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African Journal of Agricultural Research

Review

The effects of drought on rice cultivation in sub-Saharan Africa and its mitigation: A review

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Drought is the primary cause of yield loss in agriculture throughout the world, and is currently the most common reason for global food shortages. Three-quarter of the most severe droughts in the last ten years have been in Africa, the continent which already has the lowest level of crop production and drought adaptive capacity. The increased incidences of drought and erratic rainfall have thrown small holder farmers in Africa into deep poverty, hunger and malnutrition. In this paper, the drought situation in sub-Saharan Africa and its impact on rice production was reviewed. Rice is particularly vulnerable to droughts as it has higher water requirement as compared to other crops. The review has also highlighted physiological and molecular plant responses to drought, with special focus on effects of drought stress on rice grain yield and other related-traits. With climate change predicted to exacerbate the problem of water security in Africa, it is imperative that we develop robust, well-planned and informed strategies to mitigate against drought. Various drought mitigation strategies including breeding for drought tolerance and water harvesting and conservation techniques are also outlined. In order to adapt to drought, there is need for a broad based approach that includes development of appropriate policies, putting in place necessary water related investments and institutions as well as capacity building at various levels.

Key words: Drought, tolerance, rice, sub-Saharan Africa, quantitative trait loci (QTL), mitigation, adaptation.

INTRODUCTION

Drought is inadequacy of water availability including periods without significant rainfall, causing a reduction in available water, thereby affecting crop growth. It can also occur when atmospheric conditions cause continuous loss of water by transpiration or evaporation (Singh et al., 2012), also indicated as a period of dry weather that is injurious to crops. In this context, drought is related to changes in soil and meteorological conditions and not with plant and tissue hydration (Lipiec et al., 2013). Drought is defined as a situation that lowers plant water potential and turgor to the extent that plants face difficulties in executing normal physiological functions (Lisar et al., 2012). Whatever the definition given to drought, it remains perhaps the most serious natural

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hazard, affecting a larger proportion of the human population than any other hazard. It is the most significant environmental constraint for rice production in sub-Saharan Africa (SSA) (Reynolds et al., 2015). Its severity mainly depends on the level of moisture deficiency and the duration.

The challenge of drought is even greater for crops such as rice when compared with other crops such as maize and wheat, as it has relatively higher water needs (Todaka et al., 2015). Rice is sensitive to deficit in soil water content because rice cultivars have been historically grown under flood irrigation conditions where the soil matric potential is zero. About 3,000 to 5,000 L of water is required to produce 1 kg of rice seed, with less than half of that amount needed to produce 1 kg of seed in other crops such as maize or wheat (Bouman et al. 2002). Moreover, as compared to several other field crops, rice has relatively weak resistance to drought and its production systems is more vulnerable to drought than other cropping systems (O'Toole, 2004). In Africa, drought has adversely affected agriculture in different parts of the continent, with production of rice declining in many parts of West Africa due to increasing water stress (Bates and Kundzewicz, 2008). Drought has had significant negative effect on the livelihood of rainfed lowland rice farmers. The increased occurrence of prolonged droughts in SSA is a worrying trend as the region is highly dependent on rainfed agriculture. In order to enhance sustainable crop production in the face of drought and the constantly changing climatic conditions around the world, there is need for constant efforts to adapt our crops and production systems to the existing and emerging environmental challenges. In this review, the challenge of drought and specifically how it impacts rice production in SSA was discussed. Measures that can be undertaken to mitigate the effects of drought are also highlighted.

DROUGHT SITUATION IN AFRICA

The greatest challenges to agricultural production and food security in Africa is drought and climate change. Agriculture in Africa is mainly dependent on rainfall, with only about 5% of Africa's total cultivated land being under irrigation (You, 2008), meaning the region is highly vulnerable to drought. In some sort of fate, drought which continues to degrade some of the most agriculturally productive environments, is predicted to most severely affect the most vulnerable populations particularly those in SSA (FAO/PAR 2011). The recurring droughts in Africa are negatively impacting the livelihoods of a huge proportion of the population, with about 25% of the population facing serious water scarcity (Jarvis et al., 2009). Drought and climate variability are leading to the emergence of novel ecosystems where various plant populations are unable to persist. The proportion of arid and semi-arid areas continues to increase and it is projected that by 2080, ASAL areas in Africa will increase by 6 to 8% (Jarvis et al., 2009). The continued increase in ASAL areas and the emergence of novel ecosystems could render large sections of land unproductive thereby seriously impacting agricultural production in Africa.

Perhaps, the greatest factor contributing to droughts is the rapidly growing human population, with the latest World Bank projections indicating that by 2060, about 2.8 billion people will be found on the continent (Canning et al., 2015). This increase in population puts enormous pressure on the available resources. It will for example lead to opening up of agricultural lands and other productive ecosystems for human settlement, thus leading to loss of valuable biodiversity. Loss of these genetic resources will reduce the diversity of plant responses to biotic and abiotic stresses thereby reducing the resilience and sustainability of agricultural production systems.

Three-quarters of the most severe droughts in the last ten years have been in Africa, the continent which already has the lowest level of crop production. Moreover, this region has the lowest drought adaptive capacity and among the highest levels of poverty, with about 48% of the total population living on less than $1.25 a day (Ravallion et al., 2012). This means that this segment of the human population lacks not only the technical capacity to deal with drought but their financial means to address these challenges is also severely limited. Based on this sad reality and predictions of climate change models, the drought situation in Africa does not look promising. The challenge ahead is hugely enormous but with the concerted efforts of all stakeholders, it will be manageable. Successful fighting of droughts is doable.

RICE PRODUCTION AND CONSUMPTION IN AFRICA

Rice is cultivated under a broad range of environmental conditions in terms of topography, soil type, water regime (various degrees and duration of drought) and climatic factors (Khush, 1996). The persistent droughts in SSA have negatively impacted agricultural production systems, with rice production being among the worst hit systems since the crop is more sensitive to droughts than other crops. The situation is particularly worse in SSA where rice is largely grown under rainfed conditions that rely solely on precipitation, making it vulnerable to droughts. Due to this sensitivity, rice yields reduce significantly even under mild drought (Guan et al., 2010). Moreover, rice varieties planted in Africa have only relatively few adaptations to water-limited conditions and are extremely sensitive to drought, thereby worsening the situation. In Africa, the ecosystems under rice cultivation range from rainfed upland (40% of total area), rainfed lowland (38%), irrigated lowland (12%), deep water/floating (6%) to mangrove swamps (4%). Upland

and lowland rice production which constitute about 80% of the total rice production area in Africa are projected to have the greatest vulnerability to drought (Bimpong et al., 2011a).

Worldwide, more than 3.5 billion people depend on rice for more than 20% of their daily calorie intake (Ricepedia, 2011; Maclean et al., 2013). Rice production is becoming increasingly popular in SSA, especially with the recent release and promotion of new, popular varieties of NERICA (New Rice for Africa) by the Africa Rice Center (formerly known as WARDA). An annual increase in rice consumption of about 6% has been reported (Bernier et al., 2008). With the high urbanization and increase in purchasing power, West Africa is experiencing a significant increase in rice consumption in urban and rural areas.

This increased consumption has also been followed by a concomitant increase in rice production in most African countries. The last 3 decades have recorded a dramatic increase in rice production in Africa, with the production more than doubling in the period between 1982 and 2012 (FAO, 2013). However, despite the increased paddy rice production and the huge potential for rice production in terms of available land area that exists in the sub-region, massive rice imports into SSA are still recorded (Nasrin et al., 2015; AfricaRice, 2009, 2011; Futakuchi et al., 2011). Rice production in West Africa covers only about 60% of the population's needs. This has resulted in increasing rice imports from Asia. With the current trends, according to FAO estimates (Staatz and Dembele 2007), rice imports in West Africa will increase from 6.4 Mt in 2008 to 10.1 Mt in 2020. It is imperative that measures are put in place to boost rice productivity in SSA. These include use of adapted high yielding rice varieties, improved husbandry practices and adoption of various drought and climate change mitigation strategies. Local rice production, processing and marketing will permit African citizens to have access to affordable food. This will contribute to extreme poverty reduction and elimination of food insecurity within the continent, since relying on imports is no longer a sustainable strategy.

EFFECT OF DROUGHT ON YIELD AND PHYSIOLOGY OF RICE

The yield potential of a cultivar under favourable conditions is important in determining the yielding ability under water stress. Drought index which provides a measure of drought related yield loss is an important criterion that has been used for screening of drought tolerance genotypes. Evaluation of eighteen rice genotypes showed reduction in panicle number (72%) and grain yield (12%) (Swain et al., 2010). Singh et al. (2010) evaluated six generations (P1, P2, B1, B2, F1 and F) of six crosses of rice under drought and irrigated conditions and observed a reduction in several characters including grain yield under drought conditions. The intensity of drought effect on various traits varied with the genetic materials. The study indicated strong relationship between grain yield under drought, leaf rolling and leaf tip burning for moderately tolerant introgression lines and also between grain yield and leaf rolling for tolerant *Oryza glaberrima*. Similar findings were reported by Ndjiondjop et al. (2012). This explains the role of leaf rolling and leaf tip burning potential of a genotype on its development.

Yield decreases are a result of drought effect on several morphological and agronomic traits, including plant height, tillering ability and leaf area (Bocco et al., 2012). Others include various root traits (length, thickness and depth), spikelet fertility, panicle exertion, leaf greenness (SPAD), leaf temperature, time to flowering, time to maturity, leaf tip drying and leaf rolling (Ndjiondjop et al., 2010a). Ndjiondjop et al. (2010a) observed 16.9, 13.7, 6.7, 14.1 and 26.7% reduction in the number of tillers, plant height, number of leaves, leaf width and grain yield, respectively. Drought-related reduction in yield and yield components can be attributed to stomatal closure in response to low soil water content with a resultant decrease in carbon dioxide intake and subsequently a reduction in photosynthesis (Chaves, 1991; Cornic, 2000; Flexas et al., 2004). In summary, prevailing drought reduces plant growth and development, leading to hampered flower production and grain filling and thus smaller and fewer grains. A reduction in grain filling occurs due to a reduction in the assimilate partitioning and activities of sucrose and starch synthesis enzymes.

Garrity and O'Toole (1995) observed an increase in leaf temperature by 9°C due to drought and significant correlation between midday leaf temperature on the day of flowering and both grain yield and spikelet fertility. This increase in leaf temperature under drought is a result of lower transpiration rate caused by a reduction (closure) in stomatal aperture. Leaf temperature is, therefore, a very sensitive indicator of plant water status and is associated with leaf stomatal conductance (Jones, 1992). Significant variations among rice cultivars in leaf temperature increase under drought are reported. Cultivars with high drought-avoidance potential consistently remained coolest under drought (Garrity and O'Toole, 1995).

Under drought, flowering time (start, 50 and 100% flowering) and time to maturity are delayed as a result of water shortage. The length of the delays is related to the type of drought, the temperature regimes, the period of occurrence of drought and the rice genotype (Bocco et al., 2012; Wopereis et al., 1996). Spikelet fertility is also influenced by drought. The production of viable pollen, panicle exertion, pollen shed and germination and embryo development, which are involved in fertilization and initiation of grain filling, are all negatively affected by drought. This causes reduced spikelet fertility and dry weight of fertile spikelets thereby leading to grain yield loss (Liu et al., 2006; Rang et al., 2011).

DROUGHT RESISTANCE MECHANISMS

General plant responses to drought

Drought resistance mechanisms include drought escape via a short life cycle or developmental plasticity, drought avoidance via enhanced water uptake and reduced water loss, drought tolerance via osmotic adjustment and antioxidant capacity.

