep (Mean ±SE).

| Items | High-land(n=92) | Mid -land(n=92) | Over all mean(n=184) |
|---|---|---|---|
| Time of grazing of sheep | | | |
| days per week | 6.45±0.18 | 6.90±0.22 | 6.50±0.12 |
| hours per days | 6.50±0.16 | 6.90±0.21 | 6.90±0.15 |
| Wet season (%) | | | |
| Free grazing | 9.8 | 9.8 | 9.9 |
| Tethered grazing | 18.5 | 18.5 | 18.5 |
| Herded | 71.7 | 71.7 | 71.7 |
| Dry season (%) | | | |
| Free grazing | 37[a] | 32.6[b] | 34.8 |
| Tethered grazing | 43.5[b] | 59.8[a] | 51.7 |
| Herded | 19.6[a] | 7.6[b] | 13.6 |

[a, b] Values across rows differ significantly; $\chi^2_{cal,}$ P<0.05.

on crop aftermaths besides grazing the sheep on the natural pasture, fallow land and crop residues respectively. The observations are similar with those of Abule (2003), Teshome (2006), Seduce (2007) and Tesfaye (2008). According to Alemayehu (2003), as the quality and quantity of the forage is not similar the year round, the animals who do not receive the supplementation during the dry period usually lose weight and in some situation may also die. According to the key informants, aftermath is an important source of feed especially for the sheep; the same is available from the dry season till the short rainy season where after their importance declines considerably during the long rainy season.

The results (Table 5) further indicate sheep graze for six days a week and the number of hours was observed to be an average seven hours a day. The results are in agreement with that of Zewdu (2008) and Berhanu (1995) from Adiyo Kaka district and Jimma areas respectively and higher values have been reported by Abebe (1999) from Lallo-Mama district of Central Ethiopia. Management with respect to feeding or grazing was different for dry and rainy or cropping seasons. Tethered, herded and free grazing animals differ significantly (P<0.05) across the two agro-ecological sites within the dry season. While in the rainy season the majority (71.7%) of sheep owners in this study areas herded their animals where as in dry season more than half (59.8%)

**Table 6.** Reason for tethered feeding of sheep as reported by households (%).

| Reasons of tethering | High-land N(92) | Mid-land N(92) | Overall mean N(184) |
|---|---|---|---|
| To avoid crop and vegetation damage | 90 | 93.8 | 92.7 |
| To save labor | 8.3 | 3.7 | 6.1 |
| To protect from predators | 1.7 | 2.5 | 1.3 |

**Table 7.** Common water sources of sheep in the study areas (%).

| Water sources | High-land(92) | Mid-land (92) | Overall mean N(184) |
|---|---|---|---|
| River | 75.0 | 81.1 | 78.05 |
| Pond | 6.5 | 6.5 | 6.5 |
| Rain water | 7.6 | 3.3 | 5.45 |
| Deep well | 10.9 | 8.7 | 9.8 |

**Table 8.** Watering frequency of sheep in the study areas (%).

| Frequencies | High-land N(92) | Mid-land N(92) | Over all mean |
|---|---|---|---|
| Dry season | | | |
| Once a day | $6.7^{b}$ | $14.6^{a}$ | 10.65 |
| Twice a day | $47.2^{b}$ | $50^{a}$ | 48.6 |
| Three times a day | $42.6^{b}$ | $65.5^{a}$ | 54.05 |
| Four times a day | $3.5^{a}$ | $1.0^{b}$ | 2.25 |
| Wet season | | | |
| Once a day | $49.9^{a}$ | $22^{b}$ | 35.95 |
| Twice a day | $37.5^{b}$ | $60^{a}$ | 48.75 |
| Three times a day | $8.8^{b}$ | $10.5^{a}$ | 9.65 |
| Four times a day | $3.8^{b}$ | $7.5^{a}$ | 5.65 |

$^{a\ b}$ Values across rows differ significantly; $\chi^2_{cal}$ P<0.05.

of the farmers practiced tethering (Table 5).

The results in Table 6 indicate the primary reasons for practicing tethering was mainly to avoid crop and vegetation damage, protection against predators and to save labor respectively. Similar finding were observed in densely populated parts of southern Ethiopia by Tsedeke (2007).

Although tethering is labor intensive, most families use unpaid own or family labor. Access to fresh grass was provided by shifting the tethering sites. Systems that did not involve tethering were most often practiced by farmers with large flocks and sufficient grazing and labor. The limitations of tethering with regard to animal performance and grazing land condition warrant further investigations.

One of the most limiting factors in livestock production is availability of regular and clean drinking water. The results indicate that the main source of water in the study areas was from rivers followed by deep well, rain water and ponds (Table 7). The sources of water are similar in both the agro ecologies while the importance of the same varied between the agro ecologies. Similar result was also reported by Tsedeke and Endries (2011) in Wolayita and Dawro zones while Tesfaye (2008) reported that water was not a limiting factor in small ruminant production in Metama district of Amhara Regional State. However, Abule (1998) reported river was the major water source in mid rift valley area for small ruminants especially for goats.

The results indicating the frequency of watering of the livestock in the two agro ecologies have been presented in Table 8. The results indicated that the frequency of watering varied with the season while two to three times a day watering was common during the dry season when the animals are tethered and feeding on crop residues. The frequency of watering was three times in a day in the midlands which may be attributed to higher temperature when compared to the highland. Moreover as the animals are unable to access water at their will frequent watering is necessary to satisfy their thirst. In the wet season the

**Table 9.** Types of sheep houses and confined during night for protection (%).

| Type of housing | High-land N(92) | Mid -land N (92) | Overall mean (148) |
|---|---|---|---|
| In the family house | 58.7 | 65.2 | 62.0 |
| Attached to the main family house | 10.9 | 10.9 | 10.9 |
| Separate constructed house | 30.4[a] | 23.9[b] | 27.2 |
| Sheep confined | | | |
| Sheep alone | 64.1[a] | 46.7[b] | 55.4 |
| Sheep and goat alone | 3.3 | 5.4 | 4.3 |
| Sheep and other animals' together | 25 [b] | 46.7[a] | 35.9 |
| Sheep and equines | 7.6 [a] | 1.1 [b] | 4.3 |

[a b] Values across columns differ significantly; $\chi^2_{cal}$ P<0.05

**Table 10.** Average holding of ram in two agro-ecology of the study areas (%).

| Ram possession | High-land N(92) | Mid -land N(92) | Overall mean N(148) |
|---|---|---|---|
| Having breeding rams | 42.4 | 41.3 | 41.8 |
| Sources of breeding rams | | | |
| Own | 18.5 | 22.8 | 20.7 |
| Neighbors | 80.4 | 76.1 | 78.3 |
| Other sources | 1.1 | 1.1 | 1.1 |

frequency of watering is less as the animals are able to obtain water from the vegetation around as they are less dependent on dry fodder.

## Housing

The results from Table 9 indicate that most of the farmers keep the sheep within the family houses in both agro ecologies, followed by those who have separate housing for the animals and those who have constructed the sheep houses adjacent to their own. Keeping the animals within the family house may be a way to protect the animals from predators or from theft; however in doing so there is always a severe risk of zoonotic diseases which may affect the humans and animals alike. The result as assessed in the present study show similarity with the findings of Abule (1998), Samuel (2005), Endeshew (2007), Tsedeke (2007) and Zewdu (2008). However, Belete (2009) also reported that sheep and goats are sheltered in most cases in separate houses in Goma district. In these study sites, the number of sheep per households is small, so the farmers may use part of their house for sheep confinement. The results also indicated that in both the agro ecologies sheep are confined with other ruminants followed by those with the equines (in the highlands) and with the goats (in the midlands).

## Breeding management

The results of Table 10 indicate that uncontrolled mating was the common practices of mating in the study area. The study further indicate that a sizable number of farmers rear breeding rams In the absence of the breeding rams the respondents indicated that they borrowed the rams from their neighbors, indicating a high amount of inbreeding within the study area. Matings within the communal grazing area too have been reported by Tesfaye (2008) and Zewdu (2008). According to Kosgey (2004) inbreeding can be minimized by communal herding which allows breeding female from other flock to mix with breeding male of different flocks, early castration of undesired males and rotational use of breeding males.

Selection of parents of the next generation in both the rams and ewes was very common among the sampled farmers. Majority of the farmers reported that they recognize the importance of selection and practiced it to some extent however they had their own criteria for the same. In contrast to the rams, fitness and reproductive traits were more important for ewes. This is because of their belief that survival is more important than fast growth and good appearance of the lambs. Therefore, priority is given to such traits of the ewes that would ensure survival of the lambs. Respondents' index value of the study area is presented in Table 11. The results indicated that irrespective of the agro ecologies the lambing interval was considered to be of prime importance followed by mothering ability, shorter lambing interval prolificacy (twinning) and least priority was given to the coat color. The present observations are similar to those reported by Tesfaye (2008) While Zewdu (2008) indicated that the body size, color and tail formation were

**Table 11.** Overall ranking (irrespective of agro ecologies) regarding selection criteria for breeding females (%).

| Selection criteria | 1st | 2nd | 3rd | 4th | Index |
|---|---|---|---|---|---|
| Lambing interval | 35 | 23 | 76 | 13 | 0.44 |
| Mothering ability | 40 | 1 | 31 | 5 | 0.27 |
| Twining | 30.7 | 17 | 19 | 7.3 | 0.26 |
| Coat color | 3 | 0 | 5 | 4.6 | 0.03 |

Index = sum of [4 for rank 1 + 3 for rank 2 + 2 for rank 3+1 for rank 4] for particular trait divided by sum of [4 for rank 1 + 3 for rank 2 + 2 for rank 3+1 for rank 4] for all traits.

**Table 12.** Over all ranked selection criteria for breeding rams (%).

| Selection criteria | 1st | 2nd | 3rd | 4th | 5th | Index |
|---|---|---|---|---|---|---|
| Appearance or conformation | 31 | 73 | 11 | 21 | 0 | 0.28 |
| Fast growth | 31 | 19 | 68 | 19 | 2 | 0.25 |
| Tail size and shape | 65 | 17 | 6 | 2 | 25 | 0.23 |
| Mating ability | 1 | 24 | 9 | 39 | 48 | 0.13 |
| Color | 0 | 2 | 38 | 25 | 35 | 0.11 |

Index = sum of [5 for rank 1 + 4 for rank 2 + 3 for rank 3+2 for rank 4+1 for rank 5] for particular trait divided by sum of [5 for rank 1 + 4 for rank 2 + 3 for rank 3+2 for rank 4+1 for rank 5] for all traits.

**Table 13.** Reproductive performances of Wolayita sheep breed (Mean ± SD).

| Parameter | High-land | Mid -land | Over all |
|---|---|---|---|
| Age at first lambing (month) | 14.2±6.2 | 12.8±6.3 | 13.5±6.3 |
| Lambing interval (month) | 8.1±4.6 | 7.8±2.4 | 7.9±3.6 |
| Twining rate (percent) | 1.5±0.5 $^{b}$ | 1.7±0.6 $^{a}$ | 1.5±0.6 |
| Reproductive life span of female (years) | 7.04 ± 1.9 | 7.8± 2.1 | 7.4±2.0 |
| Average marketing age of male(months | 22.9±14.3 $^{a}$ | 18.8±13.7 $^{b}$ | 20.9±14.1 |
| Average marketing age of female (months) | 22.6±14.4$^{a}$ | 18.5±13.5$^{b}$ | 20.5±14.1 |
| Age at first service of male (months) | 7.1± 2.1 | 7.1 ± 2.1 | 7.11±2.1 |
| Age at first service of female (months) | 8.6 ± 2.4 $^{b}$ | 9.2 ± 2.1 $^{a}$ | 8.9±2.2 |

$^{a,b}$ means with different super scripts differ significantly across rows.

the most highly rated traits in selecting breeding females in Bonga and Horro breeds. Breeding programs should be geared towards functional traits besides adequate management practices should go in line with genetic improvement programs.

The ranking of important traits as perceived by farmers for the breeds in the two agro ecology are summarized in (Table 12). Traits like appearance or body conformation, followed by fast growth, tail size and shape, mating ability while coat color type was the one which was least preferred trait. The results are in agreement with the findings of Jaitner et al. (2001) and Zewdu (2008).

## Reproductive performances

Reproductive performances of Wolayita sheep populations are presented in Table 13. Good reproductive performance is a prerequisite for any successful livestock production program. Previous study suggested that differences exist in reproductive performance between indigenous sheep breeds and their variation allow for the selection of suitable breeds for a given environment (Mukasa- Mugerwa and Lahlou-Kassi, 1995).

### *Age at first lambing*

As indicated in Table 13, the average age at first lambing was higher in ewes reared in the highlands than those in midlands. This may be attributed to colder climate where the animals mature soon in comparison to those reared in relatively warm climate. There was no significant difference for the trait between the ewes reared in the two

**Table 14.** Overall ranking of sheep disease in Wolayita area.

| Disease of sheep | 1st | 2nd | 3rd | 4th | Index |
|---|---|---|---|---|---|
| Sheep poxes(*Fentata)* | 61 | 52 | 21 | 31 | 0.29 |
| Foot and mouth disease | 44 | 45 | 48 | 18 | 0.25 |
| Liver flock | 43 | 47 | 43 | 31 | 0.25 |
| External parasites | 34 | 22 | 45 | 62 | 0.21 |

Index = sum of [ 4 for rank 1 + 3for rank 2 + 2 for rank 3+1for rank 4] for particular trait divided by sum of [ 4 for rank 1 + 3 for rank 2 + 2 for rank 3+1for rank 4] for all traits.

agro ecologies. These findings were comparable to findings of Zewdu (2008) and Solomon (2007) for Bonga and Horro sheep and Gumuz sheep, respectively. The average AFL as observed was shorter than the values reported by Samuel (2005) and Mengiste (2008) for local Sheep in Adaa Liban, and for Washera sheep, respectively.

***Lambing interval***

The results further highlighted that there was no significant difference for lambing interval values ($p > 0.05$) between the two agro-ecological sites (Table 13). Lambing interval for High-land and Mid-land were; 8.1 and 7.8 months, respectively. The values for the trait was similar to those observed in the Bonga and Horro sheep as reported by Zewdu (2008) and longer than reported for Gumuz sheep by Solomon (2007). However, it is shorter than the values reported by Niftalem (1990) for Menz sheep and Aden (2003) for Dire Dawa sheep. The shortest lambing interval generally occurs in traditional production systems where uncontrolled breeding is the norm. Wilson and Murayi (1988) obtained a longer lambing interval for on-station managed long fat-tailed sheep in Rwanda than most of the intervals reported from African traditional systems where controlled breeding was not practiced.

The results related to the prolificacy rate as observed in the present study indicates that the average rate of twinning was higher in the mid -lands (1.7) than that of the highlands (1.5) which may be attributed to both genetic and environmental factors prevailing in the area. Similar observations had been put forward by Mukasa and Lahlou (1995) and also by Tibbo (2006). There was ($p < 0.05$) difference between the two agro-ecologies regarding this trait. However, similar values for the trait were reported by Solomon (1996) for Horro sheep. Tsedeke (2007) obtained higher values for the trait in Arsi Bale breed of sheep. The average age at first service indicated that the ewes in the highlands had matured ($P<0.01$) earlier than those of the ewes reared in the midlands which can be attributed to better nutrition and also most probably due to lack of parasitic infestation in the cold arid climate. However, no differences were observed in the trait for the rams which may not be clearly demarcated as the rams show sexual maturity at an early age especially under the uncontrolled mating system. The age at first service in this study is lower than what was reported by previously by Tesfaye (2008) in Menz and Afar sheep breeds reared by the smallholders and pastoralists. These values are however higher than the observations of Solomon (2007) for Gumuz sheep. The average reproductive life of the ewes in both the agro ecologies did not differ significantly between the agro ecologies, the values as obtained for the trait find similarity with the observations of Zewdu (2009).The average marketing ages of both sexes of sheep and in both the agro ecologies as presented in Table 13, the results indicate that rams and ewes of the mid-land mature ($P<0.05$) earlier than those in the High-land.

**Sheep health and diseases**

The results pertaining to the different economically important diseases affecting sheep in the study area are presented in Table 14. The results indicate that the sheep foxes followed by Foot and mouth disease, Liver flock and Ticks, mites and flies are reported to be major external parasites affecting the flock. The weather conditions prevailing in the study area may be the cause of the diseases affecting the external bodies. Incidences of diseases affecting livestock in different parts of the country have also been reported by Berhanu (1995), Abebe (1999), Markos (2000), Markos (2006), Solomon and Gemeda (2000), Tsedeke (2007) and Tsedeke and Entries (2011). Most farmers treat liver fluke using albendazole which is a broad anthelmentic. Animal health expert reported concerns of resistance developed owning to improper utilization of the drug by the farmers.

The majority of the respondents in the studies area shave access to veterinary services. However, the services were provided by payment and farmers complain for higher price they pay to treat their sick animals. These observations are similar to those reported by Belete (2009) farmers obtained drugs from government clinics (38%) and from both government and private clinics (52%). Some farmers, however, rely on the ethno veterinary medicines and they use the herbal

**Table 15.** Over all households rankings of causes of sheep death in Wolayita area (%).

| Causes of death | 1st | 2nd | 3rd | 4th | 5th | 6th | Index |
|---|---|---|---|---|---|---|---|
| Disease and parasite infection | 98 | 54 | 16 | 8 | 0 | 0 | 0.29 |
| Water and feed deficiency | 66 | 71 | 16 | 0 | 7 | 0 | 0.25 |
| Drought | 8 | 22 | 47 | 11 | 30 | 45 | 0.15 |
| Absence of animal clinic | 12 | 13 | 72 | 11 | 1 | 4 | 0.14 |
| Lack of adequate trained veterinarian | 1 | 8 | 6 | 64 | 18 | 9 | 0.09 |
| Absence of adequate medicines | 1 | 5 | 18 | 19 | 38 | 22 | 0.08 |

Index = sum of [6 for rank 1 + 5 for rank 2 + 4 for rank 3+3 for rank 4+2 for rank 5+1 for rank 6] for particular trait divided by sum of [6 for rank 1 + 5 for rank 2 + 4 for rank 3+3 for rank 4+2 for rank 5+1 for rank 6] for all traits

**Table 16.** Overall ranking of causes of lamb mortality at pre-weaning period (%).

| Causes of mortality | 1st | 2nd | 3rd | 4th | 5th | 6th | 7th | 8th | Index |
|---|---|---|---|---|---|---|---|---|---|
| Dry season | 104 | 11 | 10 | 6 | 2 | 10 | 2 | 19 | 0.2 |
| Disease and parasite | 30 | 7 | 54 | 37 | 13 | 2 | 1 | 0 | 0.16 |
| Dystocia and accidents | 19 | 27 | 19 | 25 | 44 | 5 | 5 | 0 | 0.16 |
| In adequate feeding supply | 2 | 45 | 49 | 23 | 3 | 5 | 4 | 0 | 0.14 |
| Cold dry season &big rains | 18 | 23 | 9 | 15 | 10 | 17 | 27 | 0 | 0.11 |
| Short rains &dry season | 2 | 43 | 4 | 10 | 16 | 19 | 23 | 5 | 0.1 |
| Killed by predators' | 3 | 8 | 11 | 28 | 23 | 1 | 4 | 8 | 0.07 |
| Wet season | 8 | 7 | 13 | 1 | 6 | 13 | 6 | 37 | 0.06 |

Index = sum of [8 for rank 1 + 7 for rank 2 + 6 for rank 3+5 for rank 4+4 for rank 5+3for rank 6+2 for rank 7+1 for rank 8] for particular trait divided by sum of [8 for rank 1 + 7 for rank 2 + 6 for rank 3+5 for rank 4+4 for rank 5+3for rank 6+2 for rank 7+1 for rank 8] for all traits.

extract of the plants *Ruta chalepensis* (tenadam), and *Allium sativum* (Garlic) for treating their sheep against liver fluke.

The results pertaining to the cause of mortality in the studied area as indicated in Table 15. The major cause of ovine mortality in the study were characterized by the index value of 0.29, 0.25, 0.15, 0.14, 0.09 and 0.08 were; disease and parasite infection, nutritional deficiency, drought, inadequacy of animal clinic service, lack of skilled man power to deliver proper livestock health services and lack of drugs in the vicinity, respectively. Causes of sheep death were similarity with the findings of Tsedeke and Endrias (2011) in Wolaita and Dawuro districts. The results as obtained in the study related to the farmers using antihelmenthic medicine without proper supervision and also the use of ethno veterinary medicine in treating diseases have also been reported by Aden (2003), Niftalem (1990) and Zewdu (2008). Therefore, for the breeding strategy to be realistic farmers should be encouraged to adopt proper and cost effective disease control measures and appropriate drugs should be available to farmers at the proper time besides strengthening the animal health services.

The results pertaining to causes leading to pre-weaning mortality as observed in the study area has been presented in Table 16. The major causes of death can be attributed to lack of proper nutrition of the dam especially during the later stages of pregnancy or post parturition (during the dry season) and during which the fast growing fetus derives nutrition from the dam and in absence of the same may lead to dystocia and retained placenta besides low birth weight of the lambs (especially if there are twins) and also mortality in the first few days of birth, the observations find similarity with the findings of Gatenby et al. (1997) and Markos (2006). During the dry season the nutrition of the nursing dam is challenged and this leads to lack of milk to the neonates and hence can lead to mortality of the same. Nutritionally challenged neonates are also weak as their immune levels are generally low and thus are susceptible to various post-partum diseases and parasites. The results as obtained in the study are in consonance with the observations of Gatenby and Humbert (1991) and Ibrahim (1998). However, as there are environmental variations between years and also seasons within years the observations are in consonance with the reports of Gatenby et al. (1997).

The results related to the post weaning mortality (Table 17) as observed in the study are also in line with the observations of the pre-weaning mortality with major causes being attributed to dry season which is followed by lack of feed and fodder and parasites/diseases etc. The weak post weaned lamb would be weak and hence vulnerable to both diseases and parasites as it would not be able to compete with its strong littermates. It would

**Table 17.** Overall ranking of causes of lamb mortality at post-weaning period (%).

| Cause of mortality | 1st | 2nd | 3rd | 4th | 5th | 6th | 7th | 8th | Index |
|---|---|---|---|---|---|---|---|---|---|
| Dry season | 82 | 12 | 26 | 2 | 14 | 19 | 5 | 5 | 0.2 |
| Inadequate feeding supply | 21 | 49 | 54 | 12 | 5 | 2 | 2 | 0 | 0.18 |
| Disease & parasite | 39 | 18 | 29 | 52 | 3 | 6 | 0 | 0 | 0.17 |
| Short rains & dry season | 9 | 35 | 14 | 6 | 13 | 30 | 12 | 6 | 0.12 |
| Cold dry season &big rains | 13 | 20 | 3 | 21 | 12 | 12 | 23 | 10 | 0.1 |
| Miss-mothering &accidence | 6 | 8 | 14 | 23 | 38 | 1 | 13 | 8 | 0.09 |
| Killed by predators | 6 | 11 | 11 | 21 | 25 | 5 | 4 | 10 | 0.08 |
| Wet season | 2 | 17 | 5 | 3 | 7 | 10 | 24 | 32 | 0.06 |

Index = sum of [8 for rank 1 + 7 for rank 2 + 6 for rank 3+5 for rank 4+4 for rank 5+3for rank 6+2 for rank 7+1 for rank 8] for particular trait divided by sum of [8 for rank 1 + 7 for rank 2 + 6 for rank 3+5 for rank 4+4 for rank 5+3for rank 6+2 for rank 7+1 for rank 8] for all traits.

**Table 18.** Overall ranking of major constraints of sheep production (%).

| Problems | 1st | 2nd | 3rd | 4th | 5th | 6th | Index |
|---|---|---|---|---|---|---|---|
| Feed and grazing land shortages | 117 | 41 | 8 | 1 | 1 | 1 | 0.3 |
| Disease | 42 | 62 | 40 | 11 | 0 | 1 | 0.24 |
| Drought | 7 | 41 | 27 | 22 | 31 | 15 | 0.16 |
| Labor shortage | 7 | 16 | 58 | 31 | 17 | 2 | 0.15 |
| Water shortage | 3 | 4 | 23 | 35 | 18 | 17 | 0.09 |
| Predators | 0 | 2 | 2 | 20 | 16 | 40 | 0.05 |

Index = sum of [6 for rank 1 + 5 for rank 2 + 4 for rank 3+3for rank 4+2 for rank 5+1 for rank 6] for particular trait divided by sum of [ 6 for rank 1 + 5 for rank 2 + 4 for rank 3+3for rank 4+2 for rank 5+1 for rank 6] for all traits.

**Table 19.** Overall ranking of reported reasons for feeds shortage in the study areas (%).

| Reason of feed shortage | 1st | 2nd | 3rd | 4th | 5th | Index |
|---|---|---|---|---|---|---|
| Cultivation settlements and protection Grazing lands | 41 | 30 | 45 | 18 | 9 | 0.23 |
| Shrinking and decline of productivity | 34 | 35 | 26 | 44 | 1 | 0.21 |
| Drought | 18 | 41 | 32 | 33 | 31 | 0.2 |
| Increase of animal population | 33 | 21 | 28 | 28 | 19 | 0.19 |
| increase of human population | 28 | 20 | 15 | 9 | 68 | 0.16 |

Index = sum of [5 for rank 1 + 4 for rank 2+3for rank 3+2 for rank 4+1 for rank 5] for particular trait divided by [5 for rank 1 + 4 for rank 2+3for rank 3+2 for rank 4+1 for rank 5] sum of for all traits.

eventually perish due to lack of nutrition, the observations finds consonance with the reports of Solomon et al. (1995), Yohannes et al. (1995), Markos (2000), Solomon and Gemeda (2000) and Tsedeke (2007).

## Constraints of sheep production

The major constraints in sheep production in the area are given in Table 18. The results indicate that feed and grazing land shortages, disease, drought, labor shortage, water shortage and loss of sheep by predators were major constraints affecting sheep production with index values of 0.30, 0.24, 0.16, 0.15, 0.09 and 0.05, respectively. These constraints as explained by the respondents are not different from those reported by others researches (Abebe, 1999; Aden, 2003; Mulugeta, 2005, Fekert, 2009).

The overall reasons contributing to the feed shortage are presented in Table 19. The table indicates that the primary reasons for the feed shortage can be narrowed down to two basic factors, the first being influx of agriculture where the earlier grazing land is being used for agrarian activities and thus is leading to overgrazing in the existing land resources thus degrading the same further as the pasture is not getting enough time to rejuvenate itself. Similar observations have also been reported from various locations within the country (Abule, 2003; Endeshaw, 2007; Getahun, 2008; Kedija, 2007; Teshome, 2006; Tesfaye, 2008, Belete, 2009).

## Conclusion

From this study it could be concluded that the general production system and sheep management system in the study areas was characterized by mixed crop-livestock production system. Sheep play an important role in the livelihoods of people in the study area, and they have potential for greater contribution through better health management and genetic improvement. Based on growth and reproductive performance, the present studies demonstrate the detailed understanding of production system in ordered to design sustainable breeding strategies and management of animal genetic resources. In the study area, there are less frequent watering points, acute shortage of feed resources especially during December to April and high mortality among the lambs during pre-weaning period.

The main feed sources in both agro-ecologies were communal or natural pasture and private pasture grazing. Feed and grazing land shortages, disease, drought, labor shortage, water shortage and loss of sheep by predators were; major constraints affecting sheep production in the study area. Further researches are needed to increase the productivity of sheep in the study area as well as there should be developed alternative strategy to deal with the acute shortage of natural feed resources especially during the dry periods. Reasonably priced and easy techniques for feed conservation and strategic supplementation schemes should be required and made available to the farmers. In line with introduction of improved forages, inventory of the available local feed resources and utilization should be carried out for their efficient utilization and improvement.

## CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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# Leaf detector box: Artificial vision system for leaf area identification

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**The detection of leaf area in plants is a very important parameter to evaluate growth rates. Nowadays, this analysis is performed manually through a process of counting, cutting and weighing leaf that involves long periods of time, providing subjective results and with low repeatability. The purpose of this research was to develop a computational tool (leaf detector box) to detect leaf area through image processing techniques. The methodology implemented based on application of image processing techniques to segment leaves. Later, morphological operations were apply to eliminate objects that were not part of leaf. Finally, the leaf area was identified through a conversion of pixel to squared centimeter. The validation results showed a determination coefficient greater than 0.97 for four species of plants analyzed with regard to manual analysis by a technical expert. In addition, it validated in a set of 40 leaves precision of algorithm implemented carrying on three measurements for each one at different positions. The results showed a variance of 0.00024 for orange tree leaves, 0.007 for lemon tree leaves, 0.008 for almond tree leaves and 0.001 for mango tree leaves, indicating precision of algorithm providing similar results when it was applied in different opportunities to one leaf. Therefore, the tool becomes a reliable technological support in process of detection leaf area allowing reducing long periods of time and subjectivity in process. Likewise, the repeatability in results were increased.**

**Key words:** Leaf area, growth rates, computational tool, image processing, morphological operations, coefficient of determination.

## INTRODUCTION

Currently, various agri-environment processes are developed in different laboratories and institutions worldwide such as Ministry of Agriculture in Colombia (Ministerio de agricultura, 2016), Food and Agriculture Organization (Organización de las Naciones Unidas para la Alimentación y la Agricultura, 2012), Universidad Del Valle (Universidad del Valle, 2016), Universidad Politecnica De Madrid (Universidad Politecnica de Madrid, 2016), among others. One of these processes is study of plant growth by measuring leaf area; this magnitude

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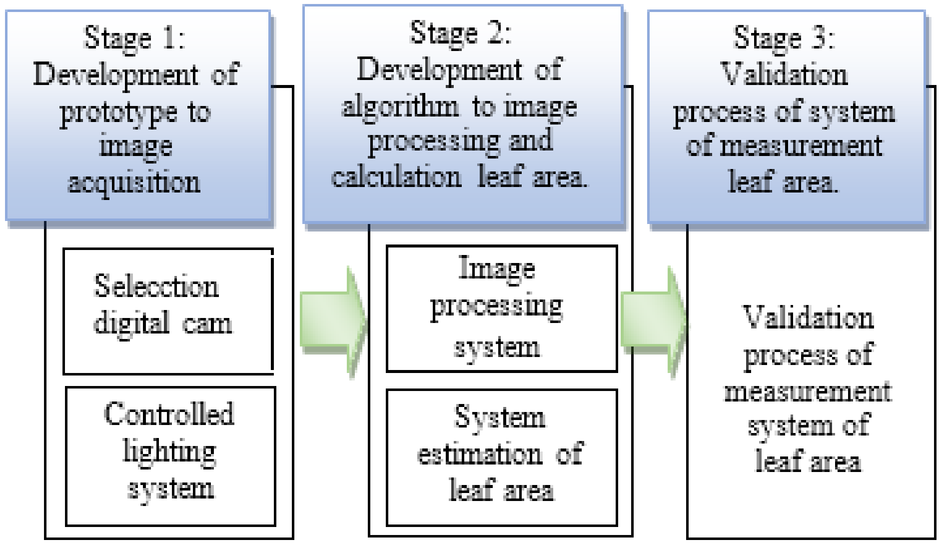


**Figure 1.** Implemented methodology. Source: Authors.

is one that defines ability to capture the photosynthetically active radiation where the plant makes the development of tissues and nutritional compounds for its own development.

Nowadays, different scientific and technological advances exist to do this measurement. One of them was develop by LI-COR company (Li-cor, 2016), in 2007, they developed a system capable of doing recognition and classification of plants of an automated way using Probabilistic Neural Networks (Gang et al., 2007). In 2009, semiautomatic systems were use making a software CAD (Rico et al., 2009) which used lines of reference to calibration of measurement. Other authors in 2011 used MatLab software (Shivling et al., 2011) to do automatic measurement of leaf area, likewise, there were some algorithms used based on invariants moments (Zalikha et al., 2011) with diverse characteristics that could be used in subsequent analyzes. In 2012, using the software ImageJ (Rincon et al., 2012) the area of semiautomatic way was determined. Also, they developed new advances on using a digital scanner (Mahdi et al., 2012) allowing progress in the detection of the rims of the leaf, due to the big precision of capturing the image with one that counts. Moreover, they developed rapid and precise algorithms in (Piyush et al., 2012), it means the conversion to another color space (CieLab), or through bonding techniques such as Zernike moments and neural networks and radial basis probabilistic (Kulkarni et al., 2013). Finally, in 2014 the segmentation was realized using the component of tone making use of the format HSI (Lin et al., 2014), this segmentation worked with an index of very low error, the last advances in the topic of the measurement of the leaf area were focusing in mobile systems principally in cell phones with operating system android.

However, in Colombia and in case of vegetal physiology laboratories at Universidad de Cundinamarca, an expert measure leaf area manually and result of measurement depends on inspection and visual experience, causing systemic errors during process that affects the reliability and repeatability of results.

The main objective of this research was development of a computational tool that allowed to quantify leaf area, through an artificial vision system based on image processing techniques using the MatLab software.

## MATERIALS AND METHODS

In Figure 1, it presents methodology used to develop measurement system of leaf area using artificial vision techniques, which consists of three main stages that are prototype development, processing image algorithms and tool validation.

First, the designs in 3D of prototype for image acquisition were developed, following analyzing of requirements and restrictions of user.

### Design of computational tool

To develop project, an artificial vision system was propose, in order to identify leaf area in plants with a maximum size of (30 × 30) cm, with controlled lighting parameters. Then, in Figure 2, show step by step of its construction in Solid Edge.

In the computational tool design, Coll Roger laminas were use, in order to fulfill requirements of user. The system was design with boxy measures of 50 cm wide, 50 cm long and 70 cm high, as is shown in Figures 2A and B. This allowed include image acquisition system (Logitech webcam C270) and lighting system (lED type) inside box to capture images up to (30 × 30) cm. In addition, a support was design to place leaf on an antireflective glass (40 × 40) cm, which helped to prevent contrast variations in image as shown in Figure 2D and C respectively. Additionally, two dampers were

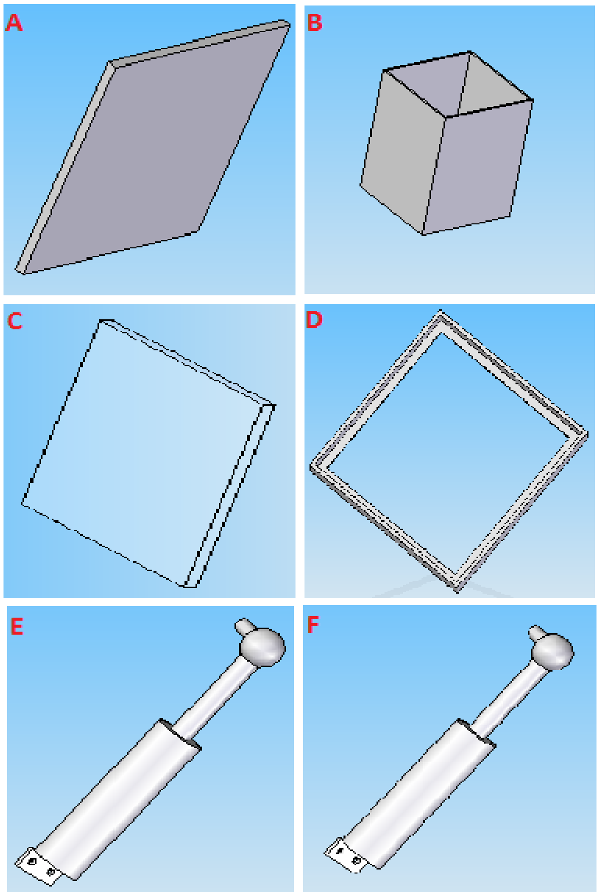


**Figure 2.** Design in 3D of computational tool. A. front view of lid; B. Side views module; C. diagonal view of glass; D. front view of glass support; E and F. front view damper.Source: Authors.

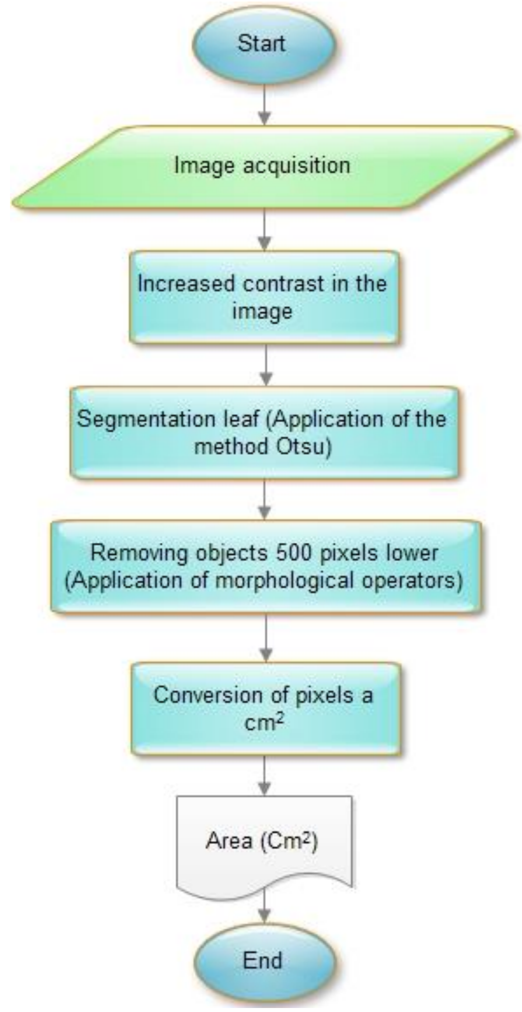


**Figure 3.** Algorithm for identification of leaf area. Source: Authors.

adapted to the sides of lid to avoid sharp blows as shown in Figure 2E and F. Finally, they were implemented four-rubber foot to give support and stability to tool, allowing displacement in laboratories. Furthermore, it is reprogrammable allowing to be apply to other projects.

### Development of algorithm for identification leaf area

The computational tool acquires images by a traditional artificial vision system. The proposed methodology identifies leaf area through application of techniques of image processing. Then, each one described in process stages of in Figure 3.

The first stage consisted in image acquisition process. Then, a contrast adjustment was applied to ease process of segmentation of leaves. Later, Otsu method and morphological operators were use, to segment objects of interest eliminating objects that were not part of leaves. Finally, leaf area was determined through a direct proportion between pixels and image centimeters.

### Acquisition and image preprocessing

The stage of image acquisition of tool, was based in a web cam Logitech c270, which obtained images with resolution of (640×480) pixels and relation 4:3 in JPG format, computer equipment presented a processor Amd c60 APU 1Ghz, memory RAM de 2Gb, with an operative system Windows 8.1. Later, edges that limit space were eliminate on images, used to limit size of the leaves. Finally, stage of preprocessing was apply to adjust contrast, allowing decreasing the variance of intensity in pixels.

### Extraction of area of the leaves

To extract leaf Otsu method and histogram analysis were implement. This method consisted in comparing values of image in gray scale with a threshold defined by colors that presented object and background of image, was assigned white color to pixels that are above threshold and black color to pixels that are below threshold.

### Detection of leaves area

The stage of area detection on leaves was realized by application of Equation 1 through of a factor relation of pixels to $cm^2$ on image.

**Figure 4.** Computational tool assembly (leaf detector box). Source: Authors.

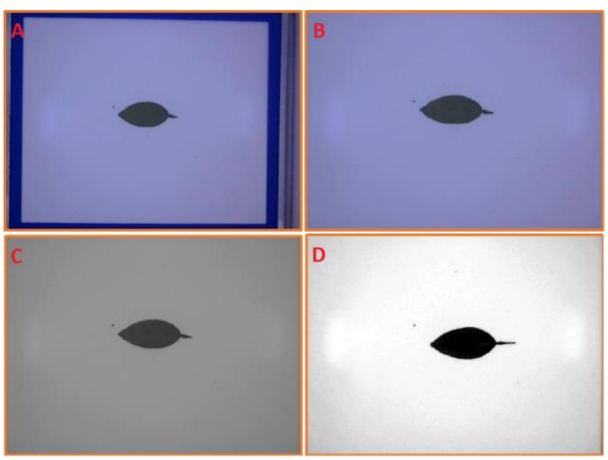


**Figure 5.** A. Original image. B. Removing of contours, C. Convertion to gray. D. contrast adjustment. Source: Authors.

$$Area(cm^2) = \frac{area\ en\ pixeles * 71{,}355}{12502} \quad (1)$$

### Validation of artificial vision system

The validation of computational tool done by a statistical correlation analysis, applying coefficient determination, to evaluate grade of concordance between methodology implemented and conventional methods used. Finally, algorithm precision was determined through a variance analysis of measurements obtained with computational tool developed, in a set of 40 images to four species of plants.

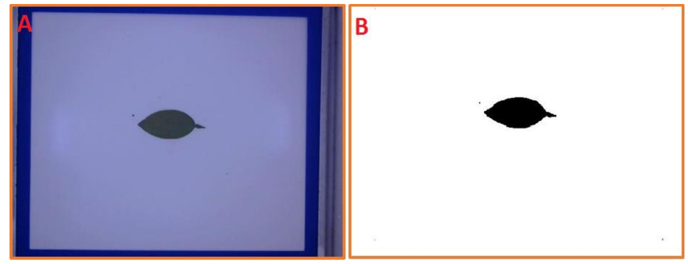


**Figure 6.** A. Original image with computational tool. B. Segmentation of leaf. Source: Authors.

## RESULTS AND DISCUSSION

In order to standardize a methodology to identify leaf area in plants, design and assembly of computational tool and each of modules that composed system of artificial vision were apply; in Figure 4, obtained results are shown. In them is evidenced that it allows to obtain the leafs area with a maximum size of (30 × 30) cm, in addition to count with parameters of lighting controlled whit lid to prevent of lighting environment unwanted situations.

The first stage tested was process of acquisition and preprocessing images, in order to get leaves area in a simple form without distortions that affect measurement. For this reason a contrast adjustment stage was implemented to diminish changes of intensity in images, then in Figure 5 are showed images acquisition results and it is preprocess respectively.

Later, extraction stage of leaf regions was evaluated, having controlled parameters of lighting without depending on external light, it was possible to apply segmentation process through a conventional technique as Otsu method and histogram analysis. Then, results of segmentation process are shown in Figure 6.

Finally, identification stage of leaf area was test by determining area of leaves, through pixels relation to $cm^2$ as shown in Equation 1. Then, in Figure 7, graph shows interface developed to visualize results.

To validate computational tool, analysis of leaf area of four different species from plants with 10 leaves was apply. Later, it was determined leaf area. The results were compared with results obtained by an expert in agronomy through conventional methods used in the Universidad de Cundinamarca.

Initially, a correlation analysis was identify between developed tool, manual measure and precision scales, demonstrated high correlation for orange tree leaves, with a coefficient of determination 0.9953 with regard to manual method and a coefficient of determination of 0.9768, with regard to method of precision scales, as shown in Figures 8 and 9, respectively.

