



# African Journal of Agricultural Research

Volume 12 Number 19 11 May, 2017

ISSN 1991-637X



*Academic  
Journals*

## ABOUT AJAR

The African Journal of Agricultural Research (AJAR) is published weekly (one volume per year) by Academic Journals.

African Journal of Agricultural Research (AJAR) is an open access journal that publishes high-quality solicited and unsolicited articles, in English, in all areas of agriculture including arid soil research and rehabilitation, agricultural genomics, stored products research, tree fruit production, pesticide science, postharvest biology and technology, seed science research, irrigation, agricultural engineering, water resources management, marine sciences, agronomy, animal science, physiology and morphology, aquaculture, crop science, dairy science, entomology, fish and fisheries, forestry, freshwater science, horticulture, poultry science, soil science, systematic biology, veterinary, virology, viticulture, weed biology, agricultural economics and agribusiness. All articles published in AJAR are peer-reviewed.

### Contact Us

**Editorial Office:** [ajar@academicjournals.org](mailto:ajar@academicjournals.org)

**Help Desk:** [helpdesk@academicjournals.org](mailto:helpdesk@academicjournals.org)

**Website:** <http://www.academicjournals.org/journal/AJAR>

**Submit manuscript online** <http://ms.academicjournals.me/>

## Editors

**Prof. N.A. Amusa**

Editor, African Journal of Agricultural Research  
Academic Journals.

**Dr. Panagiota Florou-Paneri**

Laboratory of Nutrition,  
Faculty of Veterinary Medicine,  
Aristotle University of  
Thessaloniki, Greece.

**Prof. Dr. Abdul Majeed**

Department of Botany, University of  
Gujrat, India, Director Horticulture,  
and  
landscaping.  
India.

**Prof. Suleyman TABAN**

Department of Soil Science and Plant  
Nutrition, Faculty of Agriculture,  
Ankara University,  
06100 Ankara-TURKEY.

**Prof. Hyo Choi**

Graduate School  
Gangneung-Wonju National University  
Gangneung,  
Gangwondo 210-  
702, Korea.

**Dr. MATIYAR RAHAMAN KHAN**

AICRP (Nematode), Directorate of  
Research, Bidhan Chandra Krishi  
Viswavidyalaya, P.O. Kalyani, Nadia, PIN-  
741235, West Bengal.  
India.

**Prof. Hamid AIT-AMAR**

University of Science and Technology,  
Houari Bouemdiene, B.P. 32, 16111 EL-Alia,  
Algiers,  
Algeria.

**Prof. Sheikh Raisuddin**

Department of Medical Elementology and  
Toxicology, Jamia Hamdard (Hamdard University)  
New  
Delhi,  
India.

**Prof. Ahmad Arzani**

Department of Agronomy and Plant Breeding  
College of Agriculture  
Isfahan University of Technology  
Isfahan-84156, Iran.

**Dr. Bampidis Vasileios**

National Agricultural Research Foundation  
(NAGREF), Animal Research Institute 58100  
Giannitsa,  
Greece.

**Dr. Zhang Yuanzhi**

Laboratory of Space Technology,  
University of Technology (HUT) Kilonkallio Espoo,  
Finland.

**Dr. Mboya E. Burudi**

International Livestock Research Institute  
(ILRI) P.O. Box 30709 Nairobi 00100,  
Kenya.

**Dr. Andres Cibils**

Assistant Professor of Rangeland Science  
Dept. of Animal and Range Sciences  
Box 30003, MSC 3-I New Mexico State University  
Las  
Cruces,  
NM 88003 (USA).

**Dr. MAJID Sattari**

Rice Research Institute of  
Iran, Amol-Iran.

**Dr. Agricola Odoi**

University of Tennessee,  
TN., USA.

**Prof. Horst Kaiser**

Department of Ichthyology and Fisheries Science  
Rhodes University, PO Box  
94, South Africa.

**Prof. Xingkai Xu**

Institute of Atmospheric Physics,  
Chinese Academy of  
Sciences, Beijing 100029,  
China.

**Dr. Agele, Samuel Ohikhena**

Department of Crop, Soil and Pest  
Management, Federal University of  
Technology  
PMB 704,  
Akure,  
Nigeria.

**Dr. E.M. Aregheore**

The University of the South Pacific,  
School of Agriculture and Food Technology  
Alafua Campus,  
Apia, SAMOA

## Editorial Board

**Dr. Bradley G Fritz**

Research Scientist,  
Environmental Technology Division,  
Battelle, Pacific Northwest National Laboratory,  
902 Battelle Blvd., Richland,  
Washington,  
USA.

**Dr. Almut Gerhardt** LimCo

International, University of  
Tuebingen, Germany.

**Dr. Celin Acharya**

Dr. K.S.Krishnan Research Associate (KSKRA),  
Molecular Biology Division,  
Bhabha Atomic Research Centre (BARC),  
Trombay, Mumbai-85,  
India.

**Dr. Daizy R. Batish** Department

of Botany, Panjab University,  
Chandigarh,  
India.

**Dr. Seyed Mohammad Ali Razavi**

University of Ferdowsi,  
Department of Food Science and Technology,  
Mashhad,  
Iran.

**Dr. Yasemin Kavdir**

Canakkale Onsekiz Mart University,  
Department of Soil Sciences, Terzioğlu  
Campus 17100  
Canakkale  
Turkey.

**Prof. Giovanni Dinelli**

Department of Agroenvironmental Science and  
Technology  
Viale Fanin 44 40100, Bologna  
Italy.

**Prof. Huanmin Zhou**

College of Biotechnology at Inner Mongolia  
Agricultural University,  
Inner Mongolia Agricultural University, No. 306#  
Zhao Wu Da Street,  
Hohhot 010018, P. R. China, China.

**Dr. Mohamed A. Dawoud**

Water Resources Department,  
Terrestrial Environment Research Centre,  
Environmental Research and Wildlife Development Agency  
(ERWDA),  
P. O. Box 45553,  
Abu Dhabi,  
United Arab Emirates.

**Dr. Phillip Retief Celliers**

Dept. Agriculture and Game Management,  
PO BOX 77000, NMMU,  
PE, 6031,  
South Africa.

**Dr. Rodolfo Ungerfeld**

Departamento de Fisiología,  
Facultad de Veterinaria,  
Lasplaces 1550, Montevideo 11600,  
Uruguay.

**Dr. Timothy Smith**

Stable Cottage, Cuttle Lane,  
Biddestone, Chippenham,  
Wiltshire, SN14 7DF.  
UK.

**Dr. E. Nicholas Odongo,**

27 Cole Road, Guelph,  
Ontario. N1G 4S3  
Canada.

**Dr. D. K. Singh**

Scientist Irrigation and Drainage Engineering Division,  
Central Institute of Agricultural Engineering  
Bhopal- 462038, M.P.  
India.

**Prof. Hezhong Dong**

Professor of Agronomy,  
Cotton Research Center,  
Shandong Academy of Agricultural Sciences,  
Jinan 250100  
China.

**Dr. Ousmane Youm**

Assistant Director of Research & Leader,  
Integrated Rice Productions Systems Program  
Africa Rice Center (WARDA) 01BP 2031,  
Cotonou,  
Benin.

### ARTICLES

- Berries: Cultivation and environmental factors effects on the phenolic compounds content** 1602  
Renata Adriana LABANCA, Gabriel Barbosa de OLIVEIRA and Marie ALMINGER
- Impacts of climate change on global coffee production industry: Review** 1607  
Ebisa Dufera Bongase
- Role of phytohormones (indol acetic acid, jasmonic acid, salcylic acid, and ethylene) in nematode-plant interactions** 1612  
Degife Asefa Zebire
- Superiority of Malawian orange local maize variety in nutrients, cookability and storability** 1618  
Daiki Murayama, Tomoka Yamazawa, Chandiona Munthali, Ephantus, N. Bernard, Rodney, L. Gondwe, Jiwan, P. Palta, Masayuki Tani, Hiroshi Koaze and Daigo Aiuchi
- Conservation agriculture, conservation farming and conventional tillage adoption, efficiency and economic benefits in semi-arid Zimbabwe** 1629  
Mvumi C., Ndoro O. and Manyiwo S. A.
- Influence of ascorbic acid on physiological deterioration of pieces of cassava raw pulp** 1639  
Arlindo Modesto Antunes, Túlio Natalino de Matos, Vanesa Beny da Silva Xavier Reis, Pâmella de Carvalho Melo, André José de Campos and Ivano Alessandro Devilla
- Response of bread wheat (*Triticum aestivum* L.) varieties to N and P fertilizer rates in Ofla district, Southern Tigray, Ethiopia** 1646  
Melesse Harfe
- Characterization of soybean population with sulfonylurea herbicides tolerant alleles** 1661  
Eder Eduardo Mantovani, Nara Oliveira Silva Souza, Luis Antonio Stabile Silva and Maria Aparecida dos Santos

<b>Assessment of yield loss in Rosemary (<i>Rosmarinus officinalis</i> L.) and Sage (<i>Salvia officinalis</i> L.) plants caused by <i>Fusarium oxysporum</i></b>	<b>1669</b>
Mihiret Mekonnen and Begashaw Manahile	
<b>Classification of selected white tropical maize inbred lines into heterotic groups using yield combining ability effects</b>	<b>1674</b>
Yazachew Genet Ejigu, Pangirayi Bernard Tongoona and Beatrice Elohor Ifie	

## Review

## Berries: Cultivation and environmental factors effects on the phenolic compounds content

Renata Adriana LABANCA<sup>1\*</sup>, Gabriel Barbosa de OLIVEIRA<sup>2</sup> and Marie ALMINGER<sup>2</sup>

<sup>1</sup>Food Science Laboratory - Experimental Nutrition Research Unit (BRO-UPNE), Department of Food (ALM), Faculty of Pharmacy (Fafar), Federal University of Minas Gerais - UFMG, Av. Antônio Carlos, 6627, Campus UFMG, Pampulha, CEP 31.270-010, Belo Horizonte, MG, Brasil.

<sup>2</sup>Division of Life Sciences/Food Science - Department of Chemical and Biological Engineering - Chalmers University of Technology - SE 412 96 Gothenburg, Sweden.

Received 10 January, 2017; Accepted 21 February, 2017

**Berries are rich in bioactive compounds, such as vitamins, fiber, macro and microelements and have high content of polyphenols, plant secondary metabolites. The consumption of a diet rich in polyphenols has in epidemiological studies been associated with a lower incidence of degenerative diseases including cancer, cardiovascular and other diseases. The preventive effects are often attributed, in part, to phenolic compounds and suggested to be due to their antioxidant, and anti-inflammatory effects as well as other beneficial effects. This review describes the effect of cultivation methods and environmental factors on the composition and content of phenolic compounds in some berries. Methods for extraction and analyses of phenolic compounds and reported potential mechanisms of action through which polyphenol compounds may exert protective actions are also reviewed.**

**Key words:** Berries, bioactive compounds, phenolic compounds.

### INTRODUCTION

Berries are reddish-purple fruits, normally small-sized and highly perishable. This group includes strawberry, gooseberry, blackberry, raspberry (black or red), blueberry, bilberry cranberry and other berries of minor economic importance (Manganaris et al., 2014; Hussain et al., 2016). Usually the term 'soft fruit' has been used to refer to different commodities including strawberries, blueberries and several species of the genus *Rubus*. *Rubus* is a highly diverse genus of flowering plants in the

world, with 12 subgenera, some of which group hundreds of species. *Rubus* berry species (includes e.g. both raspberries and blackberries) grow especially well in cool climate but have also been found to grow well in subtropics climates. Raspberry and blackberries are closely related to strawberries (Vicente and Sozzi, 2007).

Despite the incongruent definitions, one unifying trait among "berries" (with few exceptions) is that they contain anthocyanins, which are pigment polyphenol compounds

\*Corresponding author. E-mail: [renata@bromatologiaufmg.com.br](mailto:renata@bromatologiaufmg.com.br).

that give them their distinctive color (Joseph et al., 2014; Yamamoto et al., 2015).

## PHENOLIC COMPOUNDS

Phenolic compounds are essential for the quality of plant-derived food products through their contribution to oxidative stability and organoleptic characteristics. Phenolics are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants. Plant polyphenols have drawn increasing attention due to their potent antioxidant properties and their suggested effects in the prevention of various oxidative stress associated diseases such as cancer. Compared to other fruits, the content of phenolic compounds in berries is often higher (Balasundram et al., 2006).

Phenolics found in edible berries include flavonoids, ellagitannins, phenolic acids, and stilbenoids. Further properties that have been proposed are anti-inflammatory properties, ability to induce carcinogen detoxification enzymes, and modulate signaling pathways of cancer cell proliferation and apoptosis (Seeram, 2008). Ferulic, caffeic and p-coumaric acids and caffeoylquinic esters are the major hydroxycinnamates identified in berries; benzoic acid derivatives that have been primarily identified in berry fruits include gallic, salicylic, p-hydroxybenzoic and ellagic acids (Szajdek and Borowska, 2008).

## CULTIVATION

The most common berry fruits produced globally are strawberries, raspberries, blackberries and black/red currents and especially the rapid expansion of the blackberry industry has been remarkable. As new cultivars are developed that combine the industry's need for high quality arrivals with increased flavors and extended growth periods, the blackberry industry is expected to grow further (Clark and Finn, 2014; Da Pasa et al., 2014). This trend is similar for berries in general, and especially for blackberries, blueberries, cranberries, gooseberries, raspberries and strawberries (Hussain et al., 2014; USDA, 2016).

## ENVIRONMENTAL AND PREHARVEST FACTORS AFFECTING QUALITY OF BERRIES

Berries are highly appreciated for their intense color, delicate texture and unique flavor. Despite having a number of common attributes, the group is quite diverse and comprises simple (that is, blueberry, cranberry) and composite fruits derived from single or multiple fused fertilized ovaries (that is, strawberry, mulberry, raspberry,

blackberry) (Manganaris et al., 2014).

These varieties have a strong need for cold, requiring many hours at low temperature e.g. raspberries which requires at least 600 h at temperatures below 7°C. Other crucial factors for the cultivation and quality of these fruits refers to the soil, which must be drained and with good water retention capacity as well as the presence of organic matter (Vicente and Sozzi, 2007; Curi et al., 2014).

The harvesting need to be planned and the timing of the harvest should be determined according to the parameters of the fruit ripeness according to the further and final utilization. Harvest maturity depends on the cultivar and is mainly determined by size and color of the fruit. Other maturity indices include corking of lenticels and internal ethylene concentration. The quality assessment is based on visual aspects of the berries like the texture, flavor, uniform size and shape, bruising, scars, insect damage, as well as other defects (Szalai and Basile, 2010).

All berry fruits are susceptible to various organisms, mostly fungi, that cause fruit rot or fruit rot-like diseases. *Botrytis cinerea* is the main fungus causing rots in strawberries, raspberries and blackberries. To limit deterioration the fruit has to be harvested and taken care of and further processed or consumed as soon as possible. Normally a storage with prompt forced air cooling is needed and a storage at 0°C in 90 to 95% Relative Humidity (RH) in order to slow down the development of *Botrytis Rot* (Grey Mold), caused by *Botrytis cinerea*, which is the major cause of postharvest strawberry losses. Raspberry is considered to be the most perishable fruit among berry crops. It can only be stored for 2 to 5 days at 0±0.5°C and 90-95% RH. For Blueberry the optimum storage conditions are at 0±0.5°C and RH 90 to 95%. Shelf life at these conditions is usually two weeks. Modified atmosphere packaging for shipment with 10 to 15% carbon dioxide reduces the growth of *B. cinerea* (Szalai and Basile, 2010).

## POST-HARVEST EFFECTS ON CONTENT OF PHENOLIC COMPOUNDS

Even before the fruit enters the laboratory for phytochemical extraction and analysis their phenolic profiles and concentrations will vary because of genetic (that is, genus, species, cultivar/genotype) and environmental (that is, fruit maturity, plant age, growing season, field location) factors (Lee et al., 2012).

However, after the harvesting of fruits or berries, their concentrations of polyphenols maybe significantly affected by storage conditions, and furthermore, many compounds maybe destroyed during processing. The optimal timing of the harvest of fruits and berries is not simple when trying to optimize the levels of phenolic compounds. The ideal time of fruit and berry harvesting



occurs when fruits are not fully ripe and, hence, are also suitable for long-term storage; this stage is optimal with respect to the concentrations of flavanols and many flavonols (Kårlund et al., 2014).

In the study by Vagiri et al. (2015) Blackcurrant (*Ribes nigrum* L.) leaves were investigated as essential source of phenolic compounds as well as their variation from the leaf positions and date of collection. The content of phenolic compounds varied between harvest dates, although leaf position in the aerial part and interactions also played an important role. The content of quercetin-malonyl-glucoside, kaempferol-malonyl-glucoside isomer and kaempferol-malonyl-glucoside were higher than other phenolic compounds identified, while the minor epigallocatechin was investigated for all sheet positions and harvest dates. The content of the neo chlorogenic acid, epigallocatechin, kaempferol-3-O-rutinoside, kaempferolmalonyl-glucoside and kaempferol-malonyl-glucoside isomer was higher in June, whereas quercetin-glucoside, kaempferol-glycoside and total phenols, increase towards the end of the season. The leaf position influenced the content of myricetin-malonyl-glucoside, malonyl-glucoside yricetin-isomer, quercetin-malonyl-glucoside and kaempferol glucoside at the end of the season. Knowledge related to the influence of ontogenetic time and harvest in specific phenolic content can contribute to the adaptation of functional foods or pharmaceuticals, using blackcurrant leaves as natural ingredients.

As reported by Veberic et al. (2015), the anthocyanidin composition of *Ribers*, *Rubus*, *Vaccinium* and *Fragaria* genus or the less known species of *Crataegus*, *Morus*, *Amelanchier*, *Sorbus*, *Sambucus* and *Aronia* genus, did not differ significantly between the fruit of wild growing and cultivated species, but the total content of anthocyanidins was in general different between the different species.

## HEALTH BENEFITS

Plant-derived dietary polyphenols may improve some disease states and promote health. Berry fruits contain appreciable amounts of flavonoids, particularly anthocyanins, which are responsible for the distinctive red-blue-purple coloring of berries. A diet rich in plant flavonoids is associated with a lower risk of chronic disease development and epidemiological studies suggest that regular and long-term consumption of fruits and vegetables lowers rates of premature mortality and decreases the risk of developing chronic diseases such as heart disease, stroke and several cancers. Berries are often used as medicinal food, and the applications of berries as chemopreventive anti-inflammatory or anti-cancer agents, as anti-inflammatory remedies, as cancer treatment adjuvants, have been the subject of a large number of patents and clinical trials (Scalbert et al., 2005; McCullough et al., 2012; Joseph et al., 2014; Nile and

Park, 2014).

Oxidation is an essential process to aerobic organisms and our metabolism; free radicals are produced naturally as a result of this oxidation process, or by some biological dysfunction. In these radicals, the unpaired electron is in the oxygen or nitrogen atom, and therefore these radicals are classified as reactive oxygen species (ROS) or reactive nitrogen species (RNS) (Pham-Huy et al., 2008).

The oxidative processes that occur naturally in the human body contribute to the development of many major diseases due to an insufficient defense system. A diet rich in oxidised product components results in a reduction in antioxidant potential oxidative state or in an organism, increasing the risk of disease.

The finding that diets rich in fruits and vegetables reduce the risk of chronic diseases interfere with specific physiological targets, boosted research that identified nutrient substances and nutrients that do not interfere in the pathogenic processes of certain diseases. This evidence resulted, among other things, changes in the recommendations of the dietary guidelines, which started to indicate eating more servings of fruits and vegetables in the diet. The antioxidant action in common phenolic compounds, for example, due to the oxidation-reduction potential of certain molecules, the ability of these molecules to compete for the active sites and receptors in the different cell structures or even to modulation of gene expression encoding proteins involved in intracellular defense mechanisms against oxidative degenerative processes of cellular structures such as membranes and DNA.

Berry research has traditionally focused on their antioxidant properties. Berries (or their extracts) rank highly on *in vitro* antioxidant measures, such as oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant capacity (FRAP) analyses (Wolfe et al., 2008; White et al., 2010) and have been shown in various *in vitro* assay systems to mitigate oxidative stress (Seeram, 2008; Hurst et al., 2010). The most significant health benefits are ascribed to phenolic compounds and vitamin C (Szajdek and Borowska, 2008).

Polyphenols, including anthocyanins, have antioxidant capability; however, biologically, several studies suggest that their effects *in vivo* are not through antioxidant scavenging properties. Current hypotheses suggests polyphenols work via activation/inhibition of various cell signaling processes such as modulating kinase activity resulting in transcription factor activation (e.g., Nrf-2, NF-κB), altering receptor activation (PRR dimerization) as well as possible direct ligand activity (e.g., PPAR-γ) (Scalbert et al., 2005, Tangney and Rasmussen, 2013).

The main goal in this area is the generation through breeding programmes of novel berry cultivars with improved nutritional properties. In certain cases, increasing AOX accumulation would be beneficial from both plant and human perspectives. A study

demonstrated the successful combination of interspecies back-crosses and intra-species crosses in order to improve the nutraceutical content of strawberry fruit (Ruel et al., 2009).

From a breeder's perspective, the availability of 'highly nutritious berries' with enhanced health-promoting properties would be a strong asset, encouraging both berry producers and consumers. The antioxidant potency in combination with phenolic content of fruits has been proposed as a standardized method for the evaluation of fruit germplasms (Basu et al., 2011; Dohadwala et al., 2011).

Chiva-Blanch and Visioli (2012), in a revision of article for the periodical "Journal of Berry Research" discusses that polyphenols exhibit a wide variety of different biological effects whose quantification *in vivo* is currently hampered by the lack of robust biomarkers. Indeed, some trials do show positive modulation of surrogate markers of cardiovascular disease and cancer following the administration of defined amounts of polyphenols to human volunteers. These healthful activities are the results of manifold and complex actions of polyphenols that do extend beyond their mere antioxidant actions. All of this, obviously - applies to berries and their minor components, which play multiple roles in human physiology and should not be heralded as mere antioxidants, but, rather, as multi-functional and biologically-important compounds.

The bioactive berry components, can effectively inhibit oxidative DNA damage caused both *in vivo* and *in vitro* (Aiyer et al., 2008). Cranberry juice (2 cups/day) significantly reduces lipid oxidation and increases plasma antioxidant capacity in women with metabolic syndrome (Basu et al., 2011).

The potential of (poly)phenols to improve neurological health appears to be related to a number of mechanisms, including their ability to interact with intracellular neuronal and glial signaling, to influence the peripheral and cerebrovascular blood flow, and to reduce neuronal damage and losses induced by neurotoxins and neuroinflammation. While a number of *in vitro* experiments have suggested that (poly)phenols may influence carcinogenesis and tumor development (Del Rio et al., 2013).

## CONCLUSION

For the future of berry production, some factors necessary for expansion include the use of more efficient production practices. There are no specific studies, but, it is suggested that the quality and flavour of locally produced fruit is recognized as unique. Resistance to insect and disease-causing organisms is of immeasurable benefit to the environment, the producer and, the consumer.

Berries are rich in phenolic compounds, macro and

microelements. Several lipophilic and hydrophilic compounds are found in berries it is believed that the additional effect additive and / or synergistic result of the various components may be responsible for beneficial biological properties rather than one chemical compound or class. Currently it is believed that the combination of vitamins, minerals, antioxidants, fiber and phenolic compounds is responsible for the desired effect on health, that help combat the effects of degenerative diseases associated with aging is a major factor in the future expansion of berry crop production.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## REFERENCES

- Aiyer HS, Kichambare S, Gupta RC (2008). Prevention of oxidative DNA damage by bioactive berry components. *Nutr. Cancer Philadelphia* 60(S1):36-42.
- Balasundram N, Sundram K, Samman S (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chem. England* 99:191-203.
- Basu A, Betts NM, Ortiz J, Simmons B, Wu M, Lyons TJ (2011). Low-energy cranberry juice decreases lipid oxidation and increases plasma antioxidant capacity in women with metabolic syndrome. *Nutr. Res. Davis* 31(3):190-196.
- Chiva-Blanch G, Visioli F (2012). Polyphenols and health: Moving beyond antioxidants. *J. Berry Res. Ancona* 2(2):63-71.
- Clark JR, Finn CE (2014). Blackberry cultivation in the world. *Rev. Bras. Frutic. Jaboticabal* 36:46-57.
- Curi PN, Pio R, Moura PHA, Lima LCO, DoValle MHR (2014). Quality raspberries with and without plastic covering over the canopy in different spacing. *Rev. Bras. Frutic. Jaboticabal* 36:199-205.
- Da Pasa MS, Fachinello JC, Schmitz JD, DE Fischer DLO, DaRosaJúnior HF (2014). Performance of rabbiteye and highbush blueberry cultivars as affected by mulching. *Rev. Bras. Frutic. Jaboticabal* 1:161-169.
- Del Rio D, Rodriguez-Mateos A, Spencer JPE, Tognolini M, Borges G, Crozier A (2013). Dietary (poly)phenolics in human health: Structures bioavailability, and evidence of protective effects against chronic diseases. *Antioxid. Redox Signal. Columbus* 18(4):1818-1892.
- Dohadwala MM, Holbrook M, Hamburg NM, Shenouda SM, Chung WB, Titas M, Kluge Ma, Wang N, Palmisano J, Milbury Pe, Blumberg JB, Vita JA (2011). Effects of cranberry juice consumption on vascular function in patients with coronary artery disease. *Am. J. Clin. Nutr. Bethesda* 93(5):934-949.
- Hussain I, Assis AM, Yamamoto LY, Koyama R, Roberto SR (2014). Indolebutyric acid and substrates influence on multiplication of blackberry 'Xavante'. *Cienc. Rural* 44:1761-1765.
- Hussain I, Roberto SR, Fonseca ICB, Assis AM, Koyama R, Antunes LEC (2016). Phenology of 'Tupy' and 'Xavante' blackberries grown in a subtropical area. *Sci. Hortic.* 201:78-83.
- Hurst RD, Wells RW, Hurst SM, Mcghie TK, Cooney JM, Jensen DJ (2010). Blueberry fruit polyphenolics suppress oxidative stress induced skeletal muscle cell damage *in vitro*. *Mol. Nutr. Food Res.* 54(3):353-363.
- Joseph SV, Edirisinghe I, Burton-Freeman BM (2014). Berries: anti-inflammatory effects in humans. *J. Agric. Food Chem.* 7:3886-3903.
- Kärllund A, Salminen JP, Koskinen P, Ahern JR, Karonen M, Tiilikkala K, Karjalainen RO (2014). Polyphenols in strawberry (*Fragaria x ananassa*) leaves induced by plant activators. *J. Agric. Food Chem.* 62(20):4592-4600.
- Lee J, Dossett M, Finn CE (2012). Rubus fruit phenolic research: The

- good the bad and the confusing. *Food Chem.* 130(4):785-796.
- Manganaris GA, Goulas V, Vicente AR, Terry LA (2014). Berry antioxidants: Small fruits providing large benefits. *J. Sci. Food Agric.* 94:825-833.
- McCullough ML, Peterson JJ, Patel R, Jacques PF, Shah R, Dwyer JT (2012). Flavonoid intake and cardiovascular disease mortality in a prospective cohort of US adults. *Am. J. Clin. Nutr. Bethesda.* 95(2):454-464.
- Nile SH, Park SW (2014). Edible berries: Bioactive components and their effect on human health. *Nutrition* 30(2):134-144.
- Pham-Huy LA, He H, Pham-Huy C (2008). Free radicals antioxidants in disease and health. *Int. J. Biomed. Sci.* 4(2):89-96.
- Ruel G, Pomerleau S, Couture P, Lemieux S, Lamarche B, Couillard C (2009). Plasma matrix metalloproteinase (MMP)-9 levels are reduced following low-calorie cranberry juice supplementation in men. *J. Am. Coll. Nutr.* 28(6):694-701.
- Scalbert A, Johnson It, Saltmarsh M (2005). Polyphenols: antioxidants and beyond. *Am. J. Clin. Nutr. Bethesda* 81(1):215s-217s.
- Seeram NP (2008). Berry fruits for cancer prevention: current status and future prospects. *J. Agric. Food Chem.* 56:630-635.
- Szajdek A, Borowska EJ (2008). Bioactive compounds and health promoting properties of berry fruits: a review. *Plant Foods Hum. Nutr.* 63:147-156.
- Szalai Z, Basile S (2010). Organic fruit and vegetable productions greenfood. Project 2010-1-ES1-LEO05-20948. Europe. 36 p.
- Tangney CC, Rasmussen HE (2013). Polyphenols, inflammation, and cardiovascular disease. *Curr. Atheroscl. Reports Philadelphia* 15(5):324.
- USDA (2016). USDA AMS annual purchase summary. Available in: <<http://www.ams.usda.gov/AMSV1.0/getfile?dDocName=STELPRDC5099583>>. Accessed: 13 April 2016.
- Vagiri M, Conner S, Stewart D, Andersson SC, Verrall S, Johansson E, Rumpunen K (2015). Phenolic compounds in blackcurrant (*Ribes nigrum* L.) leaves relative to leaf position and harvest date. *Food Chem.* 172(1):135-142.
- Veberic R, Slatnar A, Bizjak J, Stampar F, Mikulic-Petkovsek M (2015). Anthocyanin composition of different wild and cultivated berry species. *LWT - Food Sci. Technol.* 60(1):509-517.
- Vicente AR, Sozzi GO (2007). Ripening and postharvest storage of 'soft fruits'. *Fruit Vegetable Cereal Sci. Biotechnol.* 1:95-103.
- White BL, Howard LR, Prior RL (2010). Polyphenolic composition and antioxidant capacity of extruded cranberry pomace. *J. Agric. Food Chem.* 58(7):4037-4042.
- Wolfe KL, Kang X, He X, Dong M, Zhang Q, Liu RH (2008). Cellular antioxidant activity of common fruits. *J. Agric. Food Chem.* 56:8418-8426.
- Yamamoto LY, Assis AM, Roberto SR, Bovolenta YR, Nixdorf SL, Garcia-Romero E, Gómez-Alonso S, Hermosín-Gutiérrez I (2015). Application of abscisic acid (S-ABA) to cv. Isabel grapes (*Vitis vinifera* × *Vitis labrusca*) for color improvement: Effects on color, phenolic composition and antioxidant capacity of their grape juice. *Food Res. Int.* 77:572-583.

Review

# Impacts of climate change on global coffee production industry: Review

Ebisa Dufera Bongase

Jimma University College of Agriculture and Veterinary Medicine, Jimma, Ethiopia.

Received 11 January, 2017; Accepted 4 April, 2017

Next to petroleum oil, coffee is the most internationally transacted commodity in the world; consumers from all around purchase and enjoy coffee in their daily activities. Climate change has emerged in recent years as one of the most critical topics at almost all actors. The impact of climate variation in all producing countries is predicted to be negative, even though within each country, it would vary a lot. Temperature and rainfall conditions are considered to be important factors in defining potential coffee yield. Both factors interfere with the crop phenology, and consequently in productivity and quality. Coffee supply chains are likely to experience significant disruption due to climate variation over the next forty years. World population would rise to nine billion by 2050 and in this scenario, coffee production is also likely to decrease globally. Coffee price varies inversely with production changes and generates the largest price increase. To overcome these problems, the mitigation of global warming involving taking actions to reduce greenhouse gas emissions is an important option.

**Key words:** Climate variation, coffee price, coffee yield, world population.

## INTRODUCTION

Coffee is one of the legal international transacted commodities of many countries following petroleum oil, consumers from all around purchase and enjoys it in their daily activities (Iscaro, 2014). In addition, Davis et al. (2012) identified during their study, that coffee is the second most transacted goods in the world. In the world, Brazil is the leading coffee producer and exporter followed by Vietnam and Colombia (DaMatta et al., 2008). In countries such as Uganda, Burundi, Rwanda and Ethiopia, coffee is the most source of revenue of their societies since the crop is the main trade

commodities of these countries with global trade sales predictable as US\$ 90 billion (DaMatta et al., 2008). Among coffee species, only two species, *Coffea arabica* L. (Arabica coffee) and *Coffea canephora* (Robusta coffee) economically dominate the world coffee trade (ICC, 2009; Damatta and Ramalho, 2006). Predominately, Arabica represents 70% of global coffee production and Robusta represents about 30% (Damatta and Ramalho, 2006; Davis et al., 2012). The production and productive of both species are largely dependent on the climate for attain high yields and quality (Killeen and Harper, 2016).

E-mail: [ebisadu2016@gmail.com](mailto:ebisadu2016@gmail.com).

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](https://creativecommons.org/licenses/by/4.0/)

Most evidence shows that climate change has appeared in recent years and immediately change common perception of many people in few years, makes looking forward the serious topics of all stake holders (Dasaklis and Pappis, 2013). Since early 1900s Climate variation has been perceived and the causes usually anthropogenic and natural drivers of climate (Masters et al., 2009). The effect of climate variation on natural systems has begun as one of the most critical issues of humankind (Jaramillo et al., 2009). Many finding proof that weather alteration is hastening at ample quicker stride than earlier that leading to irreversible changes in major earth systems and ecosystems (ITC, 2010).

According to Kasterine et al. (2010), key cause of the climate change is the burning of coal, oil, natural gas and mineralization of organic matter; these lead to increase in the carbon dioxide (CO<sub>2</sub>) content of the atmosphere. Carbon dioxide (CO<sub>2</sub>) and methane (CH<sub>4</sub>) are the most greenhouse gases that influence global climate through emissions (Masters et al., 2009). Most times, the climate change is felt through changing climate weather such as when the rainy season does not start when it is forecasted to rain, dry season lasts longer than usual, rains too much and causes flood, temperature becomes much colder or hotter than usual (Enomoto et al., 2011). This climatic variability has always been the main factor responsible for the reduction of coffee yields in the world and determines the future coffee production status in the coffee producer's countries (Kasterine et al., 2010).

Coffee requires very specific environmental conditions for successful production, depending on the coffee variety grown. But the crop is perennial, tropical crop that can grow under both humid lowlands and tropical humid/sub humid highlands. Even though the average temperatures required for coffee Arabic range between 15 and 24°C, rain fall 2000 mm per annum and altitudes between 1000 and 2000 m above sea level. Robust coffee required average temperature range between 24 and 30°C, rainfall ~2,000 mm and altitudes of about 800 m above sea level (Killeen and Harper, 2016). The objective of this review paper is to review the impacts of climate change on global coffee production industry.

## LITERATURE REVIEW

### Climate change and coffee production

Most scholars agree that the regular territories of the genus of *Coffea* are the under storey of African tropical forests (DaMatta et al., 2008). According to Hagggar and Schepp (2011), result show Arabica coffee originated in south west Ethiopia in East Africa at altitudes ranging from 1,500 to 2,800 m, where weather climate shows little seasonal. While R. coffee originated from lowland forests of the Congo River basin; with extend up to Lake Victoria in Uganda (Hagggar and Schepp, 2011). Climate factors

such as solar radiation and relative humidity influence many physiological processes of the coffee tree but are not generally thought to play an important role as thermal and rainfall conditions in defining potential yield or ecological limitations for this crop (Camargo, 2010).

Overall, influence of weather variations on coffee producing countries are predicted to be negative (Ovalle-Rivera et al., 2015). Some countries would lose area suitability while others would gain from variation in weather elements Ovalle-Rivera et al. (2015). Ovalle-Rivera et al. (2015) reveals coffee producing arears such as America, Africa, Asia and Oceania would maintain some suitability for growing Arabica coffee.

### The coffee sector and climate change

Coffee sectors contain huge ramification, includes growers, producers' associations, pulpers, buyers, certification agencies, wholesalers, transporters, retailers, roasters, exporters and consumers (Lossau, 2010). Coffee is the world's most important tropical export crop but recent studies predict severe climate change impacts on *Coffea arabica* (C. arabica) production (Craparo et al., 2015). The predicted decrease in profitability and greater economic risk of coffee production may locally and temporarily have positive environmental impacts, but overall and over the longer term these impacts will likely be severe and negative (Schroth et al., 2009). According to Potts (2003), identified global coffee production and trades were under risk because of declining forests spp.; water contamination, diminishing biodiversity to persistently uncertain revenues and makes currently an imperfect market in action.

Many scientific justifications predicted coffee sectors are likely affected due to climate variation over the next forty years (Killeen and Harper, 2016). According to Killeen and Harper (2016) reveals coffee production area changed because suitable areas becomes too warm or prone to periodic drought (Killeen and Harper, 2016). Most suitable area becomes unsuitable because of climate variation (Dekens and Bagamb, 2014). The reduction of land suitable for coffee production will influence the world coffee market and increase the price of coffee (Hagggar and Schepp, 2011). The influence of this climate variation makes the farmers to be indebted, reduce ability to invest in production and reduce their income generation (Läderach et al., 2010). About 70% of the world's coffee is produced by small-scale farmers, with over 20 million coffee farming families equivalent to more than 100 million people depending on its production for their subsistence (Vega et al., 2003). The climate variations affect coffee industry from production to export (Dekens and Bagamb, 2014). World population will rise to nine billion by 2050 and in this scenario, coffee production is also likely to decrease globally, particularly in Africa and generate the largest price rises

(International Coffee Council, 2009).

## **Influence of climate variation on coffee production**

### ***Influence of weather variation on biodiversity***

Observed changes in climate have already adversely affected biodiversity at the species and ecosystem level, and further changes in biodiversity are inevitable with further changes in climate (Secretariat of the Convention on Biological Diversity, 2009). Davis (2012) stated that the profoundly negative trend for the future distribution of indigenous Arabica coffee would be 65% reduction in the number of bio climatically suitable localities, and at worst (scenarios of almost 100% reduction, by the year 2080 under the influence of accelerated global climate change). In this study, a 90% reduction in area suitable for in situ conservation of coffee genetic resources was projected for the year 2080. Climate change is predicted to increase mean temperatures and change precipitation regimes and as a result, traditional coffee growing regions may disappear and new regions may appear (Laderach et al., 2010). The relationships between the climatic parameters and coffee production are quite complex, because it affect the growth and development of the plants at different growth stages (Camargo, 2010).

### ***Impact of climate change on coffee yield and quality***

Hagggar and Schepp (2011) revealed the potential yield and quality of coffee is determined by both temperature and rainfall condition since both ability to interfere with the phenological growth of the crop. These impacts include, for example, disrupted flowering cycles and prolonged drought periods, which ultimate result in reduced coffee quantity and quality (Masters et al., 2009). Other climate variation such as soil water balance during different growth stages of the coffee crop, can affect the available soil water and decrease of the final yield (Camargo, 2010). The Arabica coffee is more sensitive to climate variation, specifically during blossoming and fructification stage (Hagggar and Schepp, 2011). Especially, coffee flowering triggered by the first rain fall at the beginning of rain season, meanwhile if rain drops off or becomes too heavy, flowers and fruit may drop from the coffee tree (Läderach et al., 2010). The unpredictable rains will make coffee to flower at various times throughout the year, making the farmers to harvest small quantities continuously (Jassogne et al., 2013). This change will affect the crop physiology especially during the flowering and fruit filling stage (Jassogne et al., 2013).

### ***Reduction of suitable land for coffee cultivation***

The current areas coverage for growing of Arabica coffee

may be replaced by (lower value) Robusta coffee, cattle pasture and food crops in the some parts of continents (Läderach et al., 2010). It is predicted that at the year 2050, 16% of the area suitable for growing of coffee can be reduced (Läderach et al., 2010). Many scholars identified the because of climate change such as flood, land degradation, drought which can reduce the land suitability for coffee production (Läderach et al., 2011). Ovalle-Rivera et al. (2015) estimated that the acreage with aptitude for growing A. coffee would be reduced for all producing countries by 2050, moving the optimal production conditions to areas with higher altitude. Läderach et al. (2010) and Davis et al. (2012) found similar results for Central America and Ethiopia. To meet future demand in 2050, 2.5 the area that is currently available for coffee production is required. Jassogne et al. (2013) stated the climate change mapping showed that the area suitability for A. coffee in Uganda will reduce drastically in the future. The average daily maximum and minimum temperature trends revealed an increase in temperature over the 50-year period (Jassogne et al., 2013). By 2050, it is predicted that global temperatures would increase by 2°C together with some increased seasonality of precipitation. These changes would reduce climatic suitability for A. coffee at low elevations and increase suitability of higher areas (Ovalle-Rivera et al., 2015). Laderach et al. (2011) pointed out in the case study, that coffee-producing zone in Nicaragua is currently at an altitude of elevation between 800 and 1400 masl; by 2050, the optimum elevation will increase to 1200 and 1600 masl. Additional case study in Uganda reveals if temperatures increase, areas suitable for coffee will be higher in the landscape and unfortunately, the areas that will become more suitable for coffee will compete with other crops or national nature reserves (Jassogne et al., 2013).

### ***Impact of climate change on pests diseases***

Climate variation is the most favorable for increase of coffee pest disease; the loss estimate globally is 13% of yield reduction (Agegnehu et al., 2015). Major disease that occurred because of climate variation during coffee growing will increase pest and disease prevalence, expanding the altitudinal range in which the fungal disease coffee rust and the coffee berry borer can survive (Läderach et al., 2010). For example, rising temperatures will increase infestation by the Coffee berry borer (*Hypothenemus hampei*), particularly where coffee grows unshaded and the cropping is continuous throughout the year (Walyaro, 2010). Jaramillo et al. (2011) predicted that climate change would worsen pest prevalence like "broca" (berry borer) in Eastern Africa. Consequences of this event suffer viability of current high quality producers (Kasterine et al., 2010). Climate change increases need for fungicides and lead to a resurgence of certain pests and diseases on coffee (Gianessi and Williams, 2011). In

the case study of Colombia and Ethiopia, an increase in rainfall and temperature threatens the coffee at an alarming rate, respectively and is more conducive, for pests and disease prevalence (Iscaro, 2014).

### ***Increased cost of production***

The occurrence of climate variation such as sporadic and low-intensity rains during growing phase coffee or flowering period, and towards the later-phases of flower bud development, is the main reasons for unsynchronized fruit ripening (DaMatta et al., 2008). Climate change increasing atmospheric CO<sub>2</sub> concentrations tend to increase the water use efficiency of C<sub>3</sub> plants such as coffee and will tend to offset the increased evaporative demand (Schroth et al., 2009). This change makes coffee production to be under irrigated, thereby increasing pressure on scarce water resources (Kasterine et al., 2010). Harvesting often represents the majority of production costs, so if erratic flowering and ripening cycles require additional harvesting cycles, these changes could drastically and unsustainably raise costs (Läderach et al., 2010). All the above mentioned will increase the cost of production, whereas more coffee may grow and need under irrigation (Hagggar and Schepp, 2012).

### ***Mitigation strategies***

Many scholars revealed climate variation is a phenomenon that will continue to cause severe or negative effect on yield throughout the world (Iscaro, 2014). To overcome this problem, mitigation of global warming involves taking actions to reduce greenhouse gas emissions to enhance sinks aimed at reducing the extent of global warming which is important (International Coffee Council, 2009). One of the limitations to understanding the impacts of climate variability on coffee production is the lack of having precise meteorological data at coffee growing areas which is important for the development of climate-based insurance (Hagggar and Schepp, 2011). An ecosystem services payments scheme would be an ideal strategy synergizing the goals of emissions mitigation, biosphere preservation and poverty alleviation (Läderach et al., 2010). Improving new cultivars resistant to pests and diseases, more productive, well adapted to the local climatic and soil conditions, and have acceptable and desired quality for the market is very important (Enomoto, 2011). Ethiopia has a unique genetic diversity of cultivated, semi-wild and wild Arabica varieties with different types of disease resistance environmental adaptations and quality characteristics for future breeding coffee varieties opportunity that are adapted to the changed climate (Lossau, 2010).

