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**African Journal of Agricultural Research**

*Review*

# Crop abiotic stresses and nutrition of harvested food crops: A Review of impacts, interventions and their effectiveness

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**In tandem with the accelerating effects of climate change, efforts to increase agricultural productivity to feed the growing population are still being extensively rolled out in Africa. That notwithstanding, a large population in the continent remains food and nutrition insecure; rendering malnutrition the biggest public health challenge. Coupled with the increased incidences of abiotic stresses, developing countries are particularly in dire need to seek options that will sustain both yield and nutritional value of their food crops. Presently, nutritional quality deserves more attention than yield alone, hence factors perturbing it are of an immense importance. While the effects of abiotic stresses on agricultural productivity are unequivocal, their influence on nutritional quality of food crops is still hazy. In the simplest presentation of the synergy between humans, plants and the environment; man gets nutrients from plants, which source nutrients from the soil (environment). We hypothesized that abiotic factors are a double-edged sword with unclear plausible consequences on nutritional status of food crops and consequently humans. In a multifaceted approach, this review concisely presents an overview of malnutrition in Africa, intimate synergy between agriculture and nutrition, and unravels the effects of abiotic stresses on the nutrition status of harvested crops. While the effects are dynamic under many factors, the present work uncovers that abiotic stresses predominantly increase antioxidants, proteins and carbohydrates due to their contributory role in abiotic stress tolerance. It further acknowledges the promising interventions that have been implemented in this light, but in order to impact significantly on human nutrition, we call for a more collaborative approach cognizant of the complexity of this phenomenon**.

**Key words:** Abiotic stresses, nutrition, agriculture, antioxidants, climate change, breeding.

## INTRODUCTION

The fact that climate is changing needs no formal introduction. Agriculture is faced with a double role as far

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as climate change is concerned; as a major contributor and as a major victim. The associated effects include increased incidences of abiotic (drought, salinity, heat, cold) and biotic (pests and diseases, invasive species) stresses and are expected to become even more prevalent in the future decades (Wang and Frei, 2011). Whether acting individually, or synergistically, these stresses cause fundamental reductions on growth and quality of crop plants (Ashmore et al., 2006), consequently putting a wretch on global food supply systems and nutrition of human population. Contrarily, demand for food has grown tremendously in the past decades, and is expected to further escalate as population reaches 9.7 billion in 2050 (United Nations, 2015) from the present 7.6 billion. Therefore, one of the largest problems the current and future generations are confronted with is the need to meet the food demands quantitatively and qualitatively.

This challenge has resulted into a flurry of research efforts by scientists, governments, non-governmental agencies and developmental partners (Lobell et al., 2008) aimed at maintaining agricultural productivity without perturbing the food supply for the current demand. As obviously expected, release of crop cultivars tolerant to different forms of stresses has dominated the efforts. Moreover, advancements in molecular technologies have added a remarkable value to this pursuit, enabling production of tolerant cultivars, biofortified food crops and high yielding crop cultivars. Meanwhile, breeding projects are underway aimed at adapting novel crop cultivars of key cereals to heat, drought and salinity (Pinto et al., 2010; Araus et al., 2008; Fleury et al., 2010; Ren et al., 2005). While commendable progress has been made with regards to adapting key crops to the changing environmental conditions, limited focus has been diverted on the nutritional quality. Recently, a number of studies have investigated the effects of different forms of abiotic stresses on nutritional quality of food crops. Mixed results have been revealed among and within crop species and abiotic stresses; hence no affirmative conclusion can be made regarding these effects. Considering the growing awareness for a more nutrition oriented production, commonly referred to as nutrition sensitive agriculture, efforts to improve nutrient status of key crop plants, in light of prevailing environmental factors are underway.

While these efforts, advances and achievements are conspicuous and commendable, the question arises as to whether these have had a significant impact on nutrition. Moreover, what is agriculture for? Primarily, it is in our firm belief that agricultural interventions, must chiefly aim at improving nutrition status of crops, hence people. Cognizant that crop agriculture and human nutrition are intimate and inseparable, interventions to improve agriculture must be carefully regulated so as to balance with human nutritional needs. Presently, less effort has been made to study and elucidate the impact of abiotic stresses on quality of harvested crops. In a nutshell, environmental factors elicited by climate change have led to a myriad of abiotic stresses, such as changes in precipitation (high rainfall, low rainfall, truncated or prolonged rainy seasons), accumulation of salts in soils (salinity), temperature extremities (heat shocks and chilling), elevated carbon dioxide ($eCO_2$) and depletion of the ozone layer among others (Figure 1). These have varying effects on crop production, and their immediate effects *in situ* (on the field) include reductions in growth, activation, up and down-regulation of some stress responsive biochemical and physiological processes and how a crop responds to each stress varies. Initiated *on situ* but significantly impacting *ex situ* are the changes in yield and nutritional quality, which again, depend on the crop species, development stage of exposure to the stress, duration and severity of stress, etc.

This review, presents an outlook on different view points and perspectives, of how different abiotic stresses affect agricultural productivity, with particular focus on nutritional composition; briefly analyses key roles of the affected crop food nutrients in humans; and the interventions that have been made aimed at maintaining productivity and nutritional status of food crops and how effective they have been. It further highlights missed opportunities and gaps and proposes plausible interventions.

## Overview of malnutrition in the African region

In developing countries, the battle against malnutrition is far from won. Currently, under nutrition co-exists with over nutrition in Africa and even globally (FAO, 2017). Meanwhile, efforts in fighting hunger have seen an overall improvement in combating under nutrition, but increased urbanization, lifestyle changes, and other resource pressures have resulted in poor diversity in the food baskets available and consumed, thus an increment in over nutrition and micronutrient deficiencies. The focus of programmes and interventions have shifted from only tackling under nutrition to a more combined approach of fighting against what is known as ‘triple burden’ of malnutrition in Africa (FAO, 2017; De Valenca et al., 2017)

Malnutrition simply means ‘bad nutrition’ and it is used to describe a person in a state in which the physical function dwindles to an extent of inadequate capacity to maintain sufficient bodily performance processes such as growth, pregnancy, lactation, physical work and resisting and recovering from diseases and infection (Bain et al., 2013). The problem of micronutrient deficiency draws the need for action, just as under nutrition and over nutrition in Africa as it bears grave consequences to overall health. Food quality and nutrient density in foods is a result of a number of factors (both biotic and abiotic factors). Crops gain nutrients from the soil, and humans

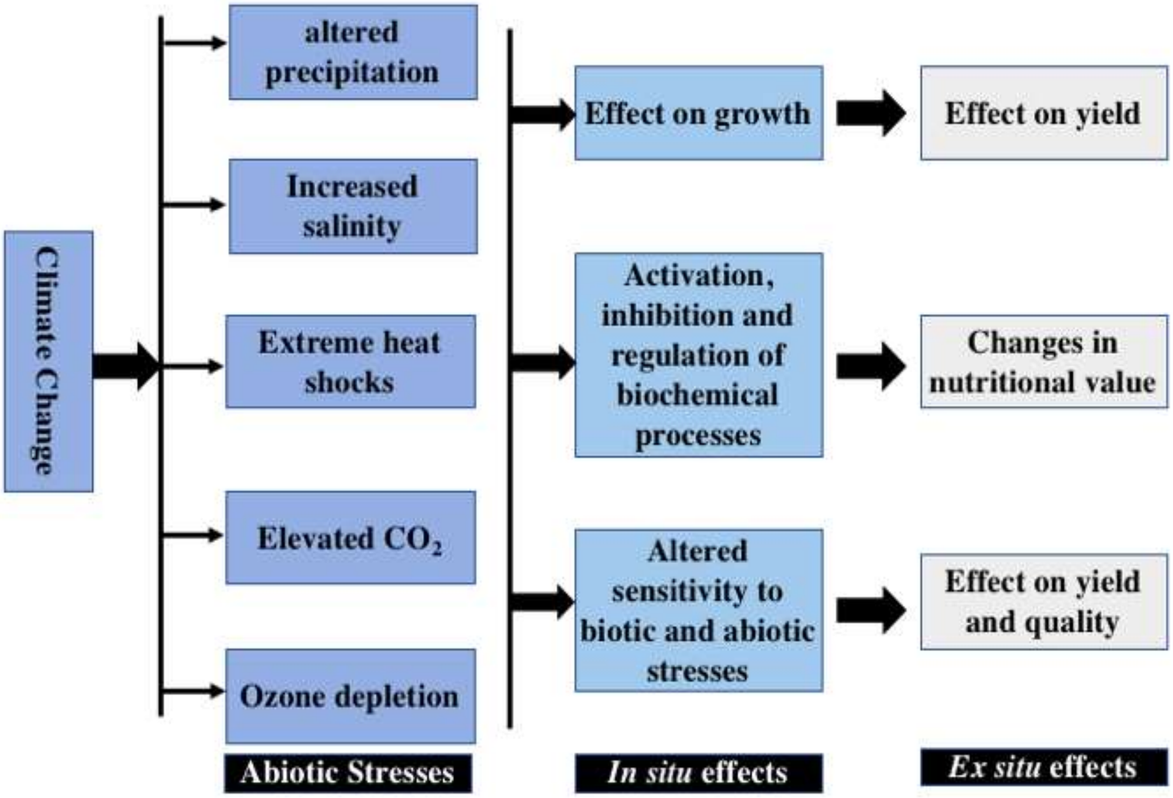

**Figure 1.** Schematic illustration of how environmental changes brought by changes in climate impacts on agricultural production and ultimately on nutritional quality and yield. Current observable consequences of climate change have altered rainfall patterns and quantities (droughts and flooding), induced accumulation of osmotically active salts in the soil, extremes in temperatures (heat shocks and chilling), high $CO_2$ levels etc. Depending on their severity, these may cause some forms of abiotic stress to crop production, whose direct effects on site may be changes in growth and ultimately yield. Physiologically, various biochemical processes will be induced, altered or inhibited, in response to these. Ultimately, these have a prominent bearing on the final nutrient content of harvestable food products.

and animals, in the simplest model, consume nutrients from such crops (Grusak and DellaPenna, 1999). Therefore, when considering the fight against both food and human nutrition issues (of public health concern), it is relevant to consider how integrated interventions can be put in place to tackle the factors that correspondingly affect the environmental resources for food availability and quality (nutrient density).

The relationship that exists between the three forms of malnutrition and their effects, gives a picture for the need of a more synergistic approach, and increased investment in malnutrition. Figure 2 shows the link that exists between under nutrition, over nutrition and micronutrient deficiencies. Considering that humans and animals get nutrients from plants, whose primary source is the soil, factors that constrain uptake, transportation, mobilization and utilization of these nutrients from the soil to the plant are of immense significance.

Under nutrition is a form of malnutrition expressed by wasting (having low weight for height), stunting (being too short for current age) and underweight. These are the anthropometric indices for the assessment of a child's nutritional status, considering that much emphasis skews towards children under the age of five in under nutrition related discussions. According to the United Nations Children Fund in 2017, nearly 50% of all deaths in children below the age of 5 were attributable to under nutrition, and this meant 'a loss of about 3 million lives of children'. The picture of stunting as a form of under nutrition is daunting in the African region, especially the sub-Saharan Africa (SSA) region, than any other region. About 38% of children in the African region were stunted and over 6% of children were wasted and there has been an increase in the absolute number of stunted children from 52 to 60 million in the period between 2000-2015 (WHO, Monitoring health for the SDGs: sustainable development goals. Geneva (2016). The Millennium Development Goals report (2015) reported that in sub-Saharan Africa, 39% of children under 5 years of age were stunted, 10% were wasted while 25% were underweight.

Children who are undernourished have an increased

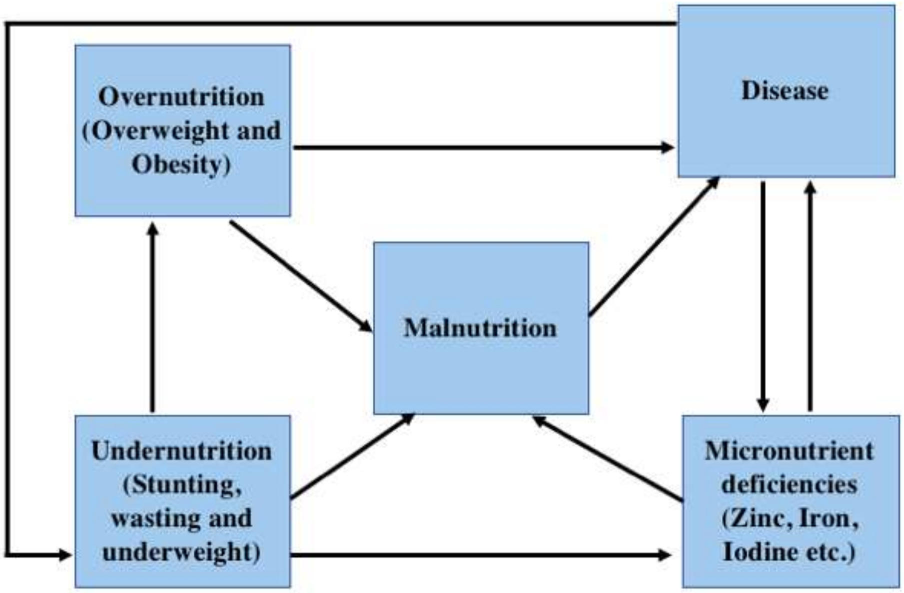

**Figure 2.** Link between the three forms of malnutrition: In the simplest presentation, undernutrition, overnutrition and micronutrient deficiencies are all forms of malnutrition, i.e. 'bad' nutrition. Undernutrition may result in micronutrient deficiencies as the body's nutrient metabolism and absorption is altered, and this can in turn result in different diseases such as Iron deficiency Anaemia (IDA) and other micronutrient deficiency disorders. Diseases such as diarrhoea can also result into micronutrient deficiencies since they alter integrity of the small intestines in nutrient absorption. Undernutrition in early childhood increases the risk of being overweight or obese later in life. Overnutrition is a risk factor for different chronic diseases such as diabetes mellitus and Cardiovascular Diseases (CVDs). Presence of disease as a result of one form of malnutrition can result into development of another form of malnutrition. For example, micronutrient deficiencies can result into disease which could in turn result into undernutrition.

risk of dying before reaching adulthood, and have poor physical and cognitive development (Jones et al., 2014). A recent meta-analysis of child malnutrition distribution in Africa using the Demographic and Health Survey (DHS) data from 32 countries found that stunting was highest in East Africa (57.7% in Burundi), and lowest in Central Africa (39.9% in Chad) (Akombi et al., 2017). Wasting was highest in Niger (18.0%) and underweight was highest in Burundi (28.8%) and Chad (28.8%) (Akombi et al., 2017). All these figures are unacceptably high, and give an overview on why under nutrition is of public health concern in SSA. Diverse factors predispose children to under nutrition. Some of the commonly reported risk factors include low mother's education, low socio-economic status, poor dietary diversity, sex of child (with male child at increased risk), low birth weight, underweight mothers, poor sanitation and hygiene, just to mention a few (Akombi et al., 2017; Akombi et al., 2017; Parkes et al., 2017). Understanding such risk factors is important in formulating interventions that target the modifiable risk factors, and thus help in combating under nutrition.

Another form of malnutrition is over nutrition. An individual is said to be overweight or obese when the Body Mass Index (BMI) is 25.0 to 29.9 kg/ $m^2$ and ≥30.0 $kg/m^2$,respectively (WHO, Waist circumference and waist-hip ratio: report of a WHO expert consultation, Geneva, 8-11 December 2008., 2011); BMI remains one of the recommended methods of assessing nutritional status of populations, especially in adults. Globally, the $21^{st}$ century has been met with an increase in overweight and obesity not only in vulnerable groups such as children and women of reproductive age, but also in men. The focus is on women in the pre-pregnant phase and the reproductive age, as they form the focal point of the continuous cycle of overweight and obesity in the children to be born. In the African region, the epidemiological transition and nutrition transition has resulted in triple burden of malnutrition; micronutrient deficiencies, over nutrition and under nutrition; hence overweight and obesity have equally become significant problems of public health concern (WHO, 2011).

A recent secondary analysis of Demographic and Health Survey data for the period from 1991-2014 in 24 African countries showed an increase in prevalence of overweight and obesity in all the 24 countries in urban women aged 15 to 49 years (Amugsi et al., 2017). The trend calls for greater attention to this issue. For example, within the period of 1991-2014, obesity in women of this age group doubled in countries like Kenya, Niger, Rwanda and Ivory coast, among others and tripled in others like Malawi, Zambia, Tanzania, Ethiopia, among other countries (Amugsi et al., 2017). A number of studies have also reported a high increase in the prevalence of overweight and obesity in young children and adolescents in different African nations (Negash et al., 2017; Ajayi et al., 2016). In South Africa, persistent low social economic status, physical inactivity, heavy alcohol use and tobacco consumption were some of the rampant risk factors (NCD Risk Factor Collaboration (NCD-RisC)–Africa Working Group, 2017). In addition, increased intake of poor diets and 'fast foods', which are characterised by more fats and less fibre, are also a significant cause of overweight and obesity and increased risk for non-communicable diseases in Africa (Welch and Graham, 2004).

As previously mentioned, malnutrition can also be presented as an imbalance or inadequate availability of micronutrients in the body; this is known as micronutrient deficiency or hidden hunger. Globally, over 2 billion people have 'hidden hunger' (Herrador et al., 2014). Particularly, African countries have exacerbated cases of micronutrient deficiencies. Micronutrient deficiencies are due to inadequate dietary intake, increased losses from the body, and/or increased requirement; and mostly affect children and pregnant women, and many other population age groups (Herrador et al., 2014). Micronutrients of known public health importance include the following: zinc, iodine, iron, selenium, copper, vitamins A, E, C, D, B2, B6, B12 and folate (Herrador et al., 2014). A study on global trends in dietary micronutrient supplies and dietary intake found that although most regions such as South East Asia have seen a decline in the prevalence of micronutrient deficiencies and increment in food micronutrient density, sub-Saharan African region has seen a decline in the micronutrient density in its food system (Beal et al., 2017). In sub-Saharan Africa, the problem is a particularly major issue, because 28 in 100 people in 2011 were consuming a diet inadequate in essential micronutrients (Beal et al., 2017). A systematic review of micronutrient status in women of reproductive age and pregnant women in African countries (that is Ethiopia, Kenya, Nigeria and South Africa) found that iron deficiency prevalence ranged from 9 to 16% in these countries, and Iron Deficiency anaemia was at 10% (Harika et al., 2017). Further, Vitamin A, zinc, and iodine deficiencies were equally more prevalent in the two age groups, indicating the magnitude of micronutrient deficiencies and inadequate intake (Harika et al., 2017).

## The intimate synergy between agriculture and nutrition

Hawkes and Ruel (Hawkes and Ruel, 2008) assert that there is a direct relationship between agriculture (food production) and food consumption, narrating that increases in food production translate into increased food availability. However, relationship between agriculture and human nutrition is multi-step and sensitive to nuisance factors, hence far more complex to establish. An argument that the primary goal of agriculture is to improve the nutritional status of the population has been put forth (Haddad, 2013). While about 5 decades ago, agriculture was predominantly considered an economic activity due tothe rising population that was consequently followed with food shortages; the focus has recently changed, aiming at maximizing agricultural nutritional potential (Hawkes and Ruel, 2006). In a gradual learning process, the attention has registered to a shift from staple food production to more micronutrient rich foods, addressing key issues of hidden hunger. Moreover, in developing countries, limited and subsistence investments in the agricultural sector imply that most agricultural produce are primarily used for food on a household, communal and national level. Thus, a number of pathways are currently known, describing the synergy between agriculture and nutrition (Yosef et al., 2015; Kadiyala et al., 2014). Yosef et al. (2015), in their study reviewing 60 articles summarized a total of 6 pathways, while Haddad et al. (2013) summarized only 5, yet more or less identical to Yosef et al. (2015) (Table 1).

The major pathway describes *Agriculture as a Source of food* in which farmers primarily produce agricultural produce to supply their households with daily food requirements. From the produce, farmers ought to obtain calories, macro and micronutrients vital for human development. It is logical to assume that households that cultivate and produce more highly-nutritious foods are likely to use a proportion of it for their own consumption (Haddad, 2013). Such households tend to be heathier. For example, production of fruits and vegetables and their consumption will invariably increase intake of essential micronutrients such as zinc, iron, Vitamin A, calcium, etc. (World Bank, 2007) under conditions that intra-household food distribution is favorable. However, increased production should be more qualitatively focused than quantitative. For an instance, during the green revolution, most governments in Africa placed emphasis on increased production of staples, such as maize, wheat, rice, etc.; consequently, reducing price of food considerably. Notwithstanding such increases in yield, production gains did not translate into nutritional gains, inasmuch as most staples lack essential micronutrients required for children, pregnant women and the sick (Hawkes and Ruel, 2006). Moreover, households could hardly access and afford the increased food supply, hence increased agricultural production for food must be geared at addressing both nutritional quality and accessibility to bear the much needed impact.

Another key pathway links agriculture with nutrition as *an Income-Oriented production,* which produces surplus for sale. The market-oriented agriculture becomes more

**Table 1.** Pathways through which agriculture relates with and contributes to nutrition.

| No | Pathway | Description | References |
|---|---|---|---|
| 1 | Agriculture as a source of Food | Production of nutritious food for household consumption | Haddad (2013); Kadiyala, et al. (2014); World bank (2007); Hawkes and Ruel (2006) |
| 2 | Agriculture as a source of Income for Food and NonFood expenditures | Proceeds derived from selling of surplus food harvests as funds for purchase of other food and non-food products | (Hawkes and Ruel (2006); Haddad (2013); Hawkes and Ruel, 2008) |
| 3 | Agriculture policy & Food prices affecting food production | Sufficient and surplus food production lowers food prices | Yosef et al. (2015); Haddad (2013); Hawkes and Ruel (2008); Torlesse et al. (2003) |
| 4 | Women in Agriculture & Intrahousehold Decision Making and Resource Alloccation | Women active in agricultural programs make sound household nutritional related decisions. | Hawkes and Ruel (2006) Yosef et al. (2015); Hawkes and Ruel (2008) |
| 5 | Female employment | | |
| 6 | Women in Agriculture & Maternal Nutrition and Health Status and agriculture Associated Health Hazards | | |

important than subsistence Agriculture, as it provides income beyond domestic food needs, such as education and health, which ultimately have a bearing on nutrition (Yosef et al., 2015). Marketing issues such as tradability, demand and supply, comparative advantage and prices inform households' decisions on crop choices (World Bank, 2007). However, it is also not straightforward, that incomes generated from agricultural livelihoods would be used to improve nutrition, whether implicitly or explicitly. Hence, intra-household factors such as education, knowledge, decision making power income control and access to use of health and sanitation services determine the subsequent translation of increased production into improved nutrition (World Bank, 2007; Berti et al., 2004).

### Women in Agriculture and Intra household decision making and resource allocation

This comes into play, cognizant of the special role women play on household nutrition, particularly among children. Studies have indicated that women who take part in horticultural programs have better nutritional outcomes. Consequently, deliberate efforts have been implemented to encourage women's participation in agricultural development strategies. For instance, households whose women participated in a gardening program produced and consumed 1.9 and 1.2 fold more fruits and vegetables, respectively, than their controls (Bushamuka et al., 2005). Furthermore, women subjected to nutritional education have better decision-making capacity with regards to distribution of agricultural produce for household consumption. As a consequence, such households increased target consumption tremendously, hence reducing Vitamin A deficiency (VAD) (Marsh, 1998). In a related scenario in Kenya, supporting women in production of orange fleshed sweet potatoes increased both consumption and nutritional outcomes when co-implemented with appropriate strategies that encourage correct child feeding and care practices (Hawkes and Ruel, 2006).

### Agricultural policy and food prices unequivocally affect food production

This pathway assumes that increasing food production adjusts prices downwards and vice versa. Reduced prices result into an affordable access to nutritious food. More nutritionally rich benefits will be derived if decreases in prices are in nutritionally rich food crops such as fruits and vegetables. It is hypothesized that macroeconomic food policies that keep food prices low are likely to impact positively on nutrition (Torlesse et al., 2003). Moreover, it is substantiated, that volatility in food prices affect calorie intake. However, self-sufficient households are more resilient to such effects (Verma and Hertel, 2009).

Other pathways described by various authors include *Female employment in Agriculture and Child Care and Feeding; Women in Agriculture and Maternal Nutrition and Health status and Agriculture-Associated Health hazards* (Yosef et al., 2015) both of which recognize the unique role women play in agriculture. Taken together, all these demonstrate an inseparable synergy between agriculture and nutrition, whether explicitly and implicitly. Therefore, in order to attain required nutritional goals in Africa, interventions made in the agricultural sector must carefully consider nutritional status of the final crop produced. It must remain considered, that if nutrition is the final impact to be achieved, then agriculture must be the starting point.

## Implications of abiotic stresses on agricultural productivity and nutritional value of food crops

As a result of climate change, there are various abiotic affects that are associated with it (Figure 1), with a considerable bearing on agriculture. When plants are exposed to an environmental stress, various physiological processes are altered, perturbed (Kamanga et al., 2018) and elicited, which may affect chemical composition; consequently, affecting the nutritional status of a harvested product (Wang and Frei, 2011). For example, drought stress reduces the photosynthetic capacity and cation uptake and translocation of tomato plants (Kamanga, Unpublished), which may limit carbohydrate synthesis. However, responses to environmental stresses are very complex, considering the multigenic nature of stress tolerance traits. In order to obtain a full picture, it is imperative to investigate interactions between plant structure, function and environment at the species, cellular and molecular levels (Barnabás et al., 2008). It is believed, that even slightest improvements in stress tolerance would improve yield and quality of crop plants in developing countries (Hoisington et al., 1999). Cognizant of the current prevailing malnutrition levels in developing countries, plant agriculture needs to be tightly monitored and regulated, considering that it is the most affordable and available nutrient source for a majority of households with limited access to animal food products and other forms of dietary supplements. Such communities are vulnerable in circumstances where abiotic stresses negatively affect agricultural productivity and nutrition. Hence, the need for a better understanding of such influences on both productivity and nutritional quality is worthwhile as it will ably inform relevant stakeholders in designing and delivering suitable interventions.

## Effects of abiotic stresses on protein content and amino acid accumulation

Both humans and animals rely on crop plants for a protein source. Despite the relatively smaller amounts of protein in crops (10 to 30% DW), plant protein foods contribute approximately 65% of per capita supply of protein worldwide (Young and Pellett, 1994), and many studies have confirmed their prime role in reducing cardiovascular diseases (Richter et al., 2015). Nutritional importance of some crops, particularly cereals, is primarily determined by their protein content.

How sensitive protein content is to abiotic stress in a crop plant, depends on its genotype, severity and duration of the stress. Generally, most crop species have responded to adverse environmental conditions with increases in protein content (Table 2). These responses are non-uniform, varying in crop species, cultivars and genotypes, some showing decreases and some registering no effect. Good et al. (1994) found a linear increase in amino acid content with advancement of drought stress in *Brassica napus* (Oil /rapeseed). However, overall reductions in protein synthesis were registered as drought progressed, with resumptions in synthesis after re-watering. Contrarily, in another study (Triboi and Triboi-Blondel, 2002), heat stress was found to increase protein concentration in the same species. Subjecting oilseed to doses of ozone significantly reduced per seed content of protein. However, at harvest, protein content increased, which was primarily ascribed to the compensation from increase in seed size as the crop progresses (Bosac et al., 1998). Yet, another study investigating the effect of ozone on protein of oilseed did not find any significant differences (Ollerenshaw et al., 1999). Such intraspecies and intra-stress differences may point to the importance of timing, duration of stress and crop development stage.