Escape

The first way for the plant to avoid drought is dodging. It is an adaptation to the environment allowing the plants to avoid the critical periods for their good development. Farmers use this plant strategy to place the crop cycle when conditions are favourable. For example, development of varieties with a shorter development cycle in order to avoid the most stressful periods of the year for plants or to shift the date of sowing and/or select varieties to prevent water deficits. This is an important mechanism for avoiding terminal drought. The shortening of growth cycle has improved the yield of many varieties in many annual crop species (Fukai et al., 1999; Turner et al., 2001). Drought evasion can be achieved through two mechanisms (i) completing the crop cycle before the occurrence of a terminal drought; (ii) Avoiding coincidence between periods of low water availability and critical or sensitive phases of crop growth where water is critically required such as flowering and grain filling.

Avoidance

The second way to avoid drought is the ability of the plant to maintain a satisfactory water state. The reduction in soil moisture may have led to lower water content in the leaves causing guard cells to lose turgor pressure and hence the size of stomatal pores are reduced (Tezara et al., 2002), causing stomatal closure (Singh et al., 2012). Avoidance allows plants to limit the effects of stress through adaptations such as wilting or leaf rolling. Drought avoidance consists of mechanisms that reduce water loss from plants due to stomatal control of transpiration, and also maintain water uptake through an extensive and prolific root system.

Drought tolerance

From a physiological point of view, drought tolerance is the ability of the plant to survive and grow under drought. From an agronomic point of view, a plant is tolerant when it is able to obtain a higher yield than sensitive plants. Tolerance allows maintenance of the essential cellular functions for survival, due to specific and targeted responses despite the deficiency of water (Passioura, 1996; Tardieu, 2003, 2005). Keeping of turgor in water deficiency can delay stomatal closure, maintain chloroplastic volume and reduce leaf wilting which confers to the plant a better tolerance to internal water deficit. This tolerance to internal water deficit in turn allows a prolonged operation of photosynthesis. The carbon products can then be used for both osmotic adjustment and root growth. Due to the unpredictability of water stress, tolerance is the most effective strategy in severe and prolonged stress situations.

Rice responses to drought stress

Rice responds and adapts to drought stress by induction of various morphological, physiological and molecular modifications, with these modifications being made according to the developmental stage (Figure 1).

Morphological and phenological modifications

In majority of the plant species, water stress is linked to changes in leaf anatomy and ultrastructure. The first and foremost effect of drought is impaired germination and poor stand establishment (Harris et al., 2002). Cell growth is considered one of the most drought sensitive physiological processes due to reduction in turgor pressure. Growth is the result of daughter-cell production by meristematic cell divisions and subsequent massive expansion of the young cells (Anjum et al., 2011). Under drought stress, plants reduce the number of leaves per plant and individual leaf size as well as leaf longevity by decreasing the soil's water potential. Leaf area expansion depends on leaf turgor, temperature and assimilates supply for growth.

Rice leaf color plays an important role in leaf photosynthesis. The reduction in photosynthetic rate in rice as a result of drought is well documented (Lauteri et al., 2014). Ndjiondjop et al. (2010a) observed an increase in leaf greenness value under drought when compared with full irrigation conditions. However, these observations contradict those of Zinolabedin et al. (2008) who reported reduced uptake of water and nutrients by plant root systems causing reduced chlorophyll concentration in plant leaves and therefore the yellowing of the leaves. Under full irrigation conditions, rice leaves normally do not roll and they do not show tip drying symptoms either. But under drought, the first response of the plant is to roll its leaves (Sié et al., 2008) to maintain a favourable internal water status. Therefore, rice genotypes with high leaf water maintenance (high leaf rolling ability) are able to out yield those with lower ability(Fukai and Cooper, 2002). This explains the relationship between leaf rolling and grain yield under drought. Leaf tip drying is also a good indicator of drought

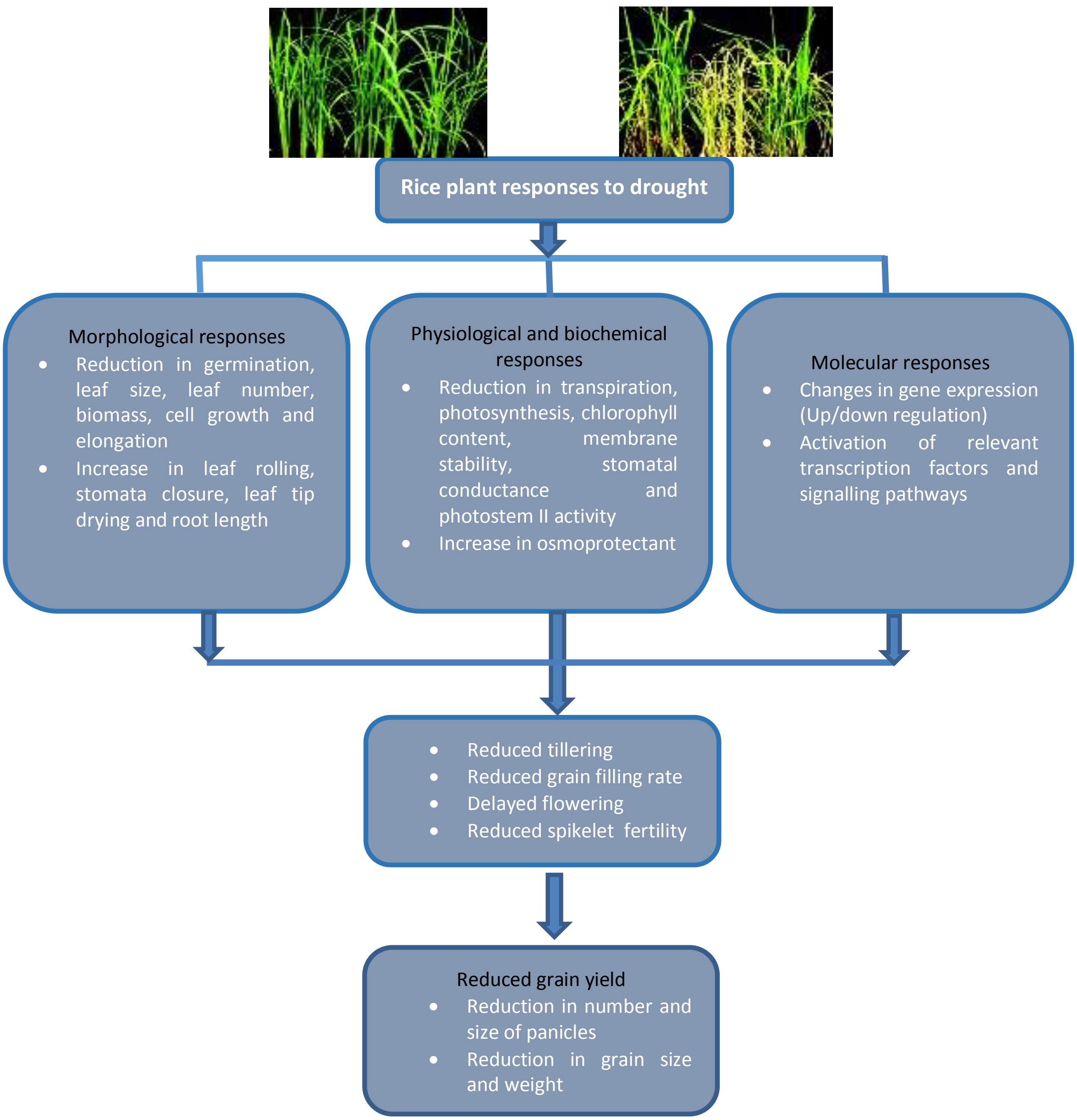

Figure 1. Schematic description of rice plant responses under drought stress.

level (Henderson et al., 1995) and just like leaf rolling, is regarded as a drought avoidance mechanism. The severity of leaf rolling and leaf tip burning is a function of the severity of drought especially on very susceptible rice genotypes. Leaf rolling is reversible but leaf tip drying is irreversible under drought.

Physiological responses

In response to water deficit, plants are able to establish a series of physiological responses that allow them to act on their own water state in order to adapt to environmental conditions. Some of the physiological responses to drought include:

Decrease in leaf size: Generally, growth decrease is one of the first drought manifestations in rice plant. Drought is manifested in the plant by a slowing down of the initiation of the new aerial organs (leaves and stems) and a reduction in the pre-existing organs (Davies and Zhang, 1991; Boyer and Kramer, 1995; Chaves et al., 2002).

These modifications, will in the long term limit the surfaces through which loss of water by transpiration can take place. Thus growth reduction is not a passive consequence of the lack of water in the cells, but rather a controlled and programmed response of the plant, the result of which is to anticipate the events of drought stress. Studies have shown that these modifications result from a decrease in the rate of division of plant cells (Granier et al., 2000) and a modification of the physico-chemical properties of the cell walls which become more rigid thereby inhibiting their growth (Cosgrove, 2005).

Root elongation: Contrary to aerial organs which are reduced under the effect of water stress, these conditions promote the development of the root system. Enhancing the development of the root system traits such as root length allows the plants to access deep ground water resources. Plant production is the function of water use (WU), water use efficiency (WUE) and harvest index (HI). It is therefore vital to understand its effect during defined developmental stage in order to design effective selection methods to improve plant production under dry environment. WUE provides the means of efficient use of water and serves as a breeding target in water saving agriculture. Traditionally, it is defined as the ratio of dry matter produced per unit of water transpired, and constitutes one of the key determinants in controlling plant production. It is also referred to as “transpiration efficiency” and it is estimated from the measures of leaf gas exchange or by using carbon isotope discrimination. Higher WUE in turn lowers photosynthetic rate due to reduced rate of transpiration and consequently slows the rate of plant growth (Condon et al., 2004). Currently, agricultural sectors are slowly moving towards use of genotypes with increased WUE and improved agronomic practices (Pereira et al., 2006).

Leaf water potential (LWP) is a measure of whole plant water status and has long been recognized as an indicator of dehydration avoidance (Pantuwan et al., 2002a). When water deficit in leaf goes beyond a certain threshold level, the stomata closes as a mechanism of lowering the rate of transpiration. Stomatas help to regulate water loss when the tissue water status becomes too low, thereby minimizing the severity of water deficiency in plants. Thus, higher LWP is maintained by stomatal closure and varietal differences in stomatal response to water status have been reported (Jongdee et al., 1998). Genotypes possessing stay-green trait maintain high photosynthetic activity and often protects the plants from premature senescence during the onset of stress. It is reported that stay-green plants assimilate more nitrogen and retain high level of nitrogen content in the leaf, thereby retaining photosynthetic capacity under water limited conditions (Borrell et al., 2001).

Molecular responses to drought stress: As soon as the stress is detected by plant receptors, a coordinated series of cellular responses is established. In fact, the physiological and morphological reactions are based on these coordinated cellular responses which induce the expression of a large number of genes. In rice, more than 5,000 genes are up-regulated and more than 6,000 are down-regulated by drought stress (Maruyama et al., 2014). Wang et al. (2011) conducted genome-wide gene expression profiling and detected 5,284 genes which were differentially expressed under drought stress, among which were under temporal and spatial regulation. Recently, it has been shown that a CO-like gene, Ghd2 (grain number, plant height, and heading date2), which can increase the yield potential under normal growth condition just like its homologue Ghd7, is involved in the regulation of leaf senescence and drought resistance. This gene is down regulated under drought conditions. Overexpression of Ghd2 resulted in significantly reduced drought resistance, while its knockout mutant showed the opposite phenotype (Liu et al., 2016).

Regulatory transcription factors involved in the response of drought stress have been extensively investigated. This allowed the discovery of two important signaling pathways of transcriptional networks under abiotic stress conditions. One involves a hormone called abscissic acid (ABA) produced when a plant undergoes water stress. Abscissic acid will initiate, at the cellular level, a cascade of signaling involving transcription factors named ABA Responsive Element Binding (AREB) (Abe et al., 1997; Uno et al., 2000). The second pathway is independent of this hormone, and involves other transcription factors, drought responsive element binding (DREB) (Yamaguchi-Shinozaki and Shinozaki, 2005). Many signaling details of ABA have been well elucidated and reviewed (Jiang and Zhang, 2002; Salazar et al., 2015; Sah et al., 2016). ABA is an important messenger that acts as the signaling mediator for regulating the adaptive response of plants to different environmental stress conditions (Sah et al., 2016).