Likewise, validation allowed demonstrating to lemon tree leaves, high correlation with a coefficient of


**Figure 7.** Interface graph of user for computational tool. Source: Authors.

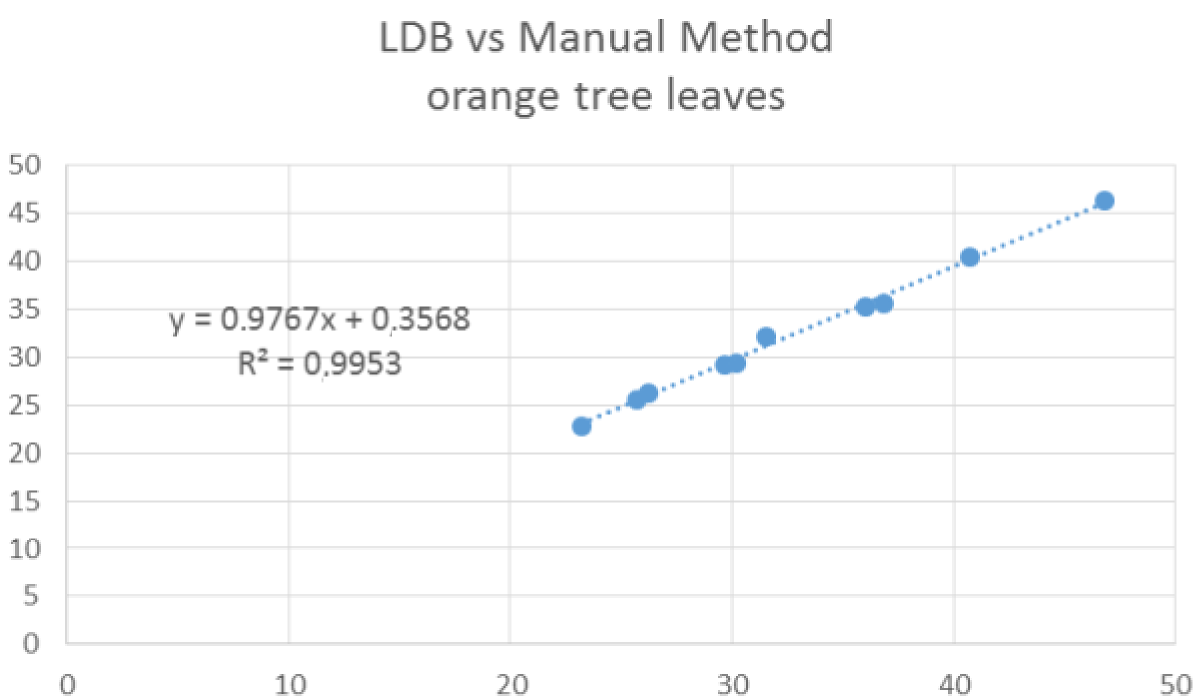


**Figure 8.** Correlation of computational tool with regard to manual method, for orange tree leaves. Source: Authors.

determination of 0.992 regarding to manual method and a coefficient of determination of 0.9894 with regard to method of precision scale, as shown in Figures 10 and 11, respectively.

Later, validation was apply for almond tree leaves and the test allowed to demonstrate high correlation with a coefficient of determination of 0.992 regarding manual method and a coefficient of determination of 0.9686 with regard to method of precision scales, as shown in Figures 12 and 13 respectively. This test evidenced that for leaves with a size and a weight relatively high differences between implemented methodology and conventional methods are most notorious but with percentages of an acceptable precision.

Finally, the validation was apply to mango tree leaves and test allowed to demonstrate high correlation with a

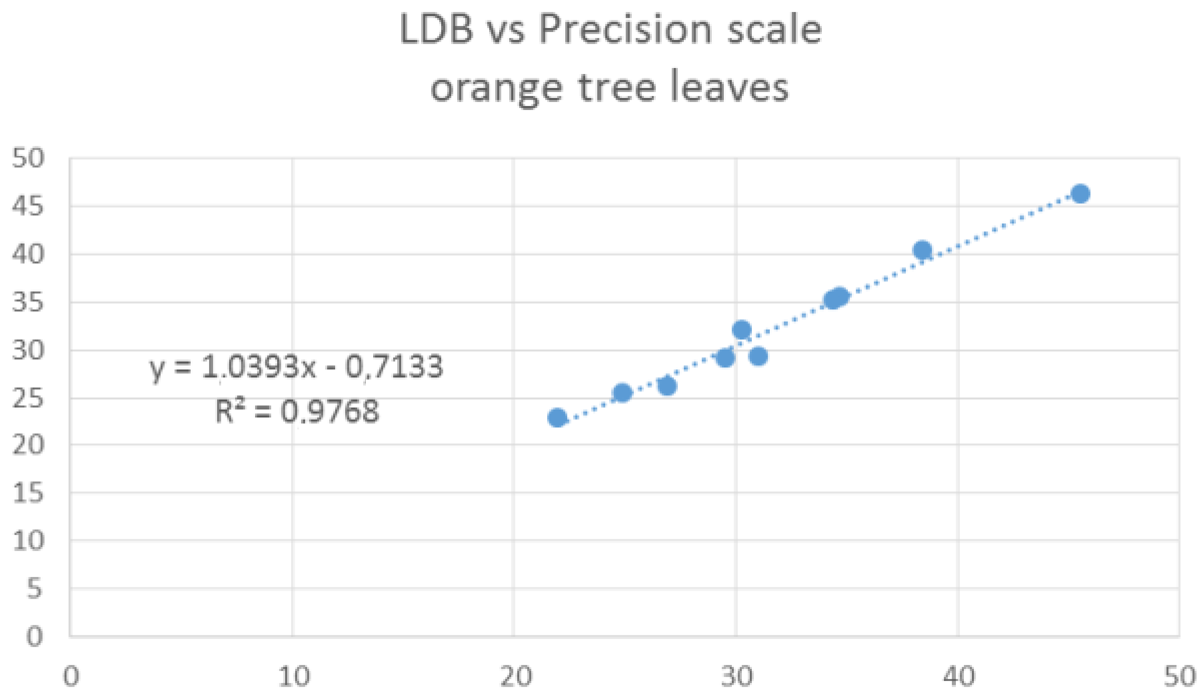


**Figure 9.** Correlation of computational tool with regard to precision scale, for orange tree leaves. Source: Authors.

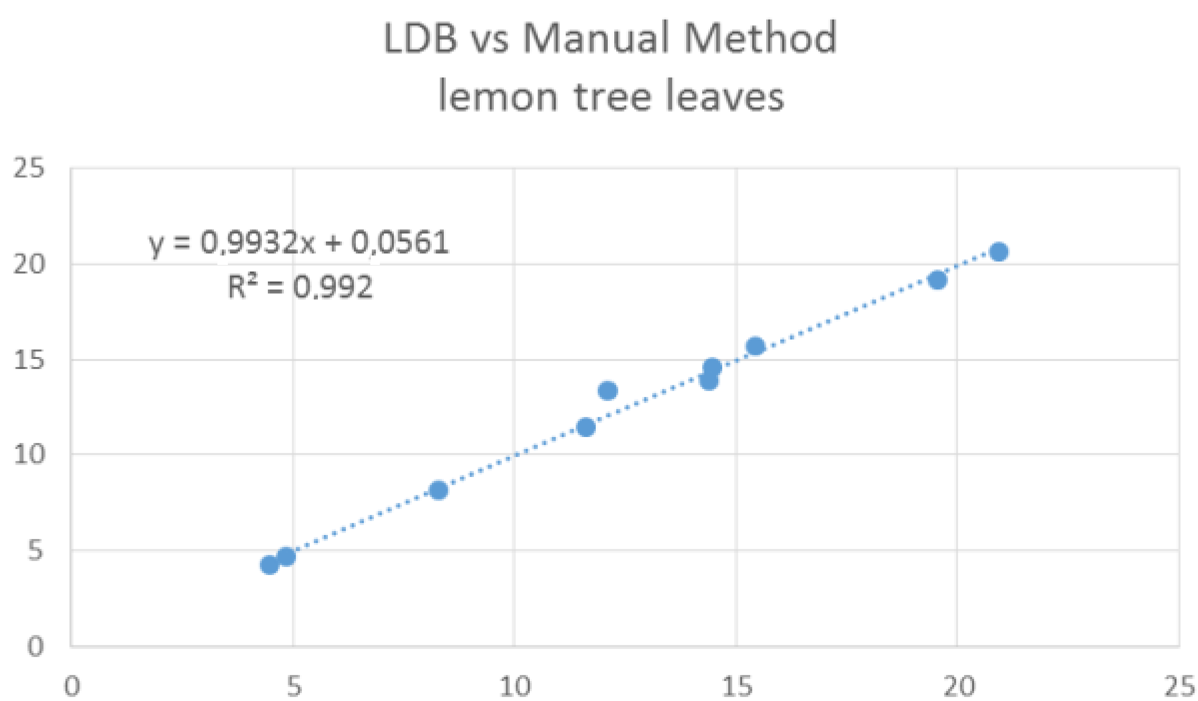


**Figure 10.** Correlation of computational tool regarding to manual method, for lemon tree leaves. Source: Authors.

coefficient of determination of 0.9997 with regard to manual method and of 0.9965 with regard to precision scales, as showed in Figures 14 and 15 respectively. The validation demonstrated that methodology implemented

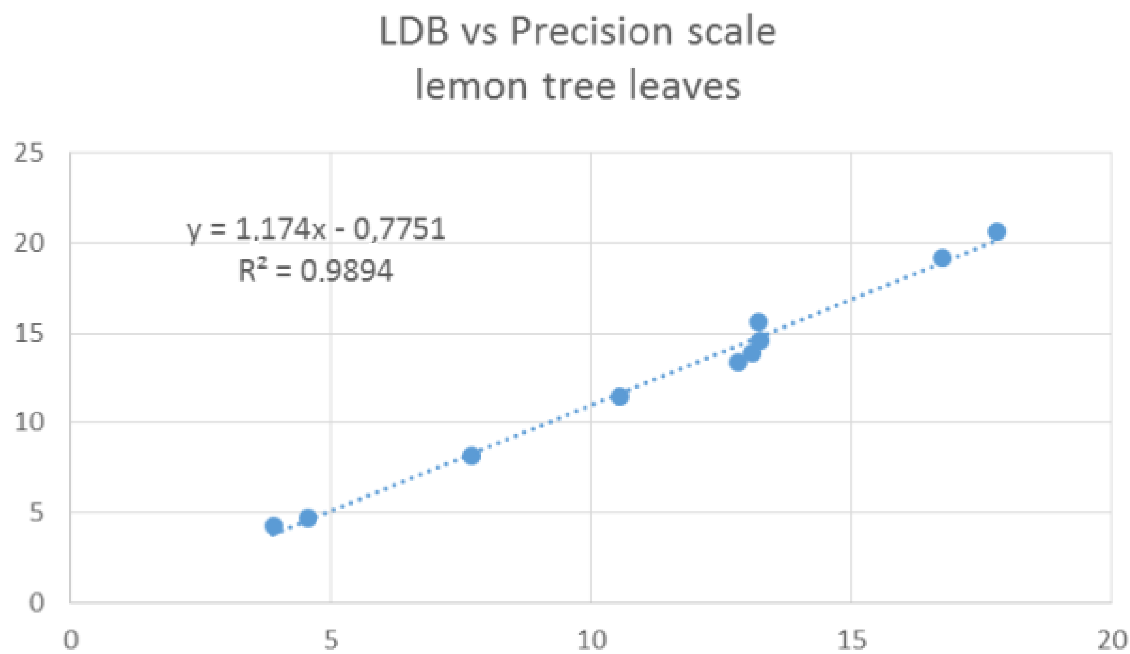


**Figure 11.** Correlation of computational tool with regard to precision scale, for lemon tree leaves. Source: Authors.

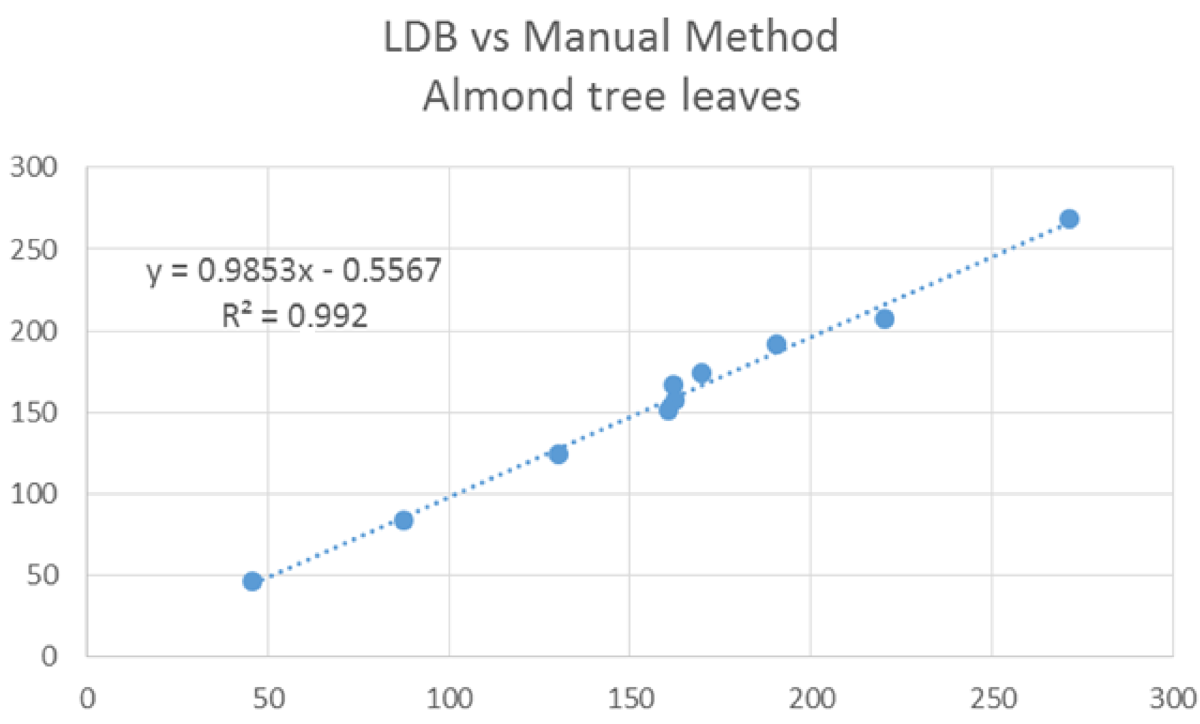


**Figure 12.** Correlation of the computational tool regarding to a manual method, for almond tree leaves. Source: Authors.

allows technological support for leaf area analysis, due to its high indexes of correlation that it was present for the four plants species.

Finally, validation of computational tool was done, by a variance in three different measurements for every leaf in a set 40 images, then, it showed the variances for every

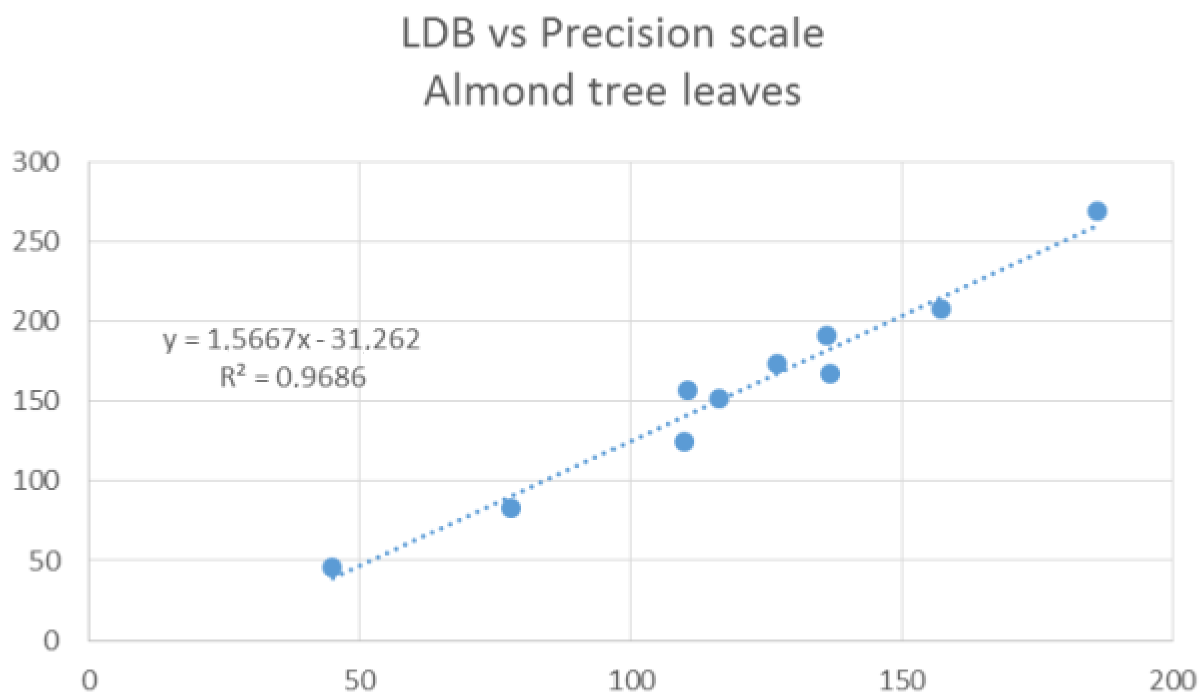


**Figure 13.** Correlation of computational tool with regard to precision scale, for almond tree leaves.

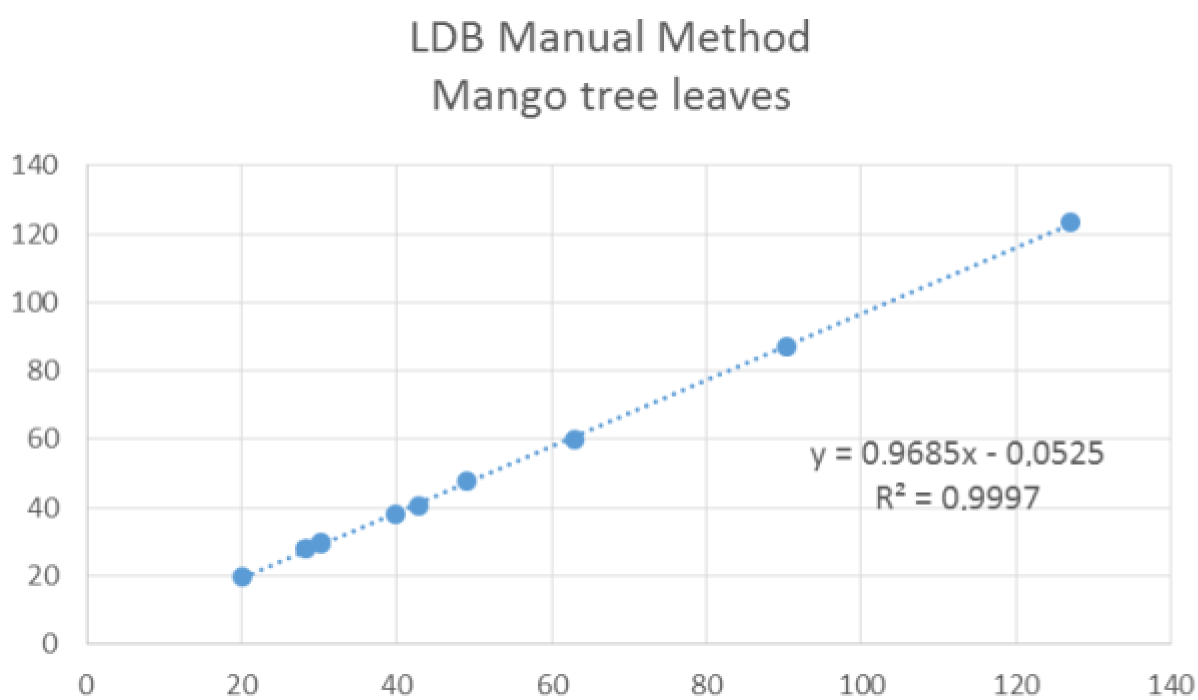


**Figure 14.** Correlation of computational tool with regard to manual method, for mango tree leaves. Source: Authors.

class in Tables 1 to 4, it was evidenced a variance of 0.00024 for orange tree leaves, 0.007 for lemon tree leaves, 0.008 for almond tree leaves and 0.001 for mango tree leaves, showing precision of algorithm to provide very similar results when it is applied in different occasions for same leaf.

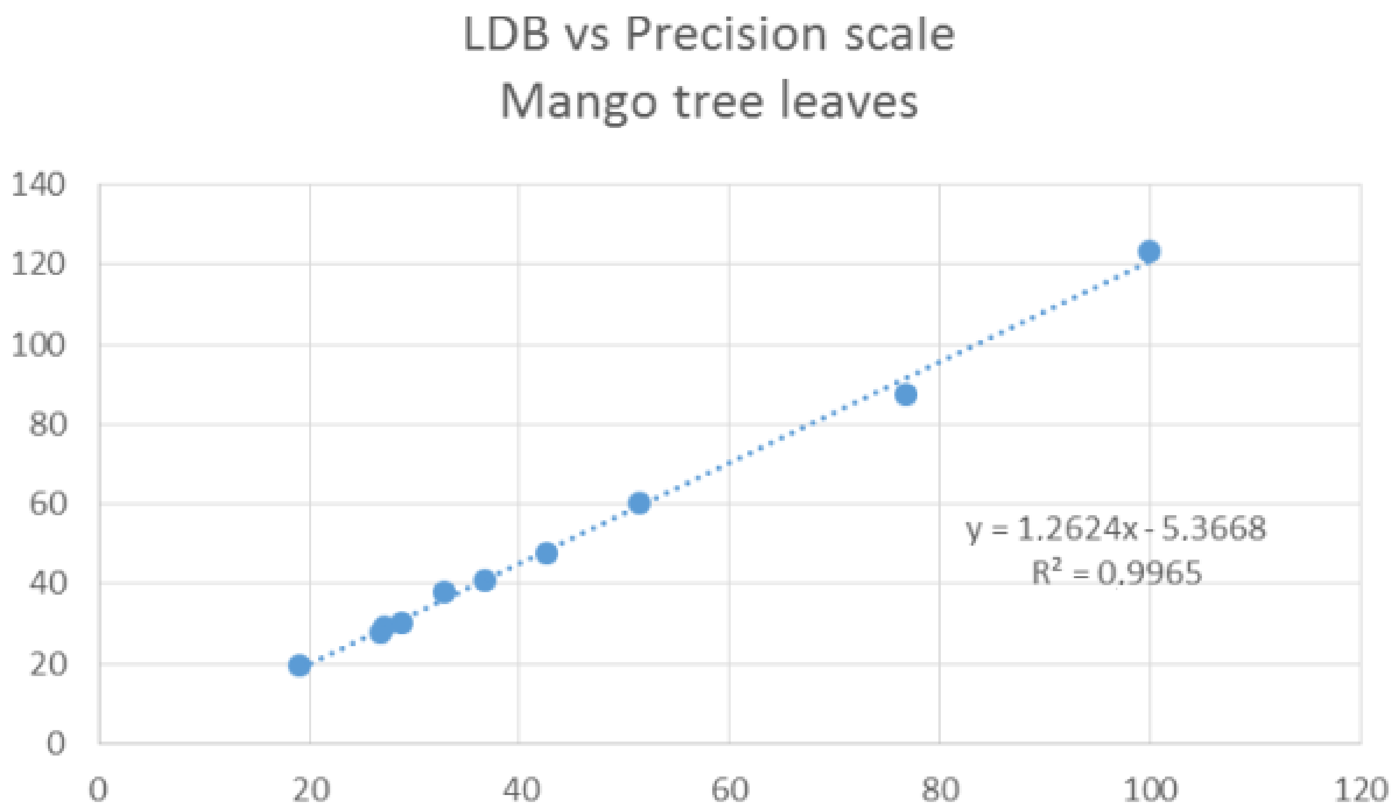


**Figura 15.** Correlation of computational tool with regard to precision scale, for mango tree leaves Source: Authors.

**Table 1.** Precision of system (Leaf detector box) for orange tree leaves.

| Orange | Module (leaf detector Box) | | | | |
|---|---|---|---|---|---|
| | **Measure 1** | **Measure 2** | **Measure 3** | **Medium** | **Variance** |
| Leaf 1 | 22.84 | 22.86 | 22.86 | 22.85 | 0.000133 |
| Leaf 2 | 25.63 | 25.59 | 25.59 | 25.60 | 0.000533 |
| Leaf 3 | 26.25 | 26.25 | 26.25 | 26.25 | 0 |
| Leaf 4 | 32.17 | 32.14 | 32.16 | 32.16 | 0.000233 |
| Leaf 5 | 29.14 | 29.19 | 29.17 | 29.17 | 0.000633 |
| Leaf 6 | 29.29 | 29.31 | 29.34 | 29.31 | 0.000633 |
| Leaf 7 | 35.57 | 35.56 | 35.55 | 35.56 | 0.0001 |
| Leaf 8 | 35.32 | 35.31 | 35.31 | 35.31 | 3.33E-05 |
| Leaf 9 | 40.4 | 40.4 | 40.4 | 40.40 | 0 |
| Leaf 10 | 46.29 | 46.29 | 46.31 | 46.30 | 0.000133 |
| | | Mean variance | | | 0.00024 |

Source: Authors.

## Conclusions

In computational tool development (Leaf Detector Box) for leaf area identification in plants, it was of vital importance adequacy of parameters of lighting, because it must ensure that in process of images acquisition, presence of unwanted objects, impurities or abrupt changes in intensity is minimized. The previous thing facilitated the extraction of leaf, since at present lighting inadequate conditions generates erroneous detections in edges, causing error in measurement. Through developed tool, leaf area was obtained with high precision compared to

**Table 2.** Precision of system (Leaf detector box) for lemon tree leaves.

| Lemon | Module (leaf detector Box) | | | | |
|---|---|---|---|---|---|
| | Measure 1 | Measure 2 | Measure 3 | Medium | Variance |
| Leaf 1 | 4 | 4.5 | 4.25 | 4.25 | 0.0625 |
| Leaf 2 | 8.16 | 8.12 | 8.16 | 8.15 | 0.000533 |
| Leaf 3 | 4.69 | 4.69 | 4.67 | 4.68 | 0.000133 |
| Leaf 4 | 13.34 | 13.35 | 13.36 | 13.35 | 1E-04 |
| Leaf 5 | 13.87 | 13.89 | 13.85 | 13.87 | 0.0004 |
| Leaf 6 | 15.67 | 15.68 | 15.63 | 15.66 | 0.0007 |
| Leaf 7 | 11.45 | 11.45 | 11.44 | 11.45 | 3.33E-05 |
| Leaf 8 | 20.63 | 20.68 | 20.62 | 20.64 | 0.001033 |
| Leaf 9 | 14.61 | 14.56 | 14.54 | 14.57 | 0.0013 |
| Leaf 10 | 19.22 | 19.22 | 19.14 | 19.19 | 0.002133 |
| | | Mean variance | | | 0.007 |

Source: Authors.

**Table 3.** Precision of system (Leaf detector box) for Almond tree leaves.

| Almond | Module (leaf detector Box) | | | | |
|---|---|---|---|---|---|
| | Measure 1 | Measure 2 | Measure 3 | Medium | Variance |
| Leaf 1 | 46.2 | 46.15 | 46.08 | 46.14 | 0.004 |
| Leaf 2 | 83.73 | 83.73 | 83.69 | 83.72 | 0.001 |
| Leaf 3 | 268.59 | 268.63 | 268.71 | 268.64 | 0.004 |
| Leaf 4 | 157.38 | 157.41 | 157.42 | 157.40 | 0.000 |
| Leaf 5 | 151.17 | 151.28 | 151.37 | 151.27 | 0.010 |
| Leaf 6 | 191.28 | 191.3 | 191.35 | 191.31 | 0.001 |
| Leaf 7 | 173.64 | 173.67 | 173.67 | 173.66 | 0.000 |
| Leaf 8 | 124.25 | 124.39 | 124.43 | 124.36 | 0.009 |
| Leaf 9 | 166.91 | 167.18 | 167.33 | 167.14 | 0.045 |
| Leaf 10 | 207.44 | 207.34 | 207.46 | 207.41 | 0.004 |
| | | Mean variance | | | 0.008 |

Source: Authors.

**Table 4.** Precision of system (Leaf detector box) for mango tree leaves.

| Mango | Module (leaf detector Box) | | | | |
|---|---|---|---|---|---|
| | Measure 1 | Measure 2 | Measure 3 | Medium | Variance |
| Leaf 1 | 19.64 | 19.63 | 19.64 | 19.64 | 0.00003 |
| Leaf 2 | 27.73 | 27.74 | 27.78 | 27.75 | 0.00070 |
| Leaf 3 | 30.02 | 30.04 | 30.02 | 30.03 | 0.00013 |
| Leaf 4 | 29.18 | 29.21 | 29.2 | 29.20 | 0.00023 |
| Leaf 5 | 38.22 | 38.22 | 38.21 | 38.22 | 0.00003 |
| Leaf 6 | 40.75 | 40.78 | 40.7 | 40.74 | 0.00163 |
| Leaf 7 | 47.58 | 47.62 | 47.58 | 47.59 | 0.00053 |
| Leaf 8 | 60.02 | 60.09 | 60.06 | 60.06 | 0.00123 |
| Leaf 9 | 87.31 | 87.3 | 87.3 | 87.30 | 0.00003 |
| Leaf 10 | 123.44 | 123.39 | 123.43 | 123.42 | 0.00070 |
| | | Mean variance | | | 0.001 |

Source: Authors.

conventional methods used in Universidad de Cundinamarca by an expert. The precision was evaluate by determination coefficients, for case of orange tree leaves, there was obtain a coefficient of determination of 0.9953 with bias of 0.3568 cm in measure, with regard to manual method and 0.9768 with bias of 0.7133 cm, with regard precision scale method. Likewise, it was apply to lemon tree leaves, obtaining a coefficient of determination of 0.992 with bias of 0.056 cm, with regard to manual method and 0.9894 with bias of 0.7751 cm, with regard to precision scale method. Later, the study was apply to Almond tree leaves, obtaining a coefficient of determination of 0.992 with bias of 0.5567cm, with regard to manual method and 0.9686 with bias of -31.36 cm, with regard to precision scale method. Finally, the mango tree leaves were evaluate with which obtained a determination coefficient of 0.9997 with bias of -0.052 cm, with regard to manual method and 0.9965 with bias of -5.36 cm, with regard to precision scale method, demonstrating good application of tool for the four leaves species. In conclusion, it was determined that methodology implemented, presented very approximate measures with regard to manual method with a max bias of 0.55, evidencing its good performance. In addition, it was determined that precision scale method has more imprecision in measure, due to cuts of known measure on leaf. Finally, results obtained with algorithm were validate with a variance analysis (ANOVA), which obtained a mean variability in measurements of 0.00024 for orange tree leaves, 0.007 for lemon tree leaves, 0.008 for almond tree leaves; and 0.001 for mango tree leaves. This demonstrating algorithm precision to provide identical results when it was apply for same leaf in different positions on measuring area, which increases repetitively in results. For future investigations, it was propose determined nutritional deficiencies in plants as information of color and present damages in leaves. As well, provide a technological support to in determination of indexes of growth.

## Conflict of Interests

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Intercropping of sweet flag (*Acorus calamus* L.) with early and late maturing cultivars of rice (*Oryza sativa* L.)

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**Field experiments were conducted to explore possibility of introducing *Acorus calamus* (Sweet flag) under rice ecosystem either as an Intercrop or main crop for optimizing maximum return. In the first experiment, Sweet flag was intercropped with two rice cultivars early IR-64 and late MTU-7029 "Swarna" in additive series where sweet flag was introduced between two normal rows of rice and in replacement series where sweet flag was planted with rice in 1:1 ratio at 20, 30 and 40 cm spacing and were compared with sole crops after one year. Further, in the second experiment, Sweet flag was intercropped only with early maturing variety (IR-64) in 20 cm spacing with a crop cycle of 1 and 1.5 year to evaluate comparative performance of Intercrops and sole crops. Results revealed that intercropping of sweet flag with rice had a significant effect on yield and yield attributing characteristics of both crops. Numbers of effective tillers in rice were increased with widening the space between intercrops as compare to sole crop of rice, while, rhizome length and width of sweet flag registered highest in sole crop and showed declined trend with decreasing spacing of intercrops. Intercropping of Sweet flag with early and late maturing cultivars of rice did not give significant effect on rhizome yield of sweet flag. Sole crop of sweet flag with a crop cycle of one year was found to be superior and gave significantly maximum equivalent yield (233.75 q ha$^{-1}$). Whereas, EQY of rice recorded from different series of intercropping were significantly at par. The inclusion of sweet flag with paddy decreased the rice yield with increasing spacing. In contrast, rhizome yield of sweet flag was in opposite trend and increased with increasing spacing from to 20 to 40 cm. Gross return and net profit incurred more from all series of intercropping compared to rice as sole crop irrespective of both early and late maturing cultivars of rice. Sweet flag planted as sole crop incurred highest gross return (Rs 248820.00 ha$^{-1}$) and net profit (Rs 187320.00 ha$^{-1}$) among different treatments. Data of another experiment revealed that sole crop of sweet flag taken for one and 1.5 year gave maximum equivalent yield (EQY) of 229.54 q ha$^{-1}$ and 347.83 q ha$^{-1}$ respectively. Intercropping of Sweet flag with rice found to be economical when it was grown as sole crop for a period of one year after rice harvest with 1.5 year of crop cycle (EQY253.23 q ha$^{-1}$). Sweet flag planted in between rows of rice and allowed to grow even after rice harvest for one year gave EQY of 268.06 q ha$^{-1}$ and at par with inter cropping of sweet flag at spacing of 20 cm with a crop cycle of 1.5 years (253.23 q ha$^{-1}$). Moreover, equivalent yield of rice calculated from different treatments was higher as compared to grain yield of sole cop of rice. Maximum gross return of Rs 431472.00 ha$^{-1}$ and net profit of Rs 364972.00 ha$^{-1}$ incurred from sweet flag taken as sole crop for a period of 1.5 years. All series of intercropping with crop cycle of 1.5 years were superior in terms of getting higher net profit.**

**Key words:** Intercrop, equivalent yield, cost benefit ratio, sweet flag.

## INTRODUCTION

Paddy (*Oryza sativa* L.) is a major staple food in Asia covers 60 million hectares with about 225 million tons of production account 37.5% of the global area and 32% of global production. This is predominantly grown in wetland and waterlogged condition since long time because of the lack of other alternative high promising crop during kharif season (Monsoon cultivation) in high moisture regime soil. However, paddy is one of the most important food crops with high income and common in India and in Asia in all groups of farmers. Since last decade, the yield of paddy is almost constant due to delayed in monsoon, infestation of disease, pest and degradation in organic manure and in other soil nutrient stocks. In the recent years farmers applying inappropriate doses of inorganic fertilizers and pesticides that are not only made the paddy cultivation non-profitable but also affect soil health (Foley et al., 2005). Due to higher cultivation cost and relatively low profit from paddy cultivation, several pulses, oil seeds and vegetables had tried as main crops and intercrops with paddy ecosystem but none of the crops was found suitable for economic point of view than paddy alone due to higher moisture level for longer period in paddy fields. This situation can be efficiently utilized by taking other crops, which can be grown at higher moisture level or under continuous submergence conditions either as inter crops with paddy or as main crop. Intercropping is the simultaneous growing of two or more crops in the same field to increase total productivity per unit area through maximum utilization of land, labour and growth resources (Takim, 2012). Yield of intercropping are often higher due to efficient use of water, light and nutrients than sole cropping system (Li et al., 2006).

Presently, there is an increasing interest among farmers towards medicinal plants as this are fetching higher income than other agricultural crops due to high demand and short supply from their natural growing areas. These species may be one of the alternative crops of paddy and may improve the economic return to farmers through crop rotation. Moreover, introduction of such medicinal plant as intercrop in paddy field may render multiple outputs through diversification, which will be helpful to conserve resources and rehabilitate degrading paddy fields. *Acorus calamus* L. (Sweet flag) is one such medicinal plant species which requires littoral environment, ability to withstand under stress aeration and grow efficiently either in submerged conditions or under high soil moisture condition (Tiwari et al., 2011). This species belongs to family Araceae with creeping rhizome commonly found on the banks of streams in swampy marshy places throughout India. It is found across globe up to 1800 m in Europe, Russia, China, Indonesia, Japan and the northern United States (Balakumbahan et al., 2010). Sweet flag has a very long history of medicinal use in Chinese and Indian herbal traditions (Gualtiero Simonetti, 1990). Commercially, the plant rhizome harvest and used widely in modern herbal medicine as its sedative, laxative and diuretic and carminative properties. In addition, the rhizome contains essential oil, which is a unique source of oxygenated sequiterpenes and responsible for antimicrobial, antifungal, insecticidal, anti-spasmodic, carminative, antihelmintic, aromatic, expectorant, nauseate, nervine, sedative, stimulant properties (Mukherjee et al., 2007). It was also used for the treatment of epilepsy, mental ailments, chronic diarrhea, dysentery, bronchial catarrh, intermittent fevers, glandular and abdominal tumors. The alcoholic extract of *A. calamus* exhibits potent antiviral activity against herpes viruses, which is HSV-1 and HSV-2. Due to its varied uses, there is a high demand for the plant in the international drug market. McAlpine and Warrier (1996) reported that sweet flag belongs among the endangered medicinal plants needs immediate conservation.

*A. calamus* is a most important wetland species mature in one year and may yield 8 to 16 tons of rhizomes in a ha land under different situations (Tiwari et al., 2012). This is commonly propagated through vegetative cutting of rhizome in a wide range of soils and climates. This species forms a shallow and compact network of rhizomes that prevents the existence of most other species (Hejny and Husak, 1978; Dykyjova, 1980). Moreover, *Acorus* displays efficient use of nutrients (N) by their uptake and internal cycling (Weber and Bandle, 1996). These characteristics have profound advantages for invasion of *Acorus* and its competition with other wetland plant species (Weber and Brandle, 1996). With increasing eutrophication in *A. calamus* field, the total amount of nitrogen not only increases, but the proportion of inorganic forms of nitrogen available for plants is also changed in the substrate (Vojtiskova et al., 2004) and total P is accompanied by its higher availability in the eutrophic water (Vojtiskova et al., 2001; Tiwari et al., 2011).

Keeping in mind the above perspectives of intercropping, the present study was undertaken aiming to find out the appropriate spacing between paddy and sweet flag based on vegetative growth, yield components and yield of both crops. Experiments conducted under present study were aimed to get significant findings on introducing sweet flag with a recommended period of crop cycle as an inter crop or sole crop under rice eco

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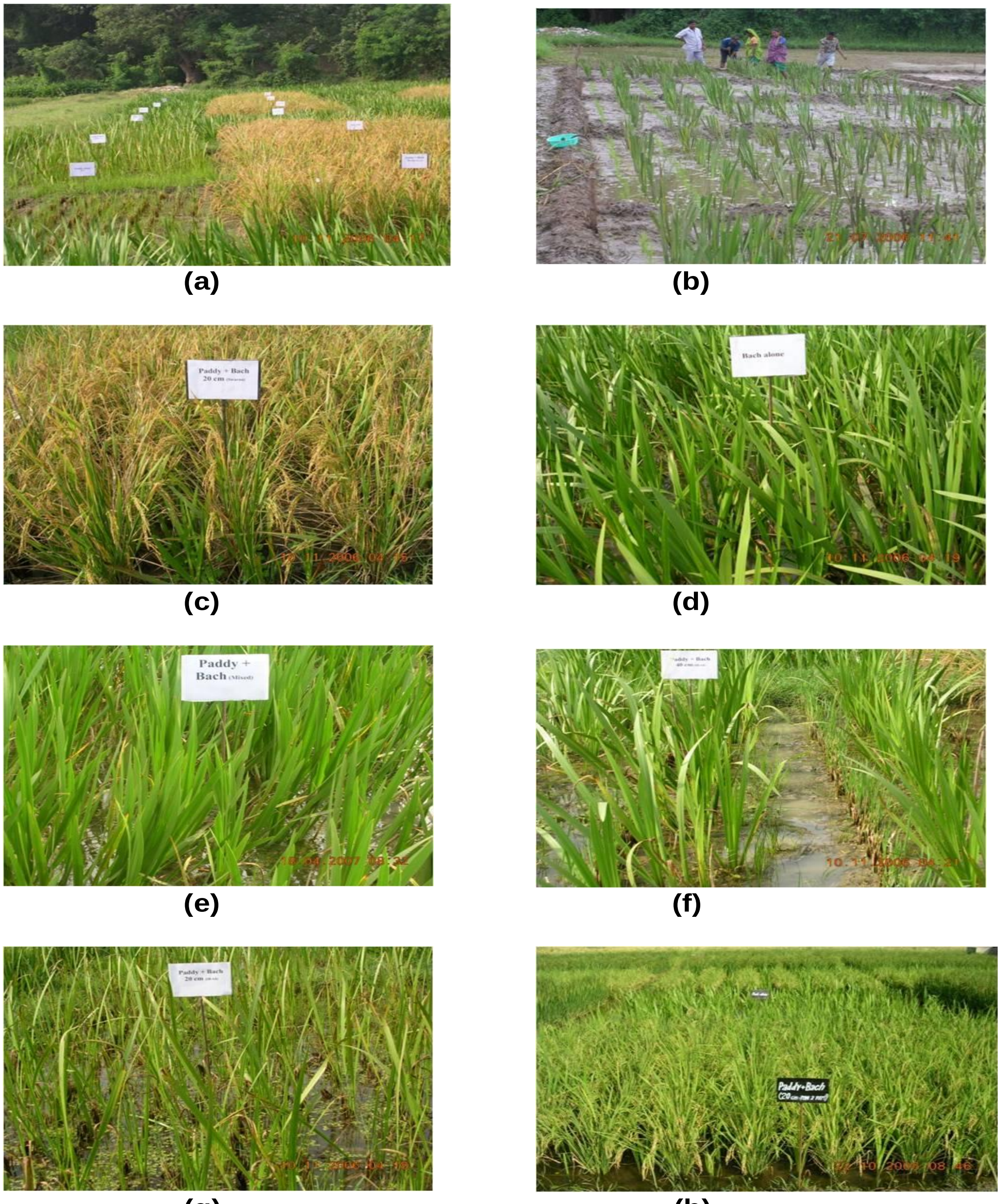


**Plate 1.** a) Experimental view at maturity stage b) Experimental view at planting stage c) Paddy+ Sweet flag 20 cm d) Sweet flag alone e) Paddy+ Sweet flag mixed f) Paddy+Sweet flag at 40 cm after paddy harvest g) Paddy+Sweet flag at 20 cm after paddy harvest and h) Paddy+Sweet flag at later stage.

system and optimum yield per unit area.