Amino acid, such as proline (Kamanga et al., 2018), content of plant products has also been widely studied under abiotic stresses. Many studies have obtained significant increases in proline content under water stress. Presently, researchers have correlated these increases with tolerance to abiotic stresses such as drought, heat and salinity. Despite the controversy brought forth by this assertion, proline accumulation in tolerant cultivars aids in osmotic adjustment that enables maintenance of turgidity. As a consequence, some transgenic plants having higher proline expression under abiotic stresses have been produced in non-food crops such as tobacco, and have exhibited higher tolerance to abiotic stress. In a study by Abid et al. (2018) in wheat, the concentration of soluble protein was severely reduced, while that of free amino acids and proline increased significantly, under drought conditions. Conversely, a greater increase in amino acids and proline was observed under drought, with the decreases being more pronounced underSevere stress than Moderate stress. Cultivar sensitivity also seemed to influence the response, with sensitive cultivars showing a lower magnitude of increase in amino acid and proline concentration and a higher reduction in soluble protein than the tolerant cultivar. Presently, proline is acknowledged as the main component of osmotic adjustment, in addition to its prime role in aiding ROS scavenging and stabilization of cell membranes (Matysik et al., 2002; Kamanga et al., 2018). In wheat grains and Soybean seeds, protein content increased as a result of induction of both drought and heat. In a study by Ozturk et al. (2004), continuous water stress in wheat increased grain protein content by nearly one fifth (Ozturk and Aydin, 2004). Water stress has been found to increase protein content in barley

**Table 2.** Effects of various crop abiotic stress factors on proteins/ Amino acids.Either all species with author name, or all without, these all not in italics. Remarkably all spp. are named by Linnaeus.

| **Stress** | **Crop species** | **Effect** | | | **Previous studies** |
|---|---|---|---|---|---|
| | | ↑ | ↓ | — | |
| Drought | *Brassica napus* L. (Oil seed) | √ | √ | | Good and Zaplachinski (1994) |
| | *Hordeumvulgare* L. (Barley) | √ | | | Savin and Nicolas (1996) |
| | *Zea mays* L. (Corn) | √ | | | Oktem (2008) |
| | *Arachis hypogeal* L. (Peanut) | √ | | √ | Dwivedi et al. (1996) |
| | *Solanum tuberosum* L. (Potato) | √ | | | Teixeira and Pereira (2007) |
| | *Triticumaestivum* L. (Wheat) | √ | | | Ozturk and Aydin (2004) |
| Heat | *Brassica napus L*. (Oil seed) | √ | | | Triboi and Triboi-Blondel (2002) |
| | | √ | √ | | Abid et al. (2018) |
| | *Helianthus annuus L*. (Sunflower) | √ | | | Triboi and Triboi-Blondel (2002) |
| | *Triticum aestivum* L. (Wheat) | √ | | | DuPont and Altenbach (2003) |
| | *Oryza sativa* L. (Rice) | √ | √ | | Lin et al. (2010) |
| Salinity | *Solanum tuberosum* L. (Potato) | √ | | | Teixeira and Pereira (2007) |
| Ozone | *Brassica napus* (Oil seed) | | √ | √ | Bosac et al. (1999) |

(↑) abiotic stress increased protein / amino acid concentration: (↓) abiotic stress decreased protein / amino acid concentration; (—) abiotic stress did not cause any significant effect on protein / amino acid concentration.

(Savin and Nicolas, 1996), corn (Oktem, 2008), Peanut (Dwivedi, et al., 1996), Potato (Teixeira and Pereira, 2007) and Soybean. Wheat is by far the most extensively studied crop species in this regard. For a more comprehensive review (Wang and Frei, 2011).

## Effect of abiotic stress on mineral content of food crops

Mineral nutrition remains among the crucial determinants of growth both in humans and plants. In plants, it is generally accepted that increased supply of crops with mineral nutrients results into increased yield and quality. As a consequence, various studies have been conducted to assess the net effect of reduced nutrient supply, through reduced fertilizer application or planting in growth medium devoid of the mineral nutrients of interest. Overall, the results have corroborated their prime role on growth, yield and quality (Taiz and Zeiger, 2010). However, a few studies have attempted to investigate the interactions between non-mineral abiotic stress such as drought, salinity, elevated carbon dioxide concentration etc, on the mineral nutrient status in edible plant organs. Presently, it is known, that water stress reduces bioavailability of nutrients in the soil and their transport to the plant organs (Oktem, 2008). However, notwithstanding the reduced uptake and bioavailability, some studies have established that severe drought stress increases concentrations of some macronutrients such as calcium and magnesium, and some micronutrients such as copper and zinc in grains of corn (Da Ge et al., 2010). The justification for the increase was related to the improved routes and transport mechanisms for the cations. In our study, investigating the physiological responses of two tomato cultivars with contrasting tolerance, to water deficit stress (Kamanga, Unpublished) however, found decreases in calcium and magnesium in non-food organs of tomato (stems and leaves), with increases in roots.

However, tomato fruits, while not assessed for their macronutrient levels, showed severe calcium deficiencies, with water soaked tissues involving cell breakdown followed by loss of turgor as described by Simon (1978). However, in another study by our group (Kamanga et al. 2018) we found increases in both calcium and magnesium in leaf tissues of tomatoes. In grains, decreases in P and K were found in corn subjected to lower moisture content (Da Ge et al., 2010), which was ascribed to their reduced bioavailability.

In addition to soil water stress, soil salinity has also been extensively studied and its relations with mineral nutrition have been elaborated (Grattan and Grieve, 1998). Overall, salinity affects crop performance and food nutritional quality, via altered nutrient availability, competitive uptake, transport and organellar partitioning of mineral nutrients within the plants. Grattan (1998) asserts that high concentrations of Na and Cl in the soil solution depresses nutrient-ion activities, leading to extreme Na/Ca, Na/K, Ca/Mg, and Cl/NO$_3^-$ratios. This increases plant’s susceptibility to osmotic and specific-ion injury as well as to nutritional disorders that may result in reduced yield or quality.Potassium is amongst key minerals affected by salinity, inasmuch as high levels of external Na interferes with K acquisition by roots, disrupts root membrane integrity and alters their selectivity (Grattan and Grieve, 1998). Studies by various researchers (Kamanga, Unpublished; Izzo et al., 1993), have shown K decreases when Na is increased, which has been implicated in growth and yield reductions in tomatoes, spinach and maize (Song and Fujiyama, 1996; Chow et al., 1990; Botella et al., 1997).The decreases in K concentration in plants subjected to higher salinity are as a result of the competition between K and Na ions, which results into lower K:Na ratios (Kamanga, Unpublished) due to excessive uptake of Na and reduced K absorption. It is reasonable, therefore, to suggest that plants that maintain a higher shoot K:Na ratio under high salinity exhibit a key tolerance mechanism, principally relying on exclusion of sodium ions from the shoots by accumulating them in the roots, and in some cases compartmentalizing them in the vacuole separate from the cytosol (Greenway and Munns, 1980).

Salinity affects ion balance of various other mineral elements in the soil, consequently their concentration in plant tissues and organs. Calcium is amongst those that have been recorded. Presently, it is known that Ca availability is sensitive to Ca supply in the soil, nature of counter ions, pH and ratio of Ca to other cations (Grattan and Grieve, 1998). Fruits are particularly sensitive to Ca deficiencies owing to the differences in transport mechanism in various plant organs (Simon, 1978). Moreover, leaves, fruits and meristematic regions act as competitive sinks for Ca, exerting an influence on its preferential distribution (Clarkson, 1984). In plants whose marketable produce is primarily a leaf enveloped head, such as cabbage and lettuce, calcium is diverted from meristematic tissues due to excessive transpiration by outer leaves (Bangerth, 1979). Increased salinity elevated the incidence and severity calcium deficiency of artichoke buds, resulting into necrosis and one fifth reduction in marketable yield (Francois et al., 1991; Francois, Salinity effects on bud yield and vegetative growth of artichoke (*Cynara scolymus* L.), 1995). In cabbages, calcium deficiencies have also been observed in salt-stressed Chinese cabbage (Osawa, 1962). In general, salinity reduces Ca availability, transport and mobility to growing plant regions resulting into reduced quality of both vegetative and reproductive organs (Grattan and Grieve, 1998). Other forms of abiotic stress, such as ozone, narrowly affected macronutrient concentration (K, P and Mg) in corn; however, it increased Zn, iron and copper (Garcia, et al., 1983). A similar study conducted with potatoes produced contrasting results, showing increases in K and Mg, while Ca remained unaffected (Piikki et al., 2007). This was ascribed to a reduction in biomass accumulation relative to macronutrient intake. In carrots, subjecting plants to drought stress at the 4-6 leaf stage reduced Mg concentration, when grown on a course sandy soil. In the same study, when drought was imposed prior to harvest, an increase in dry matter was associated with a decrease in potassium and nitrate (Sørensen et al., 1997).

## Effects of abiotic stresses on antioxidants

The current flurry of research relating to abiotic stresses has resulted into an elucidation of a myriad of physiological responses elicited by abiotic stresses. Key to the fate of these stresses, is the accumulation of antioxidants. A large amount of evidence reveals that under abiotic stresses, reactive oxygen species (ROS) production increases, which consequently results in cell death, lipid peroxidation and damage of the photosynthetic machinery (Kamanga et al., 2018). In order to scavenge such ROS, plants have evolved multiple mechanisms, including production of antioxidants; both enzymatic and non-enzymatic. While a significant amount of data is available on antioxidants accumulation under abiotic stresses, limited studies have made an effort to study the effect of such abiotic stresses on antioxidant levels in edible crop parts. In human diets, antioxidants are also a major determinant of nutritional quality of food. Fruits and vegetables are by far among key suppliers of antioxidants in human diets. In general, subjecting plants to abiotic stress invariably increases antioxidant concentrations. In a comprehensive review by Wang et al. (Wang and Frei, 2011), about two thirds of studies reviewed reported increases in concentration of phenolic compounds, one-tenth showed decreases while the remainder did not indicate any clear differences. Studies have established, that phenylpropanoid, a key enzyme in the biosynthesis of phenolics is stimulated by exposure to abiotic stresses (Oh et al., 2009; Kangasjarvi et al., 1994; Guo et al., 2008). As such, significant increases in phenolic compounds have been found in potatoes (Andre et al., 2008), grapes (Deluc et al., 2009), and rapeseed (Bouchereau et al., 1996) under drought stress; likewise, broccoli (Lopez-Berenguer, et al., 2009), raspberry (Neocleous and Vasilakakis, 2008) and strawberry (Keutgen and Pawelzik, 2007) under salinity stress, and in other crop species such as apples, grapes, lettuce, spinach and tomato (Wang and Frei, 2011).

Apart from phenolics, ascorbate (AsA), also known as Vitamin C, is also among the key antioxidants produced by plants under abiotic stress (Sharma et al., 2012). Ascorbate is a considerably abundant, yet less studied low molecular weight antioxidant and has demonstrated a key role in defense against oxidative stress caused by enhanced levels of ROS. Under abiotic stress, ascorbate is particularly useful, enabling scavenging of ROS, by reacting with superoxide radicals and hydrogen peroxide (Noctor and Foyer, 1998). Tomatoes, one of the notable suppliers of Vitamin C (ascorbate), have invariably shown increases in ascorbate content when subjected to drought stress (Zushi and Matsuzoe, 1998; Veit-Köhler et al., 1999; Favati et al., 2009). In plants, a majority of the

AsA pool results from a precursor (D-mannoseandL-galactose), dubbed the Smirnoff-Wheeler pathway, which proceeds via GDP-D-mannose, GDP-galactose, L-galactose, and L-galactono-1,4-lactone (Wheeler et al., 1998), a process not found in most animals and humans. Therefore, synthesis of these precursors de novo influences the ascorbic level in plants, required in human diets. As such, differential capacities to synthesize the necessary precursors result into differences in the plant's response under abiotic stress.

For example, in a study by Sorenson et al. (Sørensen et al., 1997), subjecting carrots to severe drought stress increased both Vitamin A (carotenes) and Vitamin C (AsA). When drought was imposed at specific growth stages, no significant changes were observed. Contrarily, reductions in carotenoid contents were observed in wheat subjected to severe and moderate water stress, with the reductions being more pronounced in sensitive cultivars (Abid, et al., 2018). Carotenoids are known to be involved in the dissipation of excess energy absorbed by photosynthetic pigments, which prevents formation of superoxide anions, an ROS, in plants receiving too much more energy than it can potentially utilize due to reduced photosynthesis (Reddy et al., 2004).Thus, maintenance of higher carotenoids in tolerant, relative to sensitive, cultivars may have enhanced photo-protection of the plant's photosynthetic apparatus. Reduced glutathione (GSH) is among the studied antioxidants produced in plants. In a study by Abid et al. (2018), both moderate and severe drought conditions increased the accumulation of GSH with the accumulation being higher in sensitive than tolerant cultivars. However, as drought period progressed, accumulation of GSH decreased. Similar results were also obtained in wheat by Herbinger et al. (2002). GSH plays an antioxidant role by directly scavenging ROS and by reducing ascorbate (Helena and Carvalho, 2008). It is therefore expected that tolerant plants may have higher scavenging ability relative to sensitive plants; hence more GSH, which contrasts with results produced by Abid et al. (2018). However, it was postulated, that tolerant cultivars, chiefly rely on upregulation of enzymatic antioxidation systems for ROS detoxification whereas the increase in GSH in sensitive cultivars might have been an attempt for the sensitive cultivars to exploit GSH (non-enzymatic) to mitigate oxidative stress.

## Effect of abiotic stresses on carbohydrate concentration and soluble sugars

Carbohydrates are a major composition of food crops, key in the supply of energy for both humans and animals. Crop plants, particularly cereals, remain the major suppliers of carbohydrates, coming in various forms such as sugars, starches and fiber. For human nutrition, the type, rather than the amount of carbohydrates, is critical for health. In plants, several studies have indicated an increase in total soluble sugars concentration when subjected to stressful conditions. It is thought, that sugar transporters ferry sugars through plasma membranes and the tonoplast to adjust the osmotic pressure under stress conditions (Barnabás et al., 2008). In grains, it is postulated that abiotic stresses that perturb plant water status and carbon assimilation, such as a case of drought and salinity stress, elicit the conversion of stem reserves into soluble sugars and the mobilization of sugars into the grains during grain filling (Blum, 1998; Blum, 2005). Moreover, recent evidence suggests that xylem-borne abscisic acid (ABA) can be transported to plant reproductive structures and influence their development, presumably by regulating the gene expression that controls cell division and carbohydrate metabolic enzyme activity under drought conditions (Barnabás et al., 2008).

In a study by Abid et al. (Abid et al., 2018) severe water stress increased total soluble sugars (TSS) and fructose concentration. The trend of increase was more in tolerant plants relative to sensitive plants, suggesting a potential role of these sugars in alleviating water stress.

Moreover, after re-watering, both TSS and fructose concentration decreased, corroborating the suggestion. Recently, another study (Kim et al., 2017) has reported diurnal changes in starch and soluble sugars including sucrose, with soluble sugar contents tending to increase while starch decreased in response to drought stress, peaking during daytime. Similar results have also been obtained (Mostajeran and Rahimi-Eichi, 2009). To the contrary, starch levels remained comparatively low at the end of the day, hinting at a possibility that changes in sugar and starch levels may playa roleas important indicators for drought response associated with diurnal rhythms in rice (Kim et al., 2017). The decrease in starch might have resulted from the starch degradation pathway that was elicited by water stress as also observed from transcriptomic analysis in tomatoes by Egea et al. (Egea et al., 2018). In a wild relative of tomato, *Lycopersicum pennellii* upregulation of *Fructose Insensitive 1 (FINS1)* gene, which codes for a cytosolic fructose 1-6 bisphosphatase and down regulation of genes related to starch biosynthesis (ADG1) were also established under drought stress (Egea et al., 2018). This suggested that the tolerant tomato species prevents allocation of carbon towards starch synthesis and utilizes it for production of sugars.

Elevated carbon dioxide concentration ($eCO_2$), is one of the major consequences of climate change. Currently, plant survival, growth and productivity will be confronted with these increases, and hence it has emerged to be a key abiotic factor of interest in agriculture. Combined with other abiotic stresses, particularly drought, they may be particularly adverse on some crop plants. However, recent studies have unravelled that $eCO_2$ enhances drought tolerance in field pea plants through stimulated increases in total soluble sugars (Jin et al., 2014). This effect was particularly enhanced under increased phosphorus application, showing a significant linear relationship between leaf inorganic P (Pi) and TSS accumulation. It is hypothesized, that high Pi facilitates translocation of triose sugars from chloroplasts thereby enhancing sugar status of plant tissues (Abel et al., 2002; Rychter and Rao, 2005).

Presently, it is known that soluble sugars are an integral component of osmotic adjustment. Additionally, reports indicate that sugars play a role in enhancing the cellular antioxidation system (Bolouri-Moghaddam et al., 2010). This plausible relationship has been investigated and confirmed by Nishikawa et al. (2005), who reported that high soluble carbohydrate production in the florets of broccoli enhanced ascorbate synthesis, which facilitated partitioning of ROS in chloroplasts (Nishikawa et al., 2004). Also, upregulation of trehaloselevels, a carbohydrate storage molecule, by manipulating the intermediate trehalose-6-phosphate, conferred drought tolerance in transgenic rice plants, achieved through sugar-signalling and carbohydrate metabolism (Redillas et al., 2012).

## The response: interventions and their effectiveness

At present time, billions of people are estimated to be micronutrient malnourished globally (Mason and Garcia, 1993). In order to sustain a healthy life, it is recommended that humans should consume 49 nutrients, failure of which results in chronic health challenges characterized by frequent and prolonged sickness, poor health, impaired development in children and consequently constraining both personal and national development (Branca and Ferrari, 2002; Grantham-McGregor and Ani, 1999). As such, efforts to increase nutritional status of food crops must be among key priorities, requiring a collaborative approach by various stakeholders. Coupled with the increased incidences of abiotic stresses associated with climate change, the need is even more exigent. While most abiotic stresses have been reviewed to increase micronutrients status in many food crops, they do adversely affect growth and yield, consequently reducing the amount of harvested food products available for consumption. This has led to a series of endless cycles of hunger and poverty, particularly in developing countries; for example, a case in Southern African countries such as Malawi following El Niño related drought effects. It is imperative, therefore, to consider elevating nutritional quality of food crops per unit of harvest, than to increase unit of harvest with limited nutritional quality.

Among the approaches to increase nutrient availability in an era of abiotic stresses may include increasing the resistance of key crop plants to maintain both nutritional quality and yield under abiotic stresses, or to simply increase their tolerance in order to maintain yield, though this may supply nutrients only minimally. Breeding, through both traditional techniques and genetic modification, is among promising tools for this aim. Various breeding criteria have been proposed for micronutrient rich staples (Welch and Graham, 2004), which include attainment of crop productivity or yield, significance of attained nutrient levels on human health, stability of the attained nutrient levels across a range of biotic and abiotic environments, proven bioavailability of the nutrients in humans and consumer acceptance. To our knowledge, numerous cases are reported on nutritionally enhanced food crops. Notable on the list include orange-fleshed sweet potato lines with high levels of β-carotene (over 200 µg/g). Also, beans with improved agronomic traits and grain type and 50 to 70% more iron have been bred through conventional means have been reported (Nestel et al., 2006). Prominently, release of golden rice (Beyer et al., 2002), a rice variety engineered to synthesize β-carotene in a way to address VAD remains amongst the largest of achievements and the noblest of pursuits in this regard. Achieved through insertion of a biosynthetic pathway de novo for synthesis of β-carotene, golden rice accumulate tremendously higher levels of carotene as a provitamin A (Beyer et al., 2002); a promising intervention particularly for developing countries. Recently, a yet new variant, golden rice 2 has been produced (Paine et al., 2005), producing 23 folds higher levels of β-carotene relative to the original Golden rice.

Moreover, nutritional enhancement is a potentially win-win approach, both for agriculture and human nutrition. Recently, improving micronutrient status in seeds of cereals has been investigated and found to enhance seed viability and seedling vigour through a more extensive and deep-rooting capacity, thus enhancing its ability to scavenge more effectively for needed nutrients, during micronutrient deficient edaphic conditions. In a trial in Bangladesh, biofortifying wheat grains for micronutrients increased wheat yield in nearly 80% of farmers' fields. Another study has also revealed that micronutrient dense seeds improve tolerance to both biotic and abiotic stress (Welch, 1986). In developed countries various success stories have been registered. Wheat varieties dense in zinc have been produced in Australia (Rengel and Graham, 1995), and have attained commercial success; In the US, iron deficiency in soils led to the development of a soybean cultivar with ability to grow and maintain high iron contents. Plants and humans have related sensitivities to micronutrient deficiencies. As such, plants low in micronutrients is usually susceptible to root diseases as previously confirmed (Graham and Rovira, 1984).

The question that arises is whether these interventions brought forth, have practical implications on health and their affordability coupled with acceptability. Indeed, various studies have been made to investigate the effects of biofortified foods on health of humans. For example, orange fleshed sweet potatoes, which have been bred for increased Vitamin A, significantly improved Vitamin A liver stores in primary school children (Van Jaarsveld et al., 2005). In another study, rice bred for high iron was found to improve serum ferritin concentrations and body iron levels in nonanemic women of reproductive age relative to control rice used locally (Haas et al., 2005). In a case of golden rice, it has demonstrated benefits beyond anticipated Vitamin A supplementation. Studies have revealed that Golden rice also supplies iron, derived from a gene from French beans which boosts iron content (Thomson, 2002). Moreover, it also contains a gene that inhibits the action of phytic acid on preventing iron absorption by the body (Gura, 1999). A prime concern that ought to be properly considered and addressed in many interventions relating to nutrient deficiency is cost effectiveness. An intervention must be duly acceptable by the target population and must be cost effective and affordable, devoid of which may render interventions unadoptable. In a study by Stein et al. (2006), it was established that introduction of Golden rice 2 as a way to combat VAD disease burden was considerably more cost effective relative to the traditional approach of using Vitamin A supplements.

## Way forward: A summary of gaps and recommendations

It is established that abiotic stresses have an immense bearing on agricultural productivity and nutritional quality of food crops (Figure 3). The synergies between agriculture and nutrition have been well demonstrated and clearly revealed. As such, stakeholders must be willing to collaborate, and invest hugely in agriculture in order to achieve better nutrition for the growing population, particularly in developing countries. Africa, in particular, is a home for a myriad of unfortunate abiotic stresses; moreover, its inadequacy in resources and knowledge constrains its capacity to cope and adapt to these. However, notwithstanding these, Africa also remains a home of huge genetic diversity due to its subtropical – tropical climate, which can be harnessed for genetic improvement in key crops for food and nutritional security. At present, the advancements and interventions brought forth are promising. Plant breeding has been duly adopted as a reliable and effective tool for achieving nutritional quality. Besides, it has also proven effective in conferring abiotic stress tolerance in many crop species. Meanwhile, the breeding goal has been to either (1) improve tolerance to abiotic stress; or (2) to improve nutritional quality. But, do the breeding for these in isolation achieve both tolerance to abiotic stress and nutritional quality? For example, can producing a genetically engineered crop for drought tolerance produce a highly nutritious crop? Similarly, will a biofortifiedcereal crop be able to survive under abiotic stresses? Presently, most interventions have hardly addressed this phenomenon. Future efforts must thus seek to maintain higher nutritional quality in food products even in situations of environmental stresses. Moreover, this may not be a hard goal to achieve considering that most abiotic stresses increase synthesis of micronutrients (Tables 2 to 4). Some key nutrients for humans such as carotenes, amino acids, sugars and some micronutrients are part of an inherent defence system in plants' response to abiotic stress. This is among the reasons for increased nutrient content under abiotic stresses. Hence, it is highly likely that crops bred for tolerance to abiotic stresses, by enhancing key pathways for biochemical processes such as antioxidants, sugars and transporters for mineral elements, may achieve both tolerance and nutritional enhancement. A remaining concern, would be to optimize and achieve stability, yield, affordability and consumer acceptance. In such instances, an optimal intervention may require yield maintenance, palatability and marketability of the edible parts. In another twist, not all abiotic stresses have had a negative bearing on agriculture. Moreover, interactive effects of some abiotic stresses have ameliorated individual effects of others. For example, elevated carbon dioxide levels enhance drought tolerance; and this has been duly investigated in field grown peas. This was also coupled with increases in sugar levels, hence achieving both nutritional quality and drought tolerance. Presently, future efforts must consider assessing relative cost effectiveness, palatability, consumer acceptance and abiotic stress tolerance in nutritionally enhanced foods. Golden rice is an optimal example of an intervention that has been duly tested for its cost effectiveness, health impact and consumer acceptability, yet its abiotic tolerance has not been clearly investigated.

## Conclusion

In total, our critical review demonstrates that agriculture and nutrition are inseparable. To our present knowledge, it is among the fewest studies, to comprehensively address issues and synergies of crop growth environmental factors and human nutrition. It has accentuated the intimacy between crop abiotic stresses and nutritional quality. Primarily, it reveals that abiotic stresses are a double-edged sword in agriculture, leaning more positively in nutritional quality and negatively on agricultural productivity (yield). Cognizant that the prime justification for agriculture is improvement of human wellbeing, with a particular focus on nutrition, factors that affect this pursuit require committed and solemn action. Notwithstanding, the auspicious interventions so far made using modern and traditional plant breeding tools, continued efforts are worthwhile, aimed at attaining both

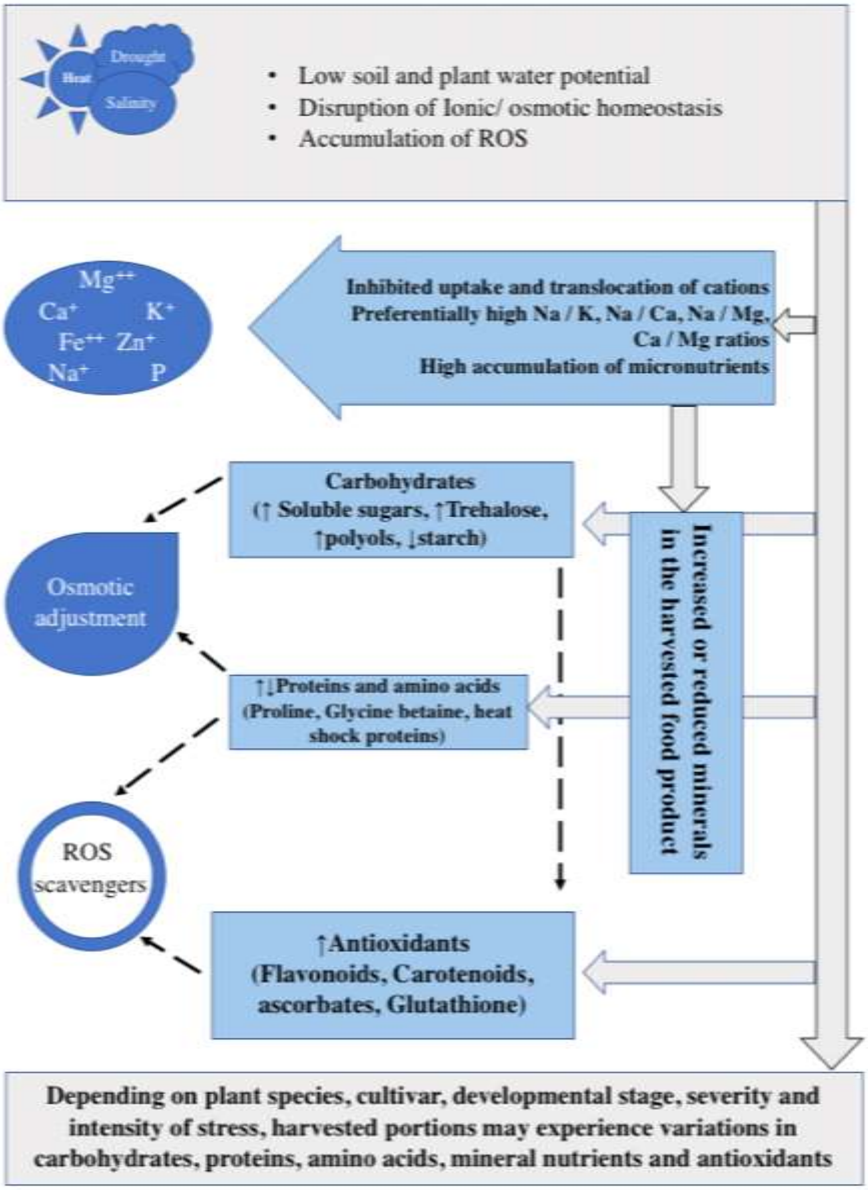

**Figure 3.** Effects of various forms of abiotic stresses on mineral nutrients, carbohydrates, proteins and antioxidants. Abiotic stress factors such as drought, salinity and heat, whether acting individually or synergistically, result into alterations in soil, and consequently plant water status. Salinity may disrupt ionic and osmotic homeostasis. Altogether, these stresses induce ROS production due to reduced photosynthetic capacity. Cumulative consequences of all these occurrences include changes in nutrient uptake, transport and distribution which affects final mineral status in harvested product, release of some soluble sugars, proteins and amino acids and antioxidants which aid in scavenging build-up of ROS and osmotic adjustments. The net effect on the final food product varies, depending on the sensitivity of the plant species, stage of development and severity of the stress.

abiotic stress tolerance and nutritional enhancement in one goal. It is imperative to explore more options and approaches towards addressing this complexity, as no single solution is apanacea in elevating crop nutritional status and yield in light of constraining environmental conditions. As scientists, researchers, farmers and development partners, we are therefore confronted with a hitch of an immense complexity and huge magnitude, calling for a multiplicity of approaches, multidisciplinary of teams and a convergence of knowledge and resources.