DETECTION OF QUANTITATIVE TRAIT LOCI (QTLS) FOR USEFUL DROUGHT TOLERANCE TRAITS

The recent development of high-density linkage maps has provided the tools for dissecting the genetic basis underlying complex traits such as drought resistance into individual components (Yue et al., 2006). Although, complex traits such as yield are routinely dissected into their component traits namely grain size, test weight and number of productive tillers per plant in rice, sometimes resulting in the development of functional markers, the same is not true in drought stress research (Prakash et al., 2016). Earlier molecular genetic analyses identified several QTLs of secondary traits important to drought tolerance such as root architecture, leaf water status, panicle water potential, osmotic adjustment and relative water content.

Genes/QTL underlying drought secondary traits

In rice, a number of physio-morphological putative traits have been suggested to confer drought tolerance (Deivanai et al., 2010). Root system architecture plays a primary constitutive role in acquisition of water and nutrient from the soil and maintains appropriate plant water status (Nguyen et al., 1997; Lafitte et al., 2001; Kato et al., 2006). Various root architecture traits among them, rooting depth, root density, root thickness and root distribution pattern (Pantuwan et al., 1996; Wade et al., 1996; Lilley and Fukai, 1994; Fukai and Cooper, 1995) enhance plant water uptake, thereby avoiding dehydration. QTLs for morphology and the index of root penetration have been identified in several rice populations (Champoux et al., 1995; Ray et al., 1996; Zhang et al., 2001; Kijoji et al., 2014; Henry et al., 2014). Liu et al. (2009) identified and cloned a gene named OsDHODH1 which encodes a putative cytosolic dihydroorotate dehydrogenase (DHODH) in rice. Overexpression of the OsDHODH1 gene in rice increased the DHODH activity and enhanced plant tolerance to salt and drought stresses.

Deep rooting is a very important trait for plants drought avoidance mechanism and it is usually represented by the ratio of deep rooting (RDR). The root growth angle (RGA) is another important trait in drought tolerance, which determines the direction of root elongation in the soil and affects the area in which roots capture water and nutrients. Courtois et al. (2009) conducted a meta-analysis of QTLs in 12 populations and detected 675 root trait QTLs. Although, many QTLs for root trait have been mapped, only 5 major QTLs for deep rooting have been reported (Kitomi et al., 2015; Uga et al., 2015, 2011) and only the DRO1 gene has been cloned (Uga et al. 2013a). DRO1 has been detected on chromosome 9 in recombinant inbred lines (IK-RILs) derived from a cross between the shallow-rooting cultivar IR64 and the deep-rooting cultivar Kinandang Patong (Uga et al., 2011). This QTL has subsequently been cloned. It has been shown that the functional allele of DRO1 introduced from Kinandang Patong (Dro1-NIL) had a significantly larger RGA and higher grain yield than the parental variety IR64, which had a non-functional allele of DRO1. The DRO1 is the first gene associated with root system architecture (RSA) that has been shown to improve the ability to avoid drought. Another major QTL for RGA named DRO2 has been identified on chromosome 4 in three F2 populations derived from crosses between each of three shallow-rooting cultivars (ARC5955, Pinulupot1 and Tupa729) and Kinandang Patong (Uga et al., 2013b).

A new QTL for RGA was recently identified on the long arm of chromosome 7. This QTL named DRO3 is involved in the DRO1 genetic pathway as its effect on RGA in plants have been detected only with a functional DRO1 allele (Uga et al., 2015). The Phosphorus Uptake 1 (PUP1) is a QTL that contributes to phosphorus (P) uptake in low P content soils. The gene underlying the QTL, later termed Phosphorus-Starvation Tolerance 1 (PSTOL1), was cloned and appeared to encode a receptor-like cytoplasmic kinase (Gamuyao et al., 2012). Recently, a novel gene, OsAHL1, was identified through genome-wide profiling and analysis of mRNAs. Analysis showed that OsAHL1 has both drought avoidance and drought tolerance mechanisms and when overexpressed, it enhances multiple stress tolerances in rice plants during both seedling and panicle development stages. Functional studies revealed that OsAHL1 regulates root development under drought condition to enhance drought avoidance, participates in oxidative stress response and also regulates the chlorophyll content in rice leaves (Zhou et al., 2016). Two QTLs for the root gravitropic response, and 4 QTLs for seminal root morphology (SRM) have been reported (Norton and Price, 2009). These 2 traits are well known to be important components of RGA. The QTL designed, quantitative trait locus for Soil Surface Rooting 1 (qSOR1) has been fine-mapped on chromosome 7, using 124 recombinant inbred lines (RILs) derived from a cross between Gemdjah Beton, an Indonesian lowland rice cultivar with soil-surface roots, and Sasanishiki, a Japanese lowland rice cultivar without soil-surface roots (Uga et al., 2012).

Liu et al. (2005) identified 2 and 6 main effect QTLs for canopy temperature and leaf water potential respectively in RILs (F9) from a cross between Zhenshan97B and IRAT109. Recently, 6 QTLs for RDR were identified using 1 019 883 single-nucleotide polymorphisms (SNPs) (Lou et al., 2015). Prince et al. (2015) identified two QTLs for canopy temperature, 1 QTL for leaf drying and 1 for SPAD under managed stress and in a rainfed target drought stress environment, respectively. The introduction of traits that contribute to drought avoidance or tolerance should improve resistance of rice to drought and this strategy therefore has considerable potential to increase rice production in areas prone to drought (Fukai and Cooper, 1995; Nguyen et al., 1997). For rice, considerable research effort has been devoted to mapping QTL for osmotic adjustment (Lilley et al., 1996), but only a few loci with major effects have been identified.

QTL for yield and yield related-traits under drought

Several studies using different mapping populations have identified QTLs for traits related to drought tolerance (Khowaja and Price, 2008). Bernier et al. (2007) identified large-effect QTLs for grain yield under drought stress. If confirmed, these identified QTLs have to be fine mapped for use in breeding programs. A drought experiment conducted by Lanceras et al. (2004) using 154 doubled haploid lines derived from a cross between two rice cultivars, CT9993-510 and IR62266-42, allowed identification of 77 QTLs for grain yield and its components under various drought intensities. Among them were 7 for grain yield, 8 for biological yield, 6 for harvest index, 5 for days to flowering, 10 for total spikelet number, 7 for percent spikelet sterility, 23 for panicle number and 11 for plant height. A recombinant inbred population obtained from a cross between high-yielding lowland rice IR64 and Cabacu was used to identify 10 QTLs for grain yield and component traits under reproductive-stage drought stress (Trijatmiko et al., 2014). The qDTY12.1 is the first reported large-effect QTL for grain yield under severe upland reproductive-stage drought conditions and was identified in a population of 436 F3-derived lines from a cross between Vandana and Way Rarem (Bernier et al., 2007). Two other large-effect QTLs, qDTY2.1 and qDTY3.1, well known to affect grain yield under lowland reproductive-stage drought, were identified in a back cross inbred line (BIL) population derived from a cross between Swarna and Apo. Both QTLs showed a very high effect (R2 = 16.3 and 30.7%) under severe lowland reproductive-stage drought. These QTLs also showed pleiotropic effects on other traits such as DTF and PHT (Venuprasad et al., 2009). Another QTL, qDTY6.1 had strong effect on yield in aerobic drought stress conditions (Venuprasad et al., 2012b).

A large-effect QTL qDTY1.1 has been identified as having an effect on grain yield under severe lowland reproductive-stage drought across F3-derived populations developed from a cross between N22 and Swarma, N22 and IR64 and N22 and MTU1010 (Vikram et al., 2011). This QTL has also been reported in CT9993-5-10-1-M/IR62266- 42-6-2 and Apo/IR64 populations (Kumar et al., 2007; Venuprasad et al., 2012a).

In the same way, qDTY2.2, qDTY4.1, qDTY9.1 and qDTY10.1 were identified to have a large effect on grain yield in BIL population from a cross between Aday Sel and IR64 (Swamy et al., 2013). Table 1 presents a summary of large effect QTLs for grain yield reported in rice.

MITIGATION AGAINST DROUGHT

Mitigating drought and climate change requires robust, well-planned and informed strategies in order to enhance agricultural sustainability and ensure that human livelihood is not negatively affected. Improved rice technologies that help reduce losses from drought can play an important role in long-term drought mitigation.

Important scientific progress is being made in understanding the physiological mechanisms that impart tolerance to drought (Blum, 2005; Lafitte et al., 2006). Similarly, progress is being made in developing drought-tolerant rice germplasm through conventional breeding and the use of molecular tools (Korres et al., 2017). Improving the resilience of rice production systems to climate change requires the development and dissemination of appropriate combinations of improved stress-tolerant rice germplasm, natural resource management strategies and creation of appropriate policy environments to help increase and stabilize yields in variable cultivation conditions.

Breeding for drought tolerance and adaptation

One of the main strategies in confronting drought is breeding for drought tolerance which helps to deliver adapted genotypes. These breeding efforts will require characterization and evaluation of diverse germplasm with the aim of identifying genotypes possessing traits that are important in enhancing drought tolerance. The replacement of diverse and adapted traditional rice varieties with genetically narrow based genotypes has significantly increased the vulnerability of the agricultural production systems. The use of a wide range of genetic resources is critical in the development of varieties that are adapted to drought. Crop wild relatives are particularly useful sources of genes for adapting crops to drought. There exists a variety of physiological traits that are associated with drought tolerance. Some of these traits include root traits, early flowering, water use efficiency, amount of water transpired, transpiration efficiency, osmotic adjustment and stay green. Breeding for increased yields under drought tolerance will require proper understanding of the various traits that are associated with yield (Pandey et al., 2015). The exact trait to target in a breeding programme in order to obtain the best response in terms of drought tolerance may not always be clear to a breeder.

Africa Rice has been spearheading efforts aimed at delivering rice varieties that are tolerant to drought. This has involved screening of a wide range of genetic resources including indigenous Africa species such as *O. glaberrima* and *Oryza barthii*. A key goal of the breeding programme has been to develop a rice variety that can escape terminal drought that frequently occurs at the end of the wet season through its short growth duration. Short duration varieties are also preferred to avoid late season fungus diseases (Jones et al., 1997). Several upland interspecific *O. sativa* × *O. glaberrima* (NERICA) varieties were evaluated at AfricaRice and it was observed that they have potential for escaping drought due to their short growth duration. The capacity of NERICA varieties to maintain growth under mild drought, their survival under severe drought, recovery from drought and their water use efficiency need to be incorporated into breeding programs (Futakuchi et al., 2011).

Exploitation of drought tolerance traits in African rice in rice breeding

African rice is one of the two independently domesticated

Table 1. Large effect QTLs reported for grain yield under drought stress conditions.