## MATERIALS AND METHODS

### Experiment site

The field experiments was carried out at the Experimental Farm of Thakur Chhedilal Barrister College of Agriculture and Research Station, Bilaspur, Chhattisgarh, India during cropping seasons of 2005-06 and 2006-07 (Plate 1). The experimental farm lies in 22°9'12" North latitude and 82° 12'12" East longitude, and at South Eastern Central zone of India with an altitude of 292.3 m MSL. The climate of Bilaspur is sub humid. The air temperature varies from a minimum of about 4.6°C (in December-January) to a maximum of about 45.0°C (in May). The relative humidity ranges from about

12.0% during May to 92.0 during July - August. Average annual rainfall is 1250 mm, of which 82% is received during June to September (Chaure et al., 2007).

#### Treatments and experimental design

Two experiments were conducted during the years, that is, 2004-2007. In the first experiment, sweet flag was intercropped with two cultivars, that is, early (IR- 64) and late (MTU-7029 "Swarna") of rice in different series of inter cropping. In additive series of inter cropping, sweet flag was planted between two rows of rice without shifting space between two rows of rice (20 cm). Whereas, in replacement series of inter cropping; sweet flag was planted along with rice in ratio of 1:1 at 20, 30 and 40 cm spacing. Rice and sweet flag as sole crops were also included as treatments for evaluation of different series of inter cropping. Low land field having clay loam soil and most suitable for rice cultivation was selected for experimentation. Field was ploughed 2-3 times in the month of May – June. Transplanting system was adopted for planting both rice and sweet flag. Filed was puddle and submerged before transplanting. Farmyard manure was uniformly (1000 kg $ha^{-1}$) applied at the time of field preparation along with basal dose of inorganic fertilizers, that is, phosphorus and potassium 60: 40 kg $ha^{-1}$. Whereas, nitrogen 100 kg $ha^{-1}$ was applied in three split doses, that is, 25% after seven days of planting followed by three times at 25% periodical application at an interval of 30 days. Nursery of rice and sweet flag were raised in nursery beds. Seeds of both varieties, that is, IR 64 and MTU 7029 (Swarna) were treated with seed dresser fungicide, that is, Carbendazim 25 SD @ 3 G /kg seed before sowing in nursery beds. Plant saplings of sweet flag were raised from small pieces of rhizomes (5 cm), allowed for sprouting in moistened gunny bags and later on planting in nursery beds. Twenty-one days old seedlings of paddy and 30 days old plant saplings were used for transplantation.

The first field experiment was laid out in all side bunded plots of 6 m × 4 m with three replications under two factor randomized complete block design for two consecutive years, that is, 2005 and 2006. Total seven treatments *viz*. Rice alone; Sweet flag + Rice (mixed) ; Sweet flag + Rice (1:1) at 20 cm row a part , Sweet flag + Rice (1:1) at 30 cm row a part, Sweet flag + Rice (1:1) at 40 cm row a part, sweet flag alone at 30 cm row a part were randomized in three replications. Treatments of intercropping were divided in two series, that is, additive series and replacement series. In additive series of intercropping, sweet flag was transplanted between two rows of rice as Sweet flag + Rice (mixed) whereas, in replacement series of intercropping, one row each of sweet flag and rice were transplanted at spacing of 20, 30 and 40 cm. Rice and sweet flag were kept as sole crops for comparative evaluation of different inter cropping series.

Looking at results of first experiment, second experiment was laid out in all side bounded plots of 6 m × 4 m with three replications under randomized block design for two consecutive years, that is, 2006 and 2007. Sweet flag was intercropped with rice for a crop cycle of one year and 1.5 years. Eight treatments *viz*. rice for two crop seasons (1 year cycle), rice for three crop seasons (1.5 year cycle), sweet flag + rice 1:1 (1 year cycle), sweet flag + rice 1:1 (1.5 years cycle), sweet flag + rice mixed (1 year cycle), sweet flag + rice mixed (1 .5 years cycle), sweet flag sole crop (1 year cycle), sweet flag sole crop (1.5 years cycle). In additive and replacement series of intercropping, rice was taken for one season and thereafter sweet flag was allowed to grow for a period of one crop season and two crop seasons with a crop cycle of 1 year and 1.5 year respectively. In additive series of intercropping, sweet flag was transplanted between two rows of rice (intra row) as Sweet flag + Rice (mixed) for one year and 1.5 years. Whereas, in replacement series of intercropping, one row each of sweet flag and rice were transplanted at spacing of 20 cm for one year and 1.5 years. Rice and sweet flag were taken as sole crops for two and three consecutive crop seasons to study comparative evaluation of different series of intercropping.

#### Yield and yield attributing characteristics of rice and sweet flag

##### *Yield and yield components of rice*

Numbers of effective tillers from each treatment were recorded tagging five plants of rice randomly leaving outer rows of plot and recorded number of effective tillers $plant^{-1}$. Crop was harvested at maturity and grain yield (q $ha^{-1}$) of rice planted as sole crop for two and three crop seasons was recorded.

##### *Yield and yield components of sweet flag*

Rhizome length (cm) $plant^{-1}$ and rhizome diameter (cm) $plant^{-1}$of sweet flag were recorded tagging five plants randomly leaving outer rows of plot at the time of harvesting of rhizome of sweet flag. Rhizomes of sweet flag were dried and yield q $ha^{-1}$ recorded from different treatments.

##### *Equivalent yield (EQY)*

The equivalent yield (EQY) was calculated on the basis of market price of rice and sweet flag @ Rs 1200 $q^{-1}$ and Rs 4000 $q^{-1}$ respectively. One year and two years data were analyzed statistically to determine pooled mean and treatment differences of both experiments.

$$Equivalent\ Yield\ of\ Rice = \frac{Rhizome\ yield\ of\ Acorus * Market\ price\ of\ Acorus}{Market\ price\ of\ Rice}$$

**Economical analysis:** Economics of different series of intercropping taken in both experiments were calculated and net profit estimated subtracting cost of cultivation from gross return from both crops, that is, rice and sweet flag. Cost of cultivation included expenditure in field preparation; planting material, human resources, irrigation, fertilizers, manures and other inter culture operations, that is, weeding, irrigation, harvesting, threshing etc. Gross return was estimated based on equivalent yield to rice obtained from different treatments of intercropping from both experiments and its market price.

## RESULTS AND DISCUSSION

### Experiment 1

### *Biometric characteristics*

The first experiment conducted on the effect of spacing on sweet flag - grown as intercrops with early (IR-64) and late "Swarna" (MTU- 7029) varieties of rice and also as sole crop are presented in Table 1 and Figures 1 to 6. Data presented in Table 1 and Figure 1 to 6 indicate the significant effect of different series of inter cropping on yield components of rice (effective tillers $plant^{-1}$) and sweet flag (rhizome length and diameter $plant^{-1}$) during both the years, that is, 2004-05 and 2005-06. Whereas, there were no significant differences between varieties of

**Table 1.** Influence of different series of inter cropping on biometric performance of rice cultivars (IR-64, MTU – 7029) and sweet flag planted at different spacing.

| Treatments (A) | Varieties (B) | 2004-05 | | | 2005-06 | | | Pooled mean | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | No. of effective tillers | Rhizome length (cm) | Rhizome diameter (cm) | No. of effective tillers | Rhizome length (cm) | Rhizome diameter (cm) | No. of effective tillers | Rhizome length (cm) | Rhizome diameter (cm) |
| Paddy alone | IR 64 | 12.06 | - | - | 10.33 | - | - | 11.19 | - | - |
| | Swarna | 11.60 | - | - | 10.33 | - | - | 10.96 | - | - |
| Sweet flag + Paddy (mixed) | IR 64 | 14.00 | 51.59 | 1.20 | 10.73 | 48.03 | 1.06 | 12.36 | 49.81 | 1.13 |
| | Swarna | 17.40 | 51.86 | 1.20 | 10.73 | 39.40 | 1.31 | 14.06 | 48.80 | 1.25 |
| Sweet flag + Paddy at 20 cm | IR 64 | 12.73 | 57.60 | 1.26 | 15.40 | 52.03 | 0.96 | 14.06 | 54.81 | 1.11 |
| | Swarna | 12.53 | 58.20 | 1.25 | 15.40 | 52.50 | 0.98 | 13.96 | 52.18 | 1.11 |
| Sweet flag + Paddy at 30 cm | IR 64 | 19.42 | 64.00 | 1.42 | 15.90 | 62.20 | 1.74 | 17.66 | 63.10 | 1.58 |
| | Swarna | 18.46 | 71.33 | 1.43 | 17.40 | 60.79 | 1.33 | 17.93 | 66.06 | 1.38 |
| Sweet flag + Paddy at 40 cm | IR 64 | 22.86 | 73.26 | 1.56 | 22.60 | 67.26 | 1.92 | 22.73 | 70.26 | 1.74 |
| | Swarna | 27.00 | 71.33 | 1.59 | 22.20 | 69.40 | 1.55 | 24.60 | 66.33 | 1.57 |
| Sweet flag alone | - | - | 91.40 | 1.86 | - | 69.40 | 1.86 | - | 80.40 | 1.86 |
| | - | - | 89.20 | 1.84 | - | 70.67 | 1.88 | - | 79.93 | 1.86 |
| C.D. at 5% A | | 6.34 | 9.04 | 0.126 | 2.36 | 3.07 | 0.408 | 5.39 | 7.60 | 0.341 |
| B | | NS | NS | NS | NS | 2.98 | NS | NS | NS | NS |
| AB | | NS | 11.93 | NS | NS | NS | NS | NS | 11.08 | NS |
| CV % | | 13.67 | 10.04 | 4.39 | 8.95 | 6.42 | 16.84 | 11.81 | 8.68 | 12.30 |

rice except in one attribute, that is, rhizome length plant$^{-1}$ of sweet flag. Numbers of tillers were in significantly increasing order with widening the row space between sweet flag and rice and registered 103.14 and 124.45% increase at 40 cm row spacing in IR-64 and Swarna, respectively. Similarly, rhizome length and rhizome diameter of sweet flag were also in increasing order and maximum at 40 cm spacing. The increment in tillers number due to increased spacing was related to the availability of more light and space through intercropping than sole crop. Whereas, number of tillers of rice in rice + sweet flag (mixed) were at par with sole crop of rice (Table 1, Figures 1 and 2). In contrast, rhizome length and rhizome diameter of sweet flag were reduced drastically in additive series (rice + sweet flag mixed) as well as closer spacing of replacement series (20 cm) (Figures 3 to 5). Above observations indicate that rice crop significantly affected the growth of sweet flag not leaving space for sweet flag to grow specially in closer spacing and intra row spacing. Sweet flag grown as sole crop had significantly higher in mean rhizome length (80.16 cm plant$^{-1}$) and diameter (1.86 cm plant$^{-1}$) compared to other treatments of intercropping. There was 62.59 and 17.38% reduction in rhizome length of sweet flag was noticed when planted with in intra row space and inter row space of 40 cm respectively compared to sole crop of sweet flag (Figure 5).

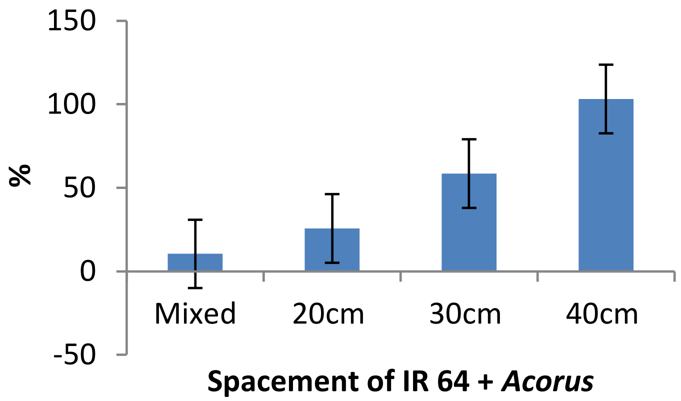


**Figure 1.** Percent increase in tiller number of IR 64 than monocrop.

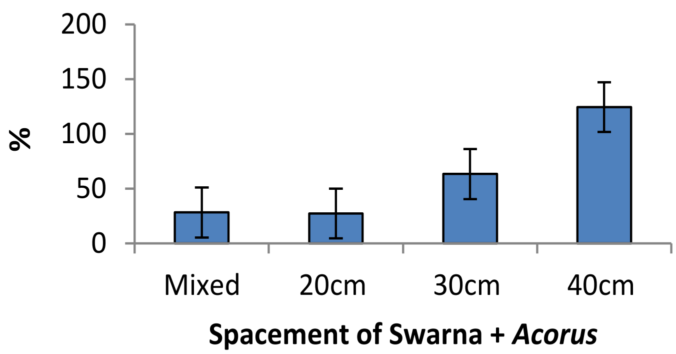


**Figure 2.** Percent increase in tiller number of Swarna than monocrop.

Similarly, sweet flag planted with rice in intra row space and inter row space of 40 cm had reduction of 56.30 and 18.18%, respectively in rhizome diameter (Figures 7 to 8).

The reason for reduction in rhizome length and diameter of sweet flag intercropped with rice might be due to faster vegetative growth of rice than sweet flag. Tiwari et al. (2011) reported that that cultivation of Sweet flag and Bramhi alone appeared to be more suitable than intercropped with rice. Intercropping with early and late cultivars of rice did not have significant effect on yield components of sweet flag.

### *Grain yield and rhizome yield q ha$^{-1}$*

The results on the effect of different series of intercropping on yield and equivalent yield of sweet flag grown as intercrops with early (IR-64) and late “Swarna” (MTU- 7029) varieties of rice are presented in Tables 2 and 3 and Figures 9 to 11. Yield data of both years indicate that, sweet flag planted in intra row space (rice + sweet flag mixed) did not affect yield of rice whereas, rhizome yield of sweet flag was affected significantly.

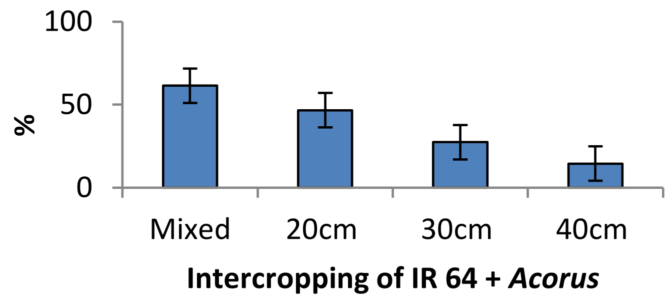


**Figure 3.** Percent decrease in rhizome length of *Acorus* compared with monocropped *Acorus.*

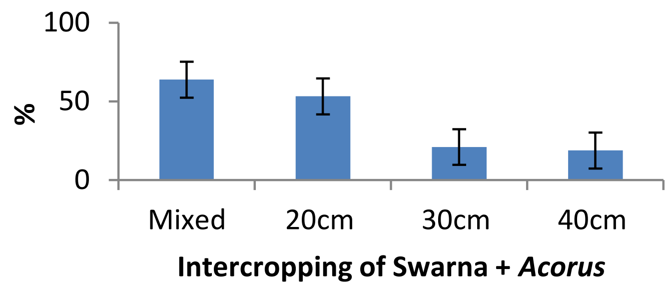


**Figure 4.** Percent decrease in rhizome length of *Acorus* compared with monocrop.

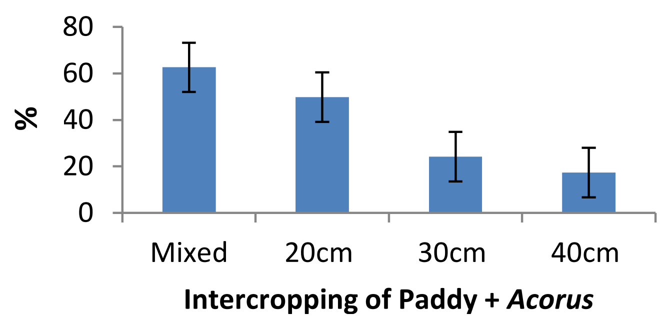


**Figure 5.** Percent decrease in mean rhizome length of both the paddy varieties compared to monocropped *Acorus.*

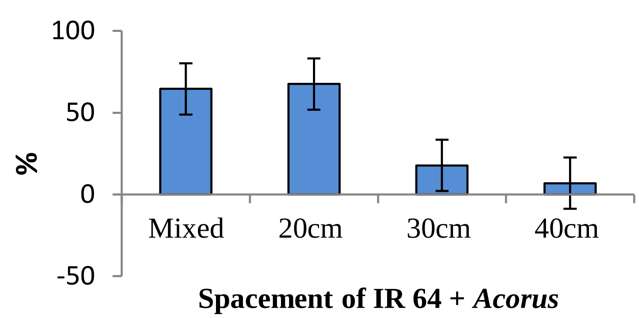


**Figure 6.** Percent decrease in rhizome diameter of *Acorus* grown with IR-64 + *Acorus*.

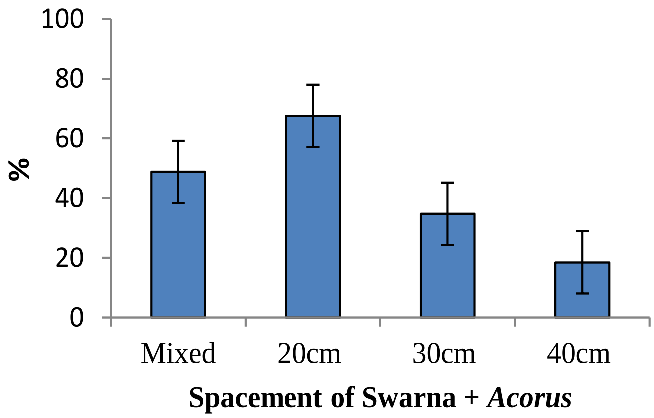


**Figure 7.** Percent decrease in rhizome diameter of *Acorus* grown with Swarna + *Acorus* than monocropped *Acorus.*

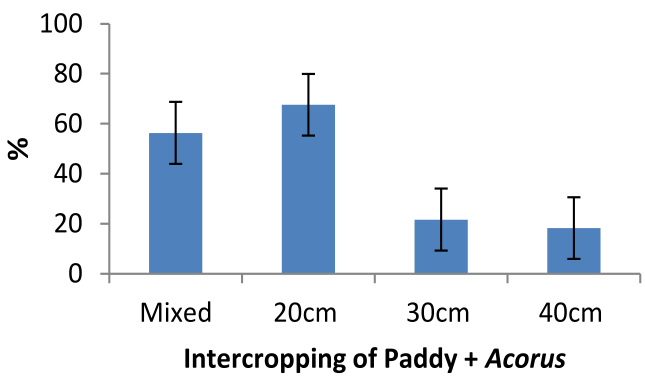


**Figure 8.** Percent reduction in mean rhizome diameter of both the paddy varieties compared to monocropped *Acorus.*

Sole crops of rice and sweet flag harvested significantly higher grain yield and rhizome yield respectively compare to different series of intercropping.

Sole cropping of IR-64 and Swarna yielded highest grain yield of 96.46 and 121.20 q ha$^{-1}$, respectively (Figure 9) but found to be at par with grain yield of rice grown in additive series of inter cropping (sweet flag + rice mixed). Whereas, both varieties of rice planted with sweet flag at spacing of 20, 30 and 40 cm had incremental decrease in grain yield (Figure 10). The maximum reduction of 246.46 and 237.51% in grain yield was recorded in varieties IR-64 and MTU- 7029, respectively under replacement series of intercropping with sweet flag at 40 cm spacing compare to sole crop of both varieties. The widening the gap between rows was resulted into declining in the paddy yield could be attributed to reducing plant density (Gabatshele et al*.,* 2012).

Sole crop of sweet flag had highest rhizome yield (70.26 q ha$^{-1}$), whereas same planted in intra row space of rice varieties IR-64 and MTU-7029 gave lowest

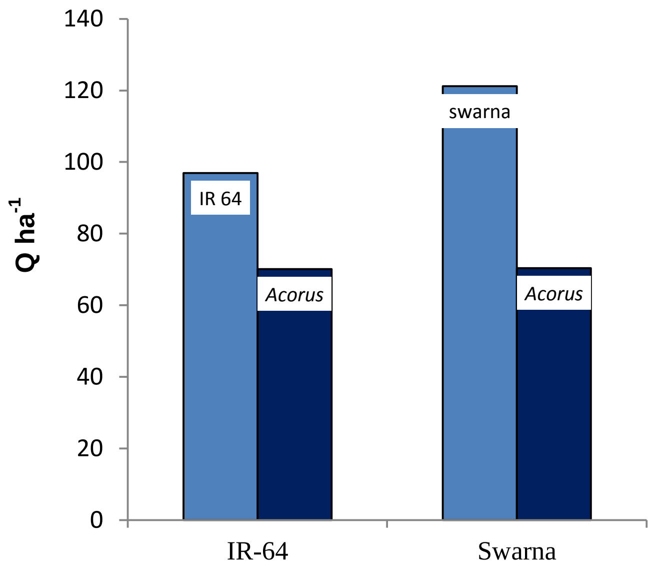


**Figure 9.** Actual yield (Q ha$^{-1}$) of IR-64, Swarna and Rhizome yield of *Acorus* grown as monocrops.

rhizome yield of 21.61 and 21.63 q ha$^{-1}$, respectively (Figures 9 to 11). There was incremental increase in rhizome yield of sweet flag planted with rice at 1:1 at 20 cm, 30 cm and 40 cm distance. Sweet flag planted at 40 cm distance had higher rhizome yield despite having less plant population compare to 20 and 30 cm distance of planting. The rhizome yield was improved significantly with the increase in spacing under both the paddy varieties. This indicate that the wider spacing provided enough space for sweet flag to have higher nutrient uptake, because their roots could reach far and deep without much competition (Lyocks et al., 2013). There was an increase in rhizome yield of sweet flag planted with IR 64 compare to MTU-7029. However, intercropping of sweet flag with early and late cultivars of rice did not give significant difference in rhizome yield of sweet flag.

It is clear from the above findings that rice yield was declined with increasing space between rice and sweet flag, while rhizome yield was in increasing trend with increasing spacing from 20 to 40 cm. This suggests that over shading of sweet flag by increasing growth of rice plants significantly affected rhizome yield at closer spacing. While, at wider spacing sweet flag plants had higher percentage of space, light and nutrient for better development of rhizome through efficient absorption of soil nutrients. This result is in agreement with earlier findings of Lyocks et al. (2013) who reported low yield of ginger in maize ginger intercrop occurred at closer spacing.

### *Equivalent yield to rice*

Equivalent yield (EQY) to rice obtained from different

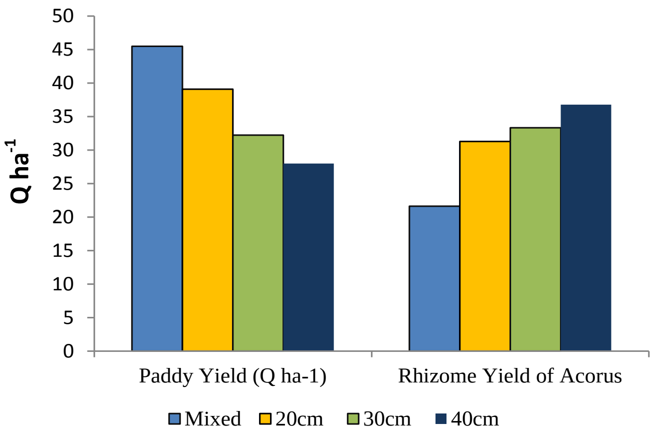


**Figure 10.** Actual yield of IR-64 and *Acorus* intercropped at different spacing.

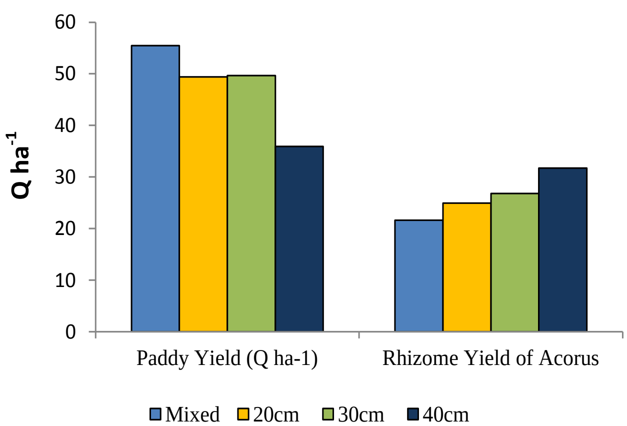


**Figure 11.** Actual yield of Swarna and *Acorus* intercropped at different spacing.

series of intercropping and sole cropping indicated that during both years, most of the treatments of intercropping had higher equivalent yield compare to sole crop of both varieties of rice grown for two successive crop seasons (Tables 2 and 3, Figure 13). However, EQY recorded from different treatments of intercropping with early and late cultivars of rice was at par with each other.

Sweet flag planted as sole crop fetched highest mean equivalent yield of 234.19 q ha$^{-1}$ compared to other treatments. Sweet flag intercropped with early variety (IR 64) gave higher equivalent yield compare to late variety (Swarna). An increase in equivalent yield of 21.18 and 54.38% was recorded when sweet flag was intercropped with IR 64 in intra row space and at 40 cm inter row space respectively compared to early rice variety (Figure 12).

Similar results were also obtained when sweet flag was intercropped with MTU-7029 (Figure 13). The intercropping is biologically more efficient than the sole crops due to enhanced land equivalent ratios which would be required to produce the same amount of paddy in sole culture.

Higher EQY from different series of intercropping indicated the possibility of intercropping of sweet flag with rice preferably with early cultivars at wider spacing (30–40 cm) and allowed to grow for one season later on after rice harvest. Highest equivalent yield of sweet flag alone (sole crop) indicated that sweet flag (*A. calamus*) could be a better substitute to rice crop under submerged as well as under aerobic conditions. Continuous cultivation of rice on same field adversely affected the soil health therefore crop rotation with sweet flag would be a very effective alternative under rice ecosystem.

### *Economics and cost benefit ratio*

Data presented in Table 4 indicated that gross return and net profit incurred more from all series of intercropping compared to rice as sole crop irrespective of both early and late maturing cultivars of rice. Whereas, sweet flag planted as sole crop incurred highest gross return (Rs 281028.00 ha$^{-1}$) and net profit (Rs 219528.00 ha$^{-1}$) among different treatments. However, cost–benefit ratio was highest under intercropping of rice + sweet flag planted at 40 cm spacing with IR 64 (1:4.83) and MTU-7029 (1:4.58) both cultivars followed by sweet flag alone (1:4.56). Sweet flag intercropped with rice in intra row space had lowest (1:2.2 – 1:2.39) cost–benefit ratio. Higher cost of cultivation under rice + sweet flag intercropping system was mainly due to higher input cost of planting material of sweet flag as well as cumulative cost of cultivation of both crops. Whereas, intercropping of sweet flag at wider spacing effectively reduced the cost of planting material of sweet flag. Moreover, sweet flag intercropped with either rice at wider spacing (40 cm) or planted as sole crop had given higher net return and cost- benefit ratio.

## Experiment 2

### *Grain yield and rhizome yield q ha$^{-1}$*

Results of second experiment conducted on effect of different series of intercropping with varying crop cycles are presented in Tables 4 and 5. Grain yield of rice and rhizome yield of sweet flag recorded from different treatments during both the years indicated that sweet flag intercropped with rice gave higher rhizome yield when it was grown as sole crop for a period of one year after rice harvest than 1.5 year of crop cycle.

Higher grain yield of rice sole crop grown for two (109.58 q ha$^{-1}$) and three (169.82 q ha$^{-1}$) successive crop seasons was recorded during both years of experimentation compared to rice grown with sweet flag either as additive series of intercropping (rice + sweet flag

**Table 2.** Grain yield of rice cultivars (IR-64 and MTU – 7029), rhizome yield of sweet flag and equivalent yield of rice as influenced by different series of inter cropping.

| Intercropping (A) | Varieties (B) | 2004-05 Actual yield (Q ha$^{-1}$) Paddy | 2004-05 Actual yield (Q ha$^{-1}$) Sweet flag | 2004-05 Equivalent yield rice (q ha$^{-1}$) | 2005-06 Actual yield (Q ha$^{-1}$) Paddy | 2005-06 Actual yield (Q ha$^{-1}$) Sweet flag | 2005-06 Equivalent yield rice (Q ha$^{-1}$) |
|---|---|---|---|---|---|---|---|
| Rice alone | IR 64 | 100.03 | - | 100.03 | 93.83 | - | 93.83 |
| | Swarna | 123.76 | - | 123.76 | 118.64 | - | 118.64 |
| Sweet flag + Rice (mixed) | IR 64 | 44.03 | 17.87 | 103.40 | 46.89 | 25.35 | 131.89 |
| | Swarna | 56.41 | 17.21 | 113.70 | 54.49 | 26.06 | 141.38 |
| Sweet flag + Rice at 20 cm | IR 64 | 36.11 | 30.06 | 136.32 | 42.01 | 32.49 | 150.32 |
| | Swarna | 50.55 | 24.86 | 133.42 | 48.21 | 24.99 | 131.35 |
| Sweet flag + Rice at 30 cm | IR 64 | 31.79 | 34.53 | 146.89 | 32.63 | 32.13 | 139.75 |
| | Swarna | 44.73 | 26.34 | 132.53 | 54.63 | 27.25 | 145.47 |
| Sweet flag + Rice at 40 cm | IR 64 | 22.51 | 34.66 | 134.32 | 33.44 | 38.44 | 161.58 |
| | Swarna | 31.32 | 33.76 | 143.88 | 40.51 | 29.75 | 139.78 |
| Sweet flag alone | IR 64 | - | 79.54 | 265.14 | - | 60.70 | 202.36 |
| | Swarna | - | 77.10 | 257.00 | - | 63.70 | 212.25 |
| CD at 5% A | | | | 20.64 | | | 20.70 |
| B | | | | NS | | | NS |
| AB | | | | 12.49 | | | 8.60 |

mixed) or as replacement series (1:1). Besides, there was three to five folds reduction in grain yield of rice intercropped with different series of intercropping. Rhizome yield of sweet flag recorded from different treatments indicated that sole crop of sweet flag gave higher rhizome yield either with crop cycle of one year (67.30 q ha$^{-1}$) or 1.5 years (104.35 q ha$^{-1}$) compared to intercropping for one year (32.48 q ha$^{-1}$) or 1.5 years (65.90 q ha$^{-1}$). Moreover, crop cycle of 1.5 years found to be most suitable for optimizing the rhizome yield of sweet flag (Table 5). Planting of sweet flag in intra row space (rice + sweet flag mixed) of rice gave higher rhizome yield (37.10 q ha$^{-1}$) compared to planting of sweet flag (27.87 q ha$^{-1}$) in inter row space (rice + sweet flag 1:1).

***Equivalent yield to rice***

Equivalent yield was calculated based on rhizome yield of sweet flag grown as sole crop as well as intercrop with rice for a period of 1 and 1.5 years and presented in Table 5. Results indicated that equivalent yield of sweet flag alone taken either a period of one year (223.97 q ha$^{-1}$) and 1.5 years (347.83 q ha$^{-1}$) gave significantly higher equivalent over other treatments. Sweet flag intercropped with rice either in additive series (rice + sweet flag mixed) or in replacement series (rice + sweet flag 1:1) had produced significantly higher EQY than rice sole crop which was grown for two (109.58 q ha$^{-1}$) and three (169.82 q ha$^{-1}$) successive

**Table 3.** Grain yield of Rice cultivars (IR-64 and MTU – 7029), rhizome yield of sweet flag and equivalent yield of rice as influenced by different series of inter cropping (pooled mean two years).

| Intercropping series Treatments (A) | Treatment (B) Varieties | | | | | |
|---|---|---|---|---|---|---|
| | IR-64 | | | MTU – 7029 | | |
| | Rice (Q $ha^{-1}$) | Sweet flag (Q $ha^{-1}$) | Equivalent yield of rice (Q $ha^{-1}$) | Rice (Q $ha^{-1}$) | Sweet flag (Q $ha^{-1}$) | Equivalent yield of rice (Q $ha^{-1}$) |
| Rice alone | 96.93 | - | 96.93 | 121.20 | - | 121.20 |
| Sweet flag + Rice (mixed) | 45.46 | 21.61 | 117.40 | 55.49 | 21.63 | 127.58 |
| Sweet flag + Rice at 20 cm | 39.06 | 31.27 | 143.32 | 49.38 | 24.93 | 132.47 |
| Sweet flag + Rice at 30 cm | 32.21 | 33.31 | 143.32 | 49.68 | 26.79 | 139.00 |
| Sweet flag + Rice at 40 cm | 27.97 | 36.75 | 149.65 | 35.91 | 31.75 | 141.83 |
| Sweet flag alone | - | 70.12 | 233.75 | - | 70.40 | 234.63 |
| CD at 5%, B, AB | A - 23.70 B – NS AB - 10.75 | | | | | |

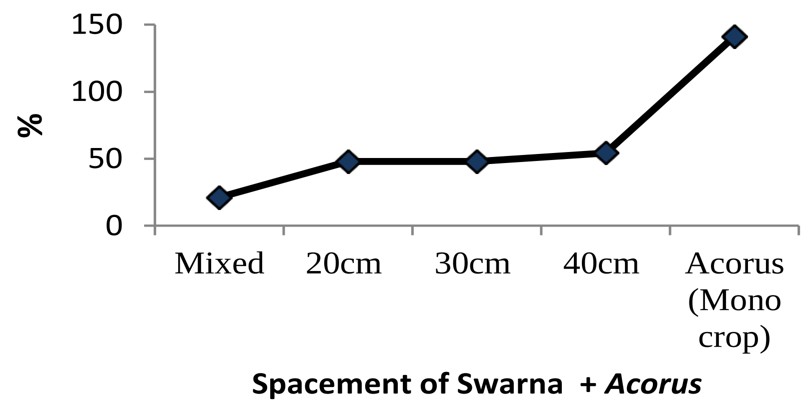


**Figure 12.** Percent increase in EQY of IR 64 + *Acorus* intercropped at different spacing compared to EQY of sole IR-64.

cropping seasons. However, EQY of sweet flag intercropped with rice in additive series (rice + sweet flag mixed) with a crop cycle of one year (165.76 q $ha^{-1}$) and 1.5 years (268.06 q $ha^{-1}$) was at par with EQY of sweet flag intercropped with rice in replacement series (132.43 and 253.23 q $ha^{-1}$). Moreover, intercropping of sweet flag and rice with a crop cycle of 1.5 years produced significantly higher equivalent yield (Figure 14) than crop cycle of one year.

***Economics and cost benefit ratio***

Data presented in Table 6 and Figure 15 indicated the significant effect of intercropping and crop cycle period on cost of cultivation, gross return,

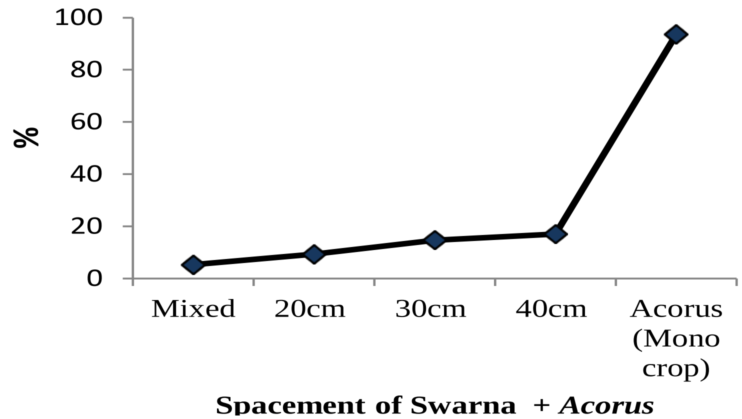


**Figure 13.** Percent increase in EQY of Swarna + *Acorus* intercropped at different spacing compared to EQY of sole Swarna.

**Table 4.** Economics of different series of intercropping of sweet flag with rice (mean of two years).

| Intercropping | Varieties | Equivalent yield to rice (q ha$^{-1}$) | Cost of cultivation (Rs ha$^{-1}$) | Gross return (Rs ha$^{-1}$) | Net return (Rs ha$^{-1}$) | Cost–Benefit ratio |
|---|---|---|---|---|---|---|
| Rice alone | IR 64 | 96.93 | 48000.00 | 116316.00 | 68316.00 | 1:2.42 |
| | Swarna | 121.20 | 48000.00 | 145440.00 | 97440.00 | 1:3.03 |
| Sweet flag + Rice (mixed) | IR 64 | 117.40 | 64000.00 | 140880.00 | 76880.00 | 1:2.20 |
| | Swarna | 127.58 | 64000.00 | 153096.00 | 89096.00 | 1:2.39 |
| Sweet flag + Rice at 20 cm | IR 64 | 143.32 | 52750.00 | 171984.00 | 119234.00 | 1:3.26 |
| | Swarna | 132.47 | 52750.00 | 158964.00 | 106214.00 | 1:3.01 |
| Sweet flag + Rice at 30 cm | IR 64 | 143.32 | 42300.00 | 171984.00 | 129684.00 | 1:4.06 |
| | Swarna | 139.00 | 42300.00 | 166800.00 | 124500.00 | 1:3.94 |
| Sweet flag + Rice at 40 cm | IR 64 | 149.65 | 37125.00 | 179580.00 | 142455.00 | 1:4.83 |
| | Swarna | 141.83 | 37125.00 | 170196.00 | 133071.00 | 1:4.58 |
| Sweet flag alone | | 234.19 | 61500.00 | 281028.00 | 219528.00 | 1:4.56 |

*Prevailing market rates:* Paddy – Rs 1200 q$^{-1}$, Sweet flag – Rs 4000 q$^{-1}$.
*Input cost:*
Planting material: Sweet flag: Rs 40000.00/ha.
Rice: Rs 2500.00/ha.
Cultivation cost for sweet flag and rice: Rs 21500.00/ha.

**Table 5.** Comparative evaluation of actual and equivalent yield of paddy and Sweet flag as influenced by intercropping for one year and one and half year of crop cycles.

| Treatments (A) | 2006-07 Actual Yield (q ha$^{-1}$) Sweet flag Rice | 2006-07 EQY of rice (q ha$^{-1}$) | 2007-08 Actual yield (q ha$^{-1}$) Sweet flag Rice | 2007-08 EQY of rice (q ha$^{-1}$) | Pooled mean EQY of rice (q ha$^{-1}$) |
|---|---|---|---|---|---|
| Rice for two crop seasons (1 year) | - 104.57 | 104.57 | - 114.59 | 114.59 | 109.58 |
| Rice for three crop seasons (1.5 year) | - 162.37 | 162.37 | - 177.27 | 177.27 | 169.82 |
| Rice + Sweet flag 1:1 (1 year) | 30.16 38.88 | 139.42 | 25.58 40.31 | 125.57 | 132.43 |
| Rice + Sweet flag 1:1 (1.5 year) | 65.65 35.88 | 254.62 | 63.75 39.20 | 251.70 | 253.23 |
| Rice + Sweet flag mixed (1 year) | 41.00 41.84 | 178.50 | 33.20 42.31 | 152.97 | 165.76 |
| Rice + Sweet flag Mixed (1.5 year) | 69.58 44.69 | 276.64 | 64.63 44.02 | 259.45 | 268.06 |
| Sweet flag sole for two crop seasons (1 year) | 67.28 - | 224.26 | 67.32 - | 223.69 | 223.97 |
| *Acorus* sole for three crop seasons (1.5 year) | 100.83 - | 336.10 | 107.87 - | 359.56 | 347.83 |
| C.D. at 5% | | 37.33 | | 47.03 | 47.91 |
| CV% | | 11.33 | | 9.74 | 10.94 |

*Market Price:* Sweet flag @ Rs 4000/q, Paddy @ Rs 1200 /q.

**Table 6.** Economics of different series of intercropping of sweet flag with rice (mean of two years).

| Intercropping | Equivalent yield to rice (q ha$^{-1}$) | Cost of Cultivation (Rs ha$^{-1}$) | Gross return (Rs ha$^{-1}$) | Net return (Rs ha$^{-1}$) | Cost-Benefit ratio |
|---|---|---|---|---|---|
| Rice for two crop seasons (1 year) | 109.58 | 48000.00 | 131496.00 | 83496.00 | 1:2.73 |
| Rice for three crop seasons (1.5 year) | 169.82 | 72000.00 | 203784.00 | 131784.00 | 1:2.83 |
| Rice + Sweet flag 1:1 (1 year) | 132.43 | 52750.00 | 158916.00 | 104166.00 | 1:2.90 |
| Rice + Sweet flag 1:1 (1.5 year) | 253.23 | 57750.00 | 303876.00 | 246126.00 | 1:5.26 |
| Rice + Sweet flag mixed (1 year) | 165.76 | 64000.00 | 198912.00 | 134912.00 | 1:3.10 |
| Rice + Sweet flag Mixed (1.5 year) | 268.06 | 69000.00 | 321672.00 | 252672.00 | 1:4.66 |
| Sweet flag sole for two crop seasons (1 year) | 223.97 | 61500.00 | 268764.00 | 204264.00 | 1:4.37 |
| *Acorus* sole for three crop seasons (1.5 year) | 347.83 | 66500.00 | 417396.00 | 350896.00 | 1: 6.27 |

*Prevailing market rates:* Paddy – Rs 1200 q$^{-1}$, Sweet flag – Rs 4000 q$^{-1}$.
*In put cost:*
Planting material: Sweet flag: Rs 40000.00/ha.
Rice: Rs 2500.00/ha.
Cultivation cost for sweet flag and rice: Rs 21500.00/ha.

net profit and cost benefit ratio. Maximum gross return of Rs 417396.00 ha$^{-1}$ and net profit of Rs 350896.00 ha$^{-1}$ incurred from sweet flag taken as sole crop for a period of 1.5 years. All series of intercropping with crop cycle of 1.5 years were superior to crop cycle of 1 year in terms of getting higher net profit. Cost of cultivation was found to be highest in rice sole crop taken for three consecutive seasons (Rs 72000.00) due to three times addition of cultivation cost. Sweet flag intercropped with rice as either intercrop (rice + sweet flag @ 1:1) or planted in intra row space for

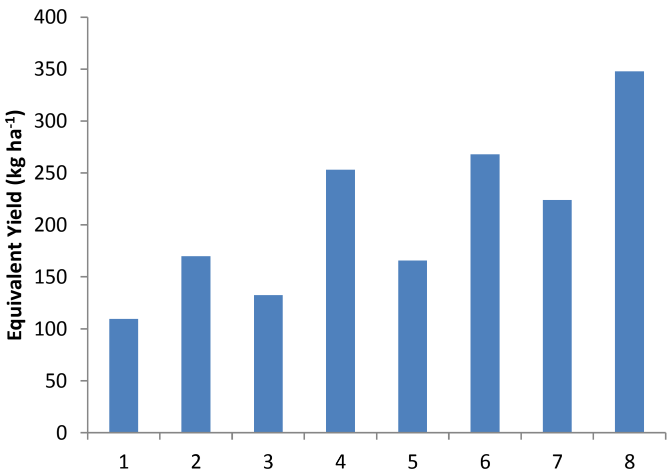


**Figure 14.** EQY (kg ha$^{-1}$) of rice intercropped with *Acorus* in 1 and 1.5 years crop cycles. Treatments :- 1 - Rice 1 Year; 2 - Rice 1.5 years; 3 - Rice+ *Acorus* (1:1) 1 year; 4 - Rice +*Acorus* (1:1) 1.5 years; 5 - Rice+*Acorus* (Mixed) 1 year; 6 - Rice+*Acorus* (Mixed 1.5 years; 7 - *Acorus* sole 1 year; 8 - *Acorus* sole 1.5 years.

