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African Journal of Agricultural Research

Review

Sustainable intensifications of African agriculture through legume-based cropping and *Brachiaria* forage systems

Tesfai M.[1*], Njarui D. M. G.[2] and Ghimire S. R.[3]

[1]Department of Soil Resources and Land use, Division of Environment and Natural Resources, Norwegian Institute of Bioeconomy Research, Fredrik A. Dahls vei 20, 1433 Ås, Norway.
[2]Kenya Agricultural and Livestock Research Organization, Katumani, Kenya.
[3]The Biosciences Eastern and Central Africa, International Livestock Research Institute, Kenya.

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Legume-based cropping system and *Brachiaria* forage system could play a significant role in enhancing food and nutrition security and sustainable intensifications of African agriculture. To reveal this potential, a comprehensive review of literatures and assessment was performed using key indicators in relation to food and nutrition quality, agro-ecological services and socioeconomic benefits. The key indicators for legumes intercropping systems include: Grain yield, soil organic matter, food availability, nutritive values of legumes, maize and millets- based foods, proportion of income from crop sale and percentage of farmers aware and/or adopting intercropping. In the case of *Brachiaria* system, the forage biomass, milk yield, availability of milk, milk nutrition contents, income from *Brachiaria* grass and milk sale and people practising the *Brachiaria* technology were considered key indicators. Both systems showed positive impacts and contribute to a range of the United Nation's sustainable development goals including 1, 2, 3, 12, 13 and 15 and other associated targets. Integrating legume-based cropping systems and *Brachiaria* forage system will enhance contributions of smallholder farmers to food and nutrition security. The necessary changes needed in technology, institutions and policies to upscale legume-based cropping systems and *Brachiaria* forage system were suggested. These changes include improved varieties, quality seeds, improved cultivation practices, market provision, effective extension and advisory services and support to the seed productions and distribution systems, among others. Yet, to fully tap the potentials of legume-based and *Brachiaria* forage systems sustainably and raise the profile of these climate smart systems, context specific research measures are necessary.

Key words: *Brachiaria* forage, climate change, food and nutrition security, legumes-based cropping systems, sustainable intensifications.

INTRODUCTION

Food and nutrition security (FNS) remain a major challenge in Africa, though some progress has been made in the last two decades, particularly in reducing the proportions of undernourished people (FAO, IFAD and WFP, 2015). The contribution of agriculture to FNS remains minimal in sub-Saharan Africa (SSA) despite the development and release of improved agricultural technologies by research institutions and others (FAO, 2015). Among the factors contributing to this situation is that the current agricultural landscape is dominated by monoculture system and there is limited use of planted forage for livestock feeding. A few crops constitute the staple diets of majority of the population in SSA, for examples, maize in eastern and southern Africa and cassava in the western African (OECD/FAO, 2016). These staple food crops are rich in carbohydrates but do not meet the recommended dietary allowance for proteins, vitamins, and minerals levels necessary for healthy life. Monoculture lessens the soil fertility and increase incidence of pests and diseases. In addition, monoculture of staple crops in large acreage causes negative impacts on the environment and ecosystem services (IPES-Food, 2016). Added to these, poor agricultural extension and advisory services, inappropriate policies, and weak institutional arrangements have aggravated the problem of FNS. Several cases have been reported on major failures of monoculture practices in different crops across the globe that includes the damage of rice crop by brown plant hopper in Indonesia in 1970s and the destruction of citrus industry by citrus greening disease in USA in 1980s (World Conservation Monitoring Centre, 1992).

A shift from monoculture to diverse agro-ecological farming can be an alternative pathway as the latter promotes sustainable agriculture intensification (SAI) and provides multiple benefits and ecosystem services from the use of the same piece of land. These benefits and services entail provision of diverse food sources to human nutrition and animal feeding, agro- biodiversity conservation, greater climate resilience, improved soil fertility, and increased income of smallholder farmers with concomitant decrease in risks of crop failure. Crop diversification with legumes and forages (e.g. *Brachiaria* grass) supply the above-mentioned benefits and services on a sustainable basis (Tables 2 and 3). *Brachiaria* grass is a tropical forage native to East Africa, which is highly palatable, nutritious to livestock, well adapted to drought and low fertility soils, and increase livestock productivity (Mutai et al., 2017; Ghimire et al., 2015).

There is a growing interest to explore the potential of the legumes-based cropping systems and *Brachiaria* forage systems for sustainable FNS and ecosystem services. To explore this potential, a project called Innovations in Technology, Institutional and Extension Approaches towards Sustainable Agriculture and Enhanced Food and Nutrition Security in Africa (InnovAfrica) funded by EU-Horizon 2020 program has been implemented in Ethiopia, Kenya, Malawi, Rwanda, Tanzania and South Africa. This consortium involves eleven institutions from Africa and five institutions from Europe (www.innovafrica.eu). Validation and upscaling of innovative sustainable agriculture intensification systems (SAIs) integrating along with novel extension and advisory services (EASs) and innovative institutional approaches (IIAs) is one of the major objectives of the InnovAfrica project. Crop diversification of maize/millets with legumes and *Brachiaria* forage interventions are two major SAIs being evaluated and promoted in the selected sites of InnovAfrica case countries. These interventions are being implemented integrating various EASs (e.g. farmer to farmer extension) and IIAs (e.g. multi-actor platforms). The multi actor platforms (MAPs) members constituted of public sectors, non-governmental organizations (NGOs), farmers organizations and small and medium enterprises (SMEs).

In this paper, we attempt to review and synthesize recent research findings on two SAIs, that is, legume-based maize/millets system and *Brachiaria* forage system for livestock production. Moreover, the paper assesses the performance of these systems in delivering food and nutrition quality, agro-ecological services and socioeconomic benefits under the smallholder farming system. Finally, the paper concludes with some possible measures to improve the legume-based cropping and *Brachiaria* forage systems thereby revealing their potentials.

Legume based cropping systems

There are several forms of crop diversification practices adopted by smallholder farmers in Africa. These include from mono-cropping to multi-storey intercropping systems (Table 1). Of the above listed cropping systems, there is enormous knowledge and rich experience on the intercropping systems. Some of the benefits and impacts of legume-based intercropping systems are listed in Table 2.

Crop diversification planting legumes with maize/millets could contribute to achieve the various SDGs including SDG 1 (Alemayehu et al., 2017), SDG 2 (Habiyaremye et al., 2017), SDG 3 (Tesfai et al., 2018), SDG 13 (FAO, 2016), and SDG 15 (Chaer et al., 2011). However, their potential to contribute to the SDGs is poorly understood and has not been fully assessed.

Brachiaria forage system

Brachiaria (*Brachiaria* spp.) is a tropical forage with productive lifespan of about 20 years. This native

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*Corresponding author. E-mail: mehreteab.tesfai@nibio.no.

Table 1. Brief definitions and concepts of the various types of crop diversification systems.

Cropping system	Definitions/concepts	Reference
Monocropping	Growing a single crop year after year on the same land also called as mono-culturing	Gebru (2015)
Intercropping	Growing two or more crops during the same growing season on the same piece of land	Ghosh et al. (2006)
Crop rotation	Planting by changing the type of crops grown in the field each season or each year	Nyambati et al. (2006)
Sequential cropping	Growing two crops in the same field, one after the other in the same year	Massawe et al.(2016b)
Strip cropping	Planting alternating strips of crops (e.g. cereals and legumes) in broad strips in the field	Szumigalski and Van Acker (2008)
Relay cropping	Growing one crop and then planting another crop in the same field before harvesting the first crop	Massawe et al. (2016a)
Multi-storey intercropping	Growing two or more crops with different heights and cultivating simultaneously on the same field.	Nimbolkar (2016)

Table 2. Benefits and impacts of legume-based intercropping.

Benefit	Impact	Reference
Increases agricultural productivity	Contribute to increased farmers income	Alemayehu et al.(2017)
Grow on residual soil moisture	Contribute to more efficient utilization of water	Mugendi et al.(2011)
Save fossil energy required to manufacture synthetic N fertilizers	Contribute to reduce emissions of nitrous oxides	FAO (2016)
Minimise risks of crop failure and market fluctuations	Increases coping strategies to climate change	Gliessman (1985)
Supply nitrogen through biological N- fixation	Contribute to increase soil organic matter and soil fertility improvement	Dwivedi et al. (2015)
Enhance pollination and provide feed to pollinators and beneficial insects	Promote agro-biodiversity	Tesfai et al. (2018)
Reduced pest and diseases incidence	Reduce cost of pesticides and chemicals	Lithourgidis et al. (2011)
Meets food preferences and/or cultural demands	Increased consumption of plant-based diets	Brooker et al. (2015)
Major source of protein and are rich in iron and zinc, excellent supplier of fibre and vitamins	Contribute to improved nutrition and alleviate malnutrition	Habiyaremye et al. (2017)
Helpful in the fight against non- communicable diseases (e.g. heart disease)	Contribute to improved human health	Tesfai et al. (2018)

African grass is well adapted to drought, marginal soils and drought stress. *Brachiaria* is the most extensively grown tropical forage in Latin America, Asia, South Pacific, and Australia (Mutai et al., 2017). The cultivation of *Brachiaria* grass for pasture production has been spurred in Africa following the pioneering collaborative work among Biosciences eastern and central Africa - International Livestock Research Institute (BecA-ILRI) Hub, Kenya Agricultural and Livestock Research Organization (KALRO) and Rwanda Agricultural Board (RAB), International Centre for Tropical Agriculture (CIAT) and Grasslanz Technology Limited (Ghimire et al., 2015). *Brachiaria* grass is used for hay production and for sale by non-livestock farmers. Some of the benefits and impacts of *Brachiaria* forage systems are listed in Table 3.

Brachiaria forage cultivation could contribute to achieve the various SDGs including SDG 1 (Kermah et

Table 3. Benefits and impacts of *Brachiaria* forage production system.

Benefit	Impact	Reference
Increases forage availability and milk productions	Contribute to increased farmers income	Kermah et al. (2017)
Ability to sequester large amounts of organic carbon	Contribute to reductions of greenhouse gas emissions	Njarui et al. (2016)
High biomass production with nutritious herbage	Hosts diverse groups of bacteria beneficial to plant growth	Mutai et al. (2017)
Improve soil fertility	Significant roles in erosion control	Ghimire et al. (2015)
Adapted to drought conditions and enhance nitrogen use efficiency	Greater climate resilience and efficient resources utilization	Arango et al. (2014)
Minimizes eutrophication and ground water pollution	Contribute to reduce nitrogen and phosphorus losses	Moreta et al. (2014)

Table 4. Challenges faced by African smallholder famers to implement legume-based intercropping and *Brachiaria* forage cultivations.

Challenges faced by African smallholder famers	Reference
Legume based intercropping	
Lack of access to improved seeds	Hauggaard-Nielsen et al. (2003)
Difficulties in farm mechanization or inputs application	Feike et al. (2012)
High cost of maintenance (e.g. labour for weeding)	Kebebew (2014)
Supply chains and markets are inadequately developed	Stagnari et al. (2017)
Inadequate policy support to legume-based intercropping	Mapfumo (2011)
Lack of awareness on long term benefits of legumes	Mapfumo (2011)
Brachiaria forage	
Lack of access to *Brachiaria* seeds	Ondabu et al. (2017)
Lack of information and awareness on *Brachiaria* grass	Njarui et al. (2016)
Inadequate policy support to *Brachiaria* forage cultivation	Njarui et al. (2016)
Upscaling repatriated commercial varieties requires caution	Ondabu et al. (2017)

al., 2017); SDGs 2 and 3 (Vendramini et al., 2014); SDG 12 (Arango et al., 2014); SDG 13 (Moreta et al., 2014); and SDG 15 (Mutai et al., 2017). However, their potentials to contribute to the SDGs is poorly understood and yet to be documented.

Despite the immense benefits and positive impacts of the *Brachiaria* grass; its potential to address the challenge of livestock feed scarcity in Africa, remain unexploited. Some of the challenges faced by African smallholder famers to implement legume-based intercropping and *Brachiaria* grass systems are presented in Table 4.

In the following sections, the multiple services delivered by legume-based cropping and *Brachiaria* forage systems are assessed and discussed using a set of key ecological, food and nutrition and socioeconomic indicators.

APPROACH

It is assumed that with integrated interventions in technology (e.g. legume-based intercropping with maize/millets plus Brachiaria forage); innovative institutional approaches (e.g. MAPs) and EASs (e.g. F2F), the combined effects of ecological and food/nutritional impacts will contribute positively to socio-economic impacts (Figure 1).

The criteria used to select these indicators include: (i) methodological soundness and base line data availability, (ii) easy to measure and sensitivity to changes in short term, (iii) relevance to objectives of the study and utility for users, (iv) capacity to monitor the indicators, and (v) usefulness of indicators for project monitoring and evaluation.

Indicators for legume-based cropping systems

Six key indicators were selected to assess the performance of maize/millets-legumes intercropping systems against sole crops. These indicators assess the ecological, food and nutrition and socioeconomic aspects of the cropping systems.

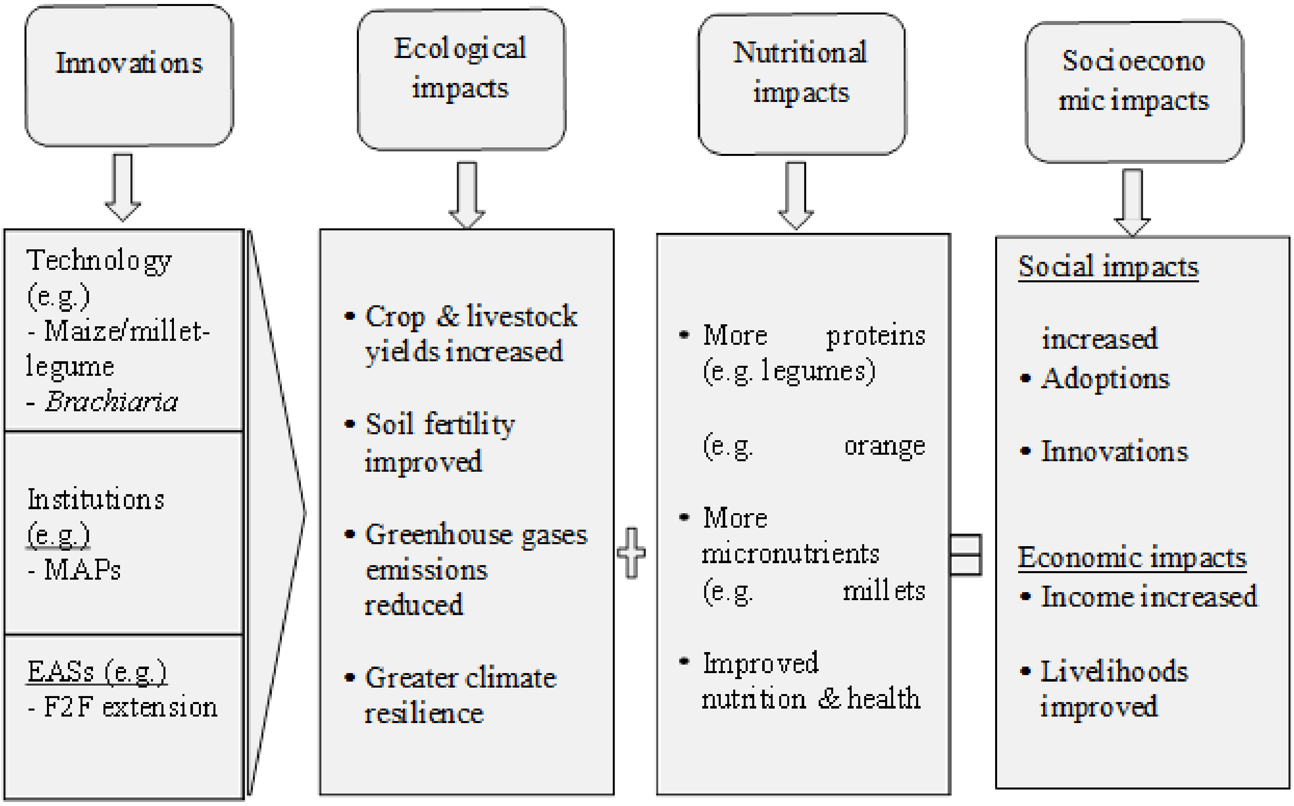

Figure 1. Innovations in technology, extension and advisory services (EASs) and institutional approach on ecological, nutritional and socio-economic conditions of a smallholder in Africa.

Crop yield

The grain yield in maize-legume intercropping was higher than sole cropping in Ethiopia (Alemayehu et al., 2017), in South Africa (Tsubo et al., 2004) and in Malawi (Mhango, 2011), and in Tanzania (Massawe et al., 2016b). The higher yield for intercrop compared with sole crop maize was due to the additional bean (dry) yield obtained from intercropping. However, the yield is lower than sole crop when computing the yield separately for intercropping. The yield penalty of intercropping maize was compensated for by yield of the companion bean crop leading to land equivalent ratio greater than one. This additional yield of bean (dry) is of great benefit to farmers for improved nutrition, as a source of cash, and also for sustainability of the cropping system.

Soil organic matter

Legumes have potential to increase soil organic matter (McCallum et al., 2004) because the nitrogen supplied by legumes through biological nitrogen fixation facilitates the decomposition of crop residues in the soil and their conversion to increase soil organic matter. Switching from monoculture to a rotation with legume crops is reported to stimulate the accumulation of 0.5 to 1.0 t/ha of soil organic carbon annually, with the legume component in the cropping sequence contributing up to 20% of the carbon gain (Wu et al., 2003). Legume intercropped with millet, the glume/chaff residues left after threshing of millet represents a potential source of reusable organic material when applied with N and P fertilizers (Issoufa, 2015).

Food availability

The level of food availability is expressed in terms of how long the food stock lasts (number of months in a year) and the household dietary diversity score (HDDS) that ranges from 0 to 12. The food stock lasts between 8 and 11 months in all the case countries. In other words, none of the countries are food secure throughout the year. The HDDS varies from 5.8 to 10 which indicate that household diets offer some diversity in both macro- and micronutrients (Table 5).

This food diversity could include cereals and pulses. Except for Malawi, diet diversity scores are mostly lower (< 4) for female-headed households than male headed

Table 5. Number of months food stock lasts and dietary diversity scores in case of study countries (average values).

Case study	Food stock lasts (No. of months)	Household dietary diversity score * ((0 – 2)
Malawi	10	8
Ethiopia	8	6
South Africa	11	10
Tanzania	9	6
Kenya	8.6	5.8

*Source: Household survey

households.

Nutrient contents of food from legumes, maize and millets

Legumes are important food crops that can play a major role in addressing future global FNS while providing multiple ecosystem services (FAO, 2016). They have important role in human nutrition, especially in the dietary pattern of low-income households in developing countries and vegetarians. Pulses are often called ‘the poor man’s meat’ for their protein source and their rich content of minerals especially iron and zinc, fibre and vitamins (Tharanathan and Mahadevamma, 2003). Except for carbohydrate, the nutrient composition of pulses in general is higher than rice and wheat (Tesfai et al., 2018). On the other hand, millets, in general are rich proteins, fiber, mineral, iron and calcium compared to rice and wheat. For example, finger millet has 7.6 times more calcium than rice while some of the other millets group contains even more calcium compared to rice and wheat (Tesfai et al., 2018).

Maize seeds are rich in various nutrients including carbohydrates and vitamins. Intercropping of unfertilized maize with grain legumes increased protein yields compared to sole maize stands (Snapp et al., 2010; Waddington et al., 2007a). Similar increments in protein are possible through some maize-grain legume rotations (Waddington et al., 2007b). This shows possible to enhance the production of protein without large investments in subsidized mineral fertilizer (Droppelmann et al., 2017). Therefore, consuming legumes with millets/maize -based diets can alleviate malnutrition that affects millions of people in Africa.

Proportion of income from crop sale

In Ethiopia, economics of the intercropping versus sole cropping system was analysed following a partial budget procedure based on the existing cost of production. Legume-based intercropping increased financial returns by 16% relative to sole crop maize (Alemayehu et al., 2017). According to this finding, the highest financial advantage was obtained from the single row intercropping plant arrangement (with 128 kg N and 20 kg P kg per ha) due to the high productivity of the component crops. Similar results were also found by Workayehu and Wortmann (2011) who reported the profitability of maize–common bean intercropping as compared with sole crop production.

Percentage of farmers aware and/or practising intercropping

The practice of intercropping (e.g. cereals with legumes) has existed over a long period of time and is embedded in the indigenous knowledge systems. Almost all the sampled farmers in the case countries were aware of intercropping principles and practices, and most of them cultivate legumes intercropped with cereals or other suitable crops to the area. There are several success stories on legume based intercropping practices in the case countries. One good example is the Malawi Farmer to Farmer Agroecology project that aimed to implement intercropping of cereal crops with legumes using a Farmer to Farmer (F2F) extension approach. Intercropping of legumes and cereals was encouraged for soil health improvement through biomass incorporation and nitrogen fixation. The incorporation of legume residues into the soil redressed the soil nutrients in areas used to apply bush burning. Moreover, the project encouraged farmers to apply compost and/or organic manure, and organic pest control methods. The maize-legumes intercropping rendered a 6% increase in yield when compared to sole cropping (Nyantakyi-Frimpong et al., 2016). However, constraints such as lack of access to improved seeds and low market prices deter farmers from fully integrating the intercropping system in their farm. In this case, the MAPs members (in each case country) are actively engaged in linking the value chain actors (from producers to consumers continuum). The MAPs members also participate in other activities of the project (www.innovafrica.eu).

Indicators for *Brachiaria* forage systems

The multiple services delivered by *Brachiaria* forage grass

Table 6. DM yield of *Brachiaria* spp. at different field management in Kenya and Tanzania.

Country	AEZs and field management	DM yield (t/ha)	Source
Kenya	With fertilizer applications	5 - 36	Bogdan (1977)
	Coastal lowlands	≥ 8.0	Ondiko et al. (2016)
	Central highlands	> 10.0	Nyambati et al. (2016)
	Semi-arid eastern region	4.0	Njarui et al. (2016)
Tanzania	With no fertilizer application	3.0	Frederiksen and Kategile (1980)
	With N fertilizer application	6.0 - 26.5	Urio et al. (1988)

are assessed using six key indicators in contrast to other forage grasses. These indicators assess the ecological, food and nutrition and socioeconomic aspects of the *Brachiaria* forage systems.

Forage biomass

There is limited information on the productivity of *Brachiaria* spp. in different agro-ecological zones (AEZ) in Africa. The dry matter (DM) yields of *Brachiaria* vary among countries and are influenced by a range of factors including variety, moisture, soil fertility, and fertilizer applications (Table 6).

Furthermore, the cultivated forages are relegated to the less fertile part of the land and degraded soils and, as a result, growth is poor, they suffer mineral deficiencies, and are low in crude protein and energy. This is primarily because forages are not the final product.

Milk yield

In Kenya, *Brachiaria* grass has shown remarkable response when fed to livestock. It is superior to Rhodes grass which is the commonly cultivated grass for livestock feed. Studies carried out with smallholder farmers showed increased milk production from 4 to 4.6 L per cow per day for low yielding animals, a 15% increase and 9 to 12.6 L per cow per day for the relatively higher yielding dairy cattle representing a 40% increase (Muinga et al., 2016).

In Rwanda, dairy cattle feed *Brachiaria* grass and supplemented with legumes reported higher daily milk yield than those based on Napier grass. Cows fed with sole *Brachiaria brizantha* cv. Piata produced 33% more milk than cows fed with sole Napier grass diets. Cows fed with *Brachiaria brizantha* cv. Piata-legume diets produced approximately 21% more milk than cows fed with Napier-legume diet (Mutimura et al., 2018).

Availability of milk

Feeding *Brachiaria* has significant positive impacts on annual milk production. Data from recent trials indicates that adoption of *Brachiaria brizantha* cultivars increased baseline milk production by up to 4 L per cow per day on participating farmers thus improving the availability of milk at both household level and for sale.

Milk nutrition contents

Milk contains numerous nutrients and it makes a significant contribution to meeting the body's needs for calcium, magnesium, selenium, riboflavin, vitamin B12 and pantothenic acid (vitamin B5) (Muehlhoff, 2013). As a concentrated source of macro- and micronutrients, milk and dairy products can play a particularly important role in human nutrition in smallholder farm that frequently lack diversity and consumption of animal-source foods. Water is the maincomponent and make up approximately 90% of milk followed by fat (or lipid) which constitute from 3.5 to 6.0% of milk. Milk is also a major source of dietary energy, protein and fat (FAOSTAT, 2012). The concentration of protein in milk varies from 3.0 to 4.0 percent or 30 to 40 g L^{-1} (Wattiaux, 1995). Milk is recommended as part of a healthy diet since it contains naturally many essential nutrients.

Income from Brachiaria grass and milk sale

Livestock are important source of income for smallholder farmers in Africa. Adoption of *Brachiaria* technology has positive implications for income generation of smallholder livestock farmers. The most important contribution of *Brachiaria* forage is their direct effect on increasing milk production which generates cash. Although no economic analysis has been conducted from the milk yield increment as a result of adoption of *Brachiaria,* it is quite clear that the extra milk produced would give high profit margin as there is no additional cost in establishment of *Brachiaria* compared with the other traditional forages. The use of *Brachiaria* for hay production also offers not only as feed resource for livestock but also an opportunity to raise income by selling the baled hay to other livestock farmers.

People practising the Brachiaria technology

The current dissemination and expansion of *Brachiaria* acreage in Africa depends on seeds imported from South America and East Asia. The seeds are not easily accessible and expensive for smallholder farmers. Despite numerous efforts to promote cultivation of forages in Africa, adoption of *Brachiaria* grass have remained slow and its expansion of acreage is low in Africa. The contributing factors could be: (i) lack of information and awareness on *Brachiaria* grass*; (ii)* small land holding size (1-2 ha) and (iii) shortage of labour. The source of labour is mainly from the family and forage production yet to be mechanised (Njarui et al., 2011). Hence, small scale farmers give preference to grow food crops than forages in general. On average, less than 10% of the households' land holdings are allocated for forage production. For example, in Kenya, the *Brachiaria* technology is practised mainly by smallholder crop-livestock farmers in the coastal lowlands, eastern region, central highlands and north western highlands. The farmers are more commercially oriented, and the main animals reared are exotic and crossed with local zebu for milk production (Njarui et al., 2011).

CONCLUSIONS AND RECOMMENDATIONS

In this paper, the benefits of growing legume-based cropping systems and *Brachiaria* forage grass and associated constraints of both systems was reviewed. Legume-based cropping systems and *Brachiaria* forage system contribute to a range of SDGs including 1, 2, 3, 12, 13 and 15 and other associated targets. Adoption of legume-based cropping systems with *Brachiaria* forage system will enhance contributions to SDGs and associated targets. Moreover, legume-based inter cropping systems and *Brachiaria* forage system showed positive impacts on the key indicators chosen for ecological, food and nutrition, and socioeconomic conditions of smallholder farmers. Despite these, adoption and expansion of legume-based cropping systems and *Brachiaria* forage system is limited and slow in SSA. Possible measures that could improve the adoption of legume-based cropping and *Brachiaria* forage systems are suggested below.

Measures to improve legume-based cropping system

Measures that could improve the adoption of legume-based cropping include the following:

1. Improved varieties and quality seeds by:

a. Increasing investments to research and development on improved varieties that are climate resilient and tolerant to abiotic and biotic stress through participatory plant breeding programs; and
b. Providing better access to quality seeds of improved varieties of legumes, maize/millets that are rich in the essential micronutrients, minerals and vitamins for human nutrition and livestock feeding.

2. Improved cultivation practices (including farm mechanization or inputs application) by:

a. Intensifying crop diversification in smallholders farming systems through crop rotations or intercropping of legumes with maize/millets or other suitable cereals; and
b. Integrating conservation agriculture practices in legume-based cropping system.

3. Making legumes-maize/millets marketing accessible and attractive to consumers by:
a. Investing in value added product innovations (for e.g. developing legume/maize/millet-based food recipes) gives the opportunity to diversify their use and reuse; and
b. Promoting access to markets by establishing effective legume/maize /millet networks that connect the different value chain actors and enhances public-private partnerships

4. Improved extension and advisory services by*:*

a. Raising awareness and promotional campaign on benefits of legumes particularly targeting women, children and youth on the health and nutrition benefits of legumes with maize and millets;
b. Providing customized trainings on seed production, multiplication, storage, and consumption of legumes with maize and millets; and
c. Stimulating the development of agribusiness services to support smallholders' access to inputs and services for e.g. by supporting legumes maize/millets seed systems (like community seed banks)

5. Creating enabling policy environment by:

a. Reforming policies that are barriers to the development of legumes and maize/millets cultivation (for e.g. insecure land ownerships laws); and
b. Developing upscaling strategies/incentives that promote legume-based cropping system in SSA.

Measures to improve *Brachiaria* forage cultivation

Measures that could improve the adoption of Brachiaria forage cultivation includes the following:

1. Improvement of Brachiaria for specific trait by:

a. Initiating breeding for drought tolerance as well as pest and diseases resistance; and

b. Capitalizing on acquisition and screening of existing germplasm that are stored in gene banks in different part of the world. For e.g. ILRI Ethiopia and CIAT-Colombia gene banks hosting about 700 accessions of *Brachiaria*.

2. Improved Brachiaria forage management by:

a. Implementing improved agronomic practices and management technologies to maximize herbage yield and improve plant persistence; and

b. Developing guidelines on forage cultivation practices (including planting, harvesting, fertilizer application) for optimum production and nutritive quality.

3. Increased Brachiaria seed production by:

a. Developing a sustainable seed production system to address seed availability at affordable costs. Research should focus on identifying optimum conditions for maximizing seed production for smallholder farmers; and

b. Developing simple and affordable seed harvesting, threshing technologies and storage structures at smallholder level.

3. Improved extension and advisory services for Brachiaria by:

a. Raising awareness program on the potential of different *Brachiaria* forage spp. for income generation of livestock farmers and identify best extension methods to increase adoption and upscaling.

4. Conducive policy and institutional environment of Brachiaria by:

a. Supporting local institutions to promote *Brachiaria* forage cultivation; and

b. Developing upscaling strategies to promote *Brachiaria* forage production and to repatriate commercial varieties as problem of pests and diseases are foreseen.

CONFLICT OF INTEREST

The authors confirm that there is no conflict of interest.

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REFERENCES

Alemayehu A, Tamado T, Nigussie D, Yigzaw D, Kinde T, Wortmann CS (2017). Maize- common bean intercropping to optimize maize-based crop production, Journal of Agricultural Sciences 155:1124-1136.

Arango J, Moreta D, Núñez J, Hartmann K, Domínguez M, Ishitani M, Miles J, Subbarao G, Peters M, Rao I (2014). Developing methods to evaluate phenotypic variability in biological nitrification inhibition (BNI) capacity of *Brachiaria* grasses. Tropical Grasslands 2:6-8.

Bogdan AV (1977). Tropical Pasture and Fodder Plants (grasses and Legumes). London: Longman, ISBN:0582466768, pp. 432-461.

Brooker RW, Bennett AE, Cong WF, Daniell TJ, George TS, Hallett PD, Hawes C, Iannetta PPM, Jones HG, Karley AJ, Li L, McKenzie BM, Pakeman RJ, Paterson E, Schöb C, Shen J, Squire G, Watson CA, Zhang C, Zhang F, Zhang J, White PJ (2015). Improving intercropping: a synthesis of research in agronomy, plant physiology and ecology. New Phytologist 206:107-117.

Chaer GM, Resende AS, de Balieiro FC, Boddey RM (2011). Nitrogen-fixing legume tree species for the reclamation of severely degraded lands in Brazil. Tree Physiology 31:139–149.

Droppelmann KJ, Snapp SS, Waddington SR (2017). Sustainable intensification options for smallholder maize-based farming systems in sub-Saharan Africa. Food Security: The Science, Sociology and Economics of Food Production and Access to Food, Springer;The International Society for Plant Pathology 9(1):133-150.

Dwivedi A, Ista D, Kumar V, Yadav RS, Yadav M, Gupta D, Singh A, Tomar SS (2015). Potential Role of Maize-Legume Intercropping Systems to Improve Soil Fertility Status under Smallholder Farming Systems for Sustainable Agriculture in India, International Journal of Life Sciences Biotechnology and Pharma Research 4:3.