QTL name	Chrom	Interval	Population	Ecosystem	R2	References
qDTY1.1	1	RM11943–RM12091	N22/Swarna	Lowland	13	Vikram et al. (2011)
qDTY1.1	1	RM11943–RM12091	N22/IR64	Lowland	17	Vikram et al. (2011)
qDTY1.1	1	RM11943–RM12091	N22/MTU1010	Lowland	13	Vikram et al. (2011)
qDTY1.1	1	RM486–RM472	Apo/IR64	Upland	58	Venuprasad et al. (2012a)
qDTY1.2	1	RM259–RM315	Kali Aus/MTU1010	Upland	7	Sandhu et al. (2014)
qDTY 1.3	1	RM488–RM315	Kali Aus /IR64	Upland	5	Verma et al. (2014)
qDTY2.1	2	RM327-RM262	Apo/Swarna	Lowland	16	Venuprasad et al. (2009)
qDTY2.2	2	RM236–RM279	Aday Sel./ IR64	Lowland	11	Swamy et al. (2013)
qDTY2.2	2	RM236–RM555	Aday Sel./ IR64	Lowland	3	Swamy et al. (2013)
qDTY2.2	2	RM236–RM555	Aday Sel./ IR64	Lowland	9	Swamy et al. (2013)
qDTY2.2	2	RM211–RM263	Kali Aus/ MTU1010	Upland	6	Sandhu et al. (2014)
qDTY2.2	2	RM211–233A	Kali Aus/ MTU1010	Lowland	16	Palanog et al. (2014)
qDTY3.1	3	RM520–RM16030	Apo/Swarna	Lowland	31	Venuprasad et al. (2009)
qDTY3.1	3	RM168–RM468	IR55419-04/TDK1	Lowland	8	Dixit et al. (2014)
qDTY3.1	3	RM168–RM468	IR55419-04/TDK1	Upland	15	Dixit et al. (2014)
qDTY 3.2	3	RM569–RM517	Aday Sel./ Sabitri	Lowland	23	Yadaw et al. (2013)
qDTY 3.2	3	RM60–RM22	N22/Swarna	Lowland	19	Vikram et al. (2011)
qDTY 3.2	3	id3000019–id3000946	Moroberekan/Swarna	Lowland	8	Dixit et al. (2014b)
qDTY 3.2	3	id3000019–id3000946	Moroberekan /Swarna	Upland	19	Dixit et al. (2014b)
qDTY 4.1	4	RM551–RM16368	Aday Sel./IR64	Lowland	11	Swamy et al. (2013)
qDTY6.1	6	RM589–RM204	Vandana/IR72	Upland	40	Venuprasad et al. (2012b)
qDTY6.1	6	RM589-RM204	Apo/IR72	Upland	63	Venuprasad et al. (2012b)
qDTY6.1	6	RM586-RM217	IR55419-04/TDK1	Lowland	9	Dixit et al. (2014)
qDTY6.1	6	RM586-RM217	IR55419-04/TDK1	Upland	36	Dixit et al. (2014)
qDTY6.2	6	RM121-RM541	IR55419-04/TDK1	Lowland	9	Dixit et al. (2014)
qDTY6.2	6	RM121-RM541	IR55419-04/TDK1	Upland	20	Dixit et al. (2014)
qGY8.1	8	RM38–RM331	MASARB25 / Pusa Basmati; HKR47/ MAS26	Upland	34	Sandhu et al. (2014)
qDTY10.1	10	MTU1010/N22	RM216–RM304	Lowland	5	Vikram et al. (2011)
qDTY10.2	10	Aday Sed./IR64	RM269–G2155	Lowland	17	Swamy et al. (2013)
qDTY11.1	11	id11002304-id11006765	Moroberekan- Swarna	Upland	25	Dixit et al. (2014b)
qDTY12.1	12	RM28166–RM28199	IR74371-46-1-1 / Sabitri	Lowland	24	Mishra et al. (2013)
qDTY12.1	12	RM28048 -RM511	Way Rarem/ Vandana	Upland	33	Bernier et al. (2007)

rice species, with its distribution being limited to West Africa. Its genetic potential in terms of resistance to both biotic and abiotic stresses has been well documented and deployed in rice improvement (Wambugu et al., 2013). Its tolerance to drought is a particularly valuable trait during these periods that are characterized by increased occurrences of drought and erratic rainfall. Some alien introgression lines derived from an interspecific cross between *O. sativa* and *O. glaberrima* under drought conditions had higher yield than the parents (Bimpong et al., 2011b). This demonstrates the potential of transferring drought related traits from African rice to Asian rice. In this study, novel QTLs for drought related traits such as yield and yield components were identified with about 50% of the beneficial alleles being contributed by African rice.

A total of 2000 African rice accessions conserved at AfricaRice genebank were evaluated by Shaibu et al. (2018) for drought tolerance in three locations in West Africa over a period of 3 years. Results of this screening showed that four *O. glaberrima* genotypes had significantly higher yields under both drought and rainfed conditions than the *O. glaberrima* check, CG14, which is considered a drought tolerant variety. Though, these genotypes were not significantly different from the *O. sativa* checks (Table 2), they will serve to widen the African rice genepool that can be used for breeding for drought tolerance. African rice has several drought avoidance mechanisms such as early flowering. It has also been reported to have thin leaves which easily roll

Table 2. Grain yield (g/m^2) of selected *Oryza glaberrima* accessions and standard checks under drought, rainfed and control conditions during 2013-14 at three locations in West Africa.

Entries	Drought			Rainfed			Control	
	Ibadan	Ibadan	Ibadan	Badeggi	Cotonou	Ibadan	Ibadan	Cotonou
	DS 2013	DS 2013	DS 2014	DS 2014	WS 2014	WS 2014	WS 2014	WS 2014
No. of *O. glaberrima* genotypes evaluated	200	285	74	74	30	30	30	30
Selected *O. glaberrima* genotypes								
TOG 7400	-	236	236	25	83	270	399	368
TOG 6520	401	-	301	7	-	349	403	455
TOG 6519-A	393		226	5	72	303	286	308
TOG 7442-B	327	-	152	7	69	251	292	443
Checks								
O. glaberrima check								
CG 14	217	238	51	6	60	104	415	339
O. sativa check								
Apo	432	397	472	9	48	242	255	401
FARO 52	472	447	216	9	-	55	-	619
IR 77298-14-1-2-B-10	363	451	298	12	56	263	418	285
Trial mean	246	161	122	6	51	171	241	310
$LSD_{0.05}$	142	142	103	5	43	130	119	149
Heritability	0.87	0.75	0.71	0.69	0.70	0.85	0.90	0.84

Adapted from Shaibu et al. (2018).

during drought to retain water, in addition to having small diameter roots which easily extract water from the soil (Dingkuhn et al., 1999). The phenological responses of African rice during times of drought have been found to be superior to those of traditional and improved *O. sativa* cultivars (Dingkuhn et al., 1999). African rice has also been found to possess the capacity to close stomata earlier in response to drought as compared to *O. sativa* (Bimpong et al., 2011c).

Challenges in breeding for drought tolerance

In most rice breeding programs, grain yield as an important trait of interest is widely used as an index for adaptation to drought stress. But several researchers have reported inconsistency in yield production by rice genotypes across environments and years (Fukai and Cooper, 1995; Pantuwan et al., 2002a, b, c). A genotype performing well in one type of drought environment may not perform well in other environments (Pantuwan et al., 2002a, c). It is unclear whether promising materials selected under drought condition will yield well in full irrigation/wet-season condition. This explains the large genotype-by-environment (GxE) interactions and the low heritability of grain yield of rainfed lowland rice under drought and the uncertainty in the selection of drought resistant genotypes (Fukai and Cooper, 1995). To accommodate the effects of GxE interactions and improve selection efficiency, a large number of multi-location trials over years in various drought intensity conditions could be a solution (Nyquist and Baker, 1991; Fukai and Cooper, 1995). Unfortunately, such evaluation processes are costly and time-demanding for making selections in the breeding program. Therefore, it has become necessary to identify more efficient breeding options based on the use of indirect selection methodology (Falconer, 1989).

Even though there is extensive evidence that selection under target stresses may accelerate breeding gains for stress environments (Atlin and Frey, 1990; Ceccarelli et al., 1992; Ud-Din et al., 1992; Bänziger et al., 1997), the difficulty of choosing appropriate selection environments, given a highly variable target environment, may limit the identification of superior genotypes. While breeding programs in high-income countries may resort to real-time GIS information for adequately weighting information from METs (Podlich et al., 1999), these opportunities rarely exist in low-income countries as there is a lack of both real-time GIS information and resources for conducting a large number of METs. Progress in improving drought resistance has been slow. This is partly due to the complexity of the drought environment, the number of different mechanisms of drought resistance exploited by rice and the interaction between the two as well as the genetic complexity of most traits.

Other drought mitigation strategies

In addition to crop improvement and selection of drought tolerant genotypes, other strategies for mitigating against long term impacts of drought include development of irrigation facilities and water harvesting structures such as dams. Development of water resources is an important area of protection against drought that is emphasized in SSA. The large-scale development of irrigation schemes that was a hallmark of the green revolution is limited now by high costs and increasing environmental concerns (Rosegrant et al., 2002). Moreover, the rationale of establishing new large scale irrigation schemes may be questioned as many such schemes have and continue to stall. The collapse of these schemes, many of which have been established in partnership with various development partners, brings to the fore critical issues such as feasibility and sustainability of such projects. In some cases, the long term availability of water for these projects is usually not guaranteed. The technical and financial capacity to maintain these projects need to be explored before their establishment. However, there are still substantial opportunities to provide some protection from drought through small and minor irrigation schemes and through land-use approaches that generally enhance soil moisture and water retention (Shah, 2001; Moench, 2002). Public-sector support for further development, maintenance and rehabilitation of small and minor irrigation schemes could make them more effective in mitigating drought. Public-sector involvement, however, should be limited to the provision of technical assistance, while the actual management of these small-scale schemes is better left to local communities (Kerr et al., 2002). Hand dug shallow wells are another option for sourcing water resources particularly for small holder farmers.

Watershed-based approaches implemented in drought prone areas of India are providing opportunities to achieve long-term drought-proofing by improving overall moisture retention within watersheds (Rao, 2000). As already stated, one of the causes of drought in Africa is habitat destruction especially due to population pressure. Most habitats in many African countries are currently severely degraded and non-productive. Consequently, one of the ways to mitigate drought is through the rehabilitation of these degraded habitats through ecological restoration. Drought forecasting and timely provision of such advice to farmers is an important drought mitigation strategy that can help reduce the overall economic cost of drought. It also helps improve preparedness, thereby helping in managing the risk more effectively. Various indicators such as the Southern Oscillation Index (SOI) are routinely used to forecast drought in several countries (Wilhite, 2000; Meinke and Stone, 2005). Forecasting is especially important in assisting farmers make more informed decisions regarding the choice of crops and cropping practices.

CONCLUSION

Drought is one of the major climatic hazards even in the sub humid rice-growing areas of Asia and Africa. It is an event that reoccurs, affecting agriculture and the livelihoods of millions of farmers and agriculture laborers. The socio-economic impact of drought is enormous. It has huge economic costs, in terms of both actual economic losses during drought years and losses arising from foregone opportunities for economic gains. Drought contributes directly to an increase in the incidence and severity of poverty.

It is therefore critical that we establish effective strategies to mitigate the effects of drought in order to ensure agricultural productivity and environmental sustainability. Use of adapted genotypes and improvement in rice production technology are some of the components of an overall strategy for effective drought mitigation. Increased moisture availability to crops through water conservation and harvesting, and watershed development is an important component. Improvements in drought forecasting and efficient provision of such information to farmers can improve their decisions regarding crop choice and input use.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Review

Integrating crop and livestock in smallholder production systems for food security and poverty reduction in sub-Saharan Africa

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The resource base that ensures food supply and the socio-economic component which depends on this resource base are the two major components that make up the food system in sub-Saharan Africa. The sequence of the food system is organized in a spatial flow framework of biomass base. The components of rural production system consist of food production biomass at homestead and farm level, and often at the communal base non-food production lands. The degree of integration between these resources base determines flows such as material cycle, energy, food and cash, and influences how the entire production system needs to be managed. The management system influences resource use efficiency and economic returns at different levels, at individual household, communities, and national levels. Efforts to developing agriculture and reducing poverty remained sectoral and focused mainly on a specific crop or individual animal level, failed to see interconnections among sub-systems and across space and time. The concept of the integrated food system has not been adequately adopted, in many sub-Saharan African countries and the agricultural system in the region continues to exhibit a low level of productivity and resource use efficiency. Hence, food insecurity and poverty remained high among smallholder farming communities producing crop and livestock despite the availability of arable land and abundance of another natural resource. This review focuses on the significance of integrated crop-livestock system in the tropics and suggests a framework to begin understanding and addressing complex problems in smallholders' production system.