**Table 3 .**Effects of various crop abiotic stresses on mineral nutrient in harvested food crops.

| Nutrient | Stress | Crop species | Effect | | | Previous studies |
|---|---|---|---|---|---|---|
| | | | ↑ | ↓ | — | |
| | Drought | *Zea mays L. (Corn),* | √ | | | Da Ge et al. (2010) |
| | | *Solanumlycopersicum L (*Tomato) | | √ | | Kamanga (Unpublished) |
| Calcium | | *Solanumlycopersicum L* (Tomato) | √ | | | Kamanga (2018) |
| | Salinity | *Cynaracarduculus* (Artichoke) | | √ | | Francois et al. (1991); Francois (1995) |
| | | *Brassica rapa L* (Chinese cabbage) | | √ | | Osawa (1962) |
| | Drought | *Zea mays L. (*Corn) | √ | | | Da Ge et al. (2010) |
| Magnesium | | *SolanumlycopersicumL* (Tomato) | | √ | | Kamanga (Unpublished) |
| | | *SolanumlycopersicumL* (Tomato) | √ | | | Kamanga et al. (2018) |
| | | *Daucuscarota* (Carrot) | | √ | | Sørensen et al. (1997) |
| Potassium | Drought | *Zea mays L.* (Corn) | | √ | | Da Ge et al. (2010) |
| Phosphorus | Drought | *Zea mays L.* (Corn) | √ | | | Da Ge et al. (2010) |
| Sodium | Drought | *Solanumlycopersicum* (Tomato) | √ | | | Kamanga (Unpublished); Kamanga et al. (2018) |
| Zinc, Copper | Drought | *Zea mays L.* (Corn) | √ | | | Da Ge et al. (2010) |
| Zinc, Iron, Copper | Ozone | *Zea mays L.* (Corn) | √ | | | Garcia et al. (1983) |
| Potassium | Ozone | *Zea mays L.* (Corn) | | | √ | Garcia et al. (1983) |
| | Ozone | *Solanum tuberosum L.* (Potato) | √ | | | Piikki et al. (2007) |
| Magnesium | Ozone | *Zea mays L.* (Corn) | | | √ | Garcia et al. (1983) |
| | Ozone | *Solanum tuberosum L.* (Potato) | √ | | | Piikki et al. (2007) |
| Calcium | Ozone | *Solanum tuberosum L.* (Potato) | | | √ | Piikki et al. (2007) |
| Phosphorus | Ozone | *Zea mays L.* (Corn) | | | √ | Garcia et al. (1983) |

(↑) abiotic stress increased mineral nutrient concentration: (↓) abiotic stress decreased mineral nutrient concentration; (—) abiotic stress did not cause any significant effect on mineral nutrient concentration.

**Table 4.** Effects of various crop abiotic stress factors on Antioxidants (Phenolics, Carotenoids, Ascorbates and Glutathione) and Carbohydrates.

| Nutrient | Stress | Crop species | Effect | | | Studies |
|---|---|---|---|---|---|---|
| | | | ↑ | ↓ | — | |
| | HS, HL, CH | *Lactucasativa* (Lettuce) | √ | | | Oh, Carey and Rajashekar (2009) |
| | Drought | *Solanum tuberosum L.* (Potato) | √ | | | Andre et al. (2008) |
| | | *Vitisvinifera (*Grape) | √ | | | Deluc et al. (2009) |
| Phenolics | | *Brassica napus* (Oil seed) | √ | | | Bouchereau et al. (1996) |
| | Salinity | *Brassica oleracea* (Broccoli) | √ | | | Lopez-Berenguer et al., (2009) |
| | | *Rubusidaeus L.* (Raspberry) | √ | | | Neocleous and Vasilakakis (2008) |
| | | *Fragaria x ananassa* (Strawberry) | √ | | | Keutgen and Pawelzik (2007) |
| | Drought | *Solanum tuberosum L.* (Potato) | √ | | | Noctor and Foyer (1998); Zushi and Matsuzoe (1998); Veit-Köhler et al. (1999) |
| | | Daucuscarota (Carrot) | √ | √ | √ | Sørensen et al. (1997) |
| | Salinity | *Brassica oleracea* (Broccoli) | | | √ | Lopez-Berenguer et al. (2009) |
| Ascorbate | Salinity | *Rubusidaeus L.* (Raspberry) | √ | | | Neocleous and Vasilakakis (2008) |
| | Salinity | *Fragaria x ananassa* (Strawberry) | | √ | | Keutgen and Pawelzik (2007) |
| | Salinity | *Solanumlycopersicum* (Tomato) | √ | √ | | Kim et al. (2008a) |
| | Heat | *Lactucasativa (*Lettuce) | √ | | | Oh et al. (2009) |
| | Drought | *Daucuscarota* (Carrot) | √ | √ | √ | Sørensen, et al. (1997) |
| Carotenoids | | *Triticumaestivum L.* (Wheat) | | √ | | Abid et al. (2018) |
| | | *Solanum tuberosum L.* (Potato) | √ | √ | √ | (Andre et al., 2008) |

**Table 4.** Contd.

| | | | | | | |
|---|---|---|---|---|---|---|
| | | *Solanum tuberosum L.* (Potato) | √ | | √ | Zushi and Matsuzoe, (1998); Favati et al., (2009) |
| | Salinity | *Lactucasativa* (Lettuce) | √ | | | Kim et al. (2008a) |
| Glutathione | Drought | *Triticumaestivum L.* (Wheat) | √ | | | Abid et al. (2018) |
| Carbohydrates | | | | | | |
| TSS | Drought | *Triticumaestivum L.* (Wheat) | √ | | | Abid et al. (2018) |
| | | *Oryza sativa L.* (Rice) | √ | | | Kim et al. (2017); Mostajeran and Rahimi-Eichi (2009) |
| | | *Solanumlycopersicum* (Tomato) | √ | | | Egea et al. (2018) |
| | $eCO_2$ | *Pisumsativum* (Field pea) | √ | | | Jin et al. (2014) |
| Starch | Drought | *Oryza sativa L.* (Rice) | | √ | | Kim et al. (2008b) |
| | | *Solanumlycopersicum* (Tomato) | √ | | | Egea et al. (2018) |

(↑) abiotic stress increased antioxidant / carbohydrate concentration: (↓) abiotic stress decreased antioxidant / carbohydrate concentration; (—) abiotic stress did not cause any significant effect on antioxidant / carbohydrateconcentration.

## CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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*Full Length Research Paper*

# Characterization of Pico Island (Azores) wood-pastures

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**Wood-pastures are gaining importance in various parts of the planet, either for their role in combating desertification, protecting endemic faunas and flora, or their role in animal welfare or nutritional value in all year round grazing systems. On Pico Island and on other islands in the Azores, farmers have been using tree pastures for a long time, especially in the winter, when there is a shortage of grass and the climate is harsh, mainly because of the wind. The studied pasture comprises a mixture of grasses, based on ryegrass (*Lolium perenne*), white clover (*Trifolum repens*), tufted grass (*Holcus lanatus*) and lambs' tongue (*Plantago lanceolata*), on the border of trees, grasses and shrubs. As trees and shrubs, there is incense (*Pittosporum undulatum*), holly (*Ilex perado*), ginger lily (*Hedychium gardnerianum*) and acacia (*Acacia melanoxylon).* The wood pastures, according to the results achieved by us, can be used for animal feed in the livestock production of Pico Island, mainly for meat production. However, further studies should be carried out to assess the impact of the use of wood pastures on the production system, on the mitigation of greenhouse gases produced by animal production and on animal welfare.**

**Key words:** Wood-pastures, unconventional forages, Pico Island, grasses, trees and shrubs.

## INTRODUCTION

Wood-pastures are part of the cultural heritage of different parts of the world, playing an important role in providing food for animals and preserving biodiversity (Bergmeier et al., 2010). Grassland with trees can be defined as forests that are systematically grazed (Rackham, 2004). This type of land use involves herding animals, trees, shrubs and grasses. Traditionally managed wood-pastures have high structural diversity, which increases the biodiversity of species that are dependent on various microhabitats, and also bring valuable information to landscape history and ecology (Szabó, 2010; Oldén, 2016).

In the Azores, especially in islands such as Pico, during periods of lack of grass, with two clear periods of scarcity in both summer (particularly August and September) and winter (November through February), especially in the winter, animals are kept in the so-called "invernadores", as a way of protecting them from the adverse conditions, especially the rain and wind (Borba et al., 2015). There, they feed on branches, mainly incense, preserved foods, hay and straw, corn cane and, more recently, silages and some concentrates: corn grain or even commercial concentrate. In these periods of lack of grass, it is normal to use unconventional fodder as sources of fiber, with

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shrubs playing a predominant role. Of the unconventional fodder used in Azorean animal feeding, *Pittosporum undulatum* (incense), *Hedychium gardnerianum* (ginger lily), *Morella faya* (beech) and *Ilex azorica* (holly) can be highlighted (Borba et al., 2015). Some of these species were introduced into the archipelago as ornamental plants (ginger lily) or as fences (incense) but, as a result of their rapid growth, a very important aspect for protection against the wind in the production of orange, they have become invasive plants (Sjögren, 1973).

Pico Island is the second largest island of the Azores archipelago, with an area of 444.89 km$^2$ and an economy that is mainly dedicated to agriculture, fisheries and livestock. About 30% of its surface is occupied by forests (SREA, 2016). In this work, the winter pastures of Pico Island (wood-pastures) will be studied, aiming to determine, in a first phase, its composition of trees, shrubs and pastures, and its nutritive value. In a second phase, Pico's wood-pastures and their productivity will be quantified.

The goal is to delimit the current wood-pasture areas on Pico Island and the potential areas that could be occupied by this type of pasture management. In doing so, a portion of this pasture will be characterized in terms of chemical composition and nutritional value of conventional and non-conventional fodder that can be grazed by the animals.

## MATERIALS AND METHODS

### Delimitation of wood-pastures areas

The areas corresponding to the wood-pastures were delimited based on the observation of the landscape, in a study visit made to the island of Pico, using the method of digitalization above the corresponding aerial photograph, creating a shape file with geo-information in a Geographic Information System where these areas are included using the WGS 1984 UTM Zone 26N georeferencing, with an additional information table that includes the area in square meters and zonal images.

Areas with potential for wood-pastures were delimited through the visualization of landscape units, taking into account the observation of aerial photography and the island's soil chart, which allowed us to better interpret the landscape. The shape file with information from recent lava flows was also used to differentiate forest areas, as well as altimetry to exclude very steep slopes and slopes less than 150 m in areas where holly does not develop.

### Forage collection and preparation

The current study was conducted in the Animal Nutrition Laboratory, Department of Agricultural Sciences, University of the Azores, Azores, Portugal. Samples of pasture, grasses, trees and shrubs were collected on the island of Pico, municipality of Madalena, which is found at 345 m altitude. This region is dominated by very fine soils from basaltic pyroclastic materials (bagacina) on lava mantle, litolic soils according to Ricardo et al. (1979). They would fit in the Lhitic Hapludands according to Soil Taxonomy (USDA, 2014).

Samples are harvested manually at three different locations, where the parts of the plantain animals are normally eaten. The pasture and the trees, grasses and shrubs used as unconventional fodder was analysed. The studied pasture consists of a mixture of grasses, based on ryegrass (*L. perenne*), white clover (*T. repens*), tufted grass (*H. lanatus*) and lambs' tongue (*P. lanceolata*), on the border of trees, grasses and shrubs, the incense (*P. undulatum*), the holly (*I. perado*), the ginger lily (*H. gardnerianum*) and the acacia (*A. melanoxylon*).

### Chemical analysis

Dried samples were then ground through a 1-mm screen using a Retsch mill (GmbH, 5657 HAAN, Germany). These ground samples were analysed for dry matter (DM, method 930.15), crude protein (CP, method 954.01) and total ash method (942.05), according to the standard methods of AOAC (1995). Briefly, the dry matter content of the forage was determined by placing samples in a forced air oven at 105°C for 24 h. Total ash was evaluated by igniting samples in a muffle furnace at 500°C for 12 h. Crude protein was determined by standard micro-Kjeldahl method, using digestion equipment (Kjeldatherm System KT 40, Gerhart Laboratory Instruments, Bonn, Germany) and an automated Kjeltec 2300 Auto-analyser apparatus for distillation and titration (Foss Electric, Copenhagen, Denmark). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) was be determined according to Goering and Van Soest (1970). *In vitro* digestibility was be determined using the Tilley and Terry (1963) method, modified by Alexander and McGowan (1966), and the juice of the rumen was obtained from a slaughterhouse, as described by Borba et al. (2001).

### *In vitro* gas production

*In vitro* gas production (GP) technique simulates the rumen fermentation process and it has been used to evaluate the potential of feeds to produce greenhouse gas. It is similar to the ruminal process as gas ($CO_2$ and $CH_4$) is produced from the carbohydrate fermentation.

Each assay was repeated three times (runs). Blanks were used for each inoculum to measure the fraction of total gas production due to substrate in inocula and these values were subtracted from the total to obtain net GP. All treatments, for each assay, were incubated simultaneously in all runs, as per Menke et al. (1979).

Rumen digesta was collected as described by Borba et al. (2001). The preparation of buffer solutions and rumen inocula was as described by Menke and Steingass (1988).

The initial gas volume was recorded after 4, 8, 12, 24, 48, 72 and 96 hours of incubation.

This gas production represents the kinetic of the rumen apparent GP and is expressed by the McDonald (1981) equation. Gas production profiles were obtained after fitting the data to the exponential equation of Ørskov and McDonald (1979):

$$p = a + b\,(1 - exp^{-c\,t})$$

Where, $p$ represents the gas production at time *t; the values of a, b and c represent constant values* in the exponential equation; a+b the total potential gas production (ml/g DM), and $c$ the rate constant

## RESULTS AND DISCUSSION

The area of wood-pastures currently used on Pico Island is 752.87 ha, as can be seen in Figure 1. In the past, this area was much more extensive, having decreased

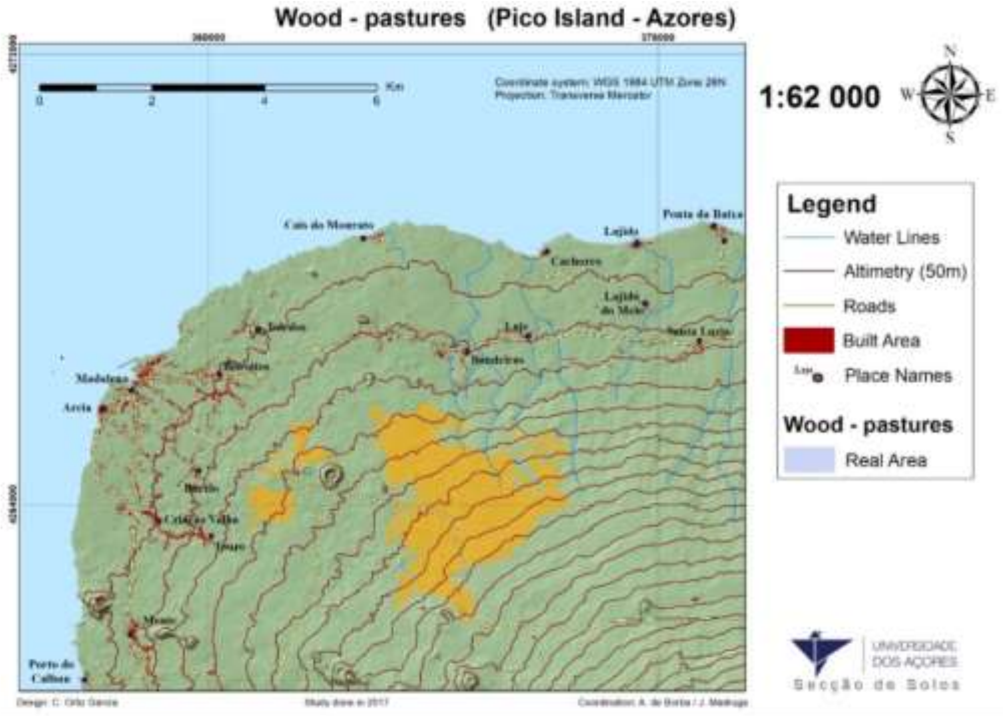

**Figure 1.** Current wood-pasture areas on Pico Island.

**Figure 2.** Potential wood-pasture areas on Pico Island.

with the intensification of the production system.

In the delineation of the potential areas for the installation of wood-pastures (Figure 2), the soil, susceptible to support pasture and the normal growth altitude of holly were taken into account, since the ginger lily and incense grow in almost all environments. For this

**Table 1.** Chemical composition and nutritive value of wood-pastures forages.

| Forage | DM (%) | 100 g DM | | | | | | DMD (%) |
|---|---|---|---|---|---|---|---|---|
| | | CP | NDF | ADF | ADL | EE | Ash | |
| Pasture | 10.73 | 22.34 | 51.53 | 30.96 | 4.72 | 5.04 | 10.42 | 64.20 |
| Holly | 30.11 | 8.76 | 50.12 | 44.72 | 21.88 | 6.46 | 5.48 | 55.36 |
| Ginger lily | 16.60 | 6.73 | 66.04 | 35.51 | 5.53 | 4.06 | 8.94 | 42.38 |
| Incense | 32.60 | 9.60 | 30.97 | 25.76 | 9.99 | 4.56 | 6.51 | 66.73 |
| Acacia | 44.24 | 15.04 | 64.84 | 53.54 | 31.18 | 3.68 | 4.75 | 31.63 |

DM, Dry Matter; CP, Crude Protein; NDF, Neutral Detergent Fiber; ADF, Acid Detergent Fiber; ADL, Acid Detergent Lignin; EE, Extract Ether; DMD, *In vitro* Dry Matter Digestibility.

**Table 2.** Equation terms for gas production, including residual standard deviations (rsd).

| Parameter | a | b | c | Lag time (h) | RSD |
|---|---|---|---|---|---|
| Pasture | -5.15 | 53.83 | 0.0472 | 2.1 | 1.23 |
| Holly | -0.96 | 33.77 | 0.0441 | 0.7 | 0.71 |
| Ginger lily | 3.46 | 57.04 | 0.0397 | 0 | 1.24 |
| Incense | -6.63 | 33.33 | 0.0647 | 3.4 | 1.29 |
| Acacia | 1.96 | 13.16 | 0.0382 | 0 | 0.31 |

reason, these areas are located at an altitude varying between 150 and 550 m, on average, although it falls occasionally down to 120 m (in the case of São Mateus), or up to 700 m (in the case of Santa Luzia).

Form the results presented in Table 1, the high crude protein content of the pasture (22.34% DM) and the high lignin content of the acacia (31.18% DM) are of note, resulting in a low digestibility of DM (31.63%). Lignin, is a molecule in biochemical terms of great complexity and almost indigestible in nutritional terms (Jung et al., 1982). Pasture presents the lowest DM value (10.73%), while acacia wood shows the highest (44.24%). Holly also exhibits a high ADL value (21.88%DM), while Ginger lily has the lowest CP value (6.73%), even lower than the 7% suggested by Lazzarini et al. (2009) as the minimum limit for normal microbial activity in the rumen. Incense has the lowest NDF value (30.97% DM), mostly made up of cellulose, which translates into the high value of DMD (66.73%).

Analysing the results obtained in Table 1, it can be noted that the holly presents similar results to the ones found by Borba et al. (2015a) for Terceira Island. Presenting a slightly higher value in crude protein (CP) and lower in NDF, those authors report a CP value of 7.48% DM and NDF 52.02% DM.

The incense presents appreciably better values than those found by Moselhy et al. (2014) for Terceira island, where they refer to CP values of 6.11% DM and 43.84% DM of NDF. The values for dry matter are lower than those reported by Moselhy et al. (2015), which refers to a value of 15.64%, a higher value in crude protein, in NDF, ADF and ADL, those authors refer to 18.66, 49.41, 27.28 and 2.68% of DM, respectively.

The contents are lower than those reported by Moselhy et al. (2015) for the ginger lily, (6.73 versus 8.05% of DM), NDF (66.04 versus 75.69% of DM), ADF (35.51 versus 48.69% of DM) and ADL (5.53 versus 8.96% of DM).

Acacia has values very similar to those reported by Singh et al. (1997), although the protein value is significantly lower than that presented by those authors (15.04 versus 20.70% of DM). In relation to Acacia, Burner et al. (2008) report the need for toxicity studies of this species, although there is no documentation to support the negative effect of this species on ruminant feed.

Regarding the digestibility of DM, it can be seen that both the incense and the holly, have a good nutritive value, being acacia the forage that presents lower values of digestibility. Other authors, namely Dynes and Schlink (2002) and Gebeyew et al. (2015) report digestibility values of acacia leaves higher than those current finding, so these authors state that acacia is a potential feed supplement for livestock Production.

Wood-pastures *in vitro* gas production results (Table 2) show that the initial time of fermentation (Lag Time) varies greatly from forage to forage, ranging from 0 hours to 3.4 hours. This variation is in line with previous findings (Tuah et al., 1996). It was observed that the acacia and the ginger lily have a Lag Time of 0 hours, while the pasture presents a Lag Time of 2.1 h and the incense of 3.4 hours. According to the gas production curves (Figure 3), acacia and incense are the least gas-producing forage, with ginger lily and pasture being the major

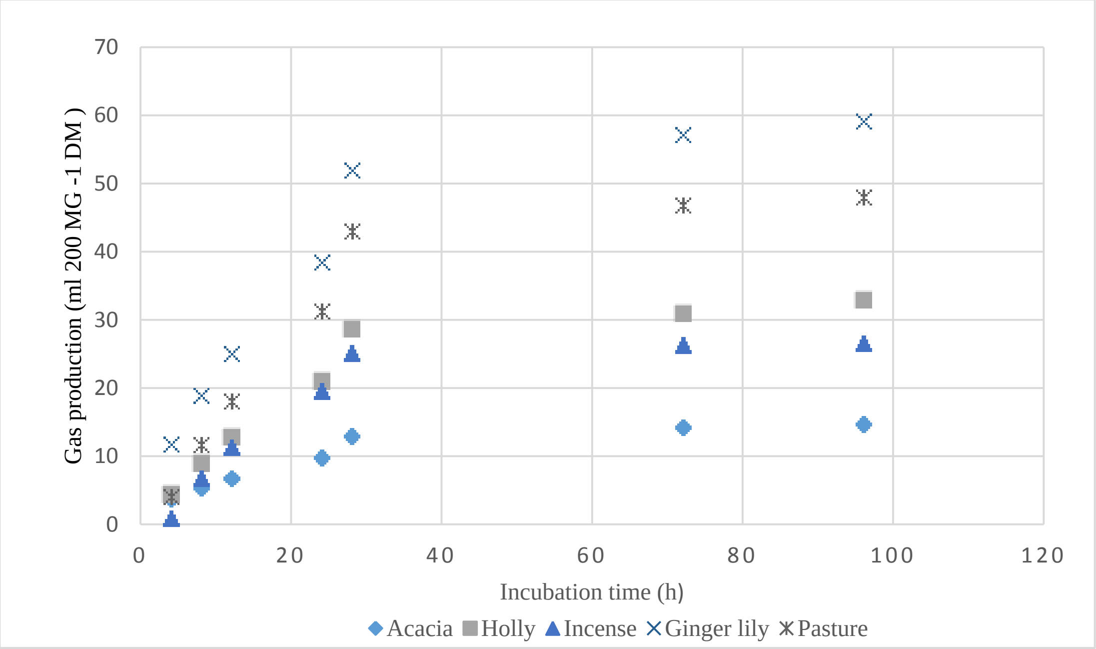

**Figure 3.** Pattern of *in vitro* gas production (fitted with exponential model) on incubation of wood pastures in buffered rumen fluid.

producers, indicating the potential inhibition of acacia gas production and of incense, already confirmed for incense by Moselhy et al. (2014), who verified a little inhibitory effect on total gas by ginger lily.

The archipelago of the Azores has a Usable Agricultural Area (UUA) of 120 400 hectares, with 16% of the UAA on the island of Pico and 19.5% of the agricultural holdings in the archipelago. Dairy cattle have strategic importance for the archipelago, especially in the islands of S. Miguel, Terceira and S. Jorge, on both the agro-industrial and territorial levels (DGIP, 2015).

The island of Pico is an island with a strong vocation for the production of beef cattle. According to the SREA (2016), beef cattle slaughtered on the island of Pico increased by 26.6% from the year 2014 to the year 2015. This type of animal production tends to increase on this island, especially the PGI meat (Protected Geographical Indication). It is on this island that about 40% of PGI Azores beef producers are located.

Wood-pastures are characteristic of traditional extensive grazing systems in forested areas (Oldén et al., 2016). The system of wood-pastures exploitation is less intensive than the conventional pastures. Its management must allow, on the one hand, for the regeneration of the trees and shrubs and, on the other hand, it must prevent the invasion by the arboreal cover. This finding is verified throughout Europe, as noted by Plieninger et al. (2015), and is the main reason for the decrease of these pasture areas in the Azores, which has significantly intensified its production system in the last 40 years.

Trees and shrubs on pasture have many agronomic functions. They provide additional forage for livestock, with complementary nutrition and fiber, and play an essential role in overcoming the lack of grass in summer and winter. Trees provide shelter for livestock from sun, rain and wind, and deposit nutrients in soil, when they lose their leaves.

Carmona et al. (2013) concluded that not only the number of animals grazing on land, but also the time of grazing, determine the condition and density of the holm oaks trees.

With very few exceptions, wood-pastures are not recognized in European Union nature conservation policies and are not protected as distinct land cover types with a special history of management, ecological and cultural value (Hartel et al., 2013). The important role they play in animal welfare, especially in the winter, when it is cold, windy and there is heavy rain, should also be highlighted.

Van Uytvanck et al. (2008) reported that due to its great value for conservation wood-pastures restoration should be encouraged, bearing in mind that the initial regeneration of the tree is an essential component of recovery, but may be hampered by grazing. In the Azores, due to the type of trees used, of great growth and burst capacity, some of them being weeds that need to be controlled, the problem of overgrazing does not arise.

The wood pastures can be used for animal feed in the livestock production of the island of Pico, mainly in the production of meat. However, further studies should be carried out to assess the impact of the use of wood pastures on the production system, on the mitigation of greenhouse gases produced by animal production and on animal welfare.

## Conclusions

It is concluded that wood-pastures are a potential system for animal production in the Island of Pico, especially for the production of beef cattle. It makes way for a sustainable animal production, with respect to the environment and animal welfare, ideal for an organic production system as recommended by the European Union, in the CAP 2014-2020, and for the IGP (Protected Geographical Indication) system for the production of "Carne dos Açores" (Meat from the Azores).