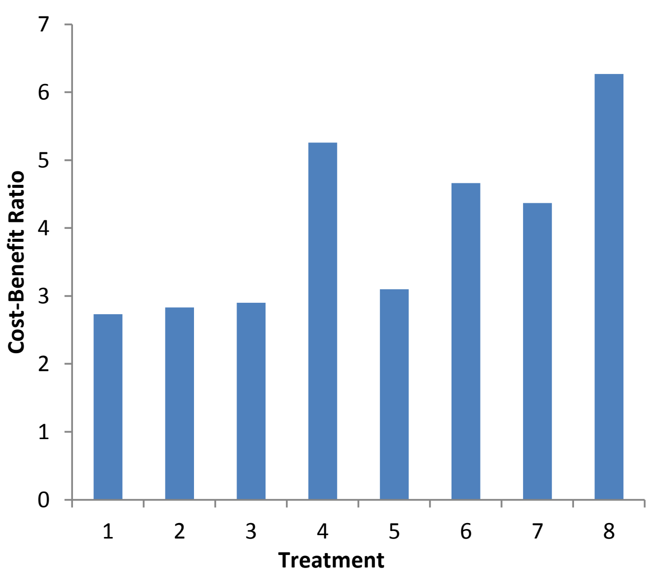


**Figure 15.** Cost-Benefit ratio of different series of intercropping of sweet flag with rice in 1 and 1.5 years of crop cycles. Treatments: 1 - Rice 1 Year, 2 - Rice 1.5 years, 3 - Rice+ *Acorus* (1:1) 1 year, 4 - Rice +*Acorus* (1:1) 1.5 years, 5 - Rice+*Acorus* (Mixed) 1 year, 6 - Rice+*Acorus* (Mixed 1.5 years, 7 - *Acorus* sole 1 year, 8 - *Acorus* sole 1.5 years.

a period of 1.5 years had given similar amount of net profit despite that gross return and net profit incurred more from all series of intercropping compared to rice as sole crop.

## Conclusion

The intercropping of *Acorus* with both paddy varieties propounded higher yield and EQY compared to mono cropping of paddy varieties under test. The spacing between intercrops was the main factor affects all parameters of plant and yield of the both intercrops and in present study 40 cm spacing was found best in terms of yield and equivalent yield of paddy and *Acorus*. The monocropping of *Acorus* either one year or 1.5 years cropping system rendered the higher yield and equivalent yield among their categories. Thus as compared to sole cropping of paddy, intercropping of *Acorus* can be more profitable to farmers due to higher equivalent yield and income.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Combining ability analysis of quality protein maize (QPM) inbred lines for grain yield, agronomic traits and reaction to grey leaf spot in mid-altitude areas of Ethiopia

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**A study was carried out to study the combining ability effects of diallel cross hybrids for grain yield, agronomic traits and reaction to grey leaf spot (GLS). Forty five experimental diallel cross hybrids made from ten quality protein maize (QPM) inbred lines with varying level of resistance to GLS were evaluated along with three checks at Bako and Jima Research Centers during 2014/2015 cropping season. Analysis of variance showed significant variation (P≤0.05) among genotypes and between environments. This depicted the existence of genetic variation among genotypes in all studied traits. Mean squares characterized by general and specific combining ability effects were significant for the most traits and this suggested that both additive and non-additive gene actions have the contribution in the expression of the traits. However, the ratio of General combining ability (GCA) to specific combining ability (SCA) sum of squares were greater than unity, this revealed that there was preponderance of additive gene action in the expression of all the traits under study. Among inbred lines studied P6 and P10 had a desirable GCA effects for grain yield whereas P1, P4 and P10 were the best general combiners for GLS resistance. Furthermore, P10 was identified as good general combiner for grain yield as well as GLS is therefore recommended to be used in breeding programs with a purpose of developing both high yielder as well as GLS disease resistant genotypes. Crosses, P2×P8 and P4×P6 showed the most desirable performances and SCA effects for grain yield. Grain yield showed positive correlation between ear per plant, plant and ear height, and ear position whereas negative correlation were observed with GLS, days to anthesis, days to silking, plant aspect and ear aspect traits. The information which is generated in this study could be helpful to develop high yielding maize varieties with good agronomic traits as well as GLS resistance.**

**Key words:** Agronomic traits, diallel, general combining ability, gray leaf spot, quality protein maize, specific combining ability.

## INTRODUCTION

Maize [*Zea mays* (L.) 2n=2x=20] is one of the major cereal crops in terms of production and productivity in

Ethiopia. It grows on 2.1 million hectares of land with total production of 7.2 million tonnes annually with the national average productivity of 3.42 tonnes per hectare (CSA, 2015). Maize is considered as a widely adaptable crop that is grown over a range of agro-climatic zones and suitable for diverse environments and can grow up to 2,600 masl from moisture deficit semi-arid lowlands, mid-altitude and highlands to moisture surplus areas in the humid lowlands, mid-altitudes and highlands of the country (Legesse et al., 2012).

In the developing countries particularly in Africa, South America and Asia, people consume maize as an important staple food and derive their protein and calories requirements from it (Prasanna et al., 2001). However, naturally maize has protein deficiency of the two essential amino acids of lysine and tryptophan. The opaque-2 gene which is responsible for the enhancement of lysine and tryptophan can be incorporated into any population, inbred lines or any other germplasm of maize through modified backcrossing recurrent selection program (Vasal et al., 1980). The modified opaque-2 maize which had hard endosperm and vitreous kernels is known as quality protein maize (QPM). Hence, nutritional superiority over normal maize is well established. Therefore, development of quality QPM maize would greatly reduce malnutrition problems of subsistence and resource poor farmers and low-income people that depend on maize as their staple food (Leta et al., 2003). Despite some efforts made so far to improve quality of maize (Twumasi et al., 2012), enhancing yield potential with having good agronomic trait and disease resistant QPM maize varieties are still the major problems that needs attention in Ethiopia.

Disease is the most important production challenge and creates a series of problems from year to year in the most part of maize growing agro ecologies of the country. Around 65 maize diseases have been recorded in Ethiopia (Tewabech et al., 2012). Among these diseases, gray leaf spot (GLS) caused by *Cercospora zeae-maydis* is the most important threat to maize production in the country (Dagne et al., 2001) and can create yield reduction of up to 37% in susceptible varieties under artificial disease pressure (Dagne et al., 2004). The most recommended control measure of the disease in maize is the use of relatively resistant or tolerant cultivars (Daniel et al., 2008). Hence, use of host plant resistance (HPR) is considered as the best option to alleviate GLS disease pressure. Identification of inbred lines which are high yielding as well as GLS resistant is a pre option to solve the problem.

Combining ability could help to provide information in the selection of inbred lines and desirable cross combination (Falconer and Mackay, 1996). The general combining ability (GCA) helps to evaluate the contribution of an inbred line to the hybrid performance and population improvement, whereas specific combining ability (SCA) is utilized to identify cross combination with superior performance. GCA indicates the additive gene action whereas SCA indicates non-additive gene actions mainly a function of dominance effects (Sprague and Tatum, 1942). So far, combining ability studies of QPM inbred lines conducted by the Gudeta (2007) and Demissew (2014) indicated both GCA and SCA effects to be significant and important for grain yield and most other studied traits. However, Melkamu et al. (2013) reported the preponderance of SCA or non-additive gene action for grain yield and most other studied traits. In Ethiopia studying combining abilities effects for newly developed QPM inbred lines to grain yield, agronomic traits and reaction to disease have been limited. This study was, therefore, undertaken to estimate magnitude of combining ability effects and the relationship of grain yield, agronomic and GLS traits.

## MATERIALS AND METHODS

### Description of the study areas

The experiment was conducted during the 2014/2015 main season at Bako National Maize Research Center (BNMRC) and Jima Agricultural Research Center (JARC). Bako National Maize Research Center is located in East Wellega Zone of the Oromia National Regional State, Western Ethiopia at an altitude of 1650 m above sea level (masl). It lies between 9°06' North latitude and 37°09' East longitude in the sub-humid agro-ecology of the country. During the experimental season, the rainy season covered the period from May to October and maximum rainfall is received in the months of July and August and the average annual rainfall was 944.4 mm. The mean, minimum and mean maximum temperature was 12.3 and 29.8°C, respectively. The soil is classified under the Nitosol order. Naturally, this area is exposed to high maize foliar disease pressure and mostly used as a hot spot area to screen new maize genotypes for the most foliar diseases.

Jima Agricultural Research Center is located in Oromia Regional State South West part of Jima Zone at an altitude of 1750 m above sea level (masl). It lies between 7°46' North latitude and 36°00' East longitude in the sub-humid agro-ecology of the country. During the experimental season, the average annual rainfall is 1536 mm. The mean minimum and mean maximum temperature is 11.2°C and 25.9°C respectively. It is the most recommended area to evaluate new maize genotypes for GLS since it is a hot spot area for the most foliar diseases.

### Experimental materials

The inbred lines used in this study were obtained from Bako National Maize Research Center, Maize Protection Research Section. Based on per se performance evaluation of GLS and other

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**Table 1.** List of parental QPM inbred lines used to generate the single cross hybrids using half diallel mating design.

| Inbred lines’ code | Pedigree | Source of inbred lines | Reaction to GLS |
|---|---|---|---|
| P1 | [CML144/Kuleni(F2)]-8-1-1-2-1-1-1 | BNMRC | R |
| P2 | CML144 | CIMMYT | S |
| P3 | CML149 | CIMMYT | S |
| P4 | [CML176/Kuleni(F2)]-4- 3-1-1-1 | BNMRC | MR |
| P5 | [CML144/144-7-b (F2)] 4-2-1-1-1-1-1 | BNMRC | R |
| P6 | CML491 | CIMMYT | S |
| P7 | CML502 | CIMMYT | S |
| P8 | CML159 | CIMMYT | S |
| P9 | CML165 | CIMMYT | MR |
| P10 | [CML142/144-7-b(F2)]-9-2-2-1-1-1-1 | BNMRC | R |

BNMRC = Bako National Maize Research Center; MR = Moderately resistant; R = Resistant; S = Susceptible; QPM = Quality protein maize; NM= Normal maize. Disease severity score based on 1 - 5 scale, R = 1 .0 - 2.0; MR = 2.0 - 2.5; S= 2.5-3.0; HS = 3.1- 5.

agronomic traits, ten inbred lines were selected (Table 1). The lines were categorized as resistant, moderately resistant, susceptible and highly susceptible. Selected lines were crossed in a 10×10 half-diallel mating design at Bako National Maize Research Center during 2014 cropping season and three commercial check hybrids (BHQPY545, BH546 and BH547) were used in the trials. All these checks are widely adapted in mid-altitude agro-ecologies. BHQPY545 is a popular quality protein maize hybrid whereas BH546 and BH547 are recently released high yielder non quality protein maize hybrids.

### Experimental procedure

The 45 $F_1$ progenies derived from the diallel cross of 10 selected inbred lines along with three commercial hybrid checks (BHQPY545, BH546 and BH547) were planted in 6 × 8 alpha-lattice designs with two replications at Bako and Jima research centers. There were two rows per plot with row length of 5.1 m spaced 0.75 m apart and plant-to-plant distance of 0.3 m. At planting, two seeds were planted per hill, and then thinned to one plant per hill after two weeks of emergence to attain a population density of 44,444 plants per hectare. Nitrogen ($N_2$) and diammonium phosphate ($P_2O_5$) fertilizers were applied at the recommended rates of 92 kg per hectare (kg/ha) and 69 kg/ha, respectively. $P_2O_5$ was applied at planting and $N_2$ was applied in two splits: At planting and knee height. All other agronomic management practices such as weeding and hoeing were done based on research recommendations for the areas.

### Data collection

#### *Yield and agronomic performance*

Data collected include: number of plants per plot was recorded at emergence and harvesting time, days to anthesis and silking (days): Taken during 50% of plants in a plot shed pollen and 50% of the plants in a plot showing 2 to 3 cm long silk emergence, anthesis-silking interval (days): It was calculated by subtracting from days of silking to days of anthesis, plant height (cm) was measured in centimeter from ten randomly selected plants as a distance from the ground level to the first tassel branch and for ear height, from the ground level to the upper most ear bearing node. Ear position (ratio): It was calculated as the ratio of ear height to plant height. At harvest time; number of ears per plant was calculated from the total numbers of ears harvested in a particular plot divided by the total number of plants harvested and grain yield (tonnes per hectare) was recorded the weight of the ears per plot and this was adjusted to 12.5% moisture content to estimate grain yield per hectare. Plant aspect was recorded by observing overall phenotypic appearance of the plant in a plot by using 1 to 5 scoring scale; where 1 = excellent and 5 = poor. Ear aspect was recorded by observing overall phenotypic appearance of the ears in a plot at harvesting time by using 1 to 5 scoring scale; where 1 = excellent and 5 = poor.

#### *Reaction to gray leaf spot*

Disease severity (scale): Disease severity was rated using 1 to 5 scoring scales based on Pusa Campus (2012) procedure. 1 = very slight to slight infection, one or two few scattered lesions on lower leaves, 2 = light infection, moderate number of lesions on lower leaves only, 3 = moderate infection, abundant lesions are on lower leaves, few on middle leaves, 4 = heavy infection, lesions are abundant on lower and middle leaves, extending to upper leaves. 5 = very heavy infection, lesions abundant on almost all leaves plants prematurely dry or killed by the disease.

### Statistical analysis

The collected data were analyzed using the lattice design procedure of SAS statistical procedure (SAS, 2004). For the analysis of variance, test genotypes were considered as fixed whereas replication and incomplete blocks within replication were considered as random. Mean separation was done using least significant difference (LSD). Analysis of combining abilities were made using the application of diallel-SAS procedure (Zhang et al., 2005) computer program following Griffing’s (1956) Method IV and Model I (fixed model). Tests of significance of the combining ability effects and their differences were made using t-test. The relationships between yield, agronomic traits and GLS were determined using the Proc Corr procedure in SAS (SAS, 2004). The correlation was determined using across location mean data. The phenotypic relationships among the traits were determined using the correlation coefficient, r and the significance of correlation coefficients was determined using t-test at alpha level of 5 and 1% probability level.

**Table 2.** Mean square of grain yield, selected agronomic traits and GLS of diallel cross hybrids evaluated at Bako and Jima in 2014/2015 cropping season.

| Traits | Bako | | Jima | | Across Location | | | | | Error (df=94) |
|---|---|---|---|---|---|---|---|---|---|---|
| | Genotypes (G)(df= 47) | Error (df=37) | Genotypes (G)(df= 47) | Error (df=37) | Location (L) (df = 1) | Rep/(L) (df =2) | Genotypes (df=47) | Crosses (df=44) | G x L (df=47) | |
| GY | 3.31** | 1.18 | 4.62** | 2.06 | 184.95** | 7.73** | 6.32** | 6.53** | 1.96 | 1.54 |
| EPP | 0.13** | 0.04 | 0.12** | 0.06 | 0.93** | 0.35** | 0.21** | 0.19** | 0.05 | 0.05 |
| DA | 16.52** | 1.94 | 14.73** | 1.86 | 1276.17** | 10.13** | 33.18** | 34.94** | 2.85 | 2.08 |
| DS | 19.78** | 2.17 | 15.05** | 2.46 | 1552.69** | 24.01** | 36.80** | 38.84** | 3.61 | 2.47 |
| ASI | 2.89* | 1.6 | 0.49* | 0.28 | 13.55** | 5.57** | 1.78** | 1.90** | 1.61* | 0.95 |
| PH | 473.60** | 133.17 | 216.77 | 327.69 | 50960.33** | 393.42 | 689.91** | 722.90** | 187.87 | 273.48 |
| EH | 309.37** | 97.35 | 294.48 | 279.73 | 20791.69** | 1120.18** | 451.84** | 476.58** | 182.45 | 226.11 |
| EP | 22.28* | 10.62 | 43.63 | 39.4 | 151.71* | 121.29* | 38.12 | 39.54 | 22.13 | 26.5 |
| PA | 0.35** | 0.1 | 0.19 | 0.16 | 3.94** | 0.11 | 0.37** | 0.38** | 0.19 | 0.13 |
| EA | 0.49** | 0.13 | 0.38 | 0.15 | 17.22** | 0.22 | 0.87** | 0.82** | 0.18 | 0.16 |
| GLS | 1.41** | 0.15 | 0.23** | 0.1 | 0.16** | 0.52* | 1.03** | 1.09** | 0.64** | 0.12 |

*, ** significant at 0.05 and 0.01 probability level respectively. GY = Grain yield; EPP = Number of ears per plant; DA = Days to anthesis; DS = Days to silking; ASI = Anthesis silking interval; EH = Ear height; PH = Plant height; EP = Ear position; PA = Plant aspect; EA = Ear aspect; GLS = Gray leaf spot, df = Degrees of freedom.

## RESULTS AND DISCUSSION

### Analysis of variance

Analysis variance of genotypes showed significant mean square for all traits at Bako and except plant height, ear height, ear position, plant aspect and ear aspect other traits also revealed significant mean square at Jima location, (Tables 2). Combined analysis of mean square was significant at P≤0.05 and highly significance at P≤0.01 among locations, genotypes and crosses in all studied traits except ear position, this indicating that the presence of genetic variability among crosses. On the other hand, mean squares due to crosses × locations interaction for all studied traits, except ASI and GLS revealed non-significant variations (Table 2). Significant mean square for grain yield, agronomic traits and GLS were reported by Dagne et al. (2008), Legesse et al. (2009), Mousa et al. (2014), Hossein et al. (2014) and Mwai et al. (2015).

### Mean performance of crosses

Mean performance of 45 experimental crosses and three commercial checks for grain yield, selected agronomic traits and GLS are presented in Table 3. The mean of grain yield (GY) for all crosses under this experiment ranged from 4.28 tonnes per hectare (P2 × P6) to 10.87 tonnes per hectare (P4×P6) with a mean value of 7.86 tonnes per hectare. Among the crosses, P4 × P6, P4 × P10, P5 × P6, P5 ×P10, P6 ×P10 and P9 ×P10 crosses showed significantly high yielder than the best check hybrid BH546 and these crosses also revealed greater than 10% yield advantages than the quality protein commercial maize hybrid BHQPY545 (Table 3). Number of ears per plant ranged from 0.94 for P3 × P8 to 1.76 for P9 × P10, with overall mean of crosses 1.34. The highest mean values for ears per plant were observed for crosses P9 × P10 and P3 × P6 as compared to the highest check BHQPY545.

The number of days to anthesis (DA) ranged from 69.0 days (P1× P3 and P1 x P5) to 79.75 days (P4 × P7) with overall mean of 76.37 days and days to silking (DS) also ranged from 72.5 days (P1 × P3) to 84.75 days (P4 × P6). Crosses, P1 × P3, P1 × P5, P1 × P7, P1 × P2, P1 × P8, P1 × P4, P1 × P6, P1 × P10 and P5 × P9 revealed significantly short days to anthesis and silking as compared to hybrid check BH546, including these crosses 21 crosses exhibited earlier as compared to quality protein hybrid check BHQPY545. Crosses which showed longer number of days to anthesis and silking could be considered as late maturing types. Conversely, crosses which had shorter days to flowering could be regarded as early maturing types. Crosses which exhibited early anthesis and silking are desirable type of crosses especially in moisture stress environments since early type crosses can escape terminal moisture stresses during the growth stages (Banziger et al., 2004).

Anthesis-silking interval (ASI) ranged from 1.75 days (P2 × P9) to 5.25 days (P4 × P6). Eight crosses showed greater days to ASI than standard checks of BHQPY545 and BH547. In general, crosses which exhibited short anthesis-silking interval, indicates that the cross had short gaps between anthesis and silking days and it is a desired character for good seed setting since these crosses may showed anthesis-silking interva below 3 days could be found within an acceptable range.

**Table 3.** Mean grain yield, agronomic performance and GLS reaction of diallel cross hybrids grown at Bako and Jima in 2014/2015 cropping season.

| Entry No. | Entries | GY (t/ha) | EPP (#) | DA (days) | DS (days) | ASI (days) | PH (cm) | EH (cm) | EP (ratio) | PA (1-5 scale) | EA(1-5 scale) | GLS (1-5 scale) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | P1× P2 | 6.2 | 1.28 | 71.25 | 74.25 | 3 | 250.8 | 141.8 | 57.96 | 3 | 2.5 | 2.25 |
| 2 | P1 × P3 | 7.53 | 1.32 | 69 | 72.5 | 3.5 | 248.8 | 121.5 | 48.93 | 2.88 | 3.38 | 2.75 |
| 3 | P1 × P4 | 7.96 | 1.09 | 72.25 | 74.75 | 2.5 | 250 | 121.3 | 48.43 | 2.63 | 2.38 | 2 |
| 4 | P1 × P5 | 7.61 | 0.96 | 69 | 72.5 | 3.5 | 251 | 118.5 | 47.04 | 2.38 | 2.5 | 2.25 |
| 5 | P1 × P6 | 8.04 | 1.32 | 72.75 | 75.75 | 3 | 250.8 | 123.8 | 49.47 | 2.38 | 2 | 2 |
| 6 | P1 × P7 | 6.56 | 1.17 | 70.5 | 73.5 | 3 | 243.3 | 112.8 | 46.63 | 2.63 | 2.63 | 2.25 |
| 7 | P1 × P8 | 5.28 | 1.06 | 71.75 | 75 | 3.25 | 254.5 | 117.3 | 45.82 | 3.38 | 3.25 | 2.13 |
| 8 | P1 × P9 | 6.3 | 1.18 | 71 | 73 | 2 | 234.8 | 124 | 52.76 | 3.25 | 3.13 | 2 |
| 9 | P1 × P10 | 8.08 | 1.16 | 73 | 75.75 | 2.75 | 271.3 | 136 | 49.81 | 2.88 | 2.75 | 2 |
| 10 | P2 × P3 | 6.51 | 1.63 | 75.25 | 78 | 2.75 | 260.8 | 143.5 | 55.08 | 3.38 | 3 | 4 |
| 11 | P2 × P4 | 8.09 | 1.38 | 78 | 81 | 3 | 273.3 | 135.5 | 49.33 | 2.75 | 1.88 | 2.5 |
| 12 | P2 × P5 | 7.68 | 1.35 | 75.75 | 78 | 2.25 | 273.3 | 140.8 | 51.31 | 3 | 2.5 | 2.63 |
| 13 | P2 × P6 | 4.28 | 1.54 | 79.5 | 82.5 | 3 | 240.5 | 125.5 | 52.17 | 3.13 | 2.5 | 3.38 |
| 14 | P2 × P7 | 7.02 | 1.49 | 77.75 | 80.25 | 2.5 | 273 | 132 | 48.26 | 2.38 | 2.38 | 2.75 |
| 15 | P2 × P8 | 8.47 | 1.02 | 78.5 | 80.75 | 2.25 | 262.8 | 115 | 43.82 | 2.5 | 2.5 | 2.5 |
| 16 | P2 × P9 | 7.87 | 1.53 | 77.75 | 79.5 | 1.75 | 277.8 | 135.3 | 48.7 | 2.88 | 3 | 3.63 |
| 17 | P2 × P10 | 8.62 | 1.23 | 78.25 | 81.25 | 3 | 279.3 | 144 | 51.19 | 2.5 | 1.88 | 2.75 |
| 18 | P3 × P4 | 7.68 | 1.39 | 76.5 | 78.75 | 2.25 | 289.8 | 137.3 | 47.3 | 3.13 | 2.88 | 3.13 |
| 19 | P3 × P5 | 8.65 | 1.42 | 75.25 | 77.25 | 2 | 279.3 | 136.5 | 48.72 | 2.5 | 2.5 | 3.63 |
| 20 | P3 × P6 | 9.08 | 1.71 | 78.25 | 80.75 | 2.5 | 258 | 123.5 | 47.73 | 2.75 | 2.88 | 3.5 |
| 21 | P3 × P7 | 7.04 | 1.57 | 78 | 81.5 | 3.5 | 269.5 | 129 | 47.81 | 2.88 | 3 | 3.13 |
| 22 | P3 × P8 | 6.42 | 0.94 | 78.25 | 81 | 2.75 | 277 | 128.8 | 46.52 | 3.13 | 3.38 | 3.75 |
| 23 | P3 × P9 | 6.81 | 1.67 | 75.5 | 78.75 | 3.25 | 256 | 119 | 46.37 | 3.25 | 3.25 | 2.88 |
| 24 | P3 × P10 | 8.46 | 1.27 | 79 | 81.75 | 2.75 | 263.5 | 131 | 49.45 | 2.88 | 3.25 | 2.75 |
| 25 | P4 × P5 | 7.68 | 1.19 | 78 | 81.5 | 3.5 | 267 | 142.3 | 53.27 | 2.5 | 2 | 2.13 |
| 26 | P4 × P6 | 10.87 | 1.42 | 79.5 | 84.75 | 5.25 | 268 | 133 | 49.93 | 2.25 | 2 | 1.88 |
| 27 | P4 × P7 | 5.89 | 1.34 | 79.75 | 83.25 | 3.5 | 252 | 116.8 | 46.2 | 2.75 | 2.88 | 3.13 |
| 28 | P4 × P8 | 8.68 | 1 | 78.5 | 81.25 | 2.75 | 270.8 | 128.3 | 47.35 | 2.63 | 2.63 | 2.63 |
| 29 | P4 × P9 | 8.43 | 1.64 | 77 | 79.5 | 2.5 | 271.8 | 140.8 | 51.69 | 2.63 | 2.5 | 2.13 |
| 30 | P4 × P10 | 9.36 | 1.29 | 79 | 83 | 4 | 288 | 159 | 55.12 | 2.38 | 2 | 2.38 |
| 31 | P5 × P6 | 10.21 | 1.45 | 77.25 | 80.75 | 3.5 | 270 | 131.8 | 48.74 | 2.13 | 2.13 | 2.25 |
| 32 | P5 × P7 | 7.91 | 1.18 | 77.75 | 80.5 | 2.75 | 291 | 144.5 | 49.56 | 2.38 | 2.5 | 2.63 |
| 33 | P5 × P8 | 7.81 | 1.04 | 78 | 82.75 | 4.75 | 281.5 | 142 | 50.4 | 2.75 | 2.5 | 2.75 |
| 34 | P5 × P9 | 7.76 | 1.66 | 74.5 | 77 | 2.5 | 253 | 129.8 | 51.26 | 2.75 | 2.75 | 2.5 |
| 35 | P5 × P10 | 9.34 | 1.32 | 77.75 | 80.5 | 2.75 | 285.3 | 153.8 | 53.42 | 2.63 | 2.38 | 2.25 |
| 36 | P6 × P7 | 8.58 | 1.41 | 78.5 | 81 | 2.5 | 279.5 | 125.3 | 44.65 | 2.38 | 2.13 | 2.75 |
| 37 | P6 × P8 | 8.2 | 1.19 | 78.75 | 82.5 | 3.75 | 276 | 128 | 46.33 | 2.63 | 2.25 | 2.63 |
| 38 | P6 × P9 | 7.87 | 1.63 | 78.5 | 81.5 | 3 | 276.3 | 124.3 | 44.97 | 2.5 | 2.25 | 3.13 |
| 39 | P6 × P10 | 10.07 | 1.47 | 79 | 82.5 | 3.5 | 275 | 140 | 50.55 | 2.38 | 1.88 | 2 |
| 40 | P7 × P8 | 7.76 | 1.26 | 77.5 | 80 | 2.5 | 258.5 | 113.8 | 44.3 | 2.5 | 3 | 2.5 |
| 41 | P7 × P9 | 7.41 | 1.6 | 76.75 | 79.25 | 2.5 | 268.5 | 132.3 | 49.33 | 3 | 2.88 | 2.5 |
| 42 | P7 × P10 | 7.2 | 1.42 | 78.75 | 81.5 | 2.75 | 275 | 148 | 53.82 | 2.5 | 1.63 | 2.25 |
| 43 | P8 × P9 | 8.23 | 1.33 | 75.25 | 77.75 | 2.5 | 265 | 124.5 | 46.92 | 2.75 | 3.13 | 2.88 |
| 44 | P8 × P10 | 8.18 | 1.05 | 77.75 | 80.5 | 2.75 | 275 | 138.8 | 50.27 | 2.63 | 2.25 | 2.38 |
| 45 | P9 × P10 | 10.09 | 1.76 | 77 | 78.75 | 1.75 | 271.5 | 146.8 | 54.04 | 2.38 | 2.63 | 2.5 |
| 46 | BHQPY545 | 8.35 | 1.7 | 77.5 | 80.25 | 2.75 | 256.3 | 128.8 | 49.96 | 2.5 | 3.13 | 2.5 |
| 47 | BH546 | 9.26 | 1.11 | 74.5 | 77.5 | 3 | 263 | 128.3 | 48.57 | 2.5 | 1.75 | 2.63 |
| 48 | BH547 | 7.38 | 0.94 | 75.5 | 78.25 | 2.75 | 260.8 | 138.5 | 52.88 | 2.38 | 2 | 2.63 |
| Entry mean | | 7.89 | 1.33 | 76.34 | 79.25 | 2.91 | 266.4 | 131.9 | 49.46 | 2.69 | 2.55 | 2.63 |

**Table 3.** Contd.

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cross mean | 7.86 | 1.34 | 76.37 | 79.29 | 2.92 | 266.8 | 131.9 | 49.39 | 2.71 | 2.57 | 2.64 |
| CV | 15.71 | 16.07 | 1.89 | 1.98 | 33.49 | 6.21 | 11.4 | 10.41 | 13.37 | 15.76 | 13.04 |
| LSD | 1.74 | 0.3 | 2.02 | 2.21 | 1.37 | 23.22 | 21.11 | 7.23 | 0.5 | 0.56 | 0.48 |

GY = Grain yield; EPP = Number of ears per plant; DA = Days to anthesis; DS = Days to silking; ASI = Anthesis silking interval; EH = Ear height; PH = Plant height; EP = Ear position; PA = Plant aspect; EA = Ear aspect; GLS = Gray leaf spot; CV = Coefficient of variation; LSD = Least significant difference.

Naturally, maize plant is proto-androus in which anthesis normally begins 1 to 3 days before the emergence of silks and 3 to 4 days after the silks emergence are ready to be pollinated (Poehlman, 1977). If, the gap between days of anthesis and silking is large, the viability of pollen would be minimized and abnormal fertilization might be takes place or fertilization may not be happened. In general, shorter ASI improves the pollen-silk synchronization, a major trait that is affected under moisture stress areas. The need for a shorter ASI to achieve high grain yield has been observed by Anderson et al. (2004), where the potential number of florets that could become grains was limited by the receptivity of the silks. In this regard, ASI has been widely used in indirect selection of higher grain yield under drought conditions (Banziger et al., 2004).

The mean value of plant and ear height for crosses varied from 234.75 cm for P1 × P9 to 291 cm for P5 x P7 and112.75 cm for P1 × P7 to 159 cm for P4 x P10 respectively. Twelve crosses showed significant plant height over check BHQPY545 and 18 crosses were showed shorter ear height than check hybrid BHQPY545. Among all the tested crosses, sixteen and seventeen crosses were showed shorter plant and ear height respectively over than high yielder check BH546. On the other hand, almost all high yielder crosses had tall plant and ear height as compared to standard checks (BHQPY545, BH546 and BH547). Ear position ranged from 43.82% for P2×P8 to 57.96% for P1×P2. In this study majority of crosses showed ear placement near to the mid part of the plant, indicating desirable character for lodging tolerance. In general, crosses which have shorter pant and ear height are desirable for lodging tolerance and to apply necessary management practices, whereas taller crosses are important to harvest high biomass yield that could be used as animal feed and source of fuel for poor farmers (Girma et al., 2015).

Plant and ear aspects are a visual evaluation of plants and ears before harvesting and at harvesting time respectively, by observing all over performance. The mean values of plant aspect ranged from 2.13 for P5×P6 to 3.38 for P2×P3 with overall mean of 2.71. Ear aspect ranged from 1.63 for P7×P10 to 3.38 for P1×P8 and P3×P8 crosses. Crosses such as P7 ×P10, P6 ×P10, P2×P10, P2×P10, P2×P4, P4×P6, P1×P6, P4×P10 and P4×P5 showed lower plant and ear aspect scores, indicating that these crosses had desirable characters such as clean, uniform and disease free.

Gray leaf spot (GLS) disease severity scores in combined analysis were showed highly significant variation. Mean value of GLS Severity was ranged from 1.88 (P4×P6) to 4.00 (P2 ×P3) with the overall mean value 2.64. Twenty four crosses were recorded higher disease severity as compared to hybrid check BHQPY545. Crosses such as P3 × P4, P3 × P7, P4 × P7, P6 × P9, P2 × P6, P3 × P6, P3 × P8 and P2 × P3 scored greater than 3 severity values, these crosses were vulnerable to GLS while P4 × P6, P1 × P4, P1 × P6, P1 × P9, P1 × P10 and P6 × P10 scored less than 2 disease severity and could be used in the development of resistant genotypes against grey leaf spot. All these resistant crosses were the results of P1, P4 and/or P10 as one of the parent and might be contribute good source of favorable genes. Generally, crosses which showed low severity values to GLS might be used as a source of resistant genes to develop GLS resistant QPM varieties, on the other hand crosses which had high severity score values considered as susceptible varieties.

## Combining ability analysis

Mean squares due to general combining ability (GCA), specific combining ability (SCA), GCA × L (Location), SCA × L and error are presented in Table 4. The combined analysis of GCA showed a significant difference ($p \leq 0.01$) in all traits while mean squares of SCA were also significant for grain yield, days to silking, anthesis-silking interval, and plant and ear height and gray leaf spot. Therefore, significant GCA and SCA indicating both additive and non-additive gene action contribute the expression of the traits. However, mean squares due to SCA was non-significant for ear per plant, days of anthesis and ear position this showed that only additive gene actions contributed to the inheritance of these traits. The mean square of GCA × Location (GCA × L) interaction was significant only gray leaf spot, days of anthesis, plant aspect and ear aspect whereas the SCA × L interaction was non-significant for all traits (Table 4). Similar finding were reported by different investigators. Shushay et al. (2013) reported significant mean squares due to GCA of lines and SCA for grain yield and plant height, ear height and days to anthesis traits. In line by tester analysis, Melkamu et al. (2013) reported lines

**Table 4.** Mean square of combined analysis of GCA and SCA for grain yield, selected agronomic traits and GLS in diallel cross hybrids evaluated at Bako and Jimma, in 2014/2015 cropping season.

| Trait | GCA (df =9) | SCA (df =35) | GCA × L (df = 9) | SCA × L (df = 35) | GCA/SCA ratio | Error (df=88) |
|---|---|---|---|---|---|---|
| GY | 15.17** | 4.31** | 1.62 | 2.12 | 3.52 | 1.48 |
| EPP | 0.75** | 0.05 | 0.05 | 0.05 | 15.00 | 0.05 |
| DA | 158.46** | 3.18 | 5.04* | 2.41 | 49.83 | 2.14 |
| DS | 170.13** | 5.08** | 3.28 | 3.617 | 33.49 | 2.57 |
| ASI | 3.06** | 1.6* | 1.57 | 1.64 | 1.91 | 1 |
| PH | 1827.3** | 438.92* | 423.83 | 130.29 | 4.16 | 272.28 |
| EH | 1464.51** | 222.54 | 305 | 149.27 | 6.58 | 226.91 |
| EP | 93.31** | 25.71 | 27.65 | 20.87 | 3.63 | 27.75 |
| PA | 0.98** | 0.23* | 0.52** | 0.12 | 4.26 | 0.13 |
| EA | 2.93** | 0.28* | 0.38* | 0.11 | 10.46 | 0.16 |
| GLS | 3.76** | 0.41** | 2.02** | 0.28 | 9.17 | 0.12 |

*=0.05 and **= 0.01 significant probability level. GY = Grain yield; EPP = Number of ears per plant; DA = Days to anthesis; DS = Days to silking; ASI = Anthesis silking interval; EH = Ear height; PH = Plant height; EP = Ear position; PA = Plant aspect; EA = Ear aspect; GLS = Gray leaf spot; df = degrees

showed significant both GCA and SCA for all traits except for days to 50% tasseling, ear length, ear diameter, grain yield per plant and number of kernels per row. Mousa (2014) reported highly significant GCA and SCA mean squares for all traits studied. In a combined analysis of variance, Hossein et al. (2014) reported significant mean squares due to GCA and SCA for days to silking, plant height, ear height, 1000-kernel weight, ear length, number of rows per ear, number of leaf and kernel yield maize. In quality protein maize diallel analysis, Demissew (2014) reported GCA mean squares were significantly different ($P < 0.01$) for grain yield and all agronomic traits except EPP while mean squares due to SCA were significantly different for all traits excluding ASI and EPP. In addition to this significant GCA and SCA effects for GLS resistance were identified, resistance was conditioned by both additive and non-additive gene actions. Similar results were reported by Menkir and Ayodele (2005), Dagne et al. (2008), Leggesse et al. (2009), Nzuve et al. (2013) and Mwai et al. (2015).

The ratio of GCA to SCA mean square was greater than unity for all studied traits. This suggests that additive gene action could be more important than non-additive gene action. The importance of additive gene effects in different traits has been reported in several investigators in different times (Dagne et al., 2008; Legesse et al., 2009; Nzuve et al., 2013; Hussien et al., 2014; Mwai et al., 2015).

## Estimates of GCA and SCA effects

Significant differences in GCA effects were detected among lines for various traits (Table 5). The highest GCA effect for grain yield was observed from parent P10 (1.08 tonnes per hectare), followed by parent P6 (0.819 tonnes per hectare), whereas the lowest GCA value was observed form parent P1 (-0.9 tonnes per hectare), followed by parent P2 (-0.759 tonnes per hectare) and Parent P7 (-0.67 tonnes per hectare). Inbred lines, P6 and P10 were good combiners for grain yield since they showed positively significant GCA effects. These inbred lines can be desirable parents for synthesis of new synthetic varieties, since they may contribute favorable alleles in the improvements of the yield. On the other hand parents P3, P6 and P8 revealed positively significant GCA effects for ear per plant as well as parent P1 was good general combiner for days of anthesis, days to silking, plant height and ear height. These lines suggesting that they might be contribute favorable genes to develop early and short type of new varieties formation. Similarly, Parent P10 had also expressed highest significant GCA effects for plant height and ear height in the positive direction. Parent P7 and P8 were good general combiners in ideal ear placement formation. Parental lines such as P1, P3 and P8 showed positive significant GCA effects to the undesirable direction for plant and ear aspect. On the other hand, P6 and P10 revealed negative GCA effect which is the desirable direction which showed good plant and ear characters.

A line with negative GCA for gray leaf spot would be expected to be a useful source of resistance to the disease. Parents with significant negative GCA may contribute resistance in their crosses. Parents such as P1, P4 and P10 showed negatively significant GCA effects, this indicating that they could be good sources GLS resistant genes. It was recommended that these parents should be used in breeding programs with a purpose of incorporating GLS resistance genes with other desired traits. Especially, parental line P10 was a poor combiner for days of anthesis, plant height and ear height, but best for grain yield and GLS.

Significant SCA effects indicated that the crosses performed better or poorer than what would be expected

**Table 5.** Estimates of general combining ability (GCA) effects for grain yield, selected agronomic traits and GLS reactions of ten QPM maize inbred line of freedom (GCA = General combining ability; SCA = Specific combining ability).

| Inbred lines | Traits | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | GY | EPP | DA | DS | ASI | PH | EH | EP | PA | EA | GLS |
| P1 | -0.90** | -0.19** | -5.86** | -5.83** | 0.03 | -18.30** | -8.82** | 0.29 | 0.13** | 0.18* | -0.51** |
| P2 | -0.75** | 0.05 | 0.58 | 0.24 | -0.34 | -1.27 | 3.24 | 1.66* | 0.14* | -0.12 | 0.33** |
| P3 | -0.32 | 0.11** | -0.29 | -0.42 | -0.13 | 0.14 | -2.16 | -0.83 | 0.30** | 0.55** | 0.72** |
| P4 | 0.49 | -0.04 | 1.39* | 1.77** | 0.38* | 3.64 | 3.34 | 0.51 | -0.09 | -0.25** | -0.23* |
| P5 | 0.48 | -0.06 | -0.51 | -0.36 | 0.16 | 6.23 | 6.56* | 1.15 | -0.17* | -0.17 | -0.09 |
| P6 | 0.81** | 0.13** | 1.83** | 2.30** | 0.47* | -0.93 | -4.04 | -1.25 | -0.23** | -0.39** | -0.03 |
| P7 | -0.67* | 0.05 | 0.99 | 0.89 | -0.09 | 1.11 | -4.13 | -1.75* | -0.12 | -0.01 | 0.02 |
| P8 | -0.21 | -0.27** | 0.86 | 0.99 | 0.13 | 2.45 | -6.38* | -2.85** | 0.07 | 0.22* | 0.05 |
| P9 | 0 | 0.24** | -0.51 | -1.08 | -0.56** | -3.36 | -1.35 | 0.19 | 0.13 | 0.30** | 0.05 |
| P10 | 1.08* | -0.01 | 1.52** | 1.49 | -0.03 | 10.29** | 13.74** | 2.89** | -0.15* | -0.31** | -0.31** |
| SE (g) | 0.21 | 0.04 | 0.38 | 0.3 | 0.21 | 3.45 | 2.93 | 0.88 | 0.12 | 0.1 | 0.24 |
| SE (gi-gj) | 0.32 | 0.06 | 0.56 | 0.45 | 0.31 | 5.15 | 4.37 | 1.31 | 0.18 | 0.15 | 0.36 |

*=0.05 and **= 0.01 Significant probability level; GY = Grain yield.; GLS = Gray leaf spot; EPP = Number of ears per plant; DA = Days to anthesis; DS = Days to silking; ASI = Anthesis silking interval; EH = Ear height; PH = Plant height; EP = Ear position; PA = Plant aspect; EA = Ear aspect; SE (gi)= Standard error of general combining ability effects; SE (gi-gj) = Standard error of the difference of general combining ability effects*=0.05 and **= 0.01 Significant probability level; GY = Grain yield.; GLS = Gray leaf spot; EPP = Number of ears per plant; DA = Days to anthesis; DS = Days to silking; ASI = Anthesis silking interval; EH = Ear height; PH = Plant height; EP = Ear position; PA = Plant aspect; EA = Ear aspect; SE (gi)= Standard error of general combining ability effects; SE (gi-gj) = Standard error of the difference of general combining ability effects.

based on GCA effects of the respective parents (Table 6). Crosses, P2 × P8 and P4 × P6 for grain yield, P3 × P5 for days of anthesis-silking interval, P2 × P6 for plant height, P2 × P8 for plant aspect and P7 × P10 for ear aspect depicted good specific combining ability effects, indicating the crosses showed top performance in each trait. In the case of GLS, P2 × P8, P3 × P9 and P4 × P6 crosses were showed significant and negative SCA effects, indicating the importance of non-additive gene action to be in the desirable direction for the improvement of the crosses against GLS reaction. Generally, crosses which revealed good SCA effects for the most studied traits could be help to choose good hybrids.