### ***Coffee adaptation strategies for climate change***

Measures to adapt coffee cultivation to climate change also contribute to reducing CO<sub>2</sub>. Other environmental benefits include enhanced water storage, the regulation of local temperatures, and biodiversity conservation (Lossau, 2010). Changes in temperature and frequency of rains are associated positively and significantly with a higher probability to implement at least one adaptation strategy to climate change (Zuluaga et al., 2015). Proven approaches build on existing indigenous practices and knowledge to maximize benefits of climate change adaptation (Dinesh and Vermeulen, 2016). As climate change becomes increasingly severe, an assessment of coffee producers' ability and willingness to adapt would be especially valuable to those hoping to create adaptation strategies and policies (Battiste et al., 2016).

Good management practices that reduce soil erosion (e.g. cover crops and contour bunds) and increase water retention (mulching, shade) will further help farmers adapt to climate change and retain the more fertile topsoil (Deressa et al., 2009; Jassogne et al., 2013). Scientists seem to agree that the best way to preserve A. coffee is through the use of shade trees (Jaramillo et al., 2009). Shade trees planted near coffee plants have the ability to block out the sun's impact on the plants. They create lower temperature, reduce up to 4°C better suited for Arabica coffee plants.

### **CONCLUSION**

Climate change has emerged in recent years as one of the most critical topics. It is predicted that rising temperatures and water shortages will negatively affect coffee production suitability at lower elevations and vice versa. The already perceived and the future predicted impacts of climate change on coffee production will not only be threat small scale farmers but also all actors involved in coffee industry including consumers. World population will rise to nine billion by 2050. In this scenario, coffee production is also likely to decrease globally, particularly in Africa. Coffee price varies inversely with production changing and generating the largest price increase. Only half the area will currently available for coffee production by 2050 G.C. 2.5-times of the current area will be needed to be meet the future demand. Reduced yields and increased prices were shown to reduce the coffee market by more than 5 million tons per year. As a result of the above reason, many authors believe that the area with aptitude for growing coffee would be reduced by 16% by 2050, especially for coffee Arabica.

Mitigation of global warming involves taking actions to reduce greenhouse gas emissions and to enhance sinks aimed at reducing the extent of global warming measures to adapt to coffee cultivation to climate change also

contributing to reducing CO<sub>2</sub>. Other environmental benefits include enhanced water storage, the regulation of local temperatures, and biodiversity conservation. Improved agronomy and sustainable management of resources including the use of drought and heat resistant varieties, irrigation, and shade cover are good first steps.

Many researchers concluded that the fluctuation of climate in the coffee growing area resulted in reduction in the yield and quality, increasing the outbreak of pest disease, increasing cost of production and reduced area of production. The consequence of the problem may make the coffee sector to have negative impact on the producers and consumers. Generally, further research will be focused on discovering climate change adaptation strategies feasible for smallholder producers for practically implement.

## CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

## REFERENCES

- Agegnehu E, Thakur A, Mulualem T (2015). Potential Impact of Climate Change on Dynamics of Coffee Berry Borer (*Hypothenemus hampei* Ferrari) in Ethiopia. Open Access Library J. 2(01):1.
- Battiste M (2016). Specialty Coffee Farmers' Climate Change Concern and Perceived Ability to Adapt (Doctoral dissertation, University of Michigan).
- Camargo MBPD (2010). The impact of climatic variability and climate change on arabic coffee crop in Brazil. *Bragantia*, 69(1):239-247.
- Craparo ACW, Van Asten PJA, Läderach P, Jassogne LTP, Grab SW (2015). *Coffea arabica* yields decline in Tanzania due to climate change: Global implications. *Agric. For. Meteorol.* 207:1-10.
- Damatta FM, Ramalho JDC (2006). Impacts of drought and temperature stress on coffee physiology and production: a review. *Braz. J. Plant Physiol.* 18:55-81.
- Damatta FM, Ronchi CP, Maestri M, Barros RS (2008). Ecophysiology of coffee growth and production. *Braz. J. Plant Physiol.* 19:485-510.
- Dasaklis TK, Pappis CP (2013). Supply chain management in view of climate change: an overview of possible impacts and the road ahead. *J. Industrial Eng. Manage.* 6(4):1139-1161.
- Davis AP, Gole TW, Baena S, Moat J (2012). The impact of climate change on indigenous arabica coffee (*Coffea arabica*): predicting future trends and identifying priorities. *PLoS One* 7(11):e47981.
- Dekens J, MAK FB (2012). Promoting an Integrated Approach to Climate Adaptation: Lessons from the coffee value chain in Uganda.
- Deressa TT, Hassan RM, Ringer C, Alemu T, Yesuf M (2009). Determinants of farmers' choice of adaptation methods to climate change in the Nile Basin of Ethiopia. *Global environmental change* 19(2):248-255.
- Dinesh D, Vermeulen S (2016). Climate change adaptation in agriculture: practices and technologies. Messages to the SBSTA 44 agriculture workshops.
- Enomoto R (2011). Climate-friendly and Productive farming Guide for Coffee smallholders in Africa.
- Gianessi L, Williams A (2011). Climate Change Increases Need for Fungicides for Coffee Trees.
- Haggard J, Schepp K (2011). Coffee and climate change. Desk study: impacts of climate change in four pilot countries of the coffee and climate initiative. Hamburg: Coffee and Climate.
- International Coffee Council (2009). Climate change and coffee 103<sup>rd</sup> Session. London, England.
- International Trade Centre (ITC) (2010). Climate Change and the Coffee Industry Geneva: ITC, vi, 28 pages (Technical paper).
- Iscaro J (2014). The Impact of climate change on coffee production in Colombia and Ethiopia. *Global Majority E-Journal*, 5(1):33-43.
- Jaramillo J, Eric M, Fernando EV, Aaron D, Christian B, Adeniran C O (2011). Some Like It Hot: The Influence and Implications of Climate Change on Coffee Berry Borer (*Hypothenemus hampei*) and Coffee Production in East Africa. *PLOS ONE* 6(9):1-14.
- Jaramillo J, Setamou M, Muchugu E, Chabi-Olaye A, Jaramillo A, Mukabana J, Maina J, Gathara S and Borgemeister C (2013). Climate change or urbanization? Impacts on a traditional coffee production system in East Africa over the last 80 years. *PLoS one* 8(1):e51815.
- Jaramillo J, Chabi-Olaye A, Kamonjo C, Jaramillo A, Vega FE, Poehling HM., Borgemeister, C (2009). Thermal tolerance of the coffee berry borer *Hypothenemus hampei*: predictions of climate change impact on a tropical insect pest. *PLoS One* 4(8):e6487.
- Jassogne L, Läderach P, Asten VP (2013). The Impact of Climate Change on Coffee in Uganda: Lessons from a case study in the Rwenzori Mountains. *Oxfam Policy and Practice: Climate Change and Resilience* 9(1):51-66.
- Kasterine A, Scholer M, Hilten JH (2010). Climate Change and the Coffee Industry. Abstract for trade information services. International Trade Centre, Palais des Nations, 1211 Geneva 10, Switzerland.
- Killeen JT, Harper G (2016). Coffee in the 21<sup>st</sup> century. Will Climate Change and Increased Demand Lead to New Deforestation?
- Läderach P, Haggard J, Lau C, Eitzinger A, Ovalle O, Baca M, Jarvis A, Lundy M (2010). Mesoamerican coffee: Building a climate change adaptation strategy. CIAT Policy Brief no. 2. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia.
- Lossau VA (2010). Agro biodiversity and adapting to climate change: The example of coffee. *Agriculture, Fisheries and Food*.
- Masters G, Baker P, Flood J (2009). Climate Change and Agricultural Commodities. CABI Position Paper.
- Ovalle-Rivera O, Läderach P, Bunn C, Obersteiner M, Schroth G (2015). Projected shifts in *Coffea arabica* suitability among major global producing regions due to climate change. *PLoS One* 10(4):e0124155.
- Potts J (2003). Building a Sustainable Coffee Sector Using Market-Based Approaches: The Role of Multi-stakeholder Cooperation.
- Schroth G, Läderach P, Dempewolf J, Philpott S, Haggard J, Eakin H, Castillejos T, Moreno JG, Pinto LS, Hernandez R, Eitzinger A (2009). Towards a climate change adaptation strategy for coffee communities and ecosystems in the Sierra Madre de Chiapas, Mexico. *Mitigation and Adaptation Strategies for Global Change*, 14(7):605-625.
- Secretariat CBD (2009). Connecting biodiversity and climate change mitigation and adaptation: Report of the Second Ad Hoc Technical Expert Group on biodiversity and climate change, Montreal. In Convention on Biological Diversity Technical Series No. 41.
- Vega FE, Rosenquist E, Collins W (2003). Global project needed to tackle coffee crisis. *Nature* 425(6956):343-343.
- Walyaro J D (2010). Climate change: potential impact on Eastern Africa coffees. Proceedings of the ASIC Conference
- Zuluaga V, Labarta R, Läderach P (2015). Climate Change Adaptation: The Case of the Coffee Sector in Nicaragua.



Review

# Role of phytohormones (indol acetic acid, jasmonic acid, salicylic acid, and ethylene) in nematode-plant interactions

Degife Asefa Zebire

Department of Plant Sciences, College of Agricultural Science, Arba Minch University, P. O. Box 21, Arba Minch, Ethiopia.

Received 27 May, 2016; Accepted 9 August, 2016

Plant parasitic nematodes are among the most destructive major pest of crop plants. Root knot nematode species and cyst nematodes are well studied species of nematode with various ranges of host. The common means of control for these pests mostly rely usually on use of chemicals like nematicides, which are environmentally unfriend and costly especially in large scale agricultural production systems. However, recent advances showed that there are other alternative strategies, such as development of resistant crop varieties and exploiting natural resistance genes of plants in conventional breeding programs. Molecular basis of biotechnological applications are also considered as an effective measure to control these pests. Many studies showed the existence of complex gene expression and hormone signaling for both compatible and incompatible interaction with the process of plant-nematode interaction. This review provides information on the overview of recent knowledges on the role of plant hormones mediating feeding site development through plant-parasitic nematodes and the role of phytohormones resistance against nematodes.

**Key words:** Plant parasitic nematode, phytohormones, nematode-plant interaction.

## INTRODUCTION

Plant parasitic nematodes (PPNs) cause huge damage in many plant species and agriculturally important crops. However, there are major differences in the suitability of particular plant species and varieties as a host for each nematode. Many studies showed that root knot nematode species (RKNs), which causes a major economic loss throughout the world, have very broad host ranges. Some

other species of nematode are limited to one or a few hosts (Djian-Caporalino et al., 2011; Gleason et al., 2008). For example, cyst nematodes usually have narrower host ranges. *Globodera rostochiensis* and *Globodera pallida* are the most common cyst nematode species damaging potato. Potato cyst nematodes reproduce mainly on potato and related Solanaceous

\*Corresponding author. E-mail: asefadegife@gmail.com.

species, whereas soybean cyst nematodes can cause huge yield losses on soybean, which are commonly limited to this crop (Lilley et al., 2007).

Parasitic nematodes can be distinguished by their different feeding habits as ectoparasitic and endoparasitic. They feed essentially on the living plant cell cytoplasm (Bar-Or et al., 2005). Sedentary plant-parasitic nematodes of the family Heteroderidae cause most of the damage. These nematodes transform differentiated plant root cells into nematode feeding sites (NFS). Sedentary endoparasites have a distinctive and complex interaction with their hosts in order to establish parasitism. RKNs and cyst nematodes cause a host cell to be stressed through suitable interaction. Once these nematode species form suitable interaction, it is easy for penetration and the establishment of a feeding site, due to their migration into the host cells (Hewezi and Baum, 2013). Plant induces defense responses during the process of nematode infection. Plant cells respond to nematode attack by the production of toxins such as super oxide and hydrogen peroxide. Compatible interaction between tomato and RKN in the cells surrounding migrating nematodes and feeding sites can be affected by hydrogen peroxide (Melillo et al., 2006).

There are several strategies that sedentary endoparasitic nematodes use to interact with their host. Sedentary endoparasitic nematodes are obligate biotrophs that can establish successful relationships with their host plants by inducing redifferentiation of root cells into specific feeding cells (Jaouannet et al., 2012). The development of nematodes is determined by their success in establishing feeding cells. Manipulation of the essential elements of cell development enables RKNs to induce feeding cell formation in a wide range of their host (Caillaud et al., 2008). Feeding cells are converted into multinucleate giant cells via simultaneous nuclear divisions in the absence of cell division. These enlarged giant cells containing many nuclei may undergo extensive endoreduplication. Extensive reprogramming of gene expression results in the formation of giant cells (Caillaud et al., 2008). Nematodes secrete effectors which play a critical role in parasitism by manipulating the recipient host plant. Nematode responsive plant cells enable RKNs to alter root development to induce and maintain giant cells (Bellafiore and Briggs, 2010).

Plant-nematode interaction influences the association between suppression of plant defenses and nematode feeding site development during gene expression. As a result, during infection of *Arabidopsis thaliana* with RKN, 70% of the genes involved in defense pathways were found to be locally repressed (Jammes et al., 2005). Resistance gene and signaling pathways overwhelms defense mechanisms in plant. For instance, salicylic acid (SA) pathways in the root can be suppressed by successful parasitism between cyst nematode and *A. thaliana*, whereas, susceptibility of plants to nematodes can be increased by ethylene signal transduction

(Wubben et al., 2008). Susceptibility of tomato to RKN is facilitated by jasmonate signaling pathway (Bhattarai et al., 2008). Thus, plant hormones play a vital role in effective interactions between plants and nematodes. The objective of this paper was to review current published articles and journals on plant hormones in plant-nematode interaction. The review mainly focuses on research papers available in the online literature database using Scopus and Google scholars searched information by using key words of the content. For instance, "nematode effectors", "plant parasitic nematode", "nematode-plant interaction", "plant hormone signaling pathway" and so forth. Research papers used in this review were not older than 2005. This paper basically reviews plant-nematode interaction, role of hormones in feeding cells and role of hormone in plant resistance to plant parasitic nematodes. It aims to provide information to people about research carried out on defense response of plants to plant parasitic nematodes and nematode effectors

## THE ROLE OF PLANT HORMONE IN FEEDING SITE ESTABLISHMENT

The cyst nematode *Heterodera schachtii* and the root-knot nematode *Meloidogyne incognita* infect the roots of *A. thaliana*. But these two nematode species are distantly related and the feeding sites they induce are dissimilar in nature. During infection, the pre-parasitic second-stage juveniles (preparasitic J2) penetrate the roots of a suitable host. The cyst and root-knot nematodes then migrate intracellularly or intercellularly, respectively towards the vascular cylinder to find a competent plant cell for the induction of a multinuclear feeding cell complex. Cyst nematodes induce syncytia, whereas root-knot nematodes induce giant cells as a feeding site. The similarities between these two nematode species are that they induce Death receptor 5 (DR5) activation patterns in *A. thaliana* root. Transcriptional regulation of synthetic auxin-responsive promoter element DR5 used to study the role of plant hormone (auxin) in induction of nematode feeding cell. DR5 promoter element was administered using a model plant *Arabidopsis thaliana* roots infected with *H. schachtii* or *M. incognita*. Strong and specific activation of DR5 using gusA reporter observed inside the feeding cells at 18 hour post inoculation (hpi), showing an increase apparent auxin concentration. Therefore, the phytohormone indol acetic acid (IAA) might give an important clue in feeding cell induction by PPNs due to an increase in apparent IAA concentration in the initial feeder cell (Caillaud et al., 2008; Karczmarek et al., 2004).

Ethylene and auxin are important plant hormones involved in the regulation of many important plant processes. For instance, cell differentiation, cell expansion, and responses of a plant to abiotic stresses

mediated by phytohormones (Davies, 2010). In addition, these hormones play vital roles in many plant-pathogen interactions, including manipulation of plant defense responses and development of symptoms. Sedentary plant-parasitic nematodes, requires the formation of a complex feeding site within the host roots as an important pre-requisite. A great change in host cell morphology and gene expression occurs during the formation of nematode-induced feeding sites. These changes are probably mediated by phytohormones (Gutierrez et al., 2009).

The complex interacting signaling networks are regulated and modulated by the hormonal balance which also dictates the responses of resistance or susceptibility. Three biological compounds, including SA, jasmonic acid (JA), and ethylene (ET) regulate the resistance gene-mediated and induced basal defense responses. Experiments on gene expression profiling of hormone signaling pathways indicate that there is a significant overlap among these pathways (Li et al., 2006). The signaling of JA and ET generally showed synergistic interactions where negative pathway crosstalk may occur between the JA and SA. The JA signaling pathway is essential for tomato susceptibility to root knot nematodes (Bhattarai et al., 2008).

Important aspects of developmental processes in plants can be facilitated by phytohormones. Phytohormones may also participate in various aspects of plant-nematode interactions. Several reports revealed that nematode infection interferes with the accumulation and transport of auxin in the plant. Auxin is an essential plant hormone involved in the formation of feeding site (Curtis, 2007). Ethylene is another plant hormone which might be involved in the modulation of many cellular processes during feeding site formation. Auxin is involved in plant-nematode interaction by acting as a signalling molecule by inducing changes on the surface of the cuticle, making it more susceptible and can also change the behavior of *Meloidogyne* species. The change in the surface of the cuticle and behavior of these nematode species might be important for infection (Curtis, 2007). Involvement of SA on *A. thaliana* mutant affects the activity of cyst nematode. (Wubben et al., 2008) reported that *A. thaliana* mutants deficient in SA were more susceptible than the wild types to cyst nematode. SA also plays a significant role in the reduction of parasitism between cyst nematode and plant (Wubben et al., 2008); can be increased by the presence of protease inhibitors, senescence associated and seed specific proteins associated with JA (Uehara et al., 2010). Ethylene-induced genes activated by ripping and allow compatible interaction between plants and PPNs (Uehara et al., 2010). Susceptibility of plants to cyst nematodes can also be facilitated by ET signaling transduction (Uehara et al., 2010; Wubben et al., 2008). Plant hormones like ET and JA have the ability to interfere with tomato SA-inducible PCN resistance pathway in susceptible cultivars. In

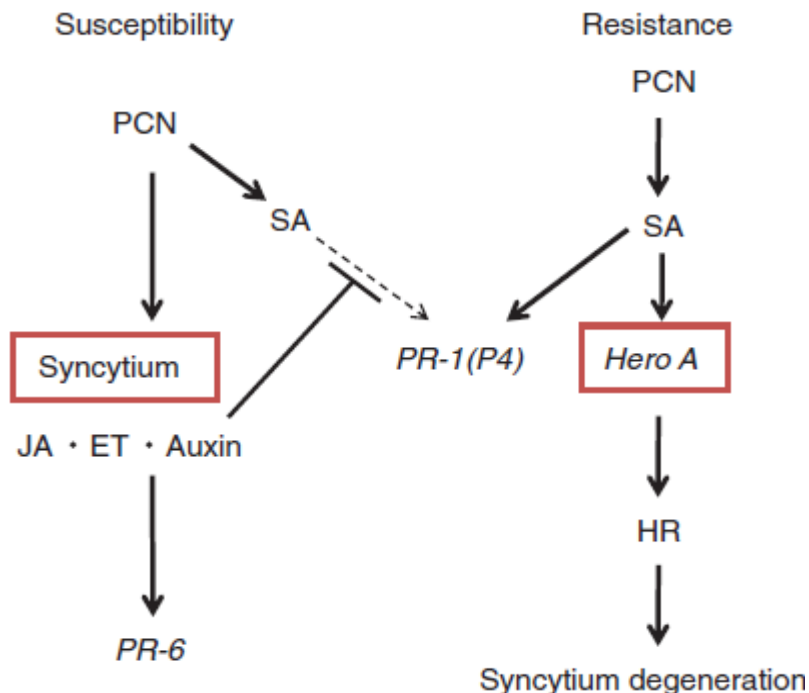
addition, auxin response factor induces the compatible interaction between plant and PCN (Uehara et al., 2010). Thus, phytohormones involved in many processes of plant nematode parasitism like the involvement of invasion plant cell and induction of syncytium.

SA triggers the resistance mechanism to plant cyst nematode. But the SA-inducible pathway in susceptible cultivars, one way or another affected by other hormones includes JA, auxin and ET (Uehara et al., 2010) (Figure 1).

Auxin and ethylene are involved in the formation of syncytia and host susceptibility. Cyst nematodes commonly complete their life cycle inside the root of the plant. They are associated with the plant by feeding on a nematode induced conglomerate of metabolically active root cells (Gutierrez et al., 2009). Cell enlargements in the plant after infection by nematodes are a common characteristic. Cell enlargement results in the formation of multicellular giant cells and this hypertrophy in the surrounding tissue in turn results in the formation of typical galls of the infected root. Enlargement and division of cell is mainly associated with increase in IAA perception. Auxin is also involved in programming the neighboring cells for integration into the developing syncytium (Karczmarek et al., 2004). The initiation and development of feeding sites of sedentary PPN is facilitated by plant signaling molecules of auxin (Grunewald, 2009). Auxin is also involved in the activation of cell division needed to keep up with the growing cells.

## THE ROLE OF HORMONES IN PLANT PARASITIC NEMATODES RESISTANCE

Naturally, plants have the ability to protect themselves from pathogen attack. Resistance mechanisms are one of the ways by which plants respond to stimuli and activate the production of defensive compounds. The hypersensitive response results from the death of host cell and blocks the development and reproduction of the pathogen, and can be involved in various types of resistances (Goggin et al., 2006). Plant resistance to the most destructive nematode species such as RKN and cyst nematodes could be based on dominant, recessive or additive effects and can be found in many crop species. Therefore, the use of well-studied genetic resistance, which is environmentally friendly, may pave the way for breeding programs aimed at controlling nematodes (Djian-Caporalino et al., 2011). Several studies revealed the requirement of hormone signaling in response to resistance. The complex interaction of signaling networks is regulated and modulated by the hormonal balance which also dictates the responses of resistance. SA, JA, and ET regulate the resistance gene-mediated and induced basal defense responses (Li et al., 2006).



**Figure 1.** Model to explain resistance and susceptibility of tomato cultivars to potato cyst nematode. R-gene-mediated resistance is induced through the SA-mediated signaling pathway. Signaling pathways mediated by other hormones such as auxin, ethylene (ET) and jasmonic acid (JA), which possibly interferes with the SA pathway, may be activated by syncytium formation because some hormones are involved in the formation of syncytia. Arrows indicate positive regulation; the blunt end indicates negative regulation (Uehara et al., 2010).

SA plays a major role in activation of defenses against biotrophic pathogens. ET provokes the SA-dependent resistance against biotrophic pathogens together with JA. Therefore, hormones such as JA and ET in the susceptible cultivars are expected to interfere with the tomato SA-inducible PCN resistance pathway (Uehara et al., 2010). Over accumulation of the defense signal molecule SA commonly displays plants morphological phenotypes. SA acid could interfere with auxin responses due to reminiscent of auxin-deficient or auxin-insensitive mutants. Wang et al. (2007) reported that SA causes global repression of auxin related genes. This results in balancing the AUX/IAA repressor proteins and inhibition of auxin responses. SA-mediated disease resistance mechanism is due to the inhibitory effect on auxin signaling.

Foliar application of systemic JA to plants induces a systemic effect that can suppress RKN infestation. This shows that jasmonate plays a significant role in foliar defenses. The results also revealed that jasmonates has an important role of in the protection of root tissues against root herbivorous. The resistance induced by JA is heat stable. In addition to this, JA treatments have the capacity to enhance the control of avirulent nematodes on resistance cultivars. The existence of this situation shows that Mi-mediated resistance and JA-induced

resistance may have an additive effect. Induction of JA through systemic means could have a significant influence in protecting crops from damage caused by nematodes. This in turn can reduce the use of hazardous nematicides which are environmentally dangerous chemicals (Cooper et al., 2005).

Synergistic interactions between the signaling molecules JA and ET is due to the negative pathway crosstalk that could occur between the JA and SA (Fujimoto et al., 2011). At the early stages of *Meloidogyne*-tomato plant incompatible interactions, SA was not found to be responsible for inhibition of catalase. Branch et al. (2004) reported that SA has a key role in the resistance response of tomato to RKN. They also investigated various resistance genes against *M. incognita* such as *Mi-1*. This SA showed positive effect of resistance response to the RKN *Meloidogyne javanica* in transformed tomato carrying *Mi-1* with construct expressing NahG. NahG encodes a bacterial enzyme salicylate hydroxylase that has the capacity to degrade SA into catechol. In addition, they produce SA, which would lead to an increase in the levels of catechol, which is toxic for both resistance and susceptible tomato lines. SA have a positive contribution both in hypersensitive reactions of plants to incompatible RKN and systemic

acquired resistance (Molinari and Loffredo, 2006). Tomato plants might not be affected by signaling of SA concerning the inhibition of catalase during early stage of incompatible interaction between *Meloidogyne* spp. However, catalase have a role in subsequent lesion formation which is part of *Mi-1*-mediated resistance to RKNs (Molinari and Loffredo, 2006).

Uehara et al. (2010) found that the induced SA-dependent pathogenesis-related genes mediate resistance in incompatible interactions between tomato and potato cyst nematode *G. rostochiensis* at 3 days-post-inoculation (dpi) when compared with compatible interaction. As direct evidence to show the link between salicylic acid and the *Hero A*-dependent *PR-1 (P4)* gene transcript, they produced *Hero A-NahG* tomato. The transcript accumulation of *PR-1 (P4)* was inhibited by the expression of *NahG* after potato cyst nematode infection of transformed tomato roots. Additionally, the reproduction of potato cyst nematodes on *Hero A-NahG* tomato was higher than on the *Hero A* tomato plants. This indicates that SA directly controls the *Hero A*-mediated potato cyst nematode resistance (Uehara et al., 2010).

Wubben et al. (2008) reported that wild type cultivars were more resistant to the cyst nematode than SA-deficient mutants. Moreover, SA application reduced parasitism by cyst nematodes. Wubben et al. (2008) demonstrated that SA plays more or less the same roles in resistance to the cyst nematode. According to Uehara et al. (2010), cultivars resistance to PCNs is elicited by SA, resulting in an elevated level of *PR-1 (P4)*, a hallmark gene for SA-inducible type of resistance. However, the SA-inducible pathway in the susceptible cultivars is somehow disturbed, possibly by other hormones such as JA, auxin and ET.

JA application in two near-isogenic lines of tomato with and without *Mi-1.2* resistance gene helps in activation of induced resistance. This result shows a significant role of jasmonate in the defense mechanism of root tissues against root herbivorous. The JA treatment gives only partial resistance to susceptible tomato cultivars. The reproductions of avirulent RKNs were almost completely inhibited by *Mi-1.2* gene response. On the other hand, the effects of *Mi*-mediated resistance response were reduced at a temperature of 32°C. Though, the most effective result was obtained in the combined treatment of JA and the presence of *Mi-1.2* gene at a temperature of 25°C (Cooper et al., 2005). Gene expressions and induced resistance response to *M. incognita* through foliar application of methyl jasmonate treatment at four different levels and timing were studied to realize the effects of exogenous optimum JA on resistance responses by Fujimoto et al. (2011). This result revealed that there was a positive correlation between the application of methyl jasmonate at 0.5 mM. This leads to a significant reduction of plant infection by RKNs.

In addition, *Mi-1* plants transfected with *NahG*, which

leads to the suppression of SA production, did not show any increase in susceptibility to RKN which is in contrast to the results obtained by Branch et al. (2004). They proposed that the low levels of basal SA, which has a crosstalk interaction with JA, could be appropriate for *Mi-1* mediated resistance to RKN because all *Mi-1 NahG* plants showed complete resistance (Bhattarai et al., 2008). These differences between the two studies can be due to different analysis techniques used in the experiment.

## Conclusion

Plant parasitic nematodes have the ability to infest several plant species by manipulation of the basic elements of plant cell development. They can also participate in establishment of feeding site, including multinucleate cell induction, which is the main source of nutrients for reproduction, multiplication and development of nematodes. There are several processes involved in establishment of feeding site and plants also have a defense mechanism to protect the pressure of plant parasitic nematodes. Phytohormones like ethylene and auxin regulate many important processes in the plants such as cell differentiation, cell expansion and responses to abiotic and biotic stresses. They also have a significant role in several plants-pathogen interactions, including regulation of plant defense responses and the development of symptoms. Sedentary parasitic plant nematodes are the world most destructive plant pathogens, need hormones for the formation of a complex feeding site within the host root system. Feeding sites induced by nematodes result in a dramatic change in the host cell morphology and gene expression. These changes are most likely mediated by phytohormones. The development of resistance cultivars through introduction natural resistance gene to the plant is the environmentally friendly way of nematode pest control. This method leads to loss of susceptibility factors in plant responses to plant parasitic nematodes. Several complex gene expressions and subsequent hormone signaling like JA and SA signaling pathways during incompatible interactions between plants and nematodes are considerable aspects to understand the mechanism underlying resistance responses as well as helping to conduct further studies for more details. Furthermore, the importance of plant hormone signaling in the determination of plant-nematode interactions is supported by several evidences. Apart from the contribution of directly defense response, plants also regulate hormone signaling pathway in order to control its vital processes for resistance. The role of phytohormones in establishment of feeding site and plant response in defense mechanisms of nematode is complex. Therefore, further investigation is needed to clearly understand this complex process.

## CONFLICT OF INTERESTS

The author declared that there is no any conflict of interest

## REFERENCES

- Bar-Or C, Kapulnik Y, Koltai H (2005). "A broad characterization of the transcriptional profile of the compatible tomato response to the plant parasitic root knot nematode *Meloidogyne javanica*" *European J. Plant Pathol.* 111:181-192.
- Bellaïf S, Briggs SP (2010). "Nematode effectors and plant responses to infection" *Curr. Opin. Plant Biol.* 13:442-448.
- Bhattarai KK, Xie QG, Mantelin S, Bishnoi U, Girke T, Navarre DA, Kaloshian I (2008). Tomato susceptibility to root-knot nematodes requires an intact jasmonic acid signaling pathway. *Mol. Plant-Microbe Interact.* 21:1205-1214.
- Branch C, Hwang C-F, Navarre DA, Williamson VM (2004). "Salicylic acid is part of the Mi-1-mediated defense response to root-knot nematode in tomato". *Mol. Plant-Microbe Interact.* 17:351-356.
- Caillaud MC, Dubreuil G, Quentin M, Perfus-Barbeoch L, Lecomte P, de Almeida Engler J, Abad P, Rosso MN, Favery B (2008). Root-knot nematodes manipulate plant cell functions during a compatible interaction. *J. Plant Physiol.* 165:104-113.
- Cooper W, Jia L, Goggin L (2005). Effects of jasmonate-induced defenses on root-knot nematode infection of resistant and susceptible tomato cultivars. *J. Chem. Ecol.* 31:1953-1967.
- Curtis RH (2007). "Do phytohormones influence nematode invasion and feeding site establishment"? *Nematology* 9:155-160.
- Davies PJ (2010). "The plant hormones: their nature, occurrence, and functions" In *Plant hormones* (pp. 1-15). Springer Netherlands.
- Djian-Caporalino C, Molinari S, Palloix A, Ciancio A., Fazari A, Marteu N, Ris N, Castagnone-Sereno P (2011). "The reproductive potential of the root-knot nematode *Meloidogyne incognita* is affected by selection for virulence against major resistance genes from tomato and pepper. *Eur. J. Plant Pathol.* 131:431-440.
- Fujimoto T, Tomitaka Y, Abe H, Tsuda S, Futai K, Mizukubo T (2011). Expression profile of jasmonic acid-induced genes and the induced resistance against the root-knot nematode (*Meloidogyne incognita*) in tomato plants (*Solanum lycopersicum*) after foliar treatment with methyl jasmonate. *J. Plant Physiol.* 168:1084-1097.
- Gleason CA, Liu QL, Williamson VM (2008). Silencing a candidate nematode effector gene corresponding to the tomato resistance gene Mi-1 leads to acquisition of virulence. *Mol. Plant-Microbe Interact.* 21:576-585.
- Goggin FL, Jia L, Shah G, Hebert S, Williamson VM, Ullman DE (2006). Heterologous expression of the Mi-1.2 gene from tomato confers resistance against nematodes but not aphids in eggplant. *Mol. Plant-Microbe Interact.* 19:383-388.
- Gutierrez O, Wubben M, Howard M, Roberts B, Hanlon E, Wilkinson J (2009). The role of phytohormones ethylene and auxin in plant-nematode interactions. *Russian J. Plant Physiol.* 56:1-5.
- Hewezi T, Baum TJ (2013). Manipulation of plant cells by cyst and root-knot nematode effectors. *Mol. Plant-Microbe Interact.* 26(1):9-16.
- Jammes F, Lecomte P, Almeida-Engler J, Bitton F, Martin-Magniette ML, Renou JP, Abad P, Favery B (2005). Genome-wide expression profiling of the host response to root-knot nematode infection in *Arabidopsis*. *Plant J.* 44:447-458.
- Jaouannet M, Perfus-Barbeoch L, Deleury E, Magliano M, Engler G, Vieira P, Rosso MN (2012). A root-knot nematode-secreted protein is injected into giant cells and targeted to the nuclei. *New Phytol.* 194(4):924-931.
- Karczmarek A, Overmars H, Helder J, Goverse A (2004). Feeding cell development by cyst and root-knot nematodes involves a similar early, local and transient activation of a specific auxin-inducible promoter element. *Mol. Plant Pathol.* 5:343-346.
- Li Q, Xie QG, Smith-Becker J, Navarre DA, Kaloshian I (2006). Mi-1-mediated aphid resistance involves salicylic acid and mitogen-activated protein kinase signaling cascades. *Mol. Plant-Microbe Interact.* 19:655-664.
- Lilley CJ, Bakhetia M, Charlton WL, Urwin PE (2007). Recent progress in the development of RNA interference for plant parasitic nematodes. *Mol. Plant Pathol.* 8:701-711.
- Melillo MT, Leonetti P, Bongiovanni M, Castagnone-Sereno P, Bleve-Zacheo T (2006). Modulation of reactive oxygen species activities and H<sub>2</sub>O<sub>2</sub> accumulation during compatible and incompatible tomato-root-knot nematode interactions. *New Phytol.* 170:501-512.
- Molinari S, Loffredo E (2006). The role of salicylic acid in defense response of tomato to root-knot nematodes. *Physiol. Mol. Plant Pathol.* 68:69-78.
- Uehara T, Sugiyama S, Matsuura H, Arie T, Masuta C (2010) Resistant and susceptible responses in tomato to cyst nematode are differentially regulated by salicylic acid. *Plant Cell Physiol.* 51:1524-1536.
- Wang D, Pajerowska-Mukhtar K, Culler AH, Dong X (2007) Salicylic acid inhibits pathogen growth in plants through repression of the auxin signaling pathway. *Curr. Biol.* 17:1784-1790.
- Wubben MJE, Jin J, Baum TJ (2008) Cyst nematode parasitism of *Arabidopsis thaliana* is inhibited by salicylic acid (SA) and elicits uncoupled SA-independent pathogenesis-related gene expression in roots. *Mol. Plant-Microbe Interact.* 21:424-432.

Full Length Research Paper

## Superiority of Malawian orange local maize variety in nutrients, cookability and storability

Daiki Murayama<sup>1</sup>, Tomoka Yamazawa<sup>2</sup>, Chandiona Munthali<sup>3</sup>, Ephantus, N. Bernard<sup>2</sup>, Rodney, L. Gondwe<sup>4</sup>, Jiwan, P. Palta<sup>5</sup>, Masayuki Tani<sup>6</sup>, Hiroshi Koaze<sup>6</sup> and Daigo Aiuchi<sup>6\*</sup>

<sup>1</sup>Division of Utilization of Bioresources, The United Graduate School of Agricultural Sciences, Iwate University, 3-18-8 Ueda, Morioka, Iwate, Japan.

<sup>2</sup>Department of Food Science, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido, Japan.

<sup>3</sup>Chitedze Agricultural Research Station, Department of Agricultural Research Services, Chitedze, Lilongwe, Malawi.

<sup>4</sup>Department of Agro-environmental Science, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido, Japan.

<sup>5</sup>Department of Horticulture, University of Wisconsin–Madison, 575 Linden Drive, Madison, WI 53706, USA.

<sup>6</sup>Research Center for Global Agromedicine, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido, Japan.

Received 10 January, 2017; Accepted 5 April, 2017

The objectives of this study were to evaluate the potential of an orange Malawian local maize variety MW5021 by comparing with a hybrid variety DKC-9089 for nutritional quality and processing properties such as proximate and mineral composition as well as pasting and thermal properties of the flour, and resistance against *Prostephanus truncatus* infestation during storage of the grain. Maize plants sampled for the experiments were cultivated under three different fertilizer applications, namely 0, 92 and 184 kg-N/ha, considering the chemical fertilizer input among Malawian smallholder farmers. Even without fertilizer input, significantly higher contents of crude protein, Mg, P, Ca, Fe and Zn were observed in MW5021, as compared with DKC-9089. Among pasting properties, setback of the flour slurry from MW5021 was significantly lower than DKC-9089 for all fertilizer treatments. Two-way ANOVA indicated that MW5021 had significantly lower number of grains damaged by *P. truncatus* than DKC-9089, throughout 12 weeks of storage experiment at 28±2°C. Thus, this study revealed that the Malawian orange local variety MW5021 retained by the smallholder farmers has considerably higher nutritional quality and *P. truncatus* resistance as well as lower retrogradation hardening after cooking the flour, than the hybrid maize DKC-9089.

**Key words:** Maize, Mineral composition, pasting properties, postharvest losses, *Prostephanus truncatus*, Malawi.

### INTRODUCTION

Maize (*Zea mays* L.) is the most important staple food among cereals, providing food and income to millions of

resource-poor smallholder farmers in the eastern and southern Africa (Tefera, 2012). In Malawi, maize-based

diets are the major source of energy accounting for more than 70% of daily calorie intake as well as nutrients (Ecker and Qaim, 2011). Thus, the Malawi government has been implementing the agricultural subsidy programs to improve maize productivity since 2005, and, as a result, the maize yield has increased by 2.9 times in 2014 (Dorward and Chirwa, 2011; FAOSTAT, 2016). Although, the smallholder farmers produce 95% of total maize production in Malawi, their maize yield is still significantly lower than the estate farmer (Mutegi et al., 2015). In addition, a large number of the smallholder farmers cannot afford to use chemical fertilizer without the subsidy (Darko and Ricker-Gilbert, 2013).

The Malawian smallholder farmers generally classify maize into local and hybrid varieties. "Local variety" is defined as the farmers' varieties that are not the direct products of the research system (Heisey and Small, 1995), while the term "hybrid variety" refers to any improved seeds including hybrid and open-pollinated varieties (Ricker-Gilbert and Jones, 2015). The local varieties are preferred by the smallholder farmers, owing to a number of favorable characteristics such as storability, poundability, flour-to-grain ratio and taste (Lunduka et al., 2012). Furthermore, Hwang et al. (2016) reported that Malawian orange local variety (MW5021) has the potential to be a remarkable natural source of carotenoids including provitamin A.

In Malawi, maize is milled into several types of dry flour for cooking staple food of *Nsima*, namely stiff maize flour porridge (Smale, 1995). Chinsinga (2011) and Lunduka et al. (2012) reported that the local varieties are preferred by the smallholder farmers in Malawi because of their taste, as compared with the hybrid maize. Texture of the maize flour porridge is known as one of important traits determining consumer preference (Bolade, 2009), and Osungbaro (2009) stated that pasting properties of maize flour can be used to predict gelling characteristics of the porridge.

After harvest, the smallholder farmers mainly store their maize in plastic sacks until its consumption (Kamanula et al., 2010). *Prostephanus truncatus* is known as the most destructive storage pest causing post-harvest losses on stored maize in the eastern and southern Africa including Malawi (Farrell et al., 1996; Denning et al., 2009). Without chemical insecticides, post-harvest losses of stored maize caused by storage pests at the household-level are reported to be in the range of 40 to 100% in Malawi (Denning et al., 2009). The farmers in Malawi believe that local varieties have higher resistance to storage pests than that of hybrid varieties (Smale, 1995; Chinsinga, 2011; Lunduka et al., 2012). Differences in poundability and flour-to-grain ratio between the local and

hybrid varieties implies existence of varietal differences in grain hardness (Blandino et al., 2010; Lunduka et al., 2012), which has long been suspected to play a role in resistance against the storage pests among others (Meikle et al., 1998).

Limited information of the local varieties is, however, available on the nutritional quality, and the processing properties of its flour as well as storability of the grains against storage pest. Therefore, we chose popular orange local variety of MW5021, and commercial hybrid maize of DKC-9089 to identify the possible varietal differences in:

- (1) Proximate and mineral composition as well as pasting and thermal properties of maize flours and
- (2) Resistance against *P. truncatus* during storage of the grains.

Maize samples subjected to the experiments were cultivated under three different fertilizer applications, namely 0, 92 and 184 kg-N/ha, in consideration with the chemical fertilizer input among Malawian smallholder farmers.

## MATERIALS AND METHODS

### Maize varieties

Two maize varieties were used in the present study, namely an orange local variety (MW5021, locally known as *Mthikinya*) obtained from Malawi Plant Genetic Resources Center in Chitedze Agricultural Research Station (CARS) and a hybrid maize (DKC-9089, DeKalb Genetics Corp., USA).

### Field experiment

To obtain maize samples, six plots were prepared as a bi-factorial experiment where the A factor was three fertilization rates, and the factor B was two maize varieties without randomization. A trial was conducted at the experimental field of CARS from December 2012 to May 2013. Each plot size was 10 m × 10 m consisted of 14 rows with 0.75 m inter-row spacing. Forty seeds were planted in each row with 0.25 m in-row spacing. Two types of chemical fertilizers, which include a compound fertilizer (N: P: K= 23: 21: 0% and 4% of S) and urea (46% of N), were applied in a ratio of 2:3 to give 0, 92 and 184 kg-N/ha, and coded as treatments A, B and C, respectively. Of these, the treatment B is the standard fertilization application recommended by the Ministry of Agriculture and Food Security in Malawi (Mutegi et al., 2015). Three plots, where the hybrid variety of DKC-9089 was planted, were coded as plots AH, BH and CH, while the rest three plots cultivated with the local variety of MW5021 were coded as plots AL, BL and CL, respectively. Maize cobs were shelled after harvesting and drying, grains were weighed for calculation of yield and stored in plastic bags. Grain yields of AH, BH, CH, AL, BL and CL were 7.7, 10.7, 11.3, 4.2, 6.4 and 6.5 Mg/ha, respectively.

\*Corresponding author. E-mail: aigo@obihiro.ac.jp. Tel: +81 155 49 5934. Fax: +81 155 49 5229.