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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# Genetic diversity of rice (*Oryza sativa* L.) accessions collected from Sudan and IRRI using SSR markers

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**The assessment of genetic diversity of the traditional rice varieties or landraces is an essential component in germplasm characterization and conservation to identify potential parents. In the present study SSR markers (588 SSR markers) were used for the assessment of genetic diversity and relatedness among 31 rice accessions. These included 18 accessions from Sudan and 13 from IRRI. Among the SSR markers used only 483 generated polymorphic patterns, and showed 1274 alleles. The number of alleles per locus ranged from 2 (about 214 markers) to 5 (RM16820 and AP3206a) with an average of 2.64 alleles per locus. The polymorphic information content (PIC) values ranged from 0.06 (RM3138, RM10671, SKC3, R1M7, R6M30, S07101 and S12041B) to 0.69 (RM7643), with an average of 0.39. The major allele frequency per locus varied from 32% (RM7643) to 97% (RM3138, RM10671, SKC3, R1M7, R6M30, S07101 and S12041B), with an average of 64%. Among the primers used in the present investigation, RM7643 was highly informative as it recorded the highest PIC value (0.69). The UPGMA resulted in allelic richness of four major clusters in which cluster I is composed of a high number of accessions. The pairwise genetic dissimilarity indices revealed the highest genetic dissimilarity of 62.3% between Pipanfary Red1 and FL478. The lowest genetic dissimilarity was found between NBGS3 and NBGS2 (4.1%), but they showed wide dissimilarity with other accessions. The study highlighted the usefulness of the application markers for efficient characterization of the Sudanese rice accessions.**

**Key words:** Rice accessions, genetic diversity, SSR markers, polymorphism.

## INTRODUCTION

Information on genetic variability within cultivated crops has a strong impact on plant breeding strategies and conservation of genetic resources (Dean et al., 1999; Simioniuc et al., 2002). This is particularly useful in the characterization of individuals, accessions and cultivars, in determining duplications in germplasm collection and for the choice of parental genotypes in breeding programme (Abu Assar et al., 2005). In Sudan, rice was introduced from Congo since 1905 (Hakim, 1963). It was also known that Oryza *punctata* Kotschy was growing wild for long time in rain-fed depressions (Mac, 1992). Since then, and after numerous introductions and evaluation, rice is now being grown under irrigation in Gezira and White Nile of Sudan and Bahr El-Ghazal, Upper Nile (Malakal), and Jonglei of South Sudan. Although rice has the largest *ex situ* germplasm in the world, which made great contribution to rice breeding (Jackson and Juggan, 1993); however, the genetic diversity of rice in Sudan is not well understood.

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**Table 1.** Code number, name, type, origin and source of the 18 accessions of rice (*Oryza sativa* L.) native to Sudan and South Sudan and 13 accessions from IRRI.

| Name | Type | Source |
|---|---|---|
| NBGS1 | Landrace | Aweil, Akuem area |
| NBGS2 | Landrace | Aweil, Aryakryak area |
| NBGS3 | Landrace | Aweil, Aulic area |
| NBGA | Landrace | Aweil, Wotding Achol area |
| BG400-1 | Landrace | Aweil, plot 8 |
| BG90-2 | Landrace | Aweil, plot 6 |
| BANBAN | Landrace | Aweil, Madwang area |
| MASURY1 | Cultivated | Kosti, Leya and Hamarya area |
| PIPANFARY RED1 | Landrace | Kosti, Leya and Hamarya area |
| JAING ARRI RED | Landrace | Kosti, Leya and Hamarya area |
| SOMMBOY | Landrace | Kosti, Leya andHamarya area |
| COMARWA | Landrace | Kosti, Leya and Hamarya area |
| PAINJLA | Landrace | Kosti, Leya and Hamarya area |
| PIPANFARY | Landrace | Kosti, Leya and Hamarya area |
| TAGMIZEDO | Landrace | Kosti, Leya and Hamarya area |
| PIPANFARY RED2 | Landrace | Kosti, Leya and Hamarya area |
| BACTING ARRI | Landrace | Kosti, Leya and Hamarya area |
| MASURY2 | Cultivated | Kosti, Leya and Hamarya area |
| IR29 | Released variety | IRRI gene bank |
| FL478 | Released variety | IRRI gene bank |
| IR64 | Released variety | IRRI gene bank |
| IR64 - SUB1 | Released variety | IRRI gene bank |
| FR13A | Released variety | IRRI gene bank |
| IR42 | Released variety | IRRI gene bank |
| KHAO HLAN ON | Released variety | IRRI gene bank |
| MAZHAN RED | Released variety | IRRI gene bank |
| DAWE | Released variety | IRRI gene bank |
| ERATIO | Released variety | IRRI gene bank |
| DSBRC222 (IRRI 154) | Released variety | IRRI gene bank |
| IRRI119 | Released variety | IRRI gene bank |
| AZUCENA | Released variety | IRRI gene bank |

Exploring diversity in a landrace collection is very important for identifying new genes and further improvement of the germplasm (Thomson et al., 2007). Therefore, detailed study on genetic diversity of the native germplasm of rice in Sudan and South Sudan is very important for the initiation of rice breeding programme that could result in selection of high yielding genotypes under normal and stress conditions. For the assessment of genetic diversity, molecular markers have been found to be generally superior to morphological markers, pedigree, heterosis and biological data (Melchinger et al., 1991). The genetic diversity is commonly assessed by genetic distance or genetic similarity. Among the DNA markers, microsatellites are the most widely used for many purposes such as diversity, genome mapping, varietal identification, determination of the genetic relationship between several sub-species etc. (Ma et al., 2011). The objective of the present study is to assess the extent of correlation and genetic similarity among rice accessions grown in Sudan and South Sudan to be used as parents for future breeding program.

## MATERIALS AND METHODS

### DNA extraction

A total of 18 rice accessions native to Sudan and South Sudan as well as other 13 genotypes from International Rice Research Institute (IRRI) gene bank were used in this study (Table 1). DNA was extracted from the leaf samples of 14 day old seedlings planted in the green house at IRRI-Philippines using the modified Miniprep Protocol of Thomson et al. (2006). The DNA was quantified using a Thermo Scientific NanoDrop ND -2000/2000C spectrophotometer (Thermo Fisher Scientific, USA). A total of 588 markers were used for the genetic diversity analysis. The motifs for these markers can be found in a public domain (http://www.gramene.org/markers/microsat/).

The polymerase chain reaction (PCR) was carried out using the SSR Programmable Thermal Cycler (MJT55L.scr) as modified by Thomson et al. (2006). The polymerase informative content (PIC) was calculated for each SSR marker as described by Anderson et al. (1993) as follows:

$$PIC_j = 1 - \sum_{i=1}^{n} p_i^2$$

Where, $P_{ij}$ is the frequency of the *j*th allele for the *i*th marker, and

**Table 2.** SSR markers, Chromosome location, major allele frequency and number of alleles per locus, gene diversity and polymorphism information content (PIC) values among 31 rice (*Oryza sativa* L.) genotypes.

| Marker | Chromosome location | Major allele frequency | Number of alleles | Gene diversity | PIC value |
|---|---|---|---|---|---|
| RM3138 | 6 | 0.97 | 2 | 0.06 | 0.06 |
| RM7643 | 1 | 0.32 | 4 | 0.74 | 0.69 |
| RM10671 | 1 | 0.97 | 2 | 0.06 | 0.06 |
| RM16820 | 4 | 0.44 | 5 | 0.71 | 0.66 |
| AP3206a | 1 | 0.47 | 5 | 0.69 | 0.65 |
| SKC3 | 1 | 0.97 | 2 | 0.06 | 0.06 |
| R1M7 | 1 | 0.97 | 2 | 0.06 | 0.06 |
| R6M30 | 6 | 0.97 | 2 | 0.06 | 0.06 |
| S07101 | 7 | 0.97 | 2 | 0.06 | 0.06 |
| S12041B | 12 | 0.97 | 2 | 0.06 | 0.06 |
| *General mean |  | 0.64 | 2.64 | 0.46 | 0.39 |

*The general mean is the average of 483 polymorphic marker

is summed over *n* alleles.

Allelic diversity of the SSRs was calculated according to the diversity index 'H' as described by Nei (1987) as follows:

$$H_E = \frac{2N}{2N-1} (1-\sum P^2i)$$

Where, Pi is the frequency of the $i^{th}$ of k allele.

Genetic similarity between the genotypes was estimated using PowerMarker ver. 3.25 "C. S. Chord, 1967" (Cavalli-Sforza and Edwards, 1967).

### Diversity analysis

Based on the DNA fragments, the clearly unambiguous bands were scored visually for their presence and absence with each primer. The scores were obtained in the form of matrix with '1' and '0' which indicates the presence or absence of bands in each accessions, respectively. SSR polymorphisms were measured in terms of major allele frequency, number of alleles per locus, gene diversity and PIC values using PowerMarker software (version 3.25; Liu and Muse, 2005). For the unrooted phylogenetic tree, genetic distance was calculated using the "C.S Chord 1967" distance (Cavalli-Sforza and Edwards, 1967) in PowerMarker with tree viewed using Tree view software.

## RESULTS

### SSR polymorphism and PIC value

Among the SSR markers used only 483 generated polymorphic patterns, and showed 1274 alleles in the 31 genotypes (Table 2), whereas primers with monomorphic banding patterns were excluded. The number of alleles per locus ranged from 2 (about 214 markers) to 5 (RM16820 and AP3206a), with an average of 2.64 alleles per locus.

SSR markers were highly informative and polymorphic as evident from its PIC value. The PIC values derived from allelic diversity calculated to estimate the informativeness of each primer and allelic frequency among the genotypes were not uniform for all the SSR loci tested. This PIC value ranged from 0.06 (RM3138, RM10671, SKC3, R1M7, R6M30, S07101 and S12041B) to 0.69 (RM7643), with an average of 0.39. The major allele frequency per locus varied from 32% (RM7643) to 97% (RM3138, RM10671, SKC3, R1M7, R6M30, S07101 and S12041B), with an average of 64%. Among the primers used in the present investigation, RM7643 was highly informative since it recorded the highest PIC value of 0.69 followed, respectively by RM149 (0.68), RM10716 (0.68), RM16820 (0.66) and RM23930 (0.65) (Figure 1). Markers that revealed the highest PIC values had highest genetic diversity and lowest allele frequency, whereas markers that showed the lowest PIC value had low genetic diversity and highest allele frequency.

### Genetic distance base analysis

The UPAGMA based-dendrogram was obtained from the binary data deduced from the DNA profiles of the samples analyzed where the genotypes that are derivatives of genetically similar types clustered together. The UPGMA resulted in allelic richness of four major clusters observed by rectangular cladogram (Figures 2) with additional sub-clusters within them. Group I is composed of 20 accessions, which can be subdivided into five sub- clusters. Sub-cluster one (GI-1) comprised eight accessions (NBGS3, NBGS2, NBGA, NBGS1, BG90-2, BG400-1, Banban and Masury1) in which NBGS3 showed a narrow genetic similarity with the other accessions (4.10 to 28.42%). Sub-cluster two (GI-2) consisted of two accessions (Pipanfary Red2 and Masury2) with genetic similarity of 15.31%. Whereas Sub-cluster three (GI-3) is composed of five accessions (IR64-Sub1, IR64, IR29, IR42 and FL478) with genetic similarity of 18.37%. Sub-cluster four (GI-4) included two accessions (IRRI119 and DSBRC222) with genetic similarity of 26.84%. On the

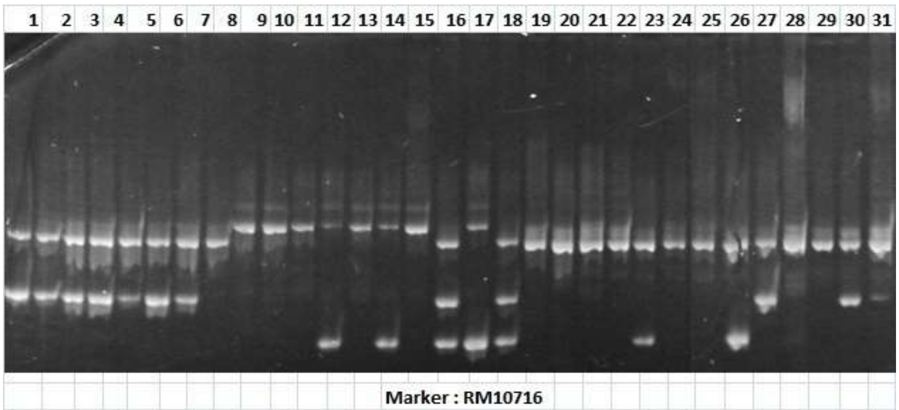

**Figure 1.** Gel photos polymorphic marker RM10716 showing the bands. Numbers from 1-31 showing the Sudanese and IRRI rice accessions.

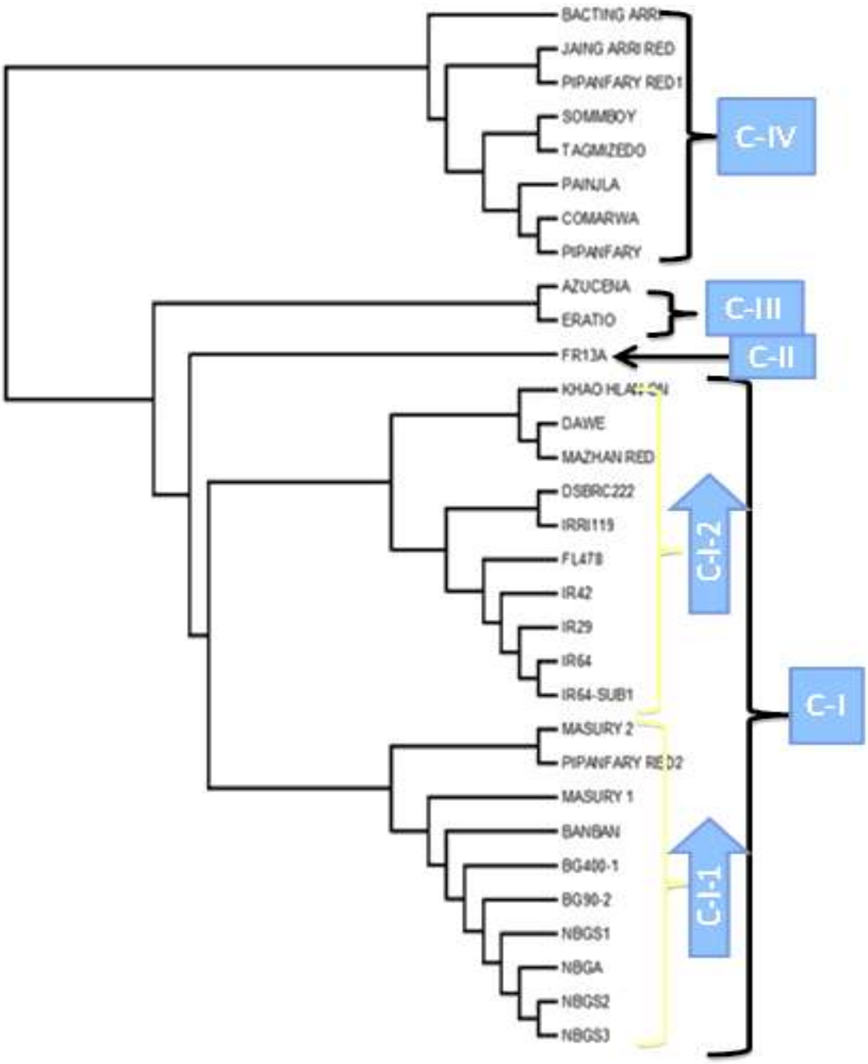

**Figure 2.** Dendrogram showing a genetic diversity of 31 rice accessions based on polymorphic SSR markers derived from UPGMA cluster using tree view (Win 32).

other hand, sub-cluster five (GI-5) exhibited three accessions (Mazhan Red, Dawe and Khao Hlan On), with a genetic similarity of 26.1%. Group II is composed of only one accession (IR13A), which was distinctly different from all three accessions (Mazhan Red, Dawe and Khao Hlan On), with a genetic similarity of 26.1%.

Group II composed of only one accession (IR13A), which was distinctly different from all other accessions examined. There were two accessions in GIII (Eratio and Azucena) with genetic similarity of 29.77%. Group IV consisted of eight accessions, which can be subdivided into four subgroups. Subgroup GIV-1 composed of three accessions (Pipanfary, Comarwa and Painjla) which revealed genetic similarity of 10.55 and 12.18%; GIV-2 consisted of two accessions (Tagmizedo and Sommboy) with 11.77% genetic similarity. Subgroup GIV-3 consisted of two accessions (Pipanfary Red1 and Jaing Arri Red) which showed 6.08% genetic similarity, and subgroup GIV-4 composed of only one accession (Bacting Arri).

## Pairwise genetic diversity

A dissimilarity matrix was used to determine the level of relatedness among the studied accessions. The genetic similarity (GS) among accessions varied from 0.041 to 0.623 (Table 3). The pairwise genetic dissimilarity indices revealed the highest genetic dissimilarity of 62.3% between Pipanfary Red1 and FL478 and the lowest genetic dissimilarity of 4.1% was found between NBGS3 and NBGS2 (Table 3).

Genetic dissimilarity between the Sudanese rice accessions and IRRI genotypes was comparatively high. Therefore, SSR markers provide an adequate power of resolution to discriminate between the accessions and it could serve as a potential tool in the identification and characterization of genetically distant accessions from different sources.

## DISCUSSION

The use of SSR markers to investigate genotypic variations among different genotypes was previously reported by some researchers (Sajib et al., 2012).

The mean alleles of 2.64 per locus detected in the present study was in accordance with the result of Wong et al. (2009) who achieved a value of 2.6 alleles per locus in analysis among 8 Barrio rice cultivars using 12 SSR primers and detecting a total of 31 alleles. Moreover, the result was in the range of 2.0-5.5 alleles per SSR locus for various classes of microsatellites as reported by Cho et al. (2000). However, our results were invariance to those reported by Sajib et al. (2012) and Hossain et al. (2012) who found 3.3, 3.57 and 3.8 alleles per locus, respectively in some rice genotypes. However, the number of alleles 2-5 obtained in this study was slightly lower than the results observed in previous diversity studies in rice genotypes (for example 3 to 9 alleles with an average of 4.53 alleles per locus (Hossain et al., 2007); 3-17 alleles with an average of 7.4 (Yu et al., 2003). Therefore, it could be concluded that the markers with the highest number of discernable alleles are the best markers for molecular characterization and diversity analysis. The variability existing in the number of alleles detected per locus in the present study might be due to the diverse germplasm used and selection of SSR primers with scorable alleles.

The average estimate of gene diversity (*H*) of 0.46 across 31 rice accessions, that was reduced to 0.43 when the analysis was performed with only 18 Sudanese accessions is lower than the estimates of H=0.68 for the rice accessions (*H* = 0.68) reported by Yu et al. (2003) and that of 0.53 reported by Onaga et al. (2013). These results indicate that upland rice accessions grown in Sudan are not sufficiently diverse, although some differences in polymorphism information content (PIC) values were obtained. The SSR marker RM7643 that attained the highest PIC value of 0.69 and high gene diversity of 0.74 was highly informative and can be used for assessing the genetic diversity of rice accessions from Sudan. Hence there was a strong relationship between the PIC value and the number of alleles detected, in which markers that had higher PIC value also had higher number of alleles. This strong positive association between gene diversity of a SSR locus and the number of alleles detected was also reported by Yu et al. (2003) and Onaga et al. (2013). Therefore, confirming that SSRs analysis has a considerable potential for studying the genetic diversity of rice (Xu et al., 2004; Jeung et al., 2005). The level of polymorphism determined by the PIC value (mean = 0.39) in this study is lower than the reported PIC value in previous works (Borba et al., 2009; Upadhyay et al., 2011) who reported an average PIC of 0.6, 0.75 and 0.78, respectively. Evidently, this might be due to the lack of knowledge about diversification of rice accessions in Sudan and it is the first time to use these markers for genetic map of Sudanese rice landraces.

The genetic dissimilarity between the rice accessions was also determined using a dissimilarity matrix. Generally, modern rice cultivars share a relatively narrow genetic background, when compared to the unexplored vast variability existing in rice landraces worldwide. For example, the pedigree of maximum IRRI rice varieties can be traced back to few Indian landraces such as Kitchili Samba, Vellaikar, Tadukan, Thekkan and Eravaipandi (Khush and Virk, 2005). Therefore, it is important not only to conserve landrace genotypes, but also to obtain the gene-pool of rice landraces and unlock valuable genes for breeding purposes (Rabbani et al., 2008).

In the present study, the large range of similarity values for cultivars exhibited by microsatellite markers provides great confidence for the assessments of genetic diversity and relationships, which can be used in future breeding programme. Hence with the aid of microsatellite makers and clustering data, different distantly related rice genotypes may be combined by intercrossing genotypes such as Aweil rice genotypes with IRRI rice genotypes from different clusters to get hybrid varieties with high heterosis. Many studies have also reported significantly greater allelic diversity of microsatellite markers than other molecular markers

**Table 3.** Pair-wise genetic distance indices among 31 rice accessions obtained from microsatellite marker analysis.

| Accessions | Azucena | Bacting Arri | Banban | BG400-1 | BG90-2 | Comarwa | Dawe | DSBRC222 | Eratio | FL478 | FR13A | IR29 | IR42 | IR64 | IR64-SUB1 | IRRI119 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Azucena | 0.000 | | | | | | | | | | | | | | | |
| Bacting Arri | 0.524 | 0.000 | | | | | | | | | | | | | | |
| Banban | 0.547 | 0.596 | 0.000 | | | | | | | | | | | | | |
| BG400-1 | 0.518 | 0.588 | 0.254 | 0.000 | | | | | | | | | | | | |
| BG90-2 | 0.529 | **0.618** | 0.214 | 0.208 | 0.000 | | | | | | | | | | | |
| Comarwa | 0.566 | 0.167 | **0.616** | 0.591 | **0.611** | 0.000 | | | | | | | | | | |
| Dawe | 0.524 | 0.545 | 0.315 | 0.315 | 0.325 | 0.575 | 0.000 | | | | | | | | | |
| DSBRC222 | 0.490 | 0.554 | 0.350 | 0.371 | 0.333 | 0.566 | 0.292 | 0.000 | | | | | | | | |
| Eratio | 0.298 | 0.537 | 0.540 | 0.503 | 0.512 | 0.573 | 0.493 | 0.490 | 0.000 | | | | | | | |
| FL478 | 0.535 | 0.566 | 0.324 | 0.330 | 0.300 | 0.605 | 0.297 | 0.290 | 0.508 | 0.000 | | | | | | |
| FR13A | 0.505 | 0.513 | 0.452 | 0.441 | 0.418 | 0.545 | 0.413 | 0.404 | 0.476 | 0.378 | 0.000 | | | | | |
| IR29 | 0.497 | 0.551 | 0.284 | 0.340 | 0.287 | 0.590 | 0.289 | 0.309 | 0.511 | 0.248 | 0.420 | 0.000 | | | | |
| IR42 | 0.504 | 0.583 | 0.309 | 0.348 | 0.297 | 0.608 | 0.301 | 0.258 | 0.498 | 0.308 | 0.409 | 0.256 | 0.000 | | | |
| IR64 | 0.482 | 0.561 | 0.325 | 0.366 | 0.308 | **0.611** | 0.311 | 0.276 | 0.515 | 0.254 | 0.424 | 0.211 | 0.226 | 0.000 | | |
| IR64-SUB1 | 0.495 | 0.549 | 0.349 | 0.379 | 0.329 | 0.590 | 0.307 | 0.286 | 0.511 | 0.272 | 0.391 | 0.228 | 0.227 | **0.096** | 0.000 | |
| IRRI119 | 0.482 | 0.568 | 0.324 | 0.322 | 0.307 | 0.566 | 0.262 | 0.268 | 0.491 | 0.326 | 0.399 | 0.287 | 0.271 | 0.292 | 0.307 | 0.000 |
| Jaing Arri Red | 0.562 | 0.195 | 0.609 | 0.594 | **0.611** | 0.156 | 0.602 | 0.572 | 0.555 | **0.616** | 0.554 | 0.606 | 0.609 | 0.595 | 0.590 | 0.574 |
| Khao Hlan On | 0.492 | 0.556 | 0.333 | 0.334 | 0.308 | 0.594 | 0.302 | 0.360 | 0.474 | 0.310 | 0.402 | 0.361 | 0.294 | 0.326 | 0.318 | 0.346 |
| Masury1 | 0.530 | 0.568 | 0.278 | 0.208 | 0.267 | 0.576 | 0.347 | 0.392 | 0.502 | 0.358 | 0.414 | 0.367 | 0.366 | 0.361 | 0.384 | 0.357 |
| Masury2 | 0.506 | 0.569 | 0.339 | 0.353 | 0.317 | 0.596 | 0.333 | 0.363 | 0.471 | 0.314 | 0.421 | 0.326 | 0.334 | 0.313 | 0.333 | 0.371 |
| Mazhan Red | 0.510 | 0.573 | 0.341 | 0.353 | 0.324 | 0.598 | 0.261 | 0.335 | 0.505 | 0.300 | 0.410 | 0.325 | 0.292 | 0.312 | 0.316 | 0.300 |
| NBGA | 0.528 | 0.596 | 0.181 | 0.175 | 0.188 | 0.604 | 0.305 | 0.357 | 0.513 | 0.296 | 0.425 | 0.303 | 0.354 | 0.347 | 0.363 | 0.324 |
| NBGS1 | 0.552 | 0.568 | 0.207 | 0.205 | 0.179 | 0.594 | 0.297 | 0.337 | 0.522 | 0.298 | 0.429 | 0.290 | 0.306 | 0.316 | 0.340 | 0.317 |
| NBGS2 | 0.537 | 0.590 | 0.216 | 0.203 | 0.202 | 0.609 | 0.304 | 0.381 | 0.513 | 0.293 | 0.421 | 0.293 | 0.360 | 0.335 | 0.353 | 0.340 |
| NBGS3 | 0.530 | 0.595 | 0.210 | 0.191 | 0.194 | 0.607 | 0.301 | 0.379 | 0.513 | 0.297 | 0.426 | 0.295 | 0.363 | 0.341 | 0.362 | 0.336 |
| Painjla | 0.570 | 0.179 | **0.615** | 0.581 | 0.604 | 0.124 | 0.582 | 0.560 | 0.559 | 0.601 | 0.563 | 0.589 | 0.594 | 0.585 | 0.576 | 0.563 |
| Pipanfary | 0.570 | 0.143 | 0.599 | 0.589 | **0.616** | 0.106 | 0.567 | 0.561 | 0.572 | 0.587 | 0.532 | 0.583 | 0.606 | 0.604 | 0.577 | 0.568 |
| Pipanfary Red1 | 0.571 | 0.205 | 0.597 | 0.589 | 0.601 | 0.170 | 0.598 | 0.585 | 0.566 | **0.623** | 0.552 | **0.611** | 0.607 | 0.606 | 0.601 | 0.584 |
| Pipanfary Red2 | 0.513 | 0.568 | 0.316 | 0.324 | 0.308 | 0.582 | 0.334 | 0.351 | 0.479 | 0.313 | 0.426 | 0.306 | 0.351 | 0.325 | 0.344 | 0.340 |
| Sommboy | 0.568 | 0.187 | 0.605 | 0.601 | **0.614** | 0.137 | 0.587 | 0.592 | 0.559 | 0.601 | 0.540 | 0.600 | **0.622** | **0.618** | 0.599 | 0.580 |
| Tagmizedo | 0.561 | 0.153 | 0.604 | 0.578 | **0.613** | 0.151 | 0.568 | 0.569 | 0.558 | 0.583 | 0.531 | 0.576 | 0.586 | 0.597 | 0.572 | 0.565 |

**Table 3.** Contd**.**

| Accessions | Jaing Arri Red | KhaoHlan On | Masury1 | Masury2 | Mazhan Red | NBGA | NBGS1 | NBGS2 | NBGS3 | Painjla | Pipanfary | Pipanfary Red1 | Pipanfary Red2 | Sommboy | Tagmizedo |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Jaing Arri Red | 0.000 | | | | | | | | | | | | | | |
| Khao Hlan On | 0.604 | 0.000 | | | | | | | | | | | | | |
| Masury1 | 0.562 | 0.335 | 0.000 | | | | | | | | | | | | |
| Masury2 | 0.607 | 0.336 | 0.352 | 0.000 | | | | | | | | | | | |
| Mazhan Red | 0.600 | 0.261 | 0.369 | 0.321 | 0.000 | | | | | | | | | | |
| NBGA | 0.600 | 0.331 | 0.261 | 0.331 | 0.328 | 0.000 | | | | | | | | | |
| NBGS1 | 0.597 | 0.295 | 0.281 | 0.320 | 0.341 | 0.168 | 0.000 | | | | | | | | |
| NBGS2 | 0.607 | 0.322 | 0.288 | 0.326 | 0.341 | **0.098** | 0.141 | 0.000 | | | | | | | |
| NBGS3 | **0.622** | 0.324 | 0.284 | 0.324 | 0.340 | **0.077** | 0.145 | **0.041** | 0.000 | | | | | | |
| Painjla | 0.130 | 0.606 | 0.577 | 0.598 | 0.605 | 0.600 | 0.583 | 0.608 | 0.601 | 0.000 | | | | | |
| Pipanfary | 0.169 | **0.611** | 0.588 | 0.590 | 0.609 | 0.598 | 0.586 | 0.604 | 0.602 | 0.122 | 0.000 | | | | |
| Pipanfary Red1 | **0.061** | 0.595 | 0.567 | **0.614** | 0.605 | 0.604 | 0.587 | 0.603 | 0.609 | 0.140 | 0.170 | 0.000 | | | |
| Pipanfary Red2 | 0.590 | 0.360 | 0.325 | 0.153 | 0.329 | 0.314 | 0.321 | 0.325 | 0.325 | 0.584 | 0.573 | **0.614** | 0.000 | | |
| Sommboy | 0.132 | 0.593 | 0.592 | 0.581 | 0.590 | 0.608 | 0.586 | 0.609 | **0.613** | 0.161 | 0.145 | 0.153 | 0.576 | 0.000 | |
| Tagmizedo | 0.141 | 0.588 | 0.577 | 0.579 | 0.590 | 0.599 | 0.588 | **0.610** | 0.603 | 0.124 | 0.114 | 0.150 | 0.570 | 0.118 | 0.000 |

(Sajib et al., 2012; Hoque et al., 2014).