FAOSTAT (2012). FAO statistical database. Available at: http://faostat.fao.org/. Accessed 12 September 2012.

FAO (Food and Agriculture Organization of the United Nations), IFAD, WFP (2015). Achieving Zero Hunger. The critical role of investment in social protection and agriculture. Rome, Italy.

FAO (Food and Agriculture Organization of the United Nations) (2016). Regional Overview of Food Insecurity in Africa: African Food Security Prospects Brighter Than Ever. FAO, Accra. FAO (Food and Agriculture Organization of the United Nations) (2016). FAO Committee on Agriculture, Twenty-Fifth Session. The International Year of Pulses: Nutritious Seeds for a Sustainable Future. COAG/2016/3. available at http://www.fao.org/3/a-mr021e.pdf.

Feike T, Doluschitz R, Chen Q, Graeff-Höonninger S, Claupein W (2012). How to overcome the slow death of intercropping in the North China Plain. Sustainability 4:2550-2565.

Frederiksen JH, Kategile JA (1980). The effects of nitrogen fertilization and time of cutting in first growth in *Brachiaria* brizantha on yield, crude protein content and in vitro digestibility. Tropical Animal Production 5:136-143.

Gebru H (2015). A review on the comparative advantages of intercropping to mono-cropping system Journal of Biology, Agriculture and Healthcare 5:1-13.

Ghosh PK, Manna MC, Bandyopadhyay KK, Ajay AKT, Wanjari RH, Hati KM, Misra AK, Acharya CL, Subba RA (2006). Interspecific Interaction and Nutrient Use in Soybean/Sorghum Intercropping System. Agronomy Journal 98:1097-1108.

Ghimire S, Njarui D, Mutimura M, Cardoso J, Jonhson L, Gichangi E, Teasdale S, Odokanyero K, Caradus J, Rao I, Djikeng A (2015). Climate smart *Brachiaria* for improving livestock production in East Africa: Emerging opportunities. In: Vijay D. Srivastava M. K., Guupta C. K., Malaviya D .R., Roy M.M., Mahanta S. K., Singh J. B., Maity A. and Ghosh P. K. (eds) Sustainable use of grasslands resources for forage production, biodiversity and environmental protection. Proceedings -23rd International Grassland Congress, Range Management Society of India, pp 361-370.

Gliessman SR (1985). Multiple cropping systems: A basis for developing an alternative agriculture. Innovative biological technologies for lesser developed countries. Office of Technology Assessment, Washington, D.C. BP-F-29:76-83.

Hauggaard-Nielsen H, Ambus P, Jensen ES (2003). The comparison of nitrogen use and leaching in sole cropped versus intercropped pea and barley Nutrient Cycling in Agroecosystems 65:289-300.

Habiyaremye C, Matanguihan JB, Guedes JD, Ganjyal GM, Whiteman MR, Kidwell KK, Murphy KM (2017). Proso Millet (Panicum miliaceum L.) and Its Potential for Cultivation in the Pacific Northwest U.S.: A Review. Frontiers in Plant Science 7:1961. https://doi.org/10.3389/fpls.2016.01961.

IPES-Food (2016). From uniformity to diversity: a paradigm shift from industrial agriculture to diversified agro-ecological systems, International Panel of Experts on Sustainable food systems. Online: www.ipes-food.org (accessed on 15 November 2018)

Issoufa BB (2015). Composting millet glume for soil fertility improvement and millet/cowpea productivity in semi-arid zone of Niger. PhD thesis, University of Science and Technology, Kumasi, Ghana.

Kebebew S (2014). Intercropping Soybean (Glycine max L. Merr.) at Different Population Densities with Maize (Zea mays L.) on Yield Component, Yield and System Productivity at Mizan Teferi, Ethiopia, Journal of Agricultural Economics, Extension and Rural Development: ISSN-2360-798X: 1(7):121-127.

Kermah M, Franke CA, Adjei-Nsiahc S, Ahiabord BDK, Abaidooc RC, Giller KE (2017). Maize-grain legume intercropping for enhanced resource use efficiency and crop productivity in the Guinea savanna of northern Ghana. Field Crops Research 213:38-50.

Lithourgidis AS, Dordas CA, Damalas CA, Vlachostergios DN (2011). Annual intercrops: an alternative pathway for sustainable agriculture. Australian Journal of Crop Science 5(4):396-410.

Mapfumo P (2011). Comparative analysis of the current and potential role of legumes in integrated soil fertility management in southern Africa. In: Fighting Poverty in Sub-Saharan Africa: The Multiple Roles of Legumes in Integrated Soil Fertility Management. Springer pp. 175-200.

Massawe PI, Mtei KM, Munishi LK, Ndakidemi PA (2016a). Existing practices for soil fertility management through cereals-legume intercropping systems World 3:080-091.

Massawe PI, Mtei KM, Munishi LK, Ndakidemi PA (2016b). Improving Soil Fertility and Crops Yield through Maize-Legumes (Common bean and Dolichos lablab) Intercropping Systems Journal of Agricultural Science 8:148.

McCallum MH, Kirkegaard JA, Green T, Cresswell HP, Davies SL, Angus JF, Peoples MB (2004). Improved subsoil macroporosity following perennial pastures. Australian Journal of Experimental Agriculture 44:299-307.

Mhango WG (2011). Nitrogen budgets in legume-based cropping systems in Northern Malawi. USA: PhD thesis, Michigan State University. Michigan.

Muehlhoff E, Bennett A, McMahon D (2013). Milk and dairy products in human nutrition. Food and Agriculture Organization of the United Nations, Rome, Italy P. 376.

Mugendi DN, Waswa BS, Mucheru-Muna MW, Kimetu JM, Palm C (2011). Comparative analysis of the current and potential role of legumes in integrated soil fertility management in east Africa, In: Bationo, A., (eds). Fighting poverty in Sub Saharan Africa: The multiple roles of legumes in integrated soil fertility management, Springer science Business Media, B.V. pp. 151-173.

Muinga RW, Njunie MN, Gatheru M, Njarui DMG (2016). The effects of *Brachiaria* grass cultivars on lactation performance of dairy cattle in Kenya. In: Njarui DMG, Gichangi EM, Ghimire SR, Muinga RW (Eds). Climate Smart *Brachiaria* Grasses for Improving Livestock Production in East Africa – Kenya Experience. Proceedings of the workshop held in Naivasha, Kenya, 14 - 15 September 2016 pp. 229-237.

Mutimura M, Ebong C, Idupulapati M, Rao IM, Ignatius V, Nsahlai IV (2018). Effects of supplementation of *Brachiaria brizantha* cv. Piata and Napier grass with *Desmodium distortum* on feed intake, digesta kinetics and milk production in crossbred dairy cows. Animal Nutrition 4(2):222-227

Mutai C, Njuguna J, Ghimire S (2017). *Brachiaria* Grasses (*Brachiaria* spp.) harbor a diverse bacterial community with multiple attributes beneficial to plant growth and development. Microbiology Open 1-11.

Moreta DE, Arango J, Sotelo M, Vergara D, Rincón A, Ishitani M, Castro A, Miles J, Peters M, Tohme J, Subbarao GV, Rao IM (2014). Biological nitrification inhibition (BNI) in Brachiaria pastures: A novel strategy to improve eco-efficiency of crop-livestock systems and to mitigate climate change, Tropical Grasslands – Forrajes Tropicales 2:88-91.

Nimbolkar P (2016). Multi Storied Cropping System in Horticulture-A Sustainable Land Use Approach International Journal of Agriculture Sciences, ISSN:0975-3710.

Njarui DMG, Gichangi EM, Ghimire SR, Muinga RW (2016). Climate smart *Brachiaria* grasses for improving livestock production in East Africa: Kenya Experience: Proceedings of a workshop, Naivasha, Kenya, 14-15 September 2016, Kenya Agricultural and Livestock Research Organization.

Njarui DMG, Gatheru M, Wambua JM, Nguluu SN, Mwangi DM, Keya GA (2011). Feeding management for dairy cattle in smallholder farming systems of semi-arid tropical Kenya. *Livestock Research for Rural Development. Volume 23, Article #111.* Retrieved from http://www.lrrd.org/lrrd23/5/njar23111.htm.

Nyambati E, Sollenberger L, Hiebsch C, Rono S (2006). On-farm productivity of relay-cropped mucuna and lablab in smallholder crop-livestock systems in northwestern. Kenya Journal of Sustainable Agriculture 28:97-116.

Nyambati EM, Ayako W, Chelimo EJ, Njarui DMG (2016). Production and nutritive quality of *Brachiaria* grass cultivars subjected to different cutting intervals in the cool sub-humid highlands of central Kenya. In: Njarui, D M G, Gichangi EM, Ghimire SR, Muinga RW (Eds). Climate Smart *Brachiaria* Grasses for Improving Livestock Production in East Africa– Kenya Experience. Proceedings of the workshop held in Naivasha, Kenya, 14 - 15 September, 2016, pp. 70-79.

Nyantakyi-Frimpong H, Kangmennaang J, Kerr RB, Luginaah I, Dakishoni L, Lupafya E, Shumba L, Katundu M (2016). Agroecology and healthy food systems in semi-humid tropical Africa: Participatory research with vulnerable farming households in Malawi, available at: europepmc.org/abstract/med/27983973, accessed on 12/12/2017.

OECD/FAO (2016). "Agriculture in Sub-Saharan Africa: Prospects and challenges for the next decade", in OECD-FAO Agricultural Outlook 2016-2025, OECD Publishing, Paris. DOI: http://dx.doi.org/10.1787/agr_outlook-2016-5-en.

Ondabu N, Maina S, Kimani W, Njarui D, Djikeng A, Ghimire S (2017). Molecular Characterizations of Kenyan Brachiaria Grass Ecotypes with Microsatellite (SSR) Markers, Agronomy pp. 7- 8.

Ondiko CN, Njunie MN, Njarui DMG, Auma E, Ngode L (2016). Effect of cutting frequency on forage production and nutritive value of Brachiaria grass cultivars in coastal lowlands of Kenya. In: Njarui DMG, Gichangi EM, Ghimire SR, Muinga RW, editors. Climate smart brachiaria grasses for improving livestock production in East Africa—Kenya experience. Proceedings of the workshop held in Naivasha, Kenya, 14–15 September 2016, P. 271.

Snapp SS, Blackie MJ, Gilbert RA, Bezner-Kerr R, Kanyama-Phiri GY (2010). Biodiversity can support a greener revolution in Africa. Proceedings of the National Academy of Sciences of the United States of America 107: 20840–20845.

Stagnari F, Maggio A, Galieni A, Pisante M (2017). Multiple benefits of legumes for agriculture sustainability: an over view, Chemical, and Biological Technologies in Agriculture 4: 2, DOI: 10. 1186/s40538-016-0085-1.

Szumigalski AR, Van Acker, RC (2008). Land equivalent ratios, light interception, and water use in annual intercrops in the presence or absence of in-crop herbicides, Agronomy Journal 100:1145-1154.

Tharanathan RN, Mahadevamma S (2003). Grain legumes a boon to human nutrition. Trends in Food Science & Technology 14: 507–518.

Tesfai M, Nagothu US, Adugna A (2018). Pulses-Millets crop diversification by smallholders and their potential for sustainable food and nutrition security, In: Nagothu US (ed.): Agricultural Development and Sustainable Intensification, Technology and Policy Challenges in the Face of Climate Change, New York: Routledge, pp. 136-161, ISBN: 978- 1-138-30059-0.

Tsubo M, Ogindo HO, Walker S (2004). Yield Evaluation of Maize-Bean Intercropping in a Semi-Arid Region of South Africa, African Crop Science Journal 12(4):351-358.

Urio NA, Sarwatt SV, Mtengeti EJ (1988). A review of the potential of *Brachiaria* species as forage crop for livestock in Tanzania. In: Dzowela, BH (Eds) African forage plant genetic resources, evaluation of forage germplasm and extensive livestock production systems, Proc. 3rd Workshop, Arusha, Tanzania, 27-30/04/1987, ILCA. http://www.fao.org/wairdocs/ilri/x5491e/x5491e0c.htm.

Vendramini JMB, Sollenberger LE, Soares AB, Da Silva WL, Sanchez JMD, Valente AL, Aguiar AD, Mullenix MK (2014). Harvest frequency affects herbage accumulation and nutritive value of Brachiaria grass hybrids in Florida. Tropical Grasslands 2:197–206.

Waddington SR, Mekuria M, Siziba S, Karigwindi J (2007a). Long-term yield sustainability and financial returns from grain legume-maize intercrops on a sandy soil in subhumid north Central Zimbabwe, Experimental Agriculture, 43(4): 489–503.

Waddington SR, Karigwindi J, Chifamba J (2007b). The sustainability of a groundnut plus maize rotation over 12 years on smallholder farms in the sub-humid zone of Zimbabwe. African Journal of Agricultural Research 2: 342–348.

Wattiaux MA (1995). Milk composition and nutritional value, Babcock Institute of International Dairy Research and Development. University of Wisconsin, Madison. http://babcock.wisc.edu/sites/default/files/de/en/de_19.en.pdf.

Workayehu T, Wortmann CS (2011). Maize-bean intercrop weed suppression and profitability in southern Ethiopia. Agronomy Journal 103(4):1058-1063.

World Conservation Monitoring Centre (1992). Global Biodiversity: Status of the Earth's living resources. Chapman & Hall, London, xx -I-p. 594.

Wu T, Schoenau JJ, Li F, Qian P, Malhi SS, Shi Y (2003). Effect of tillage and rotation on organic carbon forms of chernozemic soils in Saskatchewan. Journal of Plant Nutrition and Soil Science 166:328-335.

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Full Length Research Paper

Effect of silver nitrate on shoot multiplication, rooting induction and plantlet characteristics of St. John's wort (*Hypericum perforatum* L.) *in vitro* culture

Sitti Fatimah Syahid* and Sri Wahyuni

Indonesian Spices and Medicinal Crops Research Institute (ISMCRI), Indonesian Agency for Agricultural Research and Development (IAARD), Indonesia.

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St. John's wort (*Hypericum perforatum* L.) is one of the introduced medicinal plants that have medicinal uses for anti-depressant. Germplasm conservation of St. John's wort has been conducted at the laboratory for over ten years and plantlets showed a rosette growth. Therefore, laboratory investigations are necessary to be done to have better methods for obtaining normal growth of culture. In this study, we evaluate the effect of concentration levels of silver nitrate ($AgNO_3$) on shoot multiplication, rooting induction and visual characteristics of plant *in-vitro* culture. The study was conducted in two stages (shoot multiplication and rooting induction). For shoots multiplication, cultures were grown in Murashige and Skoog (MS) media supplemented with 0.1 mgl^{-1} N^6-Benzyl Adenine (BA) combined with various concentration levels of $AgNO_3$ as follow: MS + 0.1 mgl^{-1} BA + $AgNO_3$ (0.0, 0.1, 0,3, 0.5 and 0.7 mgl^{-1}). For rooting induction, cultures were grown in half-formula of MS media with various concentration levels of $AgNO_3$: ½ MS + $AgN0_3$ (0.1, 0.3, 0.5 and 1.0 mgl^{-1}). The results showed that the application of $AgNO_3$ combined with 0.3 mg/l BA could improve the culture characteristics and showed normal plantlets. The best rooting induction was obtained at ½ MS + 0.3 mgl$_{-1}$ $AgNO_3$. This protocol provides a technique for improving visual culture during conservation.

Key words: *Hypericum perforatum* L., shoot multiplication, protocol for improving visual culture, *in vitro*.

INTRODUCTION

St. John's wort (*Hypericum perforatum* L.) is one of the medicinal plants that have been used for over a decade (Gadzovska et al., 2012). St. John's wort is a species of the *Hypericaceae* that has many benefits and efficacy as medicinal treatments such as burns, bruises, swelling, wound healing, mild to moderate anti-depressant, antiviral, antibiotic, antioxidant and anti-cancer (Luo et al., 2004; Agostinis et al., 2002; Silva et al., 2005; Yadollah-Damarvandi et al., 2015).

St. John's wort grows optimally in the highlands. Traditionally, plant propagation can be done by separating tillers, or generatively by seeds. St.John's wort needs to be conserved because of the benefit of this plant as medicine and plant germplasm need to be maintained for future research purpose. To support the conservation of St. John's wort germplasm in the

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*Corresponding author. E-mail: ifa_sy@yahoo.co.id.

Indonesian Spice and Medicinal Crops Research Institute (ISMCRI-IAARD), propagation is done through tissue culture technique. Plantlets multiplication is done by using Murashige and Skoog (MS) media, supplemented with 0.1 mgl^{-1} BAP. In ISMCRI-IAARD, St. John's wort has been conserved as *in vitro* culture for over ten years. The culture grew on MS media supplemented with 0.1 mgl^{-1} BA, and has fairly characteristics of their acclimated plants. It showed rosette growth, small plant leaves, no roots, and the ones which are not the original characteristics of normal plants as grown in the field. Those abnormal characteristics may be due to changes during culture for a long period.

Many factors may influence plant characteristics during its growth in the *in vitro* culture, which tends to produce changes at morphological, cytologycal, physiological, biochemical or event at molecular levels (Bajaj, 1992). Changes that occur during the period of *in vitro* culture can be triggered by both internal and external factors, such as; sources of explants, media composition, type and concentration of growth regulators used (Karp, 1991; Veilleux and Johnson, 1998). In case of propagation of Big-White Ginger Cultivar of Indonesian Variety Cimanggu-1 *in vitro* by direct organogenesis, the healthy plantlet and vigorous plants were not able to produce normal rhizome after planting in the field. It is suggested that genetic alteration or epigenetic change during the *in vitro* culture and regeneration have been performed (Rostiana and Syahid, 2008).

Silver nitrate ($AgNO_3$) is an inhibitor of ethylene activity and has been widely used in *in vitro* plant propagation. This chemical compound also plays a role in the process of shoot proliferation and multiplication, as well as rooting induction in *Solanum nigrum* culture (Geetha et al., 2016), and induced shoot multiplication and rooting of vanilla (*Vanilla planifolia*) (Giridhar et al., 2001). The application of silver nitrate showed the effect to increase direct organogenesis from leaf explants of *Brassica napus* and *Sinningia speciosa* (Akasaka et al., 2005; Park et al., 2012).

The purpose of this study was to determine the effect of concentration levels of silver nitrate on shoot multiplication, root induction and visual characteristics of St. John's wort *in-vitro* culture.

MATERIALS AND METHODS

Plant materials and culture conditions

St. John's wort has been conserved as *in vitro* culture for over ten years. The *in-vitro* culture was prepared from a sterile young shoot taken from two months old of healthy St. John's wort plant. It is transferred and grown in MS medium (Murashige and Skoog, 1962) supplemented with 3% sucrose.

Shoot multiplication culture was performed on MS media supplemented with N^6-benzyl adenine (BA), in combination with concentration levels of silver nitrate ($AgNO_3$): 1) MS + 0.1 mgl^{-1} BA + 0.0 mgl^{-1} $AgNO_3$0, 2) MS + 0.1 mgl^{-1} BA + 0.1 mgl^{-1} $AgNO_3$ 0.1, 3) MS + 0.1 mgl^{-1} BA + 0.3 mgl^{-1} $AgNO_3$, 4) MS + 0.1 mgl^{-1} BA + 0.5 mgl^{-1} $AgNO_3$, and 5) MS + 0.1 mgl^{-1} BA + 0.7 mgl^{-1} $AgNO_3$. Plantlets produced a highest number of shoot multiplication then were used for rooting induction experiment. Rooting induction culture was prepared on a half-strength of MS media, in combination with concentration levels of $AgNO_3$ as follow: 1) ½ MS + 0.1 mgl^{-1} $AgN0_3$, 2) 1/2 MS + 0.3 mgl^{-1} $AgNO_3$, 3) 1/2 MS + 0.5 mgl^{-1} $AgNO_3$, and 4) 1/ 2MS + 0.5 mgl^{-1} $AgNO_3$. Number of shoots produced, shoot length, number of leaves, root number and length, and plantlets characteristics were observed during treatment. Growing plantlets of St. John's wort were maintained in *in vitro* culture at temperature 24^{o}C ± 20^{o}C, with 16 h/8h photoperiod and phot on flux density at 1000 lux.

Data analysis

The experiment was arranged in completely randomized design with ten replications. Data obtained were analyzed by using Statistical Analysis System (SAS) portable version 9.1. Further analysis was carried out by using Duncan Multiple Range Test (DMRT) at 5% of level of significant.

RESULTS AND DISCUSSION

Shoot multiplication

Application of silver nitrate at various concentrations affected the growth of St. John's wort *in vitro*. Without application of silver nitrate, culture tend to be rosette on MS + 0.1 mgl^{-1} BA as indicated in Figure 1a. Combination of silver nitrate from low concentrations (0.1 -0.5 mgl^{-1}) with BAP could improve the growth of St John's wort which is characterized by changes of leaf size as presented in Figures 1b, c and d. It indicated that culture has more normal morphological characteristics when cultured on MS + BA 0.1 mgl^{-1} in combination with 0.1-0.5 mgl^{-1} $AgNO_3$. Increasing concentration of $AgNO_3$ to 0.7 $mg/^{-1}$ showed the signs of vitrification of the culture as shown in Figure 1e. The best treatment to obtain the normal growth was reached on MS containing 0.1 mgl^{-1} BA + 0.3 mgl^{-1} AgNO3 as indicated in Tables 1 and 2 and Figure 1c.

Rooting induction

Rooting induction on St. John's wort *in vitro* showed a low response. Application of macronutrient at a half-strength concentration, in combination with silver nitrate at 0.3 mgl^{-1} were able to produce a rooted culture even though only small percentage (30%) and the roots were still very limited as indicated in Table 3 and Figure 1f.

DISCUSSION

Addition of silver nitrate into culture media could improve the quality of culture growth by inhibiting of ethylene activity in the culture. St. John's wort culture treated with a combination of 0.1 mgl^{-1} BAP + 0.3 mgl^{-1} AgNO3

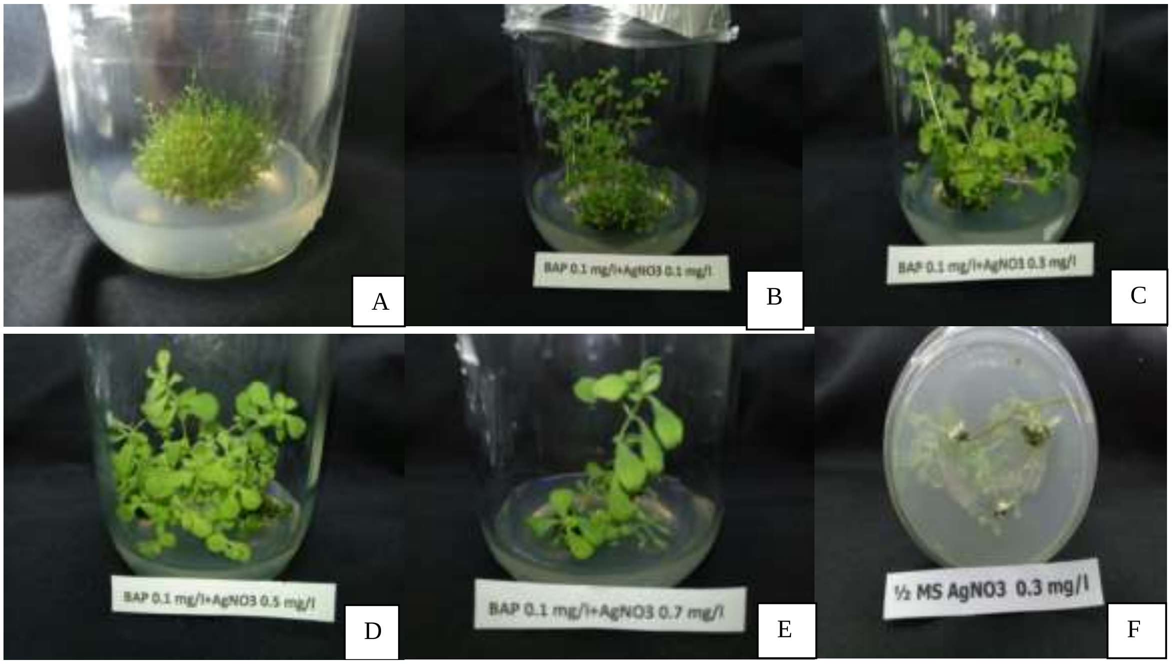

Figure 1. (A) Characteristic of St. John's wort on MS + 0.1 mgl^{-1} BA , effect of BA and $AgNO_3$ combined with several concentration levels on shoots multiplication and visual characteristics of St. John's wort *in vitro*: (B) 0.1 mgl^{-1} BA + 0.1 mgl^{-1} $AgNO_3$, (C) 0.1 mgl^{-1} BA + 0.3 mgl^{-1} $AgNO_3$, (D) 0.1 mgl^{-1} BA + 0.5 mgl^{-1} $AgNO_3$, and (E) 0.1 mgl^{-} BA 0.1 mgl^{-1} + $AgNO_3$ 0.7 mgl^{-1}. (F) Rooting induction of St. John's wort *in vitro* at ½ MS + 0.3 mgl-1 $AgNO_3$.

Table 1. Effect of combined concentration levels of BA and $AgNO_3$ on the growth of St John's wort *in vitro* at two months after culture.

Treatment (mgl^{-1})	Number of shoot	Shoot length (cm)
0.1 BA + 0.0 $AgNO_3$	9.0^b	8.40^{bc}
0.1 BA + 0.1 $AgNO_3$	9.6^b	9.00^b
0.1 BA + 0.3 $AgNO_3$	12.6^a	10.6^a
0.1 BA + 0.5 $AgNO_3$	8.4^b	8.8^b
0.1 BA + 0.7 $AgNO_3$	4.2^c	7.4^c

The numbers followed by the same letter in each column are notsignificantly different at 5% DMRT.

Table 2. Effect of combined concentration levels of BAP and $AgNO_3$ on plantlets characteristics of St. John's wort *in vitro* at two months after culture.

Treatment (mgl^{-1})	Plantlet characteristic
0.1 BAP + 0.1 $AgNO_3$ (control)	Leaves rather small and tended to rosette
0.1 BAP + 0.1 $AgNO_3$	Leaves were slightly larger than control
0.1 BAP + 0.3 $AgNO_3$	Leaves were normal
0.1 BAP + 0.5 $AgNO_3$	Leaves were rather large but tended to show vitrification symptom
0.1 BAP + 0.7 $AgNO_3$	Leaves showed signs of vitrification

showed the best growth performance compared to the others. However, higher concentration of silver nitrate may cause vitrification of St. John's wort culture. Plantlets having vitrification symptom usually produces larger leaves which contain much water and easy to be withered. Silver nitrate is known to promote multiple

Table 3. Effect of various levels of silver nitrate concentration on rooting induction of St. John's wort *in vitro.*

Treatment (mgl^{-1})	Rooting percentage (%)	Number of root	Root length (cm)
½ MS + 0.1 $AgNO_3$	0.0^b	0.0^b	0.0^b
½ MS + 0.3 $AgNO_3$	30.2^a	1.3^a	3.3^a
½ MS + 0.5 $AgNO_3$	1.9^b	0.7^b	1.2^b
½ MS + 1.0 $AgNO_3$	0.0^b	0.0^b	0.0^b

The numbers followed by the same letter in each column are not significantly different at 5% DMRT.

shoot formation in different plants. *In vitro* shoot formation was improved by incorporating silver nitrate in the culture medium.

Most plants that are propagated through tissue culture produced ethylene which may affect the growth of culture. The influence of ethylene on the growth of culture was greatly varied depending on plant sensitivity. Silver nitrate is usually applied to inhibit ethylene activity in *in vitro* propagation. Silver ions are able to prevent a variety of responses displayed by plants due to the influence of ethylene (Beyer et al., 1984).

The exact mechanism of $AgNO_3$ action on plants is unclear; however, few existing evidences suggest its interference in ethylene perception mechanism (Beyer, 1976c). In recent years, $AgNO_3$ has been employed in tissue culture studies for inhibiting ethylene action because of its water solubility and lack of phytotoxicity at effective concentrations (Beyer, 1976a). In *S. nigrum* culture, the use of silver nitrate is able to increase shoot multiplication, the same as can be observed in *Coffea arabica* and *V. planifolia* plants (Ganesh and Sreenath, 2008; Sankar et al., 2008). The addition of different concentrations of $AgNO_3$ (10, 30 and 50 M) to the medium, however, induced shoot regeneration in distal cotyledon except Suyo Long cultivar and effectively increased shoot regeneration response as well as the number of shoots per explant in proximal cotyledon and hypocotyl of all cucumber cultivars (Mohiuddin et al., 1997).

Results of preliminary research showed that the use of macronutrients at full concentration with the addition of auxin IBA has not been able to induce rooting perfectly (Syahid, 2008). Response on silver nitrate is varying in different plants. In *S. nigrum* culture, a combination of IBA 2.0 mgl^{-1} with silver nitrate 0.4 mgl^{-1} was able to produce roots up to 24.6 ± 0.26 (Geetha et al., 2016). The effect of $AgNO_3$ on rooting and shooting was evaluated in *V. planifolia*. Application of silver nitrate into the medium showed positive response not only on shoot initiation, number and growth, but also increased root number and length. Maximum number of shoots and highest shoot length was obtained on medium containing 20 µM $AgNO_3$. Application of $AgNO_3$ not only induced shoot multiplication but also influenced rooting of vanilla explants (Giridhar et al., 2001).

Conclusion

Application of silver nitrate in combination with BA was able to improve the growth of St. John's wort (*Hypericum perforatum) in vitro*. The combination of silver nitrate at concentration of 0.3 mgl^{-1} with 0.1 mgl^{-1} BA produced the highest number of shoots, longest shoot and largest quantity of leaves (10.6 leaves) within two months. Silver nitrate was able to induce normal morphological characteristics of St. John's wort which was indicated by normal stems and leaves growth during culture period. The best rooting induction was obtained on ½ MS + 0.3 mgl^{-1} $AgNO_3$.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Agostinis P, Vantieghem A, Merlevede W, De Witte PA (2002). Hypericin in cancer treatment more light on the way. International Journal of Biochemistry and Cell Biology 34:221-241.doi: 10.1016 / S1357-2725 (01) 00126-1.

Akasaka-Kennedy Y, Yoshida H, Takahata Y (2005). Efficient Plant Regeneration from Leaves of Rapeseed (*Brassica napus* L.): The influence of AgNO3 and genotype. Plant Cell Report 24:649-654.

Bajaj YPS (1992). Somaclonal variation-origin, induction, cryopreservation and implication in plant breeding. In: Bajaj. YPS (*ed*.). Biotechnology in Agriculture and Forestry 11: Somaclonal variation in crops improvement I. Springer-Verlag, Berlin pp. 3-48.

Beyer EM, Page YM, Yang SF (1984). Ethylene. In: Wilkins MB (*ed*.). Advanced Plant Physiology. Pitman, London, pp. 111-115.

Beyer EM (1976a). A potent inhibitor of ethylene action in plants. Plant

Physiology 58(3):268-271.

Beyer EM (1976c). Silver ion: a potent anti-ethylene agent in cucumber and tomato. HortScience 11(3):175-196.

Gadzovska-Simic, Tusevski O, Antevski S, Atanasova-Pancevka N, Petreska J, Stefova M, Kungulovski D, Spasenoski M (2012). Secondary metabolit production in *Hypericum perforatum* L cell suspension upon elicitation with fungal mycelia from *Aspergillus flavus*. Archives of Biological Science Belgrade 64(91):113-121.

Ganesh D, Sreenath HL (2008). Micropropagation of *Coffea arabica* Using Apical Buds of Mature Field-Grown Plants. Journal of Plant Crops 36:1-7.

Geetha G, Harathi K, Naidu CV (2016). Role of Silver Nitrate on *in vitro* flowering and shoot regeneration of *Solanum nigrum* (L.). An Important Multipurpose Medicinal Plant. American Journal of Plant Sciences 7:1021-1032.

Giridhar P, Obul RB, Ravishankar GA (2001). Silver nitrate influences *in vitro* shoot multiplication and root formation in *Vanilla planifolia* Andr. Current Science 81(9):1166-1170.

Karp A (1991). On the current understanding of somaclonal variation. Oxford Surveys of Plant Molecular and Cell Biology 7:1-58.

Luo L, Sun Q, Mao YY, Lu YH, Tan RX (2004). Inhibitory effect of flavonoids from *Hypericum perforatum* on nitric oxide synthase. Journal of Ethnopharmacology 93:221-225.