Key words: Biomass production, food security, crop-livestock systems, poverty, smallholder production systems, sub-Saharan Africa.

INTRODUCTION

Agriculture is the largest single occupation in the world, employing 40% of the global population and contributing substantially to the health and well-being of rural populations (United Nations, 2015). Approximately, more than 950 million people are found in Africa, with 60% between 15 and 24 years (Koira, 2014). The majority of the population in sub-Saharan Africa (SSA) resides in rural areas, and up to 80% are smallholder farmers (Senbet and Simbanegavi, 2017) directly or indirectly dependent on agriculture for their livelihood. Africa presents a paradox of hungry and malnourished farming families; the continent continues to be a global hotspot for food and nutrition insecurity and is home to some of the world's poorest populations; and food aid has virtually become a perennial feature, particularly in SSA (Gliessman and Tittonell, 2015). More critically, in contrast to other continents, agricultural productivity in Africa has continued to decline (van Ittersum et al., 2013). The agricultural production practices and value chains remain underdeveloped as a result engaging in agriculture in the region remains less attractive to the young generation (Ströh de Martínez et al., 2016).

The variety of resources, productive and non-productive, as well as livelihood specific assets like land and livestock including various phases of production to the consumption of food through distribution and processing in the food system, consists of a more complex adaptive system. Despite the complexity of biophysical and socio-economic components of the food system, attempts to understand and improve its efficiency in SSA remained sectoral, fragmented and simplistic, and hence have thus far been less successful. Population pressure has continued to increase, and the resources base are depleting. The challenge is compounded by climate variability and change, under development of infrastructure and markets that continue to affect people and agriculture in SSA.

The various components of the resource base (soils, crops, livestock, weather, etc.) and socio-economic elements (culture, farm management practices, knowledge systems, non-farm and off-farm income generating activities) and many other factors interact in complicated ways to influence agricultural productivity and sustainability of production systems. The development and adoption of sustainable farming systems require a better understanding of the ecology of farming systems, the socio-economic aspects of the communities managing the production systems, and capacity to identify and use options for sustainable intensification and to overcome barriers to adopt good practices. According to Tilman et al. (2002), fundamental shifts in policies, incentives, and institutions will be required in the search for, extensive adoption of sustainable agricultural practices; that search must be an on-going and adaptive process.

Most of the agricultural researches being conducted to benefit the poor in SSA are hampered by the historical lack of cross-disciplinary linkages and cross-sectoral approaches (Lenné and Thomas, 2006). Failure to address challenges in an integrated manner continues to limit adoption and use of most agricultural research results by smallholders. As a result, many continue to poorly understand and address interactions that contribute to poverty alleviation, food security, and sustainable resource use by smallholders in SSA (Mortimore, 1991; Kristjanson and Thornton, 2001).

Working at an integrated level in crop-livestock systems provide opportunities for the improvement of the two production components of sub-systems at the same time (FAO, 2010). It allows improvements in the workforce, the stability of production and reducing production related risks; greater chances of producers reaching their socio-cultural aspirations; and greater food security to meet the needs of consumers regarding the diversity and quality of products they may get at a given point in time. A high level of biodiversity (Mores et al., 2014) is maintained that further supports the sustainable agricultural systems, ensure food availability while also reducing environmental degradation and assisting agriculture to adapt to climate change.

By definition, therefore, a complex adaptive system is a system composed of many heterogeneous pieces whose interactions drive system behavior (National Research Council, 2015). Ignoring these characteristics can distort our picture of how these systems work, causing policies to be less effective or even counter-productive (Levin et al., 2013). This result in situations where research recommendations do promote either intensive cropping or livestock production in cases where farmers' objectives and resources would have supported further integration (Kassa et al., 2011). As a result, most recommendations fail to be adopted by smallholder farmers. On the other hand, Endashew (2017) description of food and nutritional security along with hunger alleviation on a global scale can only be within reach if technological innovations are accepted, promoted and implemented particularly at smallholder farm level.

The poor performance of the agricultural production that leads to food insecurity, persistent poverty, low-income levels and declining environment multi-functionality of production systems is not a mere effect of technical and financial scarcity. It is related to the lack of adequate information on area-specific resources and how the agricultural system evolves at local, regional and national levels. This is particularly true in a region where extension packages are designed and promoted assuming that smallholder farming systems are uniform and mixed farming systems need to specialize in crop or livestock production systems. That is, not only the extension systems, but the policy direction also fails to take into account the reality of the existing integrated crop-livestock production systems (Kassa et al., 2011) in many developing countries.

Most researchers and policymakers fail to realize the the available land use, biomass base, labor, draft power and manure that are utilized in a way to meet subsistence interaction levels between crop and livestock production systems in energy and nutrient links, which complement food and cash needs of the farm households.

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It is also true that all interaction effects are not always positive. Smallholders are facing food insecurity, high capital shortage, and high risks associated with agricultural production. Unless the innovation works under the real circumstances of the smallholder systems, its adoption will naturally be slow. Given the challenges, they continue to minimize risk and optimize total farm productivity than maximize a specific crop or livestock productivity. Thus, it is important to think through how their efforts can be assisted to increase productivity through increased efficiency of use of resources available (or can be made available) to them.

Smallholder farmers operating crop-livestock production systems in SSA often manage fragmented holdings and face annual and intra-seasonal variability of climate factors; they depend mainly on family labor with poor access to transport services and the market, limited availability of extension and credit services. As a result, smallholder producers hardly benefit from the growing national and global demand for agricultural products.

Designing development interventions and devising an agricultural policy that works for smallholder farmers in SSA calls for better understanding of the production objectives of smallholders and the functional and organizational structure of mixed crop-livestock farming systems. This review focuses on the description and importance of integrated biomass base crop-livestock production system building on cases and identifies entry points to enhance food security and reduce poverty amongst smallholder farmers in the SSA.

INTEGRATED CROP-LIVESTOCK PRODUCTION IN THE FOOD SYSTEM

Biomass base of integrated crop-livestock systems

In integrated systems, biomass base is defined as nutrient flows, linking crops, livestock and human components of agriculture, whereas the land is a spatial framework of the flow path. The spatial dimension is key to the concept of material cycles and energy flows, and management of integrated systems and flow paths connect a point of origin to an end by displaying a spatial distance (Poccard-Chapuis et al., 2014). The rural production system consists of a spatial structure and social scale in the concept of biomass base that integrates system components in complex ways and with interdependence. Spatial structure can matter by directly shaping the local context experienced by actors, but it also can shape impacts at a distance and affect changes in the environment over time (National Research Council, 2015). Development and adaptation of integrated systems analysis, therefore, must include different technical, social, demographic and environmental functional relations that are defined simultaneously on different hierarchical levels to gain a better framework of the complex problems of our society.

Agro-systems are complex systems of topographical sequences which usually contain a variety of distinct pathways. For instance, in Southern Mali, the landscape relief determines the soil type and its potential for production whether native or cultivated (Kante, 2001). The upland portions have more fragile soils with a coarse texture and low fertility than the lowland sections. Where in latter, soils were deeper with a higher percentage of clay and were fertile due to water flows in the watershed (Riou, 1990), and from a sociological view, village residents were sorted along the topography by inhabiting the uplands (Dufumier, 2004). Native vegetation areas are community property where the pastures and forests are used communally during the non-growing period, whereas cultivated land is managed privately during the crop production season (Poccard-Chapuis et al., 2007). A village leader defines rules for common use of the areas (Hardin, 1968). Farming practices based on the cut and burn system continued to be practiced up to the first half of the 20th century, with regeneration cycles of about 20 years as indicated in Mali situation. At that time the upland portions of the topography were preferentially cultivated due to finer vegetation that was easier to clear (Riou, 1995). The return of ashes from burned forests was compensated for such soils common low chemical fertility, which was also a technique to make the land to work easier (Poccard-Chapuis et al., 2007). As such, fields are spread across the topography, allocated by traditional authorities and family heritage management takes into account the spatial structure of the property. Thus, each property has a unique spatial distribution among the three types of fields (non-manured fields, manured fields, and bushland) and size of cattle herds (Lemaire et al., 2011).

With consolidation of permanent fields, the limited quantities of chemical fertilizers distributed by agribusiness became insufficient to correctly manage the soil; farmers were interested to utilize manure from cattle herds to improve fertility of cropland soil, such that cropland became an essential part of the fodder calendar, especially in the dry season, whereby cow dung from the grazing cattle was deposited directly on cropland (Poccard-Chapuis et al., 2007). Biomass recycling is the linchpin of maintaining and enhancing animal and plant productivity along with investment in capital and labour use.

Optimizing crop and livestock component within biomass base

According to Kumaraswamy (2012), environmental sociology is increasingly becoming indispensable in the restoration of ecological functions. Hughes (1995) and Cooke and Kothari (2001) defined environmental sociology as complex symbolic and non-symbolic reciprocal interactions between society and environment that are influenced by the cultural and social behavior while interacting with the physical and biological elements. The rural landscapes have inherent physical and functional characteristics that determine to some extent necessary spatial structure (Poccard-Chapuis et al., 2014). The functional improvements in rural landscapes can occur over time with consideration of crop and livestock components in space (Poccard-Chapuis et al., 2011). Rangeland (non-food production) biomass with native vegetations and grasses in many rural regions are still larger than the food production biomass base. Substantial refinement has not been done yet on spatial arrangements. A case study shows that the productivity gains are potentially high through the recovery of degraded pastures and formation of eco-efficient from arrangements of various components of the landscapes (Poccard-Chapuis et al., 2014). Herrero et al. (2013) reported that grasslands are sometimes considered either underused or seen as an ecosystem warranting judicious management because of their importance for protecting key regulating ecosystems services (carbon, biodiversity, and water). Farmers will need to identify characteristically different parts of their farmland, such as hill sides, plains, wetlands, river banks, etc., in order to develop an efficient spatial arrangement of land use and appropriate management practices for optimizing production and use of biomass and water. In the grassland areas, the arrangement should be thought of as a process of progressive pasture reform, with occasional diversification into other uses, and with a greater appreciation of where animal manure is deposited (that is, on pasture for temporal rotation with cropland or in the corral for collecting and spreading manure on cropland).

Integrated management and role of institutions

Two specific cases have been cited by Poccard-Chapuis et al. (2014) to compare the productive performance of the integrated systems of Mali and the Brazilian Cerrado while also highlighting similarities from an ecological but differences from a social point of view. Management by the agribusiness company in the Brazilian Cerrado certainly facilitated several factors that Mali farmers lacked: access to finance equipment technical support, quality inputs, training and complementarity among specialized components. Food production in Brazil foods mobilized public policies to support efforts, particularly public funding for infrastructure development. Attempts to enhance an integrated system of food production was facilitated through direct control of livestock and cultivated areas (e.g., prices paid to farmers) so that complementarity of feed production, meat production and other components were ensured. The company, known as Brazil Foods, could monitor and adjust crops and livestock in the territory, as well as biomass circulation in the integrated system. Even transportation is managed by the company with a fleet of trucks and a dense network of passable country roads. Brazil foods were responsible for balancing this system, economically and agronomically. However, this integrated structure was faced with the risk that farmers wanted to invest in other production systems, such as sugarcane as this had attractive prices in neighboring regions. It is possible that a decline in grain supply would lead Brazil Foods to forego the territory and move its activities to another region. This is one limiting factor in managing a large corporation: it can optimize integrated crop-livestock systems, but it can also change strategy and withdraw, compromising the development direction of the territory. Therefore, the social system may be influenced by an uncontrollable external agent, affecting the viability of integrated crop-livestock systems.