The genetic distance between the 31 rice accessions pairs that ranged from 0.04 to 0.62 indicated a high degree of dissimilarities between the accessions. The high genetic dissimilarity between Aweil accessions and Kosti accessions is an evidence that their source of origin is different from that group of Kosti, and narrow genetic distance among Aweil accessions and also among Kosti accessions may be due to the lack of genetic diversity and they were collected from the same environment. These findings are supported by the report of low genetic diversity for Japanese, Korean, and Venezuelan rice germplasm (Song et al., 2002; Hashimoto et al., 2004; Ghneim et al., 2008). Narrow genetic base of cultivated rice varieties was also reported in other regions, including Latin America (Aguirre et al., 2005) and USA (Xu et al., 2004) and Chile (Becerra et al. 2015). The narrow genetic base observed in this study is not surprising and it could be due to the fact that only a few varieties are available in the country. In addition, several cultivars are named locally and could have arisen through field out-crossing and farmer selection over the years. Thus, one might expect that genetic diversity was on one hand, enhanced by mutation and meiotic recombination, and nonetheless as suggested by Hartl and Clark (1997) curtailed by genetic drift and natural and artificial selection.

The four main clusters of UPGMA analysis in the 31 rice accessions of the present study is unlike the cluster grouping of Sajib et al. (2012) who found five clusters for aromatic landraces, and that of Onaga et al. (2013) who found three main clusters for IRRI and Ugandan rice cultivars, and Prabakaran et al. (2010) who reported six clusters for other rice landraces. The wide variability existed in this study, between the thirty-one genotypes as revealed by the microsatellite markers provides greater confidence for the assessment of genetic diversity and relationships, which may be useful in marker-assisted selection in breeding programme.

From the results it could be concluded that the DNA fingerprinting and genetic diversity of Sudanese rice accessions using SSR markers is effective. The information about the genetic diversity will be useful for proper identification and selection of appropriate parents for breeding programme including gene mapping, and ultimately for emphasizing the importance of marker assisted selection (MAS) in Sudanese rice improvement.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Seed treatment and pre-inoculation of soybean: effect of storage period and agrochemicals on the physiological quality of seed and yield

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**Industrial seed treatment of soybean [*Glycine max* (L.) Merr.] has increased over the last years. New technologies have been developed to allow the inoculation procedure, which is traditionally done at the day of sowing, to be performed with the industrial treatment days or even weeks before sowing. Since little is known about the compatibility of agrochemicals and pre-inoculation, the objective of this study was to determine whether the storage period and the combination of fungicides and insecticides could negatively affect the physiological quality and yield of soybean seeds that were pre-inoculated. Soybean seeds received fourteen seed treatments that consisted of different fungicides and insecticides and were pre-inoculated with *Bradyrhizobium elkanii.* The seeds were treated and stored for 51 days until it was sown in the field. Every 17 days the physiological quality of the seed was assessed. The results shown that pre-inoculation did not affect the physiological quality of seeds. However, some combinations of agrochemicals, as well as storage period reduced seed vigor and seed germination, while increased abnormal seedlings. The findings of this study indicated that some combinations of fungicides and insecticides can have adverse effect on the physiological quality of seed that is stored for up to 51 days before sowing, but none of them jeopardized the nodulation and soybean yield under field conditions.**

**Key words:** Biological nitrogen fixation, *Bradyrhizobium*, compatibility, fungicide, *Glycine max*, HiCoat, industrial seed treatment, insecticide, polymer.

## INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is one of the most important agricultural crops grown around the world.

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Numerous pathogens (Henning, 2005) and pests (Hoffman-Campo et al., 2000) can cause yield losses by jeopardizing seed germination and initial establishment of plants under field conditions. Hence, seed treatment has been considered as a cost-effective tool that can be used to manage pests and diseases that occur soon after sowing (Goulart, 1998; Pereira et al., 2010a).

Industrial seed treatment of soybean is a practice that has gained more prominence over the last years (França-Neto et al., 2015). Immediately after processing, seed producer companies treat, bag and then store the seeds until sowing (Conceição et al., 2016; Brzezinski et al., 2017). Since growers do not need to treat the seeds on-farm, the sowing process is optimized (Brzezinski et al., 2015), which permits to sow the crop during the most ideal period to achieve high yields (Meotti et al., 2012). In addition, there is no need for the growers to have specific machines to perform the seed treatment on-farm (Brzezinski et al., 2017), which reduces the exposure of workers to toxic products (Abrasem, 2017).

Nevertheless, industrial seed treatments still face many challenges that need to be addressed regarding the storage period of the seed and the compatibility of agrochemicals with inoculants. Previous studies demonstrated that seed treatment with fungicides and insecticides (Pereira et al., 2010a; Dan et al., 2012; Ferreira et al., 2016; Camilo et al., 2017) as well as the period of storage (Krohn and Malavasi, 2004; Dan et al., 2010; Piccinin et al., 2013) can have negative effects on physiological quality of soybean seed. As a result, seed with low vigor can decrease seedling emergence (Khaliliaqdam et al., 2012) and reduce yield (Scheeren et al., 2010), which could seriously lead to unfeasibility of industrial seed treatments.

Inoculation of nitrogen-fixing bacteria of the *Bradyrhizobium* genus is a very important component of soybean production systems (Hungria et al., 2006; Salvagiotti et al., 2008); therefore, it needs to be considered by the companies that offer industrial seed treatment to the growers. Inoculation been performed traditionally at the day of sowing; however, due to the reduction of efficiency of the sowing process; pre-inoculation has been assessed as an alternative to the conventional inoculation (Zilli et al., 2010; Silva Junior et al., 2016; Anghinoni et al., 2017). Pre-inoculation is a practice that consists of inoculating soybean seeds days or even weeks before sowing (Anghinoni et al., 2017); therefore, growers do not need to worry about rushing and inoculating the seeds at the day of sowing.

Fungicides and insecticides applied to the seeds can be harmful to *Bradyrhizobium* spp. (Campo et al., 2009; Campo et al., 2010; Pereira et al., 2010b; Zilli et al., 2010; Costa et al., 2013; Gomes et al., 2017), and this is a challenge that needs to be addressed. Thus, new technologies have been developed with the aim of enabling pre-inoculation on soybean seeds by adding polymers to the inoculant (Fernandes Júnior et al., 2009; Silva Júnior et al., 2012), which allows better survival of the bacteria without negatively affect nodulation and crop yield (Pereira et al., 2010a).

The advancement of industrial seed treatments depends on the identification of products that could be combined with pre-inoculation without compromising the symbiotic relationship of soybean with *Bradyrhizobium.* Hence, the objectives of this study were to investigate whether the storage period and the combination of fungicides and insecticides can negatively affect the physiological quality, nodulation and yield of soybean seeds that were pre-inoculated with *B. elkanii*.

## MATERIALS AND METHODS

The study was carried out in two stages: i) evaluation of the physiological quality of soybean seeds in laboratory, and ii) evaluation of nodulation and soybean yield under field conditions.

### Seed treatments with agrochemicals

All fungicides and insecticides used in this study were commercial products recommended for seed treatment (Table 1). The dose used was the one in the label recommended by the manufacturer. The products were mixed according to their respective doses, and water was added to bring the volume to 5 ml. The mixture was applied on 1.5 kg of seed of the soybean cultivar ‘Nidera 7310’ that was previously placed into a plastic bag, following by agitation to uniformly distribute the products on the seeds. The bags were maintained on a laboratory bench for one hour for the seeds to dry and then pre-inoculation was performed. A non-treated check without application of fungicide and insecticide was added to the experiment.

### Pre-inoculation procedure

Pre-inoculation was performed with commercial products manufactured and commercialized by BASF S/A using the HiCoat® technology, which consists of 300 g of peat inoculant (Adhere HC®, *B. elkanii* strain SEMIA 587 and SEMIA 5019 at 5 x $10^9$ CFU $ml^{-1}$), 300 ml of liquid inoculant (Gelfix®, *B. elkanii* strain SEMIA 587 and SEMIA 5019 at 5 x $10^9$ CFU $ml^{-1}$), 150 ml of polymer S30 and 200 ml of distilled water. A volume of 9.5 ml $kg^{-1}$ was applied on the seeds, which were maintained on a laboratory bench to dry for an hour.

### Physiological quality of seed

The experiment was carried out under controlled conditions in the Laboratory of Field Crops at the Universidade Estadual do Centro-Oeste (UNICENTRO) in Guarapuava, Paraná, Brazil. The experiment design was a completely randomized with six replications. The treatments consisted of a factorial arrangement of four periods (0, 17, 34 and 51 days after treatment) and fourteen seed treatments (Table 1). After treatment with the agrochemicals and pre-inoculation, seeds were stored in paper bags and maintained at room temperature. The physiological quality of seed was evaluated over time with samples from the same paper bag. Fifty soybean seeds were arranged in a “germitest” paper previously soaked in distilled water for a period of 24 h, and another sheet was used to cover the seeds. The paper sheets were

**Table 1.** Treatments used in this study to evaluate the effect of fungicides and insecticides associated to pre-inoculation of *Bradyrhizobium elkanii* using the HiCoat® technology in soybean seeds.

| Treatment | Active ingredient | Type of active ingredient[a] | Trade name | Dose of commercial product (ml kg$^{-1}$)[b] |
|---|---|---|---|---|
| 1 | Non-treated and non-inoculated control | - | - | - |
| 2 | Pre-inoculation (PI)[c] | - | - | - |
| 3 | PI + Thiamethoxan | I | Cruiser® | 2 |
| 4 | PI + Carbendazim + Thiram + Thiamethoxan | F + F + I | Derosal® + Cruiser® | 2 + 2 |
| 5 | PI + Carbendazim + Thiram + Imidacloprid | F + F + I | Derosal® + Gaucho® | 2 + 2 |
| 6 | PI + Carbendazim + Thiram + Fipronil | F + F + I | Derosal® + Standak® | 2 + 2 |
| 7 | PI + Imidacloprid | I | Gaucho® | 2 |
| 8 | PI + Fludioxonil + Metalaxyl-M | F + F | Metaxyl-M® | 1 |
| 9 | PI + Fludioxonil + Metalaxyl-M + Imidacloprid + Thiodicarb | F + F + I + I | Metalaxyl-M® + CropStar® | 1 + 2 |
| 10 | PI + Fludioxonil + Metalaxyl-M + Thiamethoxan | F + F + I | Metalaxyl-M® + Cruiser® | 1 + 2 |
| 11 | PI + Fludioxonil + Metalaxyl-M + Imidacloprid | F + F + I | Metalaxyl® + Gaucho® | 1 + 2 |
| 12 | PI + Fludioxonil + Metalaxyl-M + Fipronil + Thiophanate-methyl + Pyraclostrobin | F + F + I + F + F | Metalaxyl-M® +Standak® Top | 1 + 2 |
| 13 | PI + Fipronil | I | Standak® | 2 |
| 14 | PI + Fipronil + Thiophanate-methyl + Pyraclostrobin | I + F + F | Standak® Top | 2 |

[a] Type of product: I = insecticide; F = fungicide.
[b] All products were mixed and distilled water was used to bring the volume to 5 ml that was applied to one kilogram of seed.
[c] Pre-inoculation was performed with the HiCoat® Technology: a mixture of 300 g of peat inoculant (Adhere HC®*, Bradyrhizobium elkanii* strain SEMIA 587 and 5019 at 5 x $10^9$ CFU ml$^{-1}$), 300 ml of liquid inoculant (Gelfix®*, Bradyrhizobium elkanii* strain SEMIA 587 and 5019 at 5 x $10^9$ CFU ml$^{-1}$), 150 ml of polymer S30 and 200 ml of distilled water.

rolled and placed in an incubator at 25°C in the dark. Each sheet was considered as a replication. At the fifth day, seed vigor was evaluated according to Brasil (2009). The rolled papers were returned to the incubator for three more days and then germination, abnormal seedlings and non-germinated seeds were determined according to Brasil (2009). All data were expressed as percentage (%) of the total seeds placed on each paper sheet.

## Nodulation and soybean yield

The study was carried out under field conditions during the cropping season 2009/2010 at Santa Cruz Farm in Guarapuava, Paraná, Brazil. The field is located in a region with humid subtropical climate (Cfb) and at approximately 1,100 m of altitude. The soil of the experimental area is classified as a Brown Latosol (Embrapa, 2013).

The experimental design was a randomized complete block with four replications and 16 treatments. Soybean seeds that were treated, pre-inoculated and stored for 51 days were used in this experiment. In addition to the treatments used for evaluation of physiological quality of seed, one treatment with application of nitrogen fertilizer and one treatment with standard inoculation at the day of sowing were added as controls. The experimental plot was composed of four rows spaced 0.40 m apart with 5.5 m length. The useful area for evaluation corresponded to the two central rows without 0.50 m of each extremity for 3.60 m².

Desiccation of triticale (X *Triticosecale* Wittimack) was performed with glyphosate herbicide (720 g ha$^{-1}$ a.i.) 30 days before sowing, which was performed on 15 Dec 2009. Fertilization was performed at the day of sowing of the triticale crop with 14 kg ha$^{-1}$ of N, 34 kg ha$^{-1}$ of $P_2O_5$ and 60 kg ha$^{-1}$ of $K_2O$. The soybean crop was not fertilized. This protocol follows the standard procedure done by the farmer. Management of weeds, pests and diseases was performed when needed following the appropriate recommendation.

The number of plants was counted at V2 growth stage to determine the density of plants. Nodulation was evaluated at full flowering (R2) stage. Three soybean plants were selected from each plot, and the root system was collected by placing a cylindrical metal device with 10 cm diameter and 10 cm height. The soil was washed off the roots with tap water, and the nodules were collected with a metal screen and counted. The nodules were placed in paper bags that were maintained in a drying oven at 65ºC for 72 h. Dry mass of the nodules was then determined in mg per plant.

The plants in the two central rows of the experimental plot were manually harvested and threshed. The soybean seeds were weighted and seed moisture was measured.

**Table 2.** Mean square values for the effects of seed treatment and storage period on the physiological quality of soybean seeds.

| Effect | Degrees of freedom | Mean square values | | | |
|---|---|---|---|---|---|
| | | Seed vigor[a] | Seed germination[a] | Abnormal seedlings[a] | Non-germinated seeds[a] |
| Seed treatment (ST) | 13 | 2.65 ** | 1.96 ** | 7.12 ** | 0.49 ** |
| Storage period (SP) | 3 | 6.27 ** | 1.15 ** | 4.94 ** | 0.41 ns |
| ST x SP | 39 | 0.43 ** | 0.33 ** | 1.19 ** | 0.16 ns |
| Error | 280 | 0.15 | 0.11 | 0.47 | 0.16 |
| Mean | | 8.85 | 9.11 | 4.15 | 1.23 |
| Coefficient of variation (%) | | 4.42 | 3.59 | 16.52 | 32.08 |

[a]Variables in percentage were previously transformed to √x+1 for statistical analysis. [ns] non-significant and ** significant at 1% probability level.

Soybean yield was determined in kg $ha^{-1}$ at 13% moisture. A sample was taken and used to determine the thousand-grain mass by counting and weighing 300 grains.

### Data analysis

The variables expressed as a percentage were transformed to √(x + 1). All data were submitted to analysis of variance. The means of seed vigor, seed germination, abnormal seedlings and non-germinated seeds were compared by Scott Knott's test at 5% probability level, whereas the means of nodulation and soybean yield were compared by Tukey's test a 5% probability level. All the analyses were performed using the statistical program Sisvar 5.6 (Ferreira, 2000).

## RESULTS AND DISCUSSION

### Physiological quality of seed

Seed vigor, seed germination and abnormal seedlings were significantly affected by the seed treatment, the storage period and the interaction, whereas the percentage of non-germinated seeds was significantly influenced by the seed treatments (Table 2).

Vigor is one of the main characteristics regarding to the physiological quality of seed that need to be considered for an appropriate establishment of a crop in the field (Scheeren et al., 2010). Storage for 51 days did not significantly affect seed vigor for the non-inoculated and non-treated control as well as for the pre-inoculation with HiCoat® technology (Table 3). This indicates that the use of inoculant with polymer did not jeopardize the physiological quality of seed similarly to what was reported by other authors (Conceição et al., 2016).

Application of the insecticide imidacloprid associated with the fungicides fludioxonil + metalaxyl-M and with carbendazim + thiram had the lowest seed vigor (Table 3) and lowest seed germination (Table 4) compared to the other treatments previous to the storage as well as 51 days after storage of the seeds. These effects were associated with increase in the percentage of abnormal seedlings (Table 5), but did not affect the percentage of non-germinated seeds (Table 6). Application of imidacloprid without association with any of the fungicides caused significant reduction of seed vigor after 51 days of storage (Table 3). Seed treatment with carbendazim + thiram + thiamethoxan, fludioxonil + metalaxyl-M + imidacloprid + thiodicarbe, fludioxonil + metalaxyl-M + thiamethoxan also reduced seed vigor compared to the control after 51 days of storage of the seeds (Table 3). Previous studies did not observe a negative effect of application of imidacloprid only (Castro et al., 2008; Dan et al., 2013, 2012), however, the association of imidacloprid + thiodicarb reduced vigor and germination of soybean seeds (Dan et al., 2012; Bortoletto et al., 2017). Sometimes when products are combined, there can be modification in some characteristics of the mixture that become more toxic to the seeds compared to the product alone. Moreover, the effect of the toxicity might not only be due to the active ingredients but also due to some component added in the commercial product to serve as a vehicle to the active ingredient (Kintschev et al., 2014).

Several studies had shown that the application of the insecticide thiamethoxan negatively affect the physiological quality of seed (Castro et al., 2008; Piccinin et al., 2013). In this study, no significant difference regarding to seed vigor was observed from the non-inoculated and non-treated control when only thiamethoxan was applied to the pre-inoculated seeds even after 51 days of storage (Table 3). However, there was reduction in seed germination after 51 days of storage when thiamethoxan was applied isolated as well as associated with fludioxonil + metalaxyl-M (Table 4). These results are contrary to what was observed by other authors (Barros et al., 2001; Dan et al., 2012; Dan et al., 2013; Ferreira et al., 2016; Bortoletto et al., 2017), where no negative effects of thiamethoxan on physiological quality of seed occurred. The differences among studies could be due to the storage conditions, dose applied and

**Table 3**. Vigor (%)$^{a,b}$ of soybean seeds treated with different associations of fungicides and insecticides, pre-inoculated$^{c}$ with *Bradyrhizobium elkanii* and stored at room temperature for different periods of time.

| **Seed treatment** | **Storage period (days) after seed treatment** | | | |
|---|---|---|---|---|
| | **0** | **17** | **34** | **51** |
| Non-inoculated and non-treated control | 84.0 $^{aA}$ | 84.0 $^{aA}$ | 86.0 $^{aA}$ | 79.0 $^{aA}$ |
| Pre-inoculation (PI) | 87.0 $^{aA}$ | 85.7 $^{aA}$ | 84.0 $^{aA}$ | 79.0 $^{aA}$ |
| PI + Thiamethoxan | 85.3 $^{aA}$ | 77.3 $^{bA}$ | 82.0 $^{aA}$ | 72.7 $^{a B}$ |
| PI + Carbendazim + Thiram + Thiamethoxan | 77.7 $^{bA}$ | 83.0 $^{aA}$ | 73.3 $^{bA}$ | 66.7 $^{b B}$ |
| PI + Carbendazim + Thiram + Imidacloprid | 69.0 $^{c B}$ | 73.0 $^{bA}$ | 76.3 $^{bA}$ | 62.7 $^{b B}$ |
| PI + Carbendazim + Thiram + Fipronil | 91.3 $^{aA}$ | 76.0 $^{bB}$ | 75.0 $^{bB}$ | 80.3 $^{a B}$ |
| PI + Imidacloprid | 85.3 $^{aA}$ | 66.7 $^{b C}$ | 76.3 $^{bB}$ | 65.7 $^{b C}$ |
| PI + Fludioxonil + Metalaxyl-M | 84.3 $^{aA}$ | 82.7 $^{aA}$ | 83.0 $^{aA}$ | 73.3 $^{a B}$ |
| PI + Fludioxonil + Metalaxyl-M + Imidacloprid + Thiodicarbe | 79.7 $^{bA}$ | 78.3 $^{aA}$ | 79.0 $^{aA}$ | 62.7 $^{b B}$ |
| PI + Fludioxonil + Metalaxyl-M + Thiamethoxan | 81.3 $^{bA}$ | 82.0 $^{aA}$ | 85.7 $^{aA}$ | 69.7 $^{a B}$ |
| PI + Fludioxonil + Metalaxyl-M + Imidacloprid | 57.0 $^{d B}$ | 72.3 $^{bA}$ | 63.0 $^{c B}$ | 62.0 $^{b B}$ |
| PI + Fludioxonil + Metalaxyl-M + Fipronil + Tiophanate-methyl + Pyraclostrobin | 86.7 $^{aA}$ | 83.0 $^{aA}$ | 84.7 $^{aA}$ | 75.0 $^{a B}$ |
| PI + Fipronil | 88.7 $^{aA}$ | 72.7 $^{bA}$ | 83.7 $^{aA}$ | 71.0 $^{a B}$ |
| PI + Fipronil + Tiophanate-methyl + Pyraclostrobin | 90.3 $^{aA}$ | 83.7 $^{aA}$ | 82.3 $^{aA}$ | 76.0 $^{a B}$ |

$^{a}$Percentage values were previously transformed to √x+1 for statistical analysis. Back-transformed data is presented. $^{b}$Means followed by the same lower case letter in the column for seed treatment and capital letter in the line for each storage period do not differ statistically from each other by Scott Knott's Test at 5% probability. $^{c}$Pre-inoculation was perfomed with the HiCoat® technology: 300 g of peat inoculant (Adhere HC®, *B. elkanii* strain SEMIA 587 and 5019 at 5 x $10^{9}$ CFU ml$^{-1}$), 300 ml of liquid inoculant (Gelfix®, *B. elkanii* strain SEMIA 587 and 5019 at 5 x $10^{9}$ CFU ml$^{-1}$), 150 ml of polymer S30 and 200 ml of distilled water.

**Table 4**. Germination (%)$^{a,b}$ of soybean seeds treated with different associations of fungicides and insecticides, pre-inoculated$^{c}$ with *Bradyrhizobium elkanii* and stored at room temperature for different periods of time.

| **Seed treatment** | **Storage period (days) after seed treatment** | | | |
|---|---|---|---|---|
| | **0** | **17** | **34** | **51** |
| Non-inoculated and non-treated control | 84.0 $^{b A}$ | 89.0$^{a A}$ | 88.7$^{a A}$ | 83.0$^{b A}$ |
| Pre-inoculation (PI) | 88.3$^{a A}$ | 87.0$^{a A}$ | 85.7$^{a A}$ | 88.0$^{a A}$ |
| PI + Thiamethoxan | 85.3$^{b A}$ | 81.3$^{b B}$ | 87.0$^{a A}$ | 78.0$^{c B}$ |
| PI + Carbendazim + Thiram + Thiamethoxan | 78.3$^{b B}$ | 85.0$^{a A}$ | 83.7$^{b A}$ | 76.3$^{c B}$ |
| PI + Carbendazim + Thiram + Imidacloprid | 69.0$^{c B}$ | 79.7$^{b A}$ | 80.7$^{b A}$ | 73.0$^{c B}$ |
| PI + Carbendazim + Thiram + Fipronil | 91.3$^{a A}$ | 79.0$^{b B}$ | 82.7$^{b B}$ | 89.7$^{a A}$ |
| PI + Imidacloprid | 85.3$^{b A}$ | 75.3$^{b B}$ | 79.3$^{b B}$ | 73.7$^{c B}$ |
| PI + Fludioxonil + Metalaxyl-M | 85.0$^{b A}$ | 86.3$^{a A}$ | 86.7$^{a A}$ | 81.7$^{b A}$ |
| PI + Fludioxonil + Metalaxyl-M + Imidacloprid + Thiodicarbe | 80.3$^{b A}$ | 83.0$^{b A}$ | 81.3$^{b A}$ | 79.3$^{b A}$ |
| PI + Fludioxonil + Metalaxyl-M + Thiamethoxan | 83.3$^{b A}$ | 87.7$^{a A}$ | 86.7$^{a A}$ | 76.0$^{c B}$ |
| PI + Fludioxonil + Metalaxyl-M + Imidacloprid | 60.3$^{d C}$ | 79.0$^{b A}$ | 68.3$^{c B}$ | 69.0$^{c B}$ |
| PI + Fludioxonil + Metalaxyl-M + Fipronil + Tiophanate-methyl + Pyraclostrobin | 87.3$^{a A}$ | 86.3$^{a A}$ | 89.7$^{a A}$ | 82.0$^{b A}$ |
| PI + Fipronil | 88.7$^{a A}$ | 81.0$^{b B}$ | 86.3$^{a A}$ | 78.0$^{c B}$ |
| PI + Fipronil + Tiophanate-methyl + Pyraclostrobin | 90.3$^{a A}$ | 87.3$^{a A}$ | 86.0$^{a A}$ | 80.0$^{b B}$ |

$^{a}$Percentage values were previously transformed to √x+1 for statistical analysis. Back-transformed data is presented. $^{b}$Means followed by the same lower case letter in the column for seed treatment and capital letter in the line for each storage period do not differ statistically from each other by Scott Knott's Test at 5% probability. $^{c}$Pre-inoculation was perfomed with the HiCoat® technology: 300 g of peat inoculant (Adhere HC®, *B. elkanii* strain SEMIA 587 and 5019 at 5 x $10^{9}$ CFU ml$^{-1}$), 300 ml of liquid inoculant (Gelfix®, *B. elkanii* strain SEMIA 587 and 5019 at 5 x $10^{9}$ CFU ml$^{-1}$), 150 ml of polymer S30 and 200 ml of distilled water.

application procedure. This demonstrates the importance of performing multiple studies under different conditions to better assess the effect of agrochemicals in the physiological quality of seed.