Mohiuddin AKM, Chowdhury MKU, Abdullah ZC, Napis S (1997). Influence of silver nitrate (Ethylene inhibitor) on cucumber in vitro shoot regeneration. Plant Cell Tissue and Organ Culture 51:75-78.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia plantarum 15(3):473-497.

Park EH,Bae H, Park WT, Kim YB, Chae SC, Park SU (2012). Improved Shoot Organogenesis of Gloxinia (*Sinningia speciosa*) Using Silver Nitrate and Putrescine Treatment. Plant Omics 5:6-9.

Rostiana O, Syahid SF (2008). Somatic embryogenesis from meristem explants of ginger. *Biotropia* 1591:12-24.

Sankar A, Libinmary S, Vijaykumar A, Karthi RR, Raja SJ, Kohila R, Liby I, Vadivukarasi S, Ganesh D (2008). Phloroglucinol Enhances Shoots Proliferation in Nodal Explants of *Vanilla planifolia* Andr. Journal of Plant Crops 36:127-131.

Silva BA, Ferreres F, Malva JO, Dias ACP (2005). Phytochemical and antioxidant characterization of *Hypericum perforatum* alcoholic extracts. Food Chemistry 90:157-167.

Syahid SF (2008). The effect of IBA and IAA on rooting induction of Hypericum (*Hypericum perforatum*). *in vitro*. National Seminar of Medicinal Plants. XXXV. 13-14 November 2008. Serpong:75-179.

Veilleux RE, Johnson AA (1998). Somaclonal Variation: Molecular Analysis, Transformation, Interaction, and Utilization. In: J. Janick (*ed*.). Plant Breeding Reviews. Vol 16. John Willey & Sons Inc. New York, Chichester, Weinheim, Brisbane, Singapore, Toronto pp. 229-260.

Yadollah-Damavandi S, Chavoshi-Nejad M, Jangholi E, Nekouyian N, Hosseini S, Seifae (2015). Topical *Hypericumperforatum* improves tissue regeneration in full-thickness excisional wounds in diabetic rat models. Evidence-Based Complementary and Alternative Medicine 2015.

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Full Length Research Paper

Screening of rice germplasms under salt stress by phenotypic and molecular markers

Nguyen Thị Lang[1], Pham Thi Thu Ha[2]*, Nguyen Thu Tra[2] and Bui Chi Buu[3]

[1]High Agricultural Technology Research Institute (HATRI) for Mekong Delta, Vietnam.
[2]Faculty of Applied Sciences, Ton Duc Thang University, Ho Chi Minh City, Vietnam.
[3]Institute of Agricultural Science (IAS) for Southern, Vietnam.

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The evaluation of salinity tolerance of rice germplasms based on phenotype and genotype of 100 rice germplasm was identified at the seedling stage at the High Agricultural Technology Research Institute for Mekong Delta, Vietnam. Screening of the phenotype was done with 30 day old seedlings using salinized concentrations (EC = 8 and 15 dS/m). Results show that the growth of the varieties, the higher the salt concentration, the lower the survival date, the lower the tree height, the higher the root length, and the weight of the stem and roots were all significant positive correlations in this study. These indicators are also closely correlated with each other. This shows that saline conditions greatly affect the survival, growth, and development of rice. Genotypic analysis on the RM223 and RM3252 molecular markers recorded polymorphism on both indicators. Among rice germplasms, the proposed varieties including Pokkali, OM4900, HATRI144, HATRI60, OM5704, HATR162, HATRI131 and HATRI132 were found to have salt tolerance for evaluation future of the breeding program. Thus, the RM233 and RM3252 used in this study are proved available to identify salt tolerance for rice breeding.

Key words: Phenotype, genotype, simple sequence repeat (SSR) markers, salt stress, seedling stage, rice.

INTRODUCTION

Rice is one of the most important grains grown and accounts for more than one-third of the world's food (Munns, 2002; Wassmann et al., 2009). However, climate change, particularly the greenhouse effect, the atmospheric temperature warms and melting ice at the two poles, will create flooding in the lowlands. Seawater intrusion into the mainland is increasing rapidly, causing serious consequences for rice production in Vietnam and other countries in the world, especially in the Mekong Delta. Farm land is affected by the saline intrusion of over 400 million hectares, accounting for 1/3 of the world's cultivated land. It is often accompanied by the phenomenon of alkaline and wetland soils (Gale, 2002). In Vietnam, in 2008 the area of rice cultivation reached 7.4 million hectares, of which the Mekong River Delta had 2.9 million hectares with 800,000 ha of saline soil. In recent years, global urbanization and climate change have affected agricultural land, especially drought and saline intrusion. Therefore, developing high-yielding rice varieties that are salt tolerant could be the best effort for

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*Corresponding author. E-mail: phamthithuha@tdtu.edu.vn.

these areas.

Research on the development of saline tolerant in plants has been at the forefront of international projects in genetic technology. The breeding crop of salt-tolerant rice would likely be an economical improvement and overcome the problems of salty soil. With the advancement of biotechnology, molecular markers can be used in breeding. Based on molecular markers associated with salt-resistant genes, breeders can identify genotypes for resistance and infection from an early stage. Determining the main gene position by microsatellite marker analysis can be used to develop new varieties (Mondal and Ganie, 2014). And appropriate strategies for breeding salinity tolerance and crop cultivation are seen as the most effective and economical way to increase paddy production in saline areas (Buu and Lang, 1995). Significant differences in injury rates between cultivars when grown under salt stress were detected by Ali et al. (2014) and the results were used for screening purposes.

Screening is also being done on different parts of the discovery of diverse genetic potential to determine salinity tolerance of rice genotypes. Vegetative stage and reproductive stage tolerance of the rice was expressed in tolerance at the seedling stage by Hariadi et al. (2015). There were some germplasms with high salt tolerance offering. In spite of this, these common germplasms have many undesirable traits. The highly tolerant, and often used as donors in breeding for salt tolerance, was Pokkali which is tall, photosensitive, low yielding, and has red kernels (De Leon et al., 2016). Conventional breeding is time-consuming and depends on environmental conditions. Molecular markers are technologies that provide the possibility of applying the genetic map (Islam, 2004), and studying genetic diversity in germplasms. The most efficient descriptors to screen the salt tolerant genotypes were simple sequence repeat (SSR) markers RM8094, RM336 and RM8046, which have a higher polymorphic information content coupled with higher marker index value (Ali et al., 2014). Five SSR markers (RM1287, RM8094, RM3412, RM493 and RM140) and two EST markers (CP3970 and CP6224) were linked to Saltol QTL on chromosome 1 were detected by Niones (2004). The RM3412 marker has been shown to be useful for marker-assisted selection of *Saltol* QTL (Naresh et al., 2014). The present study determined the phenotype of rice germplasms used salinized conditions (EC= 8 and EC = 15 dS/m) at the seedling stage and identified salt tolerance rice germplasms from 100 varieties by SSR markers.

MATERIALS AND METHODS

Plant

Seeds of 100 rice germplasms (*Oryza sativa* L.) were collected in this study (Table 1) including one salt tolerant Indian variety “Pokkali” and one salt susceptible variety “IR28” as controls. The rice germplasms were received from Intentional Rice Research Institute (IRRI), Cuu Long Delta Rice Research Institute (CLRRI) and High Agricultural Technology Research Institute for Mekong Delta, Vietnam (HATRI).

Screening of phenotype

Rice germplasms were screened for salt tolerance at the seedling stage using IRRI standard modified by Lang et al. (2001). The evaluation was done using Yoshida et al. (1976) nutrition solution to obtain EC (8 and 15 dS/m). The experiment was conducted at the HARTI laboratory. The final scoring was observed after 30 days of salinization followed by a protocol of Gregorio et al. (1997). The characteristics as SES coring, survival day, shoot height, root length, plant dry weight, and root dry weight were observed at the seedling stage.

Genotyping using SSR markers

Modified CTAB was used to extract DNA from leaf samples of genotypes. Two primers as RM233 and RM3252 were chosen for the study (Lang et al., 2017) (Table 2). 30 µl polymerase chain reactions (PCR) reaction contained 15 ng DNA samples, 1.25 units Taq DNA polymerase, 0.2 µM primer, 1.7 mM $MgCl_2$ 0.17 mM dNTPs and PCR buffer. PCR profile was maintained as the initial denaturation at 94°C for 4 min, and then the reaction was subjected to 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 2 min with a final elongation step of 4 min at 75°C. Amplification products were resolved by electrophoresis on a 3% polyacrylamide gel within TBE 1X.

Data analysis

The data was analyzed using Excel and the software by CropStat 7.2. The correlation coefficient of among traits at the seedling stage also was calculated under salinized conditions.

RESULTS AND DISCUSSION

Screening rice germplasms by phenotype

In the first experiment, the salt tolerance of the rice germplasms in the seedling stage was assessed in two different salt concentrations (8 and 15 dS/m) (Figure 1). 100 varieties were purified in the saline phase. There were 8 varieties that died 100% before 30 days. According to SES scoring of IRRI standard protocol was modified by Gregoe et al. (1997). The scoring was used for the evaluation of the tolerance, moderate tolerance, and susceptible rice varieties. The levels surveyed after 30 days of completing the saline test were as follows: Among 92 remaining varieties, there were 10 varieties found with level 3 at 8 dS/m compared to Pokkali control variety, two varieties with level 5, 79 varieties with levels 7 and 9 (susceptible). In case of 15 dS/m, the varieties were burnt and died a lot compared to the 8 dS/m medium, only a few survived, but they also burned heavily. Most of the varieties in this environment exhibited very high levels of dry leaves, level 9 with 78 varieties, accounting for 84.78%. There are 13

Table 1. List of varieties used for the study.

No.	Name of varieties	Origin	No.	Name of varieties	Origin
1	OM4900	CLRRI	51	HATRI60	HATRI
2	OM1490	CLRRI	52	HATRI90	HATRI
3	AS 996	CLRRI	53	OM10413	CLRRI
4	M362	IRRI	54	OM10414	CLRRI
5	BASMATI	IRRI	55	OM10415	CLRRI
6	Basmati ĐB	IRRI	56	OM6614	CLRRI
7	OM6162	CLRRI	57	OM6843	CLRRI
8	Swarna Sub1	IRRI	58	OMCS2009	CLRRI
9	IR64Sub1	IRRI	59	OM5703	CLRRI
10	IRGA318-11-6-9-2B	IRRI	60	OM5704	CLRRI
11	IR78966-B-10-B-B-B-2	IRRI	61	HATRI62	HATRI
12	IR78913-B-10-B-B-B	IRRI	62	OMCS2008	CLRRI
13	IR75499-73-1-B	IRRI	63	HATRI31	HATRI
14	IR78913-B-19-B-B-B	IRRI	64	HATRI32	HATRI
15	AZUCENA	IRRI	65	OM63L	CLRRI
16	IR78933-B-24-B-B-2	IRRI	66	Pokkali	IRRI
17	IR78933-B-24-B-B-3	IRRI	67	OM71L	CLRRI
18	IR78933-B-24-B-B-4	IRRI	68	OM72L	CLRRI
19	WAB326-B-B-7-H1	IRRI	69	OM5681	CLRRI
20	IR79008-B-11-B-B-1	IRRI	70	OM6730	CLRRI
21	IR75499-38-1-B	IRRI	71	OM6014	CLRRI
22	V3M-92-1	IRRI	72	OM6778	CLRRI
23	IR75499-21-1-B	IRRI	73	OM6033	CLRRI
24	V3M-109-2	IRRI	74	OM6032	CLRRI
25	WAB272-B-B-8-H1	IRRI	75	OM6613	CLRRI
26	WAB340-B-B-2-H2	IRRI	76	OM6729	CLRRI
27	WAB176-42-HB	IRRI	77	OM62L	CLRRI
28	IR78937-B-20-B-B-1	IRRI	78	OM10279	CLRRI
29	WAB880-1-38-18-20-P_1-HB	IRRI	79	OM10280	CLRRI
30	WAB881SG9	IRRI	80	OM10418-1	CLRRI
31	IR78997-B-16-B-B-B-SB2	IRRI	81	OM10704	CLRRI
32	IR78966-B-10-B-B-B-SB1	IRRI	82	OM3673	CLRRI
33	IR78944-B-8-B-B-B	IRRI	83	OM4249	CLRRI
34	IR78941-B-16-B-B-B	IRRI	84	OM4693	CLRRI
35	IR78948-B-21-B-B-B	IRRI	85	OM4726	CLRRI
36	IR78942-B-2-B-B-2	IRRI	86	OM4796	CLRRI
37	IR78937-B-20-B-B-3	IRRI	87	OM6379	CLRRI
38	IR78985-B-13-B-B-B	IRRI	88	OM5629	CLRRI
39	IR78933-B-24-B-B-1	IRRI	89	OM6387	CLRRI
40	WABC165	IRRI	90	OM6426	CLRRI
41	IR80315-49-B-B-4-B-B-B	IRRI	91	HATRI15	HATRI
42	IR78966-B-16-B-B-B	IRRI	92	HATRI16	HATRI
43	IR78913-B-22-B-B-B	IRRI	93	HATRI33	HATRI
44	OMCS2000	CLRRI	94	HATRI20	HATRI
45	OM6161	CLRRI	95	HATRI35	HATRI
46	OM10405	CLRRI	96	HATRI28	HATRI
47	HATRI144	HATRI	97	HATRI50	HATRI
48	HATRI 1	HATRI	98	HATRI192	HATRI
49	OM10408	CLRRI	99	HATRI603	HATRI
50	IR29	IRRI	100	HATRI608	HATRI

Table 2. The primers used to study.

Primer	PCR product size (bp)	Sequence	
		Forward	Reverse
RM223	200 bp-220	GAGTGAGCTTGGGCTGAAAC	GAAGGCAAGTCTTGGCACTG
RM3252	220 bp-230	GGTAACTTTGTTCCCATGCC	GGTCAATCATGCATGCAAGC

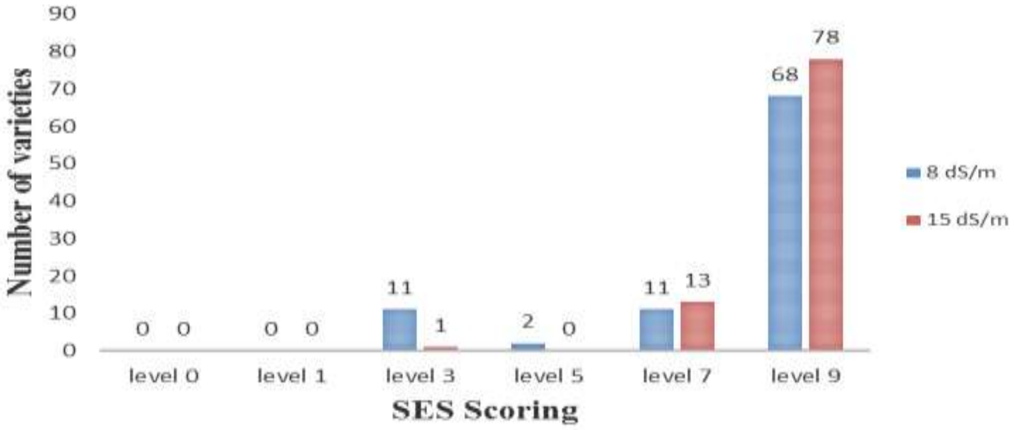

Figure 1. Performance of SES scoring of rice germplasms under salt stress.

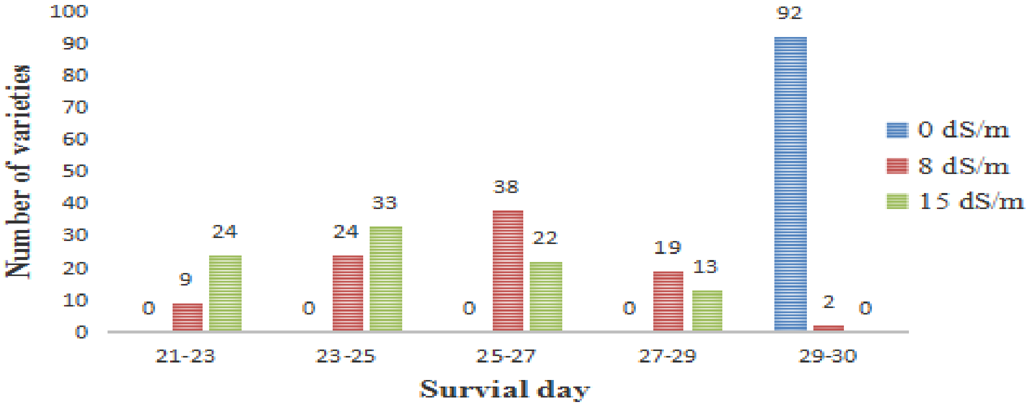

Figure 2. Performance of survival day at the seedling stage of rice germplasms under salt stress.

varieties at level 7. Only the Pokkali variety was expressed at level 3. The results in these experiments showed that the few tolerant varieties based on phenotype analysis exist.

The expression of salt tolerance also included the correlation of survival time and reduction of height seedling (Figures 2 and 3). In the saline environment with EC = 0 dS/m, all varieties survived 30 days of purification. The highest survival time in the 8 dS/m medium was 29.5 days while in the saline environment EC = 15 dS/m (28.8 days). The lowest survival time in a saline environment with EC = 8 dS/m was 22.1 days and in the environment 21 days. Saline medium with EC = 8 dS/m survived 21-23 days with 9 varieties, 23 - 25 days with 24 varieties, 25 - 27 days with 38 varieties, 27 - 29 days with 19 varieties, 29 - 30 days. The saline environment with EC = 15 dS/m survived from 21-23 days with 24 varieties, 23 - 25 days with 33 varieties, 25 - 27 days with 22 varieties, and 27 - 29 days with 13 varieties. In general, the varieties survived in EC = 0 dS/m more than in saline environments with EC = 8 dS/m, and in saline environments with EC = 15 dS/m were the most dead varieties over 30 days of salt purification in the nutrient environment.

The largest plant height reduction (from 8 to 20 cm) was observed in the three rice germplasms at EC = 8 dS/m. On the other hand, the lowest plant height (from 6 to 8

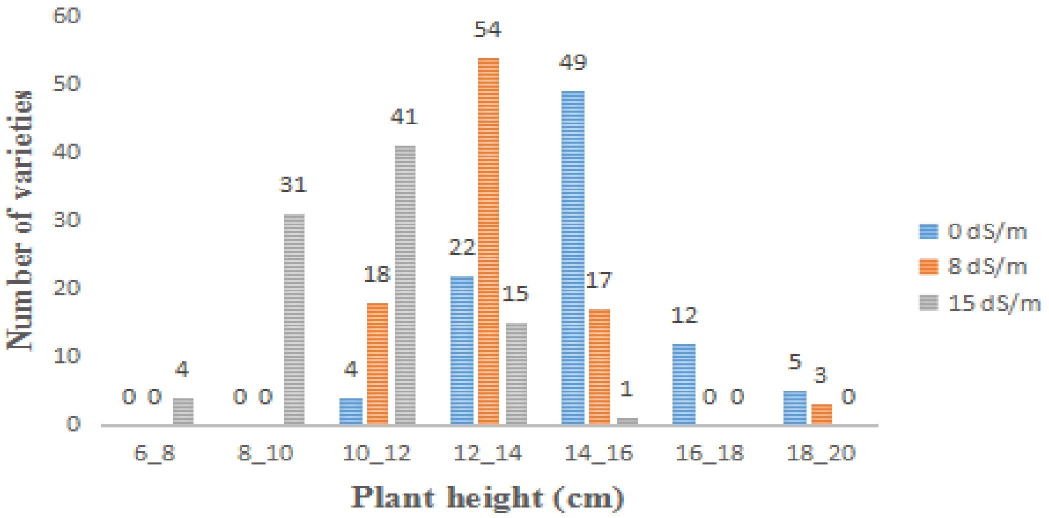

Figure 3. Performance of plant height (cm) at the seedling stage of rice germplasms under salt stress.

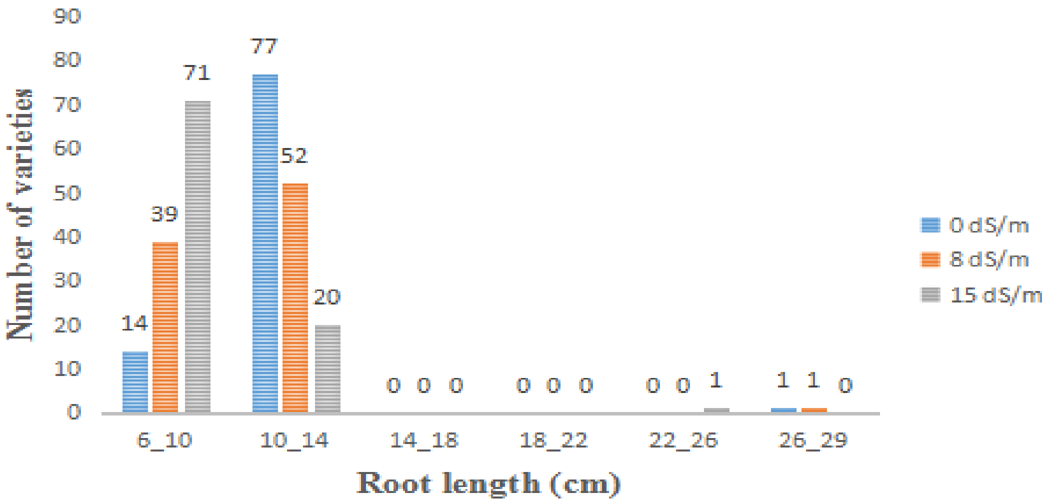

Figure 4. Performance of plant root length (cm) at the seedling stage of rice germplasms under salt stress.

cm) was observed in the five rice varieties at EC = 15 dS/m. The highest number of varieties (54 rice varieties) was found by plant height from 12-14 cm at 8 dS/m followed by 41 rice varieties from 10-12 cm at 15 dS/m. There was only one variety (16 cm) which was observed at 15 dS/m. The previous studies showed that the increase in salinity level reduced the seedling height (Javed et al., 2006; Maiti et al., 2006). These results suggested that the plant height was reduced under higher concentration of salt stress. To adapt to saline conditions, stress-tolerant rice plants will grow very quickly to get out of the sensitive phase with salt and adapt to acclimatization to harsh environments.

The roots and biomass traits were reduced in the rice seedling stage in different salt concentrations (Figures 4 to 6). Varieties have different root lengths and vary between concentrations. In this study, several rice germplasms showed higher root length reduction at 8 dS/m only one line (26 cm). At 8 dS/m, it showed the highest number of rice germplasms in 54 varieties which had roots lengths ranging from 11 to 14 cm. Almost all rice varieties also showed lower root length reduction (6-8 cm) at 15 dS/m (Figure 4). The result showed that root length decreased under salinity stress. Root length is very sensitive to salinity, so the fluctuation is complex, especially in the nutrient environment. Roy et al. (2002) reported that the number of roots per plant decreased with increasing levels of salinity. Rodrigues et al. (2002) reported that root length was reduced due to the effect of salinity, which coincided with the present study.

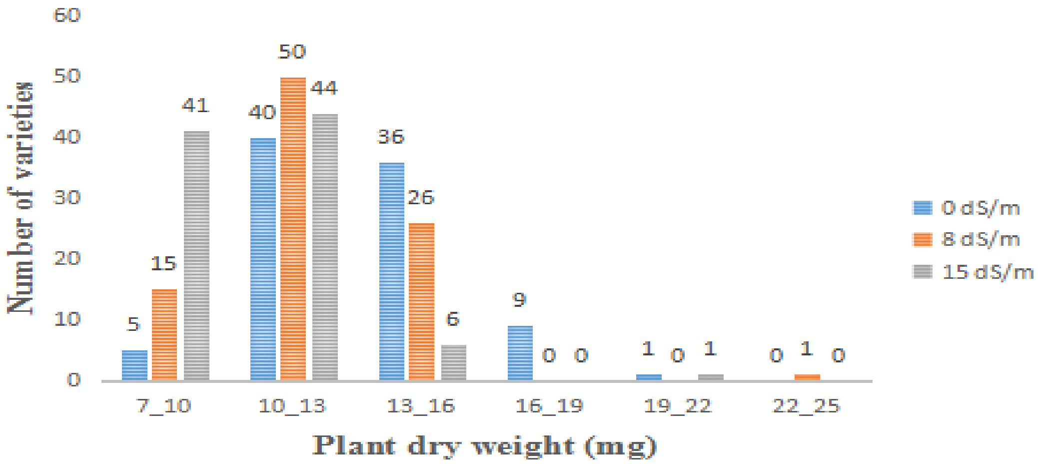

Figure 5. Performance of plant dry weight at the seedling stage of rice germplasms under salt stress.

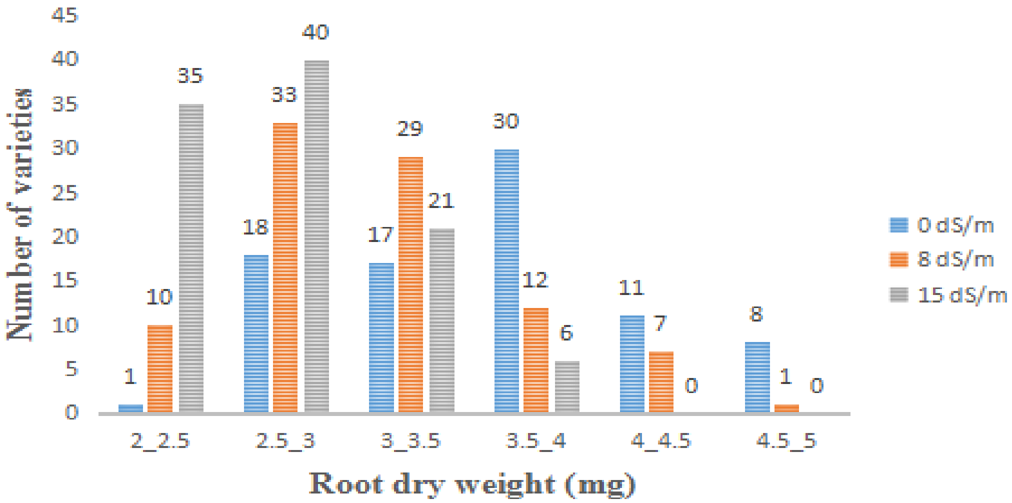

Figure 6. Performance of root dry weight (mg) at the seedling stage of rice germplasms under salt stress.

The biomass traits as plant dry weight and root dry weight are important traits to evaluate the performance of rice varieties for salt tolerance. The salinity caused a significant reduction in plant dry weight of the rice germplasms, compared with control EC = 0 dS/m (Figure. 5). The highest of plant dry weight reduction was found in one variety (26 mg) at 8 dS/m followed by one variety (26 mg) at 15 dS/m and 6 varieties (from 13 to 16 mg) at 15 dS/m after 30 days of stress (Figure 5). The lowest plant dry weight reduction was found in 41 varieties (7-10 mg).

Similar to the plant height and root length, the weight of dry shoots was greatly influenced by the saline environment. In addition, a lesser amount of dry mass in rice has been produced under salt stress (Sakina et al., 2016). Salinity reduces the ability to accumulate dry matter in rice due to the slowing down of nutrient transfer, the ability to form and accumulate dry matter in reducing the dry weight of plants. Salinity caused a significant reduction in the root dry weight compared with the control (Figure 6). The maximum number of rice germplasms recorded at 15 dS/m (40 varieties) by root dry weight varied from 2.5 to 3 mg, whereas, the minimum in one variety at 8 dS/m by root dry weight was about 4.5 mg. Morphological parameters such as plant dry weight and root dry weight are well correlated with crop salt tolerance at early growth stages and can be used as an indicator for salt tolerance. Under salt stress, reduction in biomass production is a common feature in crop plants. In this study, salinity caused a significant reduction in shoot and root dry weights of the varieties compared with control (Shereen et al., 2005); a significant reduction in seedling growth under salinity was also observed.

Table 3. The coefficient of correlation among different traits from salt stress in the seedling stage (30 days).

Trait	Survival day	Plant height (cm)	Root length (cm)	Plant dry weight (mg)	Root dry weight (mg)
EC= 0 dS/m					
Survival day	1				
Plant height (cm)	-	1			
Root length (cm)	-	0.704**	1		
Plant dry weight (mg)	-	0.968**	0.839**	1	
Root dry weight (mg)	-	0.987**	0.701**	0.971**	1
EC = 8 dS/m					
Survival day	1				
Plant height (cm)	0.960**	1			
Root length (cm)	0.681**	0.842**	1		
Plant dry weight (mg)	0.900**	0.977**	0.917**	1	
Root dry weight (mg)	0.973**	0.961**	0.727**	0.928**	1
EC = 15 dS/m					
Survival day	1				
Plant height (cm)	0.953**	1			
Root length (cm)	0.708**	0.857**	1		
Plant dry weight (mg)	0.946**	0.981**	0.884**	1	
Root dry weight (mg)	0.995**	0.962**	0.734**	0.958**	1

**Significant at the 0.01 level.

The correlation among different traits under two salinized concentrations (8 and 15 dS/m) are shown in Table 3. For salt stress, rice plants respond very differently depending on the characteristics of each variety. However, the correlation of the salt tolerant characteristics showed a significant positive correlation with each other (Table 3). A similar finding was reported by Lang et al. (2017) and Zang et al. (2004) regarding larger biomass production. In this study, the results implied that the salt tolerance germplasms had lower salt tolerance score, higher traits as plant height, root length, and dry total mater. On the other hand, the relative importance of the attributes must be decided on based on the higher condition among the traits.

Screening rice germplasms by using SSR markers

In this study, two primers RM233 and RM 3252 were used for the polymorphism survey of 100 rice germplasms. The bands were obtained from the Pokkali; a cultivar was used as salt tolerant genotype in genotype banding. With respect to RM233, eight varieties (Pokkali, OM4900, HATRI144, HATRI60, OM5704, HATR162, HATRI131 and HATRI132) were found tolerant of salt stress in Figure 7. A similar result was found by Lang et al. (2000) who found that RM233 was closely linked to the salt tolerant gene in chromosome 8. The SSR marker RM223 also was tested by Lang et al. (2017), with an accuracy of 82% between genotype and phenotype at the sexually productive stage and 92% at the seedling stage. In the case of RM3252, five rice germplasms were identified tolerant for salt stress compared to the tolerant variety Pokkali in Figure 8. These two markers showed polymorphisms in the same of four varieties (OM5704, HATR162, HATRI131 and HATRI132). Finally, salt tolerant rice germplasms were identified in both phenotype and genotype using eight varieties. The tested marker RM233 could be used efficiently to identify the study for salt tolerance in the rice breeding program.

Conclusion

Based on the results, it can be concluded that the experiment was performed to classify 100 rice germplasms into groups of tolerance, moderately susceptible and susceptible. The salinity tolerance showed a significant positive correlation to those traits (survival days, plant height, root length, plant dry weight, and root dry weight). Genotype analysis recorded the polymorphism on both markers (RM233 and RM3252). Among rice germplasms, Pokkali, OM4900, HATRI144, HATRI60, OM5704, HATR162, HATRI131 and HATRI132

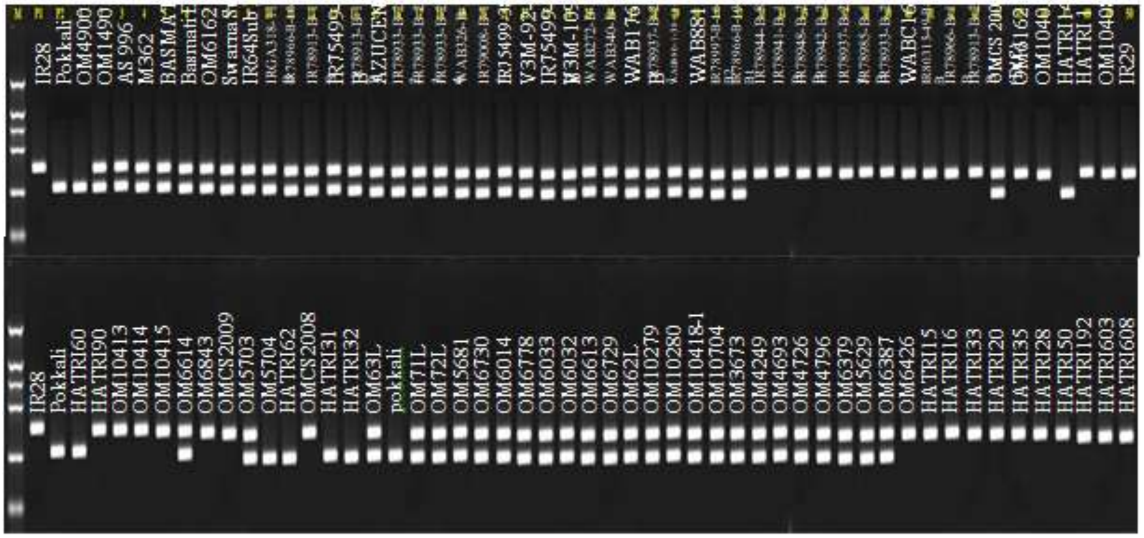

Figure 7. PCR profiles of 100 rice germplasms using RM223.