## Correlation analysis

The result of Pearson correlation analysis indicated highly significant negative and positive relationship between grain yield and other traits observed (Table 7). Grain yield showed positive correlation between EPP, PH, EH and EP whereas negative correlation observed with GLS, DA, DS, PA and EA. However, non- significant correlation was observed with anthesis silking interval (Table 7). These results agreed with the work of Dagne et al. (2008) who reported grain yield had high positively correlated with plant height while contrast correlation between grain yield and GLS disease parameters. In addition to this the results were agreed with Daniel et al. (2008) reported negative correlation grain yield with disease severity. Non-significant-correlation was shown between gray leaf spot disease with days of anthesis, days of silking, plant height and ear height indicating the disease had less impact in reduction of days of flowering and height.

## Conclusion

The combined analysis of variance showed that there was a significant variation among crosses for all studied traits. Crosses, such as P4 × P6, P5 × P6, P9 × P10, P6 × P10, P4 × P10 and P5 × P10 were high yielder hybrids as compare to the best hybrid checks BH546 and BHQPY546. Significant mean squares due to GCA for all traits and SCA for the most of the studied traits were detected, this indicating additive and non-additive gene effects contribute to the expression of the characters. Parental lines P6 and P10 were having good general combining ability for grain yield. On the other hand inbred lines P4 and P10 were good general combiner for GLS disease resistant hybrids development. Even though, there were no inbred lines that are good combiner for all traits studied, inbred line P10 is the best combiner for grain yield as well as GLS resistance. Hence, this inbred lines has a potential to be utilized for producing synthetic maize varieties which are high yielder and GLS resistant or can be used for other breeding purposes. Among the crosses P2 × P8 and P4 × P6 were found to be good specific combiners for grain yield and GLS. Hence, these parental combinations could be used for the development of high yielder as well as GLS resistant hybrids. Grain

**Table 6.** Estimates of specific combining ability (SCA) effects for grain yield, selected agronomic traits and GLS disease reactions of 45 QPM crosses evaluated at Bako and Jima in 2014/2015 cropping season.

| Crosses | Traits | | | | | | |
|---|---|---|---|---|---|---|---|
| | GY | DA | ASI | PH | PA | EA | GLS |
| P1 × P2 | 0 | 0.15 | 0.4 | 3.5 | 0.02 | -0.1 | -0.2 |
| P1 × P3 | 0.89 | -1.2 | 0.68 | 0.09 | -0.3 | 0.08 | -0.1 |
| P1 × P4 | 0.51 | 0.34 | -0.8 | -2.16 | -0.1 | -0.1 | 0.11 |
| P1 × P5 | 0.16 | -1 | 0.4 | -3.75 | -0.3 | -0.1 | 0.22 |
| P1 × P6 | 0.27 | 0.4 | -0.4 | 3.15 | -0.2 | -0.4 | -0.1 |
| P1 × P7 | 0.27 | -1 | 0.15 | -6.38 | -0.1 | -0.1 | 0.11 |
| P1 × P8 | -1.47** | 0.37 | 0.18 | 3.53 | 0.48* | 0.29 | -0.1 |
| P1 × P9 | -0.7 | 1 | -0.4 | -10.4 | 0.29 | 0.08 | -0.2 |
| P1 × P10 | 0.04 | 0.97 | -0.2 | 12.43 | 0.19 | 0.32 | 0.19 |
| P2 × P3 | -0.3 | -1.4 | 0.3 | -4.94 | 0.23 | 0.01 | 0.31 |
| P2 × P4 | 0.5 | -0.4 | 0.05 | 4.06 | 0 | -0.3 | -0.2 |
| P2 × P5 | 0.08 | -0.7 | -0.5 | 1.47 | 0.32 | 0.22 | -0.3 |
| P2 × P6 | -3.63** | 0.72 | 0 | -24.13* | 0.51** | 0.44 | 0.44 |
| P2 × P7 | 0.58 | -0.2 | 0.02 | 6.34 | -0.4 | -0.1 | -0.2 |
| P2 × P8 | 1.57** | 0.68 | -0.5 | -5.25 | -0.41* | -0.2 | -0.52* |
| P2 × P9 | 0.76 | 1.31 | -0.3 | 15.56 | -0.1 | 0.26 | 0.61* |
| P2 × P10 | 0.43 | -0.2 | 0.46 | 3.4 | -0.2 | -0.3 | 0.09 |
| P3 × P4 | -0.4 | -1 | -0.9 | 19.15 | 0.21 | 0.01 | 0 |
| P3 × P5 | 0.62 | -0.3 | -0.95* | 6.06 | -0.3 | -0.5 | 0.36 |
| P3 × P6 | 0.73 | 0.34 | -0.8 | -8.03 | 0 | 0.15 | 0.17 |
| P3 × P7 | 0.18 | 0.93 | 0.8 | 1.43 | 0 | -0.1 | -0.3 |
| P3 × P8 | -0.9 | 1.31 | -0.2 | 7.59 | 0.05 | 0.04 | 0.34 |
| P3 × P9 | -0.7 | -0.1 | 1.02** | -7.6 | 0.12 | -0.2 | -0.53* |
| P3 × P10 | -0.2 | 1.4 | 0 | -13.8 | 0.02 | 0.44 | -0.3 |
| P4 × P5 | -1.2 | 0.75 | 0.05 | -9.69 | 0.05 | -0.2 | -0.2 |
| P4 × P6 | 1.72** | -0.1 | 1.49** | -1.53 | -0.1 | 0.07 | -0.50* |
| P4 × x P7 | -1.79** | 1 | 0.3 | -19.6 | 0.26 | 0.57* | 0.70** |
| P4 × P8 | 0.54 | -0.1 | -0.7 | -2.16 | -0.1 | 0.08 | 0.17 |
| P4 × P9 | 0.08 | -0.3 | -0.2 | 4.65 | -0.1 | -0.1 | -0.3 |
| P4 × P10 | -0.1 | -0.3 | 0.74 | 7.25 | -0.1 | 0 | 0.28 |
| P5 × P6 | 1.06 | -0.4 | 0 | -2.13 | -0.2 | 0.11 | -0.3 |
| P5 × P7 | 0.24 | 0.9 | -0.2 | 16.84 | 0 | 0.11 | 0.06 |
| P5 × P8 | -0.3 | 1.28 | 1.55** | 6 | 0.15 | -0.1 | 0.15 |
| P5 × P9 | -0.6 | -0.9 | 0 | -16.7 | 0.09 | 0.05 | -0.1 |
| P5 × P10 | -0.1 | 0.37 | -0.3 | 1.9 | 0.24 | 0.29 | 0.01 |
| P6 × P7 | 0.58 | -0.7 | -0.8 | 12.5 | 0.02 | 0 | 0.12 |
| P6 × P8 | -0.3 | -0.3 | 0.24 | 7.65 | 0.09 | -0.2 | 0 |
| P6 × P9 | -0.8 | 0.81 | 0.18 | 13.72 | -0.1 | -0.2 | 0.47* |
| P6 × P10 | 0.32 | -0.7 | 0.15 | -1.19 | 0.05 | 0.01 | -0.3 |
| P7 × P8 | 0.79 | -0.7 | -0.5 | -11.9 | -0.2 | 0.22 | -0.2 |
| P7 × P9 | 0.22 | -0.1 | 0.24 | 3.93 | 0.29 | 0.02 | -0.2 |
| P7 × P10 | -1.1 | -0.1 | 0 | -3.22 | 0.07 | -0.62** | -0.1 |
| P8 × P9 | 0.59 | -1.5 | 0.02 | -0.91 | -0.2 | 0.04 | 0.14 |
| P8 × P10 | -0.6 | -1 | -0.3 | -4.57 | 0.01 | -0.2 | 0 |
| P9 × P10 | 1.14 | -0.4 | -0.6 | -2.25 | -0.3 | 0.07 | 0.12 |
| SE (sij) | 0.64 | 0.68 | 0.56 | 5.03 | 0.15 | 0.15 | 0.23 |
| SE (sij-sik) | 0.96 | 1.03 | 0.85 | 7.55 | 0.23 | 0.22 | 0.35 |
| SE (sij-skl) | 0.89 | 0.95 | 0.78 | 6.99 | 0.21 | 0.21 | 0.32 |

*=0.05 and **= 0.01 Significant probability level. GY =Grain yield; EPP =Number of ears per plant; DA =Days to anthesis; DS =Days to silking; ASI=Anthesis silking interval; EH = Ear height; PH = Plant height; EP = Ear position; PA = Plant aspect; EA= Ear aspect; GLS =Gray leaf spot; SE (sij)= Standard error of specific combining ability effect; SE (sj-sik)=Standard error of the difference of specific combining ability effects of the crosses having one parent in common and SE (sij-skl)=Standard error of the difference of specific combining ability effects of the crosses having no parent in common.

**Table 7.** Phenotype correlation among grain yield, gray leaf spot reaction and selected agronomic traits.

| Traits | GY | GLS | EPP | DA | DS | ASI | PH | EH | %EP | PA | EA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| GY | 1 | -0.257** | 0.257** | -0.207** | -0.207** | -0.060 | 0.458** | 0.372** | 0.102 | -0.572** | -0.460** |
| GLS | | 1 | 0.094 | 0.137 | 0.080 | -0.170* | 0.110 | 0.043 | -0.027 | 0.344** | 0.241** |
| EPP | | | 1 | -0.051 | -0.076 | -0.107 | 0.192** | 0.184* | 0.080 | -0.059 | -0.054 |
| DA | | | | 1 | 0.963** | 0.147* | -0.208** | -0.214** | -0.124 | 0.097 | 0.164* |
| DS | | | | | 1 | 0.406** | -0.238** | -0.241** | -0.136 | 0.078 | 0.144* |
| ASI | | | | | | 1 | -0.170* | -0.161* | -0.082 | -0.039 | -0.028 |
| PH | | | | | | | 1 | 0.720** | 0.117 | -0.240** | -0.420** |
| EH | | | | | | | | 1 | 0.768** | -0.210** | -0.445** |
| %EP | | | | | | | | | 1 | -0.068 | -0.241** |
| PA | | | | | | | | | | 1 | 0.492** |
| EA | | | | | | | | | | | 1 |

*; **. Correlation is significant at the 0.05 and 0.01 probability level respectively. GY = Grain yield.; GLS = Gray leaf spot; EPP = Number of ears per plant; DA = Days to anthesis; DS = Days to silking; ASI = Anthesis silking interval; EH = Ear height; PH = Plant height; EP = Ear position; PA = Plant aspect; EA = Ear aspect

yield showed positive correlation between EPP, PH, EH and EP traits whereas negative correlation were observed with GLS, DA, DS, PA and EA. Non-significant-correlation was showed between gray leaf spot disease with days of anthesis, days of silking, plant height and ear height indicating the disease do not have impact in days of flowering and height. Generally, this study identified inbred lines and crosses with desirable GCA and SCA effects for the studied traits. This had the possibility of developing desirable cross combinations and synthetic varieties through crossing and or recombination of inbred lines with desirable traits of interest.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.


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# Performance of cassava (*Manihot esculanta.* Cratz) clones in potential and low moisture stressed areas of Ethiopia

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**Cassava (*Manihot esculanta.* Cratz) is one of the most important food crops that constitute a considerable portion of the daily diet of the people and also serves as one of the major source of carbohydrate. Despite its importance, production of cassava in Ethiopia has different constraints and opportunities. Among which, shortage of improved varieties is the first and the most important one. It is mainly cultivated by small resource poor farmers on smallholding plots of land. Average storage root yield obtained per a given plot of cassava is as low as 100 tons per hectare despite the potential yield of 600 tons per hectare per year. This low yield might be due to the cultivation of local, low yielding, late maturing cultivars. To contribute to alleviating the problem and provide farmers other alternative varieties, Hawassa Agricultural Research Center, in collaboration with Jimma and Sekota Agricultural Research Centers, conducted evaluation of cassava clones in potential and moisture stressed agroclimatic conditions of the country. Jima and Hawassa sites represent potential agroclimatic condition, while Amaro and Sekota represent moisture stressed areas. A total of seven cassava clones namely AWC-1 (MM 96/5280), AWC-2 (MM 90/5280), AWC-3 (MM 96/7151), AWC-4 (MM96/1871), AWC-5 (MM96/3868) and Kello (standard check) were evaluated by using randomized complete block design replicated three times. The evaluation was carried out for two consecutive years, from 2012 to 2014. The combined analysis result indicated that there was statistically significant difference among the clones tested and the locations where the experiment was conducted. The clone AWC-1 (37.17 t/ha) followed by AWC-2(35.52 t/ha) and AWC-5 (35.51 t/ha) gave the highest storage root yield but there was no statistically significant difference in the total storage yield among the clones AWC-2, AWC-3 and AWC-5. In the same way, the highest dry matter content was recorded from the clones AWC-2 (51.8%), AWC-3 (48.5%) and Kello (49.1%). Among the locations tested, the best result was obtained from Amaro (35.14 t/ha) which is characterized by its low moisture stress, indicating that cassava can resist/tolerate low moisture stress and give comparative yield provided that other factors are not limited. Thus, those clones with the highest storage root yield and dry matter content were promoted for variety verification and will be released for wider production.**

**Key words:** Cassava, moisture stress, potential, storage root, dry matter.

## INTRODUCTION

Cassava is a monoecious perennial shrub having variable height ranging between 1 and 5 m, although maximum height usually does not exceed 3 m (Bernardo and Hernan, 2012). But it is extensively cultivated as an annual crop in tropical and subtropical regions for its edible starchy tuberous roots (MoC, 2014). Cassava is a very important food crop in the tropics, that is, at latitudes of 30° and from sea level to 1800 m above sea level. Also, the principal economic products are its roots, cassava leaves also have excellent potential and are extensively used in Africa and Asia, as either human food or animal feed. Cassava is the fourth most important commodity after rice, wheat and maize, and is a basic diet of many millions of people (FAO and IFAD, 2000).

In addition to the economic value of the products and byproducts obtained from cassava, it offers other recognized advantages: tolerance of drought, capacity to produce considerable yield in degraded soil, resistant to insect pests and diseases, tolerance of acid soils (which are predominant in most of the world's tropical plains), and flexibility in planting and harvesting time (Bernardo and Hernan, 2012).

Despite its enormous production potential, adaptation to a great diversity of environments, its recognized tolerance of biotic and abiotic constraints to production, and its diversity of uses, cassava has not yet been managed to fully develop its potential in tropical agriculture due to numerous factors. Among the factors that constrained the production of cassava is lack of early maturing, high yielding and low hydrogen cyanide containing varieties.

According to FAO estimates, 276,721,584 tons of cassava were produced worldwide in 2013. Africa accounted for 57%, Asia for 32%, and others 11% of the total world production. In 2013, Nigeria produced 54 million tones making it the world's largest producer followed by Thailand, Indonesia and Brazil with 30.2, 23. 94 and 21.23 million tons, respectively. In terms of area harvested, a total of 20732192 hectares was planted with cassava throughout the world in 2013; about 64% of this was in sub-Saharan Africa. The average yield in this year was 11.3 tons per hectare, but this varied from 1.3 tons per hectare in Burkina Faso to 35 tons per hectare in India. In the largest producer, Nigeria, the average yield was 14 tons per hectare (FAOStat, 2013).

In Ethiopia, It is mainly cultivated by small resource poor farmers on smallholding plots of land. It is both a food security crop and a source of household income. It is increasingly becoming a source of industrial raw material for production of starch, ethanol, waxy starch, bio-plastics, glucose, bakery and confectionery products, glue, among others (Tesfaye et al., 2013). In Ethiopia, cassava generally is being grown in almost all parts of the country. But bulk of its production is situated in south, south western and western parts of the country.

The average total coverage and production of cassava per annum in Southern region of Ethiopia is 195055 hectares with the yield of 501278.5 tones indicating the average productivity of cassava in the country is not more than 25 ton per hectare (SNNPR, BoA, 2014). Which is by far lower than the yield obtained by other tropical countries such as Nigeria that recorded 35.00 tons per hectare per year (FAOStat, 2013)

In Ethiopia, most of the varieties produced were local farmers' varieties which are low yielding, late maturing, bitter type and containing high hydrogen cyanide (Anshebo et al., 2004). To alleviate these problems, a number of research activities focusing on crop variety improvement were conducted in different agroecological locations and two out performing varieties were released in 2005 (MoA, 2005). But the varieties were late maturing and the numbers were low to provide additional alternative to the farmers and increase genetic diversity. Hence evaluation of seven cassava clones including standard and one local check were conducted at different agroclimatic condition of the country. As a result, promising varieties with regard to storage root yield per a given period from a given area of land were obtained. Therefore, this paper aimed to show the performance of cassava clones under different agro ecological conditions of the country.

## MATERIALS AND METHODS

Evaluation of cassava clones for their storage root yield and other agronomic traits was conducted in four location of the country namely Hawassa, Amaro, Jima and Sekota. Two of the locations (Hawassa and Jima) are classified as potential areas for the production of cassava, whereas the other two arbitrary are classified as low moisture stressed dry land areas. The overall description of the locations is given in the Table 1.

A total of seven cassava clones (five introduced, one standard check, and one local farmers variety) were tested in the experiment. The experiment was arranged in randomized complete block design with three replications and conducted for two consecutive years, 2012-2014, except Jimma where only one season data was availed. Gross and net plot size where the experimental units were planted were 4 x 6 and 2 x 4 m, respectively. Storage root yield and other yield related data such as root length, root girth, number of roots per plants and growth rate were taken from the net plot at harvesting except the growth rate which was taken in three months interval from planting. Data on the root length, root girth, number of

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**Table 1.** Mean annual temperature, rainfall, altitude, longitude and latitude of Hawassa, Amaro, Sekota and Jima.

| Locations | Mean annual Temptarure (°C) | | | Mean annual rainfall (mm) | Altitude (masl) | Longitude | Latitude |
|---|---|---|---|---|---|---|---|
| | Minimum | Maximum | Mean | | | | |
| Hawassa | 7.2 | 33.5 | 20.35 | 1024.2 | 1708 | 38° 28' 34"E | 7° 3' 43" N |
| Amaro | 12 | 25 | 19.5 | 800 | 1477 | 37°32"10': 38°E | 5° 3" 55': 60N |
| Jima* | 11.3 | 26 | 18.65 | 1597 | 1753 | $36^0$ E | $7^0$46' |
| Sekota | 28 | 40 | 34 | 474.5 | 1300 | $38^o$ $58^'$ 50" E | $13^o$ $14^'$ $06^{''}$ N |

*Nebyu (2006).

**Table 2.** Analysis of variance.

| Variable | df | SS | MS | MSe | F value | Pr > F |
|---|---|---|---|---|---|---|
| RD | 6 | 26.3908779 | 4.3984796 | 0.7283698 | 6.04** | <.0001 |
| RL | 6 | 982.246564 | 163.707761 | 52.22382 | 3.13** | 0.0079 |
| MRYT | 6 | 1721.069671 | 286.844945 | 62.95768 | 4.56** | 0.0005 |
| UNRYT | 6 | 98.268046 | 16.378008 | 9.875142 | $1.66^{NS}$ | 0.1411 |
| TRYT | 6 | 1994.189061 | 332.364844 | 92.76680 | 3.58** | 0.0032 |
| RNPP | 6 | 73.1054296 | 12.1842383 | 4.048105 | 3.01* | 0.0102 |

*= Significant at α value less than 0.05, **= highly significant at α value less than 0.01; RD= average root girth (cm), RL=root length (cm), MRYT= marketable root yield per hectare (tones), UNRYT = unmarketable root yield per hectare (tone), TRYT= marketable root yield per hectare (tone), RNPP=average number of roots per plant.

roots per plants and growth rate were taken from randomly selected five plants. Whereas, the storage root yield data was taken from each plot in kilogram and converted into yield per hectare in ton by using the following formula:

$$\text{Yield per hectare} = \frac{\text{Yield per plot (kg) x 10000 m}^2}{\text{8 m}^2 \text{ x 1000 (kg/t)}}$$

Note that 1 ton(t) is equals to 1000 kg.

Storage root yield was clustered into marketable, unmarketable and total yield. Marketable storage roots yield was referred to the yield of those roots weighting 100-500 g, storage roots not infected by disease and infested by insect pests, whereas unmarketable storage root yield was referred to those roots weighting more than 500 g and less than 100 g, storage roots infected by disease and infested by insect pests and miss shaped rots. Total storage root yield was the sum total of marketable and unmarketable root yields.

The dry matter content of the clones were taken after oven drying for 24 h at 110°C for consecutive dates until the weight was constant. The clones were planted by using 1 x 1 m plant and row spacing. The spacing between plots were 2 m, whereas, the space between reps were 3 m. Before carrying out the combined analysis, homogeneity of variances test for total storage root yield across locations and years was conducted by using Levene's, Welch's and Bartlet's tests. The collected data were analyzed by using SAS statistical software, 2002 version 9 and IRRISTAT statistical software, 2007.

## RESULTS

### Analysis of variances

Cassava clones performed differently at different environmental conditions. But the combined analysis of variance table indicated that there was highly statistically significant difference among clones (P<0.01) for their root girth (RD), root length (RL), marketable and total storage root yield. There was also statistically significant difference among clones for their number of storage roots per plant (RNPP). But there was no statistically significant difference among the clones for their unmarketable yield (Table 2). The test for equality of variances showed no significant difference for all Levene's, Weltch's and bartlett's test at p value ≤0.05 (Table 3).

### Storage root yield and yield related components

As far as storage root performance is concerned, the highest marketable and total storage yield was obtained from the clone AWC-1 followed by AWC-2 and AWC-5. The least score was recorded from local cassava farmers' variety followed by kello (the standard check) and AWC-4 in the increasing order. The local varieties showed the largest storage root diameter but there was no statistically significant difference among the clones for this particular trait. The cassava variety Kello followed by the clone AWC-5 and AWC-2 gave the highest storage length, 40.67, 38.25 and 37.77 cm respectively. The highest number of roots per plant was recorded from the clone AWC-2 next to AWC-5 (Table 4).

All cassava clones have higher yield advantage over both the local and standard checks. The clone AWC-1

**Table 3.** Test for homogeneity of variances.

| Source | DF | Sum of squares | Mean square | F value | Pr > F |
|---|---|---|---|---|---|
| **Levene's test for TRYT** | | | | | |
| trt | 6 | 3.82E+09 | 6.37E+08 | 1.48 | 0.189 |
| Error | 134 | 5.77E+10 | 4.30E+08 | | |
| **Welch's ANOVA for TRYT** | | | | | |
| Source | DF | F Value | Pr > F | | |
| trt | 6 | 1.91 | 0.0941 | | |
| Error | 57.64 | | | | |
| **Bartlett test for equality of variances TRYT** | | | | | |
| CHISQ | | | | Pvalue | |
| 8.6778491 | | | | 0.1925226 | |

**Table 4.** Storage root yield of cassava clones combined over location.

| Cassava clones | Marketable storage root yield (t/ha) | Unmarketable storage root yield (t/ha) | Total root storage yield (t/ha) | RD (cm) | RL (cm) | RNPP |
|---|---|---|---|---|---|---|
| AWC-1 | 31.85 | 5.322 | 37.17 | 4.831 | 32.69 | 6.879 |
| AWC-2 | 30.89 | 5.047 | 35.52 | 4.524 | 37.77 | 8.115 |
| AWC-3 | 28.63 | 4.04 | 32.67 | 4.826 | 33.81 | 7.873 |
| AWC-4 | 26.31 | 2.996 | 29.31 | 4.751 | 35.55 | 6.517 |
| AWC-5 | 30.94 | 4.591 | 35.51 | 4.557 | 38.25 | 8.515 |
| Kello | 23.86 | 4.674 | 28.53 | 4.756 | 40.67 | 7.45 |
| Local | 20.3 | 4.416 | 24.72 | 6.059 | 38.26 | 6.59 |
| CV(%) | 29.69 | 65.75 | 32.29 | 17.59 | 19.71 | 26.99 |
| LSD | 5.35 | 1.72 | 6.09 | 0.5383 | 4.55 | 1.27 |

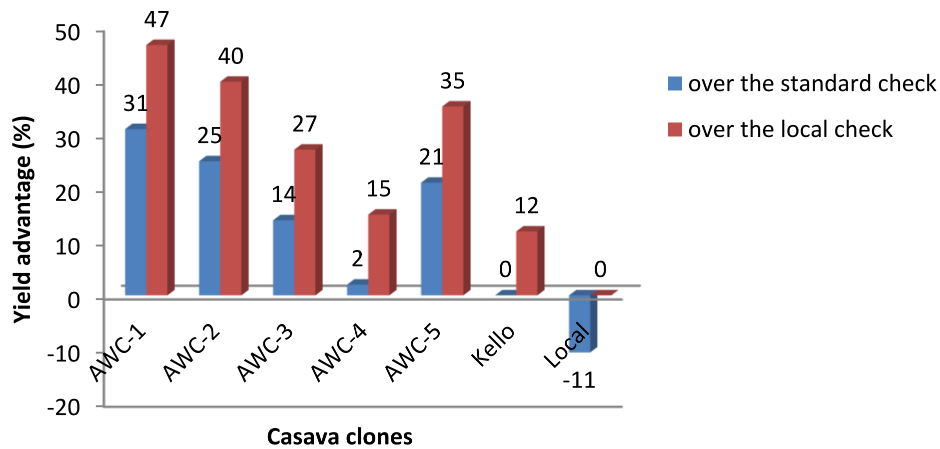


**Figure 1.** Yield advantage of cassava clones over the standard and local checks.

has 47 and 31% yield advantage over the local and the standard check followed by the clone AWC-2 which recorded 40 and 25% yield advantage over the local and standard checks, respectively. However, the yield advantage of the clone AWC-4 over the local and the standard checks was very minimal. Similarly, the yield of standard check was better than the average performance of the local farmer varieties (Local Checks). The yield advantage of the local check over the standard check was -11 as opposed the standard check over the local checks which recorded 12% yield increment (Figure 1).

The combined analysis result of the dry matter content

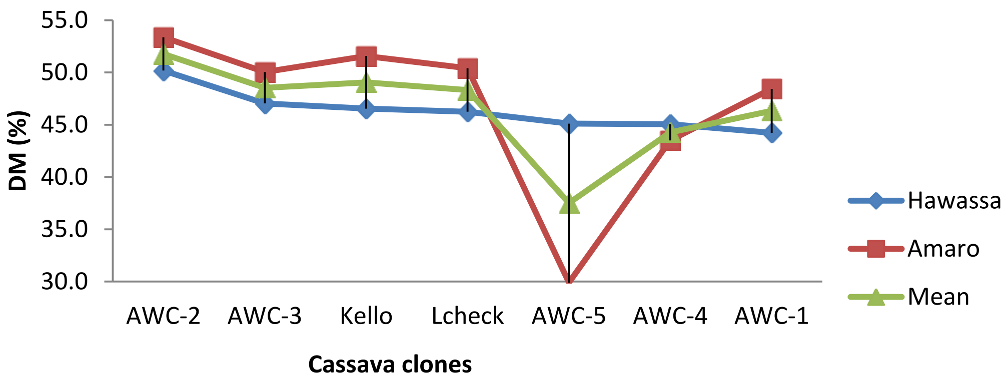


**Figure 2.** Percent dry matter content of storage root over location.

**Table 5.** Percent dry matter content of cassava clones tested across locations.

| Cassava clones | Hawassa | Amaro | Mean |
|---|---|---|---|
| AWC-2 | 50.2 | 53.4 | **51.8** |
| AWC-3 | 47.0 | 50.0 | 48.5 |
| Kello | 46.5 | 51.6 | 49.1 |
| Lcheck | 46.2 | 50.4 | 48.3 |
| AWC-5 | 45.1 | 29.9 | 37.5 |
| AWC-4 | 45.0 | 43.5 | 44.3 |
| AWC-1 | 44.2 | 48.4 | 46.3 |
| LSD | NS* | 20.3 | 10.5 |
| CV | 10.58 | 24.45 | 18.9 |

*NS= Non-significant.

of storage root of cassava clones under investigation showed the presence of statistically significant differences. The clone AWC-2 gave highest dry matter content followed by the clones AWC-3 and the standard check (Kello) (Table 5). The dry matter content also varies with the locations where the experiment was conducted as it there was no statistically significant difference among cassava clones tested at Hawassa as opposed the values obtained from Amaro (Figure 2).

## Characters' association

The correlation coefficient of most of the traits indicated positive and significant association among each other with some exceptions. Cassava storage root diameter is significantly correlated with root length (r=0.24), marketable root yield (r=0.50) and total storage root yield (r=53). But it is statistically not significantly correlated with unmarketable storage root yield and number of roots per plant. Root length was also significantly positively correlated with marketable and total storage root yields with r value of 0.40 and 0.25, respectively. In the contrary, it was negatively but significantly correlated with unmarketable storage root yield (r=-0.34) and not statistically significantly correlated with number of roots per plant. Even though marketable and unmarketable storage root yields were not statistically correlated with each other, both of them were significantly and positively correlated with total storage root yield with correlation coefficient of 0.92 and 0.41, respectively. The total storage root yield was also positively significantly correlated with root numbers per plant with r=0.41.6. It was also positively and significantly correlated with leaf yield per plant. Root weight per plant was positively and significantly correlated with dry matter content and leaf yield per plant (Table 6).

## Yield stability of cassava clones across locations

As indicated in Table 7, the performance of cassava clones tested across locations varied with agroclimatic conditions. The clone AWC-1 gave the top total storage yield (tons)/hectare at Hawassa and Amaro locations. At Sekota, the other clone, AWC-2 gave the highest storage root yield and at Jimma, the clone AWC-5 followed by the clone AWC-2 gave the highest yield (Table 7). As far as the location performance is concerned, the best mean yield of the two seasons average indicated that the location Amaro is the best area for cassava production followed by Hawassa. The value of Jimma is higher but it was the average of only one season so was not included in the comparison. The additive main effects and multiplicative interaction (AMMI) stability analysis of seven genotypes on seven environments also indicated the variability of performance of cassava clones under different environmental condition (Table 8). The clones AWC-1 and AWC-5 possessed wider adaptability as they were found close to the origin. In the contrary, farmer varieties (local checks) were able to adapt to specific environmental conditions which is far from the origin of the plot.

The interaction principal component axis (IPCA) score

**Table 6.** Pearson correlation coefficients of storage root yield and yield component of cassava clones.

| Variables | RD | RL | MRYT | UNRYT | TRYT | RNPP | RWPP | DM | LYPP |
|---|---|---|---|---|---|---|---|---|---|
| RD | 1 | 0.34337 | 0.5053 | 0.08437 | 0.492 | 0.089 | 0.557 | 0.08 | 0.377 |
| | | 0.0036 | <0.0001 | 0.4874 | <0.0001 | 0.465 | <0.0001 | 0.508 | 0.001 |
| RL | | 1 | 0.4251 | -0.40108 | 0.235 | -0.113 | 0.072 | -0.481 | 0.547 |
| | | | 0.0002 | 0.0006 | 0.05 | 0.35 | 0.551 | <.0001 | <0.0001 |
| MRYT | | | 1 | 0.03987 | 0.926 | 0.267 | 0.486 | -0.288 | 0.503 |
| | | | | 0.7431 | <0.0001 | 0.025 | <.0001 | 0.016 | <0.0001 |
| UNRYT | | | | 1 | 0.415 | 0.457 | 0.337 | 0.652 | -0.41 |
| | | | | | 4E-04 | <0.0001 | 0.004 | <0.0001 | 4E-04 |
| TRYT | | | | | 1 | 0.416 | 0.57 | -0.016 | 0.303 |
| | | | | | | 3E-04 | <0.0001 | 0.899 | 0.011 |
| RNPP | | | | | | 1 | 0.508 | 0.046 | -0.145 |
| | | | | | | | <0.0001 | 0.704 | 0.231 |
| RWPP | | | | | | | 1 | 0.312 | 0.037 |
| | | | | | | | | 0.009 | 0.76 |
| DM | | | | | | | | 1 | -0.514 |
| | | | | | | | | | <0.0001 |
| LYPP | | | | | | | | | 1 |

also indicated the stability of a clone across environments. The more the IPCA approximate zero, the more stable the clone is over all the environments tested. According to IPCA1 (Table 7), clone AWC-1 and AWC-5 had approximately zero score (0.1 and 0.27, respectively) and hence could be considered as most stable clones.

## DISCUSSION

### Storage root yield and yield related components

The highest total storage root yield obtained from the clone AWC-1 (37.17 t/ha), AWC-2 (35.52 t/ha) and AWC-5 (35.51 t/ha) was by far higher than the yield obtained from most of cassava growing countries in the world in general and East Africa in particular. FAOstat 2013 indicated that the average yield obtained from India, China, Brazil and Nigeria was 34.96, 24.55, 13.92 and 14.03 tons per hectare per year. Average storage yield obtained from East African countries such as Kenya, Uganda and Tanzania in 2013 was 15.89, 12.02, 7.50 tons per hectare, respectively. Differences in cassava tuber yield are determined by several factors, such as number of tubers, tuber length and tuber weight per plant. Ntawuruhunga and Dixon (2010) concluded that storage root number, storage root size and storage root diameter were the main yield components contributing to yield enhancement in cassava.

As far as yield related traits are concerned, the value obtained directly concedes with the report of Kenneth (2011). In his study, the highest storage root length obtained from the variety, Cuban White Stick was 40.46 cm. In the same way, the highest storage root number per plant was obtained from the variety John LaMotte (7.78) which is similar to the value recorded from the current study (8.52).

### Characters' association

The total storage root yield was positively significantly correlated with root numbers per plant, storage root length, storage root weight/plant, leaf yield per plant but negatively correlated with dry matter content. This show those traits which are positively and significantly correlated with storage root yield were important components of yield across locations. The current study finding is also in line with the report made by Ntawuruhunga et al. (2001). They indicated that storage root weight (r=0.53) and storage root number (r=0.45) are the main component of total yield per a given area per a given time. Dry matter content was negatively correlated with storage root weight, suggesting that when the storage root weight is high, the dry matter content tends to be low which is in line with the study conducted by Kenneth (2011).

### Yield stability of cassava clones across locations

The AMMI and the IPCA scores indicated the clones AWC-1 and AWC-5 seams possessed wider adaptability as they are found near the origin. In the contrary, farmer varieties (local checks) were able to adapt specific environmental conditions which is far from the origin of

**Table 7.** Mean total storage yield (ton/ha) clone ranks in seven environments.

| Clone name | Environments | | | | | | | | | | | | | | TRT means | Rank | IPCA1 SCORE | IPCA2 SCORE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Hawassa1 | Rank | Hawassa2 | Rank | Amaro1 | Rank | Amaro2 | Rank | Jima1 | Rank | Sekota1 | Rank | Sekota2 | Rank | | | | |
| AWC-1 | 460.4 | 1 | 456.2 | 1 | 416.7 | 1 | 444.6 | 3 | 351.2 | 5 | 238 | 3 | 210.8 | 3 | 368.3 | 1 | -0.10 | 0.10 |
| AWC-2 | 299.2 | 5 | 367.5 | 3 | 289.2 | 3 | 507.9 | 1 | 478.9 | 2 | 241 | 2 | 274.3 | 1 | 351.1 | 2 | 0.49 | -0.75 |
| AWC-3 | 320.8 | 3 | 327.9 | 5 | 257.9 | 5 | 474.6 | 2 | 453.1 | 3 | 190.4 | 5 | 210.7 | 4 | 319.3 | 4 | 0.37 | 0.81 |
| AWC-4 | 281.2 | 6 | 250.4 | 6 | 269.2 | 4 | 410.8 | 4 | 352.1 | 4 | 232.3 | 4 | 225.9 | 2 | 288.9 | 5 | 0.92 | -0.55 |
| AWC-5 | 352.1 | 2 | 416.7 | 2 | 236.7 | 6 | 396.2 | 6 | 531.3 | 1 | 247 | 1 | 196.2 | 6 | 339.4 | 3 | 0.27 | 0.11 |
| Kello | 318.8 | 4 | 347.9 | 4 | 326.2 | 2 | 302.9 | 7 | 285.9 | 7 | 182.6 | 6 | 202.3 | 6 | 281 | 6 | 0.69 | -0.46 |
| Lcheck | 156.2 | 7 | 161.7 | 7 | 179.2 | 7 | 407.5 | 5 | 351 | 6 | | | | | 251.1 | 7 | -0.88 | -0.98 |
| Environment mean | 312.67 | | 332.61 | | 282.16 | | 420.64 | | 400.50 | | 221.88 | | 220.03 | | 314.16 | | | |
| IPCA1 | -0.67 | | -0.55 | | -0.70 | | 0.71 | | 0.10 | | 0.95 | | 0.12 | | | | | |
| IPCA2 | 0.25 | | 0.70 | | -0.53 | | -0.56 | | 0.64 | | -0.83 | | -0.41 | | | | | |

**Table 8.** Analysis of variance for the AMMI model.

| Source | D.F. | S.S. | M.S. | F | FPROB |
|---|---|---|---|---|---|
| Treatments | 6 | 98987.1 | 16497.8 | | |
| Locations | 6 | 277624. | 46270.6 | | |
| Treatment x sites | 34 | 123804. | 3641.29 | | |
| AMMI component 1 | 11 | 78078.0 | 7098.00 | 3.570 | 0.005 |
| AMMI component 2 | 9 | 30019.5 | 3335.50 | 2.973 | 0.033 |
| AMMI component 3 | 7 | 10469.3 | 1495.61 | 1.999 | 0.190 |
| AMMI component 4 | 5 | 3374.80 | 674.960 | 0.725 | 0.666 |
| GXE residual | 2 | 1862.41 | | | |
| Total | 46 | 467901. | | | |

the plot and larger absolute value scores of IPCA (Figure 3). This indicates that the clones, AWC-1 and AWC-5 are not affected by environmental conditions. In the same way, a variety performance trial was conducted in Indonesia by using 15 genotypes at different range of altitudes, some of the clones' storage root yield was stable across locations. The clone Malang 4 (G3) and CMM 03038-7 (G8) are adaptive clones to environment at medium altitude of up to 800 masl (Noerwijati and Budionob, 2015). Noerwijati et al. (2014) indicated that environment gives the most effect (64.69%), followed by genotype-by-environment interaction effect (6.53%), and genotype effect (4.94%) on performance of a given genotype of cassava. He also indicated the most stable cassava genotype by using that GGE biplot with high yield which is in line with this study, showing the possibility of obtaining the most stable varieties across locations.

## CONCLUSION AND RECOMMENDATIONS

The clone Awc-1 gave the highest yield, followed by AWC-2 when compared with standard and local checks. As far as the yield advantage over

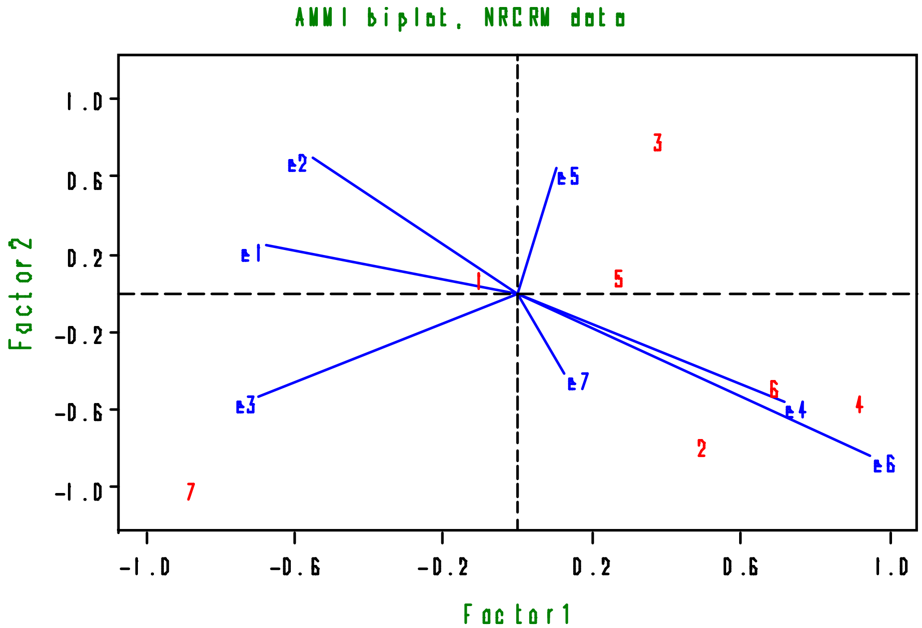


**Figure 3.** AMII biplot of seven environments and seven varieties.

the standard and local checks is concerned, the clone AWC-1 have 47 and 31% yield advantage over the local and the standard check followed by the clone AWC-2. Three clones (AWC-1, AWC-2 and AWC-3) have statistically no significant difference for their dry matter content (more than 50%). Those clones having stable and higher root yield combined with higher dry matter content were proposed for variety verification trial for a wider dissemination as well as production. One of the most important problems of cassava production is the lack of early maturing varieties. In Ethiopia, cassava generally grows in almost all parts of the country. But bulk of its production is in south, south western and western parts of the country. Most of the varieties produced were local farmers' varieties which are low yielding, late maturing, bitter type and containing high hydrogen cyanide. The existing improved and farmers' varieties take more than 18 months for full maturity. Therefore, continuous breeding and selection program is required to fill the gap due to the production of late maturing varieties.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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# Guidelines for sustainable irrigation system design and management in sub-Saharan Africa

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**Despite the fact that the economies of most countries in sub-Saharan Africa (SSA) are dominated by the agriculture sector, the productivity of the sector is still very low. One of the factors responsible for this is the fact that agriculture in SSA is mainly rain fed despite the abundant water resources in the region. Many attempts have been made in SSA to expand irrigation but most of these efforts have not been successful. The goal of this study was therefore to learn from the few irrigation success stories in SSA, and propose guidelines for sustainable irrigation development. This will thereby contribute to helping farmers adapt better to climate change, increase productivity, ensure food security and consequently eliminate hunger and poverty. The major findings of the study are that irrigation designs should be participatory, tuned to the farmers' objectives, seek to maximize water use efficiency with priority given to small-scale irrigation systems, and ensure that water supply is reliable. Furthermore, designs should provide opportunities for farmers to grow a variety of crops to ensure both food security and income generation. In addition, farmers should make a significant contribution in the implementation of irrigation projects as well as have significant management responsibility for the completed system which will increase their sense of ownership and commitment. Finally, it is crucial to create an enabling environment for irrigation development by: facilitating access of farmers to water, land, credits, other agricultural inputs, and markets; building capacity of all key stakeholders in irrigation development and facilitating the creation of functional water users associations.**

**Key words:** Successful irrigation development, design considerations, management constraints.

## INTRODUCTION

The agricultural sector is very important in sub Saharan Africa (SSA) and the economies of most countries in the region are dominated by this sector. According to UNCTAD (2016), the sector employed about 36.7% of the total workforce in SSA in 2015. Furthermore, in many countries, over 50% of the workforce is engaged in agriculture with a proportion of over 90% in some countries like Burundi. About 17% of the gross domestic product (GDP) in the region is generated by the agricultural sector (The World Bank, 2016). In some countries, agriculture generates up to 50% of the GDP, contributes over 80% of trade in value and more than 50% of raw materials to industries (FAO and UNIDO, 2008).