In the case of Mali where the large company and support services and policies do not exist, management was left to the farmers themselves and traditional authorities. Biomass production, transport, and transformations are limited due to limitations in investment in technology, low level of capital to process plants and to buy inputs such as quality seeds and fertilizers. Public policies are poorly aligned with the needs of poor producers limiting the impact on the agrarian system. Moreover, traditional management has been facing difficulties in promoting new innovations and in enforcing certain management practices such as controlling grazing on communal land. This comparison clearly shows the role of managers in innovation, public policy to mobilize resources and support efforts to promote integrated management. The social system in villages combined with the former slash and burn and forest succession system can be considered hindrances to optimizing integrated systems at a regional scale. The trend is that farmers, as seen in the Amazon case, end up developing integration individually without the collective mobilization of potentially shared resources and biomass.

Attempts are being made in Africa so that agricultural research and knowledge generation strategies involve multiple-stakeholders and promote sustainable and equitable agricultural development. The Forum for Agricultural Research in Africa (FARA) supports efforts towards integrated agricultural research for development (Adekunle et al., 2012).

Farm level integrated crop-livestock production systems

The coexistence of crop-livestock production systems in many different forms at a global scale is evidenced (Seré et al., 1995; Dixon et al., 2001). As a group of farms, they are assumed to be operating in a similar environment that provides a useful scheme for the description and analysis of crop and livestock development opportunities and constraints (Otte and Chilonda, 2002). Investment in agriculture to have a sustainable impact on food security and poverty and decisions have to be made with respect to smallholder farmers and their biophysical environment and socio-economic and cultural setting (Notenbaert et al., 2009); future scenarios modeling could be amenable ideally for these systems.

The impacts of agricultural production on the natural environment strongly depend on specific local conditions. Changes in water or nutrient cycles, for example, are related to soil conditions, terrain type and local climate condition (Lotze-Campen et al., 2005). In crop-livestock systems, the feed supply is defined to a large extent by the biomass produced on grazing lands and by crops that could be available for use as livestock feed (Fernández-Rivera et al., 2004). Estimations of feed surplus and deficit areas linked to potential stocking capacity can give an indication of current and probable future pressure on the natural resource base (Notenbaert et al., 2009). Other assessments include manure calculations, nutrient cycle, and land degradation. The value of animal traction for purposes of cultivation can legitimately be included as one of the potential assessment but information is rarely available even in countries where animal traction is predominantly used in crop production: cultivation, weeding threshing, transport, etc. It was estimated that in Ethiopia, the annual production of crop residues has increased from 6.3 million tons in 1980 to about 31 million tons due to the expansion of cultivated land and increased crop productivity (CSA, 2008). However, the use of crop residue varies from place to place in the country. A study by Amejo et al. (2017) reported that in smallholder crop-livestock systems, the feed source from grazing/browsing and from crop by products accounted for 92 and 8%, the total annual supply of livestock feed. The same study concluded that feed from rangelands biomass accounts for about 82% of the feed for livestock in the lowland areas of that study.

Earlier studies in Ethiopia indicated that about 80% of farmers use animal traction to plough their farm fields. In the Ethiopian highlands, the area under cultivation is positively associated with cattle ownership (Gryseels, 1988; Mergia et al., 2005; Bogale et al., 2009). Ploughing with cattle also increases crop output per hectare. In Oromia regional state of Ethiopia, farmers who used oxen or a combination of oxen and hand cultivation obtained higher yields of both teff and maize as compared to farmers using hand cultivation alone (Mergia et al., 2005). Assessment of livestock productivity in the mixed farming systems in Southern Ethiopia shows that cattle manure (dry matter produced in kg/year) and draught animal power accounted for 29% of the gross household income from the livestock sector (Amejo et al., 2018).

The Livestock Policy Initiative of the Intergovernmental Authority on Development (IGAD) reported that the mean weight of cow dung used for fuel by households in Ethiopia was equivalent to 293 kg per year per cattle (Behnke, 2010). The conventional methods used for agricultural GDP calculations fail to capture a wide range of economic benefits provided by livestock to the Ethiopian national economy. The IGAD policy brief recommendation asserted that in the interest of supporting more informed policies for livestock development, the Ministry of Finance and Economy Development and the Ministry of Agriculture should collaborate to supplement the standard national accounts with periodic estimations of the value of livestock goods and services that are underestimated in national accounts (Behnke and Metaferia, 2013).

Another comparative system analysis in three countries showed the net income issues from agriculture activities: the US $40 in Vihiga (Kenya), $284 in Upper West (Ghana) and $4,368 in Kandy (Sri Lanka). It demonstrated that the low incomes and the high reliance on off-farm income (92%) in Vihiga could be explained by small farm size and that the high proportion income obtained from sale of milk, on the other livestock could be a vehicle for intensifying systems without the associated effects of land-based intensification (Herrero et al., 2007).

Role of production system characterization for integrated management

Integrated crop-livestock systems are organized to maximise synergies and minimize trade-offs between crops and livestock sub-systems through the production of crops and livestock on the same area, concurrently or sequentially in rotation or succession (Moraes et al., 2014). The result of an integrated system is that the whole is greater than the sum of its parts and resulting in having emergent properties (Anghinoni et al., 2013). These integrated crop-livestock systems are produce with minimal supply of inputs and technologies (Moraes et al., 2014).

The role of research and development in integrated crop-livestock systems

Because of various constraints that smallholder farmers managing integrated crop-livestock production systems face, they have not been benefiting from research and development efforts to the extent expected. Infrastructural limitations and poor market access made farmers benefit little from growing demands for food in SSA and in the world at large. Thus research directions and development interventions need to focus on improving the policy and institutional aspects that enable farmers to increase total farm productivity and household income through improved links to technologies and services and better links to markets. More research and policy instruments are needed to improve resource use efficiency of integrated farming systems.

Efforts to improve crop-livestock systems therefore necessitate a detailed analysis of farmers' circumstances and practices of the components of production systems and their operation from various regions. White (1998) reported that opportunities for and constraints to improving the productivity, sustainability and viability of integrated farming systems are often specific to particular agro-ecological zones and socio-economic settings. Understanding the subsystem is an essential part of the bio-economic foundations of rural livelihood systems (Thornton and Herrero, 2001), which requires accounting for its component stocks, resource flows and interactions (Ashley and Carney, 1999).

FOOD SECURITY AND POVERTY IN SMALLHOLDER PRODUCTION

The concept of food security is multidimensional in nature and includes food access, availability, use, stability and even entitlement to food. The analysis of food insecurity as a social and political construct has been growing in importance (Devereux, 2000). Poverty and food insecurity continue to be highly concentrated in SSA.

Reducing hunger and poverty calls for improvements in economic conditions of households and infrastructure, the organization of food production, the provision of social services, political and institutional stability, among others (FAO, 2013). In terms of natural resources, most of SSA countries have relatively abundant agricultural land. For example, in 2008, SSA allocated 29 million ha of agricultural land (about two-thirds of global demand), for foreign investment (Deininger and Byerlee, 2011). Gomiero (2016) emphasized the greatest potential for croplands in tropical Africa given current climatic conditions (560 million ha) followed by North and South America (470 million ha). Yet currently cultivated land in SSA is under smallholders with low productivity levels and managing less than 1 ha of landholdings (Deininger and Byerlee, 2011).

In Ethiopia, total land area cultivated for grain in 2016 was 14,934,373 ha and a total of 2,998,828 tonnes of grain was produced (CSA, 2016). Smallholder farmers accounted for 95.5% of the area cultivated, whereas commercial farmers accounted for 4.2% of the area cultivated and 0.3% of small-scale irrigation user. The same is true for livestock production where 98.59% of cattle population are local breeds (CSA, 2016). Livestock products supply chain is dominated by smallholders and pastoralists except very few per-urban farmers engaged in dairy and poultry.

The existing yield gap in productivity, the growing demand for food products and shortage and in some cases, absence of large-scale competitive commercial farmers in the agriculture sector provide opportunity for market oriented agricultural development that would raise smallholder productivity in many SSA countries (Deininger and Byerlee, 2011). Given the widespread rural poverty and small-scale farming in Africa, the conventional wisdom supports a strong role for agriculture in African development (Diao et al., 2010). However, emphasis to developing the agricultural sector and enhancing its contribution in rural development in SSA remain limited due to policy distortions against agriculture and narrow focus toward higher value export crops.

In low-income countries with high dependence on agriculture, strategies that promote agricultural productivity and link producers to markets are most appropriate for making progress in poverty reduction and, by implication, improving food security (Mellor, 1995; de Janvry and Sadoulet, 2001). The links between increased production and improved food consumption of poor and food-insecure persons are mediated through complex institutional and socio-economic relations, thus one should not just think of production increases alone to positively impact food security and poverty. As undernourishment handicaps, the efforts to improve food production, feedback effects between food production and consumption should be considered. A recent sustainable development agenda recognizes the need for eradicating poverty in all its forms and dimensions. This is the greatest global challenge and an indispensable requirement for sustainable development (Resolution, 2015). Thus, reduction and ultimate eradication of poverty and hunger are the most urgent tasks facing national governments in SSA. This necessitates significant public interventions to develop the agricultural sector, supporting rapid income growth that translates to increased capacity to produce or purchase food (FAO, 2013). Agricultural development, coupled with the expansion of rural non-farm activities are the most effective means of promoting income growth.

The term undernourishment is used to describe the status of persons whose food intake does not provide enough calories to meet their physiological requirements on a continuing basis (FAO, 1999a). As recommended by FAO/WHO, the body mass index (BMI) measure (the ratio of body weight in kg to the square of height in meters) is commonly used in adults group, and the considered range for healthy adults is between 18.5 and 25. The BMI can clearly vary over an adult's lifetime, but physical stature is determined by the time an individual reaches adulthood. It is critical to note that poor anthropometric status is the outcome not only of insufficient food intake but also of sickness spells.

The economic costs of malnutrition and undernutrition, often translated to poor anthropometric status of individuals. First, this limits physical strength of an individual and his/her ability to do sustained work often required among rural communities that are dependent on agriculture which requires much manual labour. This in turn limits capacity to generate more income. Poor nutritional status leaves people more susceptible to illness. Poor nutritional status is associated with a risk of intergenerational transmission. For instance, women who suffer from poor nutrition are more likely to give birth to underweight babies. These babies thus start out with a nutritional handicap. Poor nutrition is associated with poor school performance in school-age children as prolonged and severe malnutrition are known to impair the cognitive ability of the child. People who live on the edge of deprivation do follow a policy of safety rather than to invest in agriculture. Finally, the macroeconomic performance of the whole economy will continue to suffer from the cumulative impact of all these effects.

Several studies reported that increased BMI had a significant impact on output and wages. For example, Croppenstedt and Muller (2000) found that in rural Ethiopia, an increase of 1% in BMI increased farm output by about 2.3% and wages by 2.7%. Thomas and Strauss (1997) found that a 1% increase in BMI in their sample from urban Brazil was associated with a 2.2% increase in wages. Strauss and Thomas (1998) presented a succinct and illuminating review of the impact of adult stature and BMI on productivity through an analysis of two data sets from the United States and Brazil. They found that adult stature is positively correlated with wages in both countries, but the effect is strong in Brazil and weak in the United States. The implications of the findings are profound. The loss of income to those suffering from undernutrition can be large. Thus, it appears that in Brazil, people with BMIs of 26 earn wages that are considerably higher than wages earned by those with a BMI of 22. Furthermore, people with BMIs of 26 are far more likely to find work than people with BMIs of 22.

A significant impact of increased calorie consumption on farm output and wages has also been reported. For example, a study by Thomas and Strauss (1997) found that an increase of 1% in calorie intakes increased wages by about 1.6% at calorie intake levels of around 1700 calories per day, but that this effect ceased to operate after calorie consumption levels reached around 1950 calories per day. Increased attention is being given to the role of micronutrient deficiencies in reducing labour productivity. Iron deficiency that causes anaemia was associated with a 17% loss of productivity in heavy manual labour and 5% in light blue-collar work.