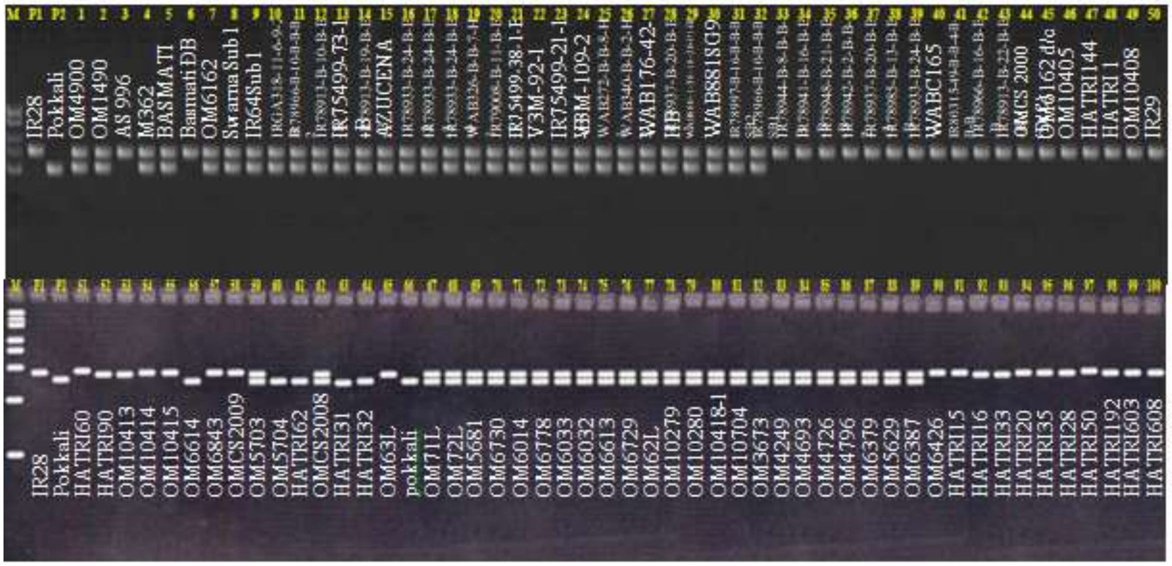

Figure 8. PCR profiles of 100 rice germplasms using RM3252.

were found to be salt tolerant at the seedling stage, which can be used in breeding rice for tolerance to salinity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Ali MN, Yeasmin L, Gantait S, Goswami R, Chakraborty S (2014). Screening of rice landraces for salinity tolerance at seedling stage through morphological and molecular markers. Physiology and Molecular Biology of Plants 20:411-423.

Buu BC, Lang NT (1995). Application of biotechnology in rice variety improvement. Agriculture Publishing House, Hanoi.

De Leon TB, Linscombe S, Subudhi PK (2016). Molecular Dissection of seedling salinity tolerance in rice (*Oryza sativa* L.) sing a high-density GBS-Based SNP Linkage Map. Rice 9:52.

Gale M (2002). Applications of molecular biology and genomics to genetic enhancement of crop tolerance to abiotic stress – a discussion document. FAO – Consultative Group on International Agricultural Research Interim Science Council. Rome, Italy.

Gregorio TL, Dharmawansa S, Mendoza RD (1997). Screening rice for salinity tolerance. IRRI, Discussion Paper Series No.22, International Rice Research Institute. Manila, The Philippines, pp. 1-30.

Hariadi YC, Nurhayati AY, Soeparjono S, Arif I (2015). Screening six varieties of rice (*Oryza sativa*) for salinity tolerance. Procedia Environmental Sciences 28:78-87.

Islam MM (2004). Mapping salinity tolerance genes rice (*Oryza sativa* L.) at productive stage. PhD dissertation university of the Philippine Los Banos College, Laguna, Philippine P. 149.

Javed MA, Ishii T, Kamijima O, Misoo S (2006). Discrepancy of two ecotypes of *Oryza Sativa* L. to salinity at germination and seedling stages. Annals of Biology 22:201-211.

Lang NT, Phuoc NT, Ha PTT, Buu BC (2017). Identifying QTLs associated and marker-assisted selection for salinity tolerance at the seedling, vegetative and reproductive stages in rice (*Oryza sativa* L.). International Journal of Environment, Agriculture and Biotechnology. 2:2927-2935.

Lang NT, Yanagihara S, Buu BC (2000). Quantitative trait loci for salt tolerance in rice via molecular markers. Omonrice 8:37-48.

Lang NT, Yanagihara S, Buu BC (2001). A microsatellite marker for a gene conferring salt tolerance on rice at the vegetative and reproductive stages. Sabrao 33:1-10.

Maiti RK, Vidyasagar P, Banejee PD (2006). Salinity tolerance in rice (*Oryza sativa* L.) hybrids and their parents at emergence and seedling stage. Crop Research Hisar 31:427-433.

Mondal TK, Ganie SA (2014). Identification and characterization of salt responsive miRNA- SR markers in rice (*Oryza sativa*) Gene 535(2):204-209.

Munns R (2002). Comparative physiology of salt and water stress. Plant Cell and Environment 25:239-250.

Naresh BN, Vinod KK, Gopala KS, Bhowmick PK, Vanaja T, Krishnamurthy SL, Nagarajan M, Singh NK, Prabhu KV, Singh AK (2014). Marker based haplotype diversity of saltol QTL in relation to seedling stage salinity tolerance in selected genotypes of rice. Indian Journal of Genetics 74(1):16–25.

Niones JM (2004). Fine mapping of the salinity tolerance gene on chromosome 1 of rice *(Oryzasativa* L.) using near isogenic lines, M. S. dissertation, College, Laguna, Philippines: University of the Philippines Los Baños, Laguna

Rodrigues LN, Ferandes PD, Gheyi HR, Viana SBA (2002). Germination and formation of rice seedlings under saline stress. Revista Brasileira de Engenharia Agrícola e Ambiental 6(3):397-403.

Roy SK, Patra SK, Sarkar KK (2002). Studies on the effects salinity stress on rice (*Oryza sativa* L.) at seedling stage. Journal of Interacademicia 6:254-259.

Sakina A, Ahmed I, Shahza A, Iqbal M, Asif M (2016). Genetic variation for salinity tolerance in Paksistan rice (*Oryza sativa* L.) germplasm. Journal of Agronomy and Crop Science 202(1):25-36.

Shereen A, Muntaz S, Raza S, Khan MA, Solangi S (2005). Salinity effects on seedling growth and yield components of different inbred rice lines. Pakistan Journal of Botany 37:131-139.

Wassmann R, Jagadish SVK, Heuer S, Ismail A, Redona E, Serraj R, Singh RK, Howell G, Pathak H, Sumfleth K (2009). Climate change affecting rice production: the physiological and agronomic basis for possible adaptation strategies. Advances in Agronomy 101:59-122.

Yoshida S, Forno DA, Cock JH, Gomez KA (1976). Laboratory manual for physiologycal studies of rice. IRRI, Los Banos, Lauguna, Philippines, pp. 61-66.

Zang ZH, Li P, Wang LX, Hu ZL, Zhu LH, Zhu YG (2004). Genetic dissection the relationships of biomass production and partitioning with yield and yield related traits in rice. Plant Science 167:1-8.

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Full Length Research Paper

Thermal comfort estimation for dairy cows in the south of Goiás State, Brazil

Larissa Mendes Cruvinel[1], Larissa Christina de Paula[1], Eliandra Maria Bianchini Oliveira[1,2]*, Tiago Neves Pereira Valente[1,2], Mateus Sobral de Araújo[1], Jeferson Corrêa Ribeiro[1,2], and Wallacy Barbacena Rosa dos Santos[1,2]

[1]Department of Animal Science, Instituto Federal Goiano, Morrinhos, Brazil.
[2]Department of Animal Science, Instituto Federal Goiano, Posse - Goiás, Brazil.

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The objective of this research was to analyze the correlation between climatic and milk composition variables. Thus, meteorological data and data on milk quality and composition (fat, protein, lactose, total solids content and defatted dry extract (DDE)) were used, as well as somatic cell count (SCC). The analysis was performed using the Pearson correlation test. After the study, it was verified that the temperature variations had a significant correlation with the fat, protein and total solids contents, as well as the temperature-humidity index (THI) also had correlation with lactose. In the analysis between the components, the correlations were between fat and protein, fat and total solids content, protein and total solids content, and lactose and fat. Moreover, SCC correlation with DDE and lactose were considered. The thermal comfort of dairy females has a direct influence on the quantity and quality of the final dairy product.

Key words: Dairy cattle, milk production, welfare.

INTRODUCTION

The dairy cattle are more sensitive and lacking more specific care to maintain good productivity due to the climatic variations that occur in the tropical climate. Thus, according to Rossarolla (2007), the management of dairy cattle to pasture leads the animals more vulnerable to higher temperatures, as in the case of countries with a warm climate. With the need to offer artificial shadows to the animals, aiming at improving thermal comfort and reducing losses in milk production by stress. High temperatures, prolonged days length and few shadows to the animals, decreases dairy cow productivity (Fagan et al., 2010).

Mastitis is an inflammation of the mammary gland. The inflammatory response are to destroy or neutralize the injurious agent and allow healing and return to normal function. The inflammation is the influx of white blood cells or leukocytes which results in an increase in the somatic cell count (SCC) of milk; although, the SCC is a usual measure of mammary gland health and milk quality (Harmon, 1995).

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*Corresponding author. E-mail: eliandra.oliveira@ifgoiano.edu.br.

The quantitative distribution of milk components informs about their quality (Noro et al., 2006). The consumer market raises its requirement for products with superior quality. Dangers of degradation of milk with eradication of contamination by undesirable bacteria and also by unusual solids should be reduced (Vallin et al., 2009).

When the animal stress increases, its welfare decreases, and hence, it is possible to conclude that the production efficiency of the cows is linked to the quality of the dairy cow environment (Rossarolla, 2007). One of the ways to evaluate the thermal comfort of milk cows is by temperature-humidity index (THI) to indicate the level of thermal comfort, to evaluate the welfare of several animals, as in this study for dairy cattle (Perissinotto and Moura, 2007).

Therefore, the objective of this study was to evaluate the determinant effect of climatic indexes on milk production in dairy farms located in the city of Morrinhos, southern region of the state of Goiás.

MATERIALS AND METHODS

Data on milk production and quality were obtained from the Company Mixed Cooperative Milk Producers of Morrinhos (COMPLEM), through a logistic information control system. Information from 94 dairy farms in the city of Morrinhos, state of Goiás, Brazil, was used for the period from March 2015 to August 2018, reaching a total of 3,948 observations. The climatic and meteorological data used in the development of the study were collected from the Meteorological Station installed at the Institute Federal Goiano - Campus Morrinhos (located at the geographic coordinates: Latitude 17º 48' 50.4" S; longitude 49º 12' 16.5" W; altitude 902 m).

In this study, total milk production, milk quality, fat, protein, lactose, defatted dry extract (DDE), total solids content and somatic cell count (SCC) were analyzed, after collected data.

Regarding the climatological factors, data of average temperature, maximum and minimum, relative humidity and temperature-humidity index (THI) were analyzed. In order to determine the THI, the following model was used: THI = T = 0.55 (1-UR) (T-58), where T = ambient temperature in °F and UR = relative humidity in decimal number (Roma Júnior et al., 2009).

The information collected was arranged in a single spreadsheet, organized in order of properties and the respective months of the year, in order to analyze the correlations between milk production, milk composition and climatic measures by means of statistical analysis. The equation for Pearson's correlation is described below in Equation 1.

$$\rho = \frac{\sum (x-\bar{x})(y-\bar{y})}{\sqrt{\sum (x-x)^2 \sum (y-y)^2}} \quad (1)$$

Pearson's correlation using the statistical program SAS, (2001) (SAS® University Edition).

RESULTS AND DISCUSSION

Milk composition

For milk production, there was no significant correlation with any environmental variables or milk composition. However, according to Bertoncelli et al. (2013), milk production is clearly associated with the duration of thermal comfort in which the dairy cow is exposed, that is, the lower the thermal stress, the greater its milk production.

For the evaluated components, protein had the highest correlation with the maximum and minimum temperature indices, with r = -0.18027 and r = -0.17489, respectively, as shown in Table 1. Nakamura et al. (2012), for protein analysis, had similar results, it was also negative correlation, r = - 0.250, for maximum temperature and r = -0.218, for minimum temperature. When the cow is subjected to high temperature stress, a change in its feed intake and digestion occurs. It results in alteration of the microorganism population with variation in the percentage of volatile fatty acids in the rumen produced results in a reduction in the amount of propionic acid, with a decrease in milk protein levels (Van Soest, 1994). In conditions of thermal discomfort, the animal naturally exhibits physiological and behavioral changes, and consequently, its productivity decrease. Thus, dairy cows have a reduction in feed intake and grazing, with grazing preference at night, as well as search for water and shade sites, increased water consumption and respiratory rate (Rossarolla, 2007).

For protein, the study also showed a negative correlation with the mean temperature (r = -0.17694). According to Vargas et al. (2014), there is a positive correlation for mean temperature with somatic cell count at a value of r = 0.34, this correlation is considered as mean.

These results with the heat-stressed animals tend to increase the SCC, had as a consequence, the reduction of the protein content of the milk produced. According to Pereira et al. (1999), the modification in the proportion of protein and in other constituents of milk is by the decrease in the efficiency of synthesizing the secretor cells. Fat, however, is the most inconstant constituent that is incorporated in milk; depends on factors such as genetics, feed management and environment (Fagan et al., 2010). The Jersey breed has its genetic selection to produce milk with the highest percentage of fat (Botaro et al., 2011). According to Reis et al. (2012), the Holstein breed is more productive, and their milk contained lower concentration of lipids and proteins when compared to the Girolando breed and cows crossbreed. The fat content values were also negative and significant, however, the correlation results for this variable are classified as low, -0.12228 for fat correlation with maximum temperature and –0.12454 for correlation with minimum temperature. Nakamura et al. (2012), with negative correlation values with mean intensity (r = -0.586 and r = - 0.619), in this order. Thus, Noro et al. (2006), pointed out the proportion of fat is higher during the winter season, when compared to the summer days, due to the change in feed intake of roughage tropical and

Table 1. Pearson's linear correlation between maximum and minimum temperatures and milk components**.**

Component of milk	Temperature maximum (°C)	Temperature minimum (°C)	P value
Fat (r)	- 0.12228	- 0.12454	0.0001
Protein (r)	- 0.18027	- 0.17489	0.0001
Total solids content (r)	- 0.13160	- 0.13113	0.0001

r: Correlation value; correlations were significant (p <0.05).

Table 2. Pearson's linear correlation between temperature-humidity index (THI) and milk components.

Component of milk	Temperature-humidity index (THI)	P value
Protein (r)	- 0.10513	0.0001
Fat (r)	- 0.13471	0.0001
Lactose (r)	0.15004	0.0001
Total solids content (r)	- 0.11576	0.0001

r: Correlation value; correlations were significant (p <0.05).

temperate by dairy cows. Dairy cows exposed to heat stress have reduced ruminal contractions, and for this reason, they are more predisposed to acidosis. This reduction in contractions reflects unfavorably on the production of saliva, causing a reduction in the pH of the rumen. This type of alteration can also be associated with a decrease in the proportion of volatile fat acids (VFAs), within the rumen in stress condition (Valente et al., 2017). Mota et al. (2010) observed that cows with a lower proportion of acetate in rumen affected fat production in milk.

Nakamura et al. (2012), in their study revealed a negative correlation between the maximum temperature and the fat level. The correlation values for total solids and for fat were negative and low, with both maximum and minimum temperature, expressing r = -0.13160 and r = -0.13113, respectively. The proportion of total solids is reduced in the summer when compared to other seasonal seasons, and is based on the decrease in the dry matter intake that occurs due to the climate with high temperatures. The result is the correlation between total solids and temperature indices (Fagan et al., 2010).

Relationship between environmental effects and somatic cell counts

The somatic cell count represents the presence of defense cells in the mammary gland. Thus, SCC of dairy cattle indicates the level of udder inflammation (Machado et al., 2000). Directly related to the appearance of mastitis, the percentage of somatic cells is used as a primordial instrument in the determination of subclinical mastitis (Araújo et al., 2012) when there is an increase in air humidity, which may contribute to higher rates of SCC and diseases of the mammary gland (Nakamura et al., 2012). Thus, dairy cows exposed to high temperatures exhibit reduced ability to protect against invasions of deleterious microorganisms (Porcionato et al., 2009). This is because it is possible to associate climatic variables with the somatic cell count.

Temperature-humidity index (THI)

The variables that affect the dispersion of body heating are air humidity and temperature. In order to better understand the influence of environmental variables on the welfare of dairy cows in lactation, THI was established, an instrument to measure the thermal disturbance, which associates the effects of the two measures mentioned above. The higher the value, the greater the animal discomfort. The THI is considered high, when it exceeds 68 points (Prado, 2018).

For the protein level, it had a negative correlation, but low intensity with THI, as shown in Table 2, against the result by Silva and Antunes (2018), which had no correlation between these two variables. However, this decrease in protein levels due to thermal stress is also associated with decreased feed intake, which will lead to a decrease in the production of microbial protein (Valente et al., 2016).

Regarding milk fat, there was a negative correlation with THI, as identified by Silva and Antunes (2018), which may be justified because, under warm conditions, there is a spontaneous decline in pasture and silage intake, leading to a reduction in fat levels in milk. Animals submitted to high THI values are 85% more likely to decrease grass grazing (Rodrigues et al., 2010; Prado, 2018).

Only for lactose had positive correlation, and in agreement with Silva and Antunes (2018), in a similar study also had a positive correlation between lactose and THI. Therefore, as the THI increases, the higher the

Table 3. Pearson's linear correlation between milk components.

Components of milk	Correlation value	P value
Fat x protein	0.23576	0.0001
Fat x total solids content	0.91426	0.0001
Protein x total solids content	0.52707	0.0001
Lactose x fat	- 0.25930	0.0001

Correlations were significant (p <0.05).

Table 4. Linear Pearson correlation between milk components and somatic cell count**.**

Components of milk	Correlation value	P value
DDE x SCC	- 0.30148	0.0001
Lactose x SCC	- 0.45169	0.0001

Correlations were significant (p <0.05).

percentage of lactose. The environmental factors reflect unfavorably on lactose and fat, noting that the elevation of the climate variables and the welfare indicators, causes the reduction of lactose and fat levels in milk.

The correlation between total solids and THI was negative and low, similar results found with Silva and Antunes (2018), obtained a negative correlation for these variables. The levels of total dry extract are higher in the winter season, and decrease in summer (Henrichs et al., 2014) confirm that the influence of temperature and humidity on these results for these compounds. A positive correlation between fat and milk protein (r = 0.23576, considered weak) in Table 3 contrary to Henrichs et al. (2014), which had a negative correlation between these variables.

The correlation between fat and milk protein was similar between the seasons and months of the year, where fat and protein appear as great indicators of milk quality, the most favorable results of measurement of these two items were obtained in the period of the lowest rainfall index and with minimum temperatures, confirming the direct influence of the environment and animal welfare on the proportion of these components in the milk (Roma Júnior et al., 2009). The reduced percentage of milk fat is a consequence of the dilution effect due to increased milk production (Fagan et al., 2010; Prado, 2018).

Already, the correlation between fat and total solids content was high, almost reaching 1, as was exposed by Henrichs et al. (2014). Zanela et al. (2006) confirms that total solids content levels increase as the fat percentage increases. This relation is related to the producing breed, for the milk produced by Jersey cows, greater percentage of fat and, consequently, greater proportion of total solids content.

The protein content is considerably relevant to determine the milk yields in the dairy industry (Henrichs et al., 2014). Casein appears to be the most important protein in dairy products. In this study, there was a positive correlation between total solids and milk protein in Table 4, a result similar to Henrichs et al. (2014). Reis et al. (2012) presented a value of r = 0.58, very similar to the value in this study. Protein and total solids have similar results when compared to the seasons, with the highest percentage in autumn and winter, decreasing during the spring and summer periods in the tropical climate (Henrichs et al., 2014). The correlation between lactose and fat in the milk was negative among the components (r = - 0, 25930). As for lactose, it is the sugar present in milk, and it is also the constituent with lower rate of change, unlike fat, which has a greater instability characteristic (Van Soest, 1994). This confirms the negative correlation result observed in the present study.

Mastitis lead to changes in the constitution of milk produced by the dairy cow (Pereira et al.,1999). The highest somatic cell count measurements occur in summer (Bueno et al., 2005). For the DDE analysis, it had a negative correlation with the SCC, with a considered average value. Montanhini et al. (2013), in their study had found a negative correlation value equal to -0.227. On the other hand, the result of Rangel et al. (2009), in his analysis was a positive correlation between SCC and DDE, for a situation of mechanically milked cows of the Holstein breed, and DDE, which does not involve the fat content of milk. The increase in SCC leads to increased fat levels (Lacerda et al., 2010; Montanhini et al., 2013). This is due to the fact that inflammation in the udder caused a decrease in the total volume of milk produced by the cow and accumulation of fat (Machado et al., 2000).

The correlation between lactose and SCC, as well as total solids, was negative for both considered of medium degree. In agreement with what was exposed by Bueno et al. (2005), which also had a negative correlation between SCC and lactose, and it is possible to conclude that lactose is the constituent with the highest decrease, and the SCC increases. Reis et al. (2012) also had found negative mean correlation for lactose and somatic cell counts. Botaro et al. (2011) stated that cows with mastitis have a decrease in lactose levels, as a result of the fall in the synthesis of this constituent under these animal health conditions.

Conclusion

Thermal comfort directly influences the quality and quantity of milk produced by dairy cows under conditions of thermal stress, milk production decreased, milk composition changed. Indirectly, the thermal comfort exerts influence in the health and the capacity of defense of these animals against pathogens.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Araújo VM, Rangel AHN, Bezerra KC, Andrade KD, Guerra MG (2012). Avaliação de testes rápidos para análises da contagem de células somáticas de leite cru de tanques. Acta Veterinaria Brasilica 6(4):321-324.

Bertoncelli P, Martin TN, Ziech MF, Paris W, Cella PS (2013). Conforto térmico alterando a produção leiteira. Revista Enciclopédia Biosfera 9(17):762-777.

Botaro BG, Cortinhas CS, Mestieri L, Machado PF, Santos MV (2011). Composição e frações proteicas do leite de rebanhos bovinos comerciais. Vet. e Zootec 18(1): 81-91.

Bueno VFF, Mesquita AJ, Nicolau ES, Oliveira NA, Oliveira JP, Neves RBS, Mansur JRG, Thomaz LW (2005). Contagem celular somática: relação com a composição centesimal do leite e período do ano no Estado de Goiás. Ciência Rural 35(4):848-854.

Fagan EP, Jobim CC, Calixto Júnior M, Silva MS, Santos GT (2010). Fatores ambientais e de manejo sobre a composição química do leite em granjas leiteiras do Estado do Paraná, Brasil. Acta Scientiarum. Animal Sciences 32(3):309-316.

Harmon R (1995). Mastitis and milk quality. In: Harding F. (eds) Milk Quality. Springer, Boston, MA. https://doi.org/10.1007/978-1-4615-2195-2_3

Henrichs SC, Macedo REF, Karam LB (2014). Influência de indicadores de qualidade sobre a composição química do leite e influência das estações do ano sobre esses parâmetros. Revista Acadêmica: Ciência Animal 12(3):199-208.

Lacerda LM, Mota RA, Sena MJ (2010). Contagem de células somáticas, composição e contagem bacteriana total do leite de propriedades leiteiras nos municípios de Miranda do Norte, Itapecurú– Mirim e Santa Rita, Maranhão. Arquivos do Instituto Biológico 77(2):209-215.

Machado PF, Pereira AR, Sarríes GA (2000). Composição do leite de tanques de rebanhos brasileiros distribuídos segundo sua contagem de células somáticas. Revista Brasileira de Zootecnia 29(6):1883-1886.

Montanhini MTM, Moraes DHM, Neto RM (2013). Influência da contagem de células somáticas sobre os componentes do leite. Revista do Instituto de Laticínios Cândido Tostes 68(392):18-22.

Mota MF, Vilela D, Santos GT, Elyas ACW, Lopes FCF, Verneque RS, Paiva PCA, Pinto Neto A (2010). Parâmetros ruminais de vacas leiteiras mantidas em pastagem tropical. Archivos de Zootecnia 59(226):217-224.

Nakamura AY, Alberton LR, Otutumi LK, Donadel D, Turci RC, Agostinis RO, Caetano ICS (2012). Correlação entre as variáveis climáticas e a qualidade do leite de amostras obtidas em três regiões do estado do Paraná. Arquivos de Ciências Veterinárias e Zoologia da UNIPAR 15(2):103-108.

Noro G, González FHD, Campos R, Dürr JW (2006). Fatores ambientais que afetam a produção e a composição do leite em rebanhos assistidos por cooperativas no Rio Grande do Sul. Revista Brasileira de Zootecnia 35(3):1129-1135.

Pereira AR, Silva LFP, Molon LK, Machado PF, Barancelli G (1999). Efeito do nível de células somáticas sobre os constituintes do leite I-gordura e proteína. Brazilian Journal of Veterinary Research and Animal Science 36(3):121-124.

Perissinotto M, Moura DJ (2007). Determinação do conforto térmico de vacas leiteiras utilizando a mineração de dados. Revista Brasileira de Engenharia de Biossistemas 1(2):117-126.

Prado DMB (2018). Temperatura corporal e comportamento de vacas leiteiras em pastejo. Trabalho de Conclusão de Curso (Graduação em Medicina Veterinária). Uberlândia, 29p.

Porcionato MAF, Fernandes AM, Netto AS, Santos MV (2009). Influência do estresse calórico na produção e qualidade do leite. Revista Acadêmica: Ciência Animal 7(4):483-490.

Rangel AHN, Medeiros HR, Silva JBA, Barreto MLJ, Dorgival Júnior ML. (2009). Correlação entre a contagem de células somáticas (SCC) e o teor de gordura, proteína, lactose e extrato seco desengordurado do leite. Revista Verde de Agroecologia e Desenvolvimento Sustentável 4(3):57-60.

Reis AM, Costa MR, Costa RG, Suguimoto HH, Souza CHB, Aragon-Alegro LC, Ludovico A, Santana EHW (2012). Efeito do grupo racial e do número de lactações sobre a produtividade e a composição do leite bovino. Semina 33(2):3421-3436.

Rodrigues AL, Souza BB, Pereira Filho JM (2010). Influência do sombreamento e dos sistemas de resfriamento no conforto térmico de vacas leiteiras. Agropecuária Científica no Semiárido 6(2):14-22.

Roma Júnior LC, Montoya JFG, Martins TT, Cassoli LD, Machado PF (2009). Sazonalidade do teor de proteína e outros componentes do leite e sua relação com programa de pagamento por qualidade. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 61(6):1411-1418

Rossarolla G (2007). Comportamento de vacas leiteiras da raça holandesa, em pastagem de milheto com e sem sombra. Dissertação (Mestrado em Zootecnia) – Centro de Ciências Rurais – Universidade Federal de Santa Maria, Santa Maria, 2007.

SAS (Statistical Analysis System) (2001). User's guide: Statistics. Cary: SAS Institute.

Silva JC, Antunes RC (2018). Efeito do tipo de ordenha e do ambiente sobre a qualidade do leite cru com base na contagem de células somáticas. Ciência Animal Brasileira 9(1-16)e-34635.

Valente TNP, Lima ES, Santos WBR, Cesario AS, Tavares CJ, Fernandes IL, Freitas MAM (2016). Ruminal microorganism consideration and protein used in the metabolism of the ruminants: A Review. African Journal of Microbiology Research 10:456-464.

Valente TNP, Sampaio CB, Lima ES, Deminicis BB, Cezário AS, Santos WBR. (2017). Aspects of acidosis in ruminants with a focus on nutrition: A review. The Journal of Agricultural Science 9(3):90-97.

Vargas DP, Nörnberg JL, Mello RO, Sheibler RB, Breda FC, Milani MP (2014). Correlações entre contagem de células somáticas e parâmetros físico químicos e microbiológicos de qualidade do leite. Ciência Animal Brasileira 15(4):473-483.

Vallin VM, Beloti V, Battaglini APP, Tamanini R, Fagnani R, Angela HL, Silva LCC (2009). Melhoria da qualidade do leite a partir da implantação de boas práticas de higiene na ordenha em 19 municípios da região central do Paraná. Semina 30(1):181-188.

Van Soest PJ (1994). Nutritional Ecology of the Ruminant. Cornell

University Press P. 476.

Zanela MB, Fischer V, Ribeiro MER, Stumpf Junior W, Zanela C, Marques LT, Martins PRG 2006. Qualidade do leite em sistemas de produção na região Sul do Rio Grande do Sul. Pesquisa Agropecuária Brasileira 41(1):153-159.

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Effects of NPK and plant tea manure (*Tithonia diversifolia*) on growth rate of amaranth (*Amaranthus cruentus* L.) in soilless growing media

Roseline Chemutai[1*], Julius Mwine[2], Richard Awichi[3] and Godfrey Bwogi[2]

[1]Department of Agriculture, Bukalasa Agricultural College, P. O. Box 174, Uganda.
[2]Faculty of Agriculture, Uganda Martyrs University, Uganda.
[3]Facaulty Science, Department of Mathematics and statistics, Uganda Martyrs University, Uganda

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Many countries are running short of agricultural land. Even where land appears to be available, soil fertility, water availability as well as nutrient mining still remains a challenge yet at the same time the world's demand for food is rapidly increasing. Millions of square meters of soil are mined each year for use in vegetable nurseries and in backyard gardens in addition to excessive use of inorganic fertilizers. There are however a number of soilless growth media and organic sources of plant nutrients that could be used to address this problem. This study investigated the effects of application of NPK (Nitrogen, Phosphorus and Potassium) and plant tea (*Tithonia diversifolia*) manure on selected soilless growing media on growth rate of *Amaranthus cruentus* L. This was to determine their suitability as an alternative growing media. A split plot design was used and the experiments carried out for two seasons. The soilless growing media investigated were: charcoal dust, saw dust, dry coffee husks, and mixture of charcoal and saw dust (1:1), saw dust and coffee husks (1:1), charcoal dust and coffee husks (4:1) and a compound mixture of charcoal dust, sawdust and coffee husks (2:2:1). The growing media were randomized in the three split blocks with eight pots each. Growth rates in terms of shoot height, number of leaves, leaf length, leaf width, stem girth and root length were measured for two seasons. Results indicated that all the growth media could support amaranth growth. However, a mixture of charcoal dust and dry coffee husk (4:1 respectively) with application of plant tea manure significantly affected the growth rate of *A. cruentus* ($p<0.05$). Mixture of charcoal dust and dry coffee husk (4:1 respectively) with application of either NPK or plant tea manure could be used as the best alternative growth media. Soilless growth media that constituted of charcoal dust and coffee husks could be explored for amaranth production in home kitchen gardens in rural areas and backyard gardens in urban areas.

Key words: Soil, soilless growing media, plant nutrients, amaranth.

INTRODUCTION

According to Wilkinson et al. (2014), surveys of tropical and sub-tropical areas of Africa, Asia, and Latin America showed unexpectedly poor field growth of seedlings after out planting, and correlated this problem with poor root development because of using soil-based media in nurseries. These nurseries could be for vegetables, fruits and trees. One of the major challenges soil-based agriculture poses is its vulnerability to pests and diseases, environmental changes such as floods, wind, drought and climate change. These changes can lead to huge losses for farmers (Bout Well, 2014).