Despite this importance, the productivity of the sector is

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low as compared to that of other developing regions in the world. For example, yields of cereals, a staple in many parts of SSA is only about 36% of that in Latin America and Caribbean and about 48% of the yields in South Asia (The World Bank, 2016). One of the factors responsible for the low productivity in SSA is the fact that agriculture is mainly rain fed. The World Bank (2016) estimates that in 2014, only about 3% of the internal renewable fresh water resources in SSA was withdrawn for all purposes. This suggests there is abundant water resources but despite this, in 2012 only about 3% of the arable land was irrigated in SSA (FAO, 2016a). This is very low as compared to Asia where the proportion of arable land that is irrigated varies from 33 to 42% and the global average proportion of arable land irrigated of 21% (FAO, 2016a). The sub-region in SSA with the lowest percentage of arable land irrigated is central Africa with 0.6%, while the highest is southern Africa with about 7%. This indicates that there is an enormous irrigation potential in SSA. FAO (2016b) has concluded that the greatest potential for expanding irrigated agriculture in the world, considering both land and water resources, is in the sub-Saharan Africa region, where only one fifth of the potential irrigable area has been developed.

Irrigated croplands have much higher yields than rain fed croplands. It is estimated that irrigated agriculture represents 20% of the total cultivated land in the world, but contributes 40% of the total food produced worldwide (FAO, 2016b). Irrigation is therefore a key input in ensuring food security in the world. Siebert and Döll (2010) estimated that global cereal production would decrease by 20% without irrigation, while Neumann et al. (2011) concluded that climate change and population growth will further enhance the role of irrigation in the future. As such, greater use of irrigation in SSA, will increase food production, help farmers adapt to climate change, play an important role in farmers transitioning from subsistence to commercial farming and ensure food security as well as reduce poverty (Tefera and Cho, 2017; Eneyew et al., 2014). FAO (2016b) documented that irrigation in conjunction with high-yielding varieties; inputs such as fertilizers and pesticides; and the use of agricultural machinery played a significant role in the green revolution in Asia. This is because, irrigation leads to the intensification of agriculture and can result to year round production. Without irrigation, farmers engaged in market gardening can grow only one crop a year whereas with irrigation, they can grow two or three crops a year.

Over the years, many attempts have been made in SSA to expand irrigation (Kadigi et al., 2012; Namara et al., 2011; Inocencio et al., 2007; Drechsel et al., 2006; Morardet et al., 2005; Fonteh, 1998). The fact that the extent of irrigation is still very small in the region indicates that most of these efforts have not been successful, leading to the perception that irrigation systems in SSA have been a failure. Hence, there is reluctance by investors to expand irrigated areas. The reality however is that there has been success stories as well as failures and that we can learn from the past to develop a more sustainable future.

The goal of this study was therefore to develop guidelines for sustainable irrigation development in SSA that can contribute to expanding irrigation, increase productivity of farmers to ensure food security. This can make a significant contribution towards the attainment of the first five sustainable development goals (UNDP, 2017) related to poverty, hunger, health, education and gender equality. Specifically, the study sought to identify critical design and management issues of successful irrigation projects in SSA, identify constraints to irrigation development in the region resulting in poor performance; and finally to recommend a way forward that can ensure that irrigation development in SSA is sustainable.

The methodology used in this paper was based on a systematic and critical review of published and unpublished literature on irrigation development in sub-Saharan Africa in particular and in other developing regions of the world in general.

## KEY DESIGN CONSIDERATIONS FOR SUCCESSFUL IRRIGATION DEVELOPMENT

The following issues which have a major bearing on the success of an irrigation system will be discussed in turn. These are: the adoptability of the system; the economic feasibility; the performance and management of the system, the sustainability and finally the enabling environment.

### Adoptability of the system

Irrigation is a socio-technical system. The technical aspects of irrigation are easier to solve but the social aspects are more complex and difficult. For example, it is much easier to design and construct an irrigation system than ensuring it functions well. Issues to resolve in the social aspects include, efficient distribution of water and with equity, maintenance of the system and managing conflicts. The social dimensions of irrigation are as important as the technical dimensions but they are often not adequately taken into consideration resulting in the non-adoption of some irrigation systems.

Three factors influence the adoption of water management technologies (Fonteh and Ajaga, 1998). Firstly, the cost from the point of view of affordability by the users not only to acquire, but also to operate and maintain the technology. Secondly, for a technology to be adoptable and sustainable, it should fit neatly into the social structure of the users and the community. As such designers should tune the objectives of the system to that of the farmers and not the reverse (Ubels and Horst, 1993). This approach results in designs that are flexible.

Thirdly, the nature of the technology. There are many aspects to this. Is it technically appropriate, that is, doing what is supposed to be done. According to Rogers (1962), the factors of the technology itself that influence its adoption are; its divisibility, its availability to potential users, relative advantage over other technologies, compatibility with existing value system and the complexity of the technology.

To ensure adoption of an irrigation system, it is critical to maximize the participation of farmers in all stages in the design. This is important in the selection of the crop to be grown, type of system to use, layout of plots, tuning the design to their social and cultural environment, designing so that operation/maintenance suits their capacity, etc. Participatory design also ensures that designs are based on informed decision, making use of valuable local information especially as many designs in SSA are often carried out with limited reliable official data.

Many studies have highlighted the need for participatory irrigation design as the key to ensuring adoptability. For example, a study by Morardet et al. (2005) concluded that if designs do not take into account actual on-the-ground operational and management capabilities of farmers, this can seriously affect the performance of irrigation systems. Merrett (2002) on his part stated that the most essential stakeholder in an irrigation system is the farmer, who, if not properly integrated in the system development, may not feel obliged to play his/her role effectively, thus jeopardizing the sustainability to the system.

Farmers, as a possible source of system design input, are still too often ignored by engineers, and as a result systems are often inappropriately designed. Giordano et al. (2012) reported that the designs that will have the best impacts are those taken into account, at the planning stage, the livelihood contexts in which smallholder farmers operate.

Understanding farmers' needs will ensure that actions to support them are beneficial. This requires involving farmers in the design process which usually results in flexible irrigation systems. Examples of flexible systems are: the use of individual low cost water lifting devices to pump water from shallow ground water or from rivers. The pump can be moved from one field to another to permit multi farm use. Another approach could be to pump water into storage tanks located in an irrigation area to enable farmers carry water from there to irrigate when they wish to irrigate. Flexible systems may also entail zoning, with farmers having plots in different zones with different soil types to facilitate growing a variety of crops.

## Economic feasibility

This depends on the cost of the system and the returns on the investments.

### *Cost of the system*

The cost is determined mainly by the scale of the irrigation and the type of irrigation system selected.

**Scale of irrigation:** Project size is the most important factor which determines unit investment cost of irrigation projects (Inocencio et al., 2007). Relatively large investment projects irrigating large areas have lower development costs/ha because they achieve significant economies of scale. Most of the irrigation systems in SSA can be grouped under two types; large-scale and small-scale. According to Carter (1989), small-scale irrigation (SSI) refers to irrigation of small plots in which private farmers have the controlling interests and using technology which they can effectively operate and maintain. The farm sizes usually do not exceed 0.2 ha. Large-scale irrigation usually refers to formal sector irrigation where the development and management is carried out in a structurally formal way, usually by the state or an agri-business concern. Large-scale irrigation systems therefore logically result in large investment projects which usually have lower development costs per unit area.

However, according to Inocencio et al. (2007), small-scale irrigation schemes offer significant performance advantages over large-scale systems within irrigation investment projects. A study by Giordano et al. (2012) concluded that smallholder farmers in sub-Saharan Africa and South Asia are increasingly using SSI to cultivate their land. Individually owned and operated irrigation technologies improve yields, reduce risks associated with climate variability and increase incomes. There is great potential for many more farmers to benefit from small-scale irrigation. In addition, in many African countries, water management by smallholders is already more important for irrigation than the public irrigation sector, in terms of the number of farmers involved, the area covered and the value of production. For example, in Ghana, private irrigation by smallholders employs 45 times more individuals and covers 25 times more land than public irrigation schemes (Namara et al., 2011). The increasing importance of SSI systems is because they are more flexible, can be more easily tuned to the farmer's context; are more adoptable and hence sustainable. SSI is intricately linked with poverty and gender issues. This is because most of the irrigation activities are carried out by women and disadvantaged groups. SSI is therefore pro poor and contributes to alleviating poverty and empowering women. In order to reduce the unit cost of irrigation development in SSA, and also have systems which perform very well, small-scale irrigation systems should be developed within the context of large-scale investment projects which have significant economies of scale and are cheaper. A large investment project comprising many small-scale systems will be able to justify recruiting qualified personnel (e.g. irrigation engineers and project managers), mobilizing the appropriate equipment for construction, and can invest sufficiently in the software components (good planning, design, project management, supervision, effective training, capacity building and institutional development among future users and managers).

Both small and large scale irrigation systems are required in SSA. According to Fonteh (1998), the key to their success is whether they are privately or publicly managed. This is because large scale systems which were performing poorly when they were state managed, started performing very well when they were privatized. Youa et al. (2011) also concluded that there is significant profitable irrigation potential for both small-scale and large-scale systems. However, if attainment of the first five SDG is an objective, then priority should be given to pro-poor SSI.

Therefore, to ensure sustainability and minimize development costs, large irrigation projects supporting many small scale irrigation systems should be designed and developed if there is demand and access to markets.

**Types of irrigation system:** There are two main types of irrigation systems in the world. These are surface irrigation (border, basin and furrow) and pressurized irrigation comprising sprinkler and drip irrigation. There is no universally technically "best" irrigation method for all situations (Burt et al., 2000). The selection of a technically appropriate method for a given context will depend upon such variables as: the soil type (infiltration rates, variability of soils and the available water content), the topography, the crop to be grown, climate conditions, local traditions and skills, the available water supply (quality, quantity and temporal distribution characteristics), and the support infrastructure for a selected technology.

In terms of worldwide use, pressurized irrigation accounts for about 14% of the total irrigated area (FAO, 2016b). This implies that surface irrigation systems account for about 85% of the total irrigated area. This domination of surface irrigation is partly due to the fact that when conditions permit and with abundant water resources, gravity operated surface methods are cheaper to construct if the construction of dams is not required. In addition, they are cheaper to maintain and operate. When surface methods are not suitable because of topographic and soil conditions, gravity operated pressurized systems are still desirable because the operation costs will be much lower. For example, in the Western Highlands of Cameroon, small scale gravity operated sprinkler systems have been use for many years, are very common and are sustainable (Ayangma, 1999; Lekeufack, 1998; Ngatchou, 1998;).

Inocencio et al. (2007) found that unit costs are significantly higher for river diversion systems with major storage capacity, and significantly lower for drainage/flood control systems as compared to simple river-diversion systems. In addition, the unit cost of irrigation projects implemented as part of multi-sectoral projects is lower than stand-alone irrigation projects.

The system selection process can be complex because there are many interrelated factors affecting the choice of methods which affects the cost. As such, irrigation systems should be designed by qualified irrigation engineers to ensure that they operate as expected.

### *Crops to be grown*

The feasibility/performance of an irrigation system greatly depends on the crops to be grown, which affects the return on investments. Expensive irrigation projects can be economically feasible if they are used to grow high value crops like fruits and vegetables. An analysis of irrigation systems in 50 different countries in the world suggests that systems designed for staple cereals, tend to have higher unit costs and lower performance (a partial exception is rice in some cases) than systems designed for such crops as fruit trees, vegetables and fodder (Inocencio et al., 2007). This is because the demand for irrigation infrastructure such as dams, reservoirs, sluices and canals, is greater for traditional staple crops than for non-cereal crops, which require much lighter infrastructure. Further, the price of cereals has been declining sharply since the mid-1980s, resulting in worsening profitability relative to crops such as fruits and vegetables, the demand for which has been increasing as economies have developed.

Since irrigation leads to intensification of agriculture with possibility of year round production; for irrigation to be sustainable, there should be access to reliable or dependable markets for agricultural products with guaranteed prices. This can be a major constraint, which, if overcome, may encourage farmers to intensify agricultural production through irrigation. The irrigation of high value market gardening crops is therefore very attractive especially in peri-urban areas due to the easy access to markets. Farmer managed surface and sprinkler irrigation systems have been very successful in the Western Highlands of Cameroon because farmers grow high value vegetables during the dry season as well as early season maize (Fonteh, 1998). In order to ensure both food security and income generation, irrigation systems should provide opportunities for farmers to grow a variety of crops on different soil types to minimize risk. Income generation crops should preferably be high value market gardening crops. This will provide the flexibility and assurance which farmers need.

## Performance and management of the system

### *Mode of operation and management of the irrigation system*

The performance of an irrigation system has been observed to be greatly affected by the mode of operation and management. Is the system managed by farmers themselves; by the state or by both the state and farmers? Inocencio et al. *(*2007) observed that systems in which operation and maintenance (O&M) is carried out by farmers performed best; followed by systems in which O&M was shared by government irrigation agencies and farmers through water users associations (WUAs). Systems where O&M was carried out only by government agencies had the least performance.

If farmers who are the most important stakeholders are to manage an irrigation system, then their involvement needs to be maximized so that designs are tuned to their social and cultural environment; and to suit their capacity to operate and maintain the system. In fact Kadigi et al. (2012) went as far as recommending that new irrigation systems should only be initiated in response to the demand from farmers because this increases the chances of local management and maintenance of the system. Stakeholder participation in the planning and decision making processes of irrigation systems is an effective way of developing systems that are most likely going to be sustainable. This should be initiated during the planning stage in order to win the support of various stakeholders.

Maximizing farmers' involvement in the development of their systems (consistent with their capacity) combined with farmers taking significant management responsibility for the completed system usually results in lower O&M costs and higher performance.

***Reliability of water supply***

For an irrigation system to be sustainable, there is need for a reliable water supply. When surface water supplies are used; to ensure a reliable water supply, adequate storage facilities are often needed which increases the cost of irrigation projects. The conjunctive use of surface water with limited storage facilities and ground water ensures a reliable water supply without major increases in the cost of the project and improves the performance of the project. Foster et al. (2010) defined conjunctive use as a situation where: both groundwater and surface water are developed to supply a given irrigation canal-command although not necessarily using both sources continuously over time nor providing each individual water user from both sources. Groundwater pumping for irrigation used in conjunction with surface water provides benefits that increase the water supply or mitigate undesirable fluctuations in the supply (Tsur, 1990) and also help to control shallow water table levels and consequent soil salinity.

A study by Inocencio et al. (2007) revealed that projects which adopted conjunctive use of surface and ground water performed better than otherwise. As such, wherever conditions are favorable, the design of irrigation schemes should provide for conjunctive use of surface water and groundwater to ensure the reliability of water supply.

***Organization of farmers***

Irrigation of farmer-managed systems requires an elaborate network of canals, sometimes passing through farmers' fields. There is therefore a need to manage the water and the system collectively and this requires functional WUAs. According to Sagardoy et al. (1986), water users associations or specialized water management organizations can be defined as social organizations aimed at an appropriate use of water for irrigation purposes among the farmers of a community. The term "appropriate" is used here to designate a timely and equitable distribution of the water. The main functions of WUAs are: the operation of the irrigation and drainage systems, the maintenance of the systems, and the assessment and collection of water charges. The management of WUAs vary depending on the scale of the irrigation. For example in Cameroon, they are either managed by farmers or there is mixed control between farmers and the state (Fonteh, 2011). Small-scale irrigation systems are usually managed mainly by farmers, while large-scale public owned surface irrigated systems usually have mixed control.

Irrigation system design should therefore include software components whose aim is to facilitate the creation of functional WUAs. This is because farmers are better able to: enforce their own rules relating to the collection of levies; maintain and repair infrastructure; ensure equitable distribution of water; and manage conflicts amongst themselves.

***Contribution of farmers to project development***

It has been observed that in general, rural development projects in which the primary stakeholders contribute in the implementation of the project perform better than otherwise. A study in Tanzania by Temba (2015) concluded that when farmers contribute towards the development of an irrigation system, the performance of the system is enhanced. It was also noted that the higher the contribution provided by farmers, the greater the performance of the irrigation system. The contribution could be in form of resource mobilization, material contribution, setting standards for monitoring the project success, collaborative partnership, consultation and information giving. This is because there is a greater sense of ownership and commitment to the project.

**Sustainability**

Sustainability concept, when viewed within the context of SSI development generally refers to the long-term ability

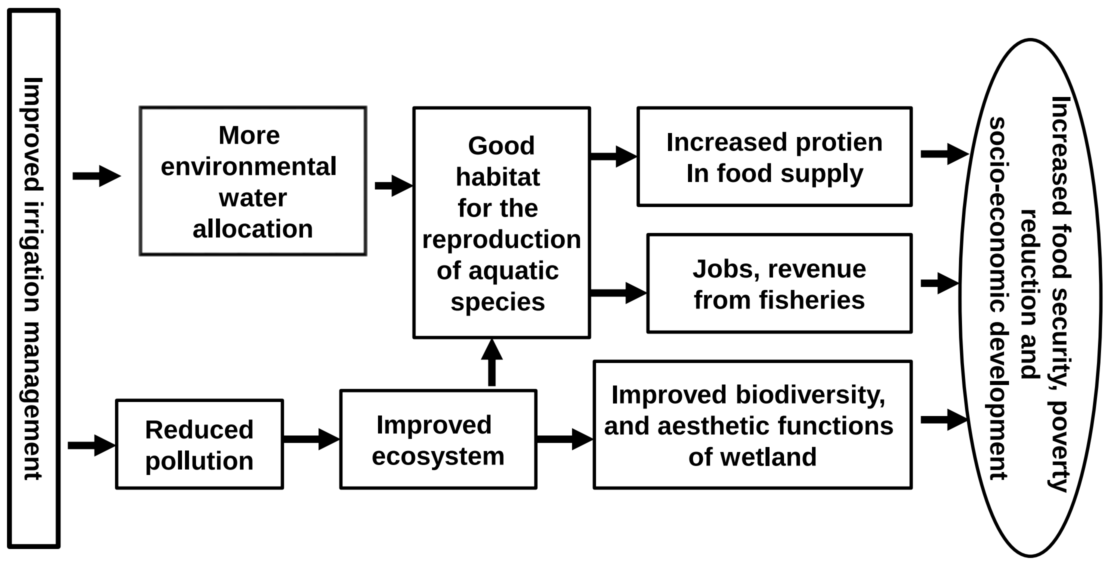


**Figure 1.** Effect of efficient irrigation water management on environmental stability, poverty reduction and socio-economic development.

of the beneficiaries to operate and maintain their schemes profitability with little or no external intervention other than the normal extension services (Giordano et al., 2012). In this light, there are two main aspects to SSI sustainability: socio-economic and environmental sustainability.

### *Socio-economic sustainability*

This is often ensured when farmers adopt a new technology because it is appropriate from the technical, social and economic points of view. SSI systems have been noted to be more sustainable from the social and economic points of view. For example, in West Africa, the urban population is now greater than the rural population and informal irrigation systems in urban and peri-urban areas are therefore taking advantage of the growing urban markets and the inadequate refrigerated transportation and storage infrastructure, to complement rural agriculture in feeding the cities with fresh vegetables (Drechsel et al., 2006). As compared to farmers involved in traditional rain fed agriculture, farmers involved in peri-urban year-round irrigated farming can earn more than twice their income despite having much smaller farm sizes. This suggests that SSI systems are more socially and economically sustainable because they are appropriate.

According to Chancellor and Hide (1997), the design of an irrigation system is one of the main issues that affects socio-economic sustainability in smallholder schemes. The major issues that need special consideration are:

1. Installation,
2. Operation and maintenance costs,
3. Mechanisms to achieve efficient and equitable water allocation and
4. The control of water losses.

### *Environmental*

Irrigation uses about 80% of all fresh water abstracted in SSA (FAO, 2016b). This can have negative impacts on the ecosystem by reducing the environmental water allocation. UN-Water (2006) estimated that the average application efficiency of irrigation in developing countries was 38% and that in addition, between 30 to 40% of water is lost in water distribution systems. Excessive withdrawals of water for irrigation can lead to: inadequate water available to meet environmental water requirements resulting in the degradation of the ecosystem; falling water tables which can lead to seawater intrusion in coastal areas, thereby polluting groundwater resources; pollution of both groundwater and surface water resources etc. Irrigation system design should therefore seek to maximize water use efficiency to ensure environmental stability which, as shown on Figure 1 also contributes to the alleviation of poverty and socio-economic development. If possible, designs should consider using new technologies which have better application efficiencies like sprinklers and low cost drip irrigation systems.

### Enabling environment

Irrigation will only thrive if there is an enabling environment. For irrigation to be sustainable, it has to be carried out within the context of an overall agricultural development strategy, which itself must fit within a rural development and a broad national development strategy. These will ensure that farmers will have access to secure land and water; access to extension services, access to loans at preferential interest rates, availability of inputs like improved seeds, fertilizers and access to markets (roads, fair price for products and demand for products).

## CONSTRAINTS TO SUSTAINABLE IRRIGATION DEVELOPMENT IN SSA

Below are some challenges confronting the development of irrigation in SSA. Measures have to be taken to overcome them before irrigation development can flourish in SSA in a sustainable manner. These constraints were identified based on findings from various parts of SSA.

1. The none-appropriateness of some systems mainly because farmers were not involved in the design and the design objectives were different from the farmers' objectives. As a result, systems do not function as well as intended because farmers operate them differently from what was planned.
2. Poorly designed and managed irrigation systems because of the limited number of local specialists in the domain resulting in systems being designed and constructed by amateurs. A study in Ghana by Namara et al. (2011) highlighted the need for competent irrigation engineers in the design of small-scale irrigation projects. The study revealed that inadequate planning and faulty design are sometimes the culprit behind the poor performance of irrigation systems. In addition, inadequate facilities and design problems are often major reasons behind inadequate distribution of water from a source to farmers' fields. Poor designs result in inefficient technologies which Ofosu et al. (2014) identified as a vital factor for successful irrigation development in SSA.
3. Inadequate capacity of farmers in irrigation management because irrigation is new in many communities and also due to poor extension services (Labassou, 2005; Tekeubeng, 1999; Ayangma, 1999; Lekeufack, 1998; Ngatchou, 1998). As such most farmers do not know when to irrigate and how much water to apply. A study by Tafesse (2003) concluded that limited knowledge in agricultural water management was one of the main constraints to irrigation development in Ethiopia, Ghana, Kenya, Tanzania and Zambia. This is consistent with the findings of Eneyew et al. (2014) in Ethiopia; and Fanadzo (2012) and Machethe et al. (2004) in the Republic of South Africa who identified inadequate capacity as an important determinant of poor irrigation system performance.
4. Poor organization of farmers. This is partly responsible for poor maintenance and repairs of irrigation infrastructure and equipment. For example in the Central African sub-region, there are very few veritable irrigation WUAs (FAO-SFC, 2009).
5. Absence of monitoring, evaluation and feedback systems. This leads to reduced management responsiveness and lack of data to improve the systems.
6. High cost of irrigation development. Inocencio et al. (2007) concluded that between the year 1965-2000, the average construction cost of irrigation systems in SSA was US$ 14,500/ha while in non-SSA countries, it was US$ 6,600/ha. However, an analysis of irrigation projects in SSA which are deemed successful indicated that these are not more expensive than in other regions of the world.
7. Poor enabling environment. This is manifested by the following: poor or non-existent extension services in irrigation due to inadequate financial and human resources; land tenure problems which makes farmers hesitant to invest in water management infrastructure; inadequate political will and commitment leading to insufficient investments in agriculture in general and irrigation in particular; poor access to credits and poor access of farmers to markets.

A number of studies have concluded that a poor enabling environment is one of the most serious challenges to developing irrigation in SSA and that the main issues are; insecure access to land and water, ineffective institutions and unfavorable policies (Ofosa et al., 2014, Fanadzo, 2012; Namara et al., 2011; Machethe et al., 2004).

## CONCLUSION AND RECOMMENDATIONS

For sustainable irrigation development in SSA that can contribute to expanding irrigation and increase productivity of farmers to ensure food security, the following guidelines should be followed:

1. Irrigation design should be participatory with the objectives of the system tuned to satisfy the farmers and not the reverse. This will result in flexible designs to suit the socio-cultural environment of farmers and enhance adoptability.
2. The development of SSI systems should be given priority in order to ensure the sustainability of irrigations systems, promote food security and alleviate poverty in SSA. However, since large-scale investments cost less per unit area; in order to reduce the unit cost of developing SSI systems in SSA, they should be designed and developed within the framework of large-scale investment projects which have significant economies of scale and are therefore cheaper.
3. When conditions permit, gravity operated surface methods are cheaper to construct if the construction of dams is not required. In addition, they are cheaper to

maintain and operate. When surface methods are not suitable because of topographic and soil conditions, gravity operated pressurized systems could be used because their operation costs will be much lower. In addition, irrigation projects should be implemented as part of multi-sectoral projects instead of stand-alone projects to reduce the unit cost.

4. Irrigation systems should provide opportunities for farmers to grow a variety of crops on different soil types to minimize risk and ensure both food security and income generation preferably from high value market gardening crops.

5. Farmers should make significant contributions in the implementation of irrigation projects as well as have significant management responsibilities for the completed system. This increases the sense of ownership and commitment and enhances the performance of the system. This usually results in lower O&M costs and higher performance.

6. The design of irrigation systems should provide for conjunctive use of surface water and groundwater to ensure the reliability of water supply wherever conditions are favorable.

7. Irrigation systems should be designed to maximize water use efficiency and to ensure environmental stability which also contributes to the alleviation of poverty and socio-economic development. If possible, designs should consider using new technologies which have better water application efficiencies like sprinklers and low cost drip irrigation systems.

8. States should strive to create an enabling environment for irrigation development by: facilitating access of farmers to water, land, credits, other agricultural inputs, and markets; building the capacity of all key stakeholders in irrigation development and facilitating the creation of functional WUAs.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Behavior and water needs of sesame under different irrigation regimes: Analysis of growth

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**The aim of this work was to evaluate the growth of sesame BRS 196 CNPA G4 in different irrigation depths (305, 436, 567 and 698 mm), applied on the basis of crop evapotranspiration– ETc (the depth of 567 mm was equal to 100% ETc). It was conducted at Embrapa Cotton, in Barbalha County, Ceará State, Brazil, 2012. The experiment was in randomized blocks, with treatments distributed in plots with three replications. The ETc was calculated by multiplying the reference evapotranspiration (ETo), determined by the Penman-Monteith method, by the crop coefficients (Kc), recommended for each phenological stage of the crop and by FAO. For the other irrigation treatments, the ETc was multiplied by 0.4, 0.7 and 1.3 (40, 70 and 130% of ETc). Primarily, height, stem diameter, leaf area and leaf area index were measured at 27, 48, 69 and 90 Days After Emergence (DAE), and then the absolute and relative growth rates in height, stem diameter and leaf area were estimated. It was concluded that height, stem diameter, leaf area and leaf area index of sesame BRS 196 CNPA G4 increased with irrigation; the highest growth occurred until 70 or 75 DAE, and the greatest growth was obtained, in general, in 698 mm of irrigation depth.**

**Key words:** *Sesamum indicum* L., evapotranspiration, growth rates, water stress.

## INTRODUCTION

Sesame (*Sesamum indicum* L.), is considered economically underused (Were et al., 2006), by the International Plant Genetic Resources Institute (IPGRI). This is because it is an alternative source of protein for consumption and enrichment of other products, in phytotherapic and phyto-cosmetic segments (Chakraborthy et al., 2008). In the Northeast, its exploration remains at subsistence levels (Andrade, 2009).

Arid and semi-arid regions are characterized by

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irregular and low rainfall and high evaporation. Therefore, since it supplements the natural rainfall, irrigation enables an increase of yield, especially in these regions (Farias et al., 2000). Sesame tolerate drought, but not waterlogging, this means that, once established, it is resistant to high water pressure in the soil more than other crops, but it does not grow well with little water (Kim et al., 2006).

Thus, it is necessary to check the crop's behavior in different applied volumes of water and identify the stages of higher consumption or critical periods where the lack or excess of water causes a growth decrease (Bernardo et al., 2009). The chronological monitoring identifies the regulatory mechanisms of periodic rhythm of growth (Abdulla et al, 2011; Leela et al., 2011). Despite using only primary data, non-destructive growth analysis is a useful and efficient tool, and may be complete if growth rates are calculated (Fontes et al., 2005; Cardoso et al., 2006; Barcelos et al., 2007).

This study aimed to evaluate the growth of sesame BRS 196 CNPA G4 in different irrigation depths applied on the basis of crop evapotranspiration (ETc).

## MATERIALS AND METHODS

The study was carried out at the Experimental Field of Embrapa Cotton, located in Barbalha county, CE State, Brazil (geographical coordinates: 07°19'S, 39º18' W and 409 m in relation to the sea level- Ramos et al., 2009) between August 4th and November 7th, 2012, in an area of Fluvic Neosoil. The experiment was installed in randomized blocks, with 4 treatments ($T_1$. 305, $T_2$. 436, $T_3$. 567 and $T_4$. 698 mm of total net depth of water applied in the cycle, corresponding to the treatment $T_3$ to 100% of crop evapotranspiration - ETc), distributed in plots with 3 replications.

The chemical characterization (0 to 20 cm) of the soil carried out at the Soils Laboratory of Embrapa Cotton, Campina Grande, PB State, Brazil, was as follows: pH 6.8; 95.3; 49.2; 2.8; 1.4 and 0.0 $mmol_c$ $dm^{-3}$ of calcium, magnesium, sodium, potassium and aluminum, respectively; 5.4 mg $dm^{-3}$ of phosphorus and 12.3 g $kg^{-1}$ of organic matter. The soil preparation consisted of plowing with chisel plow. Fertilization (123-152-30 kg $ha^{-1}$) was performed according to chemical soil analysis and technical advice from Embrapa Cotton. Sowing was done on August 8th, 2012 using 5 seeds of sesame BRS 196 CNPA G4, at each 0.20 m of the row, spaced at 0.70 m between rows.

The irrigations were performed by conventional spray, with efficiency of 75%, 0.34 MPa working pressure, using spray nozzles with 5.0 x 4.6 mm and rainfall of 10.54 mm $h^{-1}$, spaced 18 x 12 m applying water to 0.40 m from the ground, that according to Amaral and Silva (2008), matches the profile of the soil explored by the roots of sesame. The irrigations were performed every 3 or 4 days due to clayey texture of soil of the area to promote a very low water infiltration into the soil. From the beginning of the maturation stage (67 DAE), irrigations was turned weekly, considering the smaller replacement required by the irrigation culture.

At every irrigation, the net depth (ND) of replacement (ND = ETc = $ET_0$ * Kc) was function of the $ET_0$ of the period, estimated by the Penman-Monteith method using weather data from INMET Weather Station in Barbalha, CE State, Brazil, 500 m far from the experimental area and, the crop coefficients (Kc), contained in FAO 56 (Allen et al., 2006). The average Kc used were as follows (Allen et al., 2006): Phase I -

Establishment (2 to 5 DAE): 0.63 (Kc-initial); Phase II - Growth (6-32 DAE): 0.79 (Kc-intermediate); Phase III - Floration (33-66 DAE): 1.10 (Kc-medium) and; Phase IV - maturation (67-90 DAE): 0.25 (Kc-final).

Differentiation of irrigation treatments was started at 13 DAE. From then up to the last irrigation, on the other treatments, the replacement net depth (ND) calculated for the treatment based on 100% of ETc was multiplied by 0.4, 0.7 and 1.3 getting the replenishment volumes of irrigation treatments with 40, 70 and 130% of the ETc. Periodically, from 27 DAE, 4 plants were measured per plot: 1) plant height (H), distance between the base of the plant to its main pointer, in centimeters, with a tape; 2) stem diameter (SD), in millimeters, with a digital caliper, 1 cm above the soil surface and; 3) The longitudinal length of 10 leaves per plant (Severino et al., 2002), applying the equation $S = 0.3552C^2$ (Silva et al., 2002), where S = leaf area per leaf - $cm^2$ and C = longitudinal length leaf - cm, S is multiplied by the total number of leaves of the plant, resulting in the total leaf area per plant ($L_a$ - $cm^2$).

Based on measurements of these primary values in each time interval, using adapted equation of Reis and Muller (1978), some growth rates were estimated: Absolute growth rate in height - AGRH (cm $d^{-1}$), relative growth rate in height- RGRH (cm $cm^{-1}$ $d^{-1}$), absolute growth rate in stem diameter- AGRSD (cm $d^{-1}$), relative growth rate in stem diameter- RGRSD (cm $cm^{-1}$ $d^{-1}$), absolute growth rate in leaf area - AGRLA ($cm^2$ $d^{-1}$), relative growth rate in leaf area- RGRLA ($cm^2$ $cm^{-2}$ $d^{-1}$) and leaf area index- LAI ($m^2$ $m^{-2}$).

Those variables of each treatment irrigation ($Y_{305\ mm}$, …, $Y_{698\ mm}$) were subjected to several linear (polynomial: linear, quadratic, cubic and reverse from 1st to 3rd order) and non-linear (sigmoidal: sigmoid, logistic, weibull, gompertz, hill and chapman; Peak: normal log, gaussian, modified gaussian, lorentzian, pseudo-voight, weibull, etc.) functions, at 1 and 5% probability. The average data observed from those variables were adjusted only to non-linear functions sigmoidal-logistic (Equation 1) and peak-normal log (Equation 2) applied to the data by the statistical software Sigma Plot (Sigma Plot, 2011).

$Y_j$ = a / (1 + ((x / $x_0$) ^ b))………………………… [1]

and

$Y_j$ = (a/x) exp [- 0.5 ((ln (x / $x_0$) /b) ^ 2)]……………… [2]

in which: "$Y_j$"- variable analyzed in each treatment "j" of irrigation ($Y_{305\ mm}$, …, $Y_{698\ mm}$), "X"- number of Days After Emergence (DAE) of the plants, "$x_0$", "a" and "b"– model parameters of the adjusted function, where "$x_0$" is the inflection point of the equation, "a" = $Y_{max}$ – $Y_{min}$ and "b" – equation adjustment parameter.

## RESULTS AND DISCUSSION

The data of the plant height, relative growth rate in height, stem diameter and the absolute and relative growth rates in stem diameter was set to the non-linear model sigmoidal-logistic regression (Equation 1). Leaf area and leaf area index is set to non-linear regression model peak-normal log (Equation 2) (Figures 1 to 7). Growth height (H) is exponentially constant until it reaches a maximum at approximately 70 DAE from when it virtually stabilizes (Figure 1), confirming Allen et al. (2006) and Santos et al. (2010), using sesame BRS 196 CNPA G4 too, and Grilo Junior and Azevedo (2013) with sesame BRS Seda.

In the 567 mm depth, higher values were noted, over the cycle, for height followed by 698 mm (Figure 1), showing that the larger depths applied maximized the

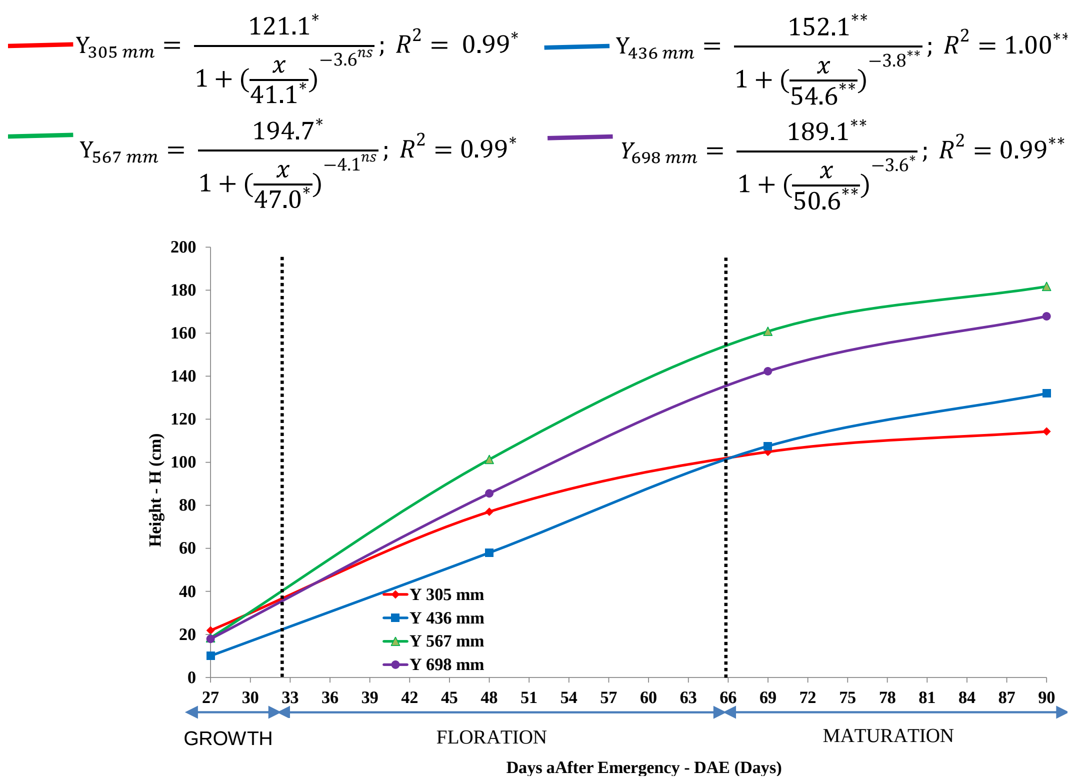


**Figure 1.** Curves and adjusted equations of sesame BRS 196 CNPA G4 growth height throughout the cycle for each of the applied irrigation depths. Barbalha, CE State, Brazil, 2012

plant height, as observed by Viana et al. (2012). For Mesquita et al. (2013), with sesame BRS Seda, the depth 150% of ETc maximized the plant height.

Non-linear regression models used did not fit the data of the absolute growth rate in height (AGRH). The non-linear regression model sigmoidal-logistic did not fit, considering the applied depth of 567 mm, to the data of the relative growth rate in height (RGRH) of sesame. In almost all applied irrigation depths, the RGRH decreased over the sesame cycle, showing the lowest decrease between 69 and 90 DAE (maturation phase) (Figure 2).

According to Abdelgadir et al. (2009) and Rashid et al. (2010), sesame growth rate at floration is usually low, making it even smaller after that stage until physiological maturity when it virtually ceases. At sufficient level of water in the soil, in case of 698 and 436 mm of irrigation depths, vegetative growth was favored, while at 305 mm of irrigation depth, water stress condition, the plants probably has reduced its vegetative growth, as evidenced by lower RGRH throughout the cycle (Figure 2).

According to Taiz and Zeiger (2009), water deficits, even moderate, lead to preferential growth of the roots towards the moist soil layers, after which the stomata closes, when the assimilated are directed to the fruits. The non-linear regression model sigmoidal-logistic did not fit the growth data of the sesame stem diameter (SD), considering the depths 436, 567 and 698 mm (Figure 3).

The stem diameter (SD) increased significantly, only for the applied water depth of 305 mm, throughout the cycle to approximately 70 DAE (initial maturation stage), corroborating statements of Allen et al. (2006) that after the Floration stage, growth virtually ceases (Figure 3). The non-linear regression model sigmoidal-logistic did not fit, considering the depths of 305, 436, 698 and of 436 and 698 mm, to the absolute growth rates (AGRSD) and relative growth rates (RGRSD) in the stem diameter, respectively (Figures 4 and 5). In the depths adjusted to the non-linear regression model sigmoidal-logistic, the AGRSD and RGRSD (Figures 4 and 5) decreased during the crop cycle, less intensely between 69 and 90 DAE (Maturation phase). This was as also reported by Abdelgadir et al. (2009) and Rashid et al. (2010).

In water stress, case of 305 mm depth (Figure 5), plants, possibly due to stomata closure, had reduced vegetative growth, initially probably investing in the growth of roots to the wetter soil layers, and later, as a survival matter, in reproduction (floration) (Taiz and Zeiger, 2009), with minor RGRSD throughout the cycle. In the leaf area ($L_a$) and the leaf area index (LAI), intense increase in values was found for all applied water depths, over the cycle, from the floration stage to approximately 70 DAE, in the depth of 305 mm, or 75 DAE, in the remaining applied irrigation depths (Figures 6 and 7).