Previous to the storage, germination was higher than the non-treated and non-inoculated control when soybean

**Table 5**. Abnormal seedlings (%)$^{a,b}$ of soybean from seeds treated with different associations of fungicides and insecticides, pre-inoculated$^{c}$ with *Bradyrhizobium elkanii* and stored at room temperature for different periods of time.

| Seed treatment | Storage period (days) after seed treatment | | | |
|---|---|---|---|---|
| | 0 | 17 | 34 | 51 |
| Non-inoculated and non-treated control | $15.7^{c\,A}$ | $11.0^{b\,A}$ | $11.3^{c\,A}$ | $15.6^{b\,A}$ |
| Pre-inoculation (PI) | $11.7^{d\,A}$ | $13.0^{b\,A}$ | $13.3^{c\,A}$ | $12.0^{b\,A}$ |
| PI + Thiamethoxan | $14.0^{d\,A}$ | $17.0^{a\,A}$ | $12.0^{c\,A}$ | $19.7^{b\,A}$ |
| PI + Carbendazim + Thiram + Thiamethoxan | $21.0^{c\,A}$ | $14.7^{b\,B}$ | $16.0^{b\,B}$ | $23.0^{a\,A}$ |
| PI + Carbendazim + Thiram + Imidacloprid | $30.7^{b\,A}$ | $20.0^{a\,B}$ | $18.7^{b\,B}$ | $26.7^{a\,A}$ |
| PI + Carbendazim + Thiram + Fipronil | $8.7^{d\,B}$ | $20.0^{a\,A}$ | $16.3^{b\,A}$ | $10.3^{c\,B}$ |
| PI + Imidacloprid | $13.7^{d\,B}$ | $22.3^{a\,A}$ | $18.3^{b\,B}$ | $25.3^{a\,A}$ |
| PI + Fludioxonil + Metalaxyl-M | $15.0^{c\,A}$ | $13.3^{b\,A}$ | $12.3^{c\,A}$ | $17.3^{b\,A}$ |
| PI + Fludioxonil + Metalaxyl-M + Imidacloprid + Thiodicarbe | $19.7^{c\,A}$ | $16.0^{a\,A}$ | $18.3^{b\,A}$ | $20.0^{b\,A}$ |
| PI + Fludioxonil + Metalaxyl-M + Thiamethoxan | $16.0^{c\,B}$ | $12.3^{b\,B}$ | $13.3^{c\,B}$ | $23.7^{a\,A}$ |
| PI + Fludioxonil + Metalaxyl-M + Imidacloprid | $39.0^{a\,A}$ | $20.7^{a\,B}$ | $31.7^{a\,A}$ | $29.7^{a\,A}$ |
| PI + Fludioxonil + Metalaxyl-M + Fipronil + Tiophanate-methyl + Pyraclostrobin | $12.3^{d\,A}$ | $12.3^{b\,A}$ | $10.0^{c\,A}$ | 17.7 |
| PI + Fipronil | $11.3^{d\,B}$ | $18.0^{a\,A}$ | $12.3^{c\,B}$ | $19.7^{b\,A}$ |
| PI + Fipronil + Tiophanate-methyl + Pyraclostrobin | $9.3^{d\,B}$ | $11.0^{b\,B}$ | $13.3^{c\,B}$ | $19.0^{b\,A}$ |

[a] Percentage values were previously transformed to $\sqrt{x+1}$ for statistical analysis. Back-transformed data is presented. [b] Means followed by the same lower case letter in the column for seed treatment and capital letter in the line for each storage period do not differ statistically from each other by Scott Knott's Test at 5% probability. [c] Pre-inoculation was perfomed with the HiCoat® technology: 300 g of peat inoculant (Adhere HC®, *B. elkanii* strain SEMIA 587 and 5019 at 5 x $10^{9}$ CFU $ml^{-1}$), 300 ml of liquid inoculant (Gelfix®, *B. elkanii* strain SEMIA 587 and 5019 at 5 x $10^{9}$ CFU $ml^{-1}$), 150 ml of polymer S30 and 200 ml of distilled water.

**Table 6**. Non-germinated seeds (%)$^{a,b}$ of soybean treated with different associations of fungicides and insecticides, pre-inoculated$^{c}$ with *Bradyrhizobium elkanii* and stored at room temperature for different periods of time.

| Seed treatment | Storage period (days) after seed treatment | | | |
|---|---|---|---|---|
| | 0 | 17 | 34 | 51 |
| Non-inoculated and non-treated control | $0.3^{aA}$ | $0.0^{bA}$ | $0.0^{aA}$ | $0.7^{aA}$ |
| Pre-inoculation (PI) | $0.0^{aA}$ | $0.0^{bA}$ | $1.0^{aA}$ | $0.0^{aA}$ |
| PI + Thiamethoxan | $0.7^{aA}$ | $1.7^{bA}$ | $1.0^{aA}$ | $2.3^{aA}$ |
| PI + Carbendazim + Thiram +Thiamethoxan | $0.7^{aA}$ | $0.3^{bA}$ | $0.3^{aA}$ | $0.7^{aA}$ |
| PI + Carbendazim + Thiram + Imidacloprid | $0.3^{aA}$ | $0.3^{bA}$ | $0.7^{aA}$ | $0.3^{aA}$ |
| PI + Carbendazim + Thiram + Fipronil | $0.0^{aA}$ | $1.0^{aA}$ | $1.0^{aA}$ | $0.0^{aA}$ |
| PI + Imidacloprid | $1.0^{aA}$ | $2.3^{aA}$ | $2.3^{aA}$ | $1.0^{aA}$ |
| PI + Fludioxonil + Metalaxyl-M | $0.0^{aA}$ | $0.3^{bA}$ | $1.0^{aA}$ | $1.0^{aA}$ |
| PI + Fludioxonil + Metalaxyl-M + Imidacloprid + Thiodicarbe | $0.0^{aA}$ | $1.0^{aA}$ | $0.3^{aA}$ | $0.7^{aA}$ |
| PI + Fludioxonil + Metalaxyl-M + Thiamethoxan | $0.7^{aA}$ | $0.0^{bA}$ | $0.0^{aA}$ | $0.3^{aA}$ |
| PI + Fludioxonil + Metalaxyl-M + midacloprid | $0.7^{aA}$ | $0.3^{bA}$ | $0.0^{aA}$ | $1.3^{aA}$ |
| PI + Fludioxonil + Metalaxyl-M + Fipronil + Tiophanate-methy + Pyraclostrobin | $0.3^{aA}$ | $1.3^{aA}$ | $0.3^{aA}$ | $0.3^{aA}$ |
| PI + Fipronil | $0.0^{bA}$ | $1.0^{aA}$ | $1.3^{aA}$ | $2.3^{aA}$ |
| PI + Fipronil + Tiophanate-methyl + Pyraclostrobin | $0.3^{aA}$ | $1.7^{aA}$ | $0.7^{aA}$ | $1.0^{aA}$ |

[a] Percentage values were previously transformed to $\sqrt{x+1}$ for statistical analysis. Back-transformed data is presented. [b] Means followed by the same lower case letter in the column for seed treatment and capital letter in the line for each storage period do not differ statistically from each other by Scott Knott's Test at 5% probability. [c] Pre-inoculation was perfomed with the HiCoat® technology: 300 g of peat inoculant (Adhere HC®, *B. elkanii* strain SEMIA 587 and 5019 at 5 x $10^{9}$ CFU $ml^{-1}$), 300 ml of liquid inoculant (Gelfix®, *B. elkanii* strain SEMIA 587 and 5019 at 5 x $10^{9}$ CFU $ml^{-1}$), 150 ml of polymer S30 and 200 ml of distilled water.

seeds were inoculated and treated with fipronil, carbendazim + thiram + fipronil, fludioxonil + metalaxyl-M

**Table 7.** Mean square values for the effects of seed treatments on yield and nodulation of soybean.

| Factor of variation | Degrees of freedom | Mean square | | | | |
|---|---|---|---|---|---|---|
| | | Yield (kg ha$^{-1}$) | Thousand grain mass (g) | Number of plants per meter | Number of nodules per plant | Mass of nodules per plant (mg) |
| Block | 3 | 120232.81 * | 199.24** | 0.8293$^{ns}$ | 190.47$^{ns}$ | 2830.68$^{ns}$ |
| Treatment | 15 | 50574.46$^{ns}$ | 30.84** | 0.9059$^{ns}$ | 278.95 * | 9283.66 ** |
| Error | 45 | 43112.09 | 15.25 | 0.4944 | 129.74 | 2878.3 |
| Mean | | 3182.42 | 146.4 | 14.16 | 59.52 | 264.89 |
| Coefficient of variation (%) | | 6.52 | 2.67 | 4.97 | 19.14 | 20.25 |

[a]Variables in percentage were previously transformed to √x+1 for statistical analysis. $^{ns}$ non-significant, * significant at 5% and ** at 1% probability level.

+ fipronil + tiophanate-methyl + praclostrobin, and fipronil + tiophanate-methyl + pyraclostrobin (Table 4). It was already reported in a previous study that seed treatment with fungicide (carboxin + thiram) increased the percentage of germinated seeds compared to the non-treated control (Brand et al., 2009). These results are contrary to what was observed by Ferreira et al. (2016) who reported that fipronil + tiophanate-methyl + pyraclostrobin had negative effect on physiological quality of seed by reducing germination whereas it increased the percentage of abnormal seedlings. It is not very clear how a fungicide could improve the physiological quality of seed, but one hypothesis is that the fungicide can provided a better control of fungi that infected seeds and this could reflect in the physiological quality during the storage period (Brand et al., 2009).

After 51 days of storage, pre-inoculation with HiCoat® and application of carbendazim + thiram + fipronil presented higher seed germination (Table 4) and lower percentage of abnormal seedlings (Table 5) than the non-treated and non-inoculated control. The application of fipronil increased the percentage of non-germinated seeds after 51 days of storage, but none of the other treatments affected these variables (Table 5). Piccinin et al. (2013) also verified that the application of fipronil reduced the physiological quality of seeds after 180 days of storage, which, according to the authors, can be due to degenerative alterations in the metabolism of cells, disruption of cell membranes.

Seed germination with the application of fludioxonil + metalaxyl-M, fludioxonil + metalaxyl-M + imidacloprid + thiodicarbe, fludioxonil + metalaxyl-M + fipronil + tiophanate-methyl + praclostrobin, and fipronil + tiophanate-methyl + praclostrobin did not differ from the control, whereas the other treatments caused its reduction (Table 4). In previous studies, seed treatment with fipronil + tiophanate-methyl + pyraclostrobin associated with polymers were not harmful to seed germination (Camilo et al., 2017; Santos et al., 2018). Similarly, treatment with carbendazim + thiram + imidacloprid + thiodicarb + micronutrient + polymer + inoculant did not cause any physical and physiological damage to the soybean seeds (Segalin et al., 2013). This indicates that these products could be applied without compromising the physiological quality of soybean seeds.

The minimum value for seed germination according to Brazilian regulations is 80% (Mapa, 2013). In this study, not all treatments provided this level of germination. This is important to be considered by companies that intend to use industrial seed treatments, otherwise the seeds cannot be commercialized.

## Nodulation and soybean yield

Soybean yield and number of plants per meter were not affected by the treatments, whereas thousand grain mass, number of nodules per plant and mass of nodules per plant were significantly influenced by the treatments (Table 7).

The application of nitrogen fertilizer significantly reduced the number and the mass of nodules per plant (Table 8) as already reported by other authors (Hungria et al., 2006; Salvagiotti et al., 2008; Anghinoni et al., 2017). The energy costs required for the biological nitrogen fixation process is usually very high (Taíz and Zeiger, 2004; Minchin and Witty, 2005), therefore, when large amounts of nitrogen are easily available in the soil, the plant supply its needs by absorbing the nutrient from the soil rather than establishing a symbiotic relationship. However, the addition of nitrogen fertilizer to the soybean crop in Brazil is usually non-profitable (Hungria et al., 2006). As a result, growers still prefer to rely on the inoculation to supply nitrogen to the plant.

Pre-inoculation did not affect the number, mass of nodules per plant, and yield when compared to the standard inoculation performed at the day of sowing (Table 8). This indicates that the use of peat inoculant + liquid inoculant + polymer maintains the viability of the nitrogen-fixing bacteria for a proper establishment of a symbiotic relationship. These results corroborate with previous reports of no adverse effect on yield due to the

**Table 8**. Soybean yield, thousand grain mass, initial number of plants per meter and nodulation of soybean for each seed treatment with fungicides and insecticides, pre-inoculation$^{c}$ with *Bradyrhizobium elkanii* and storage at room temperature for 51 days.

| **Treatment** | **Yield (kgha$^{-1}$)$^{a}$** | **Thousandgrainmass(g)$^{a}$** | **Numberofplantspermeter$^{a}$** | **Numberofnodulesperplant$^{a}$** | **Massofnodulesperplant(mg)$^{a}$** |
|---|---|---|---|---|---|
| Non-inoculatedandnon-treatedcontrol | 3207$^{a}$ | 147.8$^{ab}$ | 13.66$^{a}$ | 51.5$^{ab}$ | 238.3$^{a}$ |
| StandardInoculation | 3301$^{a}$ | 146.3$^{ab}$ | 14.66$^{a}$ | 59.0$^{ab}$ | 266.7$^{a}$ |
| NitrogenFertilization(200kgha$^{-1}$N) | 3158$^{a}$ | 139.0$^{b}$ | 14.46$^{a}$ | 33.0$^{b}$ | 93.3$^{b}$ |
| Pre-inoculation(PI) | 3315$^{a}$ | 149.0$^{ab}$ | 13.54$^{a}$ | 57.8$^{ab}$ | 268.3$^{a}$ |
| PI+Thiamethoxan | 3056$^{a}$ | 141.9$^{ab}$ | 13.00$^{a}$ | 55.5$^{ab}$ | 267.5$^{a}$ |
| PI+Carbendazim+Thiram+Thiamethoxan | 3235$^{a}$ | 147.9$^{ab}$ | 14.66$^{a}$ | 59.0$^{ab}$ | 265.8$^{a}$ |
| PI+Fludioxonil+Metalaxyl-M+Imidacloprid | 3210$^{a}$ | 146.7$^{ab}$ | 14.50a | 65.0$^{a}$ | 270.7$^{a}$ |
| PI+Carbendazim+Thiram+Fipronil | 3145$^{a}$ | 146.1$^{ab}$ | 14.58$^{a}$ | 64.2$^{a}$ | 287.5$^{a}$ |
| PI+Imidacloprid | 3192$^{a}$ | 146.7$^{ab}$ | 13.92$^{a}$ | 64.0$^{a}$ | 293.3$^{a}$ |
| PI+Fludioxonil+Metalaxyl-M | 2916$^{a}$ | 146.3$^{ab}$ | 14.63$^{a}$ | 63.5$^{a}$ | 271.7$^{a}$ |
| PI+Fludioxonil+Metalaxyl-M+Imidacloprid+Thiodicarbe | 3276$^{a}$ | 148.2$^{ab}$ | 14.08$^{a}$ | 65.7$^{a}$ | 280.0$^{a}$ |
| PI+Fludioxonil+Metalaxyl-M+Thiamethoxan | 3191$^{a}$ | 147.4$^{ab}$ | 14.33$^{a}$ | 67.0$^{a}$ | 293.3$^{a}$ |
| PI+Carbendazim+Thiram+Imidacloprid | 3147$^{a}$ | 148.7$^{ab}$ | 14.04$^{a}$ | 57.2$^{ab}$ | 270.7$^{a}$ |
| PI+Fipronil+Tiophanate-methyl+Pyraclostrobin | 3372$^{a}$ | 149.7$^{a}$ | 14.00$^{a}$ | 60.2$^{ab}$ | 277.5$^{a}$ |
| PI+Fipronil | 3147$^{a}$ | 143.4$^{ab}$ | 13.96$^{a}$ | 62.0$^{ab}$ | 297.5$^{a}$ |
| PI+Fludioxonil+Metalaxyl-M+Fipronil+Tiophanate-methyl+Pyraclostrobin | 3050$^{a}$ | 147.1$^{ab}$ | 14.46$^{a}$ | 67.5$^{a}$ | 295.8$^{a}$ |
| Mean | 3182 | 146.4 | 14.20 | 59.5 | 264.9 |
| CoefficientofVariation(%) | 6.52 | 2.67 | 4.97 | 19.1 | 20.2 |

[a] Means followed by the same letter do not differ statistically from each other by Tukey's Test at 5% probability.

[b] Standard inoculation was performed at the day of sowing with the liquid inoculant Gelfix® (*B. elkanii* strain SEMIA 587 and 5019 at $5 \times 10^{9}$ CFU ml$^{-1}$).

use of pre-inoculation with *Bradyrhizobium* spp. (Anghinoni et al., 2017; Machineski et al., 2018). All these results are contrary to what was found by Brzezinski et al. (2015) and Zilli et al. (2009), where the authors mentioned that treatment of soybean seeds with insecticide and fungicides before sowing hinders the establishment of soybean in the field and reduces nodulation of the plants. This is probably due to the toxic effect of some agrochemicals that reduces the survival of the nitrogen-fixing bacteria. However, the difference may be due to the absence of polymers in both studies and the fact that seeds were stored for 240 days before sowing in the study by Brzezinski et al. (2015). This corroborates with the study by Krohn and Malavasi (2004) who reported that seeds treated with agrochemicals and stored for more than four months led to lower emergence of seedlings in the field.

Seed treatments with fungicide and insecticides can be used in an integrated management program to control diseases and pests as a preventative tactic. According to a survey performed in Brazil, some advantages of the use of industrial seed treatments have been related to higher efficiency of the sowing process, economy of labor and time, lower risk of intoxication by growers, higher precision regarding to the dose used, more uniform coverage of the seeds, reduction of production costs, guarantee of acquisition of seeds with good quality, combat seed piracy, among others (França-Neto et al., 2015).

This finding indicates that although some seed treatments affected the physiological quality of seed, there was no significant effect on the establishment of the plants in the field and yield. Hence, the pre-inoculation of soybean seed using the HiCoat® technology could be performed on treated seeds and stored for up to 51 days without compromising yield. Some companies have added nematicides and micronutrients such as cobalt and molybdenum to the seed; therefore, additional studies are required to evaluate the association of these products with pre-inoculation. Likewise, further studies should perform the seed treatment with the machines used for industrial seed treatment in order to verify if a better coverage of the seed would have an impact in the physiological quality. Furthermore, evaluation of other soybean cultivars is necessary to verify whether different genotypes would have distinct effects regarding seeds treatments as well as the response to pre-inoculation.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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# Identification of drought selection indices of common bean (*Phaseolus vulgaris* L.) genotypes in the Southern Highlands of Tanzania

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**A study was conducted to evaluate common bean (*Phaseolus vulgaris* L.) genotypes for drought tolerance in the Southern Highlands of Tanzania. Treatments included 18 genotypes laid out in a 3×18 split plot arranged in randomized complete block design with three replications. The data on yield under water stress and non-water stress treatments were used to calculate indices that can be used for predicting tolerant genotypes. This was accomplished by ranking the yield indices within the selection indices. Results showed that, selection index, YI, identified BFS60, KG104-72 and SER16 as the most tolerant genotypes, while STI, MP and GM identified SER16, BFS60 and KG104-72. Another index, SSPI, identified RCB266, 41-EX-VAM and SER83 as most sensitive genotypes to water deficit while HM showed BFS60, SER16 and KG104-72 as genotypes tolerant to drought. In contrast, SSI discriminated 41-EX-VAM, RCB266 and PASS as most susceptible genotypes under drought. Ranking the means of yield indices, genotypes SER16, BFS60 and KG104-72 were identified as the most drought tolerant genotypes. Correlation analysis showed that Yp were highly significant ($p < 0.001$) and positively correlated with STI, SSPI, MP, HM, and GM while Ys were highly significant ($p<0.001$) and positively correlated with YI, STI, MP, HM and GM. The findings suggest that these indices are effective for discriminating genotypes with higher yields under non stress and stress conditions, respectively. Genotypes, SER16, BFS60 and KG104-72 are among the most tolerant to drought conditions therefore are recommended for cultivation in drought prone environments and subsequently as parental materials in breeding for drought tolerance.**

**Key words:** Common bean, drought, selection indices, yield indices ranks.

## INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is one of the widely cultivated crops in the Southern Highlands of Tanzania. It is considered to be one of the most important legumes for human consumptions as a source of dietary

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protein, calories, dietary fibers and minerals, especially iron and zinc (CIAT, 2008). Bean consumption provides protection from cardiovascular disease by a small depression in blood cholesterol (Kabagambe et al., 2005). In epidemiological studies of colon cancer, low incidence was observed in many Latin American countries where the consumption of common bean is high (Hangen and Bennink, 2002). Clinical studies consistently showed that when consumed solely as a carbohydrate-rich food; beans reduced postprandial glucose elevations in both diabetic and non-diabetic participants (Thompson et al., 2012).

In Tanzania, it is estimated that over 75% of rural households depend on bean for daily dietary requirements (CIAT, 2008). Despite the importance of common beans in Tanzania and other developing countries, its production mostly relies on local cultivars (Miklas et al., 2006; Tryphone et al., 2013). Like other plants, the development and productivity of the bean is adversely affected by biotic and abiotic factors (Jaleel et al., 2009). Among the abiotic factors, drought is the most limiting factor in crop production worldwide (Jones and Corlett, 1992; Sani et al., 2018) and is ranked second from insect pests and diseases that cause grain yield losses of about 60% of world bean producing areas. With the evolving phenomena of climate change, it is anticipated that drought will exert increasing impacts on crop productivity (Man et al., 2011). Drought causes reduction in yield, yield components and biomass accumulation of common beans (Munoz-Pereaet al*.,* 2006; Ambachew et al., 2015; Darkwa et al., 2016). Drought tolerance implies that the ability of a crop to grow and produce under water deficit conditions. A long term drought stress affects plant metabolic reactions associated with plant growth, water storage capacity and physiological performance of plants. In the Southern Highlands of Tanzania, the bulk of bean production is from small scale farmers who depend entirely on seasonal rainfall. In these areas, intermittent and/or terminal droughts are experienced in some years, while supplementing crops with irrigation during drought periods is uncommon and unaffordable. Therefore, variety evaluation for drought tolerance in the common bean is the most appropriate approach for plant breeders to identify superior genotypes for varieties development (Abebe et al., 1998; Darkwa et al., 2016). Selection indices, which provide a measure of drought tolerance based on loss of yield under drought and normal conditions have been used for screening genotypes (Mitra, 2001). Under water deficit conditions, crop plants resistance against damage has always been of great value and has been considered as one of the breeding objectives. In order to evaluate response of plant genotypes to drought stress, some selection indices based on a thematical relation between stress and optimum conditions have been developed. Therefore, plant breeders who are interested in genotypes which produce high yields under stressed condition came out with the use of drought tolerance selection indices for identifying high yielding genotypes. These indices include stress tolerance index (STI) (Rosielle and Hamblin, 1981), stress susceptibility percentage index (SSPI) (Moosavi et al*.,* 2008), drought resistance index (DI) (Blum, 1988), stress tolerance and mean productivity (MP) (Rosielle and Hamblin, 1981), geometric mean productivity (GMP), harmonic mean (HM) (Jafari et al., 2009), stress susceptibility index (SSI) (Fischer and Maurer, 1978) and yield stability index (YSI) (Bouslama and Schapaugh, 1984). Some of these indices, however, have not been tested under Tanzania soil and weather conditions. Thus, scanty information is available on the use of these indices for evaluating bean genotypes under drought conditions. The objective of this study was to apply drought stress selection indices and identify drought tolerant genotypes to be used for breeding purposes in the Southern Highlands of Tanzania to advance bean production.

## MATERIALS AND METHODS

The experiment was conducted at Inyala Agricultural Training Institute located at latitude 84°7'S, longitude 36° 51 E' and altitude of 1100 m above sea level (m. a. s. l). This location experiences unimodal rainfall pattern, which occurs between November and May every year. The overall average temperature is 17.5°C. The heaviest rainfall occurs from December to March. The soil characteristics of this area are loamy, slightly acidic with a pH of 5.5. Before planting, the land was cleared, ploughed and harrowed using oxen-pulled equipments. Composite soil samples were collected using a hand hoe at a depth of 15 to 20 cm and analyzed for physical and chemical characteristics at Uyole Agricultural Research Institute (ARI) soil laboratory (Table 1).

Weather data such as rainfall, minimum and maximum temperature, relative humidity and solar radiation were collected at Uyole weather station (Table 2). At planting, 18 common bean genotypes in which seventeen were known to be resistant to drought viz, SER125, MR13905-6,41-EX- VAM, BFS20, RCB233, CZ109-22, CZ104-61, KG25-21, SER82, SER83, KG104-72, SER16, KG4-30, SER45 SER124, BFS60, RCB266 and a susceptible check, PASS, were obtained from ARI-Uyole. The selection criteria of these genotypes for evaluation were based on seed size, yield, shoot types, field performance, resistance to drought and disease resistance. Fertilizers used were: triple super phosphate (TSP) (45% $P_2O_5$) and Urea (46% N). The experiment was laid out in 3 × 18 split plots arranged in a randomized complete block design (RCBD) with three replications. Plot size was 2 × 2 m and a spacing of 0.50 × 0.10 m resulting in a plant population of 200,000 plants per ha. The main plot (factor A) was water treatment at three different stress periods and sub plot (factor B) were 18 common bean genotypes. Planting was done in June 2015 by putting two seeds per hole at 5 cm depth in each row. Fertilizers were applied uniformly at a rate of 25.3 kg P/ha and 22.5 kg N/ha. Seven days after planting, seedlings were thinned to one plant per hill. Spraying with Amecron 50 EC insecticide at a rate of 2 mL/l was carried out to control bean stem maggot, termites and other insects by using a knap sack sprayer. Weeding was done three times using a hand hoe. Water stress was induced to main plots at flowering and mid pod filling when the plants had already attained 50% flowering and mid pod filling stages, respectively. The duration for water stress at both flowering and mid pod filling stages was 20

**Table 1.** Physical and chemical characteristics of soil collected from the experimental site.

| Item | Unit | Quantity | Remarks (London, 1991) |
|---|---|---|---|
| **Physical characteristics** | | | |
| Clay | % | 28.43 | |
| Silt | % | 33.01 | Sandy loam |
| Sand | % | 48.59 | |
| | | | |
| **Chemical characteristics** | | | |
| Soil pH (1:25) $H_2O$ | pH | 5.3 | Slightly acidic |
| CEC | cmol(+)/kg | 15.41 | High |
| K | cmol(+)/kg | 0.12 | Low |
| Ca | cmol(+)/kg | 4.49 | Medium |
| Mg | cmol(+)/kg | 2.14 | Medium |
| TN | % | 0.13 | Low |
| OC | % | 0.82 | Low |
| P | mg/kg | 15.3 | Medium |

**Table 2.** Summarized mean monthly weather data collected during the experimental period.

| Month | Rainfall (mm) | Temperature (°C) | | Relative humidity (RH%) | Radiation ($MJm^{-2}d^{-1}$) |
|---|---|---|---|---|---|
| | | Maximum | Minimum | | |
| May | 0 | 23.92 | 5.2 | 72.1 | 18.68 |
| June | 0 | 23.52 | 8.69 | 70.73 | 17.72 |
| July | 0 | 20.5 | 8.6 | 72.7 | 18.21 |
| August | 0 | 23.75 | 7.37 | 57.93 | 18.49 |
| September | 0 | 17.06 | 11.3 | 60.17 | 18.73 |
| October | 0 | 27.33 | 10.3 | 62.97 | 18.17 |
| November | 0 | 23.08 | 14.05 | 69.6 | 18.62 |

Source: Uyole Meteorological Station (2015).

days.

After harvest, yields of genotypes grown under non-water stress and water stress at flowering were used for calculating yield indices. These included, yield index (YI), stress tolerance index (STI), stress susceptibility percentage index (SSPI), mean productivity (MP), harmonic mean (HM), geometric mean productivity (GMP) and stress susceptibility index (SSI). They were calculated using the following relationships:

SSPI = $(Y_p - Y_s / 2 (\bar{Y}_p)) \times 100$ (Moosavi et al., 2008),
STI = $(Y_s \times Y_p) / Y^2{}_p$ (Fernandez, 1992),
YI= $(Y_s) / (\bar{Y}_s)$ (Gavuzzi et al., 1997),
MP = $(\bar{Y}s - \bar{Y}_p) / 2$ (Hossain et al., 1990),
SSI= $(1 - (Ys / Yp)) / (1 - (Ys / Yp))$ (Fischer and Maurer, 1978),
GMP = $\sqrt{YpxYs}$ (Fernandez, 1992)
HM = $\frac{2(YpxYs)}{Yp+Ys}$ according to Fernandez (1992).

where $Y_S$ and $Y_P$ are stress and non-stress (potential) yield of a given genotype, respectively. $\bar{Y}s$ and $\bar{Y}_p$ are average yields of all genotypes under stress and optimal conditions, respectively. Data analysis was carried out using GenStat 14th edition software and correlation coefficients among selection indices and grain yields under water stress and non-water stress conditions was performed.