Butler and Oebker (2006) reported that soil-based agriculture is facing some major challenges, with the advent of civilization all over the world, such as decrease per capita land availability, incidence of pests and diseases, soil degradation among others. The degraded soils require constant addition of inorganic fertilizers if any better plant growth is to be achieved. These inorganic fertilizers are relatively expensive and most local rural people may not afford them. Apart from this, due to rapid urbanization and industrialization as well as threats from climate change and its related adverse effect, the land cultivation is going to further face challenging threats (Mgbemene et al., 2016).

Over the years, vegetables have been grown on natural soil, however with the increase in population especially in urban centers, growing vegetables like *Amaranthus cruentus* is carried out in containers, sacks within the compounds and backyard gardens with natural soil as a growth media. According to Husain et al. (2014) the soil used is normally collected (mined) from other places like from the forest, swamp and gardens. This has led to soil nutrient mining which is one the greatest threats to agricultural production, decreased crop yields and per capita food production, in the mid to long term, a key source of land degradation and environmental damage according to Henao and Baanante (2006).

Besides, poor soil fertility in some of the cultivable areas, less chance of natural soil fertility build-up by microbes due to continuous cultivation, frequent drought conditions and unpredictability of climate and weather patterns, rise in temperature, river pollution, poor water management and wastage of huge amount of water, decline in ground water level, etc., are threatening food production under conventional soil-based agriculture. Under such circumstances, in near future it will become impossible to feed the entire population using open field system of agricultural production only. Naturally, soil-less culture is becoming more relevant in the present scenario, to cope-up with these challenges. In soil-less culture, plants are raised without soil. Improved space and water conserving methods of food production under soil-less culture have shown some promising results all over the World (Sengupta and Banerjee, 2012).

The recommended alternative according to Soga (2010) is to replace soil-based media whenever possible with organic-based media. If soil must be used, it should be only a small percentage of the mix, amended with other ingredients to overcome some of the problems. According to Nkonya et al. (2012) about 60% of the total land area is marginally suitable for cultivation due to limited organic matter and water retention capacity while close to 30% is considered degraded and vulnerable to erosion. Sub-Saharan Africa suffers from extensive soil degradation, threatening the livelihoods of the 70% of Africans who are involved in agriculture. The poor quality of soils is another constraining environmental factor. For example, phosphorus deficiency, low organic content, and low water infiltration and retention capacity on much of African soil have been limiting factors in agriculture. Unlike climate variability, this problem can be addressed: soil quality can be augmented through careful management and soil nutrient supplementation.

Depardieu et al. (2016) reported that due to increasing risks of water scarcity resulting from climate change and the intensification of crop production worldwide, the agricultural sector needs to improve water use efficiency for crop production. At present, soilless cultivation under protective conditions is an intense cultivation method that can provide more efficient use of water. The increasing demand for low cost, environmental friendly and highly performing soilless substrates for crop production has led to the search for alternative materials as constituents of growth media such as organic wastes from the agri-food and agriculture industries. Organic substrates are usually preferred because of their low costs, biodegradability and their high productivity potentials (Caron et al., 2015).

Soilless growing media

The origins of soilless culture go back at least to the 17th century when, in 1666, Boyle attempted to grow plants in "vials containing nothing but water", and reported that one species (spearmint, *Raphanza aquatica*) survived for nine months (Olympios, 1999). Soilless cultivation addresses the problem of dwindling farmlands, as plant roots do not need to stretch much to reach nutrients; the nutrients they need are supplied in the nutrient formula (Bout Well, 2014).

According to Olympios (1999), the main advantages of soilless culture are the most accurate control over the supply of water, nutrients, pH, root temperature, increase productivity due to easier and more accurate control of production factors, reduction of labour requirement, no need for soil sterilization and more crops per year. With soilless agriculture, plants may be cultivated indoors, where they are protected against potential destructive environmental elements. Crop yields are also stable and much higher due to the use of artificial lighting, making it possible to grow year-round. Soilless cultivation requires little-to-no pesticides or herbicides, or can get by using minimal amounts of organic options. Crops grown in protected environments are fed optimally and experience less stress. This makes them better able to resist the pests. Furthermore, weeds are not a problem in soilless

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*Corresponding author. E-mail: rosechemutai77@yahoo.com.

cultivation since they require soil to grow (Boutwell, 2014).

According to Bhardwaj (2013) and Salisa et al. (2016) in studies on effects of soilless growing media on germination of different plants, found out that growing media plays important role in seedling emergence and quality of the seedlings produced. A good growing media provides sufficient anchorage or support to the plant, reservoir for nutrients and water and permit gaseous exchange between root and atmosphere outside the root substrate (Sardoei and Shahdadneghad, 2015).

Coffee husks

According to Nguyen et al. (2013) coffee husks may be added into soil when used alone or in combination with NPK. Application of fertilizers as amendments improves the chemical characteristics of the soil. The reduction in mineral fertilizer application through their supplementation with organic sources, such as coffee husk makes the use of soil nutrient amendments affordable to small holder farmers, guarantees and improves soil life. Application of coffee waste in sandy soil increases the availability of phosphorus, retained soil water from 53 to 60%. It also promotes the retention of basic cations and immobilized manganese. Coffee waste also has the potential to be used as a liming material, NPK fertilizer and has the benefit of increasing water and nutrient retention (Kasongo et al., 2011).

Charcoal dust

Ukrfuel (2015) reported that moisture content together with the ash content are very important charcoal’s chemical properties that define its quality. Moisture lowers the heating value of charcoal. Usually fresh charcoal from the kiln contains less than 1% of moisture, however due to humidity of the air the moisture content can reach 5 to 10%. When the hygroscopicity of charcoal increased, the moisture content of charcoal can rise to 15% or even more. The reason for high cation exchange capacity on charcoal dust is due the process of pyrolysis that causes increase in the surface area after pyrolysis. Secondly, there is an increase in charge density on the surface. Generally, the higher the pyrolysis temperature the higher the charcoal dust surface area. This high surface area is in the form of micro/nanopores (Gomez-Eyles et al., 2013).

Saw dust

According to Tran (2005), sawdust holds potential as a contributing carbon source for increasing soil organic matter when applied to soil. Marinou et al. (2013) also added that sawdust is widely used as a growth medium component in areas with wood processing industries, because of its low cost, high moisture retention, and high availability.

Plant nutrients (NPK)

According to Mariana et al. (2015), soil is the main source of mineral nutrients and water for plants, but its ability to provide plant nutrients needed varies depending on the level of fertility. The removal of nutrients from the soil into the plant by sucking them through leaching or other processes related to the natural dynamics of soil, reducing entail the contents of mobile forms of nutrients and the gradual decline of production capacity of soils. For these reasons, it is necessary to applying mineral and organic fertilizers. Plants require essential nutrients for normal functioning and growth. A plant’s sufficiency range is the range of nutrient amount necessary to meet the plant’s nutritional needs and maximize growth (McCauley et al., 2011).

NPK fertilizer is primarily composed of three main elements: Nitrogen (N), Phosphorus (P), and Potassium (K), each of these being essential in plant nutrition. Among other benefits, Nitrogen helps plants grow quickly, while also increasing the production of seed and fruit, and bettering the quality of leaf and forage crops. Nitrogen is also a component of chlorophyll, the substance that gives plants their green color, and also aids in photosynthesis (Okese, 2016).

Plant tea manure made from Tithonia (*Tithonia diversifolia)*

Tithonia produces a nutrient-rich (N, K and P) biomass and its positive effect on subsequent rice and maize crops has been reported from Africa and Brazil as reported by Partey (2010). In addition, *T. diversifolia* causes immediate and sustained increase in soil pH and an immediate and sustained decrease in extractable Aluminium in soils. Its abundance and adaptability, coupled with its rapid growth rate and very high vegetative matter turnover, makes it a candidate species for soil rejuvenation and improvement, as a green manure or as a major component of compost manure. Different practices have been reported: Mexican sunflower can be left to decompose on the field, or it can be turned into green manure according to Olabode et al. (2007).

Amaranth (*A. cruentus*)

Common names: purple amaranth, red amaranth, red shank, bush greens, African spinach, Indian spinach (En.); amaranteétalée, queue de renard (Fr.); achita, bledo (Sp.). This amaranth species was domesticated as a grain in Mesoamerica and found its way to the tropics and subtropics of the Old World during colonial times. It is used for grain production (pseudo cereal), as a leafy vegetable, and for ornamental purposes. In tropical Africa it is a traditional, highly productive, nutritious and economically important leafy vegetable (Andreas, 2011). Amaranthus originated in America and is one of the oldest food crops in the world, with evidence of its cultivation reaching back as far as 6700 BC.

Most of the vegetables consumed in the urban centres in sub-Saharan Africa are grown on soil mined and collected far away from swamps, forest or gardens. The yield from these soils does not match with high demand of vegetables therefore more has to be transported from some miles away in the rural villages using motor cycles and vehicles. This leads to soil nutrient mining which is one the greatest threats to agricultural production, decreased crop yields and per capita food production. This is one of the mid to long term, a key source of land degradation and environmental damage according to Henao et al. (2006). Transporting of vegetables using motorcycles and vehicles which use the fossil fuels contributes to the carbon dioxide emissions that are responsible for the greenhouse gases and their effect on global warming and climate change. According Viljoen and Bohn (2014) when food is produced in the city, food miles are generally reduced which may as well reduce carbon dioxide emissions linked to it (Viljoen and Bohn, 2014).

Many countries are running short on agricultural land at the same time the world's demand for food is rapidly increasing. Soil and water fertility decline, nutrient mining still remain a challenge in many countries in sub-Saharan Africa. Harvesting topsoil is actually a mining operation that uses up a limited resource that took thousands of years to develop. Millions of square meters of soil are mined each year for use in, vegetable nurseries and in backyard gardens in addition to excessive use of inorganic fertilizers. However, soil fertility status has attained a saturation level, and productivity is not increasing further with increased level of fertilizer application (Sengupta and Banerjee, 2012). There are however a number of soilless growth media and organic source of plant nutrients that could be used to address this problems.

METHODOLOGY

This study was conducted in Luweero District in Central Uganda, 0.712°N, 32.1250 specifically at Bukalasa Agricultural College green house. The green house was typical standard green house for horticultural production in tropical Africa. It was well insulated with a transparent and translucent polyethene material. The inside environment was well aerated with temperature ranging from 24°C in a cool weather to 30°C on warm weather conditions. The source of water was from the taps and watering using Watering Can. This was regularly carried out early in the morning and sometimes in the evening depending on the weather conditions (Jyopti and Davidson, 2015).

Preparation of research materials

To determine the effects of application of NPK, plant tea manure on selected soilless growing media on plant growth, an experiment was conducted using *A. cruentus* L. as model plant. The different growing media were prepared by carrying out the following activities: sorting, sifting and boiling. The dry coffee husks (Robusta coffee husks) were boiled in order to soften it. The different media were measured in the right ratios, mixed and potted. Proper mixing of the media was ensured to avoid variations in container plant quality. The pots were arranged in randomized blocks in the greenhouse and watered thoroughly (Brein, 2013).

The sources of plant nutrients included NPK (17:17:17) which was purchased from the farm shops. It was applied at a rate of 2 g/pot. Tithonia (*Mexican sunflower*) was obtained from the college farm hedge. Plant tea manure was prepared from fresh Tithonia (*Mexican sunflower)* leaves (1 kg) cut into small pieces of 3 to 4 cm and soaked in 10 L of water, kept in the dark place and stirred regularly for 7 days (Nalunga, 2014; Partey, 2010). After that it was filtered to remove the residues and the filtrate is diluted at a ratio of 1 L:10 L of water for root application or 1 L:20 L for foliar application of plant tea manure and water, respectively. Each of the different sources of nutrients was applied to specific plots as shown in the research design (Koller et al., 2016).

The experimental design

A split plot design was used to carry out the investigations. Using this design enabled the control of all other factors which would have affected the research experiment. It also helped in making comparisons between the effects of different sources of nutrient (treatments) on soil and the different soilless growing media on growth rate of *A. cruentus* (O'Connor et al., 2018).

Twenty four 10 L plastic experimental pots, each perforated at the base to permit drainage of excess water each were filled with: soil, coffee husks, sawdust, and the different combinations of the soilless growth media, respectively as shown in Table 1. The distance between blocks was 1 m apart and between pots in the respective blocks was 30 cm and were randomly arranged (Koller et al., 2016; Brein, 2013).

All the pots were watered and left to rest for 48 h. *A. cruentus* seeds mixed with sand were planted in each pot with a spacing of 2 cm×10 cm and covered thinly with soil. At the growth period of 2 weeks, the plants were thinned and six plants were left to grow in each of the pots throughout the experimental period (Kamara et al., 2016).

The first group (block 1) of plants in the different growth media (T1 - T8) received no treatment and was used as a control. The second group (block 2) received NPK (17:17:17). The third group (block 3) received plant tea manure on weekly intervals (Tithonia plant tea manure). The two treatments were applied at 2 weeks after planting. The plants were allowed to grow and watered at alternate days as necessary. Subsequently, the growth parameters were recorded on weekly intervals for 5 weeks. By this time most of the plants in the different growing media and the different combinations with the different sources of nutrients had reached reasonable size for better observations and recording of data (Both et al., 2015).

Data collection methods and tools

The methods use to collect date included physical observation and measurements. The tools included camera (photographs), 30 cm ruler, and 3 m tape measure to measure leaf length, width and shoot height. Vernier calipers (12.5 cm Chinese make) were used to measure the stem girth. The shoot height was measured from the

Table 1. (a) Showing growth media composition of different plant growth media used in the research. (b) Layout of the experiment

Growth media	Growth media (% or volume ratio)
T1	Natural soil 100%
T2	Saw dust 100%
T3	Charcoal dust 100%
T4	Coffee husks 100%
T5	Saw dust + charcoal dust (1:1)
T6	Saw dust + Coffee husks (1:1)
T7	Charcoal dust + Coffee husk (4:1)
T8	Charcoal dust + Saw dust + coffee husks (2:2:1)

Control (Block 1)	NPK (Block 2)	Plant tea (Block 3)
T1	T4	T6
T2	T8	T5
T3	T7	T1
T4	T6	T3
T5	T2	T7
T6	T3	T8
T7	T1	T4
T8	T5	T2

collar region to the end of the shoot tip or foliage. The stem girth was measured at the collar region (cotyledon scar) using the vernier calipers. The root length was measured from the collar region to the end of the tap root tip (Pooter et al., 2016). The seed emergence was verified by observing the seedlings that had emerged in each pot in the first four to five days (Carta et al., 2016).

At 2 weeks after planting, the initial growth and development parameters such as number of leaves and shoot height were recorded. Thereafter as the seedlings growth progressed observations on growth indicators were recorded on weekly basis. *A. cruentus* has soft and tender stems that are quite delicate therefore the stem girth and the root length were determined at the end of the experiment. After termination of the first experiment the different growth media were left to rest for one month then fresh seeds were planted. This was used to determine whether the different soilless growing media can be reused for growing (Barrett et al., 2016).

The data recorded were analyzed using SPSS Version 16 and Genstat 2.0 software packages. For each response variable, two phases of statistical analysis were employed. The first step involved one way analysis of variance (ANOVA) to compare whether the observed response among the main factors of interest; different growing media and treatments were significant. The second phase involved further analysis in comparing means to detect the differences among the different growing media and the treatments. The means of significant response parameters were separated using the Tukey-Kramer Honestly Significant Difference (HSD) test at $p<0.05$ level of significance. This was used for separating means of different plant growth parameters and their level of significance and to determine the differences between groups (Abdi and Williams, 2017).

FINDINGS

Shoot height

Figure 2 shows clearly that the soilless growing media that had the best performance in terms of shoot height is T7 (mixture of charcoal dust and coffee husks 4:1, T3 (charcoal dust) and T4 (coffee husks). However, T7 had the highest coefficient of variation compared to all the other soilless growing media. Further analysis was conducted using ANOVA in Table 3.

There was a significant difference between means of heights of *A. cruentus* grown in different growing media at $p<0.001<0.05$. These findings confirm the observations illustrated in Figure 1. It indicated that the application of NPK and plant tea manure affects the growth rate of *A. cruentus*, shoot height in particular. From these findings therefore the null hypothesis which stated that application of NPK and plant tea manure on selected soilless media has no effect on the growth rate of *A. cruentus* was rejected.

The data on Tables 3 and 4 present sufficient evidence that there was a statistically significant difference in shoot height in different growing media and treatments. These clearly show that the different growing media and treatments have effects on the growth in height of *A. cruentus.*

Number of leaves

The data in Table 5 shows that there was statistically significant evidence that different treatments have an effect in growth in the number of leaves ($p<0.001<0.05$).

Stem girth

The data in Table 7 shows that there was statistically

Figure 1. Making observations and recording data.

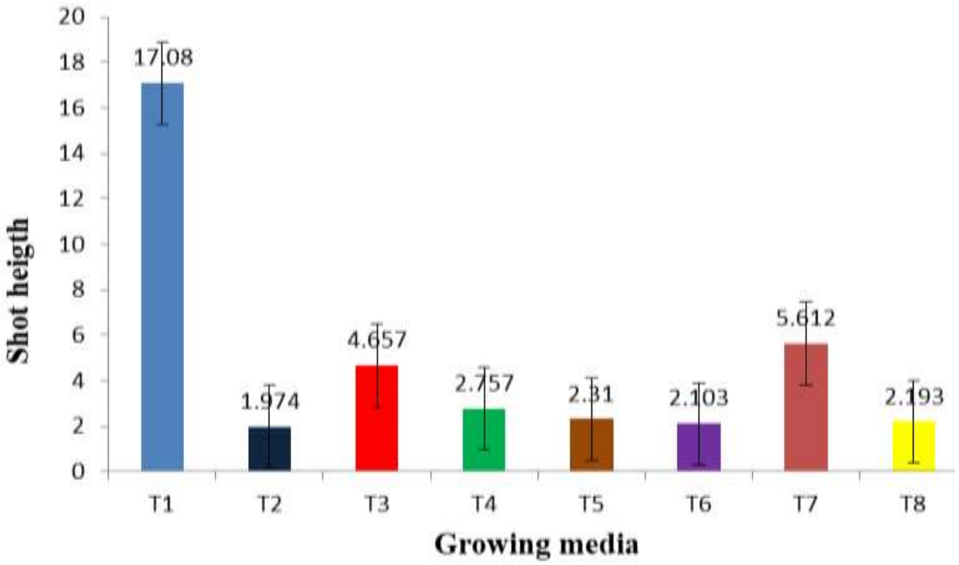

Figure 2. Shoot height in different growing media.

Table 3. Summary of ANOVA on shoot height in different growing media.

Shoot height	Sum of squares	df	Mean square	F	Sig.
Between Groups	25367.692	7	3623.956	143.662	0.001
Within Groups	28857.982	1144	25.226	-	-
Total	54225.675	1151	-	-	-

significant evidence that the stem girth of *A. cruentus* was different in the different growing media. This further confirms that application of NPK and plant tea manure has an effect on the growth rate (attributes) of *A. cruentus.* The null hypothesis which states that application of NPK and plant tea manure on soil soilless

Table 4. Summary of ANOVA on shoot height of in different treatments.

Shoot height	Sum of squares	df	Mean square	F	Sig.
Between Groups	487.295	2	243.647	5.210	0.006
Within Groups	53738.380	1149	46.770	-	-
Total	54225.675	1151	-	-	-

Table 5. Summary of ANOVA on number of leaves of in different treatments.

No. leaves	Sum of squares	df	Mean square	F	Sig.
Between Groups	1525.352	2	1525.352	67.478	0.001
Within Groups	19485.741	862	22.605	-	-
Total	21011.093	863	-	-	-

Table 6. Summary statistics for stem girth in different growing media.

Growth media	T1	T2	T3	T4	T5	T6	T7	T8
Mean	0.794	0.0233	0.35	0.058	0.0654	0.0322	0.48	0.0383

Table 7. Summary of ANOVA stem girth in different growth media.

Stem girth	Sum of squares	df	Mean square	F	Sig.
Between Groups	20.322	7	2.903	106.409	0.001
Within Groups	7.639	280	0.027	-	-
Total	27.961	287	-	-	-

Table 8. Summary of ANOVA root length in different growing media and treatments.

Parameter		Sum of squares	df	Mean square	F	Sig.
Root length	Between Groups	7710.841	7	1101.549	120.905	0.001
	Within Groups	2551.034	280	9.111	-	-
	Total	10261.875	287	-	-	-
Treatment	Between Groups	0.000	7	0.000	0.000	1.000
	Within Groups	192.000	280	0.686	-	-
	Total	192.000	287	-	-	-

growth media has no effect was therefore rejected.

Root length

Data in Table 8 clearly shows that there was a statistically significant difference in root length between groups in different soilless growing media (p<.001<0.05) (Figure 3). However, the data also showed that root length was not statistically significant in different growth media with the application of the different treatments (p>1.00>0.05).

DISCUSSION

The results indicate that the application of NPK and plant tea manure has effect on the growth of *A. cruentus* on

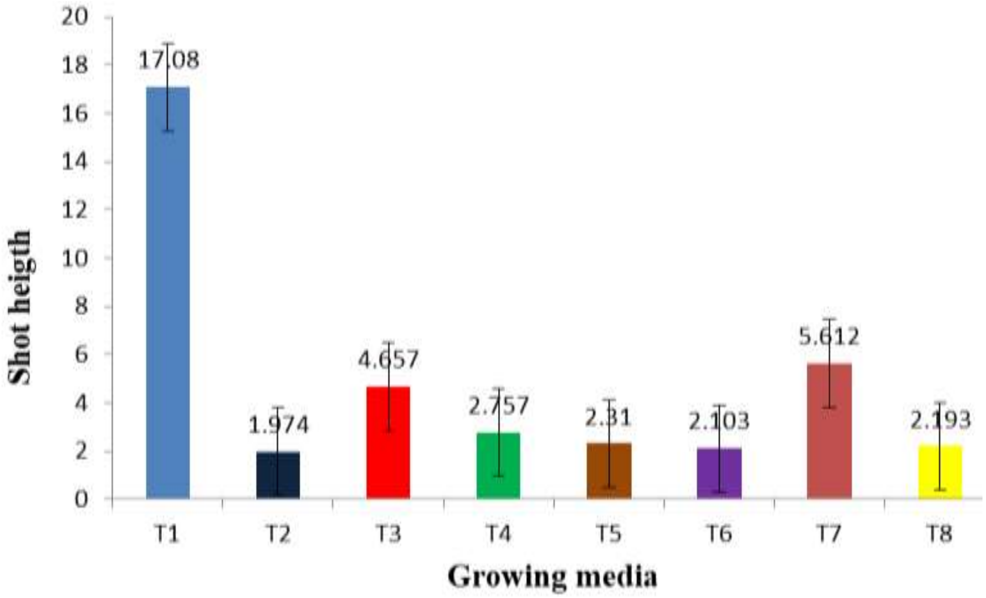

Figure 2. Shoot height in different growing media.

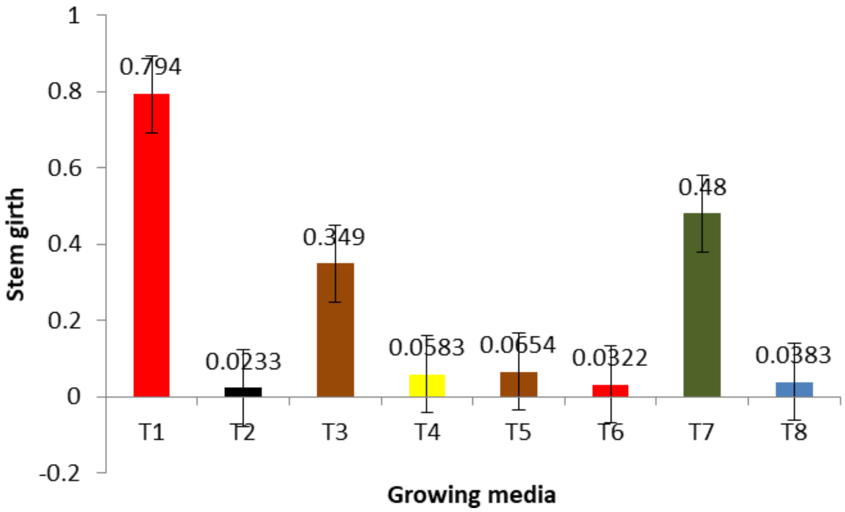

Figure 3. Stem girth in different growing media.

selected soilless growing media (Makinde et al., 2011).

Shoot height

Figures 2 and 3 reveal that the soilless media T7 had better performance compared to the rest. The mixture of coffee husks and charcoal dust had a significant effect on the shoot height of amaranth. This is in line with Rabani (2018), he found that growth media affects roots as well as shoot growth of lentils. Similar findings were also reported by Crush et al. (2005) and Olosunde et al. (2009).

Number of leaves and stem girth

As shown on Tables 5 and 6, the leaves and shoot height were similarly affected by the growth media as well as the stem girth. These findings confirm that of Chalwa and Mahta (2015) who also found that growing media affects the survival and growth of transplanted Litchi layers with better root development and therefore better leaf growth. Ibironke and Victor (2016) also reported similar results

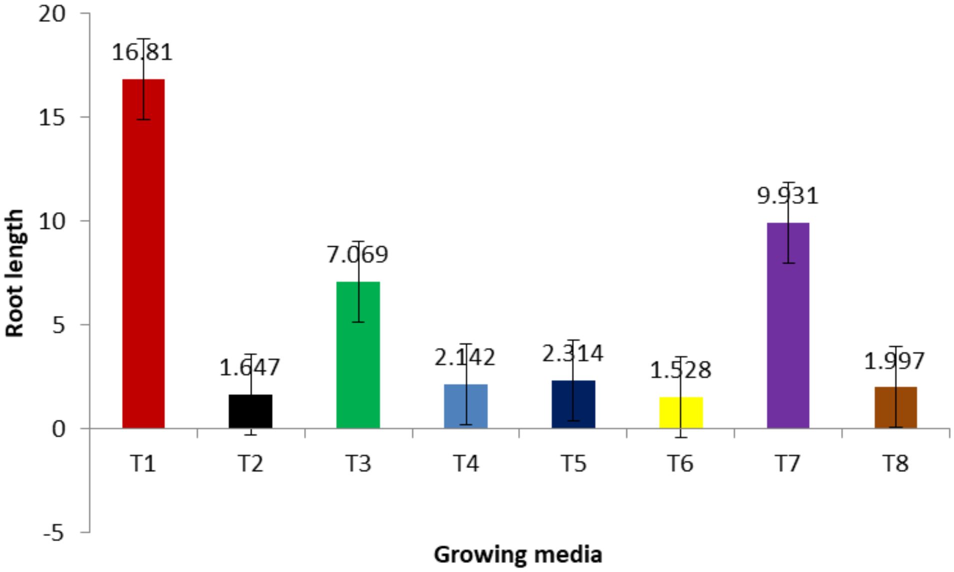

Figure 4. Root length in different growing media.

that growth media has significant influence on number of leaves per plant. However other soilless growing media showed no significance on the number of leaves and the stem girth. This could be attributed to their structural differences and ability to release nutrients required for plant growth (Olubanjo and Alade, 2018).

Root length

T7 still showed the significant effects on the root length of amaranth. These findings are also in line with the study carried out by Magesa et al. (2017) where he found that growth media affects the rooting stem cuttings of hybrid coffee. These results show that root length is determined by the media where it is grown. Furthermore Fagge and Manga (2011) found that root length in most cases is related to the growing media. Growing media that has good structure with enough pore space and ability to hold adequate water and plant nutrients for plant growth is an ideal media for proper root growth (University of Maine, 2019).

The mixture of coffee husks and charcoal dust is well aerated, porous thereby promoting rapid absorption of nutrients facilitating root penetration and eventual plant growth observed in shoot height, number of leaves, stem girth, and root length among others. Therefore, addition of either NPK or plant tea manure further enriches the mixture with plant nutrients. Fairhurst (2012) reported that coffee husks when added into the soil improve its chemical properties. These chemical properties include water holding capacity, aeration and cation exchange capacity (Mukherjee, 2013; Gomez-Eyles et al., 2013). Growing media with a high cation exchange capacity withhold cation and act as a long-term cache. In general, an abundance of small to medium-sized pores can enhance the surface area of the material. The high surface area of charcoal dust provides space for formation of bonds and complexes with cations and anions with metals and elements on its surface which improves the nutrient retention capacity. This high surface area is in the form of micro/nanopores that can tightly trap ions (e.g., NH_4^+) and water cannot flush them out (Haven, 2010). This capacity is the reason charcoal dust is able to hold water for long compared to other soilless growth media (Barrett et al., 2016).

Coffee husks are organic substrates which are highly biodegradable. Therefore, after it breaks down it avails all the nutrients for pant growth. This confirms the report by Caron et al. (2015). Kasongo et al. (2011) also reported that addition of coffee husks in soil promote the retention of basic cations and immobilized manganese as improving the pH of soil due its liming properties.

Charcoal dust is porous and hygroscopic in nature which makes it absorb moisture from the air. The porous nature also makes it well aerated therefore facilitating root penetration and eventual plant growth. These properties also make charcoal dust to hold water, regulate its temperature due to its dark colour and availability of nutrients for plant growth. This confirms the report by Ukrfuel (2015) that quality charcoal has the moisture content of around 5 to 15% of the gross weight of charcoal.

Conclusion

The findings of the study showed that NPK and plant tea manure influence significantly the growth rate of *A. cruentus* grown on a mixture of charcoal dust and coffee husks as a growing media. *A. cruentus* reached a height of 26 cm at 5 weeks from the time of planting. This growth rate is even higher than for natural soil conditions. Therefore, the best alternative soilless growing media that gave outstanding performance on the growth attributes of *A. cruentus* was the mixture of charcoal dust and coffee husks in the ratio of 4:1, respectively. It can therefore be used as an alternative growing media for growing *A. cruentus* and plant tea manure as an alternative source of plant nutrients.

RECOMMENDATIONS

Rising tree and vegetable seedlings in nurseries as well as kitchen gardening and urban farming should be carried out using the soilless growing media: a mixture of charcoal dust and coffee husks. Harvesting topsoil is not a sustainable operation that uses up a limited resource that took thousands of years to develop. Therefore, by using the soilless, soil mining, nutrient mining, food miles, soil degradation and environmental pollution could be avoided. Coffee husks as a bio-waste should be put to agricultural use by mixing it with charcoal dust so that it is properly put into useful use. This will also further help to reduce soil degradation and environmental pollution which are of great challenge in soil based agriculture and also proper management and disposal of coffee husks which present major challenges in countries where coffee is produced in large scale.

More research is needed on the different soilless growing media in open field conditions so that the findings could easily be adopted by the people even in local communities who do not have green houses. Furthermore, there is need to carry out research to determine the nutritive profile of *A. cruentus* grown on soilless growing media. Carrying out this would help in establishing the possibility of coming up with a soilless media with best quality growth conditions and *A. cruentus* with higher nutrients (higher quality) which is good for human health. This would further help the current vitamin A deficiency which is one of the major health problems in Uganda and sub-Saharan Africa at large.

Further research should also be carried out to determine the possibility of using charcoal dust as a growing media for other vegetables like tomatoes, egg plants and other African leafy vegetables that are highly nutritious as well as in flower farms to avoid the importation of cocoa peat which is expensive.

There is need to establish the possibility of popularizing amaranth as a leafy and grain vegetable as an alternative vegetable/crop which requires less inputs, easy to establish and manage for income generation in marginal areas in Uganda

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

REFERENCES

Abdi H, Williams L (2017). Tukey's Honestly Significant Difference (HSD) Test. https://www.researchgate.net/publication/237426041_Tukey's_Honestly_Signiflcant_Difierence_HSD_Test

Barrett GE, Alexandera PD, Robinson JS, Bragg NC (2016). Achieving environmentally sustainable growing media for soilless plant cultivation systems – A review. https://doi.org/10.1016/j.scienta.2016.09.030

Bhardwaj RL (2013). Effects of growing media on seed germination and seedling growth of Papaya. Indian Journal Of Agricultural Research. 47(2):163:168.

Both AJ, Benjamin L, Franklin J, Holroyd G, Incoll L, Lefsrud MG, Pitkin G (2015). Guidelines for measuring and reporting environmental parameters for experiments in greenhouses. Plant Methods 11:43.