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](https://creativecommons.org/licenses/by/4.0/)



### Analytical methods of chemical properties

Maize grains were kept in a deep freezer at  $-30^{\circ}\text{C}$  for disinfection of storage pests until use, and thawed at  $4^{\circ}\text{C}$  for 1 week to determine the chemical composition. Thawed maize grains were placed at room temperature for 1 h, and milled by a blender (New Power Mill PM-2005, OSAKA Chemical Co. Ltd., Japan) to obtain maize flour with less than  $350\ \mu\text{m}$  of particle size. The maize flour samples were stored at  $4^{\circ}\text{C}$  until use. Moisture, crude protein, crude fat and ash contents were determined using the standard methods by the Association of Official Analytical Chemists (AOAC) with triplicate determinations (AOAC International, 2005). Mineral contents of magnesium (Mg), phosphorus (P), potassium (K), calcium (Ca), iron (Fe) and zinc (Zn) were determined by an inductively coupled plasma mass spectrometry (ICP; ICPS-8100, Shimadzu Co. Ltd., Japan) with triplicate determinations based on the method reported by George et al. (2004) with slight modification. Maize flour in a porcelain crucible was preliminary ashed using a Bunsen burner until smoking had ceased, and then it was dry-ashed using a muffle furnace (FM-35, Yamato Scientific Co., Ltd., Japan) at  $550^{\circ}\text{C}$  for 8 h. The resultant ash was dissolved in 100 mL of 2N-HCl, and then diluted 10-fold using deionized water. The solution obtained was subjected to mineral analysis by ICP.

### Analytical methods of physical properties

Pasting and thermal properties of maize flours from three treatments were determined for the two varieties. Pasting properties were measured based on the method reported by Uarrota et al. (2013). Twenty-eight grams of maize flour slurry (8% dry wt.) were subjected to analysis using a Rapid Visco Analyzer (RVA-4, Newport Scientific, Inc., USA) with a paddle rotating at 960 rpm for the first 10 s, and then at a fixed speed of 160 rpm. The slurry was heated from  $50$  to  $95^{\circ}\text{C}$  at a rate of  $12^{\circ}\text{C}/\text{min}$ , held at  $95^{\circ}\text{C}$  for 2.5 min, cooled to  $50^{\circ}\text{C}$  at a rate of  $12^{\circ}\text{C}/\text{min}$ , and then kept at  $50^{\circ}\text{C}$  for 2 min. Breakdown is defined as the difference between peak and trough viscosities, and setback is the difference between trough and final viscosities.

Thermal properties were determined using a differential scanning calorimeter (DSC) (DSC7000X, Hitachi High-Tech Science Corp., Japan). A sample of 18 mg dry wt. was weighed in a DSC pan, and deionized water was added to give a suspension of 30% dry wt. The scanning temperature range was set at  $30$  to  $95^{\circ}\text{C}$ . and the heating rate was  $1.5^{\circ}\text{C}/\text{min}$ . Forty-two milligrams of water was used as a reference. Thermal transitions of maize flour samples were defined as  $T_o$ ,  $T_p$  and  $T_c$  for initial, peak and conclusion temperatures.

Maize grains kept in a deep freezer at  $-30^{\circ}\text{C}$  were used to determine hardness of maize grains after thawing at  $4^{\circ}\text{C}$  for 1 week. Thawed maize grains were dried in a hot air oven (WFO-700, TOKYO RIKAKIKI Co. Ltd., Japan) at  $40^{\circ}\text{C}$ —until they achieve  $13.0\pm 1.0\%$  of moisture content, and resultant dried maize grains were stored at  $20^{\circ}\text{C}$  for 1 week to obtain an equilibrium moisture content. After adjustment of moisture content of maize grains, grain hardness was determined using the method reported by Blandino et al. (2010). Puncture test was carried out on upper lateral surface of 25 maize grains each from 6 experimental plots using a texture analyzer (TA-XT2, Stable Micro Systems, UK) equipped with a flat probe (diameter 2 mm, P/2) and 25 kg load cell. The tests were performed at 1 mm/s of puncture speed, and grain was punctured to a depth of 2 mm. Grain hardness was expressed as break force calculated in Newton (N).

### Evaluation methods of *Prostephanus truncatus* resistance

*P. truncatus* resistance was evaluated according to the methods

reported by Abebe et al. (2009) and Tefera et al. (2011) with slight modification. Each evaluation was conducted with triplicate determinations using 6 different samples of maize grains after adjustment of moisture content with  $13.0\pm 1.0\%$ . 100 g of maize grains were placed in a glass jar covered with stainless screen lid (60 mesh). *P. truncatus* used in this study was obtained from CARS, and successively generated in the laboratory. Thirty adult *P. truncatus* (1-14 days old) were released to each jar containing maize grains, and these jars were kept in an incubator (MIR-254, Panasonic Healthcare Co., Ltd., Japan) at  $28\pm 2^{\circ}\text{C}$ . Number of adult *P. truncatus* and damaged grains were measured on 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 8<sup>th</sup> and 12<sup>th</sup> weeks after release. All *P. truncatus* were removed from maize grains using sieves with 1.0 and 4.0 mm openings, and then live *P. truncatus* were counted for determining number of adult present. For measuring number of damaged grains, 100 maize grains were randomly taken from each jar, and then damaged grains namely grains holed by *P. truncatus* were counted with triplicate. Grains and live *P. truncatus* were returned to each jar for further measurement.

### Statistical analysis

Statistical analyses were conducted using statistical package for social sciences (SPSS) for Windows (ver. 17.0). Two-way analysis of variance (ANOVA) was performed to evaluate the main effects of maize variety, and fertilization treatment as well as the interaction at 5% significance level. When significant interaction was detected between the variety and the fertilization treatment, *t*-test and Tukey's multiple-range test were used for comparison of means to assess the variety difference with the same fertilization and the amount of fertilizer application among each variety, respectively, at 5% significance level. Pearson's correlation analysis was conducted to evaluate the effects of grain hardness on resistance against *P. truncatus*.

## RESULTS

### Nutritional quality of maize flours

Nutritional quality, namely proximate and mineral compositions of MW5021 and DKC-9089 applied with the different amounts of fertilizer are shown in Table 1. Ash, crude protein and crude fat contents of the two varieties ranged between 1.11 to 1.34, 9.23 to 11.80 and 5.17 to 5.58% dry wt., respectively. Most dominant mineral for both varieties was P followed by K, Mg, Ca, Fe and Zn with 212.20-287.83, 116.36-139.79, 86.31-108.07, 4.87-5.73, 2.93-5.43 and 1.09-1.85 mg/100g on dry wt., respectively. Table 2 represents the corresponding results of two-way ANOVA. Significant effects of the variety were observed in all components, and significant main effects of the fertilization treatment were detected in all components, except for K and Zn ( $p < 0.05$ ). Significant interactions were also found in all components, excluding ash and Ca. Thus, nutritional quality components, except for ash and Ca, were compared by *t*-test for assessing the effect of variety with the same fertilization, while Tukey's test for the fertilizer application was used to compare among each variety.

Among proximate composition, MW5021 showed significantly higher contents of ash regardless the

**Table 1.** Nutritional quality of Malawian orange local maize variety of MW5021 and hybrid maize of DKC-9089 grown in Malawi.

Variable	MW5021			DKC-9089		
	Fertilizer treatments					
	A	B	C	A	B	C
<b>Proximate composition (% dry wt.)</b>						
Ash	1.27±0.01b*	1.28±0.04ab*	1.34±0.02a*	1.12±0.02b	1.11±0.01b	1.18±0.02a
Crude protein	10.20±0.05c*	11.16±0.04b*	11.80±0.09 a*	9.23±0.07c	9.77±0.03b	10.81±0.02a
Crude fat	5.32±0.03b	5.49±0.07a*	5.34±0.06b	5.24±0.06b	5.17±0.01b	5.58±0.01a*
<b>Mineral composition (mg/100g, dry wt.)</b>						
Mg	102.08±0.69b*	108.07±0.96a*	95.33±1.6c	86.31±1.55 c	92.94±0.29b	97.39±1.45a
P	276.41±2.08b*	287.83±4.35a*	261.60±3.45c	212.20±4.66 c	227.67±0.42b	244.50±5.62a
K	132.46±2.58a	132.95±2.51a	116.36±3.62b	127.92±1.94 b	129.94±1.06b	139.79±1.09a*
Ca	5.73±0.09a*	5.09±0.35a	5.32±0.06a	5.08±0.22 a	4.87±0.11a	5.31±0.38a
Fe	5.43±0.06a*	3.33±0.01b	2.93±0.15c	4.34±0.02 a	3.29±0.07c	3.76±0.08b*
Zn	1.83±0.16a*	1.85±0.12a*	1.62±0.03a*	1.09±0.05 b	1.18±0.02a	1.29±0.01a

Mean values in the same column with different letters are significantly different among each variety ( $p < 0.05$ ); Mean values in the same column with \* are significantly higher than that of the corresponding maize variety with the same fertilizer treatments ( $p < 0.05$ ); n=3; Fertilizer treatments; A, 0 kg N/ha; B, 92 kg N/ha; C, 184 kg N/ha.

**Table 2.** Two-way ANOVA for the effect of maize variety and fertilizer treatment on nutritional quality of MW5021 and DKC-9089 grown in Malawi.

Variable	Variety	Fertilizer treatment	Variety × Fertilizer	<i>p</i> value			
Proximate composition							
Ash				<0.001	<0.001		0.745
Crude protein				<0.001	<0.001		<0.001
Crude fat				0.029	<0.001		<0.001
Mineral composition							
Mg				<0.001	<0.001		<0.001
P				<0.001	<0.001		<0.001
K				0.001	0.114		<0.001
Ca				0.030	0.023		0.128
Fe				0.012	<0.001		<0.001
Zn				<0.001	0.541		0.014

Values of *p* in bold are significant ( $p < 0.05$ ).

fertilizer level than that of DKC-9089, based on the two-way ANOVA ( $p < 0.001$ ). For the same fertilizer treatment, MW5021 had significantly higher contents of crude protein than that of DKC-9089 counterparts ( $p < 0.05$ ). Main effect of the fertilizer level was observed in ash content by the two-way ANOVA, that is, the highest input of fertilizer led to significant increase in ash contents as compared with the treatments of A and B ( $p < 0.05$ ). It was observed that increase in amounts of fertilizer led to significant increase in crude protein contents of both MW5021 and DKC-9089 ( $p < 0.05$ ),

while significant increase in crude fat contents were observed only in DKC-9089 with the treatment C as compared to the treatments A and B ( $p < 0.05$ ).

For mineral composition, Ca contents were significantly higher in MW5021 than those of DKC-9089 ( $p < 0.05$ ), based on the two-way ANOVA. When compared between the two varieties for the same fertilizer treatments of A and B, Mg and P contents were consistently and significantly higher in MW5021 than that of DKC-9089 ( $p < 0.05$ ). Similarly, Zn contents were also higher in MW5021 when compared with DKC-9089 for all fertilizer

**Table 3.** Physical properties of maize grains and flours of Malawian orange local maize variety of MW5021 and hybrid maize of DKC-9089 grown in Malawi.

Variable	MW5021			DKC-9089		
	Fertilizer treatments					
	A	B	C	A	B	C
Pasting properties						
Peak viscosity (cP)	375.0±4.0a	304.3±7.8b	313.7±3.1b	544.7±4.0a*	444.0±1.7b*	428.0±4.4c*
Breakdown (cP)	7.0±1.0b	13.3±1.5a	10.0±1.7ab	83.0±4.4a*	61.3±0.6c*	75.0±3.0b*
Setback (cP)	909.3±15.9a	783.3±23.2b	802.7±6.7b	1017.3±20.0a*	913.0±7.8b*	889.7±18.0c*
Final viscosity (cP)	1277.3±19.2a	1074.3±31.6b	1106.3±10.3b	1479.0±16.5a*	1295.7±6.8b*	1242.7±20.0b*
Pasting temperature (°C)	90.2±0.1b*	90.3±0.1 b*	92.0±0.1a*	87.2±0.1b	88.5±0.4a	88.0±0.1 a
Thermal properties						
T <sub>o</sub> (°C)	69.90±0.32a	69.98±0.18a	69.92±0.07a	69.73±0.08a	69.79±0.43a	69.99±0.23 a
T <sub>p</sub> (°C)	75.87±0.11a*	75.69±0.06a*	75.93±0.14a*	75.36±0.15a	75.34±0.15a	75.49±0.13 a
T <sub>c</sub> (°C)	82.06±0.54a*	82.28±0.17a*	82.54±0.3a*	80.45±0.11a	80.43±0.61a	80.45±0.16 a
ΔH (J/g, dry wt.)	6.78±0.22a	6.29±0.07b	6.80±0.18a	7.73±0.21a*	7.06±0.05b*	6.88±0.15 b
Grain property						
Hardness (N)	246.73±53.3a*	232.56±41.9a*	239.19±54.1a*	152.58±25ab	145.74±23.8b	172.33±50.0 a

Mean values in the same column with different letters are significantly different among each variety ( $p < 0.05$ ); Mean values in the same column with \* are significantly higher than that of the corresponding maize variety with the same fertilizer treatments ( $p < 0.05$ ); Hardness,  $n=25$ ; pasting and thermal properties,  $n=3$ ; Fertilizer treatments: A, 0 kg N/ha; B, 92 kg N/ha; C, 184 kg N/ha.

treatments ( $p < 0.05$ ). The two-way ANOVA showed significant differences in Ca contents with fertilizer levels, where higher Ca content with the treatment A than that with C was observed. There were no consistent patterns for other minerals for the differences among varieties or fertilizer treatments. However, at no fertilizer treatment of A, MW5021 had consistently higher Mg, P, Ca, Fe and Zn contents as compared to DKC-9089.

### Pasting and thermal properties of maize flours

Table 3 indicates physical properties of maize grains and flours of MW5021 and DKC-9089 with the different fertilizer applications. Results of the two-way ANOVA in Table 4 showed significant effects of the variety and/or the fertilization treatments in setback, T<sub>p</sub> and T<sub>c</sub> without significant interactions ( $p < 0.001$ ).

Among pasting properties, significant effect of the variety in setback by the two-way ANOVA indicated that the values of MW5021 were consistently lower than those of DKC-9089, regardless the fertilization levels ( $p < 0.001$ ). In comparison between the two varieties, peak viscosity, breakdown, and final viscosity of DKC-9089 were significantly higher than those of MW5021 at the same fertilizer treatments ( $p < 0.05$ ). Contrary, MW5021 showed significantly higher pasting temperatures than that of DKC-9089 in all fertilizer applications ( $p < 0.05$ ). The two-way ANOVA showed significant effect of the fertilization on setback with the highest value with the

treatment A as compared with B and C ( $p < 0.05$ ). Significant decrease in peak viscosities was observed in both MW5021 and DKC-9089 with increase in the amount of fertilizer applied ( $p < 0.05$ ). Similarly, in final viscosity, significant decrease with increase in the fertilizer application was found in both varieties ( $p < 0.05$ ).

For thermal properties, T<sub>p</sub> and T<sub>c</sub> were significantly higher in MW5021 of all fertilizer treatments as compared with those of DKC-9089, with significant effects of the variety from two-way ANOVA as shown in Table 4 ( $p < 0.001$ ). Significant differences resulting from the fertilizer treatments were not observed in T<sub>o</sub>, T<sub>p</sub> or T<sub>c</sub> among MW5021 and DKC-9089 ( $p < 0.05$ ), while DKC-9089 showed significantly higher ΔH than MW5021 in the fertilizer treatments of A and B.

### Hardness and storability of maize grains

Grain hardness of two varieties for the different fertilizer treatments were also shown in Table 3. Significant effect of the variety was observed by two-way ANOVA as shown in Table 4 without significant effect of the fertilization or the interaction ( $p < 0.001$ ). This means that grain hardness of MW5021 was significantly higher than that of DKC-9089 counterparts in each fertilizer treatment.

Three-way ANOVA carried out with the sources of the variety, fertilization treatment and storage period for numbers of adult *P. truncatus* and damaged grains

**Table 4.** Two-way ANOVA for the effect of maize variety and fertilizer treatment on physical properties of MW5021 and DKC-9089 grown in Malawi.

Variable	Variety	Fertilizer treatment	Variety × Fertilizer
	<i>p</i> value		
Pasting properties			
Peak viscosity	<0.001	<0.001	<0.001
Breakdown	<0.001	<0.001	<0.001
Setback	<0.001	<0.001	0.122
Final viscosity	<0.001	<0.001	0.006
Pasting temperature	<0.001	<0.001	<0.001
Thermal properties			
T <sub>o</sub>	0.436	0.639	0.625
T <sub>p</sub>	<0.001	0.065	0.585
T <sub>c</sub>	<0.001	0.549	0.543
ΔH	<0.001	<0.001	0.001
Grain property			
Hardness	<0.001	0.155	0.267

Values of *p* in bold are significant (*p* < 0.05).

**Table 5.** Two-way ANOVA for the effect of maize variety and fertilizer treatment on *P. truncatus* resistance of MW5021 and DKC-9089 grown in Malawi.

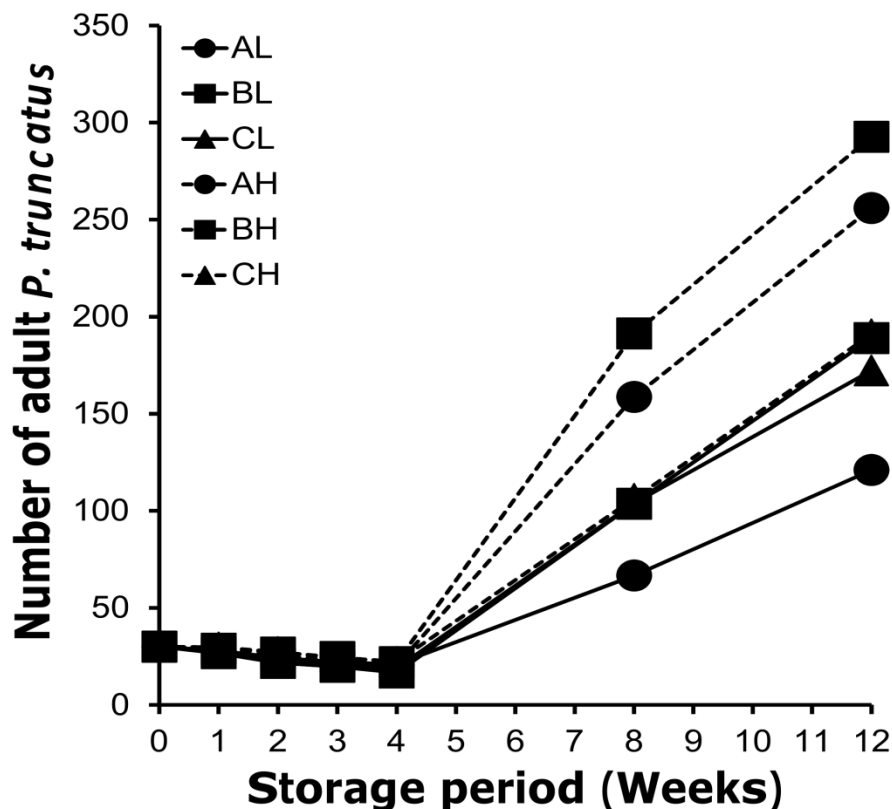
Variable	Storage period	Variety	Fertilizer treatment	Variety × Fertilizer
	(week)	<i>p</i> value		
<i>P. truncatus</i> resistance				
Number of adult <i>P. truncatus</i>	1 <sup>st</sup>	0.092	0.574	0.302
	2 <sup>nd</sup>	0.001	0.805	0.124
	3 <sup>rd</sup>	0.024	0.978	0.448
	4 <sup>th</sup>	0.079	0.672	0.239
	8 <sup>th</sup>	0.070	0.500	0.425
	12 <sup>th</sup>	0.092	0.545	0.590
Number of damaged grains	1 <sup>st</sup>	0.005	0.720	0.495
	2 <sup>nd</sup>	0.001	0.461	0.676
	3 <sup>rd</sup>	0.001	0.242	0.667
	4 <sup>th</sup>	<0.001	0.161	0.327
	8 <sup>th</sup>	0.007	0.167	0.524
	12 <sup>th</sup>	0.003	0.466	0.680

Values of *p* in bold are significant (*p* < 0.05).

showed a significant interaction for the variety with storage period (*p* < 0.001). Thus, two-way ANOVA for numbers of adult *P. truncatus* and damaged grains were performed at each storage period with the variety and the fertilization treatment as sources (Table 5).

Numbers of adult *P. truncatus* counted on 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 8<sup>th</sup> and 12<sup>th</sup> week of storage are illustrated in Figure 1. Numbers of adult *P. truncatus* slightly decreased in the first 4 weeks of storage in all samples, and then

increased steadily from 4 to 12<sup>th</sup> weeks. The highest number of adult *P. truncatus* was observed in DKC-9089 with the fertilization treatment of B as 292.7 on 12<sup>th</sup> week of storage, while in MW5021 with the treatment of A showed the lowest number of adults as 121.0. After 8 weeks of storage, DKC-9089 fertilized with the treatments A and B showed clearly higher numbers of adult *P. truncatus* among all samples. Results of two-way ANOVA are shown in Table 5, and significant effects of



**Figure 1.** Number of adult *P. truncatus* present during 12 weeks of incubation of maize grains of Malawian orange local variety of MW5021 (AL, BL, CL) and hybrid maize of DKC-9089 (AH, BH, CH). At time zero, 30 adults were released into jars containing 100 g maize grains. A, B and C refers to fertilizer treatments.

the variety were observed in numbers of adult *P. truncatus* on 2<sup>nd</sup> and 3<sup>rd</sup> weeks with lower numbers in MW5021 ( $p < 0.05$ ). On the other hand, significant effects of either the fertilization or the interactions were not observed at any storage periods.

Number of damaged grains of MW5021 and DKC-9089 during 12 weeks of infestation by *P. truncatus* is shown in Figure 2. On 12<sup>th</sup> week of storage, the highest number of damaged grains was observed in DKC-9089 with the fertilization treatment of B as 84.3 grains/100 grains, and in MW5021 with the treatment of A showed the lowest number.

During 12 weeks of storage experiment, MW5021 consistently showed lower numbers of damaged grains as compared with DKC-9089. From two-way ANOVA, significant effects of the variety were found throughout 12 weeks of storage periods ( $p < 0.001$  to  $p = 0.007$ ) with lower values for MW5021, while the significant effects of either the fertilization or the interactions were not found at any measuring date ( $p > 0.05$ ).

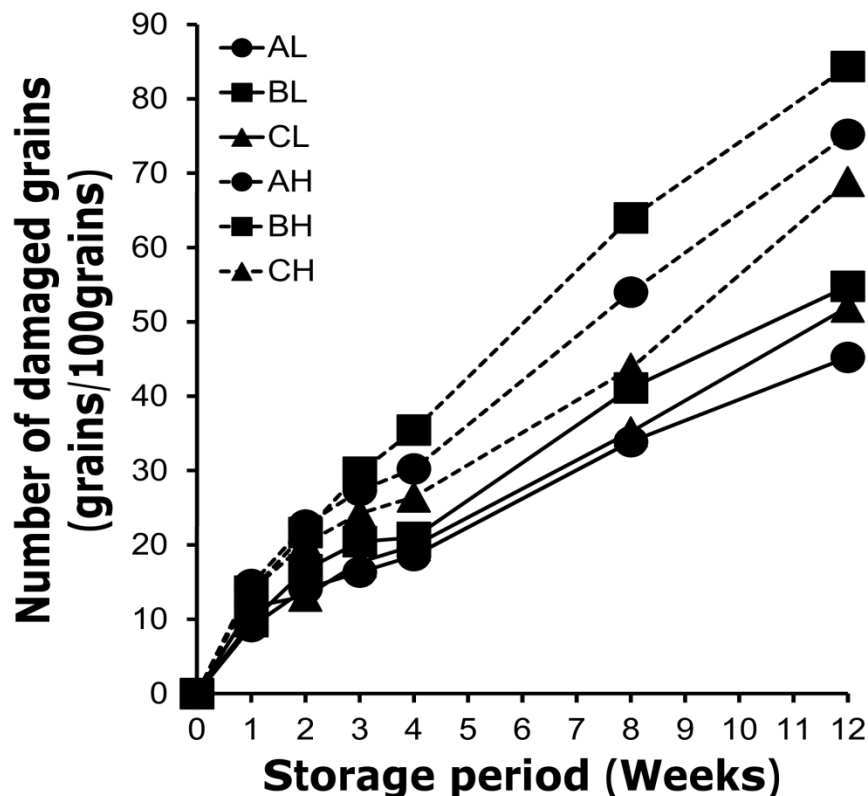
### Correlation analysis

Table 6 summarizes correlations between hardness and

*P. truncatus* resistance of maize grain measured throughout 12 weeks of storage experiment. There were significantly negative correlations between the number of adult *P. truncatus* and grain hardness on 3<sup>rd</sup>, 8<sup>th</sup> and 12<sup>th</sup> weeks ( $p < 0.05$ ). The number of damaged grains was negatively, and significantly correlated with grain hardness throughout the storage periods ( $p < 0.05$ ).

### DISCUSSION AND CONCLUSION

Widespread deficiencies of Fe and, particularly, Zn have been known in Malawi (Dickinson et al., 2014). Furthermore, high risk of mineral deficiencies such as Ca and Zn was reported by Joy et al. (2015). In the present study, without fertilizer input, Ca and Fe contents of MW5021 were significantly higher than those of DKC-9089 (Table 1). In addition, in all fertilizer treatments, Zn contents of MW5021 were significantly higher than those of DKC-9089 (Table 1), and this was also statistically confirmed by two-way ANOVA analysis (Table 3). Bioavailability of Zn contained in maize is thought to be restricted, due to the presence of phytic acid which inhibits Zn absorption by binding Zn to form an unabsorbable complex called phytate in the gut (Krebs,



**Figure 2.** Number of damaged grains present during 12 weeks of incubation of maize grains of Malawian orange local variety of MW5021 (AL, BL, CL) and hybrid maize of DKC-9089 (AH, BH, CH). At time zero, 30 adults were released into jars containing 100 g maize grains. A, B and C refers to fertilizer treatments.

**Table 6.** Correlations between grain hardness and *P. truncatus* resistances on throughout 12 weeks of incubation.

Variable	Storage period (week)	Grain hardness
Number of adult <i>P. truncatus</i>	1 <sup>st</sup>	-0.566
	2 <sup>nd</sup>	-0.800
	3 <sup>rd</sup>	-0.829*
	4 <sup>th</sup>	-0.587
	8 <sup>th</sup>	-0.860*
	12 <sup>th</sup>	-0.881*
Number of damaged grains	1 <sup>st</sup>	-0.913*
	2 <sup>nd</sup>	-0.964**
	3 <sup>rd</sup>	-0.975**
	4 <sup>th</sup>	-0.962**
	8 <sup>th</sup>	-0.909*
	12 <sup>th</sup>	-0.979**

Correlation coefficients with \* and \*\* are significant at  $p < 0.05$  and  $0.01$ , respectively.

2000). However, Miller et al. (2013) reported that dietary Ca and protein modestly enhanced Zn absorption by possible inhibition by forming complex form phytate with Zn. Thus, not only quantity but also bioavailability of Zn

contained in MW5021 might be superior to the DKC-9089, owing to significantly higher Ca and crude protein contents of MW5021 (Table 1). It was, therefore, found, that an intake of maize-based diets prepared from

MW5021 may be desirable in effectivity supplying insufficient minerals such as Ca, Fe and Zn as compared with the DKC-9089, especially for the smallholder farmers who cannot always afford fertilizer for maize cultivation (Darko and Ricker-Gilbert, 2013).

In this study, whole maize flours were used to evaluate pasting and thermal properties, since whole maize grain flour called Mgaiwa is commonly used to cook Nsima in Malawi. Significant differences in pasting properties suggested that varietal differences exist between the flour prepared from MW5021 and DKC-9089 on texture of Nsima. Maize flour prepared from DKC-9089 showed significantly higher peak viscosity, breakdown, setback and final viscosity than those of the MW5021 (Table 2), and this is may be due to relatively and significantly lower crude protein and ash contents, which indicate higher starch content (Table 1). Indeed, it is reported that starch content is prime factor determining viscosity of maize flour (Zilic et al., 2011). Further, Almeida-Dominguez et al. (1997) reported that maize flour prepared from softer grain shows higher viscosity than that of harder one, due to loosely packed starch with reduced protein-to-starch bounds in softer maize grain, and such loosely packed starch hydrates and swells more rapidly in the presence of heat and water.

During cooking of *Nsima*, continuous heating and stirring is applied to achieve uniform gelatinization of starch without formation of clumps. Thus, significantly lower breakdowns of the flours prepared from MW5021 indicate a relatively high ability to withstand heating and shear stress than those of DKC-9089, that is, higher stability of the gel during the cooking (Table 2; Newport Scientific, 1998). From the results of setback, it is clear that there are changes incurred in texture of *Nsima* after cooking, between MW5021 and DKC-9089. Setback indicates short-time retrogradation hardening of starch dominantly determined by amylose aggregation and re-association (Karim et al., 2000). In this study, setbacks of maize flours prepared from MW5021 were significantly lower than those of the MW5021 for all fertilization treatments (Table 2). Sandhu and Singh (2007) reported that the gel firming is mainly caused by retrogradation of maize starch gels during cooling. Thus, *Nsima* cooked from the flour prepared from MW5021 may be more tolerant against starch retrogradation, that is, hardening tendency of the *Nsima* made from the MW5021 flour may be being slower than that prepared from the DKC-9089 flour. Physical properties such as viscosity and retrogradation tendency may influence the differences in consumer preferences between maize flours prepared from the MW5021 and DKC-9089.

In Malawi, post-harvest losses of stored maize grain caused by storage pests have been reported to be 40 to 100%, and *P. truncatus* is known to be the major cause of such losses (Denning et al., 2009). Thus, higher storage ability, arising from lower post-harvest losses caused by *P. truncatus*, must be considered for choosing

maize varieties for cultivation not only in Malawi but also in African countries invaded by this pest. In the present study, two-way ANOVA analysis suggests that MW5021 has the higher potential of reducing post-harvest losses (Table 3) by lowering numbers of adult *P. truncatus* and damaged grains, as compared with DKC-9089 (Figure 1 and 2). Under the experimental conditions, superior storability of the orange local variety of MW5021 was observed in this study, and it enables us to further discuss the post-harvest losses of maize grains during storage. MW5021 cultivated under three different fertilizer applications showed 24.5 to 40.0 % lower number of damaged grains as compared with the corresponding DKC-9089 after 12 weeks of storage period incubation, while yields were 40.2 to 45.5 % lower in the local variety. On the other hand, the estimated amounts of wholesome maize grains after 12 weeks of storage, based on the yields and percentages of damaged grains, were 1.9, 1.7, 3.5, 2.3, 2.9 and 3.1 Mg/ha for AH, BH, CH, AL, BL and CL, respectively. Therefore, under *P. truncatus* infestation, MW5021 may have higher amount of maize grains remaining after prolonged storage as compared to DKC-9089, particularly for the smallholder farmers who cannot afford to use chemical fertilizer.

Puncture test was conducted on maize grain, and break force was defined as grain hardness in this study. It is assumed that hardness of maize grain is mainly influenced by the chemical composition of endosperm (Dombrink-Kurtzman and Knutson, 1997; Chandrashekar and Mazhar, 1999), indicating that grain hardness is compression resistance of endosperm but not hull. Santiago et al. (2013) reported that grain defenses against storage pests occur in both pre-ingestion and post-ingestion phase. In the pre-ingestion phase, host plants can limit food supplies to insects by physical barriers such as the cell wall. Therefore, grain hardness may be regarded as strength of physical barrier, and harder grain could limit food supply to *P. truncatus* in the pre-ingestion phase. This limit of food supply may result in suppressing propagation of *P. truncatus*. The observed significantly negative correlations between grain hardness and, the number of adults and damaged grains (Table 4) support this assumption. In addition, Li (1988) stated that, since *P. truncatus* lay eggs in blind-ending tunnel in maize grain, higher energy cost for tunneling through harder maize grain, leads to reduction of its fecundity and population size. Several local varieties native to Africa have been reported to have higher resistance against *Sitophilus zeamais* than hybrid varieties (Gudrupsa et al., 2001; Siwale et al., 2009), while the present study provides evidence for the superiority of the Malawian local orange variety of MW5021 over the commercial hybrid maize of DKC-9089 for losses caused by *P. truncatus* infestation during post-harvest storage.

In the present study, one genotype was chosen from each of local and hybrid varieties to obtain fundamental

information on the varietal differences. MW5021 showed superiority in:

- (1) Significantly higher contents of crude protein, Mg, P, Ca, Fe and Zn even without fertilizer application
- (2) Cookability with significantly lower setback value for all fertilizer treatments and
- (3) Storability with significantly lower number of damaged grains throughout 12 weeks of storage experiment, as compared with DKC-9089.

It appears therefore that the orange local variety of MW5021 is beneficial for low-input sustainable agriculture among the smallholder farmers with higher nutritional value, better porridge retrogradation tendency and lower post-harvest losses.

Thus, further search of local varieties with desired characteristics should be carried out to achieve food security and provide genetic sources on breeding for variety development in the eastern and southern Africa region.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge Mr. Charles Singano and Malawi Plant Genetic Resources Center for providing the maize seeds. We are grateful to Mr. Arsenio Chimphamba and Ms. Mary Chatambalala in the Chitedze Agricultural Research Station for field management during cultivation of the maize samples. The authors also wish to thank the Chitedze Agricultural Research Station and Department of Agricultural Research Services for supporting field experiments. This work was supported by JSPS KAKENHI Grant Number 26304022.

## REFERENCES

- Abebe F, Tefera T, Mugo S, Beyene Y, Vidal S (2009). Resistance of maize varieties to the maize weevil *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculionidae). *Afr. J. Biotechnol.* 8(21):5937-5943.
- Almeida-Dominguez HD, Suhendro EL, Rooney LW (1997). Factors affecting rapid visco analyser curves for the determination of maize kernel hardness. *J. Cereal Sci.* 25(1):93-102.
- AOAC (2005). Official methods of analysis of AOAC International. 18<sup>th</sup> ed. AOAC International, Gaithersburg, MD, USA.
- Blandino M, Mancini MC, Peila A, Rolle L, Vanara F, Reyneri A (2010). Determination of maize kernel hardness: comparison of different laboratory tests to predict dry-milling performance. *J. Sci. Food Agric.* 90(11):1870-1878.
- Bolade MKM (2009). Effect of flour production methods on the yield, physicochemical properties of maize flour and rheological characteristics of a maize-based non-fermented food. *Afr. J. Food Sci.* 3(10):288-298.
- Chandrashekar A, Mazhar H (1999). The biochemical basis and implications of grain strength in sorghum and maize. *J. Cereal Sci.* 30(3):193-207.
- Chinsinga B (2011). Seeds and subsidies: the political economy of input programmes in Malawi. *IDS Bull.* 42(4):59-68.
- Darko F, Ricker-Gilbert J (2013). Economic efficiency and subsidized farm inputs: Evidence from Malawi maize farmers. Invited paper presented at the 4<sup>th</sup> International Conference of the African Association of Agricultural Economists. Hammamet, Tunisia.
- Denning G, Kabambe P, Sanchez P, Malik A, Flor R, Harawa R, Nkhoma P, Zamba C, Banda C, Magombo C, Keating M, Wangila J, Sachs J (2009). Input subsidies to improve smallholder maize productivity in Malawi: toward an african green revolution. *PLoS Biol.* 7(1):2-10.
- Dickinson N, Rankin J, Pollard M, Maleta K, Robertson C, Hursthouse A (2014). Evaluating environmental and social influences on iron and zinc status of pregnant subsistence farmers in two geographically contrasting regions of Southern Malawi. *Sci. Total Environ.* 500-501:199-210.
- Dombrink-Kurtzman MA, Knutson CA (1997). A Study of maize endosperm hardness in relation to amylose content and susceptibility to damage. *Cereal Chem.* 74(6):776-780.
- Dorward A, Chirwa E (2011). The Malawi agricultural input subsidy programme: 2005/06 to 2008/09. *Int. J. Agric. Sustain.* 9(1):232-247.
- Ecker O, Qaim M (2011). Analyzing nutritional impacts of policies: an empirical study for malawi. *World Dev.* 39(3):412-428.
- FAO (2016). FAOSTAT: Statistical Database. <http://faostat.fao.org>.
- Farrell G, Hill MG, Nang'Ayo FLO, Stabrawa A (1996). A review of investigations to improve pest management of stored maize in smallholder farms in Kenya. *Integr. Pest Manag. Rev.* 1(4):251-263.
- George C, Ridley WP, Obert JC, Nemeth MA, Breeze ML, Astwood JD. (2004). Composition of grain and forage from corn rootworm-protected corn event MON 863 is equivalent to that of conventional corn (*Zea mays* L.). *J. Agric. Food Chem.* 52(13):4149-4158.
- Gudrupsa I, Floyd S, Kling JG, Bosque-Perez NA, Orchard JE (2001). A comparison of two methods of assessment of maize varietal resistance to the maize weevil, *Sitophilus zeamais* Motschulsky, and the influence of kernel hardness and size on susceptibility. *J. Stored Prod. Res.* 37(3):287-302.
- Heisey PW, Smale M (1995). Maize technology in Malawi: a green revolution in the making? CIMMYT Research Report No. 4, CIMMYT. Mexico DF, Mexico.
- Hwang T, Ndolo VU, Katundu M, Nyirenda B, Bezner-Kerr R, Arntfield S, Beta T (2016). Provitamin A potential of landrace orange maize variety (*Zea mays* L.) grown in different geographical locations of central Malawi. *Food Chem.* 196(1):1315-1324.
- Joy EJM., Bradley MR, Young SD, Black CR, Chilimba ADC, Ander EL, Barlow TS, Watts MJ (2015). Soil type influences crop mineral composition in Malawi. *Sci. Total Environ.* 505(1):587-95.
- Kamanula J, Sileshi GGW, Belmain SR, Sola P, Mvumi BM, Nyirenda GKC, Nyirenda SP, Stevenson PC (2010). Farmers' insect pest management practices and pesticidal plant use in the protection of stored maize and beans in Southern Africa. *Int. J. Pest Manag.* 57(1):41-49.
- Karim AA, Norziah MH, Seow CC (2000). Methods for the study of starch retrogradation. *Food Chem.* 71(1):9-36.
- Krebs NF (2000). Overview of zinc absorption and excretion in the human gastrointestinal tract. *J. Nutr.* 130(5):1374S-1377S.
- Li L (1988). Behavioural ecology and life history evolution in the Larger Grain Borer, *Prostephanus truncatus* (Horn). Ph.D. thesis. University of Reading, Reading, UK.
- Lunduka R, Fisher M, Snapp S (2012). Could farmer interest in a diversity of seed attributes explain adoption plateaus for modern maize varieties in Malawi? *Food Policy* 37(5):504-510.
- Meikle WG, Adda C, Azoma K, Borgemeister C, Degbey P, Djomamou B, Markham RH (1998). The effects of maize variety on the density of *Prostephanus truncatus* (Coleoptera: Bostrichidae) and *Sitophilus zeamais* (Coleoptera: Curculionidae) in post-harvest store in Benin Republic. *J. Stored Prod. Res.* 34(1):45-58.
- Miller LV, Krebs NF, Hambidge KM (2013). Mathematical model of zinc absorption: effects of dietary calcium, protein and iron on zinc absorption. *Br. J. Nutr.* 109(4):695-700.



- Mutegi J, Kabambe V, Zingore S, Harawa R, Wairegi L (2015). The status of fertilizer recommendation in Malawi: gaps, challenges and opportunities. Soil Health Consortium of Malawi. Lilongwe, Malawi.
- Newport Scientific (1998). Applications Manual for the Rapid Visco™ Analyser. Newport Scientific Pty. Ltd., Australia.
- Osungbaro TO (2009). Physical and nutritive properties of fermented cereal foods. *Afr. J. Food Sci.* 3(2):23-27.
- Ricker-Gilbert J, Jones M (2015). Does storage technology affect adoption of improved maize varieties in Africa? Insights from Malawi's input subsidy program. *Food Policy* 50:92-105.
- Sandhu KS, Singh N (2007). Some properties of corn starches II: Physicochemical, gelatinization, retrogradation, pasting and gel textural properties. *Food Chem.* 101(4):1499-1507.
- Santiago R, Barros-Rios J, Malvar RA (2013). Impact of cell wall composition on maize resistance to pests and diseases. *Int. J. Mol. Sci.* 14(4):6960-80.
- Siwale J, Mbata K, Microbert J, Lungu D (2009). Comparative resistance of improved maize genotypes and landraces to maize weevil. *Afr. Crop Sci. J.* 17(1):1-16.
- Smale M (1995). "Maize is life": Malawi's delayed green revolution. *World Dev.* 23(5):819-831.
- Tefera T (2012). Post-harvest losses in African maize in the face of increasing food shortage. *Food Secur.* 4(2):267-277.
- Tefera T, Mugo S, Tende R, Likhayo P (2011). Methods of screening maize for resistance to stem borers and post harvest insect pests. CIMMYT, Nairobi, Kenya.
- Uarrota VG, Amante ER, Demiate IM, Vieira F, Delgadillo I, Maraschin M (2013). Physicochemical, thermal, and pasting properties of flours and starches of eight Brazilian maize landraces (*Zea mays* L.). *Food Hydrocoll.* 30(2):614-624.
- Zilic S, Milasinovic M, Terzic D, Barac M, Ignjatovic-Micic D (2011). Grain characteristics and composition of maize specialty hybrids. *Spanish J. Agric. Res.* 9(1):230-241.

*Full Length Research Paper*

# Conservation agriculture, conservation farming and conventional tillage adoption, efficiency and economic benefits in semi-arid Zimbabwe

Mvumi C.<sup>1\*</sup>, Ndoro O.<sup>1</sup> and Manyiwo S. A.<sup>2</sup>

<sup>1</sup>Faculty of Agriculture, Zimbabwe Open University, Stand No. 992 C Avenue, Mutare, Zimbabwe.

<sup>2</sup>Faculty of Agricultural Science and Technology, Chinhoyi University of Technology, Private Bag 7724, Chinhoyi, Zimbabwe.

Received 14 January, 2016; Accepted 13 March, 2017

Conservation practices can be of great importance in semi-arid regions for obtaining high crop yields and income, but adoption of the conservation practices, economic efficiencies and benefits remain unknown by most smallholders. The paper presents an overview of the adoption of conservation agriculture (CA), conservation farming (CF) and conventional tillage (ConvT), their technical efficiency and economic benefits. The study was carried out in Wards 4 and 17, Chimanimani District, Zimbabwe using a cross-sectional survey of 179 farmers involving participatory was used. A Stochastic frontier analysis (SFA) was used to determine relative technical efficiencies between CA, CF and ConvT farmers. Maximum likelihood estimation (MLE) technique was used to estimate Cobb-Douglas frontier production function. Gross margin (GM) analysis was employed to determine economic benefits by farmer category. Results showed that adoption was 59% for CF 20% for CA techniques and 69% for ConvT. SFA in R revealed that CF, CA and ConvT farmers were 87, 81 and 64% technically efficient respectively. GM analysis showed that CF had the highest GM/ha of \$99.88 and 196.20 with and without family labor cost respectively. This was followed by CA with GM/ha of \$63.82 and 158.60. ConvT farmers had the least GM of -\$25.16 and 65.20 with and without family labor cost. Most communal farmers considered ConvT to be a traditional practice; this could have been responsible for high adoption of the practice. Farmers showed a negative attitude towards CA despite the high labor requirements for CF. It is recommended that, of all the three practices in semi-arid regions, farmers use CF practice as it gives highest technical efficiency and GM.