## RESULTS AND DISCUSSION

### Identification of drought tolerant genotypes through ranking the yield indices calculated from a specific drought selection index

In order to investigate suitable stress resistance indices for screening of bean genotypes for drought tolerance, grain yield response to/under both non-stressed and stressed conditions were measured. This was used for calculating different sensitivity and tolerance indices (Table 3). It was noted that YI identified BFS60 and KG104-72 as the most drought tolerant. Using STI, MP and GMP, the genotypes, SER16, BFS60 and KG104-72 were identified as best cultivars for growing under drought conditions. Thus, these indices are useful for identifying genotypes that yield best under non-stressed and severe stressed conditions. These results are in

**Table 3.** The means of yield under stress and non-stress conditions and drought tolerance selection indices of bean genotype.

| Genotype | YP (kg/ha) | YS (kg/ha) | YI (%) | STI (%) | SSPI (%) | MP (%) | HM (kg/ha) | GMP (kg/ha) | SSI (%) |
|---|---|---|---|---|---|---|---|---|---|
| SER82 | 1713 | 916 | 1.111 | 0.507 | 23.236 | 1314.5 | 1193.692 | 1252.600 | 0.888674 |
| KG104-72 | 1939 | 1041 | 1.263 | 0.653 | 26.181 | 1490 | 1354.697 | 1420.700 | 0.884586 |
| RCB266 | 2071 | 733 | 0.889 | 0.491 | 39.009 | 1402 | 1082.77 | 1232.089 | 1.234007 |
| SER125 | 1623 | 956 | 1.160 | 0.502 | 19.446 | 1289.5 | 1203.248 | 1245.630 | 0.784962 |
| KG25-21 | 1802 | 741 | 0.899 | 0.432 | 30.933 | 1271.5 | 1050.163 | 1155.540 | 1.12461 |
| SER16 | 2183 | 1015 | 1.231 | 0.716 | 34.052 | 1599 | 1385.707 | 1488.540 | 1.021952 |
| PASS | 1200 | 461 | 0.559 | 0.179 | 21.545 | 830.5 | 666.1048 | 743.770 | 1.176264 |
| SER124 | 1830 | 841 | 1.020 | 0.498 | 28.834 | 1335.5 | 1152.4 | 1240.576 | 1.032254 |
| BFS20 | 1306 | 684 | 0.987 | 0.344 | 18.134 | 995 | 897.793 | 945.148 | 0.90968 |
| CZ104-61 | 1807 | 759 | 0.921 | 0.443 | 30.554 | 1283 | 1068.989 | 1171.116 | 1.107757 |
| KG4-30 | 1240 | 859 | 1.042 | 0.562 | 11.108 | 1049.5 | 1014.921 | 1032.066 | 0.586874 |
| CZ109-22 | 1991 | 957 | 1.161 | 0.616 | 30.146 | 1474 | 1292.664 | 1380.358 | 0.991952 |
| MR13905-6 | 1347 | 961 | 1.166 | 0.419 | 11.254 | 1154 | 1121.722 | 1137.750 | 0.547345 |
| RCB233 | 1892 | 830 | 1.007 | 0.508 | 30.962 | 1361 | 1153.828 | 1253.140 | 1.072124 |
| SER83 | 1906 | 841 | 1.020 | 0.518 | 31.050 | 1373.5 | 1167.052 | 1266.075 | 1.067255 |
| BFS60 | 1999 | 1085 | 1.316 | 0.701 | 26.647 | 1542 | 1406.56 | 1472.724 | 0.873323 |
| 41-EX-VAM | 1373 | 300 | 0.364 | 0.133 | 31.283 | 836.5 | 492.4088 | 641.794 | 1.492694 |
| SER45 | 1648 | 728 | 0.883 | 0.388 | 26.822 | 1188 | 1009.886 | 1095.324 | 1.066282 |

Yp=Yield under non-stress, Ys=Yield under stress, YI=Yield index, STI= Stress tolerance index, SSPI = Stress susceptibility percent index, MP = Mean productivity, HM = Harmonic mean, GMP = Geometric mean productivity and SSI = Stress susceptibility percent index.

consistence with the findings of other authors' works (Kargar et al., 2004; Abdipour et al., 2008). On the other hand, SSPI identified RCB266, SER16 and 41-EX-VAM as the most drought tolerant genotypes. Further, HM identified BFS60, SER16 and KG104-72 as the most water stress tolerant genotypes, while SSI selected 41-EX-VAM, RCB266 and PASS as the most sensitive genotypes to drought stress. Therefore, genotype rankings by the drought indices were different from index to index. Therefore, different indices introduced different genotypes as a drought tolerant. According to previous studies, detection of drought tolerant genotypes has been suggested that by screening genotypes in non-drought conditions, both adaptability and yield potential are accessible (Kirigwi et al., 2004); but under stress environments, selection of genotypes with high yield performance can be favoured (Ceccarelli et al., 1992). According to Trethowan et al. (2002) and Fernandez, (1992), selection in alternating stress and non-stress drought environments enhance progress in development and selection of varieties. Further, selection for drought tolerance should be made using drought tolerance indices based on yield under both conditions for widely adapted genotypes (Sio-Se Mardeh et al., 2006).

## Identification of drought tolerant genotypes by ranking means of yield indices of all selection indices

Breeding for drought tolerant is challenging and time-consuming, due to the need for simultaneously considering multiple abiotic and biotic factors modulating the level of drought-tolerance. The identification of drought tolerant genotypes based on single criterion was observed to be contradictory since different indices identified different genotypes as best drought tolerant. Therefore, to determine the most desirable drought tolerant genotype according to all selection indices, the mean rank of ranks of all drought tolerance criteria was calculated (Table 4). Based on these results, genotype with lowest mean was identified as the most tolerant. Genotypes with higher yields under all conditions can be used as parental materials for breeding purposes and will be suited in areas with short rains as well as in areas with long rains. In considering ranking of the means of all genotypes, SER16, BFS60 and KG104-72 exhibited the best ranks and they were considered as the most tolerant genotypes, while 41-EX-VAM, PASS and BFS20 as most sensitive genotypes under water stress conditions. That means, under water stress condition there was a reduction in shoot dry biomass as the result of decreasing photosynthesis, increasing growth inhibitors and decreasing hormones for the sensitive genotypes to partition the assimilates. These results are supported by authors' works (Farshadfar et al., 2012; Khalili et al., 2012).

The genotypes which possess high values of STI, MP, and GMP are considered tolerant to water stress. Genotype SER16 was ranked as the best based on STI, MP, and GMP indices and was considered to be the most

**Table 4.** The ranks of drought indices and ranks of means of drought indices ($\bar{R}$) of bean genotypes.

| Genotype | Yp (kg/ha) | Ys (kg/ha) | YI (%) | STI (%) | SSPI (%) | MP (kg/ha) | HM (kg/ha) | GM (kg/ha) | SSI (%) | $\bar{R}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| SER82 | 11 | 7 | 7 | 5 | 13 | 9 | 6 | 7 | 13 | 9 |
| KG104-72 | 5 | 2 | 2 | 3 | 12 | 3 | 3 | 3 | 14 | 5 |
| RCB266 | 2 | 14 | 14 | 11 | 1 | 5 | 11 | 10 | 2 | 8 |
| SER125 | 13 | 6 | 6 | 9 | 15 | 10 | 5 | 8 | 16 | 10 |
| KG25-21 | 10 | 13 | 13 | 13 | 6 | 12 | 13 | 12 | 4 | 11 |
| SER16 | 1 | 3 | 3 | 1 | 2 | 1 | 2 | 1 | 10 | 3 |
| PASS | 18 | 17 | 17 | 17 | 14 | 18 | 17 | 17 | 3 | 15 |
| SER124 | 8 | 10 | 10 | 10 | 9 | 8 | 9 | 9 | 9 | 9 |
| BFS20 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 12 | 16 |
| CZ104-61 | 9 | 12 | 12 | 12 | 7 | 11 | 12 | 11 | 5 | 10 |
| KG4-30 | 17 | 8 | 8 | 5 | 18 | 15 | 14 | 15 | 17 | 13 |
| CZ109-22 | 4 | 5 | 5 | 4 | 8 | 4 | 4 | 4 | 11 | 5 |
| MR13905-6 | 15 | 4 | 4 | 14 | 17 | 14 | 10 | 13 | 18 | 12 |
| RCB233 | 7 | 11 | 11 | 7 | 5 | 7 | 8 | 6 | 6 | 8 |
| SER83 | 6 | 9 | 9 | 6 | 4 | 6 | 7 | 5 | 7 | 7 |
| BFS60 | 3 | 1 | 1 | 2 | 11 | 2 | 1 | 2 | 15 | 4 |
| 41-EX-VAM | 14 | 18 | 18 | 18 | 3 | 17 | 18 | 18 | 1 | 14 |
| SER45 | 12 | 15 | 15 | 15 | 10 | 13 | 15 | 14 | 8 | 13 |

Yp=Yield under non-stress, Ys=Yield under stress, YI=Yield index, STI= Stress tolerance index, SSPI = Stress susceptibility percent index, MP = Mean productivity, HM = Harmonic mean, GMP = Geometric mean productivity, SSI = Stress susceptibility percent index and $\bar{R}$= Mean of rankings.

drought tolerant and high-yielding under favourable and severe drought stress conditions (Table 4). This implies that the strong association between photosynthate assimilation and better remobilization of carbohydrates by drought-tolerant genotypes permits them to maintain high yield under water stress conditions. The findings are in agreement with that of Kargar et al. (2004), Abdipour et al. (2008) and Ilker et al. (2011) who recommended similar indices as the best in selecting high yielding wheat genotypes in both stress and non-stress conditions. Further, Jafari et al. (2009) indicated that STI was more useful in order to select favourable corn cultivars under stress and non-stress conditions. However, Khodrahmpour et al. (2011) and Khalili et al. (2012) noted that the best index to select varieties is STI as it can separate varieties which have high yield in both stress and non-stressed conditions.

### Identification of suitable selection indices

Selection based on a combination of indices may provide a more useful criterion for improving drought tolerance; therefore, a suitable index must significantly correlate with grain yield under both conditions (Farshadfar et al., 2001; Mitra, 2001; Eddie et al., 2016). Correlation analysis was carried out among grain yield and drought tolerance indices to be used for screening the best genotype and indices for drought tolerance studies. To determine the most desirable drought tolerance selection indices, correlation coefficients between Yp and Ys and other quantitative drought indices were calculated. Results of correlation analysis (r) between yield in a non-water stressed, water stressed conditions and drought indices indicated that yield in a non-water stress condition was significant and positively correlated with YI (r = 0.5008*), STI (r = 0.7196***), SSPI (r = 0.7624***), MP (r = 0.9274***), HM (r = 0.7369***) and GM (r = 0.8346***); indicating that these indices are effective in identifying genotypes that yield high in non-water stressed environments. Yield in water-stressed conditions (Ys) was significant and positively correlated with YI (r = 0.988***), STI (r = 0.9238***), MP (r = 0.8194***), HM (r = 0.9659***) and GM (r = 0.9154***), implying that, these indices are more effective for selecting genotypes with high yields under water stressed conditions (Table 5).

On the other hand, Yp and Ys were significant and positively correlated with YI, STI, MP, HM and GM. This signifies that, latter indices can be used for selecting genotypes that yield high in both non stressed and stressed conditions. These observed relationships are in consistency with other studies. Toorchi et al. (2012) showed that correlation between MP, GMP, Ys and Yp was positive and significant. Khalili et al. (2012) reported that GMP, MP, and STI were significantly and positively correlated with yield under both conditions. The correlation coefficients indicated that MP, STI, GMP and HARM were the best criteria for identifying high yielding genotypes under stress and non-stress conditions (Zare, 2012; Kargar et al., 2014; Khalili et al., 2014). Farshadfar

**Table 5.** Correlation coefficient between Yp, Ys, and drought tolerance selection indices.

| Variable | S/N | Yp | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|
| Yp (kg/ha) | 1 | - | | | | | | | | |
| Ys (kg/ha) | 2 | 0.5456* | - | | | | | | | |
| YI (%) | 3 | 0.5008* | 0.988*** | - | | | | | | |
| STI (%) | 4 | 0.7196*** | 0.9238*** | 0.9031*** | - | | | | | |
| SSPI (%) | 5 | 0.7624*** | -0.1264ns | -0.1702ns | 0.1384$^{ns}$ | - | | | | |
| MP (kg/ha) | 6 | 0.9274*** | 0.8194*** | 0.7834*** | 0.9044*** | 0.4651ns | - | | | |
| HM (kg/ha) | 7 | 0.7369*** | 0.9659*** | 0.9466*** | 0.955*** | 0.1263ns | 0.935*** | - | | |
| GM (kg/ha) | 8 | 0.8346*** | 0.9154*** | 0.8886*** | 0.9473*** | 0.2811ns | 0.9794*** | 0.9873*** | - | |
| SSI (%) | 9 | 0.2047$^{ns}$ | -0.6971** | -0.7215*** | -0.4735* | 0.7806*** | -0.171$^{ns}$ | -0.497* | 0.3583ns | - |

Ns= Non-significant, *Significant at 0.05, **Significant at 0.01, ***Significant at 0.001.

et al. (2001) reported that the most appropriate index for selecting stress tolerant cultivars is one which has partly high correlation with yield under stress and non-stress conditions. Mitra (2001) recommended that a suitable index must have a significant and positive correlation with grain yield under all conditions. The positive correlation between yields of genotypes under unstressed with SSPI implies that it is useful in identifying genotypes that yield higher under unstressed conditions. The negative correlation between Ys and SSPI implies that the latter cannot be used in selecting genotypes with high yield under stressed conditions.

## Conclusion

It is concluded that the rankings of means of ranks for yields under non-water stress, yield under stress and the used selection indices in the current study identified most tolerant genotypes as: SER16, BFS60, KG104-72, CZ109-22 and that YI, STI, MP and GM are suitable selection indices to identify genotypes with higher potential yield under both conditions. Also, SSPI and SSI are suitable for predicting genotypes that would give higher yields under unstressed condition. There is a need to investigate the presence of any physiological mechanisms and types of physiological mechanism involved in providing tolerance under limited moisture at specific plant developmental stages. This knowledge will help to improve selection criteria for drought tolerance of common bean. Genotypes, SER16, BFS60 and KG104-72, were observed to be superior in yield under water stress conditions; therefore they can be used as sources of breeding materials for drought tolerance in areas which are affected by drought at flowering. In areas where droughts occur during mid pod filling, genotypes KG4-30, RCB266, KG104-72 and SER125 should be used as sources of breeding materials for drought tolerance. In screening genotypes with high yields under non-water stress and water stress conditions, YI, STI, MP and GM indices can be used.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Comparative performance of commonly used portable coffee harvesters

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**In mountain regions due to the high slopes, the coffee harvest is carried out manually, generating higher production costs. An alternative to reducing these costs is to use portable breakers. The objective of this work was to evaluate the efficiency of portable coffee breakers in coffee harvesting. The experiment was carried out in a field of Catucaí Amarelo 24/137, 3.5 years old. In the study, six types of breakers were tested, in addition to an extra treatment, the experiment was delineated in randomized blocks with four replicates. The number of broken coffee, remaining coffee, number of broken branches that fell on the melting cloth, number of primary and secondary branches present in the plants and defoliation were evaluated. The commercial bruising Brudden promoted a greater amount of broken branches fallen in the cloth of derrick. Regarding the defoliation, it was observed that the Brudden melt promoted the highest values, followed by commercial brands AGS Dupla, Nakashi and Sthil WR9. The treatments Sthil WR6 / 2 and WR6, and Sthil associated to the rubber extensors obtained the lowest values of defoliation. Brudden was also the one that promoted the largest amount of broken branches accounted for in the plant. Based on that, results showed that harvest process using portable milling machines has high harvesting efficiency.**

**Key words:** Operational efficiency, defoliation, *Coffea arabica.*

## INTRODUCTION

Brazil is the world's largest producer of coffee for 150 years, being that this product is mainly responsible for the economic strengthening of the country. Over the years, the management of crops has been changing until reaching the molds of today, with greater population density, the coffee plants approach in the line and

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separation between lines to enable the mechanization. The profit of the activity is closely linked to the possibility of mechanization of operations (Santinato et al., 2015). In flat and undulating areas, harvesting can be done with harvesters, but in more steep areas there must be alternatives to increase the operational performance of manual operators.

In Brazil, the substitution of manual harvesting by mechanized alternatives has become evident and extensive in the last 30 years (Matiello et al., 2015). Nevertheless, it is impossible to use harvesters (trawlers or self-propelled), in some large areas with slopes, similar to what happens in other producing countries from Central America like Colombia (Cárdenas et al., 2015; Santinato et al., 2016a; Tavares et al., 2016). In addition to that, coffee harvesters (trawlers or self-propelled) are complex machines (Silva et al., 2018) with high cost and components often subjected to vibrations (Souza et al., 2018), increasing maintenance costs. Due to the high cost of alternatives for manual harvesting (Santinato et al., 2015) the use of manual harvesting machines has experienced an upsurge, which reduce labor cost and have considerable superior operational field capacity to that of harvesting by hand (Barbosa et al., 2005; Souza et al., 2005, 2006).

Plant damage is one of the drawbacks of semi-mechanized harvesting (using manual harvesters), especially for young plants (Barros et al., 1995); however, there is controversy regarding the use of portable harvesters, and there are still controversies, lacking this data. Santinato et al. (2016a) reported a substantial reduction in plant damage via the use of flexible extenders at the tip of coffee harvesters' rods. Plant damage might be further reduced by adapting such extenders at the tip of portable harvesters' rods. Consequently, the objective of this study was to evaluate the efficiency of portable harvesters used with flexible extensors and their morphological effects in coffee plants.

## MATERIALS AND METHODS

The experiment was carried out at Fazenda São Lourenço in the municipality of Manhuaçu, in the Zona da Mata region of Minas Gerais. Coffee trees of the Catucaí Amarelo cultivar 24/137 aged 3.5 years (second crop) were planted in a humic Oxisol (LVh) at 2.8 × 0.80 spacing in a slope of 18% and in dry conditions.

In this study, six types of manual harvesters (the most used portable coffee harvesters) were tested. Moreover, we included an additional treatment to test whether portable coffee harvesters with rubber extenders at the tip of rods would generate a faster, larger, safer (for the plant) harvest than that of harvesters without extenders (Figure 1).

We evaluated Shindaiwa 230 engine and double Brudem derrick (T1); Stihl KA 85 engine and Stihl WR6 / 2 derrick (T2); Stihl KA 85 engine and Stihl WR6 derrick (T3); Stihl KA 85 engine and Stihl WR9 derrick (T4); Husqvarna 226 engine and double AGS derrick (T5); Mitsubish engine and Nakashi melter (T6) and Sthil KA85 engine and Sthil WR6 more extensors (T7), and more information and technical specifications of the equipment can be obtained in their commercial catalogs. The seven treatments were carried out in randomized blocks, with four replicates and eight plant plots. The experiment began on July 3, 2017, and on this date the crop presented 6.64 L per plant. In this date, the fruits observed were in distinct stages of maturation: 26, 47 and 27% were in the green, cherry, and dry maturation stages, respectively.

**Figure 1.** Flexible extenders placed at the tip of the portable derriere rods.

The present study evaluated the amount of harvested coffee, remaining coffee, number of broken branches fallen in the harvest cloth, number of primary and secondary branches on the plant, and operational defoliation (leaves that have fallen in the harvest cloth) following the methodology of Santinato et al. (2014). Furthermore, the coffee brewing time and the harvest time per plant were measured, and then, the proportional amount of time required to brew one liter of coffee was calculated. Finally, the data were submitted to analysis of variance (ANOVA) and when applicable to Duncan's test, both were at 5% probability.

## RESULTS AND DISCUSSION

Among all of the tested harvesters, the Brudden model caused the largest number of broken branches that have fallen on the cloth, significantly higher than that of other models. This could be attributed to its structure, because the rods have bifurcations that may break the branches depending on the movements performed by the operator; increasing the oscillation angle of the adjacent plates supporting the rods might consequently overcome this issue. Notably, the differences among the other harvester models were not statistically significant. Nonetheless, there was a trend for reduced damage by the Sthil WR6 model, and a noticeable tendency for a lower number of broken branches when using rubber extenders in splitter rods' tips, which reduced the amount for 9.56 branches only.

The operational defoliation values were the highest for the Brudden harvester, followed by the AGS Dupla, Nakashi, and Sthil WR9, whereas the lowest values were obtained using harvesters with rubber extensors, namely, Sthil WR6-2 and WR6, and Sthil. This suggests that double harvesters promote greater operational defoliation than simple harvesters.

Brudden also produced the largest number of broken branches accounted for in the plant. In the background, AGS Dupla, Nakashi, and Sthil were used with rubber extensors. The Sthil harvester with the rubber extensors installed has a small distance between its rods, requiring the operator to force the harvester into the plant and therefore causing several branches to break. Despite breaking only a small number of branches off the plant, this harvester produced a large amount of broken primary branches. This was because only the green, living branches that have fallen in the cloth were considered, as the weak, dry branches in the plant were already counted.

The highest number of broken secondary branches in the plant was produced by the Nakashi harvester followed by the AGS Dupla and Brudden. The other harvesters generated insignificant amounts of broken secondary branches.

Reduction of the number of nodes reflects a reduction in productivity (Martinez et al., 2007). When measuring this, Barros et al. (1995) obtained higher values of broken branches than those of the present experiment, which demonstrates that portable harvesters have evolved and improved.

The values for functional defoliation were lower than those obtained in experiments comparing manual harvesting against mechanized harvesting (Silva et al., 2010; Santinato et al., 2015). Plant defoliation reduces the capacity of plants for active synthesis, which affects and reduces coffee productivity (DaMatta et al., 2007).

Santinato et al. (2016b) indicated that reduction of plant defoliation when harvesting with rubber extenders is caused by the materials being less rigid than those of the harvester (fiberglass or plastic rods). Damage done to the plant's bark was an observed but unmeasured aspect; because of their relatively smooth surface, rubber extenders did not damage the bark of coffee trees (Figure 2), whereas the other treatments evidently did. Damage to the branches' bark is a gateway for pests and diseases and promotes lower sap circulation, which culminates in drought of the branches or dieback (Malavolta et al., 2002). Considering that the higher the productivity, the greater the defoliation and the breakage of branches (Souza et al., 2006), it is worth noting that the experiment was carried out in a high-productivity field. Therefore, it is estimated that the values may be even lower in plants with lower crop load than those obtained herein (Table 1).

The amount of coffee harvested did not differ significantly between treatments, although it varied from 5.72 to 7.38 L per plant. This variation is attributed to the normal variability of coffee plants within the experimental area. Additionally, the amount of remaining coffee ranged from 0.14 to 0.27 L per plant. It was predominantly green and protected by branches either close to the trunk, the upper third, or the lower third, making it difficult to access and hampering the equipment's ability to harvest. The smallest amounts of remaining coffee were obtained using AGS Dupla, Nakashi, and Sthil harvesters with rubber extenders. Regarding the amount of coffee present in the feet, the amount of coffee remaining ranged from 1.97 to 4.1%. The other values of the remaining coffee were attributed to Sthil treatments, in all models. The AGS Dupla harvester generated the least remaining coffee, which consequently increased the harvesting efficiency. The utilization of rubber extensors at the rods' tip optimized the operation of Sthil harvesters, as seen in the Sthil KA85 engine and Sthil WR6 more extensors (T7 treatment); thus, it may be a viable solution to overcome this problem. Extenders may be used in other types of portable harvesters, such as those utilized in Colombia where harvesting is entirely labor-dependent (Cárdenas et al., 2013; Mejia et al., 2013; Cárdenas et al., 2015).

Barros et al. (1995) pointed out quantities of 10% remaining coffee after harvesting using markers. This fact also shows an evolution in the efficiency of the machines to see the smallest amount of coffee remaining in the plants, in all treatments, even if there were significant amount of green fruits in the plants. It is worth noting that the results could be relatively high for crops with fewer green fruits than ripe fruits, as green fruits are strongly retained in the branches (Silva et al., 2013).

Santinato et al. (2016b) described that rubber extenders increase the harvesters' efficiency because of their proximity to the fruits near the trunk of the coffee tree, so that the contact area is increased. Since the oscillation of flexible extenders is relatively high, this potentiates the operation and consequently augments the total coffee harvested in a given amount of time. When using this harvesting system, the amount of remaining coffee is small and therefore should not be considered problematic, especially considering that a well-trained operator could manually collect any remaining fruits in sight.

Double presentation produced the best results with respect to harvesting speed, while the harvester Sthil WR6/2 (T2) required the longest time. The use of rubber extenders failed to significantly reduce the amount of time required for harvest. However, all treatments were faster

**Figure 2.** Branch by the portable cutter without the rubber extenders.

**Table 1.** Number of broken branches per plant dropped on the melting cloth, operational defoliation and number of broken primary and secondary branches present in the plant after application of the treatments, Manhuaçu, 2017.

| Treatment | Number of broken branches per plant collected on the ground cloth | Defoliation | Number of broken branches per plant | |
|---|---|---|---|---|
| | | kg plant$^{-1}$ | Primary | Secondary |
| Shindaiwa 230 engine and double Brudem derrick | 22.16$^{b}$ | 0.73$^{b}$ | 2.69$^{b}$ | 4.81$^{b}$ |
| Stihl KA 85 engine and Stihl WR6 / 2 derrizer | 13.44$^{a}$ | 0.45$^{a}$ | 1.19$^{a}$ | 1.38$^{a}$ |
| Stihl KA 85 engine and Stihl WR6 derrick | 11.78$^{a}$ | 0.47$^{a}$ | 0.91$^{a}$ | 1.38$^{a}$ |
| Stihl KA 85 engine and Stihl WR9 derrizer | 12.63$^{a}$ | 0.54$^{ab}$ | 1.06$^{a}$ | 1.63$^{a}$ |
| Husqvarna 226 engine and double AGS derrick | 14.38$^{a}$ | 0.66$^{ab}$ | 2.13$^{ab}$ | 5.19$^{b}$ |
| Mitsubish engine and Nakashi melter | 14.22$^{a}$ | 0.62$^{ab}$ | 2.0$^{ab}$ | 3.31$^{ab}$ |
| Sthil KA85 engine and Sthil WR6 more extensors | 9.56$^{a}$ | 0.44$^{a}$ | 1.88$^{ab}$ | 1.88 $^{a}$ |
| CV (%) | 32.71 | 25,22 | 46,38 | 51.73 |

*Averages followed by the same letters do not differ from each other, in the columns, by the Ducan test at 5% probability.

**Table 2.** Coffee harvested and remaining per plant, remaining coffee as a function of the pending load, harvest time to harvest each plant and each liter of coffee, Manhuaçu, 2017.

| Treatment | Coffee harvested | Coffee remaining in the plant | | Break time | |
|---|---|---|---|---|---|
| | L plant$^{-1}$ | L plant$^{-1}$ | % | s plant$^{-1}$ | s L$^{-1}$ |
| 1 - Shindaiwa 230 engine and dual Brudden derrick | 7.38$^{a}$ | 0.22$^{ab}$ | 2.92$^{ab}$ | 26.38$^{a}$ | 3.57$^{ab}$ |
| 2 - Stihl KA 85 engine and Stihl WR6 / 2 derrizer | 5.72$^{a}$ | 0.23$^{ab}$ | 4.1$^{b}$ | 27.03$^{a}$ | 4.93$^{c}$ |
| 3 - Stihl KA 85 engine and Stihl WR6 derrick | 6.16$^{a}$ | 0.23$^{ab}$ | 3.91$^{b}$ | 26.19$^{a}$ | 4.42$^{bc}$ |
| 4 - Stihl KA 85 engine and Stihl WR9 derrizer | 6.94$^{a}$ | 0.27$^{b}$ | 3.92$^{b}$ | 25.06$^{a}$ | 3.68$^{ab}$ |
| 5 - Husqvarna 226 engine and double AGS derrick | 7.44$^{a}$ | 0.14$^{a}$ | 1.97$^{a}$ | 20.78$^{a}$ | 2.8$^{a}$ |
| 6 - Mitsubish engine and Nakashi melter | 6.81$^{a}$ | 0.14$^{a}$ | 2.32$^{ab}$ | 23.03$^{a}$ | 3.51$^{ab}$ |
| 7 - Sthil KA85 engine and Sthil WR6 more extensors | 6.06$^{a}$ | 0.16$^{a}$ | 2.51$^{ab}$ | 25.94$^{a}$ | 4.28$^{bc}$ |
| CV (%) | 29.89 | 29.14 | 36.57 | 25.89 | 15.59 |

*Averages followed by the same letters do not differ from each other, in the columns, by the Ducan test at 5% probability.

than manual harvesting, which takes eight times as long (Barbosa et al., 2005) (Table 2).