Boutwell M (2014). How Soilless Agriculture Differs from Soil-based Agriculture. Available from:https://www.maximumyield.com/how-soilless-agriculture-differs-from-soil-based-agriculture/2/1439 [accessed June 2018]

Butler JD, Oebker NF (2006). Hydroponics as a Hobby—Growing Plants Without Soil. Circular 844. Information Office, College of Agriculture, University of Illinois, Urbana, IL 61801.

Carta A, Hanson S, Müller JV (2016). Plant regeneration from seeds responds to phylogenetic relatedness and local adaptation in Mediterranean Romulea (Iridaceae) species. Ecology and Evolution 6(12):4166-4178.

Caron J, Price JS, Rochefort JC (2015). Physical properties of organic soil: adapting mineral soil concepts to horticultural growing media and histosol characterization. Vadose Zone Journal 14:6.

Chawla W, Mehta K (2015).Effect of different growing media on survival and growth of transplanted litchi layers. The Asian Journal of Horticulture 10(2):257-261.

Crush JR, Care DA, Gourdin A, Woodfield DR (2005). Root growth media effects on root morphology and architecture in white clover, New Zealand Journal of Agricultural Research 48(2):255-263.

Depardieu C, Prémont V, Boily C, Caron J (2016). Sawdust and Bark-Based Substrates for Soilless Strawberry Production: Irrigation and Electrical Conductivity Management. PLoS ONE 11(4):e0154104.

Fairhurst T (2012). Handbook for Integrated Soil Fertility Management. Africa Soil Health Consortium, Nairobi.

Fagge AA, Manga AA (2011). Effect of sowing media and Gibberellic acid on the growth and seedling establishment of Bougainvillea glabra, IxoracoccineaandRosa chinensis. 2: Root characters. Bayero Journal of Pure and Applied Sciences 4(2):155-159.

Gomez-Eyles J, Beesley L, Moreno-Jiménez E, Ghosh U, Sizmur T (2013). The potential of biochar amendments to remediate contaminated soils. In: N. Ladygina and F. Rineau, editors, Biochar and Soil Biota. CRC Press, Boca Raton, FL

Haven A (2010). Benefits of Using Manure Tea - Manure Tea. Available From: http://www.manuretea.com/2010/06/benefits-of-using-manure-tea/

Henao J, Baanante C (2006). Agricultural Production and Soil Nutrient Mining in Africa. Implications for Resource Conservation and Policy Development. An International Center for Soil Fertility and Agricultural Development, U.S.A. www.ifdc.org

Ibironke OA, Victor OO (2016) Effect of Media and Growth Hormones on the Rooting of Queen of Philippines (Mussaendaphilippica). Journal of Horticulture and Forestry 3:173. doi:10.4172/2376-0354.1000173

Jyopti GD, Davidson SH (2015). Best Water Saving - Watering Methods - Replacing Your Water Can in Your Garden. Google Books. Available from: https://www.gardeningknowhow.com/edible/vegetables/vgen/water-plants-vegetable-garden.htm

Kasongo RK, Verdoodt A, Kanyankagote P, Baert G, Ranst EV (2011). Coffee waste as an alternative fertilizer with soil improving properties for sandy soils in humid tropical environments. Soil Use and Management 27:94-102.

Koller M, Rayns F, Cubison S, Schmutz U (2016). Guidelines for Experimental Practice in Organic Greenhouse Horticulture. BioGreenhouse COST Action FA 1105, www.biogreenhouse.org. ISBN: 978-94-6257-534-9

Makinde EA, Samuel A, Ojeniyi S (2011). Effects of Organic, Organomineral and NPK Fertilizer Treatments on The Nutrient Uptake of Amaranthus Cruentus (L) on Two Soil Types in Lagos, Nigeria. Journal of Central European Agriculture 12:114-123. 10.5513/JCEA01/12.1.887

Magesa JM, Msogoya T, Rweyemamu CL (2017). Effects of growth media on rooting of stem cuttings of hybrid coffee varieties. African Journal of Agricultural Research 13(2):41-46

Mariana B, Daniel AC, Marian M, Mihai G (2015) Study regarding the influence of NPK fertilizers on the total nitrogen content from tomato (Lycopersicum esculentum). University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Management, 01 N. Titulescu Blvd, Calarasi, Romania.

Marinou E, Chrysargyris A, Tzortzakis N (2013). Use of sawdust, coco soil and pumice in hydroponically grown strawberry, Department of Organic Greenhouse Crops and Floriculture, Technological Educational Institute of Crete, Heraklion, Greece. http://agris.fao.org/agris-search/search.do?recordID=CZ2014000346

Mgbemene CA, Nnaji CC, Nwozor C (2016). Industrialization and its Backlash: Focus on Climate Change and its Consequences – Sci Alert Responsive Version 9(4):301-316.

Mukherjee A, Lal R (2013). Biochar Impacts on Soil Physical Properties and Greenhouse Gas Emissions. Agronomy journal ISSN2073-4395 www.mdpi.com/journal/agronomy

Nalunga J (2014). A guide for trainers, Organic Agriculture-Principles and Practices Kampala: Women in Europe for a common future. http://www.wecf.eu/english/publications/2014/Agriculture-guide.php

Nkonya E, Karsenty A, Msangi S, Souza C, Shah M, Braun J, Galford G, Park S (2012). Sustainable land use for the 21st century.Sustainable Development in the 21st Century (SD21). United Nations Department of Economic and Social Affairs Division for Sustainable Development.

Nguyen AD, Tran TD, Vo TPK (2013) Evaluation of Coffee Husk Compost for Improving Soil Fertility and Sustainable Coffee Production in Rural Central Highland of Vietnam. DOI: 10.5923/j.re.20130304.03

Olubanjo O, Alade A (2018). Growth and Yield Response of Tomato Plants Grown Under Different Substrates Culture. Available from: https://www.researchgate.net/publication/330729395_Growth_and_Yield_Response_of_Tomato_Plants_Grown_Under_Different_Substrates_Culture

O'Connor AM, Totton SC, Cullen JN, Ramezani M, Kalivarapu V, Yuan C, Gilbert SB (2018). The study design elements employed by researchers in preclinical animal experiments from two research domains and implications for automation of systematic reviews. PLoS One 13(6):e0199441

Okese KF (2016). Essential Plant Nutrients, their Functions and Fertilizer Sources, Available at http://agrihomegh.com/essential-plant-nutrients/

Olabode OS, Sola O, Akanbi WB, Adesina GO, Babajide PA (2007). Evaluation of *Tithonia diversifolia* (Hemsl.) A Gray for soil improvement. World Journal of Agricultural Sciences 3(4):503-507.

Olosunde OM, Olasantan FO, Olubode O (2009). Effect of Growth Media on Rooting of Queen of the Philippine (Mussaendaphilippica A. Rich). Nigerian Journal of Horticultural Science DOi 10.4314/njhs.v13i1.46571

Olympios CM (1999). Overview of soilless culture: Advantages, constraints and perspectives for its use in Mediterranean countries. Available from: https://www.researchgate.net/publication/267836634_Overview_of_soilless_culture_Advantages_constraints_and_perspectives_for_its_use_in_Mediterranean_countries [accessed June 08 2018].

Partey ST (2010). The Agronomic Qualities of the Mexican Sunflower (*Tithonia diversifolia*) For Soil Fertility Improvement In Ghana. A Thesis submitted to the Department of Agroforestry, Kwame Nkrumah University of Science and Technology. http://ir.knust.edu.gh/bitstream/123456789/7196/1/THE%20AGRONOMIC%20QUALITIES%20OF%20THE%20MEXICAN%20SUNFLOWER.pdf

Rabani EM (2018). The effects of drought and growth media on Lentil growth characteristics. University of Saskatchewan, Saskatoon Canada. https://core.ac.uk/download/pdf/153546287.pdf

Sardoei AS, Shahdadneghad M (2015). Effect of Different Growing Media on the Growth and Development of Zinnia (*Zinnia elegans*) under the Agro-Climatic Condition of Jiroft. Research Journal of Environmental Sciences 9(6):302-306.

Sengupta A, Banerjee H (2012). Soil-less culture in modern agriculture. pp. 103-108. https://www.researchgate.net/publication/281645564_Soil-less_culture_in_modern_agriculture

Soga DD (2010). Effects Of Five Soilless Media And Methods Of Propagation On Rooting And Growth Of Three Difficult-To-Root Ornamental Plants. Kumasi: Kwame Nkrumah University. http://dspace.knust.edu.gh/handle/123456789/402?mode=full

Tran HM (2005). Quantif ying the effects of sawdust application on soil chemical and physical properties and corn yield. Knoxville: Tennessee Research Creative Exchange https://trace.tennessee.edu/cgi/viewcontent.cgi?referer=https://www.google.com/&httpsredir=1&article=3801&context=utk_gradthes

Ukrfuel (2015). Chemical properties of charcoal.Agriwood pellets solid fuel. http://ukrfuel.com/news-chemical-properties-of-charcoal-23.html Thu-26-2015.

Viljoen A, Bhon K (2014). Second Nature Urban Agriculture: Designing productive cities. Available from: https://books.google.com/books? ISBN = 1317674510

Wilkinson KM, Landis TD, Haase DL, Daley BF, Dumroese RK (2014). Tropical Nursery Manual: A guide to starting and operating a nursery for native and traditional plants. Agriculture Handbook 732. Washington, DC: U.S. Department of Agriculture, Forest Service 376 p.

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Drought tolerance assessment of melon germplasm searching for adaptation to climate change

Haitham M. A. Elsayed[1], Rosa M. Peiró[2], Belén Picó[2] and Cristina Esteras[2]*

[1]Genetics Department, Faculty of Agriculture, Sohag University, Sohag, Egypt.
[2]Institute for the Conservation and Breeding of Agricultural Biodiversity (COMAV-UPV),
Universitat Politècnica de València, Camino de Vera s/n, 46022, Valencia, Spain.

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Shortage of irrigation water at critical melon growth stages can be the most important limiting factor in the future due to climate change, especially in the Mediterranean region. Apart from the improvement of irrigation systems and crop management, the development of drought tolerant cultivars by genetic breeding is the best solution to achieve stable yields. Screening germplasm collections is a prerequisite for that. A melon core collection was evaluated in the current work in two assays. Seven morphological traits were assessed at plantlet stage and compared under drought and standard conditions imposed. Significant differences for all traits were recorded among the sixty accessions evaluated. Clustering analysis also grouped the accessions according to their response to drought, detecting some landraces and wild types of interest, mainly of Indian and African origin, although the best behavior under drought was found in a *flexuosus* melon from Irak. Some Spanish *inodorus* landraces also showed better response than the average behavior of commercial types. The employment of this set of traits has allowed screening a large germplasm collection in an easy and non-expensive way, in one of the most sensitive developmental stages.

Key words: *Cucumis melo* germplasm, morphological seedling traits, abiotic stress, response to drought, variability

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INTRODUCTION

Melon (*Cucumis melo* L.; 2n=2x= 24), which belongs to Cucurbitaceae family, is one of the most important fruit crops worldwide. Approximately about 31 million tonnes of melons were produced worldwide with more than 1.2 million ha harvested in 2016 (FAOSTAT, 2018). In addition to China, which is the main producer country with nearly 16 million tonnes, Egypt and Spain are also important producers, ranking 4 and 8th position, with about 1 million and 660,000 tonnes produced in 2016, respectively (FAOSTAT, 2018). In fact, in 2016 Spain was the second exporter in the world after Guatemala.

Therefore, due to its economic relevance, the development of new melon cultivars adapted to different biotic and abiotic stresses and with high quality standards is required by global markets. This includes the tolerance or resistance to drought and salinity, as climate change is likely to affect many croplands, particularly in the Mediterranean region where is predicted an important increase of arid areas (Turral et al., 2011). The incidence of this stress and the resulting losses in yield for many

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*Corresponding author. E-mail: criesgo@upvnet.upv.es. Tel: +34 96 387 94 17.

crops including melon, are a major threat to economic and social stability in many societies, since developing countries probably will suffer the consequences more drastically. In addition, weed invasion can aggravate this problem in croplands with no weed management, since competition for water, light and nutrients takes place, also affecting seed germination (Yigit et al., 2016). Several studies have focused on the determination of optimum irrigation requirements in melon, as water scarcity is a growing problem nowadays in many melon-producer regions (Kusvuran et al., 2011; Lima et al., 2017). Negative effects of excessive watering in melon have been reported such as an increase in the presence of rotten fruits, flesh vitrescence or flesh sweetness loss (Sensoy et al., 2007). However, as previously said, water deficit can seriously affect yield (Sensoy et al., 2007; Sharma et al., 2016), fruit size (Fabeiro et al., 2002; Long et al., 2006), and can cause an important reduction in biomass finally leading to plant death (Kusvuran, 2010). This reduction in growth under drought and salinity is consequence of several physiological responses including modifications of ion balance, mineral nutrition and photosynthetic efficiency. The rate of photosynthetic CO_2 assimilation is reduced and generally implies oxidative stress. Additionally, metabolic disturbances and fruit quality effects due to salt stress have been described in melon, whose fruits usually become soft, wrinkled and turn brown, displaying premature ripening. In contrast, activation of secondary metabolism with positive effects on fruit quality (antioxidant compounds, aroma) also has been reported in several species in response to deficit of water (Ripoll et al., 2014).

Although plants can be affected by drought at any time of their life, presenting unique challenges to growth and productivity, one of the most critical stages are during germination and seedling growth (early-season drought). In fact, recent studies such as the one by Yigit et al. (2016) and Sevik and Cetin (2015) carried out with landscape species, have focused on the germination rate, reporting significant falls under water stress. In addition, early drought stress experienced during the seedling stage, when plants are very sensitive to this deficit, provokes significant inhibition of growth and developmental delay (Blum, 1996).

Drought tolerance is a function of several morphological traits such as reduced leaf area or stomatal density, physiological aspects such as low transpiration rate or cell membrane fluidity (Cetin et al., 2018); and biochemical composition such as proline or trehalose accumulation, which are effective indices for screening in breeding programs (Ashraf and Foolad, 2007; Cha-um and Kirdmanee, 2009). Regarding biochemical indices, studies by Dasgan et al. (2009) and Kusvuran et al. (2013) suggested citrulline as a good indicator of tolerance to salinity and drought as they reported higher accumulation in tolerant melon accessions in comparison to sensitive ones.

Characterization of these drought-tolerance-related traits under water stress conditions have been assessed in several species, including seedling traits like shoot and root weight and lengths, root/shoot ratio and coleoptiles length at seedling stage (Taiz and Zeiger, 2006; Zhang et al., 2011; Zafar and Azhar, 2015; Akinwale et al., 2017).

A few candidate genes for drought tolerance in crops have been characterized to date (Ripoll et al., 2014), and metabolic pathways and differential gene expression related to this stress are still not well understood. Recently, in the model plant *Arabidopsis*, Fàbregas et al. (2018) have overexpressed BRL3, a brassinosteroid receptor, conferring drought tolerance without affecting plant growth, and in melon, Altunoglu et al. (2017) identified several LEA genes which were up-regulated under drought conditions. Nevertheless, routine screening for drought tolerance is nowadays carried out by phenotyping the response under deficiency and not by the analysis of particular drought-related genes.

C. melo is a very polymorphic species that exhibits high levels of diversity regarding morphological, physiological and biochemical aspects, including tolerance to different abiotic and biotic stresses (Esteras et al., 2013; Pitrat, 2017). This species is divided into two subspecies, subsp. *melo* and subsp. *agrestis* (Kirkbride, 1993). Although in the last classification, Pitrat (2017) reported 19 horticultural groups, a simplified version adapted from Pitrat (2008) is usually adopted: *inodorus*, *cantalupensis-reticulatus*, *adana*, *chandalak*, *ameri*, *chate*, *flexuosus*, and *dudaim* (in subsp. *melo*), and *momordica*, *conomon*, *chinensis*, *makuwa*, *acidulus*, *tibish* and wild *agrestis* (in subsp. *agrestis*). The extant diversity in the species for drought tolerance is nowadays underexploited, as few studies have been carried out searching for drought-tolerant accessions (Kusvuran et al., 2013; Pandey et al., 2013, 2018; Ozer et al., 2015; Sharma et al., 2016; Leskovar et al., 2017).

In this context, we present the characterization of a melon germplasm collection representing most of the variability of the species, with several morphological traits easily-measured at seedling stage with the aim to search for new genotypes most adapted to the increasing lack of water. The finding of these resources may provide valuable information about potential crosses in future breeding programs to develop drought-tolerant commercial varieties. Also, the most tolerant landraces reported may be used to develop varieties better adapted to local farming systems in developing countries.

MATERIALS AND METHODS

A set of 60 melon accessions representing the huge diversity of the species and including melon varieties, from diverse origins, maintained at the COMAV's (Institute for the Conservation and Breeding of Agricultural Biodiversity) core collection at Polytechnic University of Valencia (UPV, Spain) (Esteras et al., 2013; Leida et al., 2015), and some wild accessions from India held at Germplasm Resources Information Network-National Plant Germplasm System, USDA (GRIN-NPGS) (Table 1), were analyzed for their response to early-drought in two assays.

Table 1. Accessions included in the study.

Genotype code[1]	Accession name[2]	Reference	Origin	subsp.	Variety/Hort. group	Assay
Ac-TGR1551Zimb	TGR1551	Leida et al. (2015)	Zimbabwe	*agrestis*	acidulus	1
Ac-TGR1843Zimb	PI 482429	Leida et al. (2015)	Zimbabwe	*agrestis*	acidulus	2
Ag-15591Gha	PI 185111	Leida et al. (2015)	Ghana	*agrestis*	wild melon	1
Ag-C38Nig	CO38 (CUM 287)	Leida et al. (2015)	Nigeria	*agrestis*	wild melon	1
Ag-CallInd	Callosus	Leida et al. (2015)	India	*agrestis*	wild melon	2
Ag-CallosusInd	PI 435284	Endl et al. (2018)	India	*agrestis*	wild melon	2
Ag-ChibbarInd	PI 532839	-	India	*agrestis*	wild melon	2
Ag-Cuba	Cuba	Leida et al. (2015)	Cuba	*agrestis*	wild melon	2
Ag-FadSud	Fadasi	Leida et al. (2015)	Sudan	*agrestis*	wild melon	2
Ag-HumSud	Humaid	Leida et al. (2015)	Sudan	*agrestis*	wild melon	2
Ag-KSM428Ind	PI 614465	-	India	*agrestis*	wild melon	2
Ag-KSM528Ind	PI 614518	-	India	*agrestis*	wild melon	2
Ag-KSM531Ind	PI 614521	-	India	*agrestis*	wild melon	2
Ag-MelCol	Meloncillo	Leida et al. (2015)	Colombia	*agrestis*	wild melon	1
Ag-SM2Ind_B	PI 381782 B	-	India	*agrestis*	wild melon	2
Ag-SM2Ind_A	PI 381782 A	-	India	*agrestis*	wild melon	2
Ag-TendSud	Tendelti	Leida et al. (2015)	Sudan	*agrestis*	wild melon	2
Ag-USM170Ind	PI 614307	-	India	*agrestis*	wild melon	2
Ag-VelliInd	PI 164320	Leida et al. (2015)	India	*agrestis*	wild melon	2
Ag-WChInd	Wild Chibbar	Leida et al. (2015)	India	*agrestis*	wild melon	2
Am-3584Afg	PI 125951	Leida et al. (2015)	Afghanistan	*melo*	ameri	1
Am-AfrMor	Afr-c-1	Leida et al. (2015)	Morocco	*melo*	ameri	2
Am-ApelRus	Apelsinaja	Leida et al. (2015)	Russia	*melo*	ameri	1
Am-ChandAfg	Chandalack (PI 276660)	Leida et al. (2015)	Afghanistan	*melo*	ameri	1
Am-GalaTun	Galaoui	Leida et al. (2015)	Tunisia	*melo*	ameri	2
Am-HassanTur	Hassanbey (PI 169368)	Leida et al. (2015)	Turkey	*melo*	ameri	1
Am-KafEgy	Kafr Hakim (PI 288233)	Leida et al. (2015)	Egypt	*melo*	ameri	2
Am-KizilUzbe	Kizil-uruk	Esteras et al. (2013)	Uzbekistan	*melo*	ameri	1
Am-KokUzb	Kokcha (ASI-C-5)	Leida et al. (2015)	Uzbekistan	*melo*	ameri	1
Am-NanaGeorg	Nanatri	Leida et al. (2015)	Georgia	*melo*	ameri	1, 2
Am-NesviGeor	Mucha Nesvi	Leida et al. (2015)	Georgia	*melo*	ameri	2
Am-OuzUzb	Ouzbeque	Leida et al. (2015)	Uzbekistan	*melo*	ameri	2
Am-U1715Br	CUM502		Brazil	*agrestis*	ameri	2
Can-NYIsr	Noy Israel	Leida et al. (2015)	Israel	*melo*	cantalupensis	1
Can-U1716Br	Casca de Carvalho		Brazil	*agrestis*	cantalupensis	2
Can-VedFran	Vedrantais	Leida et al. (2015)	France	*melo*	cantalupensis	1

Table 1. Contd.

Con-Co6Chi	Makuwa	Leida et al. (2015)	China	*agrestis*	conomon-makuwa-chinensis	1
Con-Pat81Ko	Pat 81	Leida et al. (2015)	Korea	*agrestis*	conomon-makuwa-chinensis	1
Con-SCKo	Songwhan Charmi (PI 161375)	Leida et al. (2015)	Korea	*agrestis*	conomon-makuwa-chinensis	2
Con-ShiroJa	Shiro Uri Okayama	Leida et al. (2015)	Japan	*agrestis*	conomon-makuwa-chinensis	1
Dud-254Afg	CUM 254	Nunes et al. (2017)	Afganistan	*melo*	dudaim	1
Dud-QPMAfg	Queen's pocket melon	Leida et al. (2015)	Afganistan	*melo*	dudaim	1
Flex-AlficozSp	Alficoz	Leida et al. (2015)	Spain	*melo*	flexuosus	1
Flex-AryaInd	Arya	Leida et al. (2015)	India	*melo*	flexuosus	1
Flex-KhiIrak	Khiar	Leida et al. (2015)	Irak	*melo*	flexuosus	1
In-AmCañSp	Caña Dulce	Leida et al. (2015)	Spain	*melo*	inodorus	1
In-BBescrSp	Blanco Escrito	Leida et al. (2015)	Spain	*melo*	inodorus	1
In-BTempSp	Blanco Tempranillo	Leida et al. (2015)	Spain	*melo*	inodorus	1
In-MaazTun	Maazoon	Leida et al. (2015)	Tunisia	*melo*	inodorus	2
In-PsPiñSp	Piel de sapo Piñonet	Leida et al. (2015)	Spain	*melo*	inodorus	1
In-RoMoch1Sp	Mochuelo	Leida et al. (2015)	Spain	*melo*	inodorus	1
In-StutzUSA	CUM 468, Stutz Supreme	Nunes et al. (2017)	USA	*melo*	inodorus	1
In-TeLVillSp	Largo de Villaconejos	Leida et al. (2015)	Spain	*melo*	inodorus	1
In-TeMollSp	Mollerusa	Leida et al. (2015)	Spain	*melo*	inodorus	1
La-Bol	Bol-84	Leida et al. (2015)	Bolivia	*melo*	indeterminate landrace	1
La-VoaMad	Voatango	Leida et al. (2015)	Madagascar	*agrestis*	indeterminate landrace	1
Mom-KhaInd	Kharbuja	Leida et al. (2015)	India	*agrestis*	momordica	1
Mom-PI124Ind	PI124112	Leida et al. (2015)	India	*agrestis*	momordica	1
	F1_PSxDud				hybrid Inodorus x dudaim	1
	F1_PSxCon				hybrid Inodorus x conomon	2

[1]Some codes employed in previous studies. [2]PI and CUM accessions were kindly provided by NPGS-USDA and IPK genebanks respectively.

Two assays were performed under greenhouse conditions in Valencia (UPV facilities) with the following conditions: average air temperature of 27.8°C and average humidity 61.5%. The first assay was conducted from June to the end of July, while the second assay was conducted from August to the end of September. Seeds were germinated in a pre-germination chamber for 24 h at a temperature 37°C. Afterwards, the uniform-sized seedlings were transferred into plastic pots (one seedling/pot, 55 ×45 cm) with commercial substrate (Huminsubstrat N3®) at the cotyledon stage. A triplicate complete randomized block design (RCBD) was used. The plantlets grew under the same conditions until the three-leaf stage. Subsequently, plants were divided into two groups, and different conditions were applied: drought and standard conditions. For drought condition, the water deficit was achieved by watering the plants with a decrease of 50% of water with respect to standard irrigation (control). The application of drought was accomplished and monitored using ECH$_2$O EC-5 capacitance sensors connected to an Em50 data logger using the ECH$_2$O Utility software (Decagon Devices Inc., Pullman WA., USA). When the humidity degree reached 15%, plants were approximately 45 days under this condition and were phenotyped. Plants (5 to 10) were evaluated per accession and condition. The seven morphological traits selected as indicators of drought tolerance at plantlet stage were: first leaf curled score, second leaf curled score, length (cm), fresh weight (g), dry weight (g), number of green leaves, and number of brown leaves. High percentage of curled leaf area is indicative of high susceptibility to drought. The leaf score employed was a 1-5 scale, where 0 is 0%, 1 is 1-5%, 2 is 5-10%, 3 is 10-15%, 4 is 15-20%, and 5 is more than 20% curled in leaf. A decrease in fresh and dry weight, and in the number of green leaves are also traits used as indicators of a decrease in total biomass in the response to drought.

A mixed model was used to analyze the data of each assay. Drought conditions (two levels; drought and standard conditions), genotype and its interaction were included as fixed effect whereas the plant was included as random effect. Significant differences were estimated by least square difference (LSD) method 95%. Correlations between traits were also estimated separately by each drought condition and assay. SAS/STAT 12.1 was used to perform all the analysis.

New parameters were calculated to measure the differences in the traits assessed with respect to control conditions in each genotype. For vine length, fresh weight, dry weight and number of green leaves, the value for control conditions were assumed to be 100% and the decrease percentage was subtracted to obtain a new parameter. For first leaf score, second leaf score and number of brown leaves the difference was calculated directly subtracting control value from drought value. A clustering analysis for each assay was also conducted with these new parameters to determine groupings of accessions with similar responses. The hierarchical dendrogram was performed with JMP v.5.1 using Ward method.

RESULTS AND DISCUSSION

Selection of drought tolerant plant species has been considered to be an economic and efficient means of alleviating agricultural problems especially in dry areas. To achieve this goal, a set of reliable drought-related traits of rapid and relatively inexpensive screening was used to assess a melon core collection which displays high levels of variability.

After the descriptive study of the dataset, a few outlier values were discarded in both assays. The analysis of the seven traits for the two subsets corresponding to both assays, at the beginning (assay 1) and at the end of summer (assay 2), were done separately, as only one accession was characterized in both assays. General data for these two subsets of accessions evaluated in drought and standard conditions are presented in Table 2.

Drought, genotype effect and its interaction were observed for all traits at 95% with the exception of the trait first leaf score which was detected at 90% in the assay performed at the beginning of summer (figures for each trait and genotype in both conditions are shown in Supplementary File 1). In addition, the drought effect for each subset of accessions for each trait is as shown in Figure 1. The effects of water availability on the traits were found significant in both assays. As expected, effects on leaves (first leaf score, second leaf score) increased with application of drought, having scores around 0 in standard conditions and over 2 (assay 1) or between 1 and 2 (assay 2) when drought was applied. The number of brown leaves also increased under drought conditions in both assays. Regarding the remaining traits, their values decreased with drought as fresh weight, dry weight, green leaves and vine length are traits directly related to biomass production. This effect was more evident in the conditions of assay 2, maybe due to the slightly increase in the temperatures in the second assay. Fresh weight was the best indicator of drought damages in both assays, with a decrease of 55.9 and 68.6% with respect to standard conditions in assays 1 and 2, respectively (Figure 2). Dry weight also suffered an important decrease of 51.9 and 65.0%, respectively, while number of green leaves (45.8 and 57.1% for assays 1 and 2, respectively) and vine length (31.2 and 41.5%, respectively) presented much moderate loss. Previous studies about salinity and drought effects on melon genotypes reported that shoot growth differed significantly among the tolerant and sensitive melon genotypes (Kusvuran, 2010; Kusvuran et al., 2011), which is in agreement with our results.

Significant differences were observed among the assayed accessions for seedling traits. As expected, any accession showed higher fresh weight under drought conditions than standard (or no drought) conditions, except for the accession Flex-KhiIrak which behaved better in this condition (Figure 2). Other accessions like Ag-C38Nig, Am-3584Afg, In-BTempSp, In-RoMoch1Sp and La-VoaMad showed similar values in both conditions (Figure 2). These results suggest that these accessions are adapted to semi-arid climates, since for example, Flex-KhiIrak, Am-3584Afg, and In-RoMoch1Sp, are grown in regions of Irak, Afganistan, and Spain, respectively, where precipitations are generally scarce. This group of accessions included a wild type and also landraces, not only from African and Asian origin, but also Spanish ones which are closer to the commercial types. These accessions, therefore, are described as not affected by drought, or even positively affected in the case of Flex-KhiIrak. La-VoaMad, together with Am-KizilUzbe, Am-KokUzb, Can-VedFran, Flex-AryaInd (assay1) and Am-NesviGeor, hybrid F1_PSxCon and Am-OuzUzb (assay 2) presented the highest fresh weight under drought conditions (Figure 2). These results under limited water conditions which inhibit plant growth suggested a sort of tolerance to drought in these high-weighed genotypes, revealing landraces such as La-VoaMad as potential genotypes for breeding. In addition, some of them also displayed one of the highest fresh values under standard conditions (Am-OuzUzb), showing the vegetative growing potential of this accession in both conditions. However, future characterization of the fruit quality will be necessary to use them in breeding programs since effects on organoleptic traits and fruit size have not been assessed in this first approach.

The correlation values (P-value < 0.01) different from zero are presented in Table 3 for each assay and condition. Similar correlations between traits were obtained in each assay. When drought conditions were evaluated, positive correlations among vine length, fresh weight and dry weight were observed in both assays. In fact, Flex-KhiIrak, In-BTempSp, In-RoMoch1Sp, La-VoaMad, Ag-C38Nig, and Am-3584Afg, accessions previously mentioned with no drought effect in fresh weight (or a positive effect in Flex-KhiIrak), presented also no differences in vine length as well as the Indian Flex-AryaInd. In both assays, positive correlation was also observed between first leaf score and second

Table 2. Mean raw effects of drought (1) and standard (2) conditions on the seedling traits analyzed in the two assays.

Assays	First leaf score		Second leaf score		Vine length		Fresh weight		Dry weight		Green leaves		Brown leaves	
	D[1]	S[2]	D	S	D	S	D	S	D	S	D	S	D	S
1	2.25	0.21	3.16	0.36	53.55	77.79	4.78	10.84	0.64	1.33	4.48	8.27	1.71	0.94
2	0.96	0.15	2.18	0.30	71.44	122.06	3.76	11.93	0.56	1.60	6.46	15.05	3.00	1.34

leaf score (Table 3). Flex-KhiIrak, La-VoaMad, Ag-VelliInd and Ag-ChibbarInd were the only accessions displaying 0% of curled leaf area (first and second leaf score) in both conditions (Supplementary file 1), suggesting again the tolerance to drought of these landraces. In addition, these two traits showed negative correlation with vine length and the number of green and brown leaves. When standard conditions were evaluated, most of the positive correlations observed in drought conditions were achieved. In addition, negative correlation between fresh weight and brown leaves were obtained.