**Key words:** Conservation agriculture, conservation farming, adoption, technical efficiency, stochastic frontier analysis.

## INTRODUCTION

Conservation practices and conventional tillage (ConvT) have been common in Southern Africa for many years. ConvT has been the first practice and regarded as a traditional method of tillage in Africa. Matthew et al.

(2012) found that ConvT led to rapid degradation of soil fertility and structure through loss of organic matter, giving higher input costs, increased runoff and excessive losses of soil and nutrients; key amongst these being soil

degradation (Sperrati and Turmel, 2015). Ngwira et al. (2012) concluded that lower yields were obtained under ConvT than under conservation agriculture (CA).

Conservation agriculture (CA) and conservation farming (CF) have great potential of solving the above mentioned production problems of smallholder farmers in the dry parts of Africa (Haggblade and Tembo, 2003; Hobbs, 2007; Rockstrom et al., 2009). They are popular alternatives which promise to utilize land and other resources in a sustainable manner (Nyamangara and Matizha, 2010). Various views are highlighted by researchers on sustainable agriculture based on CA and CF, from resource conservation, which range from low use of external inputs (Ouedraogo et al., 2001), through other various sustainable agriculture activities, to soil water and soil nutrient management (Pretty and Hine, 2000; Mashapa et al., 2013).

CA is a broader term that encompasses activities such as minimum tillage and zero tillage, tractor powered, animal powered and manual methods, integrated pest management, integrated soil and water management, and includes CF (Twomlow et al., 2008a). CF is a technology which uses planting basins and soil cover (Twomlow et al., 2008a; United Nations Food and Agriculture Organisation (FAO), 2011). It is a particular technology developed by Oldreive (1993). The CA Task Force for Zimbabwe (ZCATF) which was initiated in March 2004 outlined CA and CF components, and confirmed that digging planting basins using hoes, following principles like mulching and crop rotation, is termed CF (Protracted Relief Programme (PRP), (2005). Mazvimavi and Twomlow (2009) added that soil amendments like manure and inorganic fertilizers were incorporated precisely in the planting basins. Currently, the dominant CF technique adopted in Zimbabwe is the planting basin technique which concentrates limited water and nutrient resources applied (Thomlow, 2007). Specific examples of marked differences of CA from CF techniques include use of jab-planters, ripper tines, knife rollers and direct seeders (FAO, 2011). The principles of mulching, crop rotation and integrated pest management still apply. Farming communities' practices differ extensively. The variations can be influenced by farmer circumstances, the nature of the environmental factors prevailing in the area, and adoption. Thus, farmers may use very varied techniques to practise sustainable CA systems (Wall, 2007). This causes the interchangeable use of CA and CF.

ConvT is a standalone which is not confused with CA or CF. In this paper, ConvT involves using the mould-plough during land preparation to till the whole area to be planted, and then planting is done following behind the

plough.

History from the 1960s shows that farmers in Chimanimani started using ripper-tines as a form of CA, to open up soil for planting purposes. A share mounted on ox-drawn mouldboard plough without a mouldboard was used. Hoes were used for digging small pits to capture rainwater in fields, as a basic CF technique, and for weed control. Mulches were mostly used in vegetable gardens. These technologies were mainly promoted in the study area by Agriculture Technical and Extension Services (AGRITEX). From 2000, there has been a series of upgraded initiatives introduced by Non Governmental Organizations (NGOs), state actors and various donors in partnership with lead Ministry of Agriculture, Mechanization and Irrigation Development of Zimbabwe to promote sustainable CA practices. CA techniques which have been promoted included no-till tied ridging; mulch ripping, no-till strip cropping, clean ripping, hand-hoeing or zero till, tied furrows (for semi-arid regions) and tied ridging (Mupangwa et al., 2006, Twomlow et al., 2006). The promotions would be expected to reduce the impact of adverse environmental factors which were becoming more and more severe, and also to increase crop yields (Twomlow et al., 2008b; Mazvimavi and Twomlow, 2009). However, these CA techniques were lowly adopted (Hobbs, 2007; Derpsch 2008; Gowing and Palmer, 2008) because rural farmers were lowly mechanized; lacked appropriate implements, technical information, and appropriate soil fertility management options (Twomlow et al., 2006). These various factors prompted the need to venture into planting basins as improved initiatives of CF.

However, as long as there was enough production of staple food from CA and CF practices, the profitability aspect is usually never considered by most farmers in semi-arid regions; farmers may just have other goals like household food security, a stable income and minimizing risk of crop failure. There are not many studies known in literature done with a holistic approach to the technical efficiencies and economic benefits of CA, CF and ConvT practices and their relative adoptions in semi-arid parts of Zimbabwe. One previous study determined technical and economic efficiency of the CF techniques only, using the transcendental production function (Musara et al., 2012), while the other compared productivity and technical efficiency of CA with CF of maize using stochastic production frontier model (Mazvimavi et al., 2012). Most studies in literature simply consider CA without a further study on CF (Tsegaye et al., 2008, Gowing and Palmer, 2008; Mazvimavi et al., 2012).

The current study gives an overview of the technical efficiencies and economic benefits as well as adoption of

\*Corresponding author. E-mail: culvermvumi@gmail.com.

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](https://creativecommons.org/licenses/by/4.0/)

CA, CF and ConvT as they are all practised in most semi-arid parts of Zimbabwe. This paper addresses the shortcomings in the use of CA and CF by basing their separation on the terminology used in literature (Twomlow et al., 2008a; ZCATF, 2009; FAO, 2011).

## MATERIALS AND METHODS

The study on three practices: CA, CF and ConvT and their adoption, efficiency and economic benefits, was carried out in Chimanimani District.

### Study area

Zimbabwe has five NRs (1-V). The study was carried out in Chimanimani District, in Manicaland Province; the eastern province of Zimbabwe. Chimanimani District is characteristically known to have all the five agro-ecological or natural regions of the country. The specific wards under study were Ward 4 (Guhune) and Ward 17 (Biriiri). They fall in NR IV, which has been receiving mean annual rainfall below 500 mm in the last 10 years. The soils are sandy to sandy loams derived from granite rocks (IUSS Working Group WRB, 2007). Type of soil and gradient of an area has been known to offer incentives for adoption of soil conservation technologies due to increased danger of land degradation (Barungi et al., 2013).

### Conservation practices

In the current study, CA refers to all the farmers who used ox-drawn equipment (ripper tines, direct seeders, or jab-planters), CF farmers are those who used only hand-hoe basins, and ConvT farmers are those who used mouldboard ox-drawn plough for ploughing the whole land during land preparation and covering seed when planting.

### Research design and data

Ward 4 and Ward 17 were two wards purposively chosen for the study because of their highly variable annual rainfall of 460 to 600 mm (Moyo, 2000). The design was cross-sectional survey which included farmer-researcher-participatory action research approach. A cross-sectional survey was chosen for its low cost nature and ability to capture information at a given point in time. Pedzisa et al., 2010 found that the participatory approach encouraged greater knowledge-sharing among farmers, thus promoted grouping and adoption. A total of 2390 farming households were in the two wards and 200 farmers randomly sampled using random number tables based on the community household register intercept system (Mashapa et al., 2013). Each ward had 100 farmers sampled. Out of the total of 200 questionnaires, 179 representatives were valid for analysis because they provided all the information requested, and 21 had missing information. Structured questionnaires and face to face interviews were used to obtain data of household characteristics, technology adoption, practices, inputs, outputs, accessibility to extension services and markets. A structured questionnaire allowed data collection for quantitative analysis. A farmer could practise more than one technology and would therefore be considered as practising more than one technology. Almost all farmers in the study area were found growing maize under soil moisture conservation techniques. Although maize is a staple food crop in the area, there are other adaptable small grain

crops like sorghum, finger millet and pearl millet grown in most dry parts of Zimbabwe.

## Economic and econometric framework

### Technical efficiency analysis

Economic efficiency can be defined as comprising two components. The first is technical efficiency which is defined as production of a maximum level of output from a given bundle of inputs (Alemdar and Oren, 2006). The second being allocative efficiency, defined as a combination of inputs that maximizes profits, given input prices. Economic efficiency is then defined by the product of technical and allocative efficiency.

Two approaches can be used in determining the level of a firm's technical efficiency in empirical work. One is a parametric approach, Stochastic Frontier, and another is non-parametric, Data Envelopment Analysis (DEA).

The stochastic frontier analysis (SFA) is based on the pioneering work of Aigner et al. (1977) and Meeusen and van de Broeck (1977). SFA been widely applied in estimating farm efficiencies (Onyenweaku and Ohajianya, 2005; Hussein et al, 2012, Samuel and Kelvin, 2013). The approach allows separation of the error term into two: One is associated with factors outside the farmers' control such as weather, pest and diseases; the other relates to farm specific conditions (Mohammed et al., 2013). In the current study, particularly SFA was used because it is very suitable for analysis of data from field trials where uncertainty is high due to such factors as weather, and also because the coefficients estimated in this study directly represent elasticity of production (Abedullah and Ahmad, 2006). However, to determine technical efficiency of farmers specifically practicing CA, CF and ConvT, the protocol of Rahman (2003) was used.

The stochastic frontier production function is defined after Rahman (2003) as:

$$Y_i = f(X_i; \beta) \exp(v_i - u_i) \quad i = 1, 2, \dots, N \quad (1)$$

Where:  $Y_i$  = possible production of  $i^{\text{th}}$  farm in kg;  $f(X_i; \beta)$  = a suitable function of the vector  $X$  of inputs for the  $i^{\text{th}}$  farm, in this case a Cobb-Douglas production function was used;  $\beta$  = a vector of unknown parameters to be estimated;  $v_i$  = random error with zero mean associated with random factors;  $u_i$  = non-negative random variables associated with farm's specific factors contributing to the farm not attaining maximum efficiency of production;  $N$  = number of farms in the sample

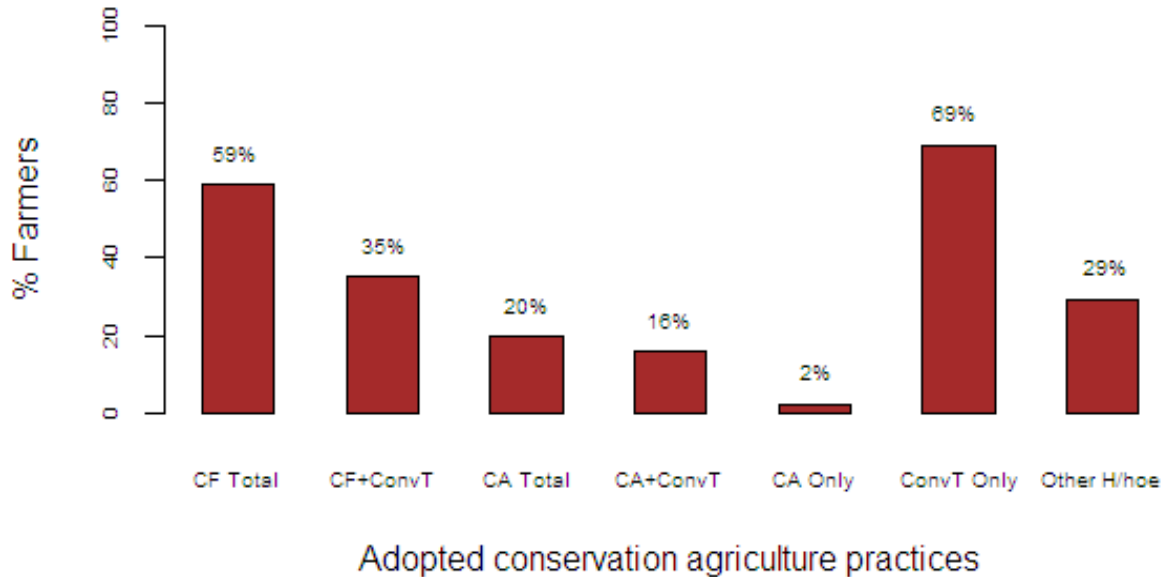
The random errors  $v_i$  are assumed to be independently and identically distributed as  $N(0, \sigma_v^2)$  random variables independent of the  $u_i$ 's which are assumed to be non-negative truncations of the  $N(0, \sigma^2)$ , that is, half-normal distribution. The Cobb-Douglas function was chosen because of its wide use in literature, easy of estimation and interpretation (Anyiro et al., 2012; Mango et al., 2015).

The following explicit function is estimated for CA, CF and ConvT farmers:

$$\ln Y_i = \beta_0 + \sum_{i=1}^N \beta_1 \ln X_i + v_i - u_i \quad (2)$$

Where, for farmer  $i$ ,  $Y$  was the total quantity or value of maize produced in metric tonnes,  $X$  was the quantity or value of input  $j$  used,  $v_i$  was the two-sided error term and  $u_i$  was the one-sided error term representing technical inefficiency effects. The inputs were:

$X_1$  = maize area cropped in ha;  $X_2$  = planted maize seed in kg;  $X_3$  = amount of basal fertilizer in kg;  $X_4$  = amount of top-dressing fertilizer



**Figure 1.** Adoptions of CA, CF and ConvT. Source: Survey data.

in kg;  $X_5$  = amount of family labor used in maize production in hours.

The inefficiency model is specified as:

$$u_i = \delta_0 + \sum_{m=1}^M \delta_m Z_m \quad (3)$$

Where  $Z_m$  are the inefficiency variables ( $m = 1, 2, \dots, M$ ), and  $\delta$  coefficients are unknown parameters to be estimated. These variables are described in the following paragraph: Education (number of years of schooling), household size (units of adult equivalents (AE)), household members away (AEs), access to credit (dummy, 1 for access and 0 for no access), gender (dummy, 1 for male and 0 otherwise), membership of a farmers' group (dummy, 1 for being a member of a group and 0, for non-member) and hired labor (dummy, 1 for use of hire labor, and 0 for non-use). A positive sign on the coefficient means the variable has a negative effect on efficiency while a negative sign of the coefficient means the variable reduces inefficiency.

Using MLE, the production and inefficiency models were estimated simultaneously in one stage using 'frontier package' in R (Coeli and Henningsen, 2013). MLE provides a consistent approach to parameter estimation problems. This means that MLE is effectively employed for use to give accurate estimates of many variables (Kadiri, 2014).

### **Economic analysis**

This study used gross margin (GM) analysis to compare performance of CA, CF and ConvT. GM was shown without considering the cost of family labor because household had limited alternative for earning an income outside their farming, and thus the opportunity cost of their labor could just be evaluated in terms of leisure. Profit is the difference between total cost and total revenue. By definition, fixed costs do not vary with the amount of maize produced, the economic optimum could be obtained by maximizing the difference between total revenue and total variable costs per ha and this difference is known as the gross margin (Sibanda et al., 2016). Although various studies use GM to compare the economic benefits of alternative technologies to farmers (Chanie et al., 2014),

not many studies have used GM analysis in evaluating CA practices, particularly in Zimbabwe. However, what can be termed accounting gross margin, which does not take account of the full opportunity costs of the resources, was used.

## **RESULTS**

The study on adoption, efficiency and economic benefits of CA, CF and ConvT which were all practised in Chimanimani District during the survey period showed varying results.

### **Adoption of practices**

#### **General practice adoptions**

Adoption of the practices is represented by a bar chart (Figure 1). The majority of farmers used ConvT only (69%), however, a sizeable proportion (59%) adopted CF. Farmers who adopted CF together with ConvT were over 30%, and there was also a group of farmers who used hand hoe but not strictly practising CF and these were 29%. CA (using ox-drawn or motorised equipment) had the least adoption rate (20%) whether as a standalone practice or in combination with ConvT.

### **Technical efficiency**

#### **Use of purchased inputs and productivity**

Use of inputs for maize, and yields for each type of technology are presented (Table 1). ConvT had the

**Table 1.** Maize production and inputs used by farmer category.

Technology	Statistic	Maize area (ha)	Seed rate (kg/ha)	Basal fertilizer (kg/ha)	Topdressing (kg/ha)	Yield (MT/ha)
CA (N= 20)	Mean	0.18	22.30	55	49	0.90
	S.D	0.07	10.66	70.87	73.29	0.32
CF (N= 77)	Mean	0.23	22.70	20	82	0.96
	S.D	0.13	7.52	45.76	79.62	0.19
ConvT (N=82)	Mean	0.49	24.90	38	50	0.44
	S.D	0.31	12.80	50.10	70.85	0.50

MT = metric tons; Source: Survey data.

highest mean maize cropped area (0.49 ha) followed by CF (0.23 ha) and CA (0.18 ha) respectively. All farmers planted using seed rates which were below the recommended rate of 25 kg/ha. Farmers used rates of basal and top-dressing far below the extension service recommended rates of 200 and 150 kg/ha respectively. Lowest yield level was obtained by ConvT farmers, whilst CF farmers had the highest (Table 1). There was a statistically significant ( $P < 0.01$ ) difference between mean yield for ConvT and the other practices (CA and CF), but the difference between mean yields for CF and CA was not statistically significant ( $P > 0.05$ ). Use of basal fertilizer was lowest among CF farmers.

### Technical efficiency model

#### Production function

CA: only basal fertilizer had a positive and statistically significant influence on maize output. A 1% increase in use of basal fertilizer resulted in 0.45% increase in output, and this output response was inelastic. An inelastic response means that a change in input use results in less than proportionate change in the quantity of output while the opposite is true for an elastic response. Maize area, seed, top-dressing and family labor had no significant influence on maize output.

CF: Maize area and seed had a positive and statistically significant impact on output for CF, while basal fertilizer and labor had a negative and significant impact. A 1% increase in the area under maize resulted in 0.48% increase in output, and a 1% increase in basal fertilizer caused an output decrease of 0.11% under CF. Both relationships were inelastic. Top-dressing fertilizer had no significant influence on maize output.

ConvT: Top dressing fertilizer had a positive and statistically significant relationship with output for ConvT, while family labor and basal fertilizer had a negative but statistically significant impact on maize output. A 1% increase in top-dressing resulted in 0.78% increase in output, while a 1% increase in family labor use and basal fertilizer use caused 0.12 and 0.43% decrease in maize

output, respectively. The output response to input use was inelastic in both cases, that is, a change in independent variables caused a less than proportionate change in the dependent variable. Maize area and seed had no significant influence on maize output under ConvT.

#### Returns to scale

A measure of returns to scale is obtained by calculating the sum of the input elasticities. Where the sum of input elasticities is equal to one, there are constant returns to scale. Increasing and decreasing returns to scale are displayed when the sum of elasticities are greater than one and less than one respectively. There were decreasing returns to scale for the three categories of farmers. However, CA and ConvT farmers had almost similar values for returns to scale (0.439 and 0.436 respectively).

#### Inefficiency model

All the inefficiency variables were not statistically significant for CA although they had different signs. Only about 11% of the 179 sampled farmers practised CA. The following variables had expected signs showing their positive effect on reducing technical inefficiency: group membership, number of non-resident members, gender (male farmers are more efficient than female farmers) and access to draft power. An increase in non-resident members improved technical efficiency since these households already had the largest size (a mean of 4.91 AE; Table 1).

For CF, all the variables in the efficiency model were statistically significant. Inefficiency reducing factors for CF were; number of family members away, gender (being male improves technical efficiency), group membership and level of household head education. Household size, gender and group membership had no influence on inefficiency for ConvT farmers. Draft power and level of education had a statistically significant reduction on

**Table 2.** MLE of the stochastic production frontier.

Variable		CA		CF		ConvT	
<i>Production function</i>		<i>Coeff.</i>	<i>S.E</i>	<i>Coeff.</i>	<i>S.E</i>	<i>Coeff.</i>	<i>S.E</i>
Intercept	$\beta_0$	0.362	0.937	2.537	1.286***	5.289	0.332***
<i>ln</i> Maize area	$\beta_1$	0.436	0.455	0.482	0.216***	0.090	0.081
<i>ln</i> Seed	$\beta_2$	-0.037	0.862	0.328	0.177***	0.120	0.078
<i>ln</i> Basal fertilizer	$\beta_3$	0.454	0.098***	-0.106	0.056**	-0.428	0.139***
<i>ln</i> Topdressing fertilizer	$\beta_4$	-0.121	0.196	-0.026	0.049	0.777	0.080***
<i>ln</i> Family labor	$\beta_5$	-0.339	0.279	-1.041	0.364***	-0.123	0.070**
<b><i>Inefficiency model</i></b>							
Intercept	$\bar{\delta}_0$	0.044	0.934	14.450	1.194***	1.755	1.000**
Hired labor	$\bar{\delta}_1$	0.115	0.286	-0.143	1.229***	-0.892	1.022
Credit access	$\bar{\delta}_2$	0.020	0.781	0.971	0.511**	8.311	1.637***
Farmer group	$\bar{\delta}_3$	-0.068	0.540	-7.140	0.684***	-2.404	1.610
Draft power	$\bar{\delta}_4$	-0.117	0.815	2.292	0.349***	-2.224	0.986**
Household size	$\bar{\delta}_5$	0.078	0.078	0.851	0.201***	-0.201	0.495
Gender	$\bar{\delta}_6$	-0.181	0.570	-6.980	0.683***	0.018	1.039
Education	$\bar{\delta}_7$	0.001	0.110	-2.323	0.157***	-0.667	0.362**
No. of members away	$\bar{\delta}_8$	-0.077	0.110	-1.590	0.254***	-1.431	0.554***
<b><i>Variance parameters</i></b>							
Sigma-squared		0.011		0.065		2.827	
$\gamma$		1.000		0.000		1.000	
$\lambda$		10000		0.0001		10000	
Log-likelihood function		25.66		-1.46		-8.817	
Mean technical efficiency		0.809		0.874		0.639	

Significance codes: 0.01\*\*\*, 0.05\*\*, 0.10\*; Source: Survey data.

inefficiency, and credit had a statistically significant increase in technical inefficiency. CF had the highest mean technical efficiency of 87% followed by CA and ConvT with mean efficiencies of 81 and 64%, respectively (Table 2).

### **Model log-likelihood ratio test**

Results of the log-likelihood ratio test rejected the no-inefficiency model in favour of presence of inefficiency effects for all the three categories of farmers (Table 3).

### **Economic benefits**

#### **GM analysis**

GM analysis was used to compare returns to the farmer for each farmer category (Table 4). CF had the highest GM/ha of \$99.88 and 196.20 with and without family labor cost respectively, while CA with GM/ha had \$63.82 and 158.60, respectively. ConvT farmers had a negative GM/ha of \$25.16 with family labor cost and a positive GM/ha of \$65.20 without family labor cost. CA and CF

gave positive (and almost the same level of) returns to purchased inputs and family labor. However, these figures were either below or equivalent to the average wage rate of \$2/Md in the study area, and this was perhaps the reason why not many farmers in the survey used hired labor (32%). ConvT had the least and negative GM/ha as well as returns to purchased inputs and family labor. However, the gross margin would become positive if the cost of family labor was removed.

### **DISCUSSION**

ConvT had the highest adoption of all the three. This was probably because it was the first known tillage practice and hence was regarded as a traditional practice by most farmers. Inevitably, the numbers of farmers practising ConvT was responsible for rapid land degradation (Abdullah, 2014). CF had higher adoption than CA (Figure 1). Lack of mechanized equipment limits adoption of some conservation practices. This outcome is confirmed by Baudron et al. (2015) who mentioned that lack of machinery has been one of the drivers of adoption of CF techniques in Southern Africa. Our study has results of 20% CA adoption which agree with Ngwira et

**Table 3.** The log-likelihood ratio test.

Category/practice	Model	DF	Log-likelihood value	DF	Chisq	P(>Chisq)
CA	1	7	12.17	10	26.66	0.000***
	2	17	25.66			
CF	1	7	-21.47	10	40.01	0.000***
	2	17	-1.46			
ConvT	1	7	-30.06	10	42.48	0.000***
	2	17	-8.82			

Model 1 = OLS (no technical inefficiency); Model 2 = Efficiency effects frontier; Significance codes: 0.01\*\*\*, 0.05\*\*, 0.10\*; Source: Survey data.

**Table 4.** GM/ha budget for farmer categories based on average farmer.

Practice	CA	CF	ConvT
<b>Gross income*</b>	270	288	132
Less expenses			
Seed	35.68	36.32	39.84
Fertiliser	59.10	60.00	50.52
Total labor	111.40	91.80	66.80
<b>Total costs that vary</b>	206.18	188.12	157.16
<b>GM (\$/ha)</b>			
With family labor cost	63.82	99.88	-25.16
Without family labor cost	158.60	196.20	65.20
<b>Returns to inputs</b>			
<i>With family labor cost</i>			
To purchased inputs (\$/\$)	0.67	1.04	-0.28
To family labor (\$/Mds)	1.15	2.18	-0.75
<i>Without family labor cost</i>			
To purchased inputs (\$/\$)	1.67	2.04	0.72
To family labor (\$/Mds)	2.85	4.27	1.95

\*A local maize price of \$300/MT, and a local hiring rate for labor of \$2/day. Source: Survey data.

al. (2014) who found an adoption rate of 18.5% among smallholder farmers in Malawi. The area under CA which is reported in our findings (0.18 ha) also agrees with his findings (0.2 ha).

Many studies in literature have used the stochastic production frontier approach to estimate efficiencies for various production systems (Geta et al., 2010; Awunyo-Vitor et al., 2013; Yegon et al., 2015). From Table 2, the positive relationship between seed and output agrees with Awunyo-Vitor et al. (2013). However, this contradicts with Mazvimavi (2012) who found a negative influence of seed on maize output and positive influence on maize output under CA. The mean seed rate for CF farmers was below the recommended rate of 25 kg/ha (Table 1) and thus additional use of the input was likely to cause an increase in output. Some studies agree with the lack of seed influence on output as observed on CA and ConvT

(Yegon et al., 2015). Using panel data in a study of smallholder maize production under CA and ConvT in Zimbabwe, Mazvimavi (2012) found a negative relationship between cropped land and output for CA, but a positive relationship for ConvT. Chirwa (2003) found a negative relationship between cropped area and output in a study of maize farmers in Malawi and notes that the relationship may be a result of area measurement errors.

Basal fertilizer had a statistically significant ( $P < 0.01$ ) influence on maize output under CA but negative influence for CF and ConvT. The results agree with studies done on CA and ConvT (Mazvimavi, 2012). The results (Table 2) confirm this where output elasticity with respect to basal fertilizer was greater and positive for CA (0.45) than for CF (-0.10) and ConvT (-0.42), which were negative and smaller. Geta et al. (2010) also found a significant influence of fertilizer on output. The negative



influence of basal fertilizer on maize output under CF and ConvT needs further research to find if factors such as difference in land preparation depths between the technologies or other factors could have any influence on the effectiveness of the fertilizers. The lack of significant influence of top-dressing fertilizer on CF and CA could be caused by the fact that farmers used a quarter to a third of recommended rates, thus causing the inputs to have little effect (Table 1). Family labor had no influence on maize output under CA and CF but statistically significant, yet negative for both CF and ConvT ( $P < 0.05$ ) (Table 2). The results concur with the findings of Yegon et al. (2015) which also showed that labor was negatively related to output. The negative response to labor under ConvT agrees with Mazvimavi (2012) who found output elasticity with respect to labor positive for CA but negative for non-conservation farming.

All the inefficiency variables for CA were not statistically significant (Table 2). This could be a result of the very small proportion of CA farmers in the sample. Household size, gender and group had no influence on ConvT inefficiency. Inefficiency reducing factors for CF were number of household members away, hired labor, gender, group membership and level of household head education. Male farmers under CF were more efficient than women perhaps due to the strenuous nature of the practice. Chirwa (2003) found that female maize farmers were more efficient than men, though gender was not important in explaining efficiency. Number of family members away, access to draft power, and education reduced inefficiency for ConvT farmers. ConvT farmers had the largest number of non-resident members and second to CA farmers in terms of household head education. It was speculated that non-resident members away could be employed somewhere and thus they support farming activities by purchasing inputs. However, the matter was not conclusive as being away might increase inefficiency by less attention to the farm.

Other studies also found similar results regarding the effect of group membership and education on reducing inefficiency (Awunyo-Vitor et al., 2013; Yegon et al., 2015). Education is believed to reduce inefficiency by enabling farmers to try new innovations. This could be enhanced by group membership, allowing sharing of knowledge. Credit increased inefficiency for CF and ConvT farmers (Table 2). Although some farmers received credit, the credit was not linked to CA and was of small amounts. Access to draft power reduced inefficiency for ConvT practice but increased inefficiency for CF. ConvT farmers used the ox-drawn mouldboard plough and so, access to draft power was likely to reduce inefficiency. CF farmers basically used the hand-hoe, and access to draft power could lead to less output as less area was likely to be cropped. Geta et al. (2010) reported that access to draft power could reduce drudgery of operations.

While the efficiency figures appear to be high for

smallholder farmers compared to other studies on technical efficiency (Table 2), they were quite plausible (Mazvimavi, 2012). Chirwa (2003) reported technical efficiencies as high as 76 and 79% for studies of smallholder maize farmers in Malawi. In the current study, the results of CF which have high technical efficiency agree with Musara et al. (2012). The technical efficiency for ConvT was within the range of 68% for both CA and ConvT (Mazvimavi, 2012). ConvT farmers were likely to be operating at a lower frontier compared to the two technologies. This could be seen from the mean yields for these technologies; ConvT maize yield was less than half of the CA technologies and the difference was statistically significant ( $P < 0.05$ ) (Table 1). This suggests that a technical change to adopt conservation agriculture would benefit the farmers (Mazvimavi, 2012).

Lack of significant influence on maize output for some variables like maize area, seed and labor on CA and variables in the inefficiency model could also be a result of small sample size for these farmers due to few farmers practising the technology in the area. Specification of the model could also limit significance tests (Coeli and Henningsen, 2013). Other variables which could be of interest were age of farmer, access to agricultural extension and level of off-farm income. The translog production function is a flexible functional form that takes care of interaction terms. However, the log-likelihood ratio test rejected the no-inefficiency model in favour of presence of inefficient effects (Table 3) for all the three technologies.

Many studies use GM analysis to evaluate impact of interventions or new technologies on farmers. Memon (2015) used GM analysis to compare hybrid rice and other rice varieties in Pakistan. Although yields for hybrid variety were high, it fetched poor prices due to low quality, and farmers would face the challenge of buying seed every year. Chanie et al. (2014) used both econometric and GM analysis to compare performance of farmers participating in a research programme and non-participants in Ethiopia. The study showed that participants in the research programme had better productivity and GMs than non-participants.

GMs and returns to factors of production were highest for CA and CF farmers and least for ConvT farmers (Table 4). Tshuma et al. (2010) found that CA gives substantially higher gross margins than ConvT. The study confirms that CF demands more labor than CA.

## Conclusions

The cross-sectional survey coupled with participatory approach revealed varied adoption results. CF had an adoption of 59% and CA had 20%. The majority of farmers (69%) engaged in convT only, and about 30% used a combination of the methods. Cobb-Douglas stochastic frontier production function was applied for the

estimation of technical efficiency of CA, CF and ConvT. CF farmers were the most efficient farmers in terms of using their available resources. Their mean efficiency was 87%, followed by CA farmers who had 81%. ConvT farmers had the least mean efficiency (64%). Main sources of inefficiency were inadequate use of inputs (both organic and inorganic fertilizers), low household head education and inadequate family labor (CF). GM analysis showed that CF had the highest GM/ha of \$99.88 and 196.20 with and without family labor cost respectively. This was followed by CA with GM/ha of \$63.82 and \$158.60 respectively. ConvT farmers had the least GM of -\$25.16 and 65.20 with and without family labor cost. CF conserves nutrients, which makes it economically efficient, conserves moisture and soil which all make it a sustainable option in semi-arid regions. Based on these findings, the study therefore recommends use of CF practice as it gives highest yields and GM of all the three practices. The study is limited to maize in one district, yet Manicaland province has 7 districts. Further economic and econometric studies on other commonly grown crops like small grains in other districts be done to determine the most economically efficient crops to grow in dry regions.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

The authors extend their thanks to an AGRITEX supervisor, Mr. S. Mutungwe, for facilitating data collection, the local authorities and farmers in the study area for the cooperation, and Zimbabwe Open University, Manicaland for the needed facilities.

## REFERENCES

- Abedullah KB, Ahmad B (2006). The technical efficiency and its determinants in potato production, evidence from Punjab, Pakistan. *The Lahore Journal of Economics*. 11(2):1-22.
- Abdullah AS (2014). Minimum tillage and residue management increase soil water content, soil organic matter and canola seed yield and seed oil content in the semiarid areas of Northern Iraq. *Soil Till. Res*. 144:150-155.
- Aigner D, Lovell CK, Schmidt P (1977). Formulation and estimation of stochastic frontier production function models. *J. Econom*. 6:21-37.
- Alemdar T, Oren MN (2006). Measuring technical efficiency of wheat production in Southeastern Anatolia with parametric and nonparametric methods. *Pak. J. Biol. Sci*. 9:1088-1094.
- Anyiro C, Emerole C, Osundu C, Udah S, Ugorji S (2012). Labor use efficiency by smallholder yam farmers in Abia State Nigeria: A labor use requirement frontier approach. *Int. J. Food Agric. Econ*. 1(1):151-163.
- Awunyo-Vitor D, Bakang J, Smith C (2013). Estimation of farm level technical efficiency of small-scale cow pea production in Ghana. *American-Eurasian J. Agric. Environ. Sci*. 13(8):1080-1087.
- Barungi M, Edriss A, Mugisha J, Waithaka M, Tukahirwa J (2013). Factors influencing the adoption of soil erosion control technologies by farmers along the slopes of Mt. Elgon in eastern Uganda. *J. Sustain. Dev*. 6(2):9.
- Baudron F, Thieffelder C, Nyagumbo I, Gerard B (2015). Where to target conservation agriculture? How to overcome challenges associated with its implementation. Experience from Eastern and Southern Africa. *Environments* 2:338-357.
- Chanie Y, Zemedu L, Teklewold T (2014). Farmers' participation in farmers research groups and its contribution on their income on rice; empirical evidence from rice production systems of Fogera district, Ethiopia. *J. Econ. Sustain. Dev*. 5:13.
- Chirwa EW (2003). Sources of technical efficiency among smallholder maize farmers in Southern Malawi. Lilongwe : University of Malawi.
- Coeli T, Henningsen A (2013). Frontier: stochastic frontier analysis, R package version 1.1-0. Retrieved from <http://CRAN.R-Project.org/package=frontier>
- FAO (2011). CATF (Conservation Agriculture Task Force). Presentation of machinery evaluation updates. Harare: FAO.
- Geta E, Bogale A, Kasa B, Elias E (2010). Productivity and efficiency analysis of smallholder maize producers in southern Ethiopia. IFPRI.
- Gowing JW, Palmer M (2008). Sustainable agricultural development in Southern Africa; the case for paradigm shift in land husbandry. *Soil Use Manage*. 24:92-99.
- Haggblade S, Tembo G (2003). Development, diffusion and impact of conservation farming in Zambia. Food security research project working paper #8. Food security research project, Lusaka.
- Hobbs PR, Sayre K, Gupta R (2008). The role of conservation agriculture in sustainable agriculture. *Philosophical Transactions of the Royal Society*. 363:543-555.
- IUSS Working Group WRB (2007). World reference base for soil resources first update. Rome : IUSS Working Group.
- Kadiri FA, Eze CC, Orebiyi JS, Lemchi JI, Ohajianya DO, Nwaiwu, IU (2014). Technical efficiency in paddy rice production in Niger Delta Region of Nigeria. *Global J. Agric. Res*. 2(2):33-43.
- Mango N, Makate C, Hanyani-Mlambo B, Sisiba S, Lundy M (2015). A stochastic frontier analysis of technical efficiency in smallholder production in Zimbabwe; The fast track land reform outlook. *Cogent Economics and Finance*.
- Mashapa C, Mhuriro-Mashapa P, Zisadza-Gandiwa P, Gandiwa E (2013). Adoption of agro-ecology techniques in semi-arid environment of Chimanimani District, Eastern Zimbabwe. *Global J. Agric. Res*. 15:1-17.
- Matthew RP, Feng Y, Githinji L, Ankumah R, Balkcom KS (2012). Impact of no-tillage and conventional tillage systems on soil microbial communities. *Appl. Environ. Soil Sci*. ID548620.
- Mazvimavi K (2013). Productivity and efficiency analysis of maize under conservation agriculture in Zimbabwe. Retrieved from ResearchGate: <http://www.researchgate.net/publication/241751236>
- Mazvimavi K, Twomlow S (2009). Socioeconomic and institutional factors influencing adoption of conservation farming by vulnerable households in Zimbabwe. *Agric. Syst*. 101:20-29.
- Meeusen W, van de Broeck J (1977). Efficiency estimation from Cobb-Douglas production functions with composed errors. *Int. Econ. Rev*. 18:435-444.
- Memon A (2015). Performance of rice hybrid and other varieties in Sindh and Balochistan. Retrieved from Researchgate: <https://www.researchgate.net/publication/265315541>
- Mohammed ST, Bila T, Amaza PS (2013). Application of stochastic frontier function in estimating production efficiency: a dual approach. *Global J. Biodiversity Sci. Manage*. 3(1):11-19.
- Moyo D (2000). Zimbabwe environmental dilemma; balancing resource inequities. Harare : Zimbabwe Research Environmental Organisation.
- Mupangwa W, Love D, Twomlow S (2006). Soil and water conservation and water harvesting in the semi-arid Mzingwane Catchment, Limpopo Basin, Zimbabwe. *Phys. Chem. Earth* 31:893-900.
- Musara JP, Chimvuramahwe J, Borerwe R (2012). Adoption and efficiency of selected conservation farming technologies in Madziva Communal Area, Zimbabwe: A transcendental production function approach. *Bull. Env. Pharmacol. Life Sci*. 1(4):27-38.
- Ngwira AR, Aune JB, Mkwinda S (2013). On-farm evaluation of yield and economic benefit of short-term maize legume intercropping

- systems under conservation agriculture in Malawi. *Field Crops Res.* 132:149-157.
- Ngwira A, Johnsen F, Aune J, Mekuria M, Thierfelder C (2014). The adoption and extent of conservation agriculture among smallholder farmers in Malawi. *J. Soil Water Conserv.* 69(2):107-119.
- Oldrieve B (1993). Conservation farming for communal, small-scale, resettlement and cooperative farms in Zimbabwe; A Farm Management Handbook. Harare: Prestige Business Services.
- Onyenweaku CE, Ohajianya DO (2005). Technical efficiency of Rice farmers in Ebonyi State, Nigeria. *J. Agric. Sci.* 3(2):175-184.
- Ouedraogo E, Mando A, Zombre NP (2001). Use of compost to improve soil properties and crop productivity under low input agricultural system in West Africa. *Agric. Ecosyst. Environ.* 84(3):259-266.
- Rahman S (2003). Profit efficiency among Bangladesh rice farmers. *Food Policy.* 28:487-503.
- Samuel OO, Kelvin MS (2013). An Analysis of technical efficiency of rice farmers in Ahero Irrigation Scheme, Kenya. *J. Econ. Sustain. Dev.* 4(10):9-16.
- Sibanda M, Mushunje A, Mutengwa CS (2016). An evaluation on the profitability of growing improved maize open pollinated varieties in the Eastern Cape Province, South Africa. *J. Dev. Agric. Econ.* 8(1):1-13.
- Sperrati BA, Turmel M (2015). Conservation Agriculture in Latin America. Retrieved from ResearchGate: <https://www.researchgate.net/publication/271965147>.
- Tsegaye W, Aredo D, Rovere L, Mwangi W, Mwabu G, Tesfahun G (2008). Does partial adoption of conservation agriculture affect crop yields and labor use? Evidence from two districts in Ethiopia Nippon IA research report No. 4. CIMMYT/SG 2000 monitoring and impact assessment (IA) project, Ethiopia.
- Twomlow S, Rhobarch D, Rusike J, Dimes J, Ncube B (2007). Spreading the word on fertiliser in Zimbabwe, in land and water management for sustainable agricultural systems. Proceedings of the EU/SADC land and water management applied research and training programmes (pp. 6-21). Lilongwe: Malawi Institute of Management.
- Thomlow S (2007). Assessment of sustainable adoption of conservation farming in Zimbabwe.
- Twomlow D, Urolov JC, Jenrich M, Oldrieve B (2008a). Lessons from the field - Zimbabwe's Conservation Agriculture Task Force. *J. SAT. Agric. Res.* 6:1-11.
- Yegon PK, Kibet LK, Lagat JK (2015). Determinants of technical efficiency in soyabean production in Bomet District, Kenya. *J. Dev. Agric. Econ.* 7(5):190-194.
- Zimbabwe Conservation Agriculture Task Force (ZCATF) (2009). *Farming for the future, a guide to conservation agriculture in Zimbabwe.* Harare: ZCATF.

Full Length Research Paper

## Influence of ascorbic acid on physiological deterioration of pieces of cassava raw pulp

Arlindo Modesto Antunes\*, Túlio Natalino de Matos, Vanesa Beny da Silva Xavier Reis, Pâmella de Carvalho Melo, André José de Campos and Ivano Alessandro Devilla

Department of Agricultural Engineering, State University of Goiás, 75 132- 400, Anápolis – GO, Brazil.

Received 18 November, 2016; Accepted 5 April, 2017

Cassava (*Manihot esculenta*, Crantz) is originally from South America and possibly Brazil, grown to more than 500 years. Its roots can be exploited in various ways for human consumption. With the focus on post-harvest cassava, this experiment was developed with the objective of evaluating the use of ascorbic acid (AA), on physiological deterioration in cassava roots minimally processed during the storage period of six days, in order to extend the life of the final product as well as to ensure their food security during distribution, marketing and consumption. The experimental design was completely randomized design (DIC) in factorial scheme 4 x 6 (concentrations x days of analysis), with three repetitions each day, for each treatment. Treatment was observed with an increased loss of mass during storage regardless of the treatment with AA being that, the larger the dose applied minor was the loss of mass. There was no significance in the interaction of concentration treatments x days of analysis, showing significant difference only in the variables and soluble solids in different concentrations of, AA and variables pH, soluble solids and titratable acidity to the days of analysis.

**Key words:** Minimally processed, *Manihot esculenta* Crantz, ascorbic acid.

### INTRODUCTION

Cassava (*Manihot esculenta*, Crantz), also known as cassava or manioc, mansa cassava (Borges et al., 2012), is originally from South America and possibly Brazil. It is cultivated for more than 500 years, initially by the Indians and later introduced in the African and Asian continents. This culture is one of the main energy food for thousands of people, especially in developing countries, where it is grown in small areas with low technological level (Nechet and Halfed-Vieira, 2010).

Cassava presents enormous versatility, since it can

make use of its aerial part in animal nutrition as well as its roots which may cause among others, flour, starch and rind, as well as being consumed in natura. (Pereira et al., 1985). However, the fate of cassava depends on their classification, considering the content of cyanide: mad or bitter cassava and cassava mansa or sweet. Cassava is destined for flour industry. Cassava also called manioc is marketed mainly in the forms: with bark; just peeled and washed; frozen; cooked or precooked. Currently, the peeled cassava has good market acceptance which is

\*Corresponding author. Email: arlindo.modesto1@hotmail.com

marketed in free fairs supermarkets and other commercial establishments, for a price of 25 to 50% higher than that of cassava with shell. But, can't be confused with minimally processed product, for failing to present food security because the product is not sanitized and not kept under refrigeration, which can increase their perishability (Cereda and Vilpoux, 2003).