The importance of subclinical vitamin A deficiency in child mortality has been recognized through meta-analysis of clinical studies (Horton, 1999). The relative risk of mortality for a child with subclinical vitamin A deficiency is 1.75 times than that for a child who does not suffer from this deficiency. Horton (1999) has provided a measure of the overall economic costs of malnutrition as a percentage of GDP for selected Asian countries. An FAO report (Arcand, 2001) has indicated a strong relationship between economic growth and nutritional factors, as measured by either the prevalence of food inadequacy or gap in the dietary energy supply per capita. The impact of nutrition on economic growth appears to be strong to operate directly, through the impact of nutrition on labour productivity and indirectly through improvements in life expectancy.

According to Fogel (1994), improvements in nutrition and health explain half the economic growth in the United Kingdom and France in the eighteenth and nineteenth centuries. An accounting approach with concepts from demography, nutrition and health sciences by the same author has stressed the physiological contribution to economic growth over the long term. A change in diet, clothing and shelter together with a reduction in the incidence of infectious diseases, increased the efficiency with which food energy was converted into work output and translated into higher economic growth.

Private income growth alone does not guarantee improvement in nutritional status. Nutritional status is the resultant of food intakes and health inputs. Thus, the solution to undernutrition is increased intakes of calories as well as improvement in micronutrients, better health and sanitation, safe drinking water, better functioning markets, etc. (FAO, 2013).

Rural poverty

Rural poverty remains entrenched among smallholders managing integrated crop-livestock production systems. More need to be done to enhance our understanding of what works in terms of reducing poverty reduction and enhancing food security. In particular, the focus should partly shift from the pursuit of win-win policies towards policy options that involve managing trade-offs and maximizing synergies between crop and livestock production systems on one hand and between agriculture and non-agricultural income generating activities on the other hand with which policymakers are more often confronted (FAO, 2013).

Smallholder farmers in SSA are engaged in largely subsistence farming and are dependent on often disconnected local food markets (Ströh de Martínez et al., 2016). The defining characteristic of most goods and services of smallholders is that they are effectively less tradable due to their marketable quality and/or volume. Most produces of smallholders are found in less accessible locations. The growth of smallholder produce is conditioned by the growth of demand in the local rural market. Devising strategy for agricultural growth that promotes productivity and income of smallholder and hence allows for greater participation of the poor is central to reducing poverty and promoting rural development in SSA (Diao et al., 2010).

PATHWAYS TO DEVELOP INTEGRATED CROP-LIVESTOCK SYSTEMS IN SUB-SAHARAN AFRICA

Co-existence of crop and livestock in traditionally integrated crop-livestock production systems has evolved from age-old practices that attempt to use available inputs and increase total farm productivity. Smallholder farmers are experienced in adapting their systems and methods of production to different circumstances, albeit slowly and with only a limited success as they have not been systematically supported by governments in terms of adoptable innovations, supportive policy instruments and market links. As circumstance change to alter one or more of the constraining factors, farmers may adopt their systems of operations.

A study in Tanzania showed that though limitations in farm size, capital and technological development and market access remain challenging, there exist means to increase agricultural production via improving technical efficiency (Hepelwa, 2010) and to use appropriate extension and other support services to better understand obstacles for scaling up (Nijbroek and Andelman, 2016).

Swanepoel et al. (2010) suggested that the institutional, market and policy-related constraints that undermine productivity and income levels of smallholder framers in SSA need to be identified and addressed in a coordinated manner. Transportation, infrastructure, markets and institutions are critical for establishing efficient markets but are often severely lacking in livestock-raising areas (PicaCiamarra, 2005). According to Moraes et al. (2014), integrated crop-livestock systems can support efforts for the sustainable intensification of agriculture. Promoting increased production of foods, fibres and energy, associated with the promotion of ecosystem services is assisted by supporting further intensification through integration of crop-livestock systems. Crop-livestock systems in SSA vary (Ruthenberg, 1971) arising from the combination of parts that have different operational features. Though a number of constraining factors limits a range and balance of resource and enterprise combinations that are found in any specific farming system, these production systems continue to adapt to and respond to demands of markets (Swanepoel et al., 2010). Focus on market-orientated smallholder production systems helps intensification that help to significantly close yield gaps in crop and livestock production, and bring about efficiency gains by reducing opportunity costs for, among others, land (Naylor et al., 2005).

It is necessary to properly consider agro-ecological, technical, social, demographic and environmental factors as attempt is made to develop integrated crop-livestock production systems on which most smallholder farmers in SSA depend for their livelihoods. Agro-ecology offers technical and organizational innovations to promote a restorative, adaptable, inclusive and resource use-efficient agricultural model at global scale, however, there are several challenges ahead. It is assumed that scaling up agro-ecology from successful isolated examples of pioneer farmers to broad-scale dissemination will be next major challenge. Investing in institutional and policy innovation will be at least as important as investing in generating new scientific knowledge on agro-ecology. The social-change aspect of agro-ecology was strongly voiced by the organizations supporting and promoting the rights and needs of food insecure and malnourished communities (Gliessman and Tittonell, 2015).

For example, policies that set the rules of the game by internalizing the environmental externalities in production costs, through preferential allocation of subsidies to low environmental impact farming; through the protection of family farmers' rights to access agro-biodiversity, which is increasingly being restricted by patents and unethical claims on property rights; and through the promotion of short commercialization circuits and local food systems, including processing, that can guarantee quality and safe food for the poorest. Policies that set the rules of the game to make agro-ecological farming as competitive and economically viable as industrial farming will be able to better inform the development of public policies to support the rural poor transition rather than policies that compel farmers to embrace agro-ecology.

Small farms could play a more significant role by complementing and reinforcing diets through the production of a large diversity of nutritious crops, rather than focusing on producing only calorie-rich crops in a context of rapidly increasing population and dwindling farm sizes. The case of smallholder rural families may constitute an exception in many situations. The average diet of people in rural areas that are well connected to markets and urban hubs, or that have access to mass communication media, is increasingly determined by demand. Yet, in regions that are less connected to markets or to mass media, or where poverty prevents people from affording external foods, the relationship between landscape and nutritional diversity is a much stronger one. The functional biodiversity that is necessary to sustain agro-ecological processes and functions also results in a greater diversity of crops and animal products that can improve the diet of farming families, aforementioned as in the case of Brazil.

It was evidenced that currently, global food production is short of vegetables by 11%, fruits by 34%, fresh milk by 50% and nuts and seeds by 58%. These nutritional gaps indicate that there is a need to diversify production through, e.g. intensive vegetable rotations and associations, crop-livestock integration, or fruit tree agroforestry, all practices that are common in agro-ecology.

Efforts should be directed towards the design of nutrition-sensitive landscapes by means of diversification. The good intention of increasing the yield of a few world commodities to reduce poverty and hunger has already shown its limitations. Particularly in smallholder family agriculture, when land sizes are as small as one acre or less, increasing the yield of staple crops will not result in families rising out of poverty. Given their small size, the

Table 1. The four dimensions of any food system and their effects.

Dimensions	Domains		
	Health	**Environment**	**Social and Economic**
Quantity	All households to an extent meet their food requirements in terms of energy (and protein), without malnutrition	Increased system productivity of biomass based values	Rising disposable income of poor household
Quality	Availability of food with adequate micro-nutrients	Rehabilitate and maintain biodiversity of natural environment and traditional agriculture scenes	Variety of affordable food for households with different income levels
Distribution	Access to a variety of food for all groups in population at all seasons	All weather condition accessible infrastructure and communication across agro-ecology, topography and river boundary	Affordable cost to move smallholders on-, off-farm supply; appropriate prices to the supply and their demand at all seasons
Resilience	Quality and healthy food in recovery of wasting, stunting and underweight	Sustainable interconnection and communication of community across agro-ecology, topography and river boundary, as well as secure access to communal resources	Community retains viability after loss either endogenic or exogenous economic source

Source: National Research Council (2015).

total income they may receive from selling their harvest, even if they produce at potential yield levels, will still be meagre. The result is that a large number of farmers in SSA regions are currently part-time farmers who are unable to pay enough attention to their farms and their landscapes. This trend will be exacerbated for future generations of family farmers unless something is done about it. It is time for agr-oecology.

Gliessman (2015) said that agro-ecology must integrate science, technology and practice, and movements for social change help to re-connect the people who grow the food and the people who eat the food in a relationship that benefits both. Food system interventions are more likely to succeed if they are informed by an understanding of the intrinsic dynamics associated with production systems, public health, environmental, and social and economic outcomes with an appreciation that their interactions are non-linear and not always readily predicted (National Research Council, 2015). Along these important dimension, Table 1 shows a summarized presentation of a conceptual framework adapted from the National Research Council (2015) to measure the effects of these important dimensions on food systems. Within an agro-ecological food system perspective with focus on localized units and from an agro-ecological standpoint, clearly the definition of system boundaries can be made explicit.

For example, integrated system analysis to ensure the roles, extents and potential demand of the resource base can confer certainty of the long-term impact of increased efficiency for food production and sufficiently high economic return in line with land capability. This approach can help planners and smallholders set future directions, and make decisions as to how to reallocate the resources without affecting existing economic and ecological basis of food production and non-food production biomass. It gives efforts to improve the efficiency of food availability, enhances resources use efficiency and attains food security without substantially degrading the natural resource base.

Government policy is an important factor that governs the development and evolution of farming systems. Government efforts also include efforts to establish and strengthen research institution and development actors at large to support rural economic development. The support provided by non-governmental organizations to help the community improve its productivity and income cannot be underestimated. The role of the private sector however remains limited. The central role of the government in coordinating development efforts to develop smallholder integrated crop-livestock systems in SSA remains central. Technical and institutional options to enhance the role of this production system to reduce poverty and food and nutritional insecurity which promote interaction of the two sub-systems, crop and livestock should be adopted, rather than attempting to increase productivity of only crops or only livestock in SSA.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Evaluation of new papaya hybrids

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To augment a narrow genetic base in papaya, this study aimed to evaluate the performance of new hybrids produced by Caliman Agrícola® S.A. The experiment was carried out in a randomized complete block design, with 12 treatments four replications and ten plants per plot. The treatments were the variety THB and the hybrids were CR1 × São Mateus, CR1 × 72/12, CR2 × São Mateus, CR3 × São Mateus, CR1 × Maradol, CR2 × Sekati, CR3 × Maradol, CR1 × UENF/Caliman 01, CR3 × Sekati, CR1 × SSAM and Baixinho (dwarf) × Pecíolo Curto (short petiole). Ten hermaphroditic plants per plot were evaluated at 8 and 12 months after transplanting. Data were recorded for sixteen characteristics related to morphology of plants and biometry of fruits harvested at maturity stage II (fruit with up to 25% yellow skin). The analysis of variance and the subsequent Scott-Knott's mean clustering test showed significant differences between cultivars for all the characteristics. The characteristic soluble solids grouped the variety THB and five medium-sized hybrids with potential for exploitation: CP1 × UENF/Caliman 01, Baixinho × Pecíolo Curto, CP1 × 72/12, CP1 × SSAM, and CP1 × São Mateus. The estimated average yield of marketable fruits in 12 months grouped the two hybrids with the highest averages: CP3 × Sekati and CP2 × Sekati. The analysis of the new hybrids revealed interesting productivity and fruit quality characteristics, suggesting that they should undergo value for cultivation and use (VCU) testing for future release as commercial hybrids.

Key words: *Carica papaya* L., plant breeding, genetic variability.