Cluster analysis was carried out in both assays and several groups of accessions were detected according to the different response to early-drought (Figure 3). In assay 1, some accessions showed lower to medium-level of damages (red, Figure 3A) displaying lower losses of weight and length and less curling in leaves. This cluster included subsp. *agrestis* types like *momordica*, *acidulus*, *conomon* and wild types, and also some intermediate types between both subspecies like *ameri* or *flexuosus*. They included types mentioned previously like the African Ag-C38Nig or the Asian Am-KizilUzbe, Am-KokUzb, and Am-3584Afg. Flexuosus melons like the Indian landrace Arya (Flex-AryaInd), also in this cluster, have been previously reported to have good adaptability to drought (Ahlawat et al., 2018). In the present work, this accession showed a good behavior although it did not group with accessions with the best response (blue cluster). This blue cluster (Flex-KhiIrak, La-VoaMad, In-BTempSp, In-RoMoch1Sp) included accessions which showed no or small difference under drought in comparison to standard conditions for weight and vine length (Figure 3A). The other two clusters (green, orange; Figure 3A) presented more severe damages and included mainly subsp. *melo* accessions (*inodorus*, *cantalupensis*, *ameri*) with the exception of two *conomon* types. The commercial Charentais and Piel de Sapo types (Can-VedFran, In-PSPiñSp), especially important in Western countries, were included in this group (green cluster), whereas Ag-MelCol and Dud-QPMAfg (orange cluster) were the ones with the higher level of brown leaves in response to drought.

Regarding assay 2, three clusters were observed, one corresponding to accessions with more severe losses in weight and length with respect to the remaining accessions assessed in this assay although a low level of curling in leaves (red, Figure 3B), a second one with intermediate accessions regarding the response to drought (blue, Figure 3B) which mainly included *ameri* types like Am-OuzUzb, and a third one with a lower level of damages (green, Figure 3B). Although most of the Indian *agrestis* accessions were among the most affected accessions (red cluster) together with the two Brazilian landraces assayed, two of them (Ag-KSM428Ind, Ag-KSM528Ind) can be highlighted due to their good response (green cluster) as well as the African wild *agrestis* Ag-HumSud and the Egypcian Am-KafEgy. India has several agro-ecological regions and therefore, maintains huge diversity in melon (Fergany et al., 2011; Roy et al., 2012), which explains the diverse response found in this work. The accessions with more damages on leaves were Am-NanaGeorg, Ag-MelCol and Dud-QPMAfg (assay1), and In-MaazTun and Am-GalaTun from Tunisia (assay 2) (Figure 3). In general, African and Indian accessions showed better results for these traits (La-VoaMad, Ac-TGR1551Zimb and Mon-PI124Ind in assay 1 and Ag-WChInd, Ag-VelliInd, Ag-ChibbarInd and Ag-USM170Ind in assay 2) (Figure 3).

The results of the evaluation of this large melon collection suggest that African and Asian continents retain an important genetic variability which should be further studied, as well as Spanish landraces which seems to be underexploited for drought-adaptation traits. In fact, several accessions were detected with better behavior than typical international commercial types belonging to *inodorus* and *cantalupensis* groups. Traditionally, exotic types belonging to *momordica* and *acidulus* groups from India have been used as sources of resistance genes to biotic stresses, but few studies have evaluated

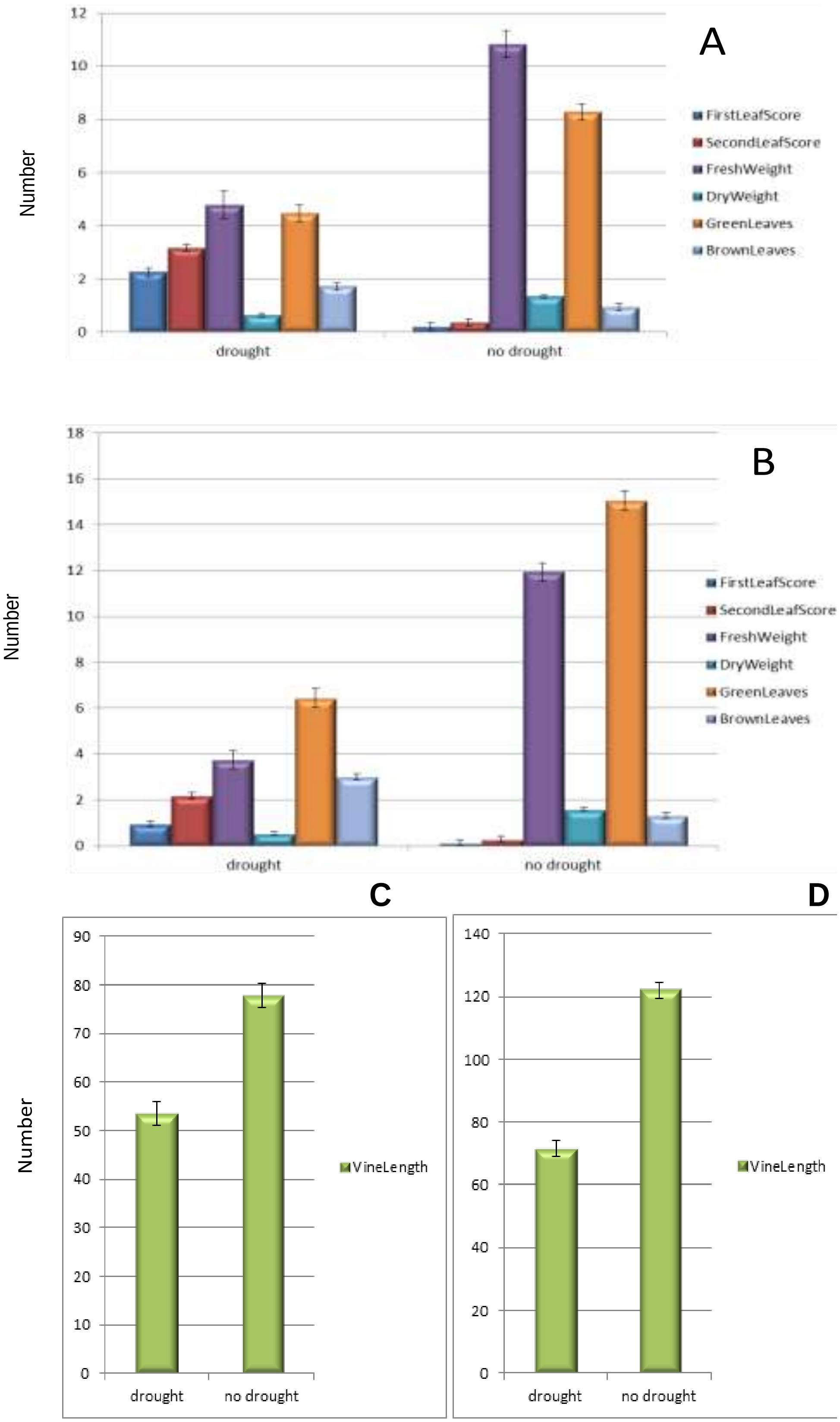
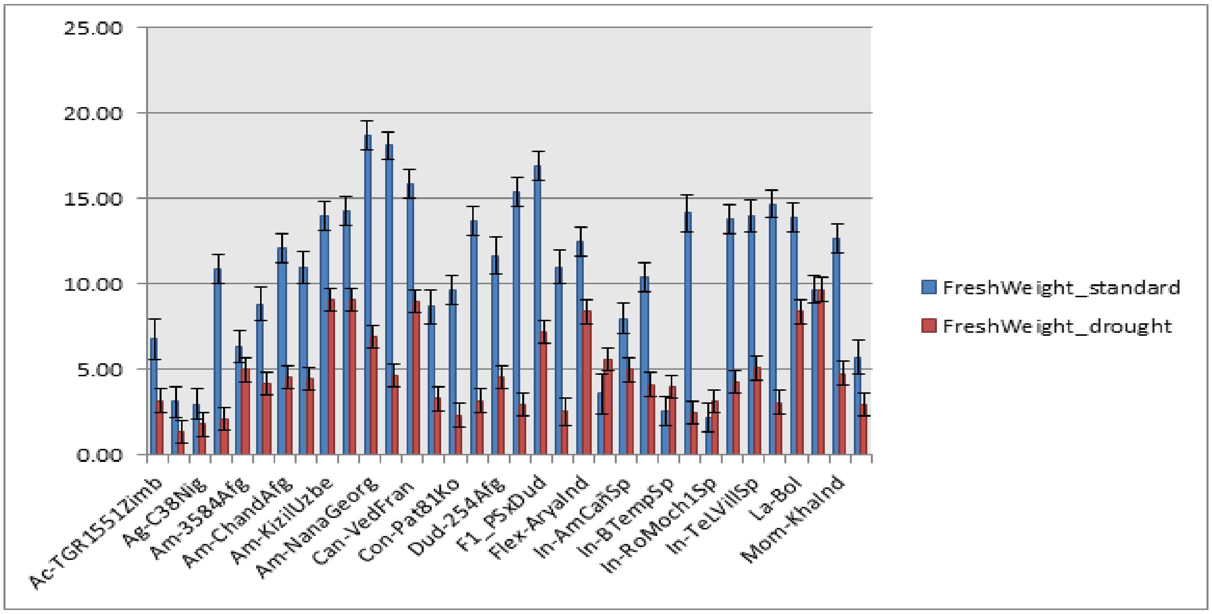


Figure 1. Effect of drought on the traits assessed in the germplasm collection. Means and standard error represented for first leaf score, second leaf score, fresh weight, dry weight, number of green leaves, and number of brown leaves evaluated in assay 1 (A) and 2 (B), and vine length evaluated in assay 1 (C) and 2 (D).

A: Assay1



B: Assay2

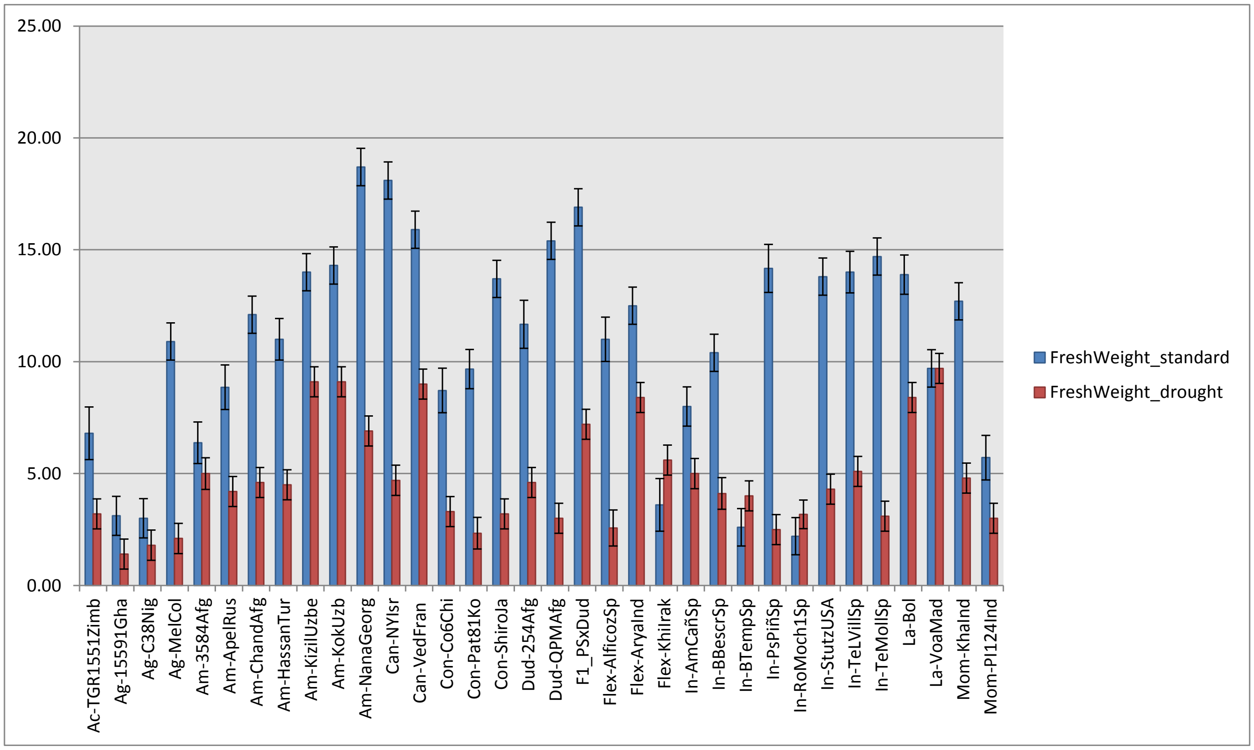


Figure 2. Mean and standard error for fresh weight for every genotype in both conditions (standard and drought). A assay1, B assay2.

drought tolerance. In fact, to date few studies have focused on the screening for drought tolerance of large melon germplasm collections (Ozer et al., 2015; Sharma et al., 2016; Leskovar et al., 2017; Pandey et al.,

Table 3. Correlations among traits evaluated under drought and standard conditions in two assays (assay 1 performed at the beginning of the summer and assay 2 at the end of the summer).

Assay 1	Drought conditions						Standard conditions					
	Second leaf score	**Vine length**	**Fresh weight**	**Dry weight**	**Green leaves**	**Brown leaves**	**Second leaf score**	**Vine length**	**Fresh weight**	**Dry weight**	**Green leaves**	**Brown leaves**
First leaf score	0.83	-0.38	-	-	-0.57	-0.36	0.73	-	-	-	-	-
Second leaf score		-0.31	-	-	-0.56	-0.30		-	-	-	-	-
Vine length			0.61	0.69	0.56	-			0.63	0.65	0.74	-
Fresh weight				0.85	0.31	-				0.89	0.36	-0.40
Dry weight					0.44	-					0.43	-
Green leaves						-						-

Assay 2	Drought conditions						No drought conditions					
	Second leaf score	**Vine length**	**Fresh weight**	**Dry weight**	**Green leaves**	**Brown leaves**	**Second leaf score**	**Vine length**	**Fresh weight**	**Dry weight**	**Green leaves**	**Brown leaves**
First leaf score	0.70	-0.29	-	-	-0.32	-0.41	0.65	-	-	-	-	0.36
Second leaf score		-0.25	-	-	-0.38	-0.42		-	-	-	-	-0.46
Vine length			0.55	0.52	0.56	-			0.21	0.33	0.55	-
Fresh weight				0.90	0.36	-				0.59	-	-0.34
Dry weight					0.41	-					-	-
Green leaves						-						-

-: Non significant correlations (P value >0.01).

2018) and most of them only include *cantalupensis* and *inodorus* types. Regarding exotic types, some accessions like the Turkish Kav-248 have been described as drought-tolerant (Ozer et al., 2015; Torun et al., 2018). Also, some Kachri melons from India, described as semi-domesticated large *agrestis* probably evolved from Wild Chibber and *momordica* melons, have been reported to show drought tolerance (Pareek and Samadia, 2002; Roy et al., 2012; Pitrat, 2017), since they can be grown in semi-desert areas. The present results are coherent with this, as the two Wild Chibbar types tested herein (Ag-WChInd, Ag-ChibbarInd) showed a good behavior (green cluster, Figure 3). Moreover, some other Indian accessions presented a notable good response under this stress, confirming again the importance of Indian germplasm in the genetic breeding of this crop. However, the most adapted melon to drought conditions was a *flexuosus* type from Irak (Flex-KhiIrak), reinforcing Near East as an important area to search for germplasm to use in breeding.

Since the screening of a core collection is the first step for breeding for adaptation to the new scenario with more severe and frequent periods of drought due to climate change (Turral et al., 2011), the results presented in this work can be very useful. As a conclusion, the accessions presented in this work as more tolerant to water scarcity might play a significant role for the incorporation of drought-tolerance genes in landraces to improve production in local-farming systems and under ecological farming, or even in commercial types. Traditionally, the development of such cultivars has been hampered by the complex nature of drought adaptation, genotype × environment interactions and the difficulty of having an effective drought screening method (Verulkar et al., 2010). However, the parameters measured in this work have allowed a first rapid and low-cost screening to select genotypes with

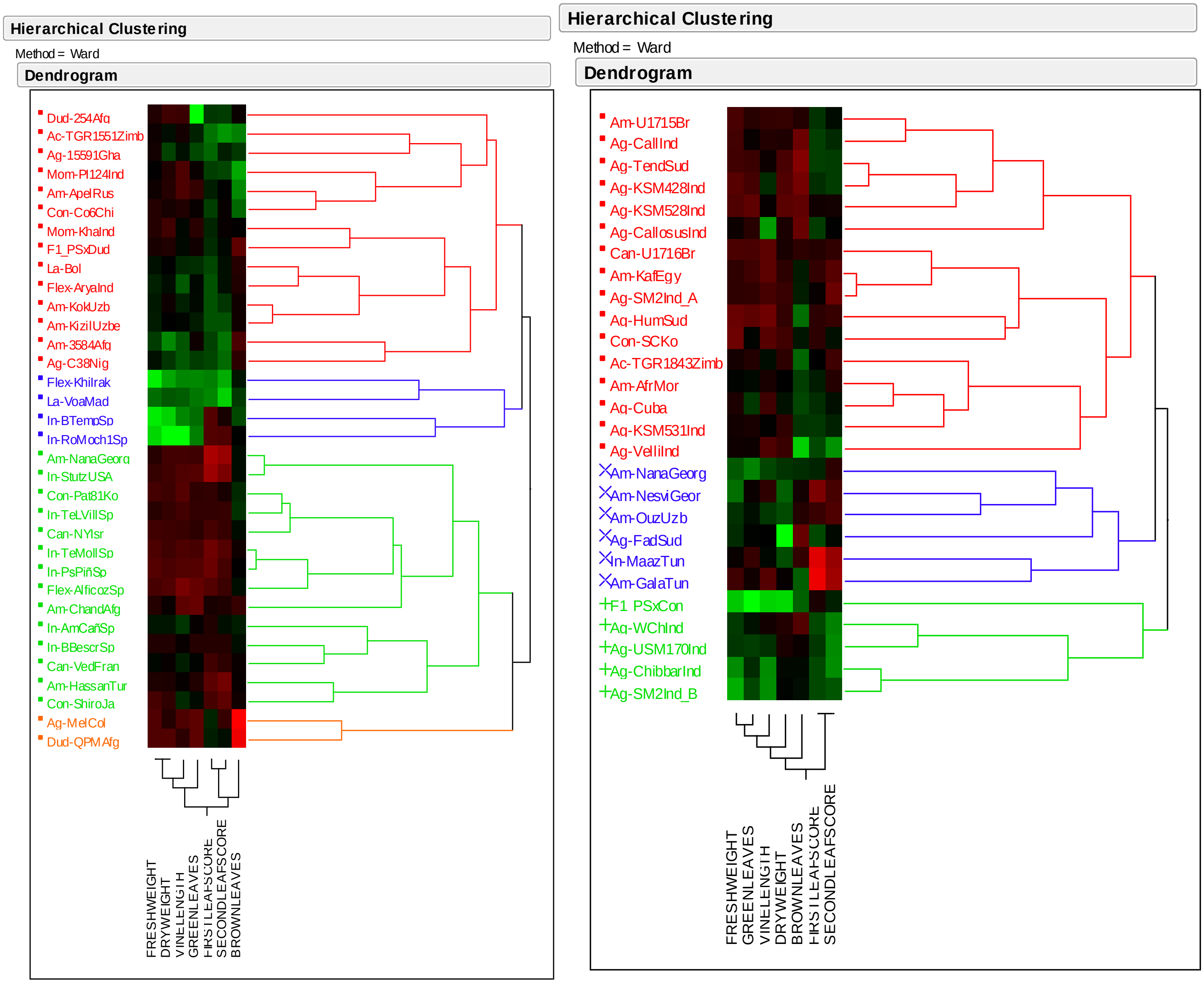


Figure 3. Dendrogram for assay 1 (A) and assay 2 (B) showing several groups based on the drought response of the 60 accessions screened. The new parameters calculated for each trait were used. Hierarchical clustering was performed using JMP V.5.1 and Ward method.

the best behavior in early stages of plant growth in order to further assess fruit yield and quality in future assays. Therefore, herein we report, not only some genotypes of potential interest for melon breeding such as some landraces and wild types from India and Africa as well as a *flexuosus* type from Irak, but also a useful and straightforward set of traits for early-drought tolerance screening.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Ahlawat SP, Pandey A, Malav PK, Bhardwaj R, Dhariwal OP (2018). A less-known vegetable melon landrace ‘‘Arya’’ (Cucumis melo L.) from

Rajasthan and Haryana, India: morphological, biochemical and taxonomic study. Genetic Resources and Crop Evolution 65:2037–2047.

Akinwale RO, Awosanmi FE, Ogunniyi OO, Fadoju AO (2017). Determinants of drought tolerance at seedling stage in early and extra-early maize hybrids. Maydica 62:1.

Altunoglu YC, Baloglu MC, Baloglu P, Yer EN, Kara S (2017). Genome-wide identification and comparative expression analysis of LEA genes in watermelon and melon genomes. Physiology and Molecular Biology of Plants 23(1):5-21.

Ashraf M, Foolad MR (2007). Role of glycine betaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany 59:206-216.

Blum A (1996). Crop responses to drought and the interpretation of adaptation. Plant Growth Regulation 20:135–148.

Cetin M, Sevik H, Yigit N, Ozel HB, Aricak B, Varol T (2018). The variable of leaf micromorphogical characters on grown in distinct climate conditions in some landscape plants. Fresenius Environmental Bulletin 27(5):3206-3211.

Cha-um S, Kirdmanee C (2009). Proline accumulation, photosynthetic abilities and growth characters of sugarcane (Saccharum officinarum L.) plantlets in response to ıso-osmotic salt and water-deficit stress. Agricultural Sciences in China 8(1):51-58.

Dasgan HY, Kusvuran S, Abak K, Leport L, Larher F, Bouchereau A (2009). The relationship between citrulline accumulation and salt tolerance during the vegetative growth of melon (Cucumis melo L.). Plant, Soil and Environment 55(2):51-57.

Esteras C, Formisano G, Roig C, Díaz A, Blanca J, Garcia-Mas J et al. (2013). SNP genotyping in melons: genetic variation, population structure, and linkage disequilibrium. Theoretical and Applied Genetics 126:1285-1303.

Fabeiro C, Martín F, de Juan JA (2002). Production of muskmelon (Cucumis melo L.) under controlled deficit irrigation in a semi-arid climate. Agricultural Water Management 54:93-105.

Fàbregas N, Lozano-Elena F, Blasco-Escámez D, Tohge T, Martínez-Andújar C, Albacete A, Osorio S, Bustamante M, Riechmann JL, Nomura T, Yokota T, Ana Conesa, Pérez Alfocea F, Fernie AR, Caño-Delgado AI (2018). Overexpression of the vascular brassinosteroid receptor BRL3 confers drought resistance without penalizing plant growth. Nature Communications 9:4680.

FAOSTAT (2018). Food and Agriculture Organization Corporate Statistical Database. [Online]. URL http://www.fao.org/faostat/en/#data/QC, Database Results 2016 (accessed November 2018)

Fergany M, Balvir Kaur, Monforte AJ, Pitrat M, Rys C, Lecoq H, Dhillon NPS, and Dhaliwal SS (2011). Variation in melon (Cucumis melo) landraces adapted to the humid tropics of southern India. Genetic Resources and Crop Evolution 58(2):225-243.

Kirkbride JH (1993). Biosystematic monograph of the genus Cucumis (Cucurbitaceae). Parkway, Boone, NC.

Kusvuran S (2010). Relationships between physiological mechanisms of tolerances to drought and salinity in melons. Department of Hortıculture Instıtute Of Natural and Applıed Scıences Unıversıty of Çukurova, Ph.D. Thesis, 356 pp.

Kusvuran S, Dasgan HY, Abak K (2013). Citrulline is an important biochemical indicator in tolerance to saline and drought stresses in melon. The Scientific World Journal ID 253414, 8pp.

Kusvuran S, Dasgan HY, Abak K (2011). Responses of different melon genotypes to drought stress. Y¨uz¨unc¨u Yıl Universityi Journal Agricultural Science 21(3):209-219.

Leida C, Moser C, Esteras C, Sulpice R, Lunn JE, de Langen F, Monforte AJ, Picó B (2015). Variability of candidate genes and genetic association for sugar accumulation and climacteric behavior in melon (Cucumis melo L.). BMC Genetics 16:28.

Leskovar DI, Sharma SP, Crosby KM, Volder A (2017). GxExM strategies for melons in water limited environments. Acta Horticulturae 1151:229-235.

Lima EMC, Carvalho JA, Viol MA, Almeida RC, Rezende FC (2017). Gália melons production in protected environment under different irrigation depths. Journal of the Brazilian Association of Agricultural Engineering 37(1):75-83.

Long RL, Walsh KB, Midmore DJ (2006). Irrigation scheduling to increase muskmelon fruit biomass and soluble solids concentration. Hortscience 41(2):367-369.

Ozer A, Hamurcu M, Turkmen O, Sari N, Solmaz I (2015). Determination of drought tolerance levels of some melon genotypes via physiological methods. In: V ISHS International Symposium on Cucurbits, Cartagena, Spain, P. 62.

Pandey S, Ansari WA, Atri N, Singh B, Gupta S, Bhat KV (2018). Standardization of screening technique and evaluation of muskmelon genotypes for drought tolerance. Plant genetic Resources-Characterization and Utilization 16(1):1-8.

Pandey S, Ansari WA, Jha, A, Bhatt, KV, Singh, B (2013). Evaluation of melons and indigenous Cucumis spp. Genotypes for drought tolerance. Acta Horticulturae 979:335-339.

Pareek OP, Samadia DK (2002). Promising indigenous cucurbit varieties. Indian Horticulture 47:15–18.

Pitrat M (2017). Melon genetic resources: phenotypic diversity and horticultural taxonomy. In: Genetics and genomics of the Cucurbitaceae (ed. Grumet R, Katzir N, Garcia-Mas J, Springer, New York, USA), pp. 25-60.

Pitrat M (2008). Melon (*Cucumis melo* L.). In: Handbook of crop breeding. Vol. I: Vegetables (ed. Prohens J, Nuez F, Springer, New York, USA), pp. 283-315.

Ripoll J, Urban L, Staudt M, Lopez-Lauri F, Bidel LPR, Bertin N (2014). Water shortage and quality of fleshy fruits - making the most of the unavoidable. Journal of Experimental Botany 65(15):4097-4117.

Roy A, Bal SS, Fergany M, Kaur S, Singh H, Malik AA, Singh J, Monforte AJ, Dhillon NPS (2012). Wild melon diversity in India (Punjab State). Genetic Reources and Croip Evolution 59:755-767.

Sensoy S, Ertek A, Gedik I, Kucukyumuk C (2007). Irrigation frequency and amount affect yield and quality of fieldgrown melon (*Cucumis melo* L.). Agricultural Water Management 88:269-274.

Sevik H, Cetin M (2015). Effects of water stress on seed germination for select landscape plants. Polish Journal of Environmental Studies 24(2):689-693.

Sharma SP, Leskovar DI, Crosby KC, Ibraim AMH, Volder A (2016). Differential response of muskmelon (Cucumis melo L.) cultivars to deficit irrigation. Acta Horticulturae 1112: 201-207.

Taiz L, Zeiger E (2006). Stress physiology. In: Plant physiology (ed. Taiz L, Zeiger E, Sinauer Associates, Inc., Sunderland, MA), pp. 671-681.

Torun A, Hamurcu M, Sarı N (2018). Physiological responses of melon genotypes with known drought tolerance levels. In: Cucurbitaceae 2018 Cucurbit2018.ucdavis.edu, Davis, California, USA, P. 42.

Turral H, Burke J, Faurès JM (2011). Climate change, water and food security. FAO Water Reports No. 36, Food and Agriculture Organization of the United Nations.

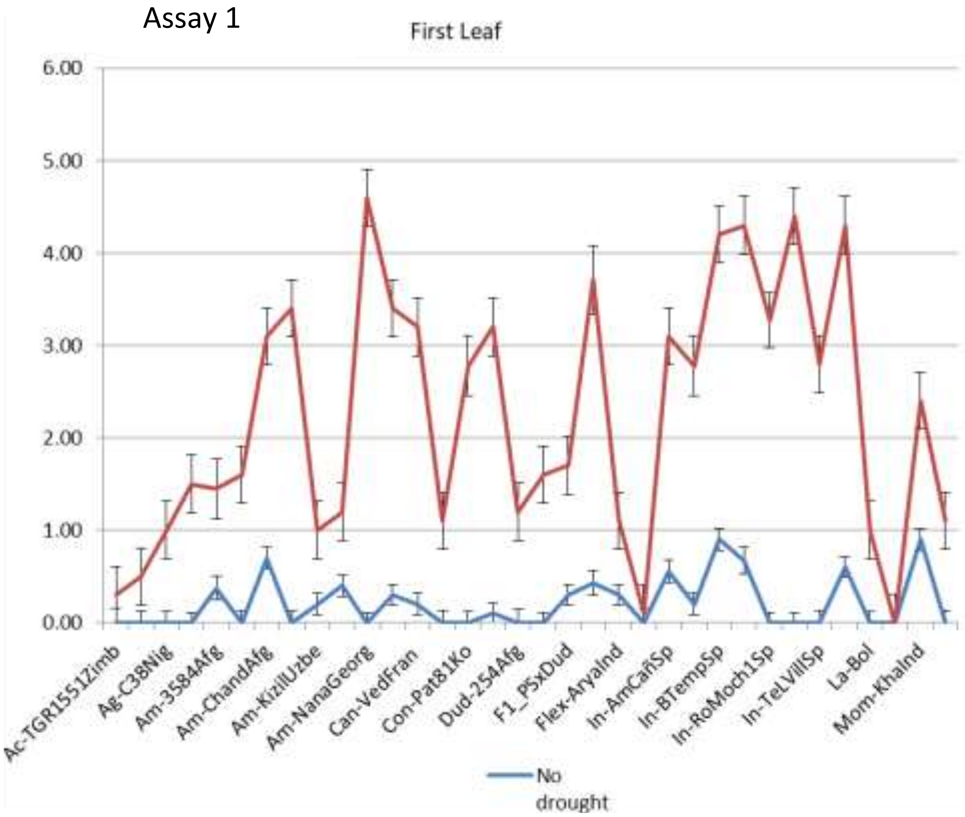
Verulkar SB, Mandal NP, Dwivedi JL, Singh BN, Sinha PK, Mahato RN, Dongre P, Singh ON, Bose LK, Swain P, Robin S, Chandababu R, Senthil S, Jain A, Shashidhar HE, Hittalmani S, Cruz VC, Paris T, Raman A, Haefele S, Serraj R, Atlin G, Kumar A (2010). Breeding resilient and productive genotypes adapted to drought-prone rainfed ecosystem of India. Field Crops Research 117:197-208.

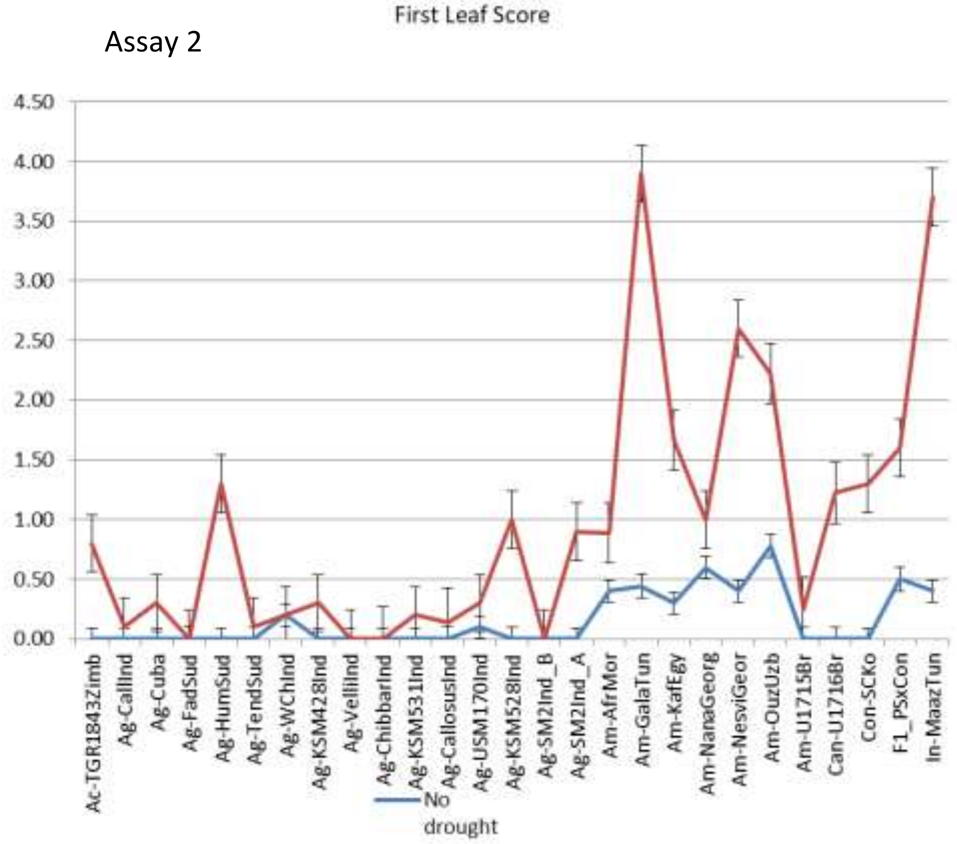
Yigit N, Sevik H, Cetin M, Kaya N (2016). Chapter 3: Determination of the effect of drought stress on the seed germination in some plant species. In: Water stress in plants (ed. Mofizur IM, Begum ZA, Hasegawa H, Intech Open), pp. 43-62.