Peeling and slicing are common practices before consumption of cassava, which makes it a little convenient product for the consumer. To see to that, after the harvest the roots of the cassava feature very short shelf life, and can become useless for marketing after few days of storage at room temperature. This high perishability of the roots generates storage difficulties, which has aroused the interest for the diversification of its use in processed form, once processed immediately such as roots, reduce post-harvest losses and offer new products that expand the possibilities of increase of consumption and production (Rinaldi et al., 2015; Vieites et al., 2012).

To do so, minimally processed products become increasingly popular for convenience, for the benefits of a fresh natural product, product quality being packaged in small portions can mainly be ready for consumption (Alves et al., 2010).

It is known that, the handling of the product during the minimum processing, mechanical product injury promotes the physiological and biochemical responses. These injuries decrease the quality and service life of the product since they promote increased respiratory rate and ethylene synthesis, total synthesis of phenolic compounds, loss of cellular integrity on cut surface with consequent descompartmentalization of enzymes and their substrates, promoting increased activity of the enzyme phenylalanine ammonia lyase, polyphenoloxidase and peroxidase. These enzymes directly involved with postharvest physiological deterioration with observed changes in color, taste, texture and nutritional quality, determining its acceptance by consumers or not (Menolli et al., 2008).

One of the ways to avoid this deterioration is the use of resistant varieties. However to date, this material was not developed, thus the most affordable option is the use of conservation techniques in natura fresh roots (Borges et al., 2002). One of the most researched include antioxidants ascorbic acid (AA), being one of the most important vitamins (vitamin C) that can maintain the normal functions of the human body which is involved in several processes of cellular metabolism. This is used in trade due to its ability to reduce quinone and phenolic, preventing the formation of dimming (Pineli et al., 2005).

This used in the assessment of nutrients as an antioxidant in fruits, vegetables and juices. Chemical treatments in AA has been touted to be effective in prevention of browning of minimally processed products as in banana in dark just minutes after cutting and stripping being a process associated with elevated

activity of polyphenoloxidase enzymes and peroxidase (Melo and Vilas Boas, 2006).

The aim of this study is to assess the physicochemical effects of AA on physiological deterioration in minimally processed cassava roots, during the storage period of six days, in order to extend the life of the final product as well as to ensure their food security during the distribution, marketing and consumption. In the development of the study, the quality and storage of cassava minimally processed when submitted to different concentrations of Ascorbic acid, by analyzing the physical-chemical parameters such as: loss of mass, firmness, pH, soluble solids and total titratable acidity was rated.

## MATERIALS AND METHODS

### Plant material

Cassava roots type table cultivar (BRS400), were produced in a private property located in the municipality of Anápolis, Goiás. The roots with about 20 months of age, were harvested by hand, packed in plastic boxes and then transported to the minimum processing unit at the State University of Goiás, where they were immediately processed.

### Processing flowchart

For realization of the experiment, the following processes is described by means of a flow chart (Figure 1). This experiment was conducted in the laboratory of a physical properties of plant products, Agricultural Engineering course in the University Unit for science and Technology of the Universidade Estadual de Goiás-UEG, during the month of July 2016.

We used cassava properly sanitized with solution of sodium hypochlorite (NaClO) 1% for 10 min, then peeled, cut in chunks of 0.05 to 0.06 meters, separated into lots and immersed for 15 min in a solution of ascorbic acid (AA) at concentrations of 1, 2 and 3%. Witness was used in the procedure of immersion in batch in distilled water. Later packed in expanded polystyrene (EPS) + film of polyvinyl chloride (PVC) and stored in refrigerated incubator stove type BOD, 10°C, 80 to 90% RH, for 6 days.

### Analysis

Physical-chemical parameters were analysed as loss in weight, firmness, pH, soluble solids and titratable acidity. For such, procedure of the solution prepared was from cassava dough and distilled water at a ratio of 1:1.

### Mass loss

During storage, the packages of minimally processed cassava were 0, 2, 4, 6 days of conservation (±10°C and 80 to 90% RH). The loss of fresh mass in the roots was determined in relation to the initial weight of the cassava in time zero, by gravimetry in BL 3200H precision balance (BL 3200H ±0.5 g, Shimadzu, Japan). The percentage of loss in mass was studied from the equation 1:

$$PM (\%) = [(P_i - P_j) / P_i] * 100 \quad (1)$$

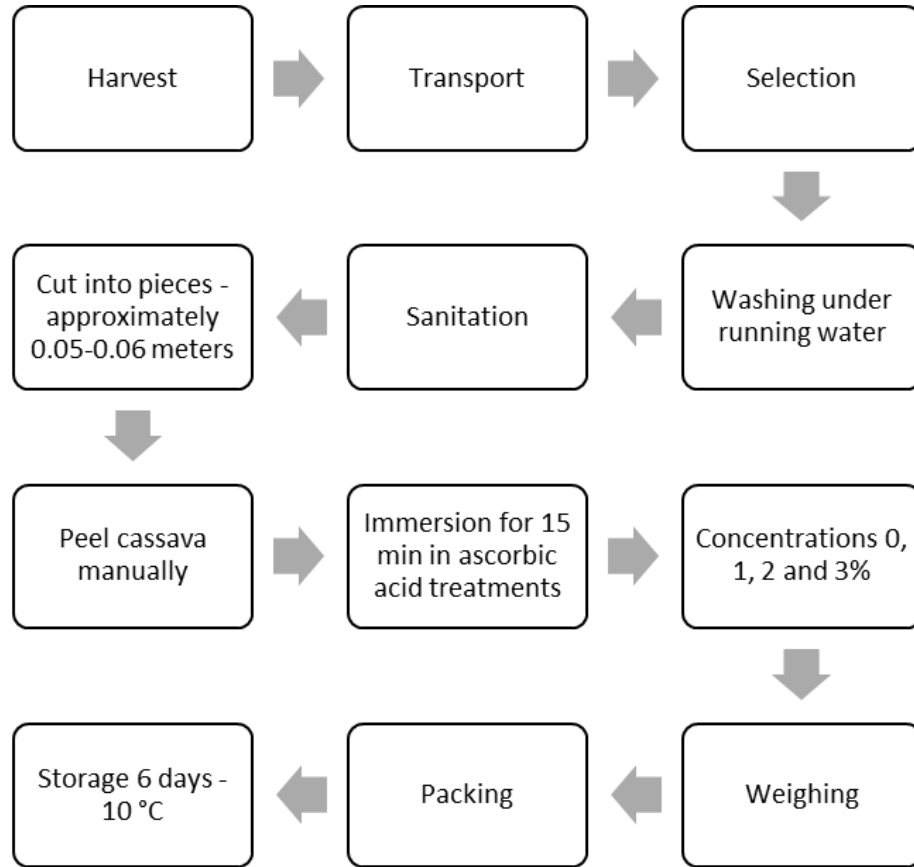


Figure 1. Processing flowchart.

Being:

PM = mass loss (%).

PI = initial weight of the fruit (g).

PJ = root weight in the subsequent period the Pi (g).

**Firmness**

This was used in texturometer brookfield-texture analyser CT3 50 k (USA), with the depth of penetration of 2 mm and speed of penetration of 6, 9 mm/s. The unit of measurement was used in centiNewtons (cN).

**Hydrogen potential (pH)**

This was carried out by potentiometry, using DMHP model pH meter-2 Digimed, as technique described by IAL (2008).

**Soluble solids (SS)**

This was held by reading, refratométrica in °Brix and Abbe refractometer (origin, accuracy) with digital brand bench quimis. As a recommendation of IAL (2008).

**Titrateable acidity (AT)**

Form the content, titrateable acidity was measured in 5 ml of the

sample (cassava pulp) in a beaker of 1 to 10 ml, supplementing with 95 ml of distilled water, with standardized solution of 0.1 M sodium hydroxide and alcoholic solution of phenolphthalein indicator (three drops). The sample was titrated till rosy coloring appeared, as a recommendation of IAL (2008). The acidity was calculated using equation 2:

Acidity in molar percent solution is

$$\left(\frac{V}{m}\right) = \frac{v.F.100}{P.c} \tag{2}$$

Being:

V = number of millilitres of sodium hydroxide solution 0.1 M required.

F = factor of sodium hydroxide solution 0.1 M.

P = number of grams of the sample used for the titration.

c = correction for solution, 10 to 0.1 M NaOH solution.

**Minimum processing**

The process consist of steps; pre-wash, wash, peel, cut in cubes, rinse, final rinse, sanitation, drainage and packaging. The temperature of the product was monitored during all stages of process, with a digital infrared thermometer (HANNA-HI Model 99551). The temperature in the processing environment was ±25°C. All utensils and equipment used for the minimum processing

steps have been sanitized.

#### Pre-wash

In order to remove the dirt from the coarser field, the cassava pre-washed was immersed in water for 10 min.

#### Selection

The cassava were selected and those attack by pathogens and pests, were discarded.

#### Washing

The roots were washed in running water to remove any dirt attached to the roots which is very difficult.

#### Cut and peel

The cassava was cut in approximately 5 to 6 rowlocks cm long with a metal blade, subsequently to decortication. Shortly after peeling, the rowlocks suffered a longitudinal and transverse cutting.

#### Ascorbic acid treatments

In this step, the roots of cassava were immersed in a plastic container properly sanitized, with percentages of AA (concentration 99,9%), solution 1, 2 and 3% being the witness immersed only in distilled water.

#### Packaging

The cassava were wrapped in packages of expanded polystyrene (EPS) + film of polyvinyl chloride (PVC).

#### Conservation

The conservation was held under refrigeration for a period of 6 days. The temperature and relative humidity were 10°C and 80 to 90% RH, respectively.

#### Statistical design

The statistical design was completely randomized design in factorial scheme 4 x 6 (concentrations x days of analysis), with three repetitions each day, for each treatment. Data were subjected to analysis of variance (ANOVA), where significant results for the F test ( $P < 0.05$ ) were subjected to the comparison test of medium for the qualitative variables, and the regression for quantitative variables. In all the statistical procedures described SISVAR 5.1 program was used.

## RESULTS AND DISCUSSION

The loss of mass is a very important factor in storage vegetables. It occurs due to the length of time in storage and in respiration. The loss of mass is related to the loss of water, which is the main cause of deterioration and

**Table 1.** R<sup>2</sup> in regression analysis.

Treatment	R <sup>2</sup>
C0	0.9596 <sup>ns</sup>
C1	0.9347*
C2	0.9064*
C3	0.9643*

ns: not significant F test ( $P < 0.05$ ), \* significant at F test ( $P < 0.05$ ).

results in quantitative losses, losses in appearance (wilting and wrinkle), the textural qualities (softening, loss of freshness and juiciness), and on nutritional quality (Kader, 1992; Carvalho and Lima, 2002). Deterioration was analyzed from the physico-chemical aspects, being the loss of mass, titratable acidity, pH, soluble solids and firmness.

This observed an increase in mass loss during storage, regardless of the treatment with AA being that the larger the dose applied minor was the loss of mass. The only treatment that showed no significance was the witness in accordance with Table 1. The small loss in mass by the witness can be explained by the use of the packaging, as this restricts in gas exchange of the product within the middle (Carvalho and Lima, 2002).

The results of the analysis of variance of the physico-chemical attributes of cassava are presented in Table 2. It is observed in this Table that there was no significance in the interaction of concentration treatments x days of analysis. There were significant differences between the following variables, firmness and soluble solids in different concentrations of AAd and these variables: pH, soluble solids and titratable acidity of the days of analysis. The decrease in firmness is related to the loss of the integrity of the cell wall, with its enzymatic hydrolysis due to the action of enzymes, such as pectinolytic polygalacturonase (PG) and pectinamylesterase (SMES) (Chitarra and Chitarra, 2005). The function of PG act in breach of glycosidic pectic substances to form galacturonic acid, having its activity related to SMES since it dependent on the product of the reaction of this last.

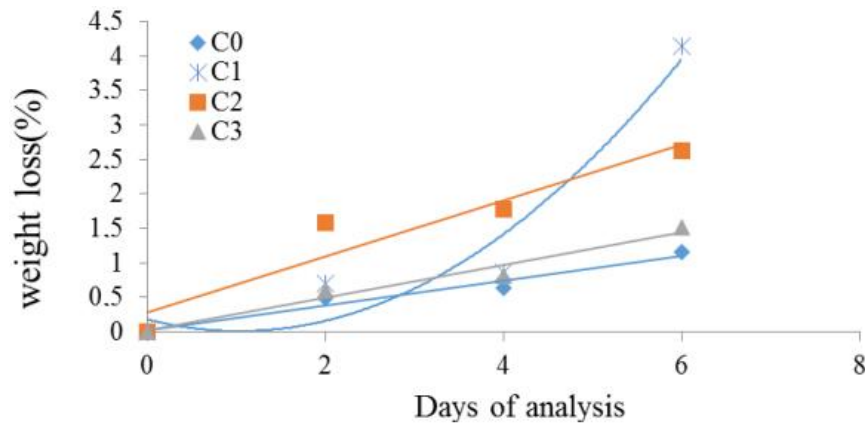
According to Figure 2, the treatments with different concentrations presented a variation on the firmness, and the treatments with concentrations 1 and 3% showed highest firmness, when compared to others (2869.16 and 3029.58 cN, respectively) (Figure 3).

pH showed significant difference only to the variation in the days of analysis. According to Table 3, the treatment shows an average pH declined with the passage of time, until day 4 had an increase on the sixth day which dose not have differentiating initial pH. The behavior obtained from titratable acidity (AT) was reflected in the results obtained for pH. For pH, there were difference between the treatments in the days of analysis, and the values were lower when the acidity was higher and vice versa.

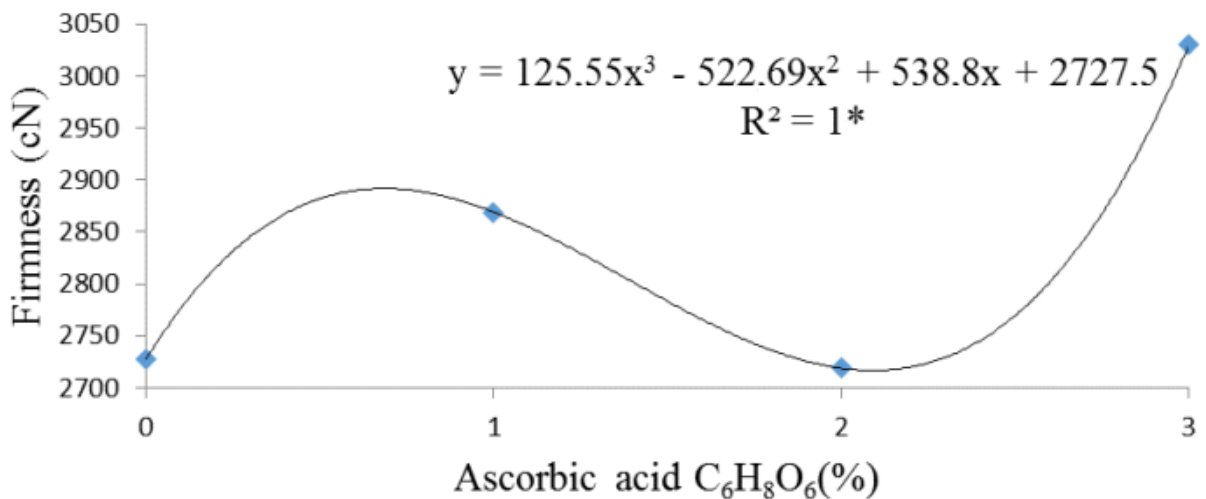
**Table 2.** Mean values obtained in analysis of variance for studied variables.

FV	GL	Firmness	pH	Soluble solids	Titrateable Acidity
Concentration	3	256376.388*	0.012414	3.712641*	0.026474
Day	3	50431.944	0.379069*	3.74203*	0.380552**
Concentration x Day	9	89490.74	0.077142	1.417845	0.023274
Residual	32	60018.229	0.042277	0.92094	0.013279
	CV(%)	8.64	3.36	27.34	13.43
	Overall Average	2836.25	6.110	3.510625	0.8581667

\* significant at 5% de probability for the F-test, \*\* significant at 1% de probability for the F-test. CV(%): (standard deviation/mean)\*100.



**Figure 2.** Mass loss rate with 0, 1, 2 and 3% of ascorbic acid during 6 days of storage at cassava type table.



**Figure 3.** Regression of the values of steadfastness 0 concentrations, 1, 2 and 3% of Ascorbic acid in cassava.

Bezerra et al. (2002) also obtained an increased AT for a period of storage for butter cassava, minimally processed when they made the same assessment subject to the

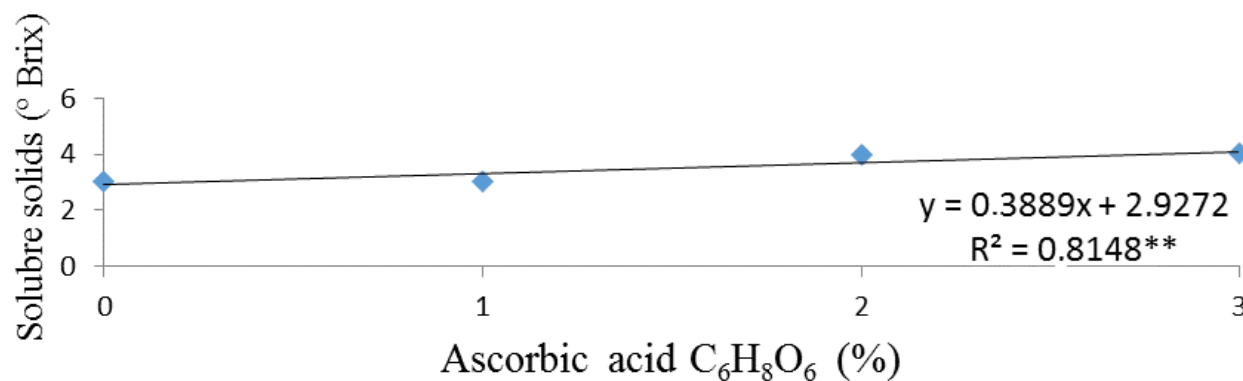
bleaching and packed in polyethylene packages. According to Dias et al. (2007), the increase of AT can be due to bacterial fermentation process with oxygen



**Table 3.** Average values of pH, soluble solids and titratable acidity of cassava table type evaluated during six days of storage at 10°C.

Days of analysis	pH	Titratable acidity (AT) (%)	Soluble solids (°Brix)
0	6.21ab	1.022a	3.133b
2	6.00bc	0.647c	3.534ab
4	5.92c	0.988a	4.291a
6	6.30a	0.775b	3.083b

Values followed by the same letters in the column do not differ among themselves by tukey test at 5% probability.



**Figure 4.** Regression of the values of steadfastness 0 concentrations, 1, 2 and 3% of ascorbic acid in cassava.

consumption and production of organic acids such as butyric, lactic, acetic, among others. Thus, the reduction of pH is related to the increase of AT.

Statistical analysis for variable soluble solid showed significant effect, which show an elevation of this variable during the experiment. Araújo (2010) reports similar behavior in minimally processed products studies caused mainly by the loss of water from the product. Still in Table 3 it can be observed that until the fourth day, there has been an increase in soluble solids, which is expected since there was also loss of mass during storage and also an increase in the concentration of soluble solids. On the sixth day, storage fell on this value that gives the degradation of sugars presented by metabolic processes, that occurred throughout the store.

The variable soluble solids was also significant for the treatment with different concentrations that obtained results expressed in Figure 4. According to the graph there was an increase in the soluble solids which increased the concentration of AA, showing values that ranged from 3.0 °Brix (witness) to 4.0 °Brix (Dose of 3%). Based on the data obtained, it was possible to notice that AA treatment was not effective for conservation of minimally processed cassava. The AA treatment was not effective in experiment performed with minimally processed butter cabbage using the same concentrations of 0, 1, 2 and 3% for Damatto Jr. et al.[s.d.].

## Conclusion

According to the results, we can conclude that the AA did not have efficiencies in the preservation of the physical and chemical characteristics, desirable in minimally processed cassava. We also observed that there was no interaction of AA concentration with the days of analysis, and that the soluble solids increased with an increasing dose of AA.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## REFERENCES

- Alves A, Cansian RL, Stuart G, Valduga E (2005). Alterações na qualidade de raízes de mandioca (*Manihot esculenta* Crantz) minimamente processadas. Ciênc. Agrotecnol. 29:330-337.
- Alves JA, Boas EVBV, Souza EC, Boas BMV, Piccoli RH (2010). Vida útil de produto minimamente processado composto por abóbora, cenoura, chuchu e mandioquinha-salsa. Rev. Ciênc. Agrotecnol. Lavras 1:182-189.
- Araújo FMMC, Machado AV (2010). Caracterização de parede celular de melão minimamente processado armazenado sob atmosfera modificada. Verde de Agroecologia e Desenvolvimento Sustentável, Mossoró - RN 5:421-427.
- Bezerra VS, Pereira RGFA, Carvalho VD, Vilela ER (2002). Raízes de

- mandioca minimamente processadas: efeito do branqueamento na qualidade e conservação. *Ciênc. Agrotecnol. Lavras* 26:564-575.
- Borges MF, Fukuda VMG, Rossetti AG (2002). Avaliação de variedades de mandioca para consumo humano. *Pesqui. Agropecu. Bras.* 37:1559-1565.
- Carvalho AV, Lima LCO (2002). Qualidade de kiwis minimamente processado e submetidos a tratamento com ácido ascórbico, ácido cítrico e cloreto de cálcio. *Pesqui. Agropecu. Bras. Bras.* 37:679-685.
- Cereda MP, Vilpoux O (2003). Conservação de raízes. In: *Tecnologia, usos e potencialidades de tuberosas amiláceas latino americanas*. São Paulo. Fundação Cargill 711:13-29.
- Damatto Jr, Manoel L, Campos AJ, Vieites RL (s.d). Utilização de ácido cítrico, ácido ascórbico e cloreto de cálcio na qualidade de couve-manteiga "minimamente processados" e armazenada sob refrigeração. Depto. Gestão e Tecnologia Agroindustrial, UNESP – FCA, Botucatu, SP [s.d.].
- Dias ARG, Elias MC, Oliveira M, Helbig E (2007). Oxidation of fermented cassava and corn staches: development of the expansion property. *Ciênc. Technol. Aliment. Campinas* 27:794-799.
- IAL-Instituto Adolfo Lutz (2008). Métodos físico-químicos para análise de alimentos. Coordenadores Odair Zenebon, Neus Sadocco Pascuet e Paulo Tinglea – São Paulo: Instituto Adolfo Lutz pp. 10-1020.
- Kader AA (1992). *Postharvest Technology of Horticultural Crops*. Division of Agriculture and Natural Resources, Oakland, California 1:94-608.
- Melo AAM, Vilas Boas EVB (2006). Inibição do escurecimento enzimático de banana maçã minimamente processada. *Ciênc. Technol. Aliment. Campinas* 26:110-115.
- Menolli LN, Finger FL, Puiatti M, Barbosa JM, Barros RS (2008). Atuação das enzimas oxidativas no escurecimento causado pela injúria por frio em raízes de batata-baroa. *Acta Sci. Agron. Maringá* 30:57-63.
- Nechet KL, Halfeld-Vieira BA (2010). Comunicado Técnico 53. Identificação e Manejo das Doenças da Mandioca em Roraima. EMBRAPA: Boa Vista 5 p.
- Pereira AS, Lorenzi JO, Valle TL (1985). Avaliação do tempo de cozimento e padrão de massa cozida em mandioca de mesa. *Rev. Bras. Mandioca Cruz das Almas* 4:27-32.
- Pineli LLO, Moretti CL, Almeida GC, Nascimento ABG, Onuki ACA (2005). Associação de atmosfera modificada e antioxidante reduz o escurecimento de batatas "Ágata" minimamente processadas. *Hort. Bras. Bras.* 23:993-999.
- Rinaldi MM, Vieira EA, Fialho JF, Malaquias JV (2015). Effect of diferente freezing forms on cassava roots. *Braz. J. Food Technol. Campinas* 93:101.
- Vieites RL, Daiuto ER, Carvalho LR, Garcia MR, Lozano MG, Watanabe LM (2012). Mandioca minimamente processada submetida a radiação gama. *Rev. Ciênc. Agrár. Londrina* 33(1):271-282.

## Full Length Research Paper

# Response of bread wheat (*Triticum aestivum* L.) varieties to N and P fertilizer rates in Ofla district, Southern Tigray, Ethiopia

Melesse Harfe

Mehoni Agricultural Research Center, Ethiopian Institute of Agricultural Research, Tigray, Ethiopia.

Received 19 October, 2015; Accepted 12 November, 2015

A field experiment was conducted during the 2006 cropping season in Ofla district in southern Tigray to assess the response of bread wheat (*Triticum aestivum* L.) varieties to nitrogen and phosphorus fertilizer rates. Factorial combinations of two wheat varieties (HAR1685 and Shehan), four N rates (0, 23, 46 and 69 kg N/ha), and four P rates (0, 10, 20 and 30 kg P/ha) were laid out in split plot design (varieties in the main plot and N-P fertilizer rates to the sub plots) with three replications. The variety HAR1685 exhibited significantly higher values for grain yield, harvest index, total N uptake, grain P uptake, N apparent recovery, and agronomic efficiency but had shorter plant height and maturity period. Shehan on the other hand, exhibited higher values for tiller number per plant, plant height, shoot dry weight at 50% flowering and at physiological maturity, biomass yield, straw yield. Application of N and P fertilizers significantly ( $P < 0.01$ ) influenced plant height, shoot dry weight at physiological maturity, and tiller number per plant. The variety-P-N interactions were highly significant for grain yield, biomass yield, harvest index, and straw yield. The increase and decrease in shoot dry weight at 50% flowering, grain yield, biomass yield harvest index, straw yield and available soil phosphorus at harvesting did not show a consistent trend. However, an increasing trend was observed when the rate of N increased from 0 to 69 kg N/ha. Non-significance difference due to the interaction of P and N with respect to plant height, number of tillers per plant, initial plant stand, 1000 kernels weight, days to 50% flowering and total soil nitrogen at harvest was observed. The grain and straw N and P contents and uptakes, increased with N and P levels but the apparent recovery and agronomic efficiency of N and P fertilizers decreased with increasing N and P rates in both varieties. Grain and biomass yields were significantly and positively correlated with most of the agronomic parameters, grain N and P contents, grain N and P uptakes, and total N and P uptakes. Moreover, grain and straw N and P contents and their uptakes were strongly and positively correlated with applied N and P respectively. Even though it is one year in one location study, it can be concluded that the released variety HAR1685 had high grain yield potential, strong stems, medium plant height, heavier kernel weight, higher harvest index, and shorter maturing period and responsive to higher N and P rates. The shortcoming of the experiment was it was conducted in a single location. It should be repeated in more than one location.

**Key words:** Bread wheat, nitrogen, phosphorous, soil, physical, chemical.

## INTRODUCTION

Wheat is one of the major cereal crops in the Ethiopian highlands that lie between latitude 6° and 16°N and

longitude 35° and 42°E and is widely grown from 1500 to 3000 masl. The most suitable areas for wheat production however fall between 1900 and 2700 masl (Hailu, 1991). Wheat production in the country is adversely affected by low soil fertility and suboptimal use of mineral fertilizers in addition to diseases, weeds, erratic rainfall distribution in lower altitude zones, and water-logging in the Vertisol areas (Amanuel et al., 2002). Among the major plant nutrients, N is the most essential for successful wheat production in most agro-ecological zones.

In Tigray Regional State, particularly in the southern part of the region, wheat is a dominant crop in the medium and highland areas. Currently, nationally released wheat varieties are introduced into the region and some of the varieties get acceptance by farmers due to their adaptability, higher yield, relatively high grain price, good bread and other food quality, and the straw for their livestock. From the wheat varieties, HAR1685 is a major one which is currently produced widely in the area particularly in Ofla district. In the area, there are also land races which are widely cultivated by farmers locally named as 'Bani/Shehan' and 'Tselim Sinday'. The landrace Bani (Shehan) is a white colored which is used for making bread, 'Kita', porridge, and consumed as roasted and boiled.

In order to alleviate the soil fertility problem in the area, the Bureau of Agriculture and Natural Resources of the Region has introduced chemical fertilizers particularly DAP (diammonium phosphate) and urea fertilizers in each district of the zone. However the fertilizer rate which is being used by farmers is 'blanket' recommendation throughout the region. Therefore, the objective of the study was to see the effect of each fertilizer rate on: (1) yield and yield components of bread wheat varieties; (2) NP concentrations in plant tissues; (3) NP uptake by bread wheat varieties; (4) fertilizer N and P recovery and use efficiency; (5) the interactions of N and P in affecting yield and yield components of bread wheat.

## MATERIALS AND METHODS

### Description of the study area

The study was conducted in Tigray Regional State in the southern zone at Ofla district (Figure 1) in the experimental field of Alamata Agricultural Research Center during 2006 cropping season (Figure 2).

Ofla district is one of the six districts of Southern Tigray zone. It is located about 620 km away from Addis Ababa to the north part of the country and about 150 km to the south of Mekelle town. The district is located on the geographic coordinates of 12°31' North latitude and 39°33' East longitude. The altitude varies between 1700 and 2800 masl and the slope ranges from 8 to 15%. Traditionally the district is classified into three agro-ecological

zones, namely, *Dega*, *Waina Dega*, and *Kolla*. The *Waina Dega* covers the largest part which accounts about 42% of the total 133,300 ha while both the *Dega*, and *Waina Dega* cover 29% (Ofla District BoANRD, 2005). The average land holding in the district is about 0.5 ha per household and estimated total population of 132,491 (Ofla District BoANRD, 2005).

### Experimental design and procedures

Two wheat varieties (HAR1685 a released variety and Shehan local variety), four nitrogen fertilizer rates (0, 23, 46 and 69 kg N/ha) and four rates of phosphorus fertilizer (0, 10, 20 and 30 kg P/ha) were used in a 2x4x4 factorial split-plot design with three replications. The varieties were assigned to the main plot and the fertilizer combination treatments were assigned to the sub-plots.

The experimental field was prepared 3 times before planting by the conventional tillage practice. Urea and diammonium phosphate (DAP) were used for the source of N and P. The total area used in the experiment was 55.5 × 18 m (999 m<sup>2</sup>). Nitrogen was applied in split doses; that is one-third at planting and two-third at mid-tillering while phosphorus was applied in full dose at planting time. The two wheat varieties were sown at the recommended seed rate of 150 kg/ha in rows by using manual row maker on July 18, 2006. The spacing between plants, rows, sub-plots, main plots, and blocks were 5, 20, 50, 100 and 150 cm, respectively.

Each sub-plot had 2 m × 3 m (6 m<sup>2</sup>) gross size and 0.4 m × 3 m (1.2 m<sup>2</sup>) net size. The row length was 3 m and there were 10 rows in each sub-plot. The middle two rows were used for agronomic data collection, the four rows were used for destructive sampling, and the four rows served as border.

### Data collection

#### *Soil sampling and determination of soil physical and chemical properties*

Soil samples were taken using auger from the experimental area (5 spots from each block) after final land preparation from a depth of 0-30 cm. The sub samples were composited to get a representative sample for analysis of soil texture, organic matter, pH, CEC, total nitrogen and available phosphorus. At maturity, one representative soil sample (0-30 cm depth) was taken from every plot with auger and the same treatments were composited making 32 samples for the analysis of available phosphorus and total nitrogen.

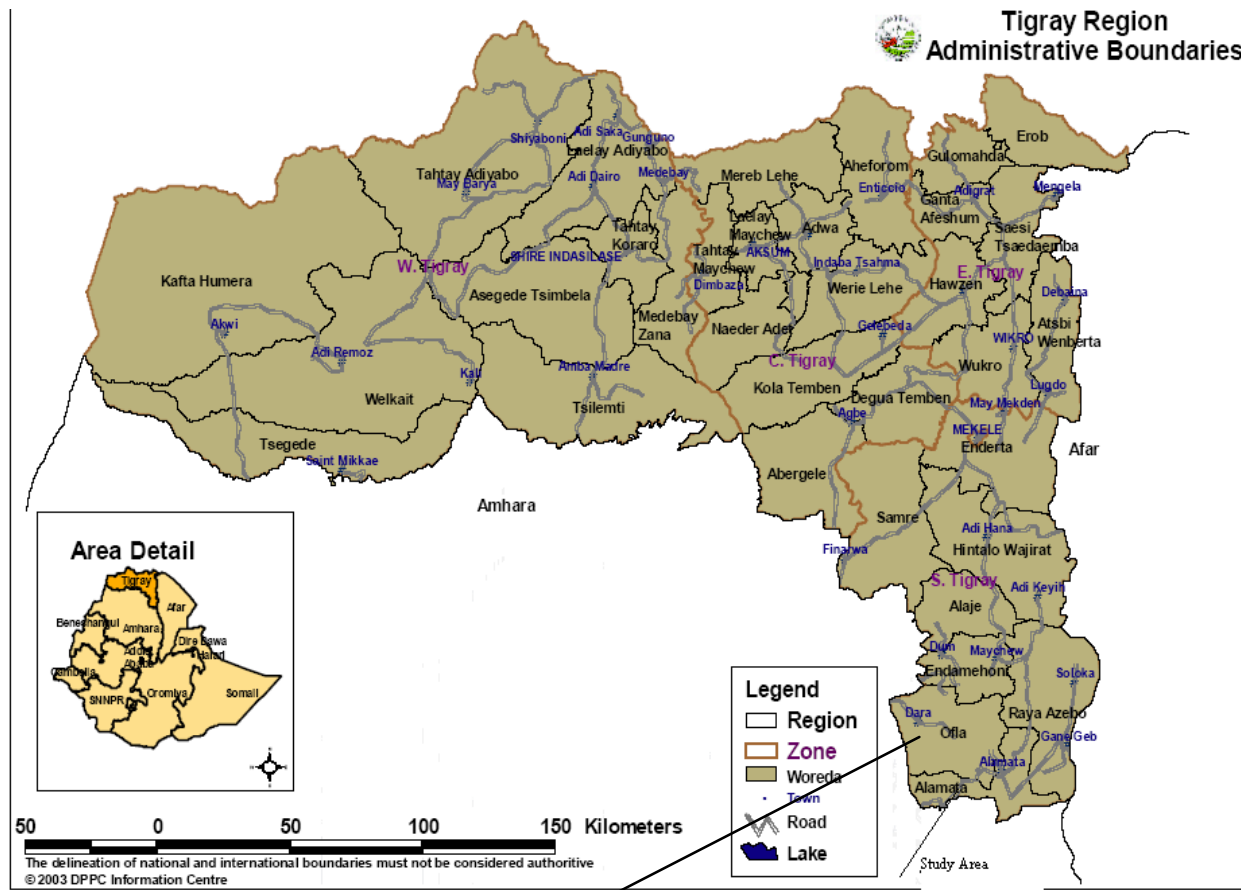
Soil texture was determined using the Bouyoucos hydrometer method (Day, 1965). The pH of the soil was measured potentiometrically in the supernatant suspension of a 1: 2.5 soil to water ratio using a pH meter and organic carbon was determined following Walkely and Black wet oxidation method as described by Jackson (1967). Available P was extracted with a sodium bicarbonate solution at pH 8.5 following the procedure described by Olsen et al. (1954). Total nitrogen was determined using Kjeldahl method as described by Jackson (1967).

#### *Plant sampling and analysis for nitrogen and phosphorus content*

After maturity, five non-boarder wheat plants per plot were randomly

E-mail: melesse2005@gmail.com.

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](https://creativecommons.org/licenses/by/4.0/)



Ofla District (the study area)

Figure 1. Map of the study area.

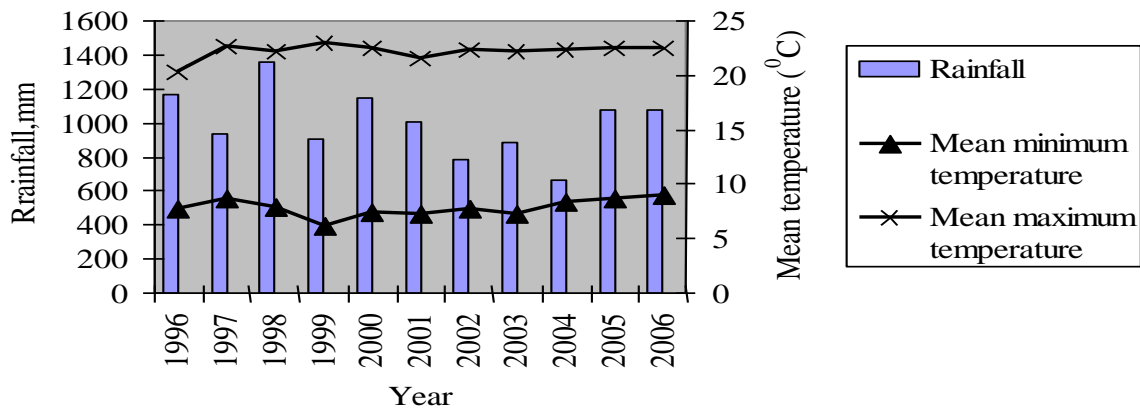


Figure 2. Eleven years' Annual rainfall, mean minimum and maximum temperatures in the study area (1996-2006).

taken and partitioned into grain and straw. Samples collected from each replication of a treatment were bulked to give one composite plant tissue sample. The grain and straw samples were separately air dried and oven dried at 70°C to constant weight. Before grinding, the straw samples were washed with distilled water to clean the samples from contaminants such as dust. After washing

with distilled water, it was dried in oven at 70°C for 24 h. After drying, the plant tissue sample was ground and passed through 1 mm sieve for laboratory analysis.

The grain and straw contents of N and P were determined by Kjeldahl method and Olsen method respectively. Sample digestion was carried out by using a fume hood. This digest was used for the

**Table 1.** Selected physical and chemical properties of the soils of the experimental site.

pH	CEC (cmol/kg)	Total N (%)	Available P (ppm)	OM (%)	Particle size distribution (%)			Textural class
					Sand	Silt	Clay	
7.04	44.41	0.134	19.9	2.23	36	33.64	30.36	Clay loam

analysis of both N and P. Total N and P uptake by straw and grain were calculated by multiplying N and P contents by respective straw and grain yield/ha. Total N or P uptake, by whole biomass, was calculated by summing up the N uptake and P uptake of grain and straw. Finally, applied N or P nutrient recovery was computed as the difference of the values of N or P nutrient (kg/ha) and the plant uptake values (kg/ha) as described by Pal (1991), agronomic and physiological efficiency as suggested by Mengel and Kirkby (1987, 1996).

#### **Agronomic data collection**

Days to 50% crop emergence, days to heading, days to 50% flowering, and days to 90% physiological maturity were recorded at the respective growth stage of the crop. Data were collected from the middle rows for initial plant stand at 15 days after sowing (DAS) and number of tillers per plant. Samples for shoot dry weight per 0.5 m row length at 50% flowering and at harvest were taken from the rows selected for destructive sampling. Dry matter of the shoot at 50% flowering and physiological maturity was determined by oven drying the samples at 70°C to constant weight. Yield and yield components and related traits such as plant height, 1000 kernel weight, total above-ground biomass, grain yield and harvest index were recorded during the study time. Thousand grain weight and grain yield were determined after adjusting the moisture level of grain to 12.5%. The harvest index was calculated by dividing the grain yield with total aboveground biomass.

#### **Data analysis**

The data were subjected to statistical analysis. Analysis of variance (ANOVA) was carried out using MSTATC computer software programs. Significant difference between and among treatment means was assessed using the least significant difference (LSD) at 0.05 level of probability (Gomez and Gomez, 1984). Correlation between agronomic parameters, N and P contents and uptakes was carried out by SPSS software program.

## **RESULTS AND DISCUSSION**

### **Selected physical and chemical properties of the soil of the experimental site**

The results of the laboratory analysis of some physical and chemical properties of the soil of the experimental site are presented in Table 1. The results indicated that the experimental soil was clay loam in texture, with pH (H<sub>2</sub>O) of 7.04, organic matter content of 2.23%, total N of 0.134%, available P of 19.9 mg/kg, and CEC of 44.41 cmol/kg soil (Table 1).

Sahlemedihn (1999) ranked 0.1 to 0.2 total N of soil as

low and, 3 to 5% organic matter (OM) as high and more than 5% as very high. In line with this, the OM content of the experimental soil was in the medium range and the total N is in low range. Cation exchange capacity (CEC) is an important parameter of soil; because it gives an indication of the type of clay mineral present in the soil and its capacity to retain nutrients against leaching. According to Sahlemedhin (1999), CEC by sodium acetate at pH 8.2 or ammonium acetate at pH 7.0 with values between 25 and 40 cmole/kg are high to medium and satisfactory for agriculture with the use of fertilizer and CEC > 40 as high to very high and needs only small amounts of lime and potassium fertilizers. Therefore, the CEC of the experimental soil (44.41 cmol/kg) was in the range of high to very high. According to Mengel and Kirkby (1996) the optimum pH range for wheat production is from 4.1 to 7.4. Thus, the pH of the experimental soil was optimum for wheat production.

### **Crop phenology**

More than 90% crop emergence occurred in all plots regardless of the N and P rates. Crop emergence up to 50% was recorded in all plots within eight days. This delay might be due to the delayed rainfall after planting.

The days to 50% flowering recorded significant difference ( $p < 0.05$ ) due to N rates (Appendix Table 2). As the N level increased from 0 kg/ha to 69 kg/ha, the days to 50% flowering were delayed from 67.58 to 70.04 days (Table 2). This shows higher rate of N delays flowering. This result is supported by Legesse (2004) who reported that the heading of teff was significantly influenced by applied fertilizer N rate. He indicated that N fertilization at the rate of 23 and 46 kg N/ha significantly delayed the heading stage of teff by five days as compared to the control.

There was no significant difference between the two wheat varieties and among the different P rates with respect to 50% flowering (Appendix Table 2). Similarly Legesse (2004) showed that there was no significant difference on heading stage of teff due to the application of P and its interaction with N. However, fertilizer N is reported to promote leaf growth and leaf area thereby increasing the amount of radiation intercepted and dry matter production (Russel, 1988). This might have promoted greater vegetative development for longer period of time before reproductive phase begins and hence might have caused delay in flowering.