The results obtained (Figures 6 and 7) are similar to

$$Y_{305\,mm} = \frac{0.1^{ns}}{1 + (\frac{x}{38.0^{ns}})^{3.8^{ns}}};\ R^2 = 0.99^*$$

$$Y_{436\,mm} = \frac{461087.6^{ns}}{1 + (\frac{x}{0.1^{ns}})^{2.4^{ns}}};\ R^2 = 0.99^*$$

$$Y_{698\,mm} = \frac{0.5^{ns}}{1 + (\frac{x}{22.6^{ns}})^{2.7^{ns}}};\ R^2 = 0.99^*$$

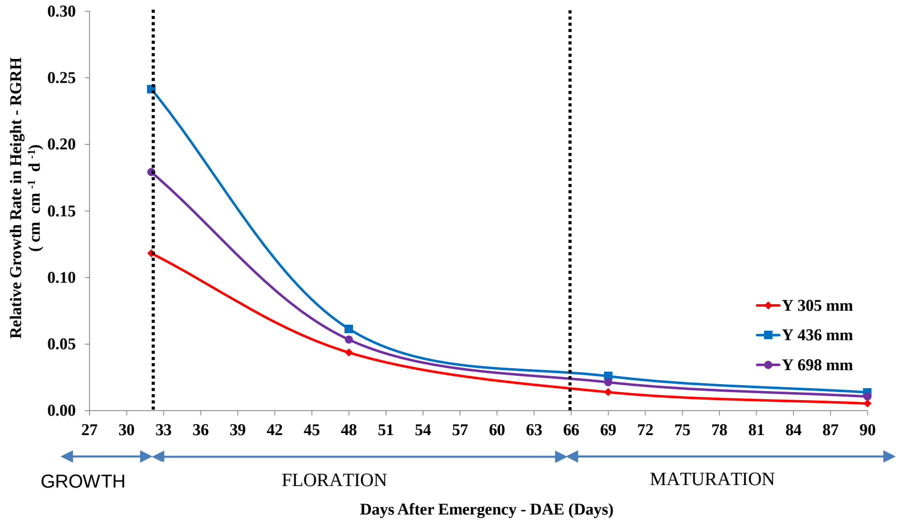


**Figure 2**. Curves and adjusted equations of the relative growth rate in height of sesame BRS 196 CNPA G4 throughout the cycle for each of applied irrigation depths. Barbalha, CE State, Brazil, 2012.

$$Y_{305\,mm} = \frac{17.2^{**}}{1 + (\frac{x}{29.8^{**}})^{-2.5^*}};\ R^2 = 0.99^*$$

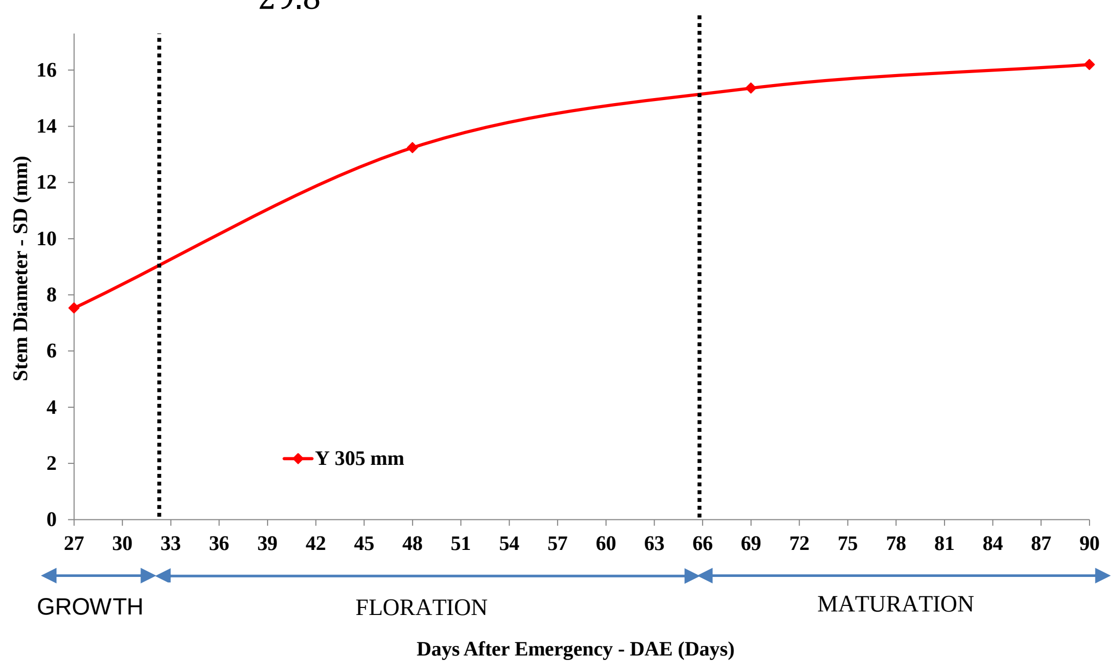


**Figure 3.** Curve and adjusted equation of the stem diameter growth of the sesame BRS 196 CNPA G4 throughout the cycle for each of applied irrigation depths. Barbalha, CE State, Brazil, 2012

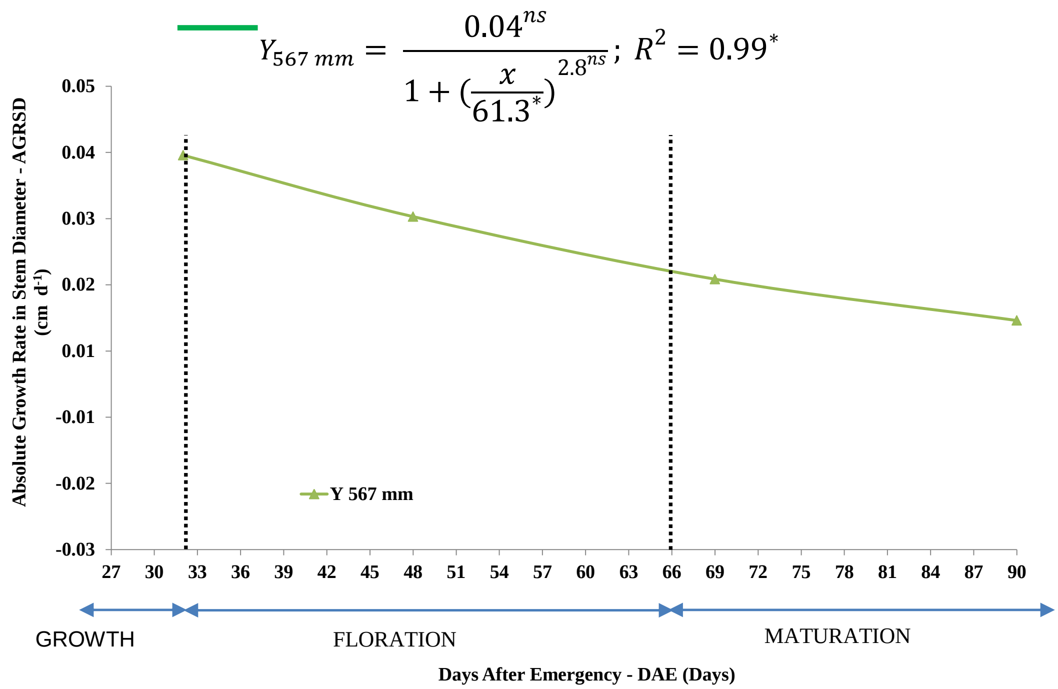


**Figure 4.** Curve and adjusted equation of the absolute growth rate of sesame BRS 196 CNPA G4 stem diameter throughout the cycle for each of the applied irrigation depths. Barbalha, CE State, Brazil, 2012.

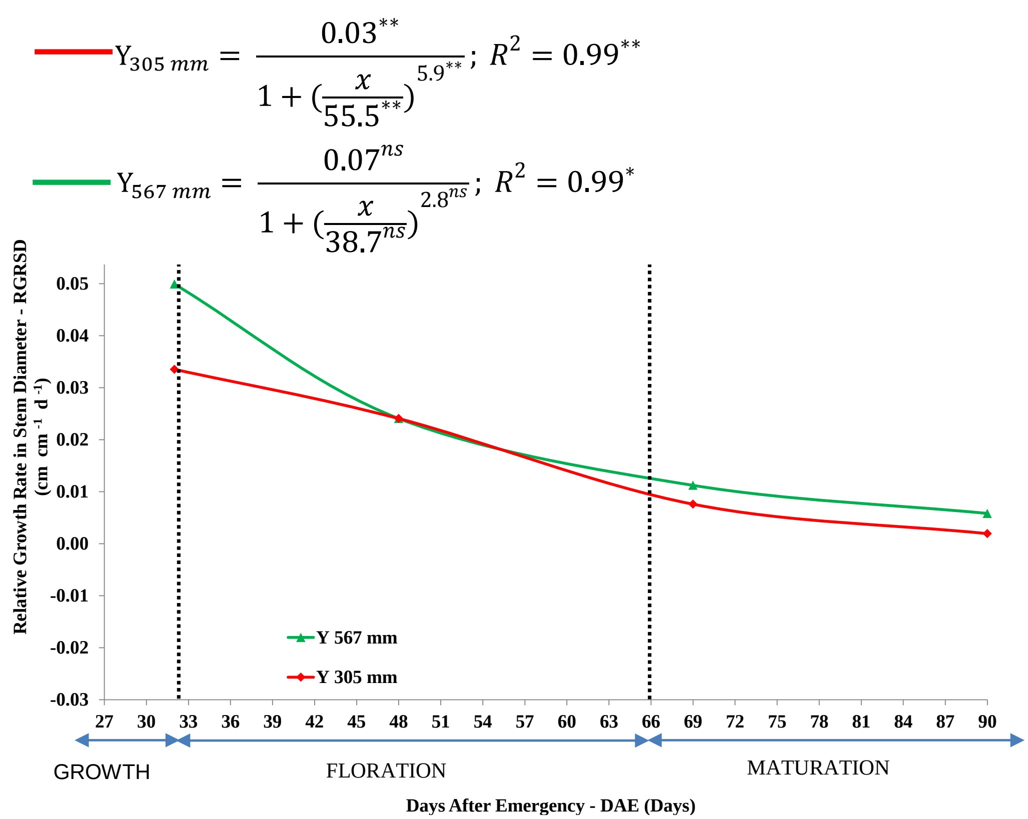


**Figure 5.** Curves and adjusted equations of the relative growth rate of sesame BRS 196 CNPA G4 stem diameter throughout the cycle for each of the applied irrigation depths. Barbalha, CE State, Brazil, 2012

$$Y_{305\,mm} = \frac{666456.2^{**}}{x}\exp\left[-0.5\left(\frac{\ln\left(\frac{x}{72.3^{**}}\right)}{0.2^{**}}\right)^2\right], R^2 = 0.99^{*}$$

$$Y_{436\,mm} = \frac{135835.6^{**}}{x}\exp\left[-0.5\left(\frac{\ln\left(\frac{x}{78.3^{**}}\right)}{0.2^{**}}\right)^2\right], R^2 = 0.99^{**}$$

$$Y_{567\,mm} = \frac{1454389.2^{**}}{x}\exp\left[-0.5\left(\frac{\ln\left(\frac{x}{76.9^{**}}\right)}{0.2^{**}}\right)^2\right], R^2 = 0.99^{**}$$

$$Y_{698} = \frac{234940.0^{**}}{x}\exp\left[-0.5\left(\frac{\ln\left(\frac{x}{78.3^{**}}\right)}{0.2^{**}}\right)^2\right], R^2 = 0.99^{**}$$

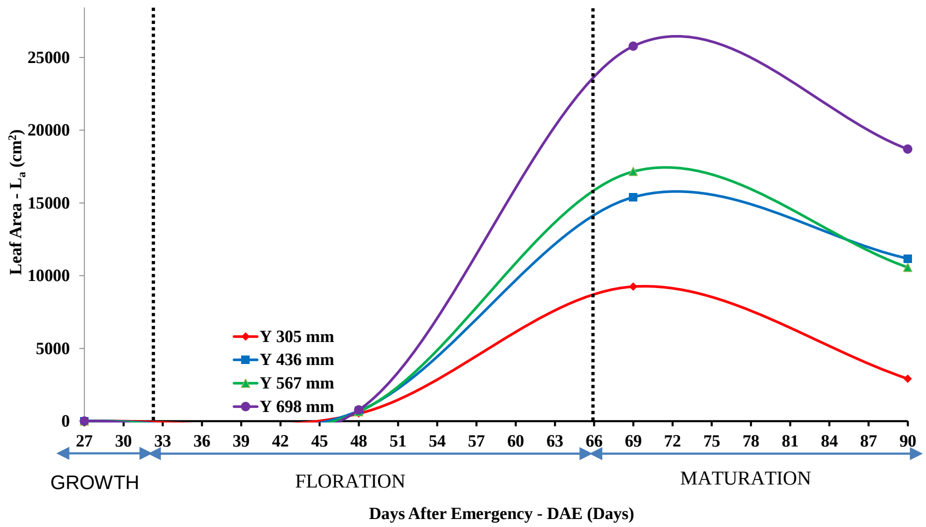


**Figure 6.** Curves and adjusted growth equations of growth of leaf area of sesame BRS 196 CNPA G4 over the cycle for each of applied irrigation depths. Barbalha, CE State, Brazil, 2012.

$$Y_{305\,mm} = \frac{952.1^{**}}{x}\exp\left[-0.5\left(\frac{\ln\left(\frac{x}{72.3^{**}}\right)}{0.2^{**}}\right)^2\right], R^2 = 0.99^{*}$$

$$Y_{436\,mm} = \frac{1940.5^{**}}{x}\exp\left[-0.5\left(\frac{\ln\left(\frac{x}{78.3^{**}}\right)}{0.2^{**}}\right)^2\right], R^2 = 0.99^{**}$$

$$Y_{567\,mm} = \frac{2077.7^{**}}{x}\exp\left[-0.5\left(\frac{\ln\left(\frac{x}{76.9^{**}}\right)}{0.2^{**}}\right)^2\right], R^2 = 0.99^{**}$$

$$Y_{698\,mm} = \frac{3356.3^{**}}{x}\exp\left[-0.5\left(\frac{\ln\left(\frac{x}{78.3^{**}}\right)}{0.2^{**}}\right)^2\right], R^2 = 0.99^{**}$$

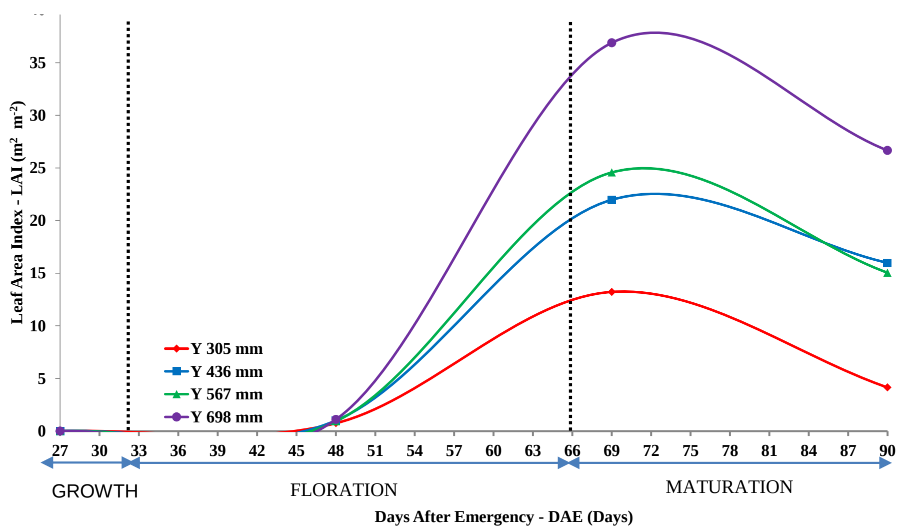


**Figure 7.** Curves and adjusted equations of leaf area index of sesame BRS 196 CNPA G4 throughout the cycle for each of the applied irrigation depths. Barbalha, CE State, Brazil, 2012

Severino et al. (2002), with sesame CNPA G4, and Grilo Junior and Azevedo (2013), with sesame BRS Seda. For Abdelgadir et al. (2009) and Rashid et al. (2010), the GRLA and the LAI, of sesame, which occur from the Floration to physiological maturity, is greater, and after that period it decreases sharply.

The evolution of the LAI (Figure 7) followed the pattern of annual plants, recommended by Allen et al. (2006), which is a slow initial phase followed by a rapid growth phase until another one with a sharp decrease after the physiological maturity, probably due, according to Benincasa (2003) and Grilo Junior and Azevedo (2013), to the senescence and the subsequent leaves fall.

An increase was noticed, from the floration, proportional to the irrigation in $L_a$ and in the LAI with higher values in the depths of 698, 567, 436 and 305 mm, respectively (Figures 6 and 7). These results are similar to Kassab et al. (2005) in Egypt, with sesame Giza 32. The growth pattern was also found with corn (Meneghetti et al., 2008). It was also found that, in the smaller depth, the decreases in $L_a$ and LAI started earlier, about 5 days before the other depths (Figures 6 and 7). This is probably due to water stress suffered by plants in that depth. According to Taiz and Zeiger (2009), if the plants suffer water stress after substantial development of leaf area, the leaves get old and fall.

None of the non-linear regression models used was fitted to the data of absolute and relative growth rates in leaf area (AGRLA and RGRLA). Finally, the applied irrigation depths did not alter the normal growth pattern in plant height, stem diameter and leaf area expected for sesame, but they differed from each other in plant height, RGRH, stem diameter, AGRSD, RGRSD and leaf area values throughout the sesame cycle (Figures 1 to 7).

## Conclusions

1) Height, stem diameter, leaf area and leaf area index of sesame BRS 196 CNPA G4 increased with irrigation.
2) The highest growth occurred until 70 or 75 DAE;
3) The highest growth was obtained, in general, in 698 mm of irrigation depth.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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*Full Length Research Paper*

# Integration of climate change information into drylands crop production practices for enhanced food security: A case study of Lower Tana Basin in Kenya

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**Climate change and variability poses the greatest challenges to agricultural production in the developing countries and especially drylands. Across Africa, yields of staple crops such as maize, wheat, sorghum and a variety of fruit crops have significantly decreased in the recent years, thus, widening food insecurity gaps. In the dryland areas of Kenya, the situation is further aggravated by low adaptive capacities and highly fragile productive systems. The, understanding of the links between climate change and agricultural production is critical to scientists, policy makers and farmers in an attempt to mainstream agricultural adaptation and mitigation measures. Climate change information provides the opportunity for efficient resource utilization, increase in agricultural production and enhancement of farmer resilience. The Lower Tana Basin in Kenya is one of the highly vulnerable regions with increasing shocks of climate change and variability that threatens the lifeblood of community livelihoods and biodiversity integrity. human survivals. This research focused on rainfall characteristics of the Lower Tana Basin in relation to maize production and food security of the region. Observed rainfall and temperature records from weather stations located in the Lower Tana Basin were collected and analyzed. The findings of this study showed evidence of increased return period of extreme drought events from a frequency of 4-5 years to 2 or less years in the last two decades. Impacts of the changing climate on maize production is evidently significant and farmers need to emphasize agricultural diversification with the adoption of other alternative crops such as mangoes and cassava that yield well, are climate resilient and likely to minimize future food security risks.**

**Key words:** Climate change, drylands, food security, climate information, planning.

## INTRODUCTION

Climate change impacts are among the most important environmental and scientific challenges facing humanity in the 21$^{st}$ century (Rahman and Mallick, 2005; IPCC, 2014). In the drylands of developing countries, drylands and the poor in society are considered highly vulnerable due to their low capacities to effectively cope with the potential impacts associated with decreased agricultural yields (Shah et al*.,* 2008; Nellemann et al*.,* 2009).

Information regarding the potential impacts of climate change and their adaptation strategies ought to be integrated while developing future policies (IPCC, 2007). Such information and their associated uncertainties should be communicated to end-users and policy-makers, so that adequate policy decisions to respond to climate change can be taken in a fully informed way. In the developing countries, drylands constitutes over 40% of the earth's land surface and home to approximately 2.5 billion people (FAO, 2011). These drylands "struggle" to provide sufficient food to the rapidly growing population amidst other daunting physical and demographic challenges such as high poverty levels and unemployment, rapid urbanization, severe water scarcity, and land degradation (Reynolds et al*.,* 2007; World Bank, 2014). Climate change is exacerbating the dryland problems and constraints leading to increased out-migration, insecurity, damaged social fabrics, low levels of productivity and limited economic growth (Keane, 2009). Therefore, there is need to urgently respond to the emerging dryland challenges that may determine the fate of these insecure regions in the years to come.

This study describes the contribution of climate information in addressing climate change adaptation strategies in the agricultural production systems to achieve food security. The observed changes in rainfall and temperature conditions (Christensen et al*.,* 2007) are causing major impacts on food security for the rural households especially in drylands which largely rely on rainfed agriculture as a source of livelihood. In Kenya, it is estimated that over 10 million people suffer from chronic food insecurity and poor nutrition with about 1 to 2 million people dependent on emergency food aid (GoK, 2010). Although there has been an increase in knowledge accumulated on climate change among stakeholders, the extent of the impact still remains uncertain for the ASALs where climate projections are nebulous. Generally, the impacts of climate change on agriculture and food security in regions are evident through changes in crop yields, water availability, pests and diseases, animal health and other biophysical factors (Hatfield et al*.,* 2011). The broad objective of this study was to mainstream climate change information into crop production systems decision making processes for enhanced food security. Specifically, rainfall and temperature in Kenya's Lower Tana Basin were analyzed to decipher salient patterns and evidence of climate change, described major crop yield characteristics and their correlation with seasonal rainfall and finally generated useful climatic information for supporting decision making to enhance crop production systems to minimize food security risks.

## METHODOLOGY

The study was conducted in Kenya's Lower Tana Basin with specific focus on Tana River County (Figure 1). The County is located 150
km north of Malindi, covers a total surface area of 38,437 km$^2$ and accounts for 6.61% of Kenya's total surface area (Mireri et al*.,* 2008).

Climate and agricultural production data for Tana River County were collected and analyzed. Climate datasets were limited to only rainfall and temperature due to their direct and significant influence on agricultural productivity and other livelihood sources in the region. Climate data were collected from weather stations located in the County namely Witu, Tana, Kipini, Garsen, Bura and Garissa. Climate data quality control tests were done using single mass curves to verify their consistency and continuity. The missing climate data records were estimated using cross correlation approach with nearby stations with highest correlation coefficient to the station with the missing data in the same period.

Study agricultural datasets comprised of crop types and their corresponding yields (tons/acre), types and population of livestock. Agricultural data were sourced from Tana River County sub-counties' agricultural and livestock extension offices namely Tana North, Tana River and Tana Delta sub-counties. The major crops focused during the study were those grown under rain-fed conditions which comprised of maize, green grams, cassava, mangoes and rice. Data were analyzed using various statistical tools namely Xcel, Instat+ and GenStat. Seasonal rainfalls were correlated with respective crop yields to establish relationships which in turn used for projecting future scenarios. Other statistical tools such as box plots scatter plots, standardized anomalies and time series graphs were used to illustrate data patterns and the underlying structures in climate and crop yield datasets. Time series graphs were also used to demonstrate variability and trends of historical rainfall, temperature and crop yield datasets. Pearson's correlation measure was used to determine the strength and direction of the two study variables. Similarly, rainfall anomaly graphs were used to illustrate trends of extreme events in the region namely drought and floods. Long-term term data records from each weather station were collected and varied according to time of establishment.

## RESULTS AND DISCUSSION

### Distribution of weather stations in the Lower Tana Basin

Assessment of the distribution of weather stations in the Lower Tana Basin showed a few and sparsely distributed weather stations (majority being rainfall recording stations) with majority being located along River Tana channel (Figure 2). A total of six stations were found in the region namely Witu, Tana, Kipini, Garsen, Bura and Garissa.

The distribution network of weather stations in the region provides essential information for understanding the potential threats posed by climate change to various

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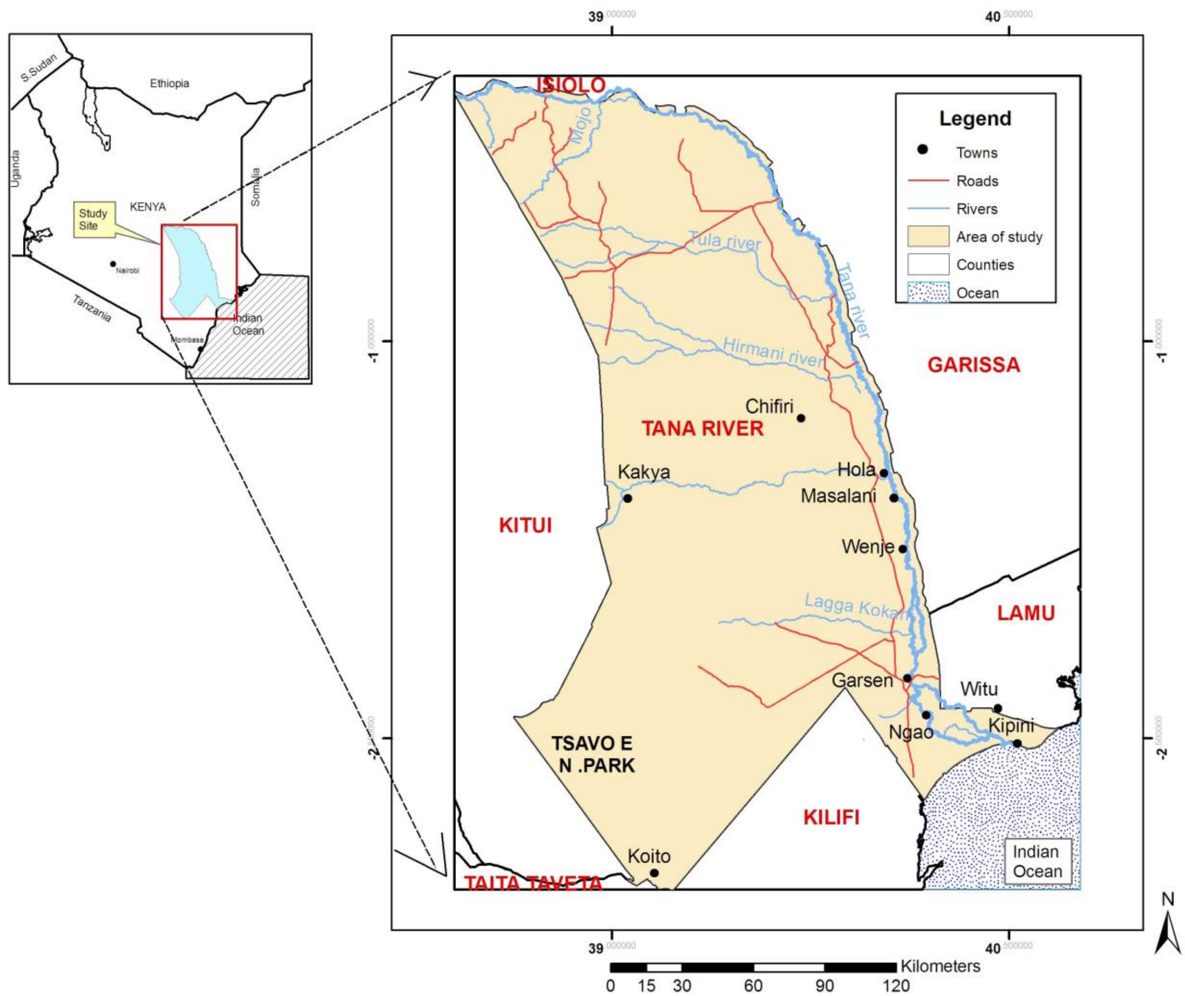


**Figure 1.** Map showing the location of study area.

economic sectors. To address the limited weather stations in the region, the study found that Kenya Meteorological Services (KMS) had initiated major programmes to increase weather observatory points (mainly rainfall records) by involving local primary schools and government institutions in data collection.

## Climate data homogeneity tests

Quality controls for climate datasets done using March – May (MAM) rainfall and December- February (DJF) minimum temperature seasons for Kipini and Garissa weather records respectively are illustrated in Figures 3 and 4.

The results show straight lines fitting on the data patterns, a clear evidence of data continuity and consistency, hence, good quality records for the study. Less than 10% of missing data records was estimated using cross correlation approach with nearby stations. Therefore, seasonal rainfall homogeneity tests affirm that weather stations records in the Lower Tana Basin were correctly recorded and could be used to make inferences regarding projected climate change trends and corresponding implications on crop yield variations. The results of quality control test formed the foundation for further analyses undertaken to investigate various specific objectives of the study.

## Rainfall characteristics in the Lower Tana Basin

Analysis of rainfall across the Lower Tana Basin showed that the mean annual rainfall in the region varied from a low of 300 mm in Garissa to highest slightly above 1100 mm in Witu (Figure 5).

The rainfall characteristics results demonstrated that Witu and Kipini are good agricultural potential zones in the region under rainfed production. These areas are also close to Indian Ocean and periodically receive convectional rain that further boosts this agricultural potential. Correspondingly, hinter-land areas such as

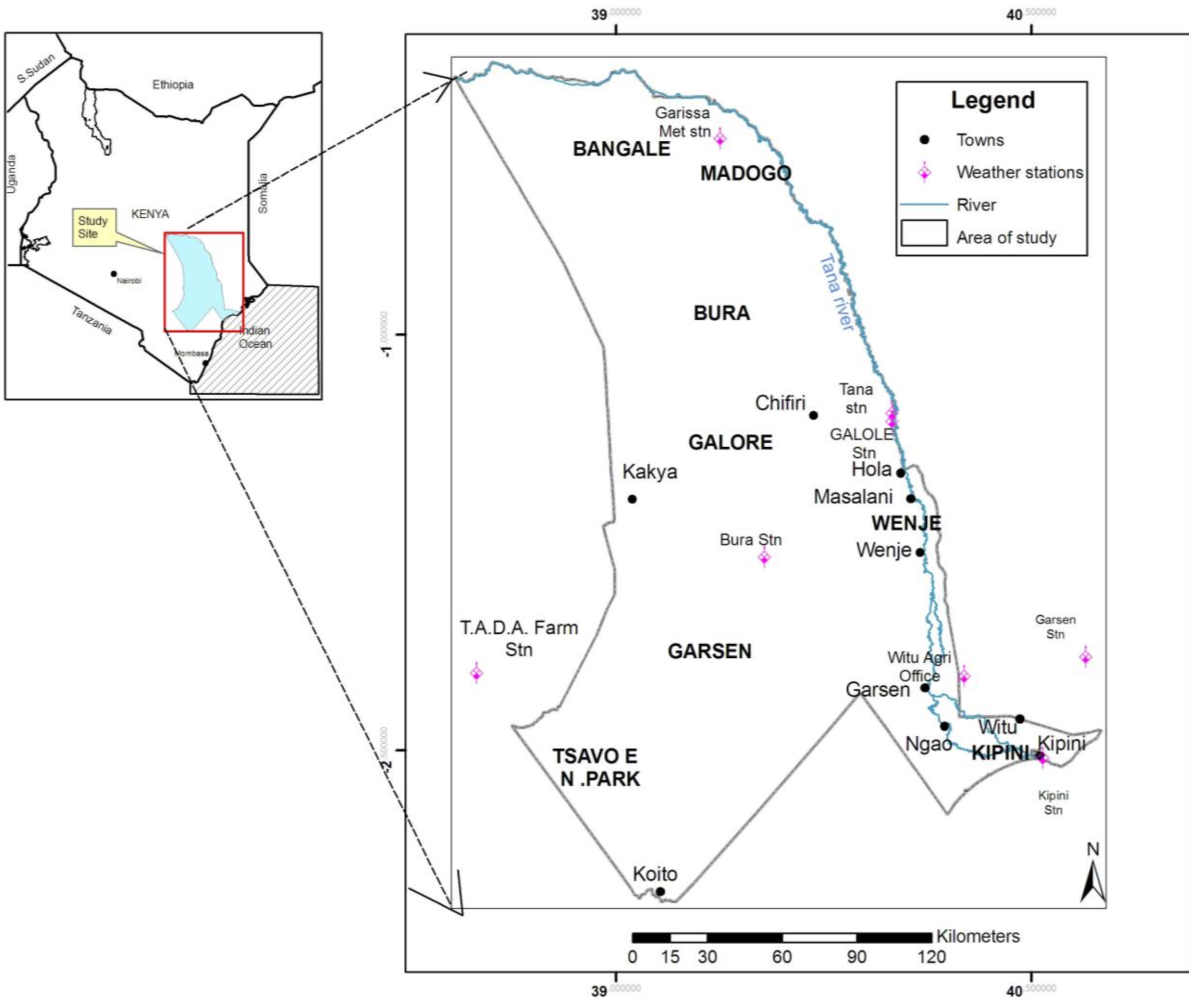


**Figure 2.** Location of weather stations in the Lower Tana Basin.

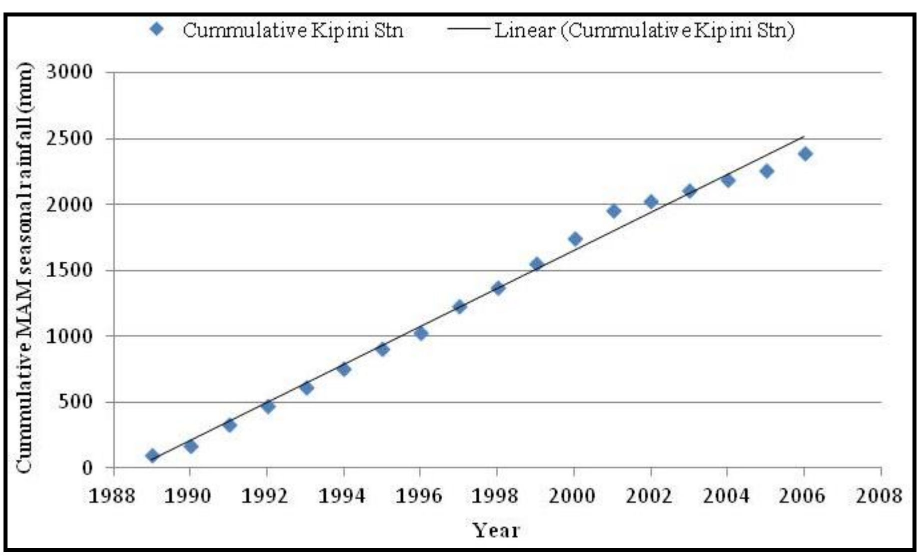


**Figure 3.** Cumulative single mass curve for March - May (MAM) seasonal rainfall over Kipini station.

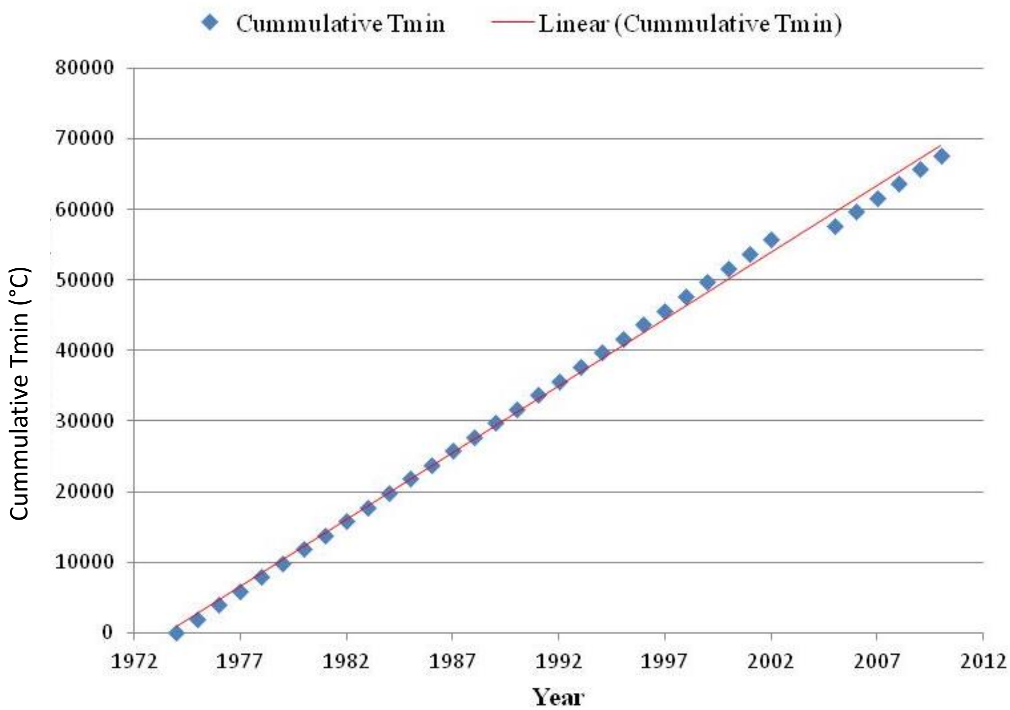


**Figure 4.** Cumulative single mass curves for January-December mean minimum (Tmin) surface temperature over Garissa station.

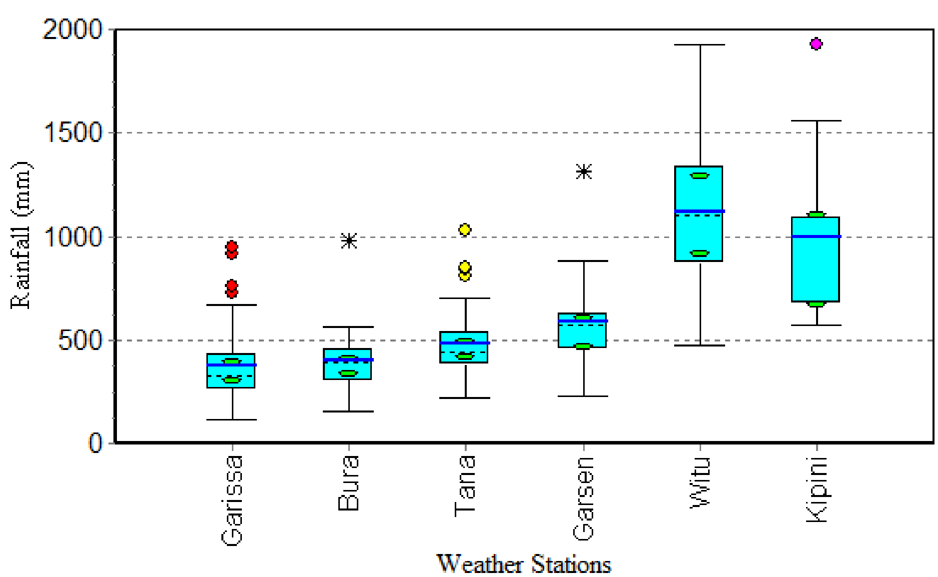


**Figure 5.** Mean annual rainfall variation across the Lower Tana Basin.

Garissa, Bura and Tana receive depressed rainfall of below 400 mm and explain why crop production activities under rainfed conditions in these areas are unsuitable and highly vulnerable. Further assessment of maize production potential during two main seasons; March-April- May (MAM) and October-November- December (OND) seasons, revealed that only the MAM season in Witu and Kipini met the required threshold for successful maize production under rainfed conditions (Figure 6). This is because according to Food and Agriculture Organization (FAO) study of 1986, maize production within a season needs a mean rainfall of between 500 to

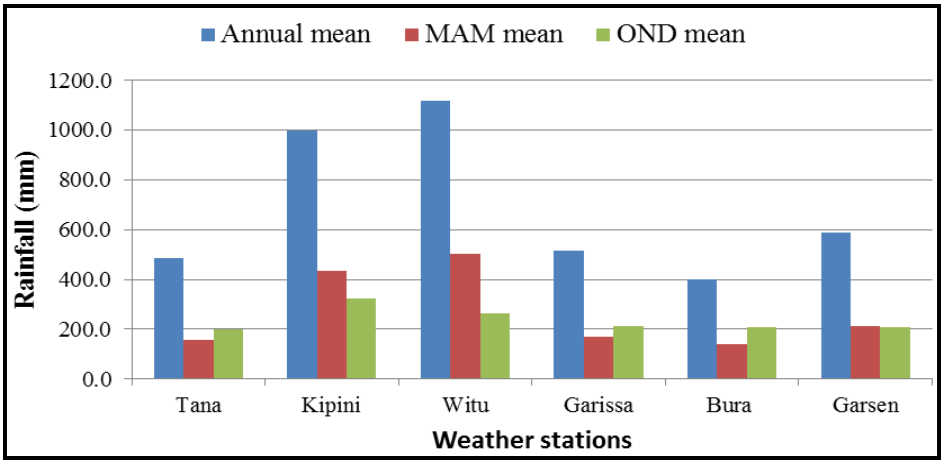


**Figure 6.** Annual and seasonal mean rainfall in the lower Tana.

800 mm.

In Kenya and most parts of eastern Africa, maize is the main staple food crop and relatively a food security measure for most communities/households. Therefore, agricultural managers can utilize the mean seasonal rainfall findings to optimize rainfed maize production at Kipini and Witu during the MAM seasons. However, this maize production potential exists only within the minimum threshold requirement. Under the changing climatic conditions, these agricultural potential zones in the region risk being rendered unsuitable for maize production. Therefore, there is need to mainstream key adaptation measures during planning of the agricultural production systems in the region.

### Temperature characteristics in the Lower Tana Basin

Assessment of temperature changes in the lower Tana showed general increases in both the minimum and maximum mean temperatures (Figure 7). However, there is an enhanced increase in the minimum temperature as compared to the maximum temperature. The results implies the existence of a wide and suitable temperature range for optimal crop yields in the region and hence, the impacts of the significant minimum temperature increase on most crops in the region may be negligible. However, the observed significant increase in the minimum temperature may increase the percent probability of Tsetse prevalence which will create unfavorable impacts on livestock production in the region.

### Crop yield characteristics in the Lower Tana Basin

Crop yield analyses in the region produced under rain-fed conditions showed maize with low yield potential (less than 1 tons/acre) as compared to cassava and mangoes that yields highest (above 7 tons/acre) despite the changing climate (Figure 8).

A correlation analysis done between MAM seasonal rainfall and various crop yields in the region generally showed low correlation values. In Kipini, maize yields showed relatively strong negative correlation of 0.45 with MAM seasonal rainfall implying the strong contribution of other factors of production which must be considered during production planning. A rainfall regression model on maize values showed that only 8% of variance in maize yields was explained. This means that other factors other than rainfall explain 92% of the maize yield variation curve. A consideration of these other agrotechnical factors which may include soil fertility, seed genotypes, planting dates, pest and disease incidence among others) is critical during agricultural planning to achieve future food security.

### Decadal seasonal rainfall changes in the Lower Tana Basin

Assessment of decadal seasonal rainfall changes across the region during MAM and OND seasons shows an increased positive change of rainfall (enhanced rainfall) during the MAM season of between -10 to 30 mm/decade and a negative change during the OND season of -30 to -11 mm/decade (Figures 9). This slight rainfall increase in the region will not have significant gains in crop production due to the resultant significant increase in temperature that will increase transpiration and evaporation rates, hence negative gains.