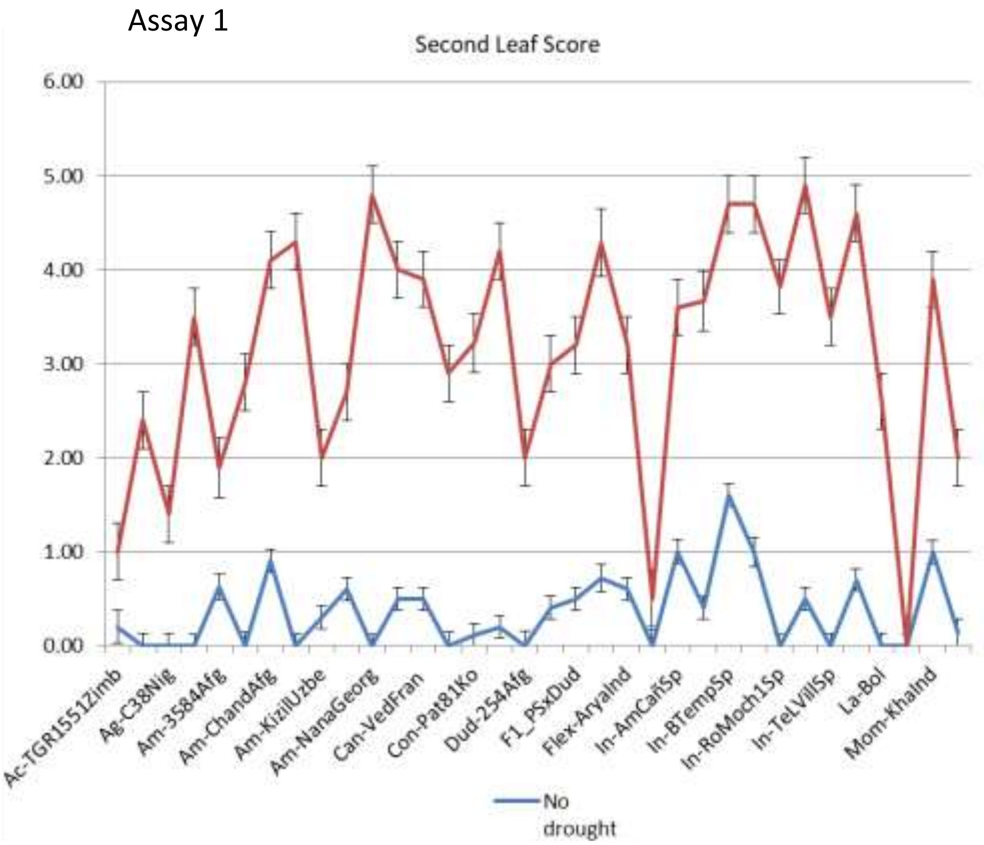
Zafar S, Azhar MT (2015). Assessment of variability for drought tolerance in Gossypium hirsutum L. at seedling stage. Pakistan Journal of Agricultural Sciences 52(2):301-307.

Zhang H, Gong G, Guo S, Ren Y, Xu Y (2011). Screening the USDA watermelon germplasm collection for drought tolerance at the seedling stage. HortScience 46:1245-1248.

SUPPLEMENTARY



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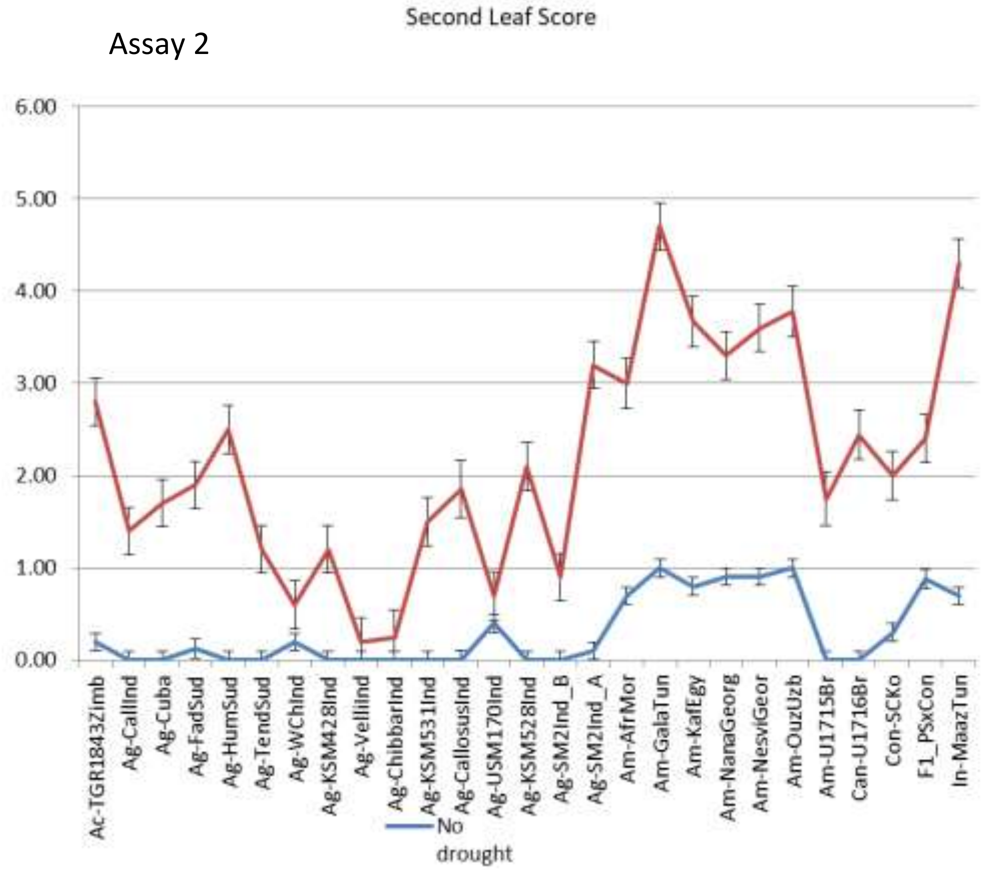
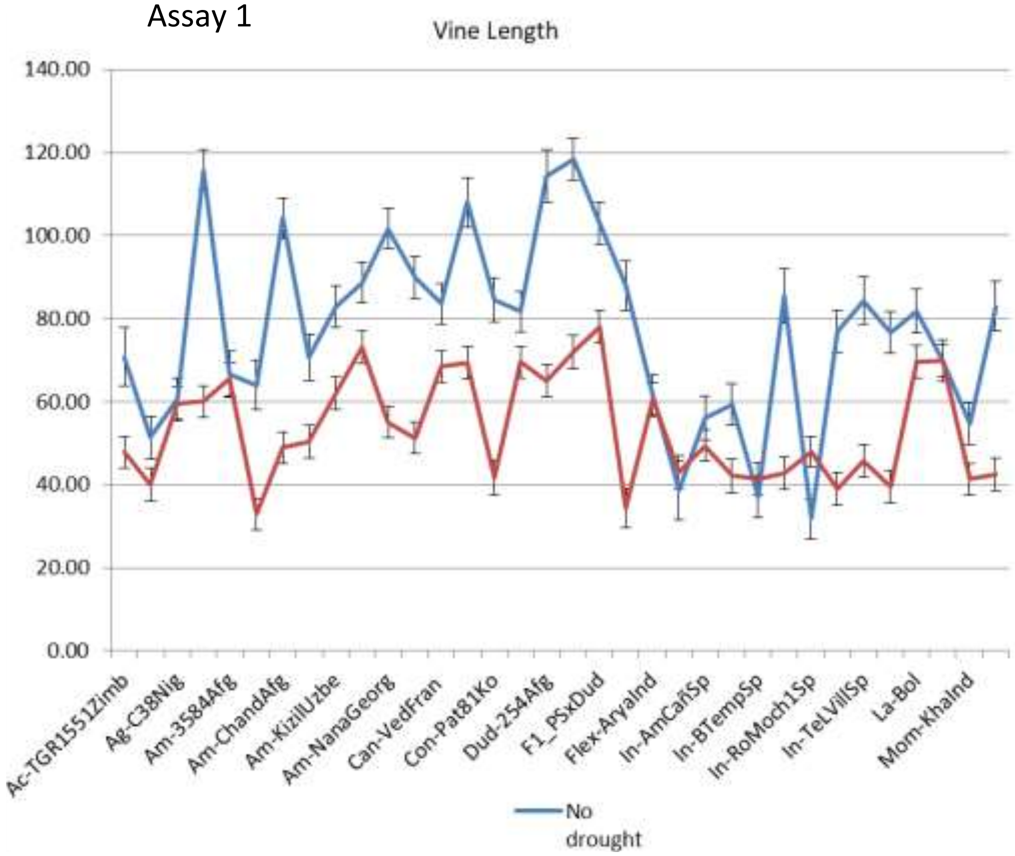
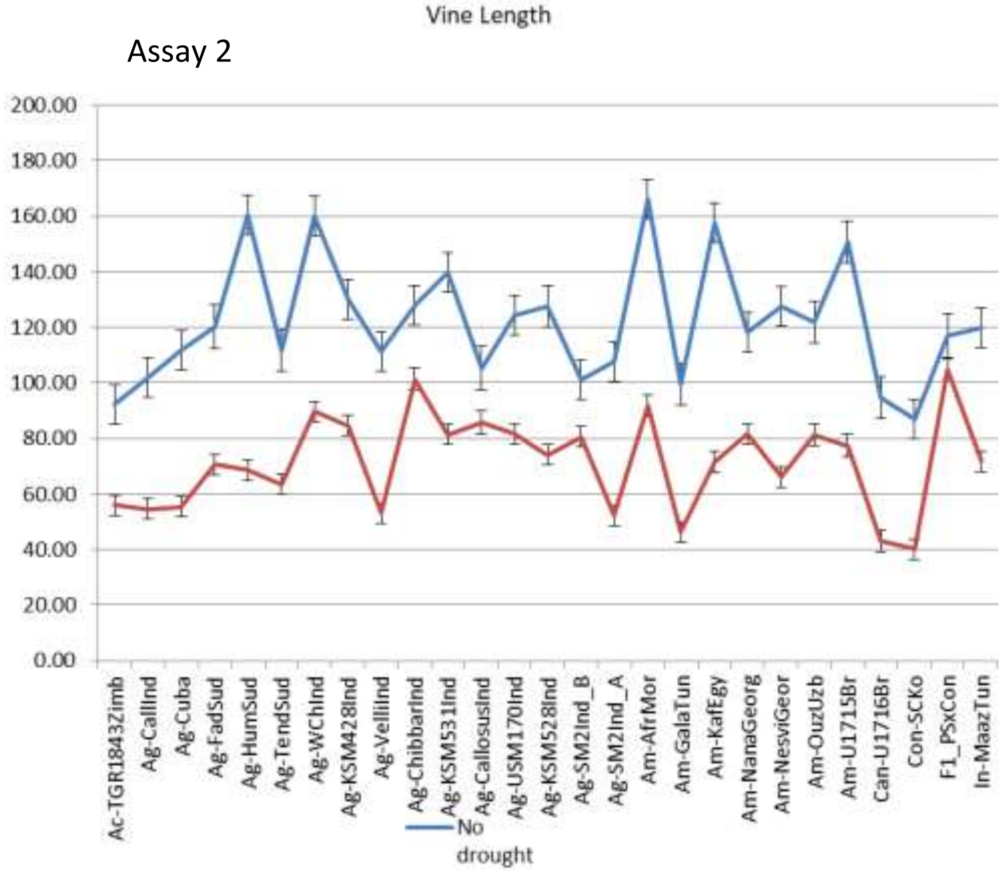
Second Leaf Score
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Ac-TGR1551Zimb
Ag-C38Nig
Am-3584Afg
Am-ChandAfg
Am-KizilUzbe
Am-NanaGeorg
Can-VedFran
Con-Pat81Ko
Dud-254Afg
F1_PSxDud
Flex-AryaInd
In-AmCafSp
In-BTempSp
In-RoMoch1Sp
In-TelVilSp
La-Bol
Mom-KhaInd
No drought

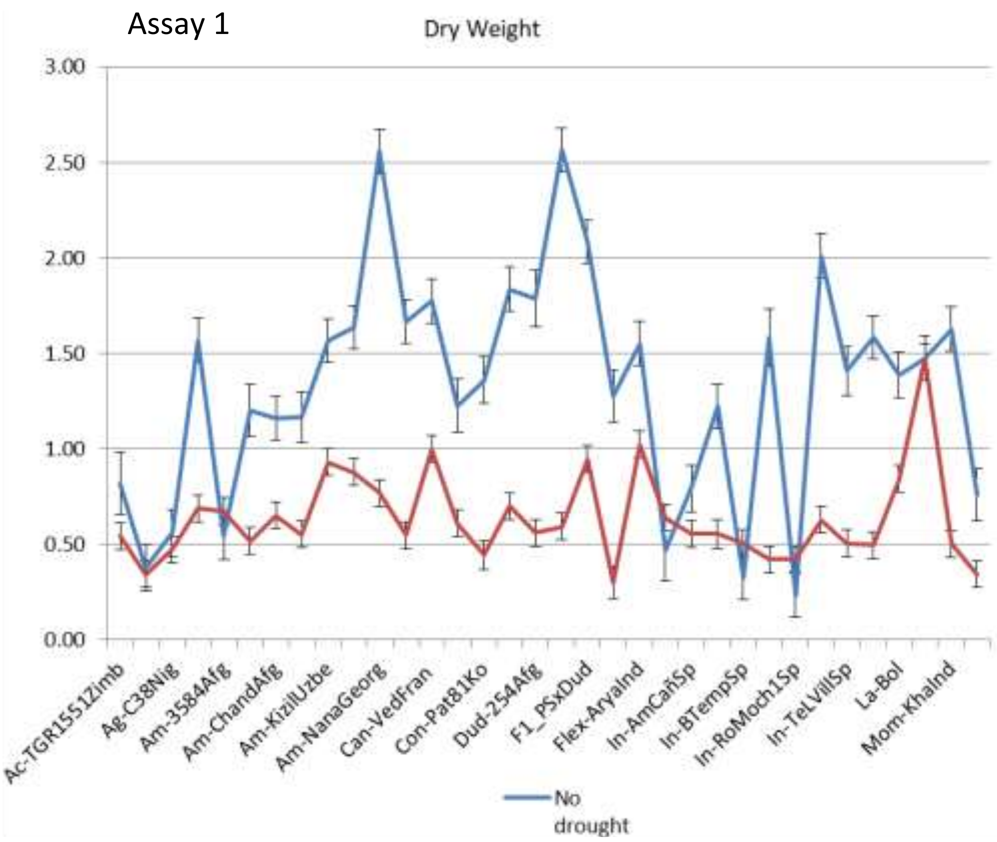

Assay 2
Second Leaf Score
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Ac-TGR1843Zimb
Ag-CallInd
Ag-Cuba
Ag-FadSud
Ag-HumSud
Ag-TendSud
Ag-WChInd
Ag-KSM428Ind
Ag-VelIInd
Ag-ChibbarInd
Ag-KSM531Ind
Ag-CallosusInd
Ag-USM170Ind
Ag-KSM528Ind
Ag-SM2Ind_B
Ag-SM2Ind_A
Am-AfrMor
Am-GalaTun
Am-KafEgy
Am-NanaGeorg
Am-NesviGeor
Am-OuzUzb
Am-U1715Br
Can-U1716Br
Con-SCKo
F1_PSxCon
In-MaazTun
No drought

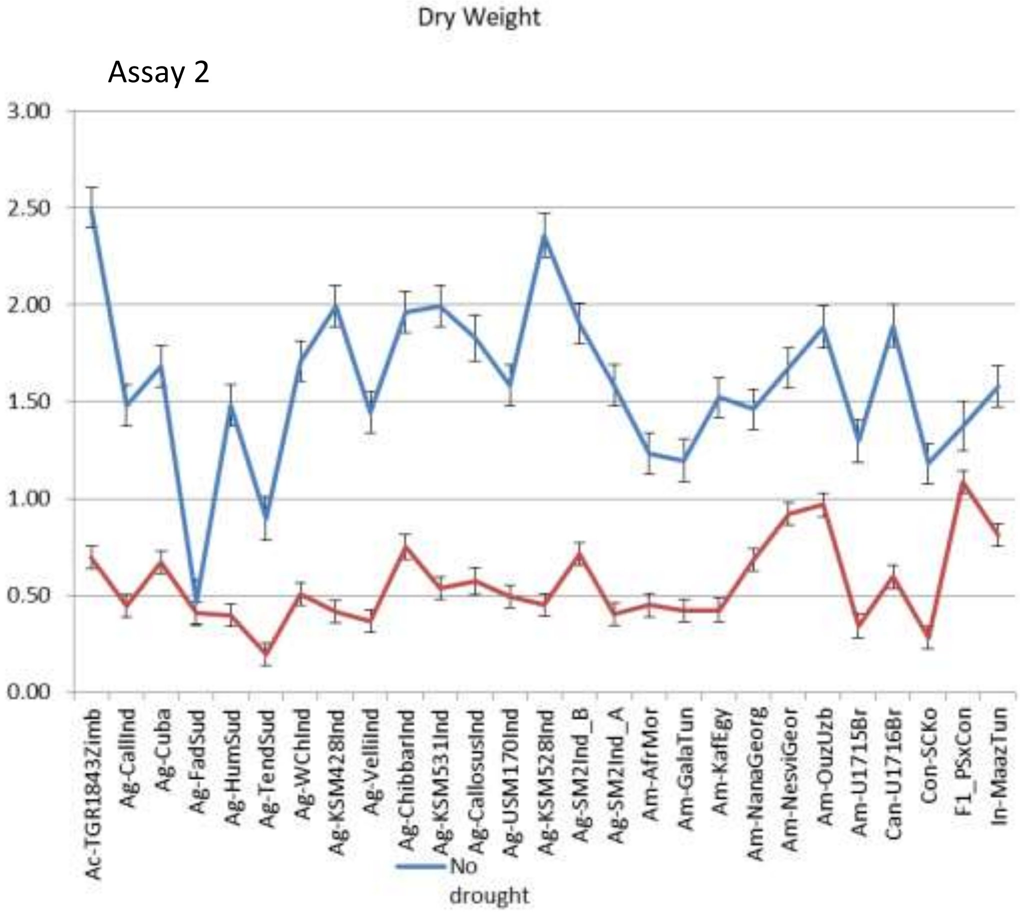
Assay 1

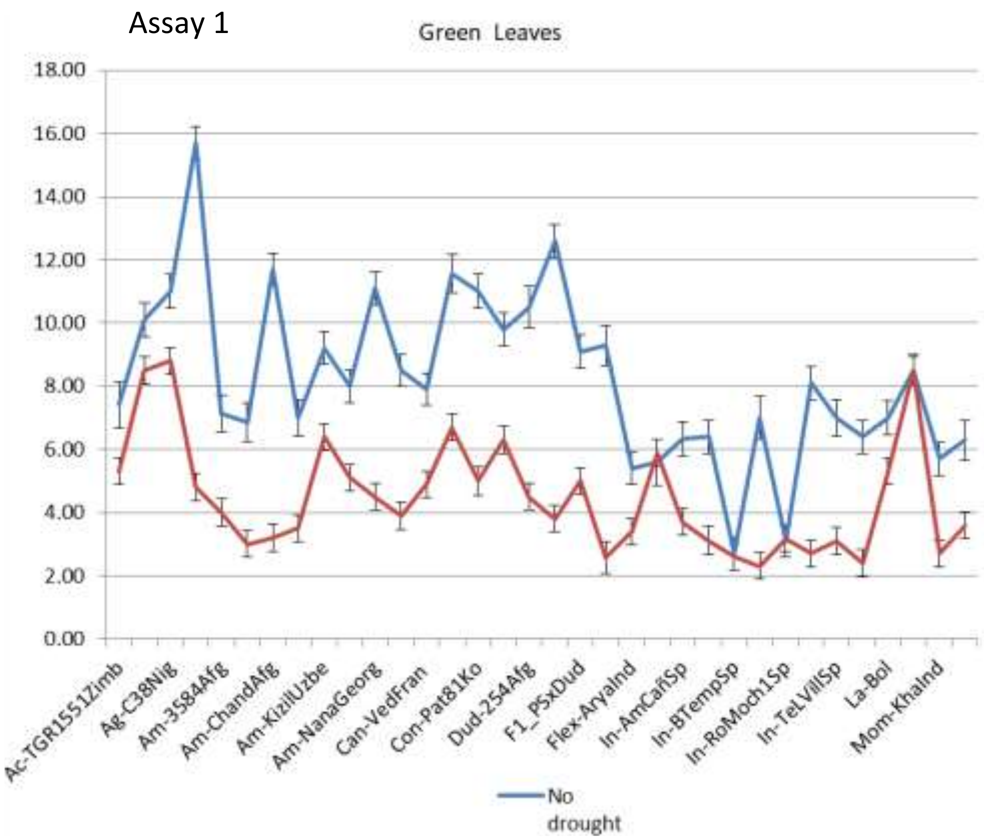


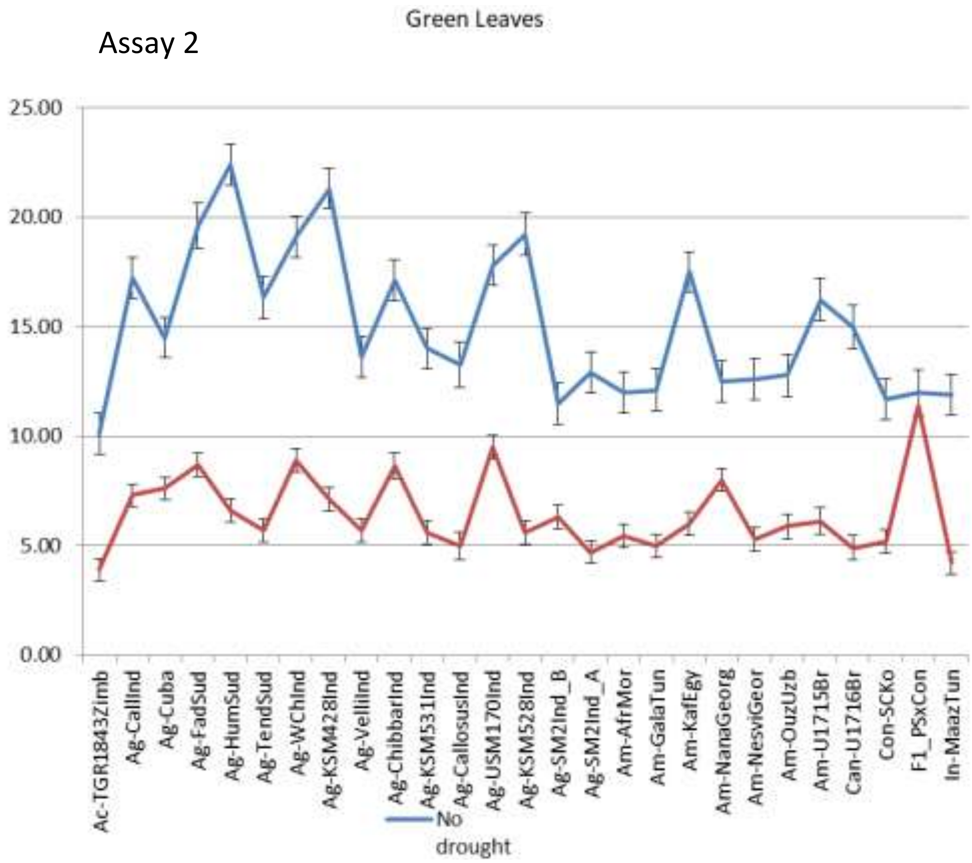
Assay 2

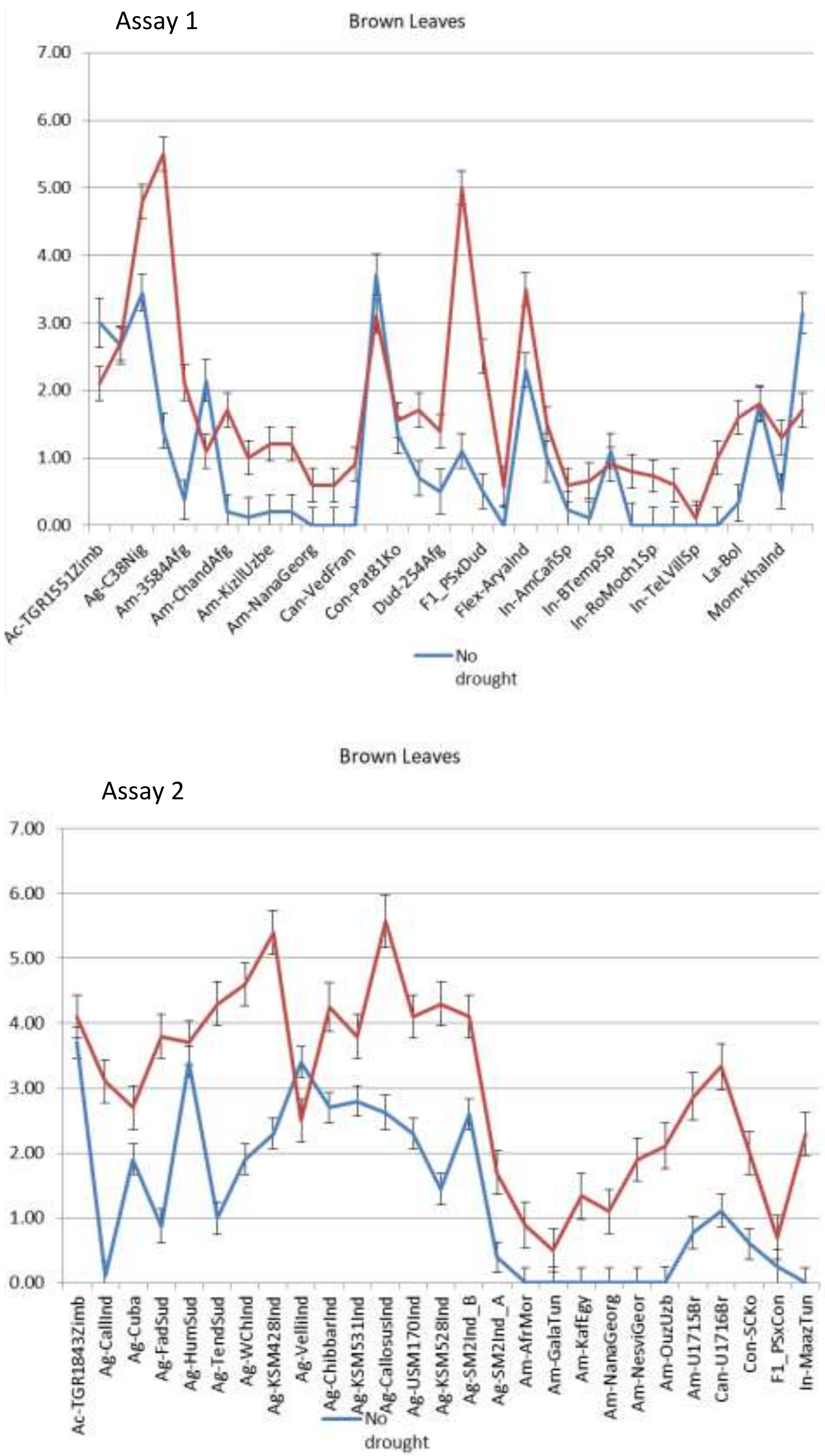



Assay 1
Dry Weight
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Ac-TGR1551Zimb
Ag-C38Nig
Am-3584Afg
Am-ChandAfg
Am-KizilUzbe
Am-NanaGeorg
Can-VedFran
Con-Pat81Ko
Dud-254Afg
F1_PSxDud
Flex-AryaInd
In-AmCañSp
In-BTempSp
In-RoMoch1Sp
In-TeLVillSp
La-Bol
Mom-KhaInd
No drought


Dry Weight
Assay 2
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Ac-TGR1843Zimb
Ag-CallInd
Ag-Cuba
Ag-FadSud
Ag-HumSud
Ag-TendSud
Ag-WChInd
Ag-KSM428Ind
Ag-VelliInd
Ag-ChibbarInd
Ag-KSM531Ind
Ag-CallosusInd
Ag-USM170Ind
Ag-KSM528Ind
Ag-SM2Ind_B
Ag-SM2Ind_A
Am-AfrMor
Am-GalaTun
Am-KafEgy
Am-NanaGeorg
Am-NesviGeor
Am-OuzUzb
Am-U1715Br
Can-U1716Br
Con-SCKo
F1_PSxCon
In-MaazTun
No drought

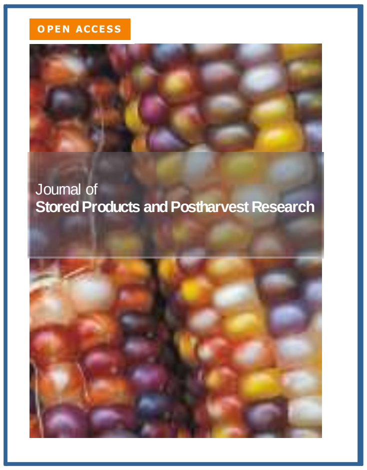

Assay 1
Green Leaves
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Ac-TGR1551Zimb
Ag-C38Nig
Am-3584Afg
Am-ChandAfg
Am-KizilUzbe
Am-NanaGeorg
Can-VedFran
Con-Pat81Ko
Dud-254Afg
F1_PSxDud
Flex-AryaInd
In-AmCañSp
In-BTempSp
In-RoMoch1Sp
In-TeLVillSp
La-Bol
Mom-KhaInd
No drought

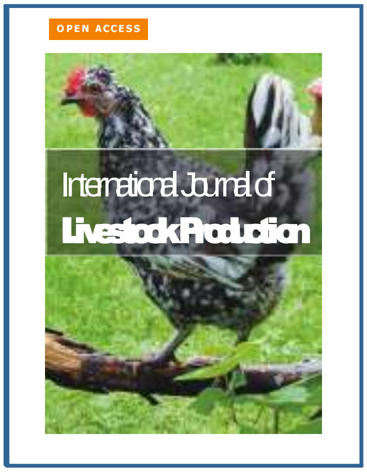

Assay 2
Green Leaves
25.00
20.00
15.00
10.00
5.00
0.00
Ac-TGR1843Zimb
Ag-CalIInd
Ag-Cuba
Ag-FadSud
Ag-HumSud
Ag-TendSud
Ag-WChInd
Ag-KSM428Ind
Ag-VelIInd
Ag-ChibbarInd
Ag-KSM531Ind
Ag-CallosusInd
Ag-USM170Ind
Ag-KSM528Ind
Ag-SM2Ind_B
Ag-SM2Ind_A
Am-AfrMor
Am-GalaTun
Am-KafEgy
Am-NanaGeorg
Am-NesviGeor
Am-OuzUzb
Am-U1715Br
Can-U1716Br
Con-SCKo
F1_PSxCon
In-MaazTun
No drought

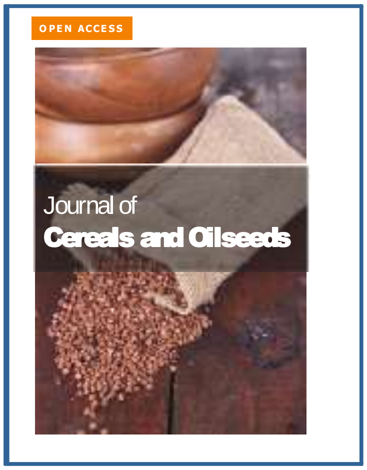


Supplementary file 1. Plots showing means and standard errors per genotype for each trait assessed in both assays under drought/no drought conditions.

Related Journals:







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