The two varieties and N rates showed highly significant

**Table 2.** Effect of variety, P and N on some agronomic parameters

Main effect				Variable			
Variety	IPS	NTP	DF	DM	PH	SDWH	TKW
HAR1685							
Shehan	415.92	5.84	71.10	129.60	103.75	208.76	40.32
<b>SE(±)</b>	<b>2.42</b>	<b>0.06</b>	<b>0.57</b>	<b>0.52</b>	<b>0.05</b>	<b>3.35</b>	<b>0.95</b>
<b>LSD(0.05)</b>	<b>43.54</b>	<b>ns</b>	<b>ns</b>	<b>6.97</b>	<b>0.74</b>	<b>15.14</b>	<b>ns</b>
<b>N(kg/ha)</b>							
	<b>IPS</b>	<b>NTP</b>	<b>DF</b>	<b>DM</b>	<b>PH</b>	<b>SDWH</b>	<b>TKW</b>
0	389.63	5.00	67.58	123.87	82.63	168.88	45.46
23	390.04	5.50	68.33	124.87	87.08	184.87	45.74
46	376.29	6.29	69.08	125.74	91.17	198.17	45.53
69	386.00	6.63	70.04	127.00	94.18	218.17	45.29
<b>SE(±)</b>	<b>4.13</b>	<b>0.09</b>	<b>0.28</b>	<b>0.37</b>	<b>0.97</b>	<b>6.52</b>	<b>0.86</b>
<b>LSD(0.05)</b>	<b>ns</b>	<b>ns</b>	<b>0.80</b>	<b>1.05</b>	<b>2.74</b>	<b>18.45</b>	<b>ns</b>
<b>P(k/ha)</b>							
	<b>IPS</b>	<b>NTP</b>	<b>DF</b>	<b>DM</b>	<b>PH</b>	<b>SDWH</b>	<b>TKW</b>
0	388.83	5.71	68.79	125.63	86.41	171.90	44.86
10	381.75	5.79	69.21	125.75	88.16	196.20	45.37
20	387.54	6.04	68.42	124.92	89.93	204.54	45.54
30	383.83	5.87	68.63	125.13	90.56	210.42	46.25
<b>SE(±)</b>	<b>4.13</b>	<b>0.09</b>	<b>0.28</b>	<b>0.37</b>	<b>0.97</b>	<b>6.52</b>	<b>0.86</b>
<b>LSD(0.05)</b>	<b>Ns</b>	<b>Ns</b>	<b>Ns</b>	<b>Ns</b>	<b>2.74</b>	<b>18.45</b>	<b>Ns</b>
<b>CV(%)</b>	<b>5.25</b>	<b>7.61</b>	<b>2.01</b>	<b>1.44</b>	<b>5.34</b>	<b>16.32</b>	<b>9.31</b>

IPS = Initial plant stand; NTP = Number of tillers per plant; DF = Days to 50% flowering; DM = Days to 90% physiological maturity; PH = Plant height; SDWH = Shoot dry weight at harvest; TKW = thousand kernel weight.

difference in relation to the days to 90% physiological maturity (Appendix Table 2). Table 2 indicates that the variety HAR1685 matured earlier compared to the local variety Shehan. The highest doses of N (69 kg/ha) delayed physiological maturity of the two wheat varieties. It increased the days to maturity from 123.87 to 127.00 days. Similarly Gurmessa (2002) indicated that fertilizer N beyond 46 kg/ha delayed the physiological maturity of wheat. Furthermore, Temesgen (2001) and Legesse (2004) have reported that N fertilization delayed the physiological maturity of teff. This delay might be due to extended vegetative growth instead of reproductive growth. Plants treated with N, particularly with the highest level of N, remained slightly green for longer duration while those plants without N showed yellow spike, leaf and stem indicating early physiological maturity which might have been due to depression of cytokinin synthesis or increased production of abscisic acid (ABA) under low N supply (Marshner, 1995). According to the author, amino acids are required for the synthesis of cytokinins so that cytokinin metabolism is low at low N status of the soil.

## Yield and yield components

### Plant height

The ANOVA table (Appendix Table 1) shows a highly

significant difference between the two wheat varieties. The local variety Shehan significantly taller than the improved variety HAR1685 (Table 2). Addis (2003) also reported that from 20 genotypes tested in four different locations of Tigray, Shehan had the highest plant height compared to HAR1685 and the other 18 genotypes. There was also highly significant ( $P < 0.01$ ) difference among the different rates of N and P fertilizer rates (Appendix Table 1). As the nitrogen fertilizer rate increased from 0 to 69 kg/ha, the plant height increased from 82.63 cm to 94.18 cm (Table 2). As the P rate increased from 0 to 30 kg/ha the plant height increased from 86.41 to 90.56 cm. Similarly Amsal et al. (2000) observed a positive and linear response to applied fertilizer to plant height in the central highlands of Ethiopia. Several studies in Ethiopia also exhibited dramatic plant height enhancement in response to each increment of fertilizer N doses (Zewdu et al., 1992; Tilahun et al., 1996; Damene, 2003). Many other researchers also have reported that application of higher dose of N fertilizer increased plant height (Evans et al., 1975; Hari et al., 1997; Behera, 1998). The variety-P-N interaction was found non-significant (Appendix Table 1).

### Number of tillers per plant

The analysis of variance showed that there was non-significant difference between the two varieties, due to N

**Table 3.** Shoot dry weight at 50 % flowering as influenced by N and P fertilizer rates.

P applied (kg/ha)	N applied (kg/ha)									
	Shoot dry weight (g/ 0.5 m)									
	HAR1685					Shehan				
	0	23	46	69	Mean	0	23	46	69	Mean
0	42.87	53.76	56.54	58.53	52.92	65.86	82.73	86.82	89.94	81.34
10	43.00	59.23	109.21	114.71	81.54	56.39	62.77	68.77	102.71	72.66
20	79.05	85.40	90.32	126.73	95.37	94.94	100.14	106.00	111.6	103.17
30	74.96	79.56	83.25	84.39	80.54	114.3	109.57	117.17	118.54	114.89
Mean	59.97	69.49	84.83	96.09	77.59	82.87	88.80	94.69	105.70	93.02
Interaction	V	P	VxP	N	VxN	PxN	VxPxN			
SE(+)	1.58	1.04	1.48	1.08	1.48	2.09	2.95			
LSD(0.05)	ns	2.95	4.17	2.95	4.17	5.91	8.35			
CV(%)	5.99									

and P rates (Appendix Table 1). However, as the N fertilizer rate increased from 0 to 69 kg/ ha, the tiller number increased from 5 to 6.63 (Table 2). In line with this several reports (Archer, 1988; Mossedeq and Smith, 1994; Ortiz- Monastrio et al., 1997; Genene, 2003) indicated greater tillering as well as higher percentage of survival of the tillers due to higher N application. Botella et al. (1993) reported that stimulation of tillering with application of N may be due to its effect on cytokinin synthesis.

### Thousand kernels weight

Non-significant difference was observed between the two wheat varieties and among the different N and P in relation to 1000 kernel weight (Appendix Table 1). However, HAR1685 had heavier 1000 kernel weight (Table 2). Gurmessa (2002) also indicated neither the main effect of N and P nor their interaction brought about significant change in 1000 grain weight. In similar other reports, Mooleki and Foster (1993), Gooding and Davies (1997), Mekonen (1985), Lemma et al. (1992) and Zewdu et al. (1992) have reported non-significant effect on 1000 kernel weight due to different doses of N fertilizers.

### Effect of N and P on initial plant stand

Plant stand was counted at 15 days after planting. The results revealed that there was significant difference ( $P < 0.01$ ) between HAR1685 and Shehan (Appendix Table 1). Shehan had more number of plants per four rows compared to HAR1685 (Table 2). This might be due to small seed size in Shehan which has more number of seeds per kilogram of seed compared to HAR1685. The ANOVA table indicates non-significant difference ( $P < 0.05$ ) among the P and N fertilizer rates and their interactions in terms of plant stand (Appendix Table 1).

### Effect of applied N and P on shoot dry weight at 50% flowering

The N rates, P rates and variety-N-P interactions showed significant ( $P < 0.05$ ) difference for shoot dry weight at 50% flowering (Appendix Table 1). As the P rates increased from 0 to 30 kg P/ha, shoot dry weight at N rates of 0, 23 and 69 kg N/ha for variety HAR1685 increased up to 20 kg P/ha and then decreased at 30 kg P/ha. At N rates of 46 kg N/ha shoot dry weight for this variety increased at 10 kg P/ha then it decreased. For variety Shehan the trend was different at all N rates (except 69 kg N/ ha); 0 kg P/ha gave high yield than 10 kg P/ha. The highest shoot dry weights at 50% flowering were found in 69-20 N-P kg/ ha interaction in HAR1685 (126.73 g/ 0.5 m) and in 30-69 P-N kg/ha interaction in Shehan (118.54 g/ 0.5 m). However, the lowest shoot dry weights at 50% flowering were observed in the interaction 0-0 N-P kg/ ha in HAR1685 (42.87 g/ 0.5 m) and in Shehan (56.39 g/ 0.5 m) in the interaction 10-0 P-N kg/ ha interaction (Table 3).

### Shoot dry weight of wheat at harvesting

The analysis of variance table (Appendix Table 1) shows that there were highly significant differences ( $P < 0.01$ ) due to varieties, P rates and N rates. Shehan gave more shoot dry weight than HAR1685 (Table 2). For both N and P as the rate increased, the shoot dry weight at physiological maturity increased.

### Effect of N and P rates on grain yield

Appendix Table 1 indicates there was highly significant difference between the varieties, the N rates, P rates and their interactions in relation to grain yield. Table 4 shows that the highest grain yield for HAR1685 was at 0 kg P/ha



**Table 4.** Grain yield as influenced by N and P fertilizer rates.

P applied (kg/ha)	N applied (kg/ha)									
	Grain yield (kg/ha)									
	HAR1685					Shehan				
	0	23	46	69	Mean	0	23	46	69	Mean
0	2333	3708	4596	4741	3844	2966	3114	3191	3628	3225
10	3671	4011	4266	4682	4157	2351	3227	3400	3903	3220
20	3710	3852	4109	4408	4020	2925	3181	3378	3542	3256
30	3217	4175	4258	4164	3954	2575	3501	3580	3819	3369
Mean	3233	3936	4307	4499	3994	2704	3256	3387	3723	3268
Interaction	V	P	VxP	N	VxN	PxN	VxPxN			
SE(+)	21.89	20.89	29.55	20.89	29.55	41.78	59.10			
LSD(0.05)	235.10	59.10	83.58	59.10	83.58	118.20	167.71			
CV(%)	2.82									

**Table 5.** Biomass yield as influenced by N and P fertilizer rates.

P applied (kg/ha)	N applied (kg/ha)									
	Biomass yield (kg/ha)									
	HAR1685					Shehan				
	0	23	46	69	Mean	0	23	46	69	Mean
0	6967	10592	10708	10992	9815	9236	10358	10325	12017	10484
10	8375	9783	9975	10625	9689	8152	9392	9642	11517	9676
20	8675	10167	10417	10667	9982	9644	9797	9975	10525	9985
30	8049	9658	10508	11042	9814	8917	11169	11233	11233	10638
Mean	8016	10050	10402	10832	9825	8987	10179	10294	11323	10196
Interaction	V	P	VxP	N	VxN	PxN	VxPxN			
SE(+)	2.30	2.86	4.05	2.86	4.05	5.73	8.10			
LSD(0.05)	33.98	8.10	11.45	8.10	11.45	16.20	22.90			
CV(%)	0.14									

and 69 kg N/ha and for Shehan it was at 10 kg P/ha and 69 kg N/ha. The increase and decrease in grain yield did not show a consistent trend. For example for HAR1685 there was yield decrement at 69 kg N/ha as P rate increased from 0 to 30 kg P/ha, but for Shehan there was fluctuation. For HAR1685 at 46 kg/ha, relatively the highest yield was obtained at the lowest P rate, but for Shehan it was vice versa.

The fertilized treatments when compared with unfertilized treatments (control), showed yield increment in both varieties. Yield increment of 1831 kg/ha (78.5%) and 853 kg/ha (28.75%) were obtained in the highest (30-69 N-P kg/ha) interaction in both HAR1685 and in the local variety Shehan over the control respectively (Table 4). Even though there was yield increment in both varieties, higher grain yield increment was found in HAR1685 due to the interaction of 69-0 N-P kg/ha (4741 kg/ha) and followed by 46-0 N-P kg/ha (4596 kg/ha). This indicates application of N fertilizer had significant effect

on grain yield compared to P fertilizer. This might be due to high initial P content in the soil. Moreover, the highest grain yield in Shehan was recorded in 69-10 N-P kg/ha interaction which was 3903 kg/ha. In contrast to this, the lowest grain yields in HAR 1685 (2333 kg/ha) and in Shehan (2966 kg/ha) were in 0-0 N-P kg/ha and in 10-0 N-P kg/ha interactions respectively. It was visually observed that wheat plants in the control plots were very thin with small spike size, short and light in color whereas plants in the fertilized plots particularly in the highest N levels had thick and strong stems, green in color, large spike size. Particularly this visual difference was evident clearly in HAR1685 variety.

#### **Effect of N and P rates on biomass yield of wheat**

The variety, P rates, N rates and all interactions were highly significantly different (Appendix Table 1).

**Table 6.** Harvest Index as influenced by N and P fertilizer rates

P applied (kg/ha)	N applied (kg/ha)									
	Harvest index									
	HAR1685					Shehan				
	0	23	46	69	Mean	0	23	46	69	Mean
0	0.34	0.35	0.43	0.43	0.39	0.32	0.30	0.31	0.30	0.31
10	0.44	0.41	0.43	0.44	0.43	0.29	0.34	0.35	0.34	0.33
20	0.43	0.38	0.39	0.41	0.40	0.30	0.33	0.34	0.34	0.33
30	0.40	0.43	0.41	0.38	0.41	0.29	0.31	0.32	0.34	0.32
Mean	0.40	0.39	0.42	0.42	0.41	0.30	0.32	0.33	0.33	0.32
Interaction	V	P	VxP	N	VxN	PxN	VxPxN			
SE(+)	0.0014	0.002	0.009	0.002	0.009	0.0013	0.041			
LSD(0.05)	0.060	0.020	0.026	0.020	0.026	0.037	0.115			
CV(%)	2.60									

**Table 7.** Effect of N and P fertilizer rates on straw yield.

P applied (kg/ha)	N applied (kg/ha)									
	Straw yield (kg/ha)									
	HAR1685					Shehan				
	0	23	46	69	Mean	0	23	46	69	Mean
0	4633	6883	6112	6251	5970	6259	7250	7134	8386	7256
10	4704	5773	5709	5944	5533	5802	6165	6241	7613	6455
20	4968	6315	6307	6258	5962	6723	6619	6597	6983	6731
30	4831	5483	6250	6878	5861	6342	7390	7651	7415	7199
Mean	4784	6114	6095	6333	5831	6282	6855	6906	7599	6910
Interaction	V	P	VxP	N	VxN	PxN	VxPxN			
SE(+)	20.53	20.95	29.62	20.95	20.62	41.89	59.24			
LSD(0.05)	537.34	59.25	83.80	59.25	83.80	118.50	167.60			
CV(%)	1.61									

Application of 69 kg N/ha gave the highest mean biomass yields in HAR1685 (10832 kg/ha) and in Shehan (11323 kg/ha). Compared to the control plots, mean biomass increment of 2816 kg/ha in HAR1685 and 2336 kg/ha in Shehan were found due to the application of 69 kg N/ha. This might be due to more plant height and number of tillers in Shehan (Table 5). The highest biomass yield for HAR1685 was at the highest P and N rates (Table 5) similar to grain yield, there was fluctuation. The only consistent trend was for HAR1685 at 0 kg N/ha to 20 kg P/ha and then decreased at 30 kg P/ha.

#### **Effect of N and P on harvest index**

There was highly significant ( $P < 0.01$ ) difference among the variety, N rate, P rate and their interactions in relation to harvest index (Appendix Table 2). Table 6 shows that the decrease and increase in harvest index does not

show any trend in any of the N and P rates. In terms of numerical comparisons, it could be said that in general harvest index of HAR1685 is higher than that of Shehan at every N and P rates.

Generally as the N and P rates increased from 0 kg/ha to 30 kg P/ha and 69 kg N/ha, the harvest indices were increased from 0.34 to 0.38 in HAR1685 and from 0.32 to 0.34 in Shehan (Table 6). The highest harvest indices were observed in 10-69 P-N kg/ha (0.44) followed by 0-69 P-N kg/ha (0.44) in HAR1685 and in 10-46 P-N kg/ha in the local variety Shehan. The variety HAR1685 had greater mean harvest index (0.41) than Shehan (0.32). This might be due to the higher grain and straw yields in HAR1685 compared to Shehan.

#### **Effect of N and P on straw yield of bread wheat**

The ANOVA (Appendix Table 2) indicates there was

**Table 8.** Available soil phosphorus as influenced by N and P fertilizer rates.

P applied (kg/ha)	N applied (kg/ha)									
	Available soil P									
	HAR1685					Shehan				
	0	23	46	69	Mean	0	23	46	69	Mean
0	8.48	9.57	10.59	9.43	9.62	8.81	9.64	8.75	8.95	9.04
10	11.49	8.63	9.63	9.56	9.83	10.68	11.12	10.52	10.55	10.72
20	15.57	11.36	11.44	13.55	12.98	11.61	11.80	12.65	13.37	12.36
30	16.36	13.81	12.62	14.62	14.35	14.64	13.9	14.82	16.78	15.04
Mean	12.97	10.84	11.07	11.79	11.67	11.44	11.62	11.68	12.41	11.79
<b>Interaction</b>	<b>V</b>	<b>P</b>	<b>VxP</b>	<b>N</b>	<b>VxN</b>	<b>PxN</b>	<b>VxPxN</b>			
SE(+)	0.011	0.047	0.066	0.047	0.066	0.094	0.133			
LSD(0.05)	ns	0.133	0.188	0.133	0.188	0.266	0.376			
CV(%)					1.44					

highly significant ( $P < 0.01$ ) difference between varieties, among N rates, P rates and their interactions with respect to straw yield. The only observable trend (Table 7) is that for HAR1685 in which at 0 kg N/ ha the straw yield increased as P rate increased from 0 to 20 kg P/ha then the straw yield decreased. In terms of numerical comparison, in general straw yield of Shehan was higher than that of HAR1685 at every N and P rates. This could help to understand farmers' preference to Shehan variety for animal feed. The mean straw yield of HAR1685 increased with increase in P up to 20 kg/ ha whereas Shehan did not show any definite trend. However mean straw yield was highest for both varieties at the highest rate of N (Table 7).

#### **Effects of N and P application on available soil phosphorus at harvesting**

The analysis of variance table (Appendix Table 2) shows there was highly significant ( $P < 0.01$ ) difference among the P and N rates and their interactions. The decrease and increase in available soil P was not consistent (Table 8). The available soil P at harvest in HAR1685 at 0 kg P/ha increased as the N rate increased from 0 to 46 kg N/ha but at 30 kg P/ha, available soil P for same variety decreased as N increased from 0 to 46 kg ha. But such type of decrease and increase due to different rates of P and the same rate of N is not observed for Shehan.

Generally as the P and N rates increased from 0 to 30 kg P/ ha and 69 kg N/ ha, the available soil phosphorus at harvesting increased from 8.48 to 14.62 ppm in HAR 1685 and from 8.81 to 16.78 ppm in Shehan (Table 8) even though the amount is less than the available soil P at planting which was 19.9 ppm. The reduction of soil available P at harvesting compared to the initial might be due to uptake by the wheat crop and fixation in soil. The lowest available soil P at harvesting was observed in 0-0

P-N kg/ ha interactions. The highest available soil P was recorded in the interaction of 30-69 P-N kg N/ha in HAR 1685 (14.62) and in Shehan (16.78 ppm).

#### **Effect of N and P application on total soil nitrogen at harvesting**

There was non-significant difference ( $P > 0.05$ ) in both the main and interaction effects with respect to total soil nitrogen at harvesting. The result is supported by Damene (2003) and Gurmessa (2002) who described that the values of total N content of the soil, analyzed from composite sample made per treatment tended to remain almost the same irrespective of different rates of N application.

#### **Crop lodging and disease incidence**

In relation to crop lodging and disease/ insect pest incidence the local variety Shehan was logged almost completely except at the control treatments (data is not given). Moreover, from the visual observation this variety was highly affected by the fungal disease rust particularly the plots with the highest N level. In contrast to this the improved variety HAR-1685 did not show any lodging as well as rust infestation even at the highest N level (69 kg/ha). The highest lodging percentage in Shehan might be due to the vigorous plant height and weak stalks it possesses.

#### **Content, uptake, apparent fertilizer recovery and nutrient use efficiency of nitrogen and phosphorus**

The grain and straw N content increased with rates of N. Maximum N content of grain were 2.2% in HAR 1685 and

**Table 9.** Nitrogen and phosphorus contents of grain and straw, uptake, apparent recovery, and N and P use efficiency at harvest in HAR1685 variety as affected by N and P fertilizers.

Treatment (kg/ha)	Content (%)		Uptake			Apparent recovery	Nutrient use efficiency	
	Grain	straw	Grain	Straw	Total		Agronomic	Physiological
<b>N rates</b>								
0	1.65	0.27	53.36	12.89	66.25	122.69	30.66	0.25
23	2.00	0.26	78.74	15.73	94.47	107.65	23.38	0.22
46	2.20	0.34	94.76	21.01	115.77	51.78	18.36	0.35
69	1.90	0.26	85.52	16.46	101.98	122.69	30.66	0.25
<b>P rates</b>								
0	0.083	0.078	3.19	4.66	7.85	-	-	-
10	0.083	0.100	3.45	5.53	8.98	4.94	31.30	6.34
20	0.085	0.105	3.42	6.26	9.68	3.98	8.80	2.21
30	0.090	0.116	3.56	6.82	10.37	3.66	3.67	1.00

**Table 10.** Nitrogen and phosphorus content of grain and straw, uptake, apparent recovery, and N and P use efficiency at harvest Shehan variety.

Treatment (kg/ha)	Content (%)		Uptake			Apparent recovery	Nutrient use efficiency	
	Grain	straw	Grain	Straw	Total		Agronomic	Physiological
<b>N rates</b>								
0	1.80	0.25	48.66	15.70	64.36	1.80	-	-
23	1.88	0.28	61.21	18.95	80.16	1.88	68.70	24.02
46	1.88	0.31	63.67	21.41	85.08	1.88	45.04	14.89
69	1.83	0.32	68.00	24.07	91.07	1.83	40.16	14.79
<b>P rates</b>								
0	0.070	0.065	2.26	4.72	6.97	0.070	-	-
10	0.080	0.088	2.58	5.68	8.26	0.080	5.58	- 0.50
20	0.085	0.089	2.77	5.99	8.76	0.085	3.88	1.55
30	0.089	0.105	3.00	7.56	10.56	0.089	5.19	4.80

1.88% in Shehan at the fertilizer rates of 23 and 46 kg N/ha (Tables 9 and 10). Grain contained 87.3% of the total N of the crop and the remaining 12.7% was found in straw of HAR1685. However, in Shehan grain had 86.43% of the total N in the crop, and the remaining 13.57% of the total N was found in the straw. At 46 kg N/ha, grain and straw contents of N increased by 33.3 and 25.93% in HAR 1685 and 4.44 and 24% in Shehan over the control respectively. Abdoulaye and Marienville (1999) and Mekonen (2005) also reported that the grain N content and total N content were generally greater with applied N.

Nitrogen uptake of grain and straw increased with levels of N and maximum uptake was obtained at 46 kg N/ha in HAR1685 and at 69 kg N/ha in Shehan. The grain and straw uptake increased by 77.58 and 62.99% in HAR1685 in 46 kg N/ha and 39.75 and 53.3% in 69 kg N/ha in the local variety Shehan respectively. The total uptake also increased with increasing N rates in both varieties (Tables 9 and 10). The highest total N uptakes

were 115.77 kg/ha in HAR1685 and 91.07 kg/ha in Shehan.

Grain and straw P contents increased with increasing of P rate in both varieties. Grain P content ranged from 0.083 to 0.09% in HAR1685 and from 0.07 to 0.089% in Shehan. Straw P content also ranged from 0.078 to 0.116% in HAR1685 and from 0.065 to 0.105 % in Shehan. Grain and straw P uptake increased with rate of P application in both varieties. As the P rate increased from 0 to 30 kg P/ha, the grain P uptake increased from 3.19 to 3.56 kg/ha in HAR1685 and from 2.26 to 3 kg/ha in Shehan. Moreover, the straw P uptake increased from 4.66 to 6.82 kg/ha in HAR1685 and from 4.72 to 7.56 kg/ha in Shehan. Grain P uptake was higher in HAR1685 than in Shehan but the reverse happened for straw P uptake (Tables 9 and 10).

The apparent N recovery decreased with increasing rates of N application in both varieties. As the N rate increased from 23 to 69 kg N/ha, the apparent recoveries decreased from 122.69 to 51.78% in HAR 1685 and from

**Table 11.** Correlations between some agronomic parameters

Parameter	P	N	IPS	SDWF	SDWH	TKW	DF	DM	GY
N	0.000								
IPS	-0.031	-0.083							
SDWF	0.545**	0.474**	0.340						
SDWM	0.415*	0.412*	0.537**	0.578**					
TKW	0.093	-0.016	-0.904**	-0.288	-0.541**				
DF	-0.056	0.351*	0.813**	0.406*	0.632**	-0.910**			
DM	-0.059	0.253	0.846**	0.390*	0.643**	-0.955**	0.948**		
GY	0.059	0.664**	-0.598**	0.175	-0.084	0.566**	-0.301	-0.383*	
BY	0.044	0.817**	0.122	0.506**	0.391*	-0.170	0.440*	0.353*	<b>0.608**</b>

\*\*, \* Correlation is significant at 0.01 and 0.05 level. SY = Straw yield, SP = Straw phosphorus, GP = Grain phosphorus content, SPU = Straw phosphorus uptake, GPU = Grain phosphorus uptake, TPU = Total phosphorus uptake, P = Applied phosphorus, N = Applied nitrogen, GY = Grain yield, SY = Straw yield, HI = Harvest index, BY = Biomass yield.

**Table 12.** Correlations between applied N and contents, uptake, grain yield, biomass yield, straw yield and harvest index.

Parameters	P	N	GY	SY	SNC	GNC	SNU	GNU	TNU
GY	0.059	0.664**							
SY	0.013	0.554**	0.051						
SNC	0.284	0.061	-0.042	0.108					
GNC	-0.136	0.070	0.055	0.056	0.009				
SNU	0.210	0.343	0.003	0.605**	0.851**	0.036			
GNU	-0.074	0.470**	0.669**	0.084	-0.029	0.778**	0.026		
TNU	-0.021	0.535**	0.646**	0.226	0.176	0.759**	0.264	0.971**	
HI	0.055	0.188	0.780**	-0.573**	-0.089	-0.0022	-0.360*	0.466**	0.363*
BY	0.054	0.817**	0.601**	0.828**	0.057	0.076	0.481**	0.442*	0.541**

\*\*, \* Correlation is significant at 0.01 and 0.05 level. SY = Straw yield, SP = Straw phosphorus, GP = Grain phosphorus content, SPU = Straw phosphorus uptake, GPU = Grain phosphorus uptake, TPU = Total phosphorus uptake, P = Applied phosphorus, N = Applied nitrogen, GY = Grain yield, SY = Straw yield, HI = Harvest index, BY = Biomass yield.

68.70 to 40.16% in Shehan (Tables 9 and 10). The maximum and the minimum apparent recoveries of N were recorded at 23 and 69 kg N/ha respectively. Furthermore, the highest P apparent recoveries were obtained at the lowest P rates in both varieties. The highest P apparent recoveries in HAR1685 (4.94) and in Shehan (5.58) were found at 23 kg P/ha.

Agronomic efficiency decreased with increasing N rate in both varieties. As the P level increased from 23 to 69 kg N/ha, the agronomic efficiency decreased from 30.66 to 18.36 in HAR 1685 and from 24.02 to 14.79 in Shehan. The highest agronomic efficiencies were observed at 23 kg N/ha both in HAR 1685(30.66) and Shehan (24.02). However, highest physiological efficiency was at the highest N rate (69 kg/ ha) in both HAR1685 (0.35) and Shehan (0.37).

The highest agronomic efficiency in HAR1685 (31.3) was recorded at application rate of 10 kg P/ha in HAR1685 and in Shehan (4.82) at 30 kg P/ha. However, the lowest agronomic efficiency of P in HAR1685 (3.67) was at 30 kg P/ha, and the lowest agronomic efficiency in

Shehan (-0.5) was at the lowest P rate (10 kg P/ha). This might be due to the higher grain yield in the control than in 10 kg P/ha of Shehan variety. According to Craswell and Godwin (1984), high agronomic efficiency would be obtained if the yield increment per unit applied is high.

Highest physiological efficiency of P in HAR1685 (6.34) was obtained with the lowest P rate (10 kg P/ha) but in Shehan (40.17) it was found at the highest P rate (30 kg P/ha). Generally, physiological efficiency of P decreased in HAR1685 but increased in Shehan with application of P rates (Tables 9 and 10).

#### Functional relationships between agronomic parameters and N and P contents and uptakes

The simple correlation between the agronomic parameters is given in Tables 11. The table indicates grain yield was significantly and positively correlated with applied nitrogen ( $r=0.664$ ), biomass yield ( $r=0.608$ ), and thousand kernel weight ( $r=0.566$ ) but negatively correlated with initial plant

**Table 13.** Correlations between applied P and contents, uptake, grain yield, biomass yield, straw yield, and harvest index

Parameters	P	N	GY	SY	SP	GP	SPU	GPU	TPU
N	0.000								
GY	0.059	0.664**							
SY	0.013	0.554**	0.051						
SP	0.659**	-0.068	0.224	-0.229					
GP	0.363*	0.026	0.130	-0.136	0.172				
SPU	0.668**	0.239	0.225	0.333	0.833**	0.096			
GPU	0.299	0.458**	0.760**	-0.045	0.263	0.735**	0.216		
TPU	0.671**	0.395*	0.517**	0.248	0.788**	0.401*	0.902**	0.616**	
BY	0.054	0.807**	0.608**	0.828**	-0.049	-0.037	0.402*	0.389*	0.496**
HI	0.055	0.188	0.780**	-0.573**	0.331	0.190	-0.022	0.647**	0.268

\*\*, \* Correlation is significant at 0.01 and 0.05 level. SY = Straw yield, SP = Straw phosphorus, GP = Grain phosphorus content, SPU = Straw phosphorus uptake, GPU = Grain phosphorus uptake, TPU = Total phosphorus uptake, P = Applied phosphorus, N = Applied nitrogen, GY = Grain yield, SY = Straw yield, HI = Harvest index, BY = Biomass yield.

stand ( $r=-0.598$ ). The biomass yield also significantly and positively correlated with applied N ( $r=0.817$ ), straw yield ( $r=0.828$ ) (Table 12) and with plant height ( $r=0.393$ ).

Table 11 also indicates harvest index was strongly and positively correlated with grain yield ( $r=0.78$ ) but negatively correlated with straw yield ( $r=-0.573$ ). The table also shows the correlation between contents of N, uptake and some agronomic parameters. It indicates that grain nitrogen uptake was correlated positively and significantly with applied N ( $r=0.47$ ), grain N content ( $r=0.778$ ), and with grain yield ( $r=0.669$ ) but negatively correlated with applied P ( $r=-0.074$ ) and straw N content ( $r=-0.029$ ).

Total N uptake was significantly and positively correlated with applied N ( $r=0.535$ ), grain yield ( $r=0.646$ ), grain N content ( $r=0.759$ ) and grain N uptake ( $r=0.971$ ) as shown in Table 12. Moreover, the table shows that harvest index was strongly and positively correlated with grain yield ( $r=0.78$ ), grain N uptake ( $r=0.466$ ) and with total N uptake ( $r=0.363$ ), but it had high negative correlation with straw yield ( $r=-0.573$ ) and with straw N uptake ( $r=-0.36$ ). The table also indicates that biomass yield was significantly and positively correlated with applied N ( $r=0.817$ ), grain yield ( $r=0.608$ ), straw yield ( $r=0.828$ ), straw N uptake ( $r=0.481$ ), grain N uptake ( $r=0.442$ ), and with total N uptake ( $r=0.541$ ).

The correlation between applied P, concentrations, uptake and some agronomic parameters is indicated in Table 13. It shows that significant and positive correlations between straw P content and applied P ( $r=0.659$ ), straw yield and applied N ( $r=0.554$ ), straw P uptake and applied P ( $r=0.668$ ). The table also indicates that grain P uptake was significantly and positively correlated with applied N ( $r=0.458$ ), grain yield ( $r=0.0760$ ), and grain P content ( $r=0.735$ ). Furthermore, biomass yield was correlated positively with straw P uptake ( $r=0.402$ ), grain P uptake ( $r=0.389$ ), and positively and significantly correlated with total P uptake ( $r=0.496$ ).

The harvest index was correlated strongly and positively with grain P uptake ( $r=0.647$ ).

## Conclusions

In Tigray Regional State, particularly in the Southern part of the region, wheat is a dominant crop in the medium and highland areas. In this area like other parts of Ethiopia, wheat production is adversely affected by low soil fertility, suboptimal use of mineral fertilizers in addition to diseases, weeds, and erratic rainfall distribution.

In order to alleviate the soil fertility problem in the study area, the Bureau of Agriculture and Natural Resources of Tigray has introduced chemical fertilizers in each district of the zone. However the fertilizer rate which is being used by farmers is blanket recommendation throughout the region.

Two wheat varieties (HAR1685 and Shehan /Bani), four nitrogen fertilizer rates (0, 23, 46, and 69 kg N/ha) and four rates of phosphorus fertilizers (0, 10, 20 and 30 kg P/ha) were used in a  $2 \times 4 \times 4$  factorial arranged in split-plot design with three replications. Surface soil samples (0-30 cm depth) were collected before planting and analyzed for selected physical and chemical properties. Analysis of soil samples indicated that the experimental soil was clay loam in texture, with pH ( $H_2O$ ) of 7.04, organic matter content 2.23%, total N 0.134%, available P of 19.9 mg/kg, and CEC of 44.41 cmol/kg soil.

The analysis of variance revealed that there was highly significant differences ( $P<0.01$ ) between the two wheat varieties with respect to plant height, initial plant stand, shoot dry weight at 90% physiological maturity, grain yield, biomass yield, harvest index, and days to 90% physiological maturity. However, no significant difference was observed between the varieties in relation to tiller number per plant, days to 50% flowering, 1000 kernels

weight, shoot dry weight at 50% flowering and available soil P at harvesting. The local variety Shehan was taller than the improved variety HAR-1685. With respect to initial plant stand, Shehan had more number of plants compared to HAR1685.

Application of nitrogen fertilizer affected significantly ( $P < 0.01$ ) plant height, shoot dry weight at physiological maturity, days to 50% flowering and days to 90% physiological maturity. As the nitrogen level increased from 0 to 69 kg/ha, the plant height increased from 82.63 to 94.18 cm. The number of tillers per plant also increased from 5 to 6.63 when the nitrogen rate increased from 0 to 69 kg/ha. The shoot dry weight at 90% physiological maturity increased from 168.88 to 218.17 g/0.5 m with nitrogen increment from control to 69 kg/ha. The highest nitrogen application delayed days to flowering and days to maturity in both varieties. It lengthened the days to flowering from 65.5 to 67.17 days in HAR 1685 and from 69.67 to 72.92 days in Shehan. Furthermore, it increased the days to maturity from 119.75 to 123.1 days in HAR 1685 and from 128 to 130 in Shehan.

Significant difference was observed among the different phosphorus fertilizer rates with respect to plant height, shoot dry weight at 90% physiological maturity. As the phosphorus rate increased from 0 to 30 kg/ha, the plant height increased from 72.21 to 74.97 cm in HAR 1685 and from 100 to 106.15 cm in Shehan. Furthermore, the shoot dry weight at 90% physiological maturity increased from 167.89 to 187.28 g/0.5 m in HAR1685 and from 175.02 to 233.55 g/0.5 m in Shehan.

The P rates, N rates and their interactions were highly significant in terms of shoot dry weight at 50% flowering, grain yield, biomass yield, harvest index, straw yield, and available soil P at harvesting. The increase and decrease in shoot dry weight at 50% flowering, grain yield, biomass yield harvest index, straw yield and available soil phosphorus at harvesting did not show a consistent trend but generally as the N rates increased from 0 to 69 kg N/ha and these parameters increased. The highest shoot dry weights at 50% flowering were found at 69-20 N-P kg/ha interaction in HAR1685 and at 30-69 P-N kg/ha interaction was in Shehan. The highest grain yield, biomass yield and harvest index were observed in the lowest P rate and at the highest N rate (69 kg N/ha). For HAR1685 grain yield was highest at 0 kg P/ha and 69 kg N/ha and for Shehan it was at 10 kg P/ha and 69 kg N/ha. The highest biomass yield was recorded at 30-69 P-N kg/ha interaction in HAR1685 and at 0-69 P-N kg/ha interaction in Shehan. The highest harvest indices were observed in 10-69 P-N kg/ha followed by 0-69 P-N kg/ha in HAR1685 and in 10-46 P-N kg/ha in the local variety Shehan. But non-significant difference due to the interaction of P and N with respect to plant height, number of tillers per plant, initial plant stand, 1000 kernel weight, days to 50% flowering and total soil nitrogen after harvesting was observed.

The grain and straw N and P content, and uptake,

increased with N and P levels but the apparent recovery and agronomic efficiency of N and P fertilizers decreased with increasing N and P rates in both varieties.

Grain yield and biomass yield were strongly and positively correlated with most of the agronomic parameters, grain N and P contents, grain N and P uptakes, and total N and P uptakes. Moreover, grain and straw N and P contents and their uptakes were strongly and positively correlated with applied N and P respectively.

From this study, it can be concluded that the improved variety HAR1685 had high grain yield potential, medium plant height, heavier kernel weight, higher harvest index, and shorter maturing period and responsive to higher N and P rates. Therefore, in Korem district, the variety HAR1685 could be preferred over the local variety Shehan. Application of N and P rates up to 30 kg P/ha and 69 kg N/ha respectively increased grain yield and most yield components of the two wheat varieties. In contrast to these, the lowest levels of P and N fertilizers (0-0 P-N kg/ha) produced the lowest grain yield and yield components. However, the study was conducted at a single location for season; therefore, further detailed studies across locations and seasons are required to recommend agronomically optimum and economically feasible levels of N and P fertilizers for bread wheat.

## CONFLICT OF INTERESTS

The authors has not declared any conflict of interests.

## REFERENCES

- Abdoulaye T, Marienville JW (1999). Nitrate reductase activity of diverse grain sorghum genotypes and its relationship to nitrogen use efficiency. *Agron. J.* 91:863-869.
- Addis A (2003). Genotype by Environment Interaction of Bread Wheat (*Triticum aestivum* L.) Genotypes under Dryland Growing Conditions of Tigray. M.Sc. Thesis in Agriculture (Plant Breeding), Alemaya University.
- Amsal T, Tanner DG, Taye T, Chanyalew M (2000). Agronomic and economic evaluation of the on-farm N and P response of bread wheat grown on two contrasting soil types in central Ethiopia. *In: The Eleventh Regional Wheat Workshop for Eastern, Central, and Southern Africa.* CIMMYT, Addis Ababa, Ethiopia.
- Amanuel G, Kuhne RF, Tanner DG, Vlek PLG (2002). Recovery of 15-N labeled urea applied to wheat in the Ethiopian Highlands as affected by P fertilization. *J. Agron. Crop Sci.* 189:30-38.
- Archer J (1988). Phosphorus. *In: John Archer (ed.). Crop nutrition and fertilizer use.* Farming Press Ltd. USA pp. 57-64.
- Behera AK (1998). Response of Scented rice (*Oryza sativa*) to nitrogen under transplanted condition. *Indian Agron. J.* 43(1):64-67.
- Botella MA, Cerda AC, Lips SH (1993). Dry matter production, yield and allocation of carbon-14 assimilate by wheat as affected by nitrogen source and salinity. *Agron. J.* 85(5):1044-1049.
- Craswell ET, Godwin DC (1984). *In: Mengel, K. and E.A. Kirkby, 1996. Principles of plant nutrition.* Panima Publishing Corporation, New Delhi, India.
- Damene D (2003). Yield response of bread wheat (*Triticum aestivum* L.) to applied levels of N and P fertilizers on Nitisol of Dawro Zone, Southwestern Ethiopia. M.Sc. Thesis in Agriculture, Alemaya University.
- Day PR (1965). Hydrometer method of particle size analysis. *In: Back,*

- C.A. (eds.), Methods of soil analysis. American Society of Agron. J. 9(2):562-563.
- Evans LT (1975). Crop physiology, Cambridge University press, London
- Genene G (2003). Yield and quality response of bread wheat varieties to rate and time of nitrogen fertilizer application in Kulumsa, Southeastern Ethiopia. An M.Sc. Thesis Presented to the School of Graduate Studies of Alemaya University, Alemaya.
- Gomez KA , Gomez AA (1984). Statistical analysis for agricultural research. John Willy and Sons Inc pp.120-155.
- Gooding MJ, Davies WP (1997). Wheat production and utilization, systems quality and the environment, CAB international, UK
- Gurmassa L (2002). Response of wheat (*Triticum aestivum*) to fertilizer N and P in Borona Zone, Ethiopia. An MSc Thesis Presented to the School of Graduate Studies of Alemaya University.
- Hailu G-M (1991). Wheat production and research in Ethiopia.pp.1-15. *In* : Hailu Gebre-Mariam, Tanner, D.G., and Mengistu Hulluka (eds.) Wheat Research in Ethiopia: A Historical perspective, IAR/ CIMMYT, Addis Ababa, Ethiopia
- Hari DM, Katyal SK, Ohiman SP (1997). Effect of nitrogen and seed rate in nursery on growth and yield of hybrid rice (*Oryza sativa*). Indian J. Agron. 42(2):275-277.
- Jackson ML (1967). Soil chemical analysis. Practice Hall of India. New Delhi.
- Lemma ZY, Tanner DG, Eyasu E (1992). The effects of nitrogen fertilizer rates and application timing on bread wheat in Bale Region of Ethiopia.. *In*: D.G. Tanner and W. Mwangi (eds.) Seventh Regional Wheat workshop for Eastern, Central, and Southern Africa. Addis Ababa, Ethiopia.
- Marshner H (1995). Mineral nutrition of higher plant. 2<sup>nd</sup> edition. Academic Press, London. New York.
- Mekonen A (2005). Response and uptake of barley (*Hordeum irregulare* L.) to different rates of Orga- P and nitrogen fertilizers on Nitisols of Gozamin district, Ethiopia, M.Sc. Thesis, Alemaya University
- Mengel K, Kirkby EA (1987). Principles of Plant nutrition. International Potash Institute, Bern, Switzerland.
- Mengel K, Kirkby EA (1996). Principles of Plant Nutrition, Panimo Publishing Corporation, New Delhi, India
- Mossedeq F, Smith DH (1994). Time of nitrogen fertilizer application to enhance spring wheat yields in the Mediterranean climate. Agron. J. 86(2):221-226.
- Mooleki SP, Foster RK (1993). Effects of N and P rates and proportional timing of N application on rainfed wheat in Zambia. Plant Soil 149:73-86.
- Ofla District BoANRD (2005). Unpublished Document.
- Olsen SR, Cole CV, Watanabe FS, Dean LA (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USA Circular 939:1-19
- Ortiz-Monasterio JI, Sayre KD, Rajaram S, McMahon M (1997). Genetic progress in wheat and nitrogen use efficiency under four rates. Crop Sci. 37(3):898-904.
- Pal UR (1991). Effects of source and rate of nitrogen and phosphorus on yield and nutrient uptake and apparent fertilizer recovery by maize in the Southern Guinea Savana. J. Agric. Sci. Technol. 1:21-24.
- Russel EW (1988). Soil condition and plant growth. Eleventh edition. Bath Press, Great Britain.
- Sahlemedhin S (1999). Draft guideline for regional soil testing laboratories. NFIA, Addis Abeba, Ethiopia.
- Temesgen K (2001). The effect of sowing date and nitrogen fertilizer on yield and yield traits of tef [ *Eragrostis tef* (Zucc.) Trotter ]. M.Sc. Thesis, Alemaya University.
- Tilahun G, Tanner DG, Tekalign M, Getinet G (1996). Response of bread and durum wheat to source, level and timing of nitrogen fertilizer at two vertisol sites in Ethiopia. *In*: Tanner, D.G., T.Payne, and O.S., Abdalla (eds.). Proceeding of Ninth Regional Wheat Workshop for eastern, Central and South Africa. CIMMYT, Addis Ababa, Ethiopia.
- Zewdu Y, Lemma Z, Tanner DG (1992). A study of several factors limiting wheat yields on farmers' fields and on station in Bale of Ethiopia. pp. 510-516. *In*: Tanner, D.G. and Mwangi W. (eds.). Proceeding of seventh regional wheat workshop for Eastern, Central and Southern Africa. CYMMYT. Addis Ababa, Ethiopia.