## Conclusions

1. In general, all portable harvesters have a high harvesting efficiency. They leave a maximum of 4.1% of the load at the feet, which could be manually picked by the operator simultaneously; thus, transfer is not required. The use of rubber extenders at the rods' tips increases harvesting efficiency.
2. Damage caused by portable harvesters is extremely variable among different models. From this study, it could be inferred that double-handed hammers cause more damage than single-handed hammers. The Brudden harvester produced the greatest damage as indicated by most of the parameters evaluated in this study. The use of rubber extenders at the rods' tips reduced plant damage.
3. Double-handed harvesters reduced the amount of time required to harvest coffee.
4. The use of rubber extenders at the end of the harvesters' rods prevented most of the damage to the bark of the branches, which would have favored plant disease.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Adjustments for axial and sieves systems of coffee harvesters

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**During the mechanized harvesting of the coffee, there is a fall of fruits in the order of 10 to 20%. This coffee must be later collected. For that, it is necessary to use harvester adjustments that promote a greater picking efficiency. Therefore, the purpose of this work was to define the best adjustments for the axial and sieves system for coffee harvesters. The experiment was carried out using an axial system harvester (Miac) and a sieves system harvester from Mogiana, in a crop with 6 sc ben ha$^{-1}$ present on the soil. The design of randomized blocks in a 3x4 factorial scheme was used, with three ground speeds: 500, 1100 and 1500 m h$^{-1}$ and four rotations speed: 1400, 1600, 1800 and 2000 rpm. The amount of coffee which remains on the surface on the soil was evaluated after the harvester passage, reap and cleaning efficiency. For the axial system harvester, there was reap efficiency close to 94% (better conditions) and 99% in cleaning efficiency. The reap efficiency for the sieves system harvester was close to 90% and the cleaning efficiency close to 67%. For the axial system harvester, it is recommended that it be operated using the tractor at 1100 m h$^{-1}$ and 2000 rpm; and for the sieves system harvester, the recommendation is 1100 m h$^{-1}$, from 1400 to 1800 rpm rotation.**

**Key words:** Mechanization, mechanical reap, coffee harvest, *Coffea arábica*.

## INTRODUCTION

The mechanical harvesting of coffee is a recurrent practice which increases every year, in the Cerrado of Minas Gerais and in Brazil, in general (Ortega and Jesus, 2011). The harvest of the fruits of the coffee tree is based on six operations: harrowing, threshing, sweeping, reap, sieving and transport. The harvesters' adjustments to remove the highest number of fruits are usually performed by attempts, varying the vibration from 650 to 950 cycles min$^{-1}$ (Silva et al., 2008).

For the coffee-threshing stage, there are several studies (Giraldo et al., 2017; Junior et al., 2016; Santinato et al., 2016; Silva et al., 2010, 2015; Villibor et al., 2016) which show results of the selective harvesting, better adjustments, quality of the operation, among other

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researches. However, there is just few researches for the reap stage operation, and there are no approaches in literature regarding adjustments, effects and losses, among others. The importance of studying this operation is due to the mechanized harvesting of the coffee hardly having crop efficiency close to 100%, since normally 10 to 20% of the coffee of the plant falls during the mechanized harvesting operation. This amount is currently acceptable due to the system of the present harvesters (Matiello et al., 2010).

According to Santinato et al. (2015a), the losses are concentrated in the harvesting system of the harvester, which is the main failure. This is because the collectors open and close slightly as the harvester moves, allowing the fruit to fall in the spaces left. In this way it is assumed that coffee will always be dropped after the plant harvesting operation.

In addition, there is also the natural detachment of the fruits, which only adds up and increases the amount of coffee fallen under the canopy of the coffee tree. Sun exposure is an important factor that influences the natural fall of the fruit. The fruits present in the upper third of the plant ripen faster than the fruits of the middle and lower thirds, falling on the soil with higher intensity (Santinato et al., 2014; Silva et al., 2010).

This coffee, however, is not considered lost, since the producer can and should collect it. It is important to emphasize that coffee that falls on the soil can undergo conditions that deteriorate it, affecting the sensorial form of the product, as well as the value paid for it (Batista and Chalfoun, 2006; Oliveira et al., 2007).

In order to facilitate the harvesting of the coffee sweeping, it is essential that the management of the coffee between lines is adequate, keeping weeds under control and at ground level to facilitate reap (Matiello et al., 2010).

The mechanized reap of the sweeping coffee is composed of two operations, the first consisting of a blower/harrow, responsible for blowing all material to the center of the street, and the second is the reap as the harvester passes, collects and separates the coffee from impurities inside the machine (Matiello et al., 2010).

The mechanized harvesting operation is complex, as reap and separation efficiency is influenced by factors such as soil texture and material plant present on the soil (branches, stumps and leaves). In sandy soils, there is a facility for collecting fruits that have fallen from the plants; in clay soils, due to the higher water content, it is difficult to collect the material present on the soil. Therefore, the operational speed and rotation of the power take-off adopted in the operation influence reap and cleaning efficiency directly.

Due to the few studies on the reap operation, it is important to understand the regulation that is closest to the ideal that aims at higher product quality with minimum loss. As a result, the aim of this study was to define the best adjustments for axial and sieves system coffee harvesters.

## MATERIALS AND METHODS

The experiment was carried out at Fazenda Paraíso 1, located in the city of Carmo do Paranaíba, MG, Brazil, at the geodesic coordinate 19°01'09 " South latitude and 46°14'22 " West longitude, with average altitude of 1000 m and average slope of 8%.

The variety used was the 15-year-old Catuaí Vermelho IAC 144 cultivar with a four meters space between rows and 0.5 m between plants, totaling 5000 plants ha$^{-1}$. The coffee was lined in the center of the streets along with impurities such as soil, stone, and branches among others.

Mechanical harvesting of fallen coffee was carried out by axial system (Miac Master Café 2) and sieves system's (Mogiana Spirlandelli 25A) harvester, the most used harvesters for coffee, both powered by a New Holland TT3880F 4 x 2 TDA coffee tractor with 55.0 kW (75 hp) in the engine. The operation was performed with the economic power take-off activated and rotations that varied according to the treatments studied.

The characteristics of the machines are shown in Table 1, describing their functions and specificities, demonstrating that the cleaning system is specific to each brand and model.

The randomized block design was analyzed in a 3×4 factorial scheme with three operational speeds: 500, 1100 and 1500 m h$^{-1}$, and four rotations speed (1400, 1600, 1800 and 2000 rpm), with 10 replications, in plots of 6 m² (2×3 m). The experiment was performed individually and equally for each of the two harvesters.

Initially, the amount of the initial material was evaluated. For that, all materials present on the soil was collected and the coffee separated using a sieve and manual selection. Subsequently, the coffee was measured in a graduated container. Samples of this variable were collected only for characterization of the area.

The harvester was operated and the residual material was collected after its operation. From this material, only the coffee was separated by using sieve and manual selection, which was subsequently weighed and the volume measured, thus becoming the remaining coffee.

The amount of initial coffee was subtracted by the remaining coffee to obtain the amount of coffee collected. The reap efficiency (%) was obtained by means of the Equation 1 (Tavares et al., 2015).

$$RE = \frac{(Ci - Cr)}{Ci} \times 100 \quad (1)$$

where $RE$ = reap efficiency (%); $Ci$ = Initial coffee quantity (g m$^{-1}$); and $Cr$ = Amount of coffee remaining (g m$^{-1}$).

Inside each harvester, a sample of the material was collected directly from the machine storage after each treatment. The sample was manually separated into coffee and other impurities. After being separated, the samples were weighed and the values transformed into percent, obtaining the purity and impurity of the sample, respectively, as the percentage of purity of the cleaning efficiency of the harvester.

$$CE = \frac{(Sc)}{Sc \times Mm \times Mv} \times 100 \quad (2)$$

where $CE$ = Cleaning efficiency (%); $Sc$ = Sample coffee batter (g); $Mm$ = Mass of the mineral impurity of the sample (g); $Mv$ = Mass of the vegetable impurity of the sample (g).

In possession of the data, the analysis of variation was done and, when appropriate, the Tukey and regression test was applied on each factor at the significance level of 5%.

**Table 1.** Characteristics of axial and sieves system for coffee harvesters.

| Characteristic | Axial system | Sieves system |
|---|---|---|
| Brand and model | Miac Master Café 2 | Mogiana Spirlandelli 25A |
| Linkage | Drawbar and power take-off 540 rpm | Hydraulic bar and power take-off 540 rpm |
| Working width | 1400 mm | 1200 mm |
| Cleaning system | Axial cylinder and suction turbines | Sieves and fans |
| Grain transport | Bucket elevator | Bucket elevator |

*Brands and models do not indicate authors' suggestions.

**Table 2.** Significance levels of the F test (p-values) for reap efficiency (RE) in the axial and sieves systems harvesters.

| Parameter | Degree of Freedom | $F_{\text{Axial system}}$ | $P_{\text{Axial system}}$ | $F_{\text{Sieves system}}$ | $P_{\text{Sieves system}}$ |
|---|---|---|---|---|---|
| GS | 2 | 62.409** | <0.0001 | 322.09** | <0.0001 |
| RS | 3 | 136.10** | <0.0001 | 36.525** | <0.0001 |
| Blocks | 9 | $1.2341^{ns}$ | 0.2832 | $0.8974^{ns}$ | 0.5307 |
| GS × RS | 6 | 29.684** | <0.0001 | 50.125** | <0.0001 |
| Resídue | 99 | - | - | - | - |
| Total | 119 | - | - | - | - |

GS: Ground speed (m h-1); RS: rotation speed (rpm); **Significant at 1% probability; ns: not significant at 5% probability.

## RESULTS AND DISCUSSION

The average volume of the initial coffee (fallen coffee) was on average 360 kg ha$^{-1}$ (6 sc ha$^{-1}$) in the studied area. For the variable reap efficiency, there was interaction between the speed and rotation factors, for both the axial and sieves system harvesters (Table 2), with this variable unfolding as follows.

The 500 and 1100 m h$^{-1}$ ground speeds presented lower reap efficiencies in the rotation speeds of 1400 rpm compared to the larger rotations, a result which was already expected, since it presents the lowest GS and RS ratio for the axial system harvester, obtaining between 35 and 47% of reap efficiency compared to the others, which varied between 84 and 94% for the other regulation combinations (Table 3). This fact is similar to the results found by Tavares et al. (2015), where the increase in RPM influenced the reap efficiency.

At the highest speed (1500 m h$^{-1}$), it was not possible to harvest coffee mechanically at 1400 and 1600 rpm. There was a jam in the machine that in just a few meters stopped working. This fact also occurred in an experiment by Santinato et al. (2015b) that did not obtain an answer from the axial system and sieves system harvesters working at 2200 m h$^{-1}$.

However, for the sieves system harvester, it was observed that the worst reap efficiency (44%) was in the 2000 rpm rotation at the highest speed (1500 m h$^{-1}$). For all other speed combinations and rotation, the reap efficiency was considered good, varying between 80 and 90% (Table 4).

The increase in speed does not change the reap efficiency for all rotations, except for the lowest rotation of 1400 rpm, which presented a linear equation (y = 0.019 + 25). However, this rotation presents the worst values of reap efficiency compared to the larger rotations for the axial system harvester (Figure 1a).

Likewise, we can observe the regressions for the sieves system harvester (Figure 1b), which showed there were no increments of reap efficiency when the speed was increased, except for the rotation of 2000 rpm, where there was a considerable decrease in the highest speed.

Thus, it can be concluded that the axial system harvester does not admit low speed and rotation and sieves system high speed and rotation. For axial system, the ideal rotation is greater than 1600 rpm and for sieves system speeds greater than 2000 m h$^{-1}$ and rotation of 2000 rpm are not meant to be used.

For the cleaning efficiency variable, there was interaction between the speed factors and the rotation, both for the axial system and sieves system harvesters (Table 5), with this variable being shown as follows.

Minor speeds (500 and 1100 m h$^{-1}$) in rotations starting from 1800 to 2000 rpm perform better cleaning efficiency, from 86 to 99%, which are considerably better than 1400 and 1600 rpm, which achieved efficiency of only 29 and 61%, respectively, for the axial system harvester (Table 6). However, it is important to note that the intermediate speed (1100 m h$^{-1}$) at 2000 rpm also showed good cleaning efficiency (91%), which is considered an interesting regulation, since it allows more than twice the speed of displacement, allowing better field operation

**Table 3.** Depth of reap efficiency (RE) in the interaction speed and rotation of the axial system harvester.

| Ground speed ($m\ h^{-1}$) | Rotation speed (rpm) | | | |
|---|---|---|---|---|
| | 1400 | 1600 | 1800 | 2000 |
| 500 | $35.12^{aB}$ | $83.98^{aA}$ | $87.09^{aA}$ | $86.66^{aA}$ |
| 1100 | $46.69^{aB}$ | $86.26^{aA}$ | $92.72^{aA}$ | $88.96^{aA}$ |
| 1500 | - | - | $94.37^{aA}$ | $88.56^{aA}$ |

*Means followed by different lowercase letters in the columns and upper case in the lines differ from each other by the Tukey test for a 5% probability level.

**Table 4.** Deployment of reap efficiency (RE) values in the interaction between speed and rotation for sieves system harvester.

| Ground speed ($m\ h^{-1}$) | Rotation speed (rpm) | | | |
|---|---|---|---|---|
| | 1400 | 1600 | 1800 | 2000 |
| 500 | $82.49^{aA}$ | $90.42^{aA}$ | $85.43^{aA}$ | $87.72^{aA}$ |
| 1100 | $88.34^{aA}$ | $90.62^{aA}$ | $82.71^{aA}$ | $80.41^{aA}$ |
| 1500 | - | - | $83.55^{aA}$ | $44.38^{bB}$ |

*Means followed by different lowercase letters in the columns and upper case in the lines differ from each other by the Tukey test for a 5% probability level.

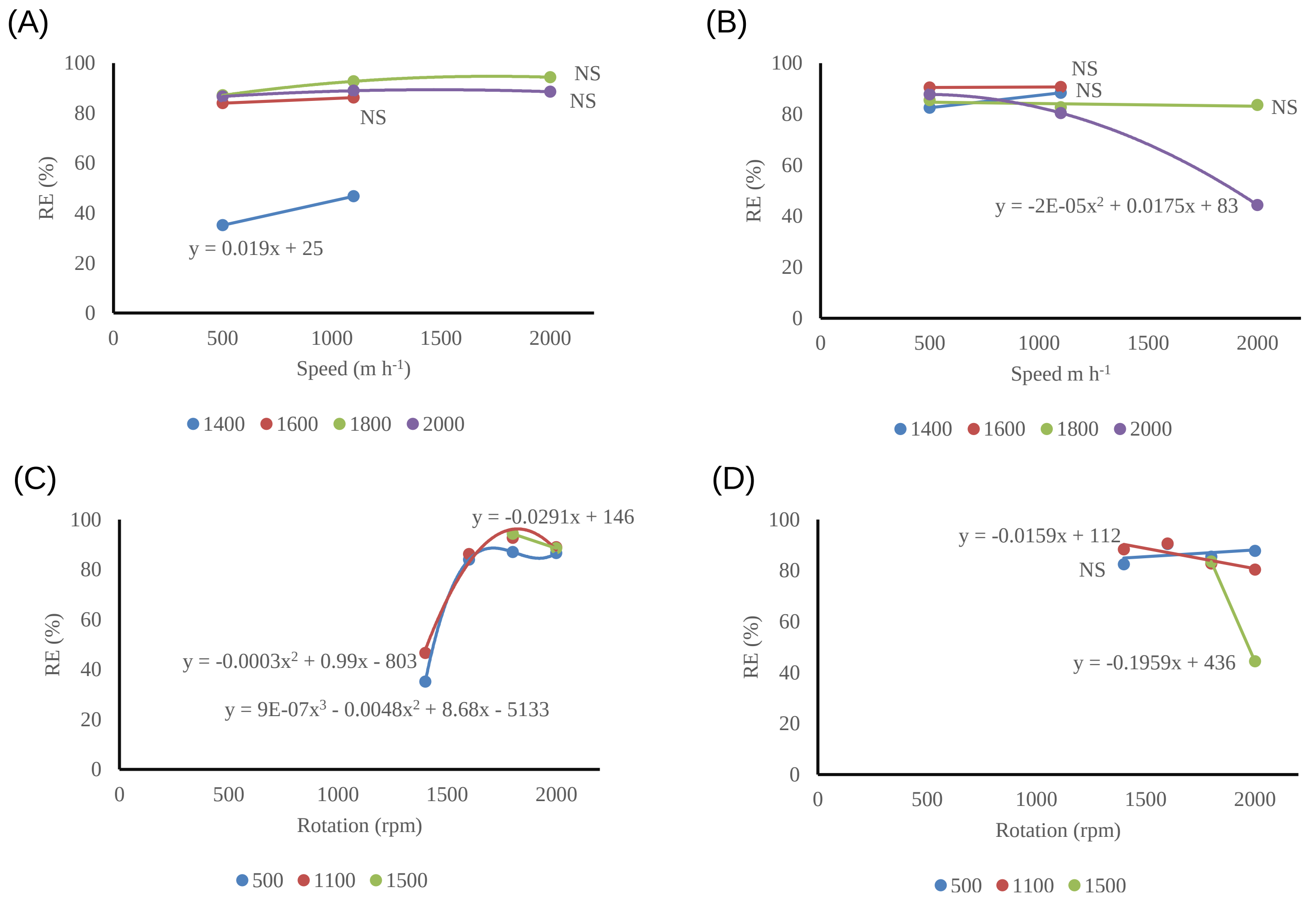

**Figure 1.** Regression efficiency of reap efficiency, as a function of speed factor for Axial system (A), Sieves system (B) and rotation for Axial system (C) and Sieves system (D).

**Table 5.** Significance levels of the F test (p-values) for cleaning efficiency (CE), on axial and sieves systems harvesters.

| Parameter | Degree of Freedom | $F_{\text{Axial system}}$ | $P_{\text{Axial system}}$ | $F_{\text{Sieves system}}$ | $P_{\text{Sieves system}}$ |
|---|---|---|---|---|---|
| GS | 2 | 39.44** | <0.0001 | 76.92** | <0.0001 |
| RS | 3 | 76,68** | <0.0001 | 3.61* | 0.0205 |
| Blocks | 9 | $0.087^{ns}$ | 0.9862 | 3.21* | 0.0213 |
| GS × RS | 6 | 2.44* | 0.0401 | 14.13** | <0.0001 |
| Residue | 44 | - | - | - | - |
| Total | 59 | - | - | - | - |

GS: Ground speed (m $h^{-1}$); RS: rotation speed (rpm); **Significant at 1% probability; ns: not significant at 5% probability.

**Table 6.** Deviation of the cleaning efficiency (CE) values in the interaction speed and rotation of the axial system harvester.

| Ground speed (m $h^{-1}$) | Rotation speed (rpm) | | | |
|---|---|---|---|---|
| | 1400 | 1600 | 1800 | 2000 |
| 500 | $28.78^{aC}$ | $60.80^{aB}$ | $86.48^{aA}$ | $99.48^{aA}$ |
| 1100 | $32.70^{aC}$ | $23.58^{bC}$ | $66.72^{abB}$ | $91.50^{aA}$ |
| 1500 | - | - | $50.72^{bA}$ | $68.74^{bA}$ |

*Means followed by different lowercase letters in the columns and upper case in the lines differ from each other by the Tukey test for a 5% probability level.

**Table 7.** Deviation of the cleaning efficiency (CE) values in the interaction speed and rotation of the motor for sieves system harvester.

| Speeds (m $h^{-1}$) | Rotation | | | |
|---|---|---|---|---|
| | 1400 | 1600 | 1800 | 2000 |
| 500 | $62.40^{aA}$ | $51.66^{aAB}$ | $35.44^{bB}$ | $57.80^{aA}$ |
| 1100 | $67.10^{aA}$ | $58.20^{aA}$ | $63.84^{aA}$ | $49.74^{abA}$ |
| 1500 | - | - | $41.60^{bA}$ | $38.48^{bA}$ |

*Means followed by different lowercase letters in the columns and upper case in the lines differ from each other by the Tukey test for a 5% probability level.

efficiency without impairing product quality.

For the Mogiana collector, lower values are observed in the harvesting efficiency compared to the Miac harvester. First, the velocity factor within the rotations was observed to be at a lower speed (500 m $h^{-1}$), the rotations of 1400 and 2000 rpm were better not differing from 1600 rpm, and the rotation of 1800 was lower (Table 7).

For the sieves system harvester, lower values are observed in the harvesting efficiency compared to the axial system harvester. When analyzing the velocity factor within the rotations, it was observed that at a lower speed (500 m $h^{-1}$) the rotations of 1400 and 2000 rpm were better, not differing from 1600 rpm, and the rotation of 1800 was lower.

For the rotation factor within the speeds, the 1400 rpm rotation showed that the 500 and 1100 speeds presented similar efficiency, not differing from one another, at reasonable values (greater than 60%). For the 1800 rpm rotation, the best speed was the intermediate speed (1100 m $h^{-1}$); finally, the 2000 rpm rotation showed the best cleaning efficiency at the lowest speed (500 m $h^{-1}$).

Therefore, the sieves system harvester does not show an ideal adjustment. There is no linear or quadratic curve that allows inferring the point of maximum cleaning efficiency. The values do not find an increasing trend, aside from being very low values, providing a coffee of lower quality. This is confirmed by regression graphs, where the axial system harvester (Figure 2A and 2C) presented decreasing equations with increasing speed or decreasing rotation, but for the regressions of the sieves system harvester (Figures 2B and 2D) the relationship between the curves for both the speed and the rotation factor is not observed.

The facts verified in this experiment make the necessity of this type of study for each type of harvester evident, since they have different systems. For the grain reap system, the axial system harvester has a 20-to-lifter roller system. For the sieves system machine, the system

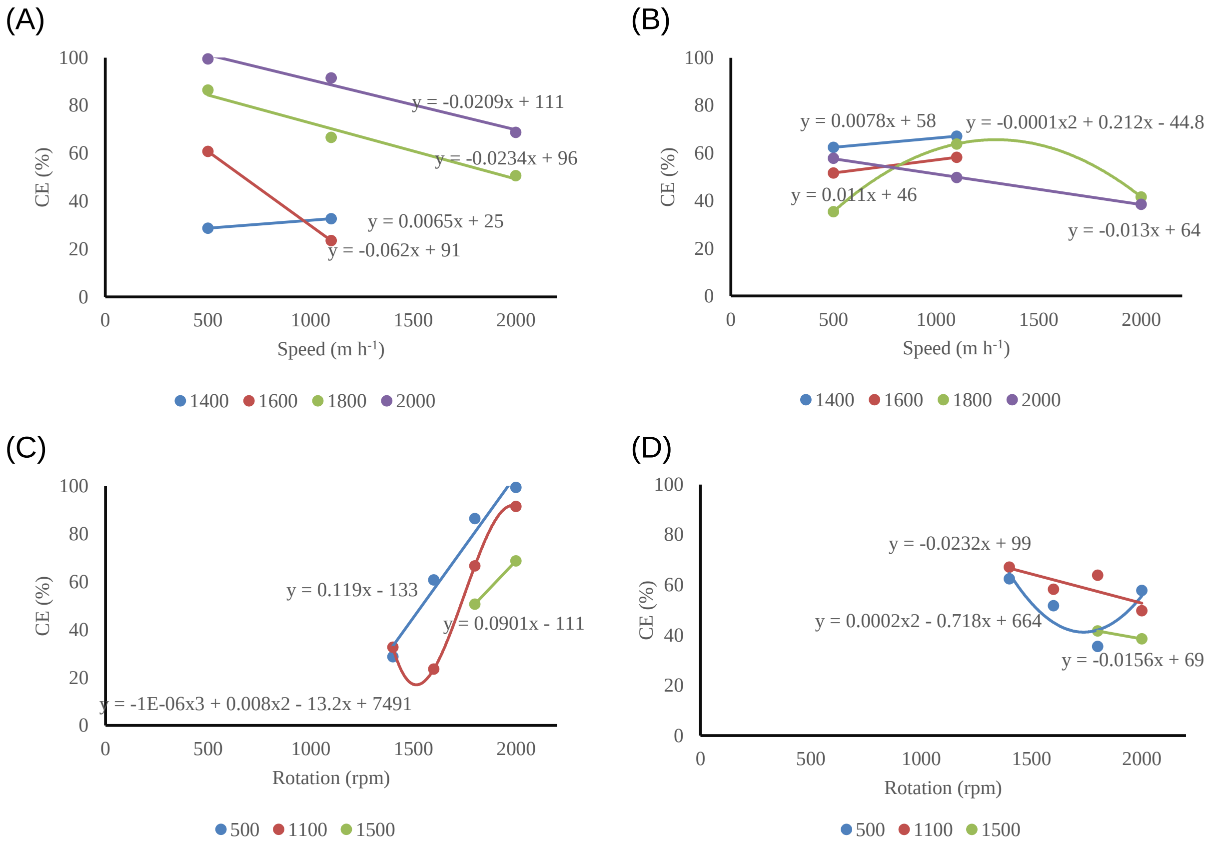

**Figure 2.** Regression of cleaning efficiency, as a function of speed factor for Axial system (A), Sieves system (B) and rotation for Axial system (C) and Sieves system (D).

captures the coffee with the aid of sweeping blades. The cleaning system, the main differential between the collectors, consists of the presence of a turbine that performs the suction of plant material in the axial system machine, in comparison to the sieves system that presents a sieving system. Both present a shaking of sieves system.

In general, it is possible to operate the axial system harvester at a speed of 500 m h$^{-1}$ regulated with 1800 to 2000 rpm or speed of 1100 m h$^{-1}$ set at 2000 rpm, obtaining a good reap and cleaning efficiency. The higher speed offers twice the field efficiency, so it is the main recommendation. For sieves system's harvester, the speed of 1100 m h$^{-1}$ and rotation from 1400 to 1800 rpm is recommended, since it offers higher operating efficiency without compromising the cleaning.

## Conclusion

To operate the axial system harvester, it is recommended the speed of 1100 mh$^{-1}$ and rotation of 2000 rpm be used. The efficiency of reap and cleaning are close to 90%. Regarding the sieves system harvester, it is recommended that it be operated at 1100 m h$^{-1}$, and rotation from 1400 to 1800 rpm. The reap efficiency is between 80 and 90% and the cleaning efficiency is close to 60%.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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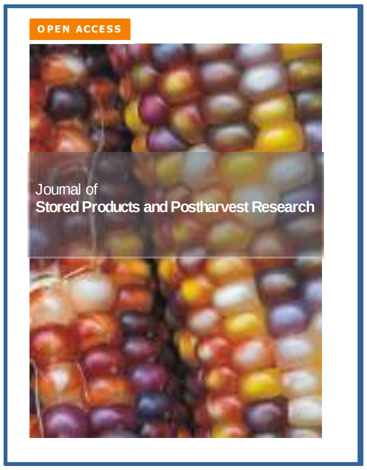

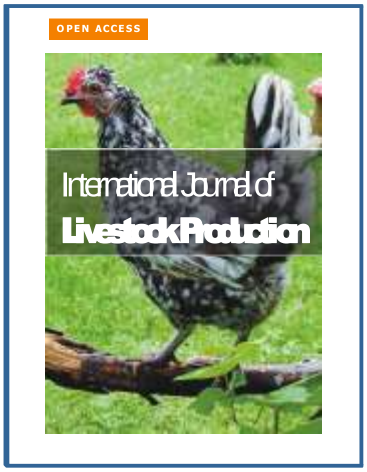

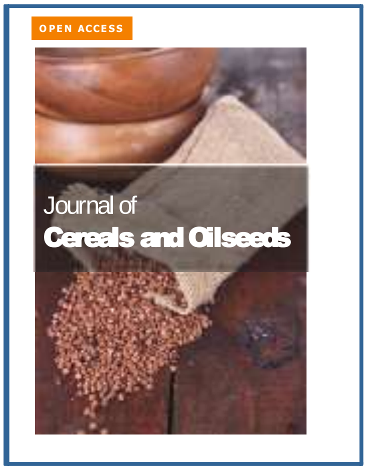

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