The decadal rainfall change findings constitutes a key strategic planning information that agricultural managers and policy makers in Tana River County can apply in addressing long-term agricultural and food security measures in the region. The result indicates that MAM seasonal rainfall is expected to increase along the region

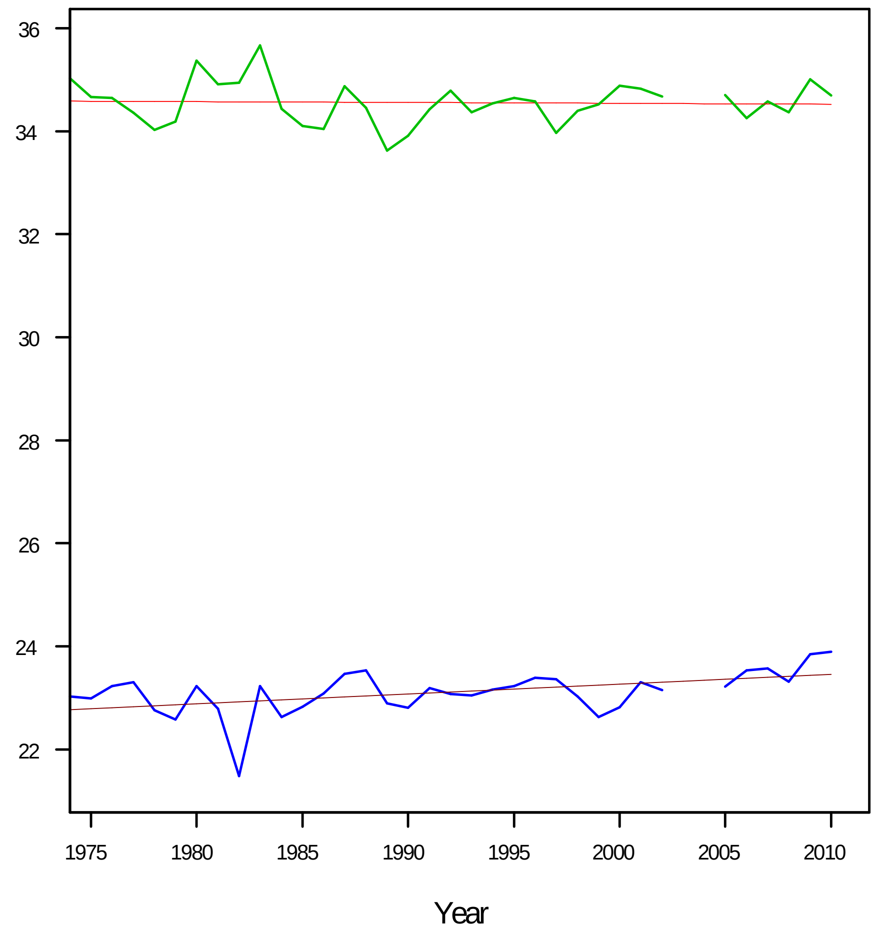


**Figure 7.** Mean minimum and maximum temperature (°C) trends in the Lower Tana Basin.

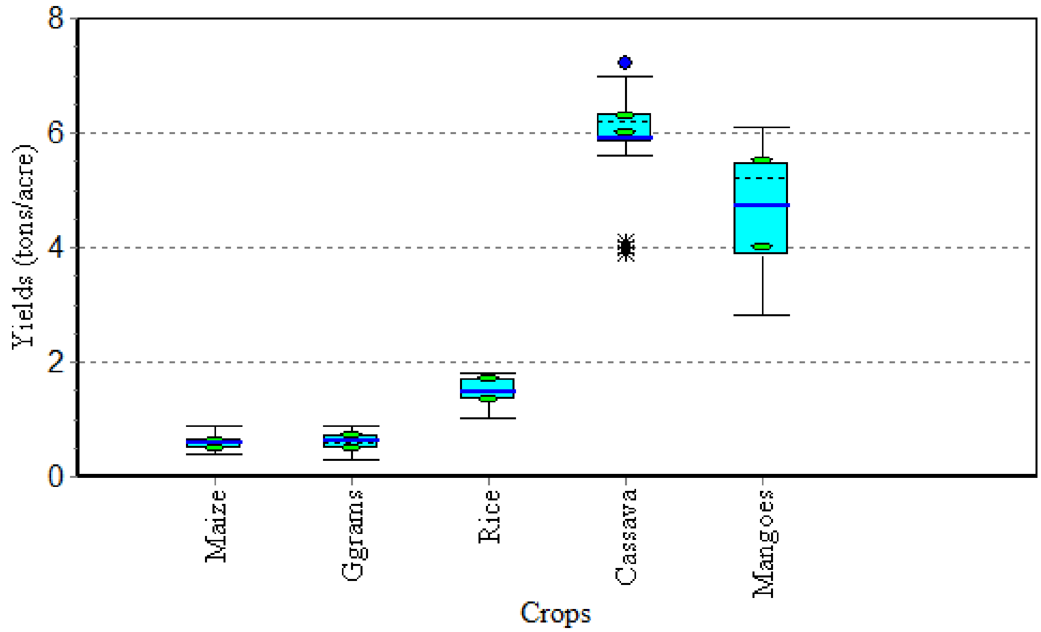


**Figure 8.** Yield potential for widely grown crops in the Lower Tana Basin.

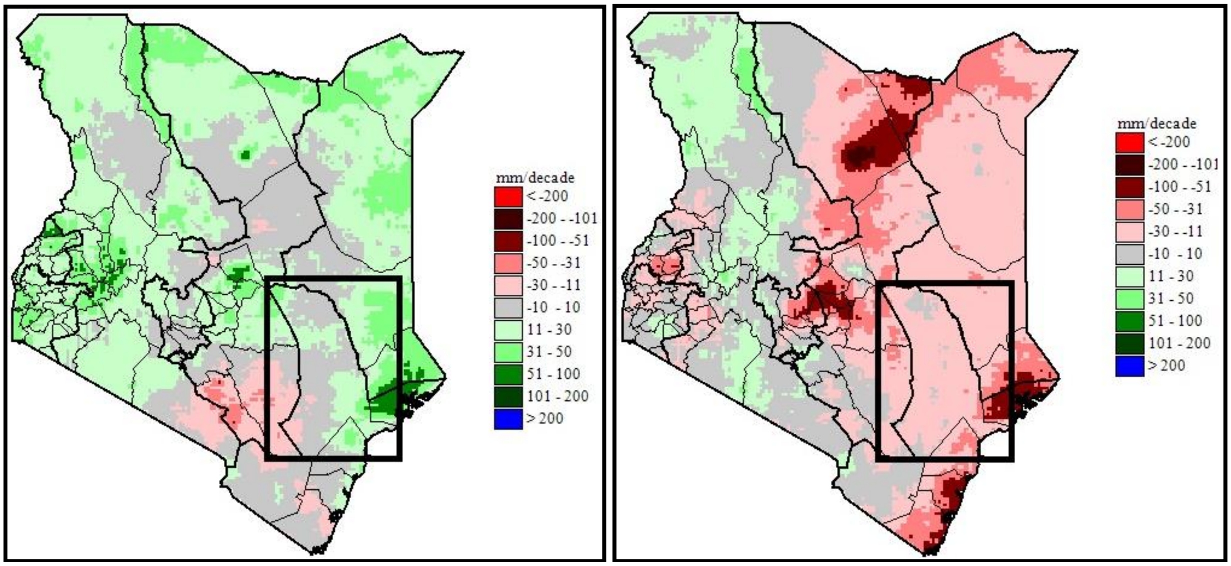


**Figure 9.** MAM and OND decadal rainfall variability change.

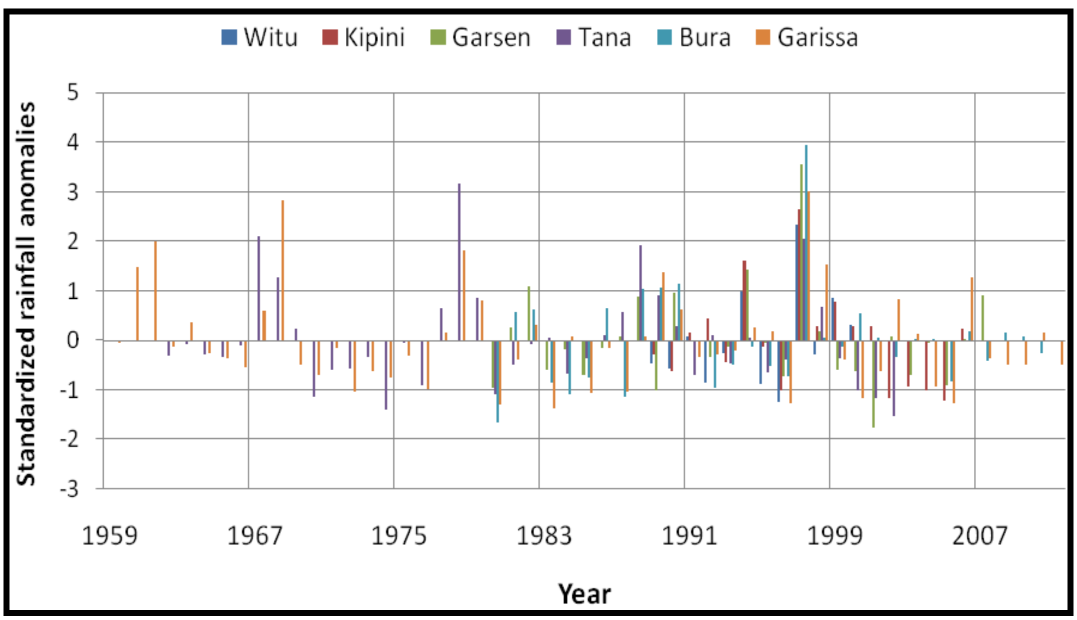


**Figure 10.** Rainfall anomalies in the Lower Tana Basin showing depressed and enhanced years.

and concurs with the projected IPCC findings on eastern Africa.

## Extreme events in the Lower Tana Basin

Assessment of rainfall anomalies in the region reveals a regular occurrence of extreme depressed and enhanced rainfall periods (Figure 10). The return period of above average rainfall event (Floods) is 8-10 years while the below average rainfall event (prolonged drought incidence) is about 4 years. For instance, some of the extreme flood events in the region occurred in 1997/98 while the prolonged droughts which led to severe famines was observed in 1983/84, 1995/96, 1999/2001 and 2004/2005 respectively (Figure 10).

The frequency of drought and intensity in the region increased from the year 2000 compared to flood events (Figure 10). This constitutes evidence that informs planning and decision makers in the region engaged on agricultural production activities to invest in key adaptation measures such as more investment in water harvesting and storages structures in order to minimize potential impacts.

## Conclusion

Rainfed crop production in the Lower Tana Basin is under threat from climate change and variability. The maize production zones in the region that are critical for food security achievement are currently producing within the minimum threshold requirements and the changing climate may render these zones unproductive. Consequently, agricultural managers in the region should strongly consider other factors of crop production in their planning beside rainfall (such as the type of soil, soil fertility, and a range of farm management practices by farmers (control of weeds, pests control and timely planting/harvesting) as this study found that 92% of the variance in maize is attributed to these other factors. Against this background, it is prudent for policy makers and planners to mainstream climate information and appropriate adaptation measures in developing agricultural plans if food security is to be achieved in the region. Further, the government should consider strengthening food production initiatives as well as, value addition and marketing strategies for mangoes and cassava crops, which have demonstrated strong resilience to climate change.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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# Effect of arbuscular mycorrhizal fungi on survival and growth of micropropagated *Comanthera mucugensis* spp. *mucugensis* (Eriocaulaceae)

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**The use of micropropagation technique has been an alternative to conservation of endangered species, *Comanthera mucugensis* subsp. *mucugensis* (popular namely sempre viva de Mucuge); however, there is no information on the effect of arbuscular mycorrhizal fungi (AMF) on the acclimation process of micropropagated plants. This study evaluated the survival, growth and nutritional aspects of the species, *C. mucugensis* subsp. *mucugensis* inoculated with native AMFs in greenhouse condition. The design of the experiment consisted initially of 80 sampling units divided into four treatments: plants inoculated with native AMF, with microbiota filtrate from soil, with AMF plus filtrate and control (non-inoculated plants). At three and eleven-month-old, the plants were collected for evaluation of growth, nutrition and mycorrhizal colonization. After eleven months of experiment, survival rate of AMF and AMF plus filtrate plants were 62.5 and 87.5%, respectively, and only one microbiota filtrate and one control plants survived. AMF inoculation also provided increase in n dry matter of rosettes and permitted obtaining flowering ten-month-growth plants. Rates of mycorrhizal colonization were high at three (aproximately 64.9%) and eleven (aproximately 94.5%) months for AMF and AMF plus filtrate plants. Number of spores in rhizosphere soil of mycorrhizal plants was also high (1599 per 100 dm$^3$ of soil) and seven diferent species of AMF were identified at the end of experiment. Data set evidenced mycortrophic character of *C. mucugensis* subsp. *mucugensis* and the importance of AMF inoculation for acclimation and survival of microprogagated plants which is essential for conservation of this endangered plant.**

**Key words:** Micropropagation, nutrition, arbuscular mycorrhiza fungi, sempre viva de Mucuge, aclimatation.

## INTRODUCTION

The Eriocaulaceae family comprises eleven genera and ca. 1200 species, has a pantropical distribution (Echternacht et al., 2010), and presents its diversity center on the Espinhaço Range between Minas Gerais and Bahia (Giulietti and Hensold, 1990; Sano, 2004). About 70% of the total Brazilian species of Eriocaulaceae occur at Espinhaço Range, 85% are endemic and often are restricted to a single mountain (Giulietti et al., 2005; Costa et al., 2008). The species, *Comanthera mucugensis* subsp. *mucugensis* is one of this microendemic Eriocaulaceae plants that occured on municipality of Mucuge (Bahia) at eastern side of the Chapada Diamantina region. This species is popularly known as sempre viva de Mucuge (evergreen of Mucuge) and its inflorescence remains with the same color and shape when their scapes, chapters and flowers are collected for making dried floral arrangements. At region of rupestrian field on Mucuge where these plants occur naturally, they were one of the main sources of income for local inhabitants at the mid-twentieth century, and each year were sold tons of flowers, especially to Europe and the United States (MMA/PNMA, 1996), whichreduced the natural population since the flowers are still at anthesis when collected to be sold as ornamental (Giulietti et al., 1988; Cerqueira et al., 2008).

Recently, *C. mucugensis* subsp. *mucugensis* was prohibited from being collected because their exploitation has been carried out without planning and without any control or cultivation (Lima-Brito et al., 2016), and currently, this plant is on the Official List of Species of the Brazilian Flora Endangered (MMA, 2008). Some tentatives of plant management are already being developed at Parque Municipal de Mucuge, as to protect *C. mucugensis* subsp. *mucugensis* populations and promote the propagation and cultivation, seeking alternative sources of income to the population of the municipality (Paixão-Santos et al., 2003; Ramos et al., 2005; Teixeira and Linsker 2005).

With the aim to increase *C. mucugensis* subsp. *mucugensis* populations in Mucuge region, the micropropagation technique has been used as a viable option for the production of seedlings of this species (Lima-Brito et al., 2011; Pêgo et al., 2013). Despite the advantages in using this technique, there are still some obstacles to their wide application, especially as regards acclimation, that is, the conditions to be transplanted *in vitro* to greenhouse, since mortality rate of *C. mucugensis* subsp. *mucugensis* micropropagated plants is high.

The absence of beneficial soil microorganisms can result to negative effects on the plant acclimation process due low adaptation to new environmental conditions imposed (Borkowska, 2002). Studies on the association of arbuscular mycorrhizal fungi (AMF) with some agronomic and ornamental plants demonstrate benefits of these microorganisms as plant growth regulators and their importance to management and acclimation (Rocha et al., 2006; Yadav et al., 2013; Moreira et al., 2015; Villarreal et al., 2016). Arbuscular mycorrhizal fungi (AMF) are an important microbial group of the soil, which form a mutualistic symbiosis with the roots of plants affecting several processes and functions in the ecosystem such as nutrient cycling, plant productivity and competition (Hazard et al., 2013). This microorganism have been used as an alternative to increase the resilience of many species during the acclimation process, stimulating the autotrophic stage of transition from *in vitro* to soil and influencing morphogenesis and architecture of root, ensuring a health formation and development of root system after transplanting (Zemke et al., 2003; Kapoor et al., 2008; Stancato and Silveira, 2010). Apart from this, AMF can act as biological controller of some pathogens and to reduce tensions as nutrition, availability of water and salinity involved on micropropagation (Schubert et al., 1990; Jaizme-Vega and Azcón, 1991).

In the present study, the authors evaluated native AMF and microbiota inoculation on acclimation of *C. mucugensis* subsp. *mucugensis* micropropagated plants, analysing survival and nutritional status with goal to contribute to process of population restoration of this endangered plant.

## MATERIALS AND METHODS

### *In vitro* culture

In the experiment, 120 days old micropropagated plants of the *C. mucugensis* subsp. *mucugensis* obtained from the Vegetable Tissue Culture Laboratory of the Horto Florestal Experimental Unit, belonging to the Biological Sciences Department of the Feira de Santana State University, in the municipality of Feira of Santana, Bahia were used. The chemical characterization of the *in vitro* plants was carried out at the Laboratory of Analysis of Vegetable Tissues of the Cocoa Research Center (CEPEC) of the Executive Committee for Cocoa Plantation Planning (CEPLAC). The results were: N = 42.18 $g.Kg^{-1}$; P = 1.98 $g.Kg^{-1}$; K = 22.92 $g.Kg^{-1}$; Ca = 2.29 $g.Kg^{-1}$; Mg = 1.28 $g.Kg^{-1}$; Cu = 2.33 $mg.Kg^{-1}$; Fe = 38.12 $mg.Kg^{-1}$; Mn = 44.3 $mg.Kg^{-1}$; Zn = 46.84 $mg.Kg^{-1}$.

### Obtaining plant material

The experiment was conducted in a greenhouse at the University of Santa Cruz (Ilheus, BA) under natural conditions of temperature and luminosity. Micropropagated seedlings of *C. mucugensis* (Giul.) L.R.Parra & Giul. subsp. *mucugensis* were provided by the Tissue Culture Laboratory of the State University of Feira de Santana (UEFS), and grown in plastic pots containing 0.4 $dm^3$ of soil collected at rupestrian field on Parque Municipal de Mucugê (Mucugê, Bahia, Brazil; 12°59'27''S, 41°20'11''W and 980 a.s.l). This native soil was previously sterilized at 121°C for two cycles of 1 h with 48 h interval, and after reaching ambient temperature, the resulting pH (measured in water) was 2.8 and it was not adjusted. Previous experiments liming on soil and substrate (coarse and fine

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sand) indicated that this plant do not tolerate (die) soil pH reaching 5.

The soil of rupestrian field collected for the experiment presented a sandy texture class with 84.80% sand, 15.06% silt and 0.14% clay. The chemical characterization of the soil was performed by the Chemical Analysis Laboratory of the Department of Soil Science College of Agriculture "Luiz de Queiroz", University of São Paulo (USP-ESALQ) following Raij et al. (2001) method and presented the following results: pH, 2.7 (in $CaCl_2$); organic matter, 76 g $dm^{-3}$; P, 5 mg $dm^{-3}$; S, 2 mg $dm^{-3}$; K, 0.4 $mmol_c$ $dm^{-3}$; Ca, 2 $mmol_c$ $dm^{-3}$; Mg, 2 $mmol_c$ $dm^{-3}$; Al, 19 $mmol_c$ $dm^{-3}$; H + Al, 386 $mmol_c$ $dm^{-3}$; Cu, 0.1 mg $dm^{-3}$; Mn, 1.1 mg $dm^{-3}$; Fe, 30 mg $dm^{-3}$; Zn, 1.8 mg $dm^{-3}$.

Ten plants of *C. mucugensis* subsp. *mucugensis* were collected on field (from natural population at Mucuge) and evaluated for nutrient composition aiming to prescribe a nutritional fertilization previously to perform the experiment. Dry matter of the rosettes (leaves) was chemical characterized (Raij et al., 2001) and results were: N, 10.78; P, 0.27; K, 1.53; Ca, 0.55; Mg, 1.40; S 0.76 (all maconutrients at g $kg^{-1}$); B, 4.67; Cu, 0.80; Fe, 26.20; Mn, 8.10; Zn, 4.60 (all micronutrients at mg $kg^{-1}$).

### Experimental design

The experimental design was completely randomized and initially 80 sampling units divided among the control and three treatments: plants inoculated with native AMF, with microbiota filtrate from soil, with AMF plus microbiota filtrate. In 20 replicates from each treatment, 12 were collected at three month plant growth to investigate the initial mycorrhiza establishment at the acclimatization phase. The remaining eight plants were collected at 11 month of plant growth. The spores of native AMF used as inoculum were obtained from the multiplication pot using *C. mucugensis* subsp. *mucugensis* as host plant, since there was low sporulation on the previous attempt using a conventional host plant (*Brachiaria decumbes*).

Spores were isolated from 100 g of soil using the technique of wet sieving of Gerdemann and Nicolson (1963) and centrifugation in 50% sucrose using the technique of Jenkins (1964). To simulate the natural microbial composition of soil, a filtrate was prepared using a suspension of field soil with autoclaved distilled water (1:10 m/v), which was stirred for 24 h (Sudová and Vosátka, 2008). Subsequently, the material was passed through a glass funnel containing filter paper (Whatman no. 1) with the aid of a vacuum pump retaining the solid part and mycorrhizal propagules.

After transplantation from *in vitro* condition to the platic pots in a greenhouse, the micropropagated plants (85 days old), and according to the treatment, received 10 ml suspension containing: mycorrhizal inoculum with 470 spores, microbiota filtrate and mycorrhizal inoculum and filtrate.

### Fertilization of plants in pots

Every week, the plants were irrigated at intervals of 48 h with 30 mL of ¼ ionic strength nutrient solution adapted from Hoagland and Arno (1950). The irrigation with distilled water of the same volume was interspersed with nutrient solution. The complete nutrient solution (in mg $L^{-1}$) consisted of: N, 70.00; P, 5; K, 45.36; Ca, 50; Mg, 12.16; S, 64.00; Zn, 0.01; B, 0.11; Cu, 0.005; Fe, 0.25; Mn, 0.11; Mo, 0.002 as $H_3BO_3$; $MnSO_4$; $ZnSO_4$; $CuSO_4.5H_2O$; $(NH_4)_6Mo_7O_{24}.4H_2O$; Fe-EDTA; $KH_2PO_4$; $(NH_4)_2SO_4$; $K_2SO_4$; $Ca(NO_3)_2$; $MgSO_4$ salts.

### Dry biomass and nutritional analysis

For the analysis of biomass, rosettes (leaves) were dried at 60°C in an oven with forced air circulation until constant weight. Dry matter was obtained and due to the small volume of plant material, only one sample (the sum of all replicates) per treatment was sent to the Laboratory of Mineral Nutrition of Plants USP - ESALQ for nutritional analysis. The methodologies used in this analysis were: P: colorimetry (ammonium metavanadate method), S: colorimetry (turbidimetric barium sulfate), K, Ca and Mg by atomic absorption spectrophotometry, Cu, Fe, Mn and Zn: absorption spectrophotometry atomic; sulfuric digestion for total N, B: colorimetry (Azomethine H method).

### Assessment of AMFs colonization

To estimate the percentage of mycorrhizal colonization, *C. mucugensis* subsp. *mucugensis* roots were blenched in 10% KOH and stained using trypan blue according to the methodology described by Phillips and Hayman (1970). The estimate of colonization of root segments was based on the method of intersection enlarged (McGonigle et al., 1990).

### Extraction and quantification of spore production

Spores of rhizosphere soil samples were extracted following the technique of decanting and wet sieving of Gerdemann and Nicolson (1963) combined with the technique of centrifugation in sucrose solution at 50% of Jenkins (1964). The isolated spores were quantified in a Petri dish and stored in tubes, kept in the refrigerator until analysis of taxonomic characteristics needed for identification.

### Taxonomic identification of AMFs

The spores were previously isolated in separate groups of morphotypes under a stereomicroscope and then mounted on slides with permanent PVLG resin and Melzer reagent (Morton et al., 1996). Spores preserved on slides were observed under an optical microscope (magnification of 1000x) and morphological characters such as size (in µm), shape, color, structure and decoration of wall, type of hyphae and spore germination mode, were recorded for comparison with the related literature. The identification was carried out by using Schenck and Perez (1988) manual and current avaliable literature.

### Statistical data analysis

The data obtained for rosette dry mass, spore number and percentage of mycorrhizal colonization were compared by a one-way ANOVA/Tukey multiple comparison or a t-test when appropriate. The analyzes were performed in the statistical package STATISTICA 8.0 (Statsoft 2002).

## RESULTS

Of the total 12 sample units for each treatment collected after three months (Figure 1A, B and C) of growth in greenhouse, 100% of *C. mucugensis* subsp. *mucugensis* plants inoculated with native AMF and inoculated with AMF plus microbiota filtrate survived. Three plants from microbiota filtrate treatment and four from control died. At nine months of growth plants initiate scape (flowering) production (Figure 1D) and some flowers were obtained at the end of eleven month of growth at greenhouse


**Figure 1.** Partial view of experiment and mycorrhizal colonization in roots of an *C. mucugensis* subsp. *mucugensis* plants inoculated with AMF. (A) Partial view of experiment with *C. mucugensis* subsp. *mucugensis* plants inoculated with AMF after three months of growth in greenhouse. (B) Partial view of experiment showing plants with their floral scapes (arrows) developed after eleven months of growth. (C) Detail of a rosette from an AMF inoculated plant with three months of growth. (D) Detail of a rosette from an AMF inoculated plant with some initial flower scapes developing (nine months of growth in greenhouse).

(Figure 1B). At the end of the experiment from the eight remaining sampling units, five plants from mycorrhiza treatment and seven plants from mycorrhiza plus filtrate treatment survived. On the other hand, seven plants from filtrate treatment and seven plants from control died.

## Aboveground biomass

Rosette dry mass from three month growth plants of *C. mucugensis* subsp. *mucugensis* presented significant differences ($p \leq 0.05$) among mycorrhiza treatments and non mycorrhizal (control and microbiota filtrate) plants evidenced the strong influence of AMF on biomass production (Table 1). The mean values of rosette biomass of eleven months growth plants from AMF inoculated and AMF plus microbiota filtrate did not statistically differ (*t* test $p \leq 0.05$) because only one plant from control and microbiota filtrate treatments survived; statistical analysis was not carried out, but the diference from mycorrhiza treatments was evident (Table 1).

## Mycorrhizal colonization

The mean values of mycorrhizal colonization in AMF inoculated and AMF plus filtrate plants did not differ significantly from each other in both collection times; however, there was an increase in the percentage of colonization of these two treatments when comparing the three and eleven months palnt (Table 1). AMF inoculated plants showed the highest percentages of colonization in root fragments of plants evaluated at three and eleven months of growth. In roots of non-inoculated control and microbiota filtrate inoculated plants, no signal of mycorrhizal structures were observed in both periods (Table 1). During qualitative evaluation with microscope, intraradical hyphae (Figure 2A) and vesicles (Figure 2B) were observed, however arbuscules were the structures more frequently observed (Figure 2C and D).

## Nutritional diagnosis

The levels of macro and micronutrients observed in composed samples of rosette dry biomass *C. mucugensis* subsp. *mucugensis* at three and eleven months of growth are presented in Table 2. The yield of dry matter of filtrate and control plants in eleven months old plants was insufficient for chemical analysis, therefore are not presented in Table 2. In general, there was no large variation on nutrient levels among plants from

**Table 1.** Biomass of rosettes and mycorrhiza of *C. mucugensis* subsp. *mucugensis* micropropagated plants inoculated with native AMF, microbiota filtrate, AMF plus microbiota filtrate and control plants after three and eleven months of growth in greenhouse conditions.

| Plant growth (months) | Parameter | Treatment | | | |
|---|---|---|---|---|---|
| | | Control | AMF | Microbiota filtrate | AMF plus Microbiota filtrate |
| Three | Rosette (leaves) dry weight (mg) | 82±18b | 145±28a | 80±14b | 137±18[a] |
| | Mycorrhiza colonization (%) | 0 | 67.7±12.8 | 0 | 62.1±13.8 |
| | Number of spores (per 100 g of soil) | 182±31 | 1118±212 | 36±14 | 1025±116 |
| Eleven | Rosette (leaves) dry wieght (mg) | 280 | 616±117 | 310 | 491±111 |
| | Mycorrhiza colonization (%) | 0 | 94±5.89 | 0 | 95±3.46 |
| | Number of spores (per 100 g of soil) | 190 | 1749±306 | 25 | 1449±74 |

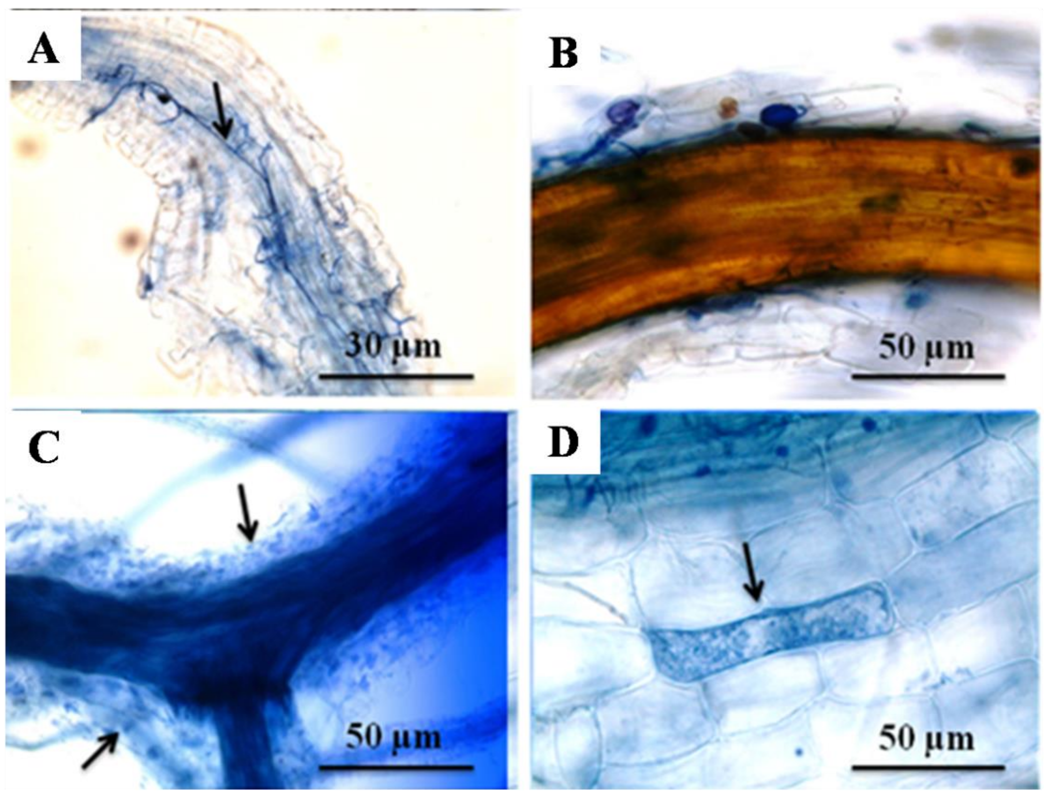


**Figure 2.** Partial view of experiment and mycorrhizal colonization in roots of an *C. mucugensis* subsp. *mucugensis* plants inoculated with AMF. (A) Mycorrhizal colonization in roots of *C. mucugensis* subsp. *mucugensis* AMF plus microbiota filtrate inoculated plants. Arrow indicate a extraradical hypha; (B) detail of some vesicles in the cortex of an AMF plus microbiota filtrate plant; (C) general view of a densely arbuscules occupied cortical cells (arrows); (D) detail of an arbuscule (arrow) in the cortical cell of an AMF inoculated *C. mucugensis* subsp. *mucugensis* root segment.

differet treatments.

## Quantification of spores

The evaluation of the number of AMF spores of soil rhizosphere demonstrated, as expected, mycorrhiza and mycorrhiza plus microbiota filtrate plants presented significat differences when compared with filtrate and control plants, but not significately different between them (Table 1).

Quantification performed for eleven month old plants presented mean values not statiticaly different between filtrate plus mycorrhiza and mycorrhiza plants (Table 1).

**Table 2.** Macronutrients and micronutrients concentrations in rosettes (leaves) of *C. mucugensis* subsp. *mucugensis* with native AMF, microbiota filtrate, AMF plus microbiota filtrate and control plants after three and eleven months of growth in greenhouse conditions.

| Plant (months) | Treatment | Macronutrient (g Kg$^{-1}$) | | | | | | Micronutrient (mg Kg$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | N | P | K | Ca | Mg | S | Cu | Fe | Mn | Zn |
| Three | Control | 32.16 | 3.82 | 58.91 | 2.10 | 2.10 | 3.96 | 19.11 | 85.70 | 142.10 | 61.40 |
| | AMF | 34.02 | 4.06 | 17.34 | 2.10 | 1.80 | 3.73 | 18.25 | 73.70 | 125.20 | 67.80 |
| | Microbiota filtrate | 36.75 | 3.85 | 28.05 | 2.05 | 1.90 | 3.56 | 17.22 | 76.80 | 132.00 | 53.70 |
| | AMF plus Microbiota filtrate | 33.96 | 4.03 | 27.03 | 2.30 | 2.20 | 4.15 | 20.66 | 76.20 | 114.30 | 55.00 |
| Eleven | AMF | 35.15 | 3.66 | 11.47 | 2.91 | 3.0 | 6.80 | 3.80 | 89.8 | 109.1 | 49.9 |
| | AMF plus Microbiota filtrated | 34.00 | 3.60 | 10.90 | 2.46 | 3.1 | 6.6 | 3.71 | 100.8 | 94.1 | 45.8 |

Statistical analysis was not performed on the control and filtrate plants due to death of plants.

## Taxonomic identification of AMFs

Spores isolated from rhizosphere soil from plants of *C. mucugensis* subsp. *mucugensis* inoculated with mycorrhizae and mycorrhiza plus filtrate used to identify seven species of AMFs listed below:

1. *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüßler
2. *Glomus macrocarpum* Tulasne & Tulasne
3. *Glomus microaggregatum* Koske, Gemma & Olexia
4. *Glomus microcarpum* Tulasne & Tulasne
5. *Glomus* sp.
6. *Scutellospora dispurpurascens* J.B.Morton & Koske
7. *Scutellospora spiniosissima* C.Walker & Cuenca

The *Claroideoglomus etunicatum* and *Glomus macrocarpon* species were the only species found in both treatments. These spores are shown in Figure 3.

## DISCUSSION

High rates of root colonization by native AMF was observed in *C. mucugensis* subsp. *mucugensis* micropropagated plants. These rates influenced growth responses of plants and showed the mycorrhizal dependence (mycotrophism) of *C. mucugensis* subsp. *mucugensis* since non-inoculated AMF plants, even with frequent nutrient solution fertilization on the natural soil, did not grow but died. Our results clearly pointed that *C. mucugensis* subsp. *mucugensis* is a mycotrophic plant with rate of mycorrhizal colonization of eleven old months higher than those observed by Pagano and Scotti (2009) on *Paepalanthus bromelioides* and Aristizabal et al. (2004) in roots of *Paepalanthus* sp., two Eriocaulaceae species. This rate of colonization by AMFs is also seen in other studies with plants of semi-arid environments (with low water availability) which showed a high symbiotic effectiveness between AMF and plant species (Yamato et al., 2008; Estrada et al., 2013).

The effectiveness of the symbiosis between the micropropagated plants of *C. mucugensis* subsp. *mucugensis* and native AMFs was also verified by the production of extensive arbuscules, hypha and spores (completing life cycle of the fungus). Spore density in soil of three-month-old mycorrhized plants of *C. mucugensis* subsp. *mucugensis* growth at greenhouse was similar to those observed by Borba and Amorim (2007) in rhizosphere soil (1014 spores 100 g $^{-1}$ soil) from natural plant population of same plant collected in Mucuge. Number of spores observed in mycorrhizal plants of *C. mucugensis* subsp. *mucugensis* can be considered high, demonstrating the dependence of this plant species on AMF for their development. Pagano and Scotti (2009) studying *Paepalanthus bromelioides* reported 139 spores per 100 g of rhizosphere sandy soil collected from field.

It was possible to isolate and identify seven species of native AMF from mycorrhizal plants of *C. mucugensis* subsp. *mucugensis*, and with the exception of *Scutellospora spiniosissima*, all other AMF identified were reported in massive study of Carvalho et al. (2012) that identified and listed 49 species of AMFs collected in rupestrian field of Minas Gerais.

Nutrient analyses of *C. mucugenis* var. *mucugensis* rosette demonstrated that mycorrhizal plants presented concentration of macro and micronutrients similar to those non-AMF inoculated plants, despite markedly difference in the plant growth. As known, probably, this is the first report on nutrient staus of a Eriocaulaceae plant, so, it is difficult to compare nutrients concentration on leaves of *C. mucugenis* var. *mucugensis* micropropaged plants with other poales plants for example. When we compare leaf nutrients between plants collected on field and from greenhouse experiment, it is observed that concentration of some nutrients such as N and P were higher (three-fold and ten-fold, respectively) in greenhouse plants than field collected plants due to frequent irrigation with nutrient solution.

The presence of DSF in the roots of *C. mucugensis* subsp. *mucugensis* observed in AMF treatments possibly occurred during inoculation, the same being adhered to

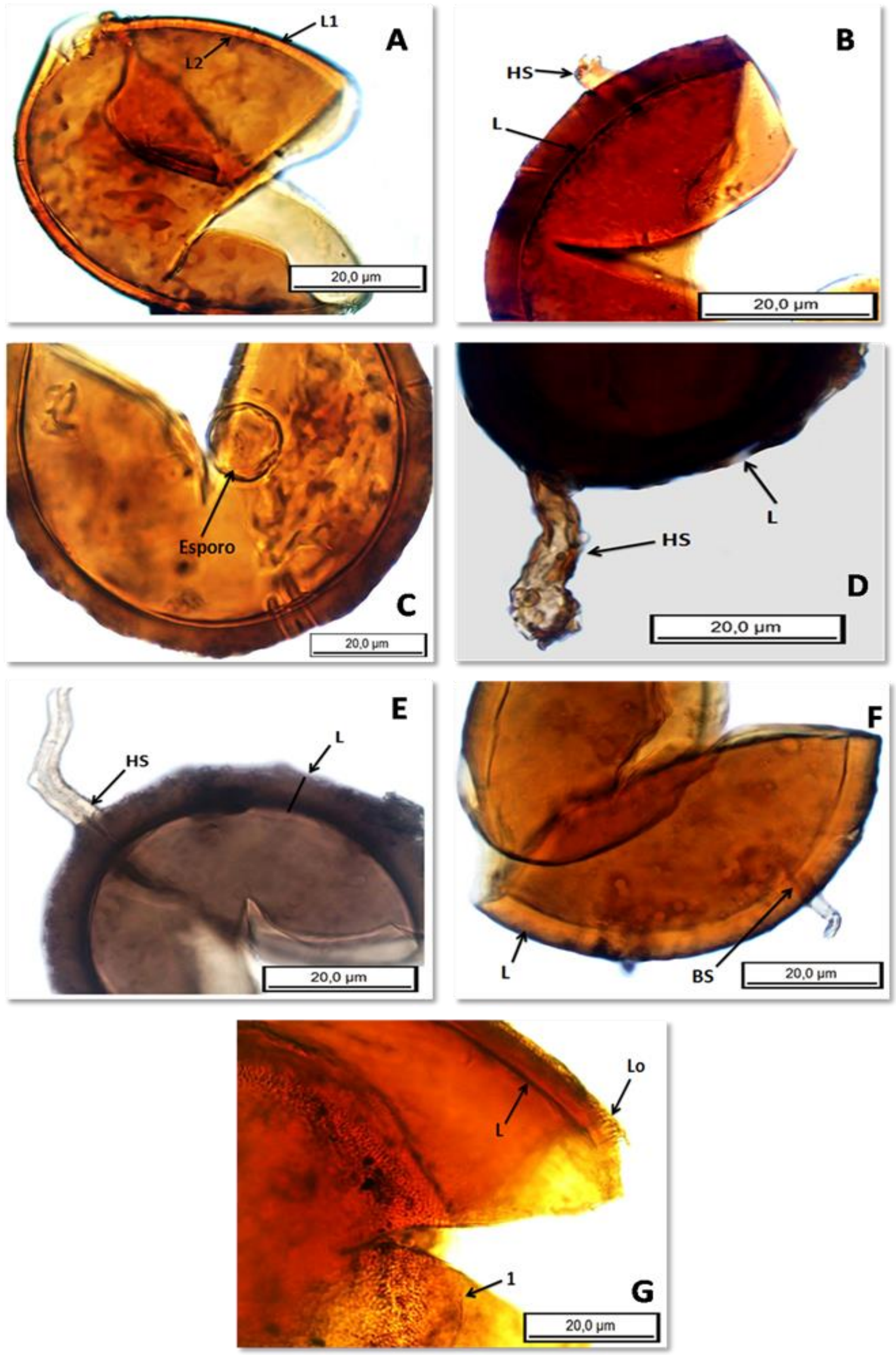


**Figure 3.** Morphological characterization of AMF spores. (A) Photo of the spore of the species, *Glomus etunicatum* found in the soils of the treatment M and M + F; (B) Image of the spore of the *Glomus macrocarpum* species found in soils of both treatments, M and M + F; (C) Photo of the characteristic spore of the species *Glomus microaggregatum*, found in the soil of the M + F treatment; (D) Photo of the spore of the *Glomus microcarpum* species found in the treatment soil M; (E) Photo of the spore of the species *Glomus* sp. found in the soil of the M + F treatment; (F) Photo of the spore of the species *Scutellospora dispurpurescens* found in the treatment soil M; (G) Photo of the spore of the species *Scutellospora spiniosissima* found in the soil of the treatment M. L, wall layer; Lo, ornamental layer; HS, support hyphae; BS, suspensoroid bulb; 1, illustration of the wall layer; Seta, characteristic structure of the species.

AMFs spores were isolated from soil samples. Reports of the coexistence of DSFs and AMFs in the roots of plants stressed environments (arid environments, acidic and nutrient-poor soils) have become increasingly common in studies involving symbiotic associations with fungi (Lingfei et al., 2005; Porras-Alfero et al., 2008; Schmidt et al., 2008).

The filtrate of soil microorganisms combined with the native AMFs also had favorable responses on survival and acquisition of dry matter of micropropagated *C. mucugensis* subsp. *mucugensis* plants*.* However, when inoculated alone, microbiota filtrate did not promote plant growth and reduced the plant survival as observed in the control plants. The influence of soil microbiota on plant development as well as possible interactions between the microbial communities present in the rhizosphere and their consequent contribution to plant productivity are widely discussed in the literature (Walker et al., 2003; Artursson et al., 2006; Bonfante and Anca, 2009; Smith and Smith, 2011).

Native AMFs inoculated in *C. mucugenis* var. *mucugensis* were essential for plants survival and growth, permitting the acclimatization at greenhouse on natural soil. The establishment of *in vitro* grown seedlings in soil is hampered by weak root system at the beginning of acclimation, however, the symbiotic association between AMF and plant roots increases the survival rate of plant to strengthen the root system (Yadav et al., 2012). This strengthening can reflect the importance of AMF for nutrients and water uptake at low fertilized environments, defense against pathogens, decreased water stress improving some important characteristics for plant acclimation (Joshee et al., 2007; Pindi, 2011; Singh et al., 2012; Yadav et al., 2013).

In this study, a relatively high amount of organic matter was observed in the soil (76 g $dm^{-3}$), one of soil characteristic that may have influenced the number of AMF species found. Borba and Amorim (2007) justified the increased number of species of mycorrhizal fungi in the rhizosphere soil, possibly due to a greater accumulation of soil organic matter. Moreover, the species richness from the rhizosphere soil of potted *C. mucugensis* subsp. *mucugensis* may have been influenced by soil type and growing conditions. According to Carvalho (2012), the high diversity of AMF on rupestrian fields can be explained by the heterogeneity of habitats in this environment and the occurrence of AMF species influenced by soil physical properties and also tolerance of these species to low humidity, as shown in some quantitative studies (Conceição and Pirani, 2005).

## Conclusion

In this study, the authors reported on native AMF populations inoculated on *C. mucugensis subsp. mucugensis* plants, but the influence of one determined fungi species was not tested and is a subsequent step to evaluate the influence of mycorrhiza inoculation. The study shows that AMF inoculation is undoubtedly an important biotechnological tool and encourages the use of these microorganisms in conservation programs of endangered *C. mucugensis subsp. mucugensis*.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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