## APPENDIX

**Appendix Table 1.** The effect of experimental factors and their interactions on agronomic parameters

Source of Variation	Mean Square							
	PH	NTP	IPS	SDWF	SDWH	TKW	GY	BY
Replication	119.7**	0.542	400.6	116.232	13.936	31.6	53.02	3282.9**
Variety(V)	21557.7**	0.667	88877.5**	5707.100	25306.8*	2579.4	182135.7**	47530.9**
Error (a)	0.133	0.167	281.885	120.261	538.314	2.828	22733.704	80.907
Phosphorus	83.7*	0.486	256.955	5973.9**	7893.2**	7.88	1559.6**	20056.2**
VxP	11.507	0.278	793.122	2351.7**	3288.3	1.08	2224.7**	16612.2**
Nitrogen	605.5**	13.125	981.483	3948.7**	10458.3**	0.80	82811.2**	407568.2**
VxN	24.8	0.083	493.760	271.6**	962.8	1.70	2324.8**	19085.4**
PxN	5.7	0.162	461.538	694.5**	155.8	0.40	3490.9**	14424.9**
VxPxN	8.7	0.287	630.372	316.6**	60.6	1.20	5763.3**	12581.6**
Error (b)	22.5	0.199	408.905	26.1	22.8	11.91	150.8	160.374
CV (%)	5.34	7.61	5.25	5.99	2.48	7.58	2.82	1.05

Key: - \*\* and \* significant different at 0.01 and 0.05 significance level respectively.

PH= Plant height, NTP= number of tillers per plant, IPS= Initial plant stand 15 days after planting, SDWF= Shoot dry weight at 50% flowering, SDWH= Shoot dry weight at harvesting.

**Appendix Table 2.** Mean Square for harvest index, Straw yield, days to 50% flowering and 90% physiological maturity and available soil phosphorus after harvesting

Source of variation	Mean Square				
	HI	SY	DF	DM	ASP
Replication	0.001	38783.393	3.510	6.292	0.052
Variety (V)	0.176**	27943940.461**	527.34	1734.00**	0.333
Error(a)	0.001	20225.107**	15.594	12.875	0.005
Phosphorus(P)	0.004**	1813243.764**	2.71	3.792	142.478**
VxP	0.001**	462917.639**	2.538	2.278	3.631**
Nitrogen (N)	0.003**	8687947.057**	26.5**	40.934**	5.908**
VxN	0.001**	791890.644**	3.12	2.278	7.383**
PxN	0.001**	460837.950**	0.71	1.125	3.311**
VxPxN	0.003**	603279.908**	0.65	1.435	3.332**
Error (b)	0.001	10529.353	1.92	3.272	0.053
CV (%)	2.94	1.61	2.01	1.44	1.96

Key: - \*\* and \* significant different at 0.01 and 0.05 significance level respectively.

HI= Harvest index, SY= straw yield, DF= Days to 50% flowering, DM= Days to 90 % physiological maturity, V= variety, P= phosphorus, N= nitrogen, ASP= available soil phosphorus at harvesting.

## Full Length Research Paper

## Characterization of soybean population with sulfonylurea herbicides tolerant alleles

Eder Eduardo Mantovani<sup>1\*</sup>, Nara Oliveira Silva Souza<sup>2</sup>, Luis Antonio Stabile Silva<sup>3</sup> and Maria Aparecida dos Santos<sup>3</sup>

<sup>1</sup>DuPont Pioneer, Cx. Postal 08283, CEP 73301-970, Planaltina, DF, Brazil.

<sup>2</sup>University of Brasília, FAV, Cx. Postal 04508, CEP 70910-900, Brasília, DF, Brazil.

<sup>3</sup>DuPont Pioneer, Cx. Postal 1344, CEP 77500-000, Porto Nacional, TO, Brazil.

Received 22 February, 2017; Accepted 11 April, 2017

With the introduction of commercial soybean genotypes with *Als1* and *Als2* alleles that confer tolerance to different active ingredients of sulfonylurea group, this work aims to test soybean populations for the presence/absence of *Als1* and *Als2* alleles and evaluate the agronomic impact of these alleles addition. These trials were conducted in experimental stations of DuPont Pioneer at Sorriso, Mato Grosso state and Planaltina, Federal District. Four populations were evaluated with 40 genotypes each; 10 genotypes without *Als1* and *Als2* (null), 10 genotypes containing *Als1*, 10 containing *Als2* and 10 genotypes containing both alleles. These populations were tested for different traits. The grain yield average at Planaltina and Sorriso were 2888 and 2456 kg ha<sup>-1</sup>, respectively. Yield for the genotypic classes null, *Als1*, *Als2* and *Als1+Als2* were 2672, 2671, 2631 and 2657 kg ha<sup>-1</sup>, respectively, and they were not statistically different from each other. Also, the other traits indicated similar behavior among classes. As the studied populations were developed for this study, they were inferior than the checks. This work demonstrated that in the four studied populations, the addition of *Als1* and/or *Als2* alleles did not cause significant differences in the evaluated traits.

**Key words:** *Glycine max* L., *Als1*, *Als2*, grain yield.

### INTRODUCTION

During the development of agriculture in Brazil, several species of weeds were selected due to a continuous exposure to herbicides with a similar mode of action. This occurred in conventional soybeans and corn crops, and thereafter due to overuse of glyphosate in genetically modified soybeans. The selection of resistant species is associated with genetic changes in the population under

a selection pressure for such products. Therefore, the rotation of herbicide with different action modes is of fundamental importance in production areas (Powles, 2008).

Sulfonylureas are herbicides that block the synthesis of essential amino acids by inhibiting the acetolactate synthase (ALS) enzyme. ALS is the first enzyme to act on

\*Corresponding author. E-mail: [eder.mantovani@pioneer.com](mailto:eder.mantovani@pioneer.com).

the biosynthesis of the amino acids valine, leucine and isoleucine. It catalyzes two parallel reactions: condensation of 2 moles of pyruvate forming acetolactate, and condensation of 1 mol of pyruvate with 1 mol of 2-oxybutyrate forming aceto-hydroxybutyrate (Eberlein et al., 1997). The inhibition of this enzyme disrupts the production of proteins, interfering with cell growth and consequently resulting in the death of the plant. Sulfonylureas have been widely used in more than 80 countries and approximately 25 crops. There is a wide variety of sulfonylureas. Some are not selective or effective in the control of all plants, while other products are selective, acting in some species and being tolerated by other species that metabolize the product and detoxify before undergoing a significant damage due to inhibition of ALS activity (Green, 2007).

Having proof the soybean capacity to tolerate some active ingredients of sulfonylureas, such as ethyl chlorimuron, through its fast metabolic inactivation, this active ingredient has become widely used in soybean crops (Zawoznik and Tomaro, 2005). Currently, in Brazil, chlorimuron ethyl is used during pre and post-emergence for the control mainly of weeds resistant to glyphosate. However, higher resistance to this component and to other sulfonylureas was given to soybean through specific mutations in the ALS genes, causing this enzyme to be less susceptible to inhibition by sulfonylureas and maintaining its active vital capacity (Walter et al., 2014).

By using mutagenic techniques and conventional breeding, the cultivar W20 was developed in the 1980s. It derived from Williams and presented a resistance to sulfonylurea herbicides (Sebastian et al., 1989). This was the first cultivar of the group commercially known as STS<sup>®</sup> (sulfonylurea tolerant soybean). This technology provided a greater flexibility in the use of different sulfonylureas with a wider weed control action spectrum, and was widely used by different companies in North and South America. After a period without use due to the introduction of genetically modified cultivars with resistance to glyphosate, the emergence of weeds resistant to this active ingredient reactivated the use of the STS<sup>®</sup> technology, reappearing in the market combined with the glyphosate resistance gene (Green and Owen, 2011). Later, the mutant allele used in the STS<sup>®</sup> technology became known as *Als1*. Its wild version is the *als1* (Walter et al., 2014).

After the incorporation of the allele *Als1* into modern cultivars, a new cycle of changes began aiming to develop mutants even more tolerant to sulfonylurea. The line W4-4 was then created. It underwent a second mutagenic event, giving rise to a second independent allele called *Als2* (Walter et al., 2014).

The type of gene mutation that occurred with the *Als1* and *Als2* alleles was base substitution. For the allele *Als1*, located on the chromosome 4, there was the substitution of proline for serine at the position 178 of the soybean protein. For the allele *Als2*, located on the

chromosome 6, tryptophan was replaced for leucine at the position 560 of the soybean protein (Walter et al., 2014).

Soybean containing *Als1* and/or *Als2* alleles were developed as an alternating tool to control weeds showing herbicide resistance especially to glyphosate. Herbicides from sulfonylurea group are intended to auxiliary in the dicotyledonous weed control such as *Conyza* spp. which is glyphosate resistant. It is mentioned in the literature (Vargas et al., 2007; Moreira et al., 2007; Lamego and Vidal, 2008) that *C. bonariensis* and *C. canadensis* are glyphosate resistant.

Given the possibility of using soybean lines containing *Als1* and *Als2* alleles, tolerant to different active ingredients of sulfonylureas and presenting a higher tolerance to the current used sulfonylureas, it is necessary to conduct tests to prove that the addition of such mutant alleles into new soybean genotypes do not cause agronomic losses to the crop. Therefore, this study aims to test different soybean populations regarding the presence/absence of *Als1* and *Als2* and evaluate the agronomic impact of these alleles addition.

## MATERIALS AND METHODS

### Obtaining the families

This study was conducted using recombinant inbred lines from four populations. The development of these genotypes using modified bulk method started during the 2011/2012 season, when crosses involving the donor of *Als1* and *Als2* alleles (CD250RRSTS) with genotypes adapted to central Brazil (BG4277, 98Y30, YB84C12 and XB85C12) were made. In the winter of 2012, F<sub>1</sub> seeds were sown and the confirmation of the crosses was made by molecular analysis using markers according to Walter et al. (2014). During the next season (2012/2013), seeds were sown as F<sub>2</sub>, and in the 2013 winter as F<sub>3</sub>. When advanced to F<sub>4</sub>, during the 2013/2014 season, another molecular analysis (Walter et al., 2014) was made to classify and select homozygous plants considering the presence/absence of *Als1* and *Als2* alleles. In the 2014 winter, F<sub>4:5</sub> recombinant lines from the four populations were sown (Table 1). The development of the populations was made at the DuPont Pioneer research center of Planaltina, Federal District (DF), except for F<sub>3</sub> and F<sub>4:5</sub> generations, whose developments were conducted at DuPont Pioneer research center of Palmas, Tocantins (TO) state.

For each population, four classes of genotypes (genotypic classes) were obtained: Null genotypes (without *Als1* and *Als2* alleles), genotypes containing *Als1* allele, genotypes containing *Als2* allele, and genotypes containing both alleles (Table 1). All evaluated ALS alleles were homozygous. 10 F<sub>4:6</sub> recombinant lines from each class were selected among the four populations based on agronomic characteristics, uniformity, maturity and germination. They were tested during the 2014/2015 season, into which four variety checks without *Als1* and *Als2* alleles were included. Variety checks; 97R21, 97R73, 98Y12 and 98Y30; were included in the statistical analysis as the fifth genotypic class.

### Field evaluation

Forty genotypes of each population were sown during the 2014/2015 season, being 10 genotypes of each class plus the four

**Table 1.** Number of genotypes obtained from each class and population after marker assisted selection.

Population		Number of genotypes				
Parental	Code	Null	Als1	Als2	Als1+Als2	Total
BG4277/CD250RRSTS	Pop001	69	73	162	183	487
98Y30/CD250RRSTS	Pop002	14	19	20	31	84
YB84C12/CD250RRSTS	Pop003	78	164	32	129	403
XB85C12/CD250RRSTS	Pop004	164	131	142	123	560
<b>Total</b>		325	387	356	466	1534

checks. The experiments were conducted at DuPont Pioneer research centers of Sorriso, Mato Grosso (MT) state, and Planaltina (DF). Although both sites have soils classified as Latosol, both locations represent two distinct environments. The Sorriso site is located at the center-northern part of MT at 12°44'39.63''S and 55°49'54.23''W with an altitude of 398 m. The Planaltina site is located at the northeastern part of the DF at 15°43'18.12''S and 47°36'10.21''W with an altitude of 1,163 m.

The experimental design was randomized blocks with three replications. Each population was planted and randomized separately. Each plot consisted of four rows of five meters long with 0.50 m between rows. The two center rows were harvested. Plant populations were 240,000 and 280,000 plants ha<sup>-1</sup> at Planaltina and Sorriso, respectively. Experiments were sowed in Sorriso on November 6<sup>th</sup> and in Planaltina on November 21<sup>th</sup>, 2014. Field management was done following EMBRAPA soja (2001) recommendation. Crop field was desiccated at the R7.3 stage with Gramoxone, which contain 200 g L<sup>-1</sup> of active ingredient Paraquat, using product dosage of 2 L ha<sup>-1</sup>. Subsequently each population was harvested with mechanical combine according to maturity level.

The evaluated agronomic traits were: Seedling emergence (EMG), which consists in a visual percentage of emerged seedlings at V2 stage; plant height (PH), measured the distance in cm from the soil surface until the apex of a representative plant and evaluated during the maturity stage of the treatment in R8; maturity (MAT), it is the number of days from planting to the date when 95% of the treatment reached the R8 maturity stage; plot evaluation (PLEV), a percentage score for the experimental unit aiming to measure the plot quality based on the number of plants and their distribution during the maturity stage in R8; visual treatment evaluation (VTE), percentage score for visual appearance of the treatment at R8 stage based on desired agronomic characteristics; non-lodging (NL0D), which consists in the percentage of plants that did not incline more than 45% during R8 stage; and grain yield (GY) that is the seed weight of a plot converted to kg ha<sup>-1</sup> and corrected to 13% moisture. The soybean stages were classified according to Fehr et al. (1971).

The collected data were analyzed using the statistical program R (R Core Team, 2016). The adopted statistical model was mixed with locations and blocks as random effects, and populations and gene genotypic classes as fixed effects. An analysis of variance was performed for each location, followed by a Bartlett's test (homogeneity of variances) at 0.005 probability in order to validate a combined analysis of variance (Ramalho et al., 2012). The means were compared by Tukey test at 5% probability.

## RESULTS AND DISCUSSION

As the errors of the variances were homogeneous in both locations for all evaluated traits in the experiments, a combined variance analysis was performed (Table 2).

The means of the experiments, populations, gene genotypic classes, all interactions and Tukey test performed for traits with significant difference are shown in Table 3.

During the crop cycle of the 2014/2015 season, the climatic conditions of Planaltina were favorable to the development of the crop, except during the final phase of the vegetative stage, when rainfalls decreased. At the Sorriso station, the weather conditions were inadequate at the beginning of the vegetative stage due to low rainfalls, complicating early crop growth and significantly compromising final grain production (Figure 1). Therefore, the yield average of the experiment in Planaltina was 2,888 kg ha<sup>-1</sup>, and in Sorriso was 2,456 kg ha<sup>-1</sup>. The average of the two experiments was 2,674 kg ha<sup>-1</sup> (Table 3).

The difference in yield between the two locations can be explained partly by a decrease in seedling emergence and plot evaluation. The average emergence score of Planaltina was 91%; in Sorriso, such score was 76% (Table 3). For the trait evaluation of the plot, the average in Planaltina was 96%, and in Sorriso 61% (Table 3).

According to grain yield data, the only significantly different sources of variation were population and location\*class (Table 2). Pop002 population was significantly better than Pop003 and Pop004 populations, but it did not differ statistically from Pop001. In the interaction, checks were superior than genotypic classes in Sorriso (Table 3). Checks had better capability to overcome the adversity conditions in Sorriso due to the whole breeding and selection process they went through, different of the populations that were developed for this type of study. The means of the genotypic classes null, Als1, Als2 and Als1+Als2 had similar values and they were not statistically different. The other sources of variation for grain yield did not show significant differences, indicating that the addition of *Als1* and/or *Als2* alleles did not change the most important characteristic in soybeans (Table 3).

For seedling emergence, the significantly different sources of variation were class and location \*population\* class (Table 2). Checks were better than null and Als1 classes, and did not vary from Als2 and Als1+Als2. The values in Sorriso were inferior than in Planaltina (Table 3). The adverse conditions during emergence and early

**Table 2.** Summary of the combined analysis of variance with the evaluated traits in the experiments conducted at Planaltina, DF and Sorriso, MT during 2014/2015 season.

Source of variation	DF	GY	EMG	PH	PLEV	NLOD	VTE	MAT
Local	1	-	-	-	-	-	-	-
Rep(Local)	4	-	-	-	-	-	-	-
Pop	3	6060313*	859 <sup>ns</sup>	5652 <sup>ns</sup>	409 <sup>ns</sup>	9238 <sup>ns</sup>	1140 <sup>ns</sup>	2262 <sup>ns</sup>
Class	4	727597 <sup>ns</sup>	568*	10179*	464 <sup>ns</sup>	5005**	3258 <sup>ns</sup>	2151*
Pop*Class	12	304056 <sup>ns</sup>	177 <sup>ns</sup>	650**	160 <sup>ns</sup>	1229**	156 <sup>ns</sup>	142 <sup>ns</sup>
Local*Pop	3	240059 <sup>ns</sup>	135 <sup>ns</sup>	1227**	422**	1127**	1501**	410**
Local*Class	4	463476**	70 <sup>ns</sup>	972**	704**	64 <sup>ns</sup>	836**	156**
Local*Pop*Class	12	186123 <sup>ns</sup>	338*	110 <sup>ns</sup>	179**	229 <sup>ns</sup>	186*	73**
Error	999	113723	155	107	59	192	100	17
CV (%)		12.6	14.9	11.4	9.8	16.1	14.2	3.3

<sup>ns</sup>Non significant; \* and \*\* significant at the 0.05 and 0.01 probability levels, respectively. DF = degrees of freedom; GY = grain yield; EMG = seedling emergence; PH = plant height; PLEV = plot evaluation; NLOD = non-lodging; VTE = visual treatment evaluation e MAT = maturity.

**Table 3.** Means of evaluated traits for locations, populations, genotypic classes, and all interactions in the experiments conducted at Planaltina (Plan), DF and Sorriso (Sorr), MT, in 2014/2015 season (Tukey test was applied for traits with significant difference).

Description	Variable	GY (kg ha <sup>-1</sup> )	EMG (%)	PH (cm)	PLEV (%)	NLOD (%)	VTE (%)	MAT (days)
Mean	Both locations	2674	84	91	79	86	71	124
Loc. mean	Planaltina	2888	91	91	96	87	69	140
Loc. mean	Sorriso	2456	76	91	61	85	72	107
Four populations means	Pop001	2755 <sup>bc</sup>	82	91	77	91	71	126
	Pop002	2835 <sup>c</sup>	86	84	79	90	72	120
	Pop003	2492 <sup>a</sup>	84	94	80	78	68	126
	Pop004	2615 <sup>ab</sup>	82	94	79	85	71	123
Five genotypic classes means	Null	2672	82 <sup>a</sup>	94 <sup>b</sup>	78	83 <sup>a</sup>	68	125 <sup>b</sup>
	Als1	2671	82 <sup>a</sup>	91 <sup>b</sup>	79	87 <sup>b</sup>	70	125 <sup>b</sup>
	Als2	2631	85 <sup>ab</sup>	95 <sup>b</sup>	78	82 <sup>a</sup>	70	125 <sup>b</sup>
	Als1+Als2	2657	84 <sup>ab</sup>	91 <sup>b</sup>	79	87 <sup>b</sup>	70	124 <sup>b</sup>
	Checks	2832	87 <sup>b</sup>	72 <sup>a</sup>	83	98 <sup>c</sup>	81	115 <sup>a</sup>
Populations and classes interaction means	Pop001xNull	2765	79	94 <sup>bcde</sup>	75	91 <sup>cde</sup>	68	126
	Pop001xAls1	2844	82	92 <sup>bcd</sup>	75	90 <sup>cde</sup>	70	127
	Pop001xAls2	2635	85	93 <sup>bcde</sup>	77	87 <sup>bcde</sup>	72	130
	Pop001xAls1+Als2	2723	82	92 <sup>bcd</sup>	78	95 <sup>de</sup>	71	125
	Pop001xChecks	2889	86	76 <sup>a</sup>	84	98 <sup>e</sup>	82	115
	Pop002xNull	2878	88	86 <sup>b</sup>	81	92 <sup>de</sup>	72	121
	Pop002xAls1	2761	84	86 <sup>b</sup>	78	88 <sup>bcde</sup>	70	121
	Pop002xAls2	2836	85	86 <sup>b</sup>	77	88 <sup>bcde</sup>	42	121
	Pop002xAls1+Als2	2826	86	86 <sup>b</sup>	78	89 <sup>bcde</sup>	70	120
	Pop002xChecks	2934	88	70 <sup>a</sup>	85	98 <sup>e</sup>	82	115
	Pop003xNull	2474	81	99 <sup>de</sup>	78	66 <sup>a</sup>	64	129
	Pop003xAls1	2395	83	91 <sup>bcd</sup>	81	83 <sup>bcd</sup>	68	126
	Pop003xAls2	2527	85	99 <sup>de</sup>	79	78 <sup>abc</sup>	66	127
	Pop003xAls1+Als2	2449	87	96 <sup>cde</sup>	79	78 <sup>abc</sup>	67	128
	Pop003xChecks	2797	87	74 <sup>a</sup>	82	97 <sup>e</sup>	81	116
	Pop004xNull	2582	80	97 <sup>cde</sup>	78	83 <sup>bcd</sup>	69	125
Pop004xAls1	2686	80	97 <sup>cde</sup>	80	88 <sup>bcde</sup>	72	124	

Table 3. Contd.

	Pop004xAls2	2527	84	102 <sup>e</sup>	78	76 <sup>ab</sup>	68	123
	Pop004xAls1+Als2	2631	82	88 <sup>bc</sup>	78	88 <sup>bcd</sup>	72	123
	Pop004xChecks	2707	87	70 <sup>a</sup>	81	99 <sup>e</sup>	81	114
Locations and populations interaction means	PlanaltinaxPop001	2933	90	93 <sup>cd</sup>	93 <sup>b</sup>	92 <sup>de</sup>	71 <sup>bc</sup>	140 <sup>f</sup>
	PlanaltinaxPop002	3063	93	85 <sup>a</sup>	96 <sup>bc</sup>	93 <sup>e</sup>	73 <sup>c</sup>	137 <sup>e</sup>
	PlanaltinaxPop003	2689	91	95 <sup>de</sup>	99 <sup>c</sup>	77 <sup>a</sup>	63 <sup>a</sup>	144 <sup>g</sup>
	PlanaltinaxPop004	2870	90	91 <sup>bc</sup>	96 <sup>bc</sup>	88 <sup>cde</sup>	69 <sup>b</sup>	139 <sup>f</sup>
	SorrisoxPop001	2572	74	89 <sup>b</sup>	60 <sup>a</sup>	91 <sup>de</sup>	71 <sup>bc</sup>	111 <sup>d</sup>
	SorrisoxPop002	2606	79	83 <sup>a</sup>	62 <sup>a</sup>	87 <sup>cd</sup>	72 <sup>bc</sup>	103 <sup>a</sup>
	SorrisoxPop003	2294	78	94 <sup>cde</sup>	61 <sup>a</sup>	80 <sup>ab</sup>	72 <sup>bc</sup>	109 <sup>c</sup>
	SorrisoxPop004	2357	74	97 <sup>e</sup>	61 <sup>a</sup>	83 <sup>bc</sup>	73 <sup>c</sup>	106 <sup>b</sup>
		PlanaltinaxNull	2888 <sup>bc</sup>	90	95 <sup>d</sup>	96 <sup>c</sup>	84	65 <sup>a</sup>
Locations and classes interaction means	PlanaltinaxAls1	2901 <sup>c</sup>	90	93 <sup>cd</sup>	96 <sup>c</sup>	89	69 <sup>abc</sup>	141 <sup>e</sup>
	PlanaltinaxAls2	2854 <sup>bc</sup>	92	95 <sup>d</sup>	97 <sup>c</sup>	84	67 <sup>ab</sup>	141 <sup>e</sup>
	PlanaltinaxAls1+Als2	2899 <sup>c</sup>	92	91 <sup>cd</sup>	97 <sup>c</sup>	89	69 <sup>abc</sup>	141 <sup>e</sup>
	PlanaltinaxChecks	2921 <sup>c</sup>	93	66 <sup>a</sup>	95 <sup>c</sup>	97	85 <sup>e</sup>	129 <sup>d</sup>
	SorrisoxNull	2454 <sup>a</sup>	74	93 <sup>cd</sup>	60 <sup>a</sup>	82	71 <sup>bc</sup>	108 <sup>c</sup>
	SorrisoxAls1	2440 <sup>a</sup>	75	90 <sup>c</sup>	61 <sup>a</sup>	86	71 <sup>bc</sup>	108 <sup>c</sup>
	SorrisoxAls2	2408 <sup>a</sup>	77	95 <sup>d</sup>	59 <sup>a</sup>	81	72 <sup>c</sup>	109 <sup>c</sup>
	SorrisoxAls1+Als2	2407 <sup>a</sup>	76	90 <sup>c</sup>	60 <sup>a</sup>	86	71 <sup>bc</sup>	106 <sup>b</sup>
	SorrisoxChecks	2743 <sup>b</sup>	81	78 <sup>b</sup>	70 <sup>b</sup>	98	78 <sup>d</sup>	100 <sup>a</sup>
Locations, populations and classes interaction means	PlanxPop001xNull	2893	86 <sup>bcd</sup>	98	89 <sup>e</sup>	93	68 <sup>bcd</sup>	140 <sup>jk</sup>
	PlanxPop001xAls1	2958	90 <sup>defgh</sup>	94	93 <sup>ef</sup>	93	70 <sup>bcd</sup>	142 <sup>kl</sup>
	PlanxPop001xAls2	2922	91 <sup>efgh</sup>	95	94 <sup>ef</sup>	86	69 <sup>bcd</sup>	141 <sup>kl</sup>
	PlanxPop001xAls1+Als2	2936	93 <sup>gh</sup>	96	94 <sup>ef</sup>	95	70 <sup>bcd</sup>	140 <sup>jk</sup>
	PlanxPop001xChecks	2984	94 <sup>h</sup>	69	97 <sup>ef</sup>	97	86 <sup>h</sup>	129 <sup>i</sup>
	PlanxPop002xNull	3122	92 <sup>fgh</sup>	87	96 <sup>ef</sup>	95	72 <sup>cde</sup>	138 <sup>j</sup>
	PlanxPop002xAls1	3085	91 <sup>efgh</sup>	88	96 <sup>ef</sup>	89	69 <sup>bcd</sup>	138 <sup>j</sup>
	PlanxPop002xAls2	2970	95 <sup>h</sup>	88	96 <sup>ef</sup>	93	73 <sup>defg</sup>	138 <sup>j</sup>
	PlanxPop002xAls1+Als2	3073	93 <sup>gh</sup>	86	97 <sup>ef</sup>	93	73 <sup>defg</sup>	138 <sup>j</sup>
	PlanxPop002xChecks	3067	95 <sup>h</sup>	67	97 <sup>ef</sup>	98	86 <sup>h</sup>	129 <sup>i</sup>
	PlanxPop003xNull	2676	92 <sup>fgh</sup>	102	99 <sup>f</sup>	61	55 <sup>a</sup>	145 <sup>l</sup>
	PlanxPop003xAls1	2590	90 <sup>defgh</sup>	93	99 <sup>f</sup>	82	64 <sup>abcd</sup>	144 <sup>kl</sup>
	PlanxPop003xAls2	2740	92 <sup>fgh</sup>	100	99 <sup>f</sup>	77	61 <sup>ab</sup>	144 <sup>kl</sup>
	PlanxPop003xAls1+Als2	2690	92 <sup>fgh</sup>	96	99 <sup>f</sup>	77	62 <sup>abc</sup>	145 <sup>l</sup>
	PlanxPop003xChecks	2843	90 <sup>defgh</sup>	65	95 <sup>ef</sup>	98	84 <sup>h</sup>	129 <sup>i</sup>
	PlanxPop004xNull	2860	89 <sup>cdefgh</sup>	94	98 <sup>f</sup>	88	65 <sup>abcd</sup>	141 <sup>kl</sup>
	PlanxPop004xAls1	2970	90 <sup>defgh</sup>	96	96 <sup>ef</sup>	90	70 <sup>bcd</sup>	141 <sup>kl</sup>
	PlanxPop004xAls2	2784	91 <sup>efgh</sup>	98	97 <sup>ef</sup>	78	66 <sup>bcd</sup>	140 <sup>jk</sup>
	PlanxPop004xAls1+Als2	2895	90 <sup>defgh</sup>	86	96 <sup>ef</sup>	90	70 <sup>bcd</sup>	140 <sup>jk</sup>
	PlanxPop004xChecks	2791	93 <sup>gh</sup>	64	93 <sup>ef</sup>	97	83 <sup>gh</sup>	129 <sup>i</sup>
	SorrPop001xNull	2628	72 <sup>a</sup>	91	59 <sup>a</sup>	90	68 <sup>bcd</sup>	109 <sup>defg</sup>
	SorrPop001xAls1	2730	75 <sup>ab</sup>	89	57 <sup>a</sup>	87	68 <sup>bcd</sup>	112 <sup>g</sup>
	SorrPop001xAls2	2349	79 <sup>abcde</sup>	90	60 <sup>a</sup>	87	76 <sup>efgh</sup>	118 <sup>h</sup>
	SorrPop001xAls1+Als2	2494	70 <sup>a</sup>	88	60 <sup>a</sup>	95	71 <sup>bcd</sup>	108 <sup>defg</sup>
	SorrPop001xChecks	2795	78 <sup>abcde</sup>	82	70 <sup>cd</sup>	99	78 <sup>fgh</sup>	100 <sup>a</sup>
	SorrPop002xNull	2634	83 <sup>abcde</sup>	84	65 <sup>abcd</sup>	90	72 <sup>cde</sup>	103 <sup>abc</sup>
	SorrPop002xAls1	2437	77 <sup>abcd</sup>	84	61 <sup>ab</sup>	87	72 <sup>cde</sup>	103 <sup>abc</sup>
	SorrPop002xAls2	2699	74 <sup>ab</sup>	84	57 <sup>a</sup>	83	71 <sup>bcd</sup>	103 <sup>abc</sup>
	SorrPop002xAls1+Als2	2578	79 <sup>abcde</sup>	85	60 <sup>a</sup>	84	68 <sup>bcd</sup>	103 <sup>abc</sup>

Table 3. Contd.

SorrPop002xChecks	2801	80 <sup>abcdefg</sup>	73	72 <sup>d</sup>	98	78 <sup>fgh</sup>	99 <sup>a</sup>
SorrPop003xNull	2272	71 <sup>a</sup>	96	57 <sup>a</sup>	71	72 <sup>cdef</sup>	111 <sup>fg</sup>
SorrPop003xAls1	2199	76 <sup>abc</sup>	89	63 <sup>abc</sup>	85	71 <sup>bcdef</sup>	108 <sup>defg</sup>
SorrPop003xAls2	2313	79 <sup>abcdef</sup>	100	59 <sup>a</sup>	79	71 <sup>bcdef</sup>	110 <sup>efg</sup>
SorrPop003xAls1+Als2	2209	82 <sup>abcdefgh</sup>	97	62 <sup>abc</sup>	79	71 <sup>bcdef</sup>	110 <sup>efg</sup>
SorrPop003xChecks	2752	83 <sup>abcdefgh</sup>	82	69 <sup>bcd</sup>	96	78 <sup>fgh</sup>	102 <sup>ab</sup>
SorrPop004xNull	2303	70 <sup>a</sup>	101	57 <sup>a</sup>	78	72 <sup>cdef</sup>	108 <sup>defg</sup>
SorrPop004xAls1	2392	70 <sup>a</sup>	98	64 <sup>abcd</sup>	86	73 <sup>defg</sup>	107 <sup>cdef</sup>
SorrPop004xAls2	2280	77 <sup>abcd</sup>	107	59 <sup>a</sup>	75	70 <sup>bcdef</sup>	106 <sup>bcde</sup>
SorrPop004xAls1+Als2	2347	74 <sup>ab</sup>	91	59 <sup>a</sup>	87	73 <sup>defg</sup>	105 <sup>bcd</sup>
SorrPop004xChecks	2624	82 <sup>abcdefgh</sup>	75	69 <sup>bcd</sup>	100	78 <sup>fgh</sup>	99 <sup>a</sup>

Means with the different letter in the column within a source of variation are different according to Tukey test (P<0.5). GY = grain yield; EMG = seedling emergence; PH = plant height; PLEV = plot evaluation; NLOD = non-lodging; VTE = visual treatment evaluation; MAT = maturity.

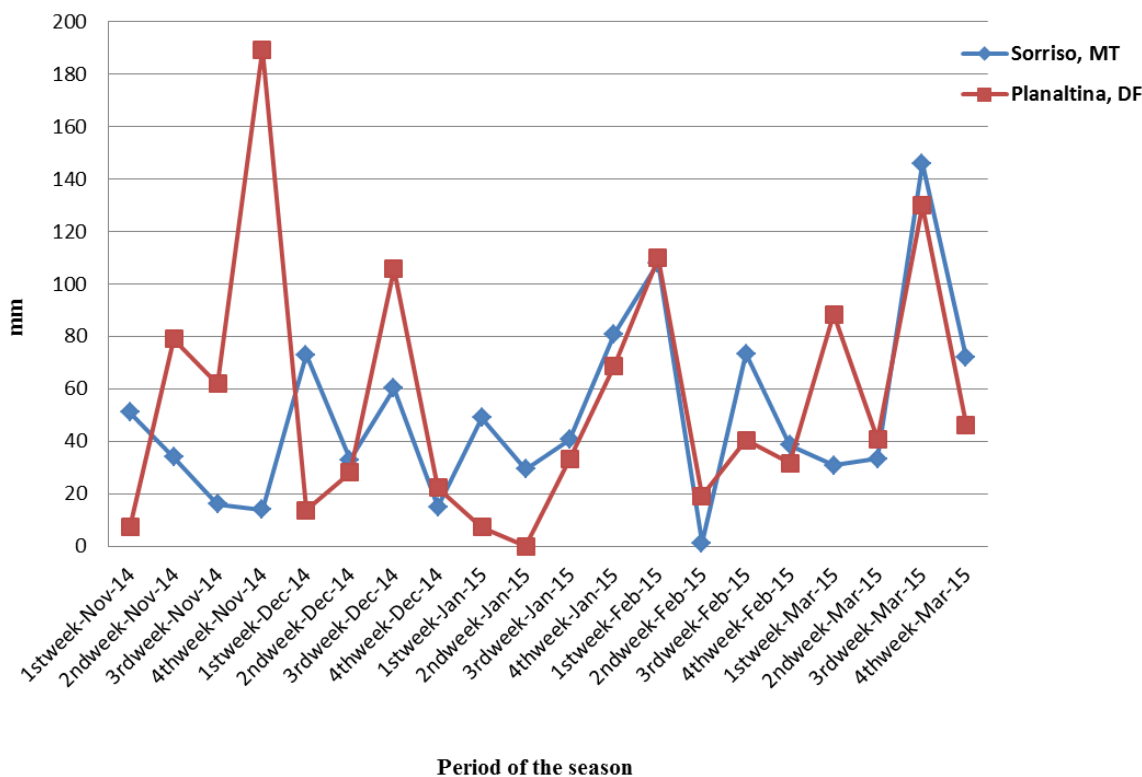


Figure 1. Rainfall at DuPont Pioneer research centers of Sorriso, MT and Planaltina, DF during 2014/2015 season. Data collected by DuPont Pioneer weather station located at the research sites.

growth of the crop in Sorriso are because of the lack of rain in the beginning of the crop cycle.

Plant height was statistically different regarding the sources of variation class, population\*class, location\*population and location\*class (Table 2). Checks were lower than the genotypic classes of *Als1* and/or *Als2* alleles (Table 3). This difference is highly associated with the cycle of the evaluated genotypes. The checks

reached maturity significantly earlier than the other genotypic classes. Comparing populations, Pop002 was significantly lower than other populations at Sorriso and Planaltina. In Planaltina, the highest population was Pop003. In Sorriso, the highest populations were Pop004 and Pop003 (Table 3). These higher populations were therefore more subject to lodging.

Another trait that reflected the environmental conditions

was plot evaluation, which was significantly different for location\*population, location\*class and location\*population\*class (Table 2). The populations and classes in Sorriso were statistically inferior than the populations and classes in Planaltina. In the Sorriso experiment, the checks were more tolerant to adverse conditions and had higher scores when compared with genotypic classes (Table 3). Checks, once again, showed superiority when in unfavorable conditions due to all testing and selection process they passed by during years before become commercial varieties different of the genotypic classes.

Three sources of variation were statistically different for non-lodging: Class, population\*class and location\*population (Table 2). Due to the low plant height of the checks, they lodged less than the genotypic classes. Among populations, those that stood out were Pop001 and Pop002 (Table 3).

Regarding the visual evaluation of the treatment, there were differences for location\*population, location\*class and location\*population\*class (Table 2). In Sorriso, populations had similar appearance and behavior, and were statistically equal. However, in Planaltina, Pop002 was significantly superior to Pop003 and Pop004 populations (Table 3). In Planaltina, Pop001 and especially Pop002 stood out visually if compared to the other populations. Pop002 was highly homogeneous and its genotypic classes were very similar. Thus, it was difficult to mark any visual difference between them. Despite the lower homogeneity of the other populations, they also showed a high visual similarity between the four gene genotypic classes (Table 3).

For the maturity trait, the genotypes in Sorriso completed the cycle faster than in Planaltina (Table 3). This is due to the geographical position of the evaluated locations. Latitude and altitude affect day length and temperature during the day and the night, causing soybean genotypes in Sorriso to accelerate the cycle if compared to Planaltina. The sources of variation class, location\*population, location\*class and location\*population\*class were significantly different regarding this trait (Table 2). The checks completed the cycle faster than genotypic classes both in Sorriso and Planaltina. Genotypic classes did not differ among themselves in each location, except for Als1+Als2 in Sorriso, which had a slightly earlier cycle than the others. Among populations, the Pop002 cycle was significantly earlier than the other populations. In Sorriso, the Pop001 cycle was delayed, while in Planaltina the Pop003 cycle required the longest time to reach maturity (Table 3). Once more, the genotypic classes of the alleles were very similar, however with a longer cycle than the average of the four checks.

The breeding process, to which the checks were submitted, played an important role. Checks presented better ability to excel under unfavorable conditions compared to the populations that were developed aiming

to evaluate *Als1* and *Als2* alleles. The genotypic classes of the alleles were very similar overall, however it is necessary that the best lines containing one or both alleles go through a whole breeding process before become a commercial variety or entry in a breeding cross.

There are no reports in the literature comparing the effects of adding *Als1* and *Als2* alleles on the agronomic characteristics of soybean genotypes. In transgenic, which involves the inclusion of a generally exogenous gene into a given genotype, there is a great concern about whether their inclusion could cause agronomic concerns, such as a decrease in yield, and change crop cycle, plant height, germination, flowering and other traits. This is due to the inclusion of a gene which may be added to an undesired region of a chromosome, disrupting endogenous genes, preventing the formation of essential proteins or causing fusion of undesirable proteins. These changes may result in phenotypes with undesirable agronomic characteristics (Que et al., 2010).

Some studies (Minor, 1998; Elmore et al., 2001) reported that the addition of a transgenic gene resistant to the first-generation of glyphosate in soybeans decreased grain yield. However, another line (Carpenter, 2001) stated that this happened because the transgenic gene was added to genotypes that were not superior, and the decrease in yield was due to the genotype and not to the transgenic gene; thus, with the introduction of new superior cultivars containing the transgenic gene, that difference in productivity would decrease until inexistent. In addition, Hungria et al. (2014) reported no yield drag on genetic modified soybean and EFSA (2010) concluded that the transgenic version is agronomically equivalent to its conventional counterpart.

Mutagenic induction aiming to generate variability and hence the appearance of new forms of a gene may also raise suspicion that such mutant allele may cause changes in the formation of essential proteins, development of improper proteins and emergence of undesirable phenotypes.

In soybeans, the mutant gene *FAD2-1A*, found in the cultivar M23, which provides a high-quality oil to soybeans seeds by increasing the oleic acid, is often associated with decrease in grain yield (Scherder and Fehr, 2008; Clemente and Cahoon, 2009).

In tomato crops, the impact of a mutation known as *ovate*, which promotes a drastic change in the tomato fruit shape, also caused negative changes in the phenotype. This mutation resulted in a decrease in soluble solids, average fruit and seed weight, fruit fixation and productivity (Faria, 2014).

On the other hand, other mutations did not significantly change agronomic characteristics. Spano et al. (2003) worked with four mutants in durum wheat with the capability of delay leaf senescence. They concluded that the extended period of flag leaf photosynthetic competence in the mutant lines generated higher seed



weights and grain yield per plants in the mutant lines compared to their parental lines.

In fig trees, the mutation process, induced by irradiation with gamma rays aiming to increase genetic variability, generated five mutant lines, which were then evaluated in performance tests and compared to other commercial cultivars. The results showed that the mutants had a performance similar to the commercial cultivars, and that the mutant PI-189 was superior to the commercial cultivars regarding important characteristics such as number of fruits per plant, average weight per fruit and yield (Rodrigues et al., 2009).

The type of gene mutation that generated *Als1* and *Als2* alleles was base substitution. Only a single amino acid was changed in each gene (Walter et al., 2014). The results of this study show that such a minimal change in amino acids, in general, did not generate statistically significant differences between the four classes of *Als1* and *Als2* alleles.

Further studies evaluating the addition of *Als1* and/or *Als2* alleles, involving populations or commercial varieties with a similar germplasm and in different environments, become crucial to confirm that the addition of such alleles does not change the agronomic characteristics in soybeans.

## Conclusions

For the four studied populations, Pop001 (BG4277/CD250RRSTS), Pop002 (98Y30/CD250RRSTS), Pop003 (YB84C12/CD250RRSTS) and Pop004 (XB85C12/CD250RRSTS), the incorporation of *Als1* and/or *Als2* alleles aiming a greater resistance to herbicides from the sulfonyleurea group did not cause significant changes in the evaluated agronomic traits.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## REFERENCES

- Carpenter JE (2001). Comparing Roundup Ready and conventional soybean yields: 1999. National Center for Food and Agricultural Policy, Washington, DC.
- Clemente TE, Cahoon EB (2009). Soybean oil: genetic approaches and modification of functionality and total content. *Plant Physiol.* 151(3):1030-1040.
- Eberlein CV, Guttieri MJ, Thill DC, Baerg RJ (1997). Altered acetolactate synthase activity in ALS - inhibitor resistant prickly lettuce (*Lactuca serriola*). *Weed Sci.* 45(2):212-217.
- EFSA – European Food Safety Authority (2010). Scientific opinion of the panel on Genetically Modified Organisms on applications (EFSA-GMO-RX-40-3-2) for the renewal of authorization for the continued marketing of (1) food containing, consisting of, or produced from genetically modified soybean 40-3-2; (2) feed containing, consisting of, or produced from soybean 40-3-2; (3) other products containing or consisting of soybean 40-3-2 with the exception of cultivation, all under Regulation (EC) No 1829/2003 from Monsanto. *EFSA J.* 8(12):1-38.
- Elmore RW, Roeth FW, Nelson LA, Shapiro CA, Klein RN, Knezevic SZ, Martin A (2001). Glyphosate-resistant soybean cultivar yields compared with sister lines. *Agron. J.* 93(2):408-412.
- EMBRAPA Soja (2001). Tecnologias de produção de soja – Região Central do Brasil – 2001/2002. Londrina: Documentos 167:267.
- Faria JPB (2014). Alterações hormonais no mutante ovate de tomateiro (*Solanum lycopersicum* L. cv Micro-Tom) e seu impacto na morfologia, qualidade do fruto, produtividade e partenocarpia. 68 f. Masters dissertation (Master in Plant Physiology and Biochemistry). Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba.
- Fehr WR, Caviness CE, Burmood DT, Pennington JS (1971). Stage of development descriptions for soybeans, *Glycine max* (L.) Merrill. *Crop Sci.* 11(6): 929-931.
- Green JM (2007). Review of glyphosate and ALS-inhibiting herbicide crop resistance and resistant weed management. *Weed Technol.* 21(2):547-558.
- Green JM, Owen MDK (2011). Herbicide-resistant crops: utilities and limitations for herbicide-resistant weed management. *J. Agric. Food Chem.* 59(11):5819-5829.
- Hungria M, Mendes IC, Nakatani AS, Reis-Junior FB, Morais JZ, Oliveira MCN, Fernandes MF (2014). Effects of glyphosate-resistant gene and herbicides on soybean crop: Field trials monitoring biological nitrogen fixation and yield. *Field Crop. Res.* 158(1):43-54
- Lamego FP, Vidal RA (2008). Resistance to glyphosate in *Conyza bonariensis* and *Conyza canadensis* biotypes in Rio Grande do Sul, Brazil. *Planta Daninha* 26(2):467-471.
- Minor H (1998). Performance of GMOs vs. traditional varieties: a southern perspective. In: Proceeding of the 28<sup>th</sup> Soybean Seed Research Conference, 1998, Chicago, IL. American Seed Trade Association, Washington, DC. pp. 1–9.
- Moreira M, Nicolai M, Carvalho SJP, Christoffoleti PJ (2007). Resistência de *Conyza canadensis* e *C. bonariensis* ao herbicida glyphosate. *Planta Daninha* 25(1):323-326.
- Powles SB (2008). Evolution in action: glyphosate-resistant weeds threaten world crops. *Outlooks on Pest Management* 19(6):256-259.
- Que Q, Chilton MDM, Fontes CM, He C, Nuccio M, Zhu T, Wu Y, Chen JS, Shi L (2010). Trait stacking in transgenic crops: challenges and opportunities. *GM Crops Food* 1(4):220-229.
- R Core Team (2016). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria. <https://www.R-project.org/>
- Ramalho MAP, Ferreira DF, Oliveira AC (2012). Experimentação Em Genética E Melhoramento De Plantas. 3.Ed. Lavras: UFLA 305 p.
- Rodrigues MGF, Correa LS, Boliani AC (2009). Avaliação de seleções mutantes de figueira cv. Roxo-de-Valinhos. *Rev. Bras. Frutic.* 31(3):771-777.
- Sebastian SA, Fader GM, Ulrich JF, Forney DR, Chaleff RS (1989). Semidominant soybean mutation for resistance to sulfonyleurea herbicides. *Crop Sci.* 29(6):1403-1408.
- Scherder CW, Fehr WR (2008). Agronomic and seed characteristics of soybean lines with increased oleate content. *Crop Sci.* 48(5):1755-1758.
- Spano G, Di Fonzo N, Perrotta C, Platani C, Ronga G, Lawlor DW, Napier JA, Shewry PR (2003). Physiological characterization of 'stay green' mutants in durum wheat. *J. Exp. Bot.* 54(386):1415-1520.
- Vargas L, Bianchi MA, Rizzardi MA, Agostinetto D, Dal Magro T (2007). Buva (*Conyza bonariensis*) resistente ao glyphosate na região sul do Brasil. *Planta Daninha* 25(3):573-578.
- Walter KL, Strachan SD, Ferry NM, Albert HH, Castle LA, Sebastian SA (2014). Molecular and phenotypic characterization of *Als1* and *Als2* mutations conferring tolerance to acetolactate synthase herbicides in soybean. *Pest Manage. Sci.* 70(12):1831-1839.
- Zawoznik MS, Tomaro ML (2005). Effect of chlorimuron-ethyl on *Bradyrhizobium japonicum* and its symbiosis with soybean. *Pest Manage. Sci.* 61(10):1003-1008.

## Full Length Research Paper

# Assessment of yield loss in Rosemary (*Rosmarinus officinalis* L.) and Sage (*Salvia officinalis* L.) plants caused by *Fusarium oxysporum*

Mihiret Mekonnen<sup>1\*</sup> and Begashaw Manahile<sup>2</sup><sup>1</sup>Wondo Genet Agricultural Research Center, EIAR, P. O. Box 198, Shashemene, Ethiopia.<sup>2</sup>Hawassa University, College of Computational and Natural Sciences, P.O. Box 128, Shashemene, Ethiopia.

Received 24 August, 2016; Accepted 18 January, 2017

*Rosmarinus officinalis* and *Salvia officinalis* wilt caused by *Fusarium oxysporum* could lead to yield losses, under various conditions. This study was therefore focused on determination of yield loss caused by *F. oxysporum* on *R. officinalis* and *S. officinalis* plants. A greenhouse experiment was conducted at Wondo Genet College of Forestry and Natural Resources (WGCFNR), Ethiopia, from 2015 to 2016. Mixed soil of animal manure, sand and clay loam (1:1:2 ratio), respectively, was autoclaved at 121°C for 2 h and sterilized soil was filled into plastic pots with three replications. Two plants were transplanted into each pot and regularly watered and maintained in the greenhouse at 26 ± 2°C and 50 to 60% relative humidity. Disease severity was recorded at weekly interval, from the first appearance of symptoms till harvest. Fresh leaf and stem weights, essential oil content and yield were recorded at the time of harvesting. Generally, fresh leaf and stem weights and essential oil content and yield of both plants from treated soil significantly increased compared to the untreated ones. Essential oil yield decreased by 53.86 and 35.36% in *R. officinalis* and *S. officinalis*, respectively compared to the treated ones.

**Key words:** Essential oil yield, *Fusarium oxysporum*, Rosemary, Sage, Yield loss

## INTRODUCTION

Rosemary (*Rosmarinus officinalis* L.) is an aromatic and medicinal shrubby herb that belongs to the family Labiatae (Türe et al., 2009). It is endogenous to Europe, Asia and Africa, originally grows in southern Europe (Pintore et al., 2002). Its herb and oil are mostly used as spice and flavoring agents in food processing for its desirable flavor, and use folk medicinal value as antimicrobial agent (Lo et al., 2002). Rosemary is used traditionally for relieving visceral muscle spasms in renal

colic, menstrual pain, bronchial asthma and gastrointestinal colic, all over the world (Singh et al., 2009; Tironi et al., 2009). In Ethiopia, rosemary, most of the time is used for flavoring food. It has also some therapeutic value in the treatment of disorders like, peptic ulcers, inflammatory diseases, hepatotoxicity, atherosclerosis, ischemic heart disease, cataracts, and cancer (Valenzuela et al., 2004; Katerinopoulos et al., 2005).

\*Corresponding author. E-mail: hanamihiret0@gmail.com or mihiretmekonnen@yahoo.com.

As well, *Salvia officinalis* L. is a perennial low shrub aromatic and medicinal plant belonging to the family Lamiaceae which has about 900 species from genera *Salvia*, native to Mediterranean region (Zervoudakis et al., 2012). Essential oil is added as supplement to meat, sausage, poultry stuffings, fish, soups, canned foods and other food products. Its essential oil is applied in different pharmaceutical, cosmetics, sanitary and food industries owing to its odour and biological effects such as antiseptic, antibacterial, antioxidant activity, etc., (Bakkali et al., 2008).

However, Antracnosis, Ascochitosis, and root rot, caused by *Colletotrichum dematium*, *Ascochyta sclarea* and *Rhizoctonia solani*, respectively are economically important disease of sage in European countries (Subbiah et al., 1996; Voltolina, 2001). Similarly, *Phomopsis sclarea*, *Phodosphaera inequalis*, *Erysiphe polygoni* and *Sclerotinia sclerotiorum* are economically important yield loss causing pathogens to the plant in Italy, Spain, California and USA. Massive dying out of sage seedlings infected by *Fusarium oxysporum* was also observed in these countries (Subbiah et al., 1996). *R. solani*, *Phytophthora citrophthora* and *P. cinnamoni* were reported as main root rot causal soil borne pathogens (Alvarez et al., 2007; Verhoeven et al., 2008). Therefore, integrated pest management development requires precise and accurate information on damage caused by pests. Disease damage reduces both quantity and quality of yield. Disease and crop loss assessments are necessary for identification of economic impact of a disease and to develop management and control strategies.

*Fusarium* species are the main soil borne plant pathogens that is economical important disease in agricultural productions all over the world (Saremi, 2000). The primary symptom of *Fusarium* wilt is characterized by discoloration of the vascular tissue which mostly starts from the ground and expands to the above parts, wilting the stem and leaves, sometimes followed by leaf abscission and plant death (Schwartz et al., 2005). *Fusarium* wilt caused by *F. oxysporum* is economically an important soil borne disease which affects plant yield severely in susceptible varieties. Depending on crop age losses due to *Fusarium* wilt disease vary from 0 to 100% (Kannaiyan and Nene, 1981). Similarly, *Fusarium* wilt is an economically important prioritized disease of Rosemary and Sage plants that significantly affects the cultivation of these plants under various conditions at Wondo Genet. This study was therefore focused on determination of yield loss caused by *F. oxysporum* on *R. officinalis* and *S. officinalis* using sterilized and unsterilized soils under greenhouse conditions.

## MATERIALS AND METHODS

### Experiment site and yield loss estimation

The greenhouse experiment was conducted at Wondo Genet

College of Forestry and Natural Resources (WGCFNR), Ethiopia, from 2015 to 2016.

Mixed soil of animal manure, sand and clay loam (1:1:2 ratio), respectively was autoclaved at 121°C for 2 h and 3 kg of sterilized soil, with pH 6.8, was filled into plastic pots (20 × 15 cm) with ten replications. Unsterilized soil of the same amount was used as control for both experiments. Two seedlings of each plant species were transplanted into the individual pot and regularly watered and maintained in greenhouse at 26 ± 2°C and 50 to 60% relative humidity. The plants were regularly observed for *Fusarium* wilt symptom development, characterized by discoloration of the vascular tissue. The symptom mostly starts from the ground, wilting the stem and leaves, sometimes followed by leaf abscission and plant death. Data were recorded on disease severity at weekly interval from the first appearance of symptom till harvest, 3 months after planting date. The disease severity was assessed based on visual assessment key (0 - 4 scale), where 0, 1, 2, 3 and 4 represent 0 to 24%, 25 to 49%, 50 to 74%, 75 to 99% and 100% (dead plant), respectively of wilted leaves. Plant height, branch number, fresh leaf and stem weights and essential oil content and yield were recorded at the time of harvesting. Percent EO content was determined on fresh weight (w/w) basis from 250 g of fresh composite leaves and EOY was calculated by multiplying the fresh weight biomass and the essential oil content. Essential oil was extracted by hydro distillation as illustrated by Guenther (1972).

The percent essential oil yield losses were calculated by the formula:

$$EOY \text{ loss (\%)} = \frac{\text{Yield of healthy plant} - \text{Yield of diseased plant}}{\text{Yield of healthy plant}}$$

### Statistical analysis

The data were statistically analyzed using analysis of variance (ANOVA) and differences between treatments means was assessed using Duncan's Multiple Range Test, using SAS statistical software (SAS, 2002).

## RESULTS AND DISCUSSION

The soil treatment had significant effects on essential oil content and yields and growth parameters of both Sage and Rosemary plants. Analysis of variance and mean comparison revealed significant differences ( $P < 0.05$ ) in plant heights, branches numbers and essential oil content and yield between plants from treated and untreated soils in both experiments (Tables 1 and 2). Average plants heights from unsterilized soil were reduced by 19.7 and 14.9 cm in *R. officinalis* and *S. officinalis*, respectively. Similarly, branch numbers from untreated soil were reduced by 84.33 and 36.73% in *R. officinalis* and *S. officinalis*, respectively on unsterilized soil. Likewise, fresh leaf weights reduced by 17.3 kg/p (60.28%) and 7.21 kg/p (27.99%) in *R. officinalis* and *S. officinalis*, respectively compared with plants from treated soil (Tables 1 and 2).

On the other hand, 14.65 kg/p (46.14%) and 14.99 kg/p (64.64%) less essential oil yields were recorded in *R. officinalis* and *S. officinalis*, respectively from unsterilized soil. In the same way, less essential oil content, 40% in *R. officinalis* and 44.44% in *S. officinalis*, were recorded

**Table 1.** Effect of Fusarium wilt on growth parameters, essential oil content and yield on *R. officinalis*.

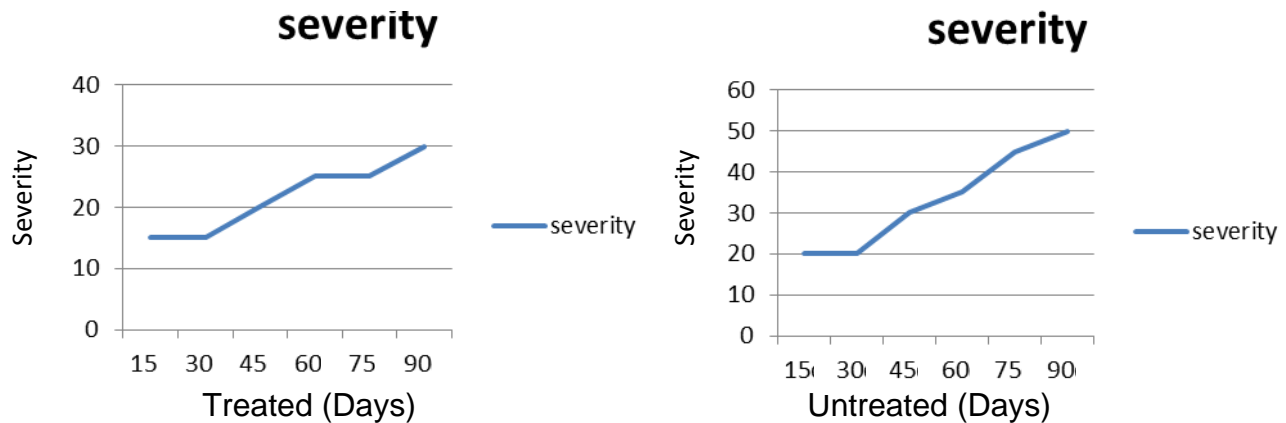
Treatments	Growth parameters				Oil yield	
	PH (cm)	BN	FLW (kg/p)	FSW (kg/p)	EOC (%)	EOY (kg/p)
Sterilized soil	52.5	45	28.7 <sup>a</sup>	24.11 <sup>a</sup>	2.5 <sup>a</sup>	31.75 <sup>a</sup>
Unsterilized soil	32.5	7	11.4 <sup>b</sup>	14.09 <sup>b</sup>	1.5 <sup>b</sup>	17.1 <sup>b</sup>
SD	42.5	26	20.05	19.1	2	24.43

Means with the same letter within the same column are not statistically different ( $p < 0.05$ ). PH: Plant height, BN: branch number, FLW: fresh leaf weight, EOC: essential oil content, EOY: essential oil yield.

**Table 2.** Effect of Fusarium wilt on growth parameters, essential oil content and yield on *S. officinalis*.

Treatments	Growth parameters				Oil yield	
	PH (cm)	BN	FLW (kg/p)	FSW (kg/p)	EOC (%)	EOY (kg/p)
Sterilized soil	70.71	27.66	25.77 <sup>a</sup>	27.76 <sup>a</sup>	0.9 <sup>a</sup>	23.19 <sup>a</sup>
Unsterilized soil	56.25	17.5	18.56 <sup>b</sup>	16.41 <sup>b</sup>	0.5 <sup>b</sup>	8.2 <sup>b</sup>
SD	63.48	22.58	22.17	22.09	0.7	15.69

Means with the same letter within the same column are not statistically different ( $p < 0.05$ ). PH: Plant height, BN: branch number, FLW: fresh leaf weight, EOC: essential oil content, EOY: essential oil yield.

**Figure 1.** Disease severity of *R. officinalis* on sterilized and unsterilized soils.

in this treatment (Tables 1 and 2). Siddiqui and Akhtar (2007) reported 15 to 20% yield losses of chili caused by *F. oxysporum* in Pakistan. Similarly, significant losses in yield of Watermelon had been reported in Maryland due to the prevalence of Fusarium wilt (Zhou et al., 2003b). Losses can approach 100% if susceptible cultivars are planted in heavily infested fields (Egel et al., 2007).

Much higher disease severity levels (50%) were observed on both *R. officinalis* and *S. officinalis* than the severity levels (30%) of plants from treated soil (Figures 1 and 3). During the first two weeks, no disease symptoms were observed on both *R. officinalis* and *S. officinalis* from sterilized soil. However, after 20 days of planting disease severity gradually increased until the time of harvest. On the other hand, disease severity increased

exponentially on *S. officinalis* after the first week of planting on untreated soil (Figures 1 and 2). This finding was in agreement with previous works (Sharma, 1994; Navas et al., 2000; Wharton et al., 2006; Saremi and Amiri, 2010).

For example, the disease severity rapidly progressed from 20 to 50% on both *R. officinalis* and *S. officinalis* on untreated soil. On the other hand, the disease severity increased gradually from 15 to 30% and from 10 to 30% on *R. officinalis* and *S. officinalis*, respectively on sterilized soil (Figures 1 and 3). The occurrence of Fusarium wilt disease on plants cultivated on sterilized soil may most likely be because of the spread of *F. oxysporum*, by irrigation water and contaminated management equipments, from adjacent plants cultivated



Figure 2. Sage and Rosemary under greenhouse condition.

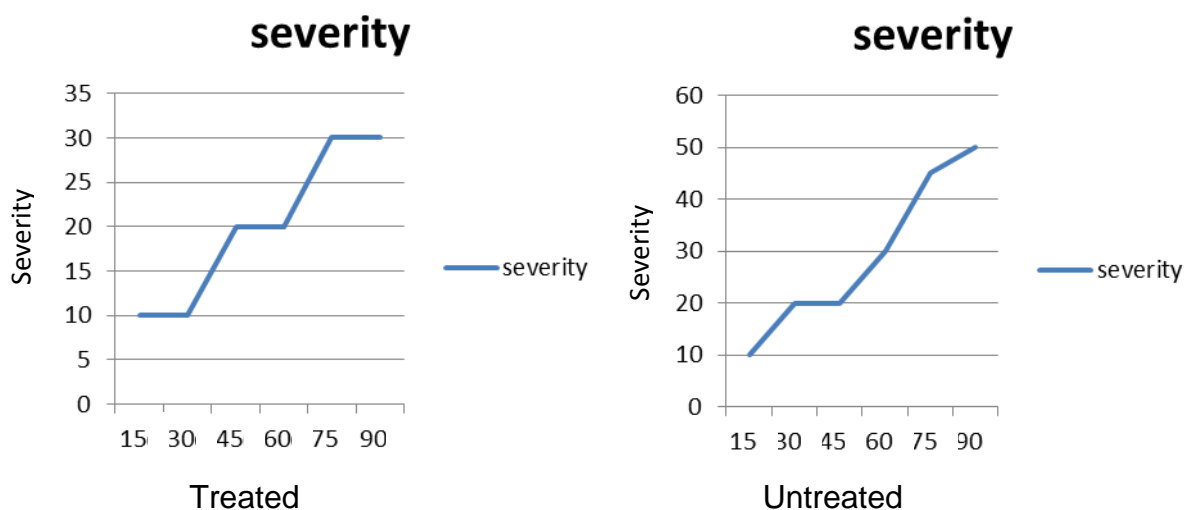


Figure 3. Disease severity of *S. officinalis* on sterilized and unsterilized soils.

on unsterilized soil. Similar result had been reported on *Chrysanthemum* Fusarium wilt, caused by *F. oxysporium*. Fusarium wilt was reported as the most widely spread and destructive disease, causing infection and yield loss from nursery to flowering stage on this plant (Locke et al., 1985).

### Conclusion

The result of this study had revealed that soil treatment had a significant effect on growth parameters, essential oil content and yield of *R. officinalis* and *S. officinalis*. Essential oil yield reduced by 53.86 and 35.36% in *R. officinalis* and *S. officinalis*, respectively from untreated soil compared to the treated ones. Therefore, designing an integrated Fusarium wilt management method as

alternative control measure for this disease is necessary.

### CONFLICT OF INTERESTS

The authors have not declared any conflict of interest

### REFERENCES

- Alvarez LA, Perez-Sierra A, Armengol J, Garcia-Jimenez J (2007). Characterization of *Phytophthora nicotianae* isolates causing collar and root of Lavender and Rosemary in Spain. *J. Plant Pathol.* 89(2):261-264.
- Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008). Biological effect of essential oils – A review. *Food Chem. Toxicol.* 46(2):446-475.
- Egel DS, Martyn RD (2007). Fusarium wilts of watermelon and other cucurbits. Online. The Plant Health Instructor. doi:10.1094/PHI-I-2007-0122-01 Available at:

- <http://www.apsnet.org/edcenter/intropp/lessons/fungi/ascomycetes/Pages/FusariumWatermelon.aspx>
- Guenther E (1972). The Essential oils; History origin in plants production analysis. Robert E. kriger publishing Co., Malabar, Florida 1:427.
- Kannaiyan J, Nene YL (1981). Influence of wilt at different growth stages on yield loss in Pigeonpea. *Trop. Pest Manage.* 27:141.
- Katerinopoulos HE, Pagona G, Afratis A, Stratigakis N, Roditakis N (2005). Composition and insect attracting activity of the essential oil of *Rosmarinus officinalis*. *J. Chem. Ecol.* 31(1):111-122.
- Lo AH, Liang YC, Lin-Shiau SY, Ho CT, Lin JK (2002). Carnosol, an antioxidant in rosemary, suppresses inducible nitric oxide synthases through down-regulating nuclear factor in mouse macrophages. *Carcinogenesis* 23:983-991.
- Locke JC, Marois JJ, Papavizas GC (1985). Biological controls of Fusarium wilt of greenhouse-grown Chrysanthemums. *Plant Dis.* 69:167-169.
- Navas-Cortes JA, Hau B, Jimenez-Diaz RM (2000). Yield loss in chickpea in relation to development to Fusarium wilt epidemics. *Phytopathology* 90:1269-1278.
- Pintore G, Usai M, Bradesi P, Juliano C, Boatto G, Tomi F, Chessa M, Cerri R, Casanova J (2002). Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. oils from Sardinia and Corsica. *Flav. Fragr. J.* 7:15-19.
- Salman A, Khan MA, Mumtaz H (2006). Prediction of yield losses in wheat varieties/lines due to leaf rust in Faisalabad. *Pak. J. Phytopathol.* 18(2):178-182.
- Saremi H (2000). *Plant Diseases Caused by Fusarium Species*. Jihad Daneshgahi, Ferdowsi Mashhad University, Iran p. 160.
- Saremi H, Amiri ME (2010). Exploration of potato cultivar resistant to the major fungal pathogen on potato wilting disease in Iran. *J. F. A. Environ.* 8(2):821-826.
- SAS (Statistical Analysis System) (2002). SAS/ STAT. Guide version 9. SAS, Institute Inc. Raleigh, Vorth Carolina, USA.
- Schwartz HF, Steadman JR, Hall R, Forster RL (2005). *Compendium of bean diseases*. 2nd ed. St. Paul: American Phytopatological Society, 120p. SILVA, C.C. da; DEL PELOSO, M.J. (Ed.).
- Sharma PN, Sharma OP (1994). Growth and yield attributes of French bean as affected by virus and fungal infections. *Plant Dis. Res.* 9:157-159.
- Siddiqui ZA, Akhtar MS (2007). Biocontrol of a chickpea root-rot disease complex with phosphate solubilizing micro-organisms. *J. Plant Pathol.* 89(1):67-77.
- Singh RP, Dilworth AD, Ao X, Singh M, Baranwal VK (2009). Citrus exocortis viroid transmission through commercially-distributed seeds of Impatiens and Verbena plants. *Eur. J. Plant Pathol.* 124(4):691-694.
- Subbiah VP, Riddick M, Peele D (1996). First report of Fusarium oxysporum on clary sage in North America. *Plant Dis.* 80:1080.
- Tironi V, Tomás M, Añón M (2009). Lipid and protein changes in chilled sea salmon (*Pseudoperca semifasciata*): effect of previous rosemary extract (*Rosmarinus officinalis* L.) application. *Int. J. Food Sci. Technol.* 44(6): 1254-1262.
- Türe H, Eroğlu F, Özen B, Soyer F (2009). Physical properties of biopolymers containing natamycin and rosemary extract. *Int. J. Food Sci. Technol.* 44(2):402-408.
- Valenzuela A, Sanhueza J, Alonso P, Corbari A, Nieto S (2004). Inhibitory action of conventional food-grade natural antioxidants and of natural antioxidants of new development on the thermal induced oxidation of cholesterol. *Int. J. Food Sci. Nutr.* 55(2):155-162.
- Voltolina G (2001). *Salvia sclarea* L. *Plante Officinali* 2:1-12
- Verhoeven JT, Jansen CC, Roenhorst JW (2008). First report of pospiviroids infecting ornamentals in the Netherlands: Citrus exocortis viroid in *Verbena* sp., Potato spindle tuber viroid in *Brugmansia suaveolens* and *Solanum jasminoides*, and Tomato apical stunt viroid in *Cestrum* sp. *Plant Pathol.* 57:399.
- Wharton PS, Tumbalam P, Kirk WW (2006). First Report of Potato Tuber Sprout Rot Caused by *Fusarium sambucinum* in Michigan. *Plant Dis.* 90:1460-11464.
- Zervoudakis G, George S, George K, Eleni K (2012). Influence of light intensity on growth and physiological characteristics of common cage (*Salvia officinalis* L.). *Braz. Arch. Biol. Technol.* 55(1):89-95.
- Zhou XG, Everts KL (2003b). Races and inoculum density of Fusarium oxysporum f. sp. niveum in commercial watermelon fields in Maryland and Delaware. *Plant Dis.* 87(6):692-698.

*Full Length Research Paper*

# Classification of selected white tropical maize inbred lines into heterotic groups using yield combining ability effects

Yazachew Genet Ejigu<sup>1,2\*</sup>, Pangirayi Bernard Tongoona<sup>2</sup> and Beatrice Elohor Ifie<sup>2</sup>

<sup>1</sup>Ethiopian Institute of Agricultural Research, Ethiopia.

<sup>2</sup>University of Ghana, Accra, Ghana.

Received 26 October, 2016; Accepted 1 February, 2017

**A line x tester mating design involving sixteen white maize inbred lines as females and two testers as males generated thirty-two single crosses. These hybrids plus three checks were evaluated using a 5 x 7 alpha lattice design replicated twice at the University of Ghana, WACCI research farm during 2015/16 offseason using drip irrigation. The objective of the study was to: assign the tropical inbred lines into heterotic groups. Based on the SCA effect for grain yield, the lines were separated into two heterotic groups. The lines L1, L3, L4, L8, L11 and L14 belonged to tester group 1368, while L2, L5, L6, L7, L9, L10, L12, L13, L15 and L16 belonged to heterotic group of CML 444. This is useful for the development of hybrids and synthetic varieties. Thus, the information generated in the present study will be useful for breeders who want to improve yield and yield-contributing traits of maize.**

**Key words:** Hybrids, heterotic group, line x tester (LxT), maize, yield.

## INTRODUCTION

Knowledge on the genetic heterogeneity and progeny performance, are significant for deciding breeding schemes, assigning the parental lines, defining heterotic groups, and predicting future hybrid performance. Thus, information on genetic diversity among genetic materials has an utmost importance for hybrid maize breeding programmes for development of lines, the assigning of lines into different heterotic groups and the preference of testers for hybrid combinations (Xia et al., 2004). Thus, assigning of maize lines into different heterotic group is very vital for hybrid breeding programmes in giving

information about the germplasms (Hallauer et al., 2010). Melchinger (1999) proposed that, when a large number of inbred lines is available and proven testers exist, the relative performance of the lines in testcrosses with proven testers can be used as a main criterion for grouping of the lines. Vasal et al. (1992a, b) used this approach to evaluate the performance of testcrosses of 92 tropical and 88 subtropical maize inbred lines with two dent and two flint tester lines. In developing countries, use of available genetic materials and application of available crop improvement methods to improve yield

\*Corresponding author. E-mail: yazachewgenet@gmail.com.

and yield stability are required to meet the increasing demand of improved maize hybrids (Dhliwayo et al., 2009; Morris et al., 1999).

Heterotic effects of the maize lines and their allocation into well-known heterotic groups is the secret for the success of a maize breeding programme, which would give utmost exploitation of heterosis. The objective of the study was to: classify the tropical inbred lines into heterotic groups.

## MATERIALS AND METHODS

### Description of experimental area

The experiment was carried out during 2015/16 offseason using drip irrigation at, West Africa Centre for Crop Improvement Research Field, University of Ghana. The University is located at 5.6508° N, latitude and 0.1869° W longitude and an altitude 97 m above sea level (m.a.s.l.).

### Genetic materials used for the study

Eighteen white tropical maize inbred lines with diverse genetic backgrounds were selected from the pool of inbred lines at the West Africa Centre for Crop Improvement (WACCI). This comprises of ten lines from the International Institute for Tropical Agriculture (IITA), six from International Maize and Wheat improvement Center (CIMMYT) and the two testers 1368 from IITA and CML 444 from CIMMYT maize breeding programmes. The 16 inbred lines were crossed to the two testers using the line by tester method (Table 1) and it generated 32 (16 x 2) cross combinations.

### Experimental design and field evaluation

The 32 F<sub>1</sub> crosses including the hybrids between the two testers, one popular open pollinated variety and a standard hybrid checks were evaluated for their agronomical performance using a 5 x 7 alpha lattice design at WACCI Research Field, the University of Ghana under irrigation system during 2015/2016. The genotypes were planted in two- rows plots, 5 m long with spacing of 0.75 m between rows and 0.5 m spacing between plants within a row. Three seeds were planted per hill, and then thinned to two plants per hill after three weeks of planting, giving 22 plants per row or 44 plants plot<sup>-1</sup>, to get a total plant density of 53333 plants ha<sup>-1</sup>. The experiment was managed using normal agronomic practices (planting, irrigation, thinning, fertilization, weeding and insect controls) from sowing to maturity.

### Data analysis and procedures

Analysis of variance for all agronomic parameters studied was calculated using the PROC GLM procedure and test for significant differences among the genotypes was performed using SAS software (SAS, 2002). Traits that showed significant differences among genotypes were further partitioned into crosses, checks and check vs. crosses using (SAS, 2002). Traits that showed significant differences among crosses were partitioned into three components, namely females in crosses, males in crosses and female x male in crosses (Kempthorne, 1957; Singh and Chaudhary, 1985). The crosses means were adjusted for block effects as analyzed according to lattice design (Singh and Chaudhary, 1985) and used

**Table 1.** List of parents and testers used for the study.

Code no.	Female parents	Male parents	
		1368 (T1)	CML 444 (T2)
L1	TZMI 763	X	X
L2	TZMI746	X	X
L3	TZMI749	X	X
L4	CML15	X	X
L5	CML 24	X	X
L6	TZMI740	X	X
L7	CML16	X	X
L8	TZMI-Unknown	X	X
L9	TZ-STR-133	X	X
L10	TZIL41	X	X
L11	CML10	X	X
L12	9006	X	X
L13	CML 05	X	X
L14	TZIL 39	X	X
L15	CML12	X	X
L16	TZMI760	X	X

to perform combining ability analysis.

## RESULTS

Genetic variability of genotypes, crosses, crosses vs check, lines, testers and line x testers and heterotic grouping of tropical white inbred lines are given in Table 2 and Table 3, respectively.

The result showed that genetic variability of GCA for lines were highly significant at  $P \leq 0.001$ , for days to 50% anthesis and silking, plant height, ear height, plant aspect, ear length, number of kernel rows ear<sup>-1</sup> and number of kernels row<sup>-1</sup>. Highly significant differences at  $P \leq 0.01$  were detected for anthesis-silking interval, maize streak virus disease, ear rot and grain yield. In addition, significant differences ( $P \leq 0.05$ ) were observed for husk cover.

The mean squares due to GCA for testers were significant  $P \leq 0.001$ , for days to 50% anthesis and ear length, significant at  $P \leq 0.01$  were mean squares for anthesis-silking interval, plant aspect and number of kernel rows ear<sup>-1</sup>, and significant at  $P < 0.05$  were days to 50% silking, plant and ear height. The GCA mean squares for testers were not significant for husk cover, ear rot and number of kernels row<sup>-1</sup>.

The line x tester (SCA) mean squares showed significant differences at  $P \leq 0.001$  for plant height, ear length and number of kernels row<sup>-1</sup>, and significant differences at  $P \leq 0.05$  for days to 50% silking, ear height, number of kernel rows ear<sup>-1</sup> and grain yield. No significant differences was observed for days to 50% anthesis, anthesis-silking interval, plant aspect, maize streak virus disease score, husk cover and ear rot.



**Table 2.** Genetic variability of genotypes, crosses, crosses vs check, lines, testers and line x testers for grain yield and yield contributed traits of maize at university of Ghana, WACCI research farm in 2015/16.

Source of variation	DF	MS						
		AD	SD	ASI	MSD	PH	EH	PLASP
Rep	1	2.41*	0.91	0.23	0.32	73.64	10.73	0.13
B(rep)	12	2.97***	2.70***	0.18	0.08	185.38***	75.13*	0.39
Lines (L)	15	7.03***	6.23***	1.10*	0.35**	268.68***	153.84***	1.00***
Testers (T)	1	12.60***	2.11*	4.71**	0.02	199.12*	184.63*	1.91**
L x T	15	0.57	0.94*	0.18	0.16	154.15***	78.38*	0.40

Source of variation	DF	MS					
		HC%	E rot%	EL	NKRE	NKR	Yld (kg ha <sup>-1</sup> )
Rep	1	0.53	1390.45***	2.41	0.06	10.4	219.73
B(rep)	12	30.87	80.53	0.96	1.10	6.36	484481.53*
Lines (L)	15	303.97***	301.19***	7.31***	6.07***	28.1***	958743.06***
Testers (T)	1	0.67	189.95	18.0***	8.05**	5.86	1558855.12**
L x T	15	128.40*	109.63	3.85***	2.39*	25.9***	466294.02*

\*, \*\* and \*\*\*=Mean squares significant at  $P \leq 0.05$ ,  $P \leq 0.01$  and  $P \leq 0.001$ , respectively. MS= mean squares, DF= degree of freedom, Rep = replications, B(rep) = block within replications, AD = days to anthesis, SD = days to silking, ASI = anthesis- silking interval, MSD = maize streak disease, PH = plant height, EH = ear height, plAsp = plant aspect, HC% = husk cover, RL%= root lodging, SL%= shoot lodging, E rot= number ear rot, EL =ear length, NKRE =number of rows ear-1, NKR =number of kernels row-1 and Yld = grain yield.

**Table 3.** Heterotic grouping of tropical white inbred lines based on SCA values with two testers: 1368 and CML 444.

Lines	GCA	SCA of grain yield		Heterotic grouping
		1368 (A)	CML444 (B)	
L1	595.33*	-491.32*	491.32*	A
L2	295.72	163.38	-163.38	B
L3	-594.96*	-620.53**	620.53**	A
L4	11.22	-214.33	214.33	A
L5	181.44	108.63	-108.63	B
L6	-522.95*	423.92	-423.92	B
L7	105.30	108.63	-108.63	B
L8	549.73*	-902.78***	902.78***	A
L9	246.73	476.46*	-476.46*	B
L10	-996.23***	173.86	-173.86	B
L11	-201.23	-137.35	137.35	A
L12	-268.84	175.99	-175.99	B
L13	260.87	233.12	-233.12	B
L14	-817.39***	-722.69**	722.69**	A
L15	288.31	322.19	-322.19	B
L16	866.95***	568.98*	-568.98*	B
<b>SE</b>	<b>223.39</b>		<b>223.39</b>	

Heterotic group A= tester 1368 and heterotic group B = tester CML 444.

### Clustering of tropical white maize inbred lines into heterotic groups

Assigning inbred lines into heterotic groups is a vital step in a hybrid-breeding programme, which can provide maximum heterosis exploitation. The relative

performance of inbred lines in crosses with divergent testers of known origin has been commonly used to assign maize inbred lines into heterotic groups (Hallauer et al., 1988). Significant GCA and SCA effects for grain yield were detected among the tropical white maize inbred lines. The SCA effect for grain yield was

considered to be a major criterion for classifying the inbred lines. The testers were able to classify 16 of tested inbred lines into two heterotic groups based on SCA effects. Therefore, inbred lines under this study were assigned into two heterotic groups based on SCA effects of mean grain yield (Menkir et al., 2004; Vasal et al., 1992a,b). An inbred line, which had negative SCA effect with tester 1368, was assigned to heterotic group A (1368) whereas, an inbred line which had negative SCA effect with tester CML 444 was assigned to heterotic group B (CML 444). All the inbred lines under study were assigned to two heterotic groups. Among the sixteen tropical white inbred lines, six inbred lines L1, L3, L4, L8, L11 and L14 were grouped into heterotic group A (1368) while ten inbred lines L2, L5, L6, L7, L9, L10, L12, L13, L15 and L16 were grouped into heterotic group B (CML 444). Thus, in order to exploit genetic diversity and then heterosis during hybrid variety development when using these inbred lines, one parent should come from the six inbred lines belonging to tester heterotic group A while the other parent should come from the ten inbred lines belonging to tester heterotic group B. Therefore, inbred lines with same heterotic groups and positive GCA effects can be used in the development of synthetic varieties while those in different heterotic groups can be used in the development of hybrid varieties to maximize on heterosis.

## Conclusions

The two testers included in the study separated effectively inbred lines into two heterotic groups. Among sixteen inbred lines included in the study, six were assigned into tester heterotic group 1368, while ten were assigned to tester heterotic group CML 444. This will be useful for developing hybrids and synthetic varieties in future breeding. Breeding programmes can take advantage of this information on combining ability to find best breeding strategy for developing high yielding lines and hybrids.

## RECOMMENDATION

Inbred lines assigned into two opposite heterotic groups should be used as parental lines for researchers who want to develop hybrid varieties and inbred lines with same heterotic group with positive GCA should be used for synthetic variety development.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

The first author is thankful for the scholarship award from Crop Scientist for African Agriculture (SCAA) project for this work to be done. A special thanks to Mr. Amos Rutherford Azinu and the field workers at West African for Crop Improvement Centre for the support rendered during field evaluation.

## REFERENCES

- Singh RK, Chaudhary BD (1985). Biometrical methods in quantitative genetic analyses. Kalyani Ludhiana, New-Delhi.
- Dhliwayo T, Pixley K, Menkir A, Warburton M (2009). Combining Ability, Genetic Distances, and Heterosis among Elite CIMMYT and IITA Tropical Maize Inbred Lines. *Crop Sci.* 49:1201-1210.
- Hallauer AR, Marccelo J, Carena JB, Mirnda F (2010). Quantative genetics in maize breeding. (M JC. Prohens, Jamie, Fernando Nuez, Ed.) (6th ed.). Springer, New York Dordrecht Heidelberg London.
- Hallauer AR, Russell WA, Lamkey KD (1988). Corn breeding. In: Sprague GF, Dudley JW (eds) Corn and corn improvement. Agronomy Monograph no. 18, 3rd edn. ASA-CSSA-SSSA, Madison, Wis. pp. 463-565.
- Kemphrone O (1957). An introduction to genetic statistics, John Wiley and Sons, New York. John Wiley and Sons.
- Melchinger AE (1999). Genetic diversity and heterosis. In: Coors JG, Pandey S (eds) The genetics and exploitation of heterosis in crops. CSSA-SP, Madison, Wis. pp. 99-118.
- Menkir A, Melake-Berhan A, Ingelbecht CI, Adepoju A (2004). Grouping of tropical mid-altitude maize inbred lines on the basis of yield data and molecular markers. *Theor. Appl. Genet.* 108:1582-1590.
- Morris ML, Tripp R, Dankyi AA (1999). Adoption and Impacts A Case Study of the of Improved Ghana Grains Maize Production Development Project Technology: Adoption and Impacts of Improved Maize Production Technology: A Case Study of the Ghana Grains. Economics Program paper 99- 01. Mexico, D.F.: CIMMYT.
- SAS (2002). SAS Institute, Inc., SAS users' guide. Version 9.0<sup>th</sup> ed. Cary, NC. 27513, USA. Cary, NC.
- Vasal SK, Srinivasan G, Pandey S, Cordova HS, Han GC, Gonzalez FC (1992a). Heterotic patterns of ninety-two white tropical CIMMYT maize lines. *Maydica* pp. 259-270.
- Vasal SK, Srinivasan GS, Han GC, Gonzalez FC (1992b). Heterotic patterns of eighty-eight white subtropical CIMMYT maize lines. *Maydica* pp. 319-327.
- Xia XC, Reif JC, Hoisington DA, Melchinger AE, Frisch M, Warburton ML (2004). Genetic Diversity among CIMMYT Maize Inbred Lines Investigated with SSR Markers: I. Lowland tropical maize. *Crop Sci.* pp. 2230-2237.



# African Journal of Agricultural Research

## Related Journals Published by Academic Journals

- *African Journal of Environmental Science & Technology*
- *Biotechnology & Molecular Biology Reviews*
- *African Journal of Biochemistry Research*
- *African Journal of Microbiology Research*
- *African Journal of Pure & Applied Chemistry*
- *African Journal of Food Science*
- *African Journal of Biotechnology*
- *African Journal of Pharmacy & Pharmacology*
- *African Journal of Plant Science*
- *Journal of Medicinal Plant Research*
- *International Journal of Physical Sciences*
- *Scientific Research and Essays*

**academicJournals**