INTRODUCTION

Papaya (*Carica papaya* L.) is one of the most important and widely distributed crops in tropical and subtropical countries. Brazil's production stood out among the world's largest in 2016 and was concentrated in an area of

30,372 ha, mainly distributed in southern Bahia, northern Espírito Santo, Ceará, and Rio Grande do Norte, with the first two considered the main producing regions (IBGE, 2016).

The crop has a narrow genetic base (Kim et al., 2002; Ma et al., 2004; Silva et al., 2008; Silva et al., 2017), which is one of the main threats to its sustainability. A feasible approach for increasing the number of commercial varieties and hybrids is to expand the genetic base of papaya by exploiting the variability existing in germplasm banks (Quintal et al., 2012; Vivas et al., 2015; Silva et al., 2017) and creating new hybrids in breeding programs (Pereira et al., 2002). Efforts should be made to broaden the genetic base and develop cultivars that meet the requirements of domestic and foreign markets and are less susceptible to pests and diseases and more resistant to biotic and abiotic stresses (Vivas et al., 2012, 2014, 2015).

Recent efforts in Brazil have studied hybrids of crosses between the groups Solo and Formosa (Silva et al., 2007; Ide et al., 2009; Dantas et al., 2015; Luz et al., 2015). In addition, Vivas et al. (2013) found variability and possible hybrid combinations within the Solo group.

New hybrids are also important to increase yield and production of fruits with potential to meet the domestic and international markets. The search for cultivars with good sensory qualities are expanding strongly with the purpose of stimulating papaya consumption (Santana et al., 2004), as well as to provide the farmers with new cultivars with commercial characteristics demanded by the market.

Therefore, the objective of this work was to carry out the agronomic evaluation of new hybrids of papaya from Caliman Agrícola SA, for the purpose of selecting superior genotypes to be included into the papaya production system in Brazil.

MATERIALS AND METHODS

The study was carried out at Santa Terezinha Farm (Caliman Agrícola SA), 19° 11' 49' S latitude and 40° 05' 52" W longitude, 30 m altitude in the municipality of Linhares, Espírito Santo, between July 2012 and July 2013. The climate of the region is type AWi (tropical humid), with rainy summer and dry winter (Rolim et al., 1999).

The experiment was arranged in a randomized block design, with 12 treatments consisting of 11 new hybrids and one commercial variety (THB) in four replicates of 10 plants per genotype. The hybrids derive from crosses between parents from the germplasm bank of Caliman Agrícola® SA (CP1, CP2, and CP3) and cultivars already exploited and adapted to the conditions of northern Espírito Santo and with characteristics of interest to domestic and international markets: CP1 × São Mateus; CP1 × 72/12; CP2 × São Mateus; CP3 × São Mateus; CP1 × Maradol; CP2 × Sekati; CP3 × Maradol; CP1 × UENF/Caliman 01; CP3 × Sekati; CP1 × SSAM; and Baixinho × Pecíolo Curto. Cultivars São Mateus, 72/12, Baixinho, and Pecíolo Curto belong to the "Solo" group.

The hybrid seeds were obtained from crosses performed by collecting hermaphroditic flowers before anthesis and transferring pollen manually to the stigma of female flowers, also before anthesis. The plants, previously labeled, and their flowers were individually protected with waterproof paper bags to prevent contamination with undesirable pollen and crosses were identified with plastic labels. Fruits were harvested at 135 to 150 days after pollination at maturation stage 1 (1/4 of the fruit was yellow) and stored for 7 to 10 days at room temperature, according to Martins et al. (2006) and Aroucha et al. (2005), with enough time to allow seeds to reach the point of total physiological maturity and maximum germination and vigor.

Seedling production was carried out in a nursery covered with polyolefin screens (50% shade). Seeds were sown, 2 seeds per cell, in 96-cell plug trays (50 cm^3) filled with Bioplant® substrate fortified with 10 kg of Basacot mini 3M® per m^3 of substrate, according to Paixão et al. (2012).

After acclimatization, about 40 days after sowing, seedlings (12 to 15 cm in height) were transplanted to the field, in July. Three seedlings were planted per hole to ensure a greater number of hermaphrodite plants. For each treatment, holes were spaced 3.6 m between rows and 1.5 m within rows. The soil of the experimental area is classified as red-yellow podzolic with clay-sandy texture. Sexing of the papaya trees was initiated three months after transplanting, and one seedling was maintained per hole, preferably a hermaphroditic plant.

The evaluations were performed at 8 and 12 months after transplanting, using 10 hermaphrodite plants per plot. At 8 months, the following variables were evaluated: plant height in cm (PH) from ground level to the insertion point of the newest leaf; first fruit insertion height in cm (FFIH) from ground level to the peduncle of the first fruit; and stem diameter in cm (SD) taken at 20 cm from ground level using a caliper. The following characteristics were measured at 8 and 12 months: total number of marketable fruits (TNMF), the sum of all fruits complying with marketing standard per plant at 8 and 12 months; fruit mass in grams (FRM), measured on precision scale with three decimal places; fruit length in cm (FRL); fruit equatorial diameter in cm (FRD); smallest thickness of fruit in cm (STP); greatest thickness of fruit in cm (GTP); equatorial diameter of the fruit cavity in cm (DFC), measured on a cross section of the fruit in the central region; soluble solids in °Brix at 8 months (SS-8) and at 12 months (SS-12) measured at maturation stage II (fruits with up to 25% yellow skin) by bench refractometer; internal fruit firmness in kg cm^{-2} at 8 months (FIRM-8) and at 12 months (FIRM-12), determined by cross-sectioning the fruit and measuring the resistance of the pulp at three points spaced equidistantly around the circumference using a penetrometer (Instrutherm, model PTR-100) with a 7.9 mm diameter tip. All fruit-related characteristics (FRM, FRL, FRD, STP, GTP, DFC, SS and FIRM) were derived from measurements of ten fruits, taking one fruit from each of 10 plants per plot. Subsequently, the products of TNMF and FRM were used and stand to obtain the estimated average yield of marketable fruits in t ha^{-1} during 12 months of production (YIELD).

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Table 1. Analysis of variance of the characteristics evaluated with the respective means and coefficient of variation (CV) for 12 cultivars of *Carica papaya* L.

Characteristics[1]	Mean square			Overall mean	CV (%)
	Block	Cultivar	Error		
PH	463.97	1730.27**	99.78	174.64	5.72
FFIH	284.98	534.64**	47.88	80.11	8.64
SD	0.27	3.52**	0.28	10.10	5.20
TNMF	56.90	2514.50**	57.04	50.79	14.87
FRM	28514.22	2749993.24**	61045.95	1558.85	15.84
FRL	0.50	113.61**	1.69	21.89	5.94
FRD	0.62	15.48**	0.35	11.39	5.20
FRL/FRD	0.01	0.20**	0.01	1.91	5.95
GTP	0.053	0.75**	0.03	3.18	5.75
STP	0.05	0.54**	0.13	2.36	15.06
DFC	0.17	4.24**	0.20	6.26	7.21
SS-8	1.03	3.97**	0.60	10.04	7.74
FIRM-8	0.50	2.14**	0.61	12.08	6.48
SS-12	2.33	9.14**	1.56	12.25	10.20
FIRM-12	4.07	10.18**	4.33	10.69	19.47
YIELD	310.04	2333.39**	366.35	114.89	16.66

**Significant at 1% by the F test. Degree of freedom: Block = 3; Cultivar = 11; and Error = 33. [1]PH: Plant height, cm; FFIH: first fruit insertion height, cm; SD: stem diameter, cm; TNMF: total number of marketable fruits; FRM: fruit mass, grams; FRL: fruit length, cm; FRD: fruit diameter, cm; FRL/FRD: fruit length and fruit diameter ratio; GTP: greatest thickness of fruit, cm; STP: smallest thickness of fruit, cm; DFC: diameter of fruit cavity, cm; SS-8: soluble solids at 8 months, ºBrix; FIRM-8: fruit firmness at 8 months, kg cm^{-2}; SS-12: soluble solids at 12 months, ºBrix; FIRM-12: fruit firmness at 12 months, kg cm^{-2}; and YIELD: estimated average yield of marketable fruits in 12 months, t ha^{-1}.

Data were examined by analysis of variance followed by the Scott-Knott (1974) mean clustering test, at 5% probability. The analyses were performed using the computational resources of the Genes software program (Cruz, 2016).

RESULTS AND DISCUSSION

The analysis of variance of the characteristics showed significant differences between the means of the 12 cultivars evaluated at alpha level of 1% (Table 1). The coefficients of variation (CV) were between 5.20 and 19.47% and are considered low to medium for the variables (Ferreira et al., 2016).

The means of the characteristics were compared by the Scott-Knott test (Table 2). Four groups were formed for plant height (PH). The group with the lowest means was formed by the variety THB and the crosses CP3 × Sekati and CP3 × Maradol, ranging from 140.65 to 154.00 cm. The other cultivars had higher means, ranging from 167.15 to 209.60 cm. It is desirable that the plant grow with shortened internodes and less space between the fruits, resulting in a longer harvestable life and greater yield. Papaya breeding aims to decrease plant height by selecting shorter genotypes that maintain vigor (Marin et al., 2003).

The Scott-Knott method formed four groups for the characteristic FFIH, with means ranging from 58.05 cm (CP3 × Maradol) to 94.10 cm (CP1 × 72/12). The low insertion height of the first fruit may be interesting because it can be associated with precocity (Storey, 1953; Dias et al., 2011), if flower initiation occurs earlier after production of fewer vegetative nodes. This allows a longer harvest season and, thus, a greater production per plant and the exploitation of longer cycles of the crop (Dantas and Lima, 2001). Therefore, the selection of cultivars that initiate the insertion of the first flower at a lower height is preferable (Alonso et al., 2008). In the selection of cultivars of the Solo group for the growing conditions of northern Espírito Santo, Marin et al. (1989) established the insertion height of the first flowers to be below 70 cm in the winter and up to 90 cm in the summer, with production capacity of over 80 perfect fruits per plant.

The characteristic SD had an overall mean of 10.11 cm, ranging from 8.37 to 11.29 cm and formed four groups, in agreement with the mean range found by Rodolfo Jr. et al. (2007) of 10.95 cm (Formosa) and 8.68 cm (Solo). Rodríguez and Rosell (2005) argued that this characteristic is positively correlated with vigor and is an important relationship to be considered in cultivar selection.

TNMF ranged from 27.36 to 112.75 and formed four groups. The hybrid Baixinho × Pecíolo Curto (TNMF = 112.75), representing group "a" with the highest mean,

Table 2. Means of the characteristics evaluated in 12 cultivars of papaya (*Carica papaya* L.).

Cultivar	Characteristic[1]					
	PH	**FFIH**	**SD**	**TNMF**	**FRM**	
CP1 × São Mateus	171.29^{c2}	80.19^{b}	11.29^{a}	37.54^{d}	1249.83^{e}	
CP1 × 72/12	207.00^{a}	94.1^{a}	10.90^{a}	51.15^{c}	1054.36^{e}	
CP2 × São Mateus	178.90^{c}	81.60^{b}	11.12^{a}	35.35^{d}	1644.36^{d}	
CP3 × São Mateus	175.35^{c}	79.15^{b}	10.50^{b}	42.70^{d}	1585.38^{d}	
CP1 × Maradol	173.19^{c}	87.19^{b}	9.59^{c}	34.21^{d}	2434.61^{b}	
CP2 × Sekati	167.15^{c}	71.35^{c}	10.22^{b}	28.80^{d}	2572.25^{b}	
CP3 × Maradol	140.65^{d}	58.05^{d}	8.57^{d}	27.36^{d}	3056.40^{a}	
CP1 × UENF/Caliman 01	191.54^{a}	85.61^{b}	10.75^{a}	52.49^{c}	1100.77^{e}	
CP3 × Sekati	151.55^{d}	67.25^{c}	8.37^{d}	41.80^{d}	2072.25^{c}	
CP1 × SSAM	209.60^{a}	98.7^{a}	10.22^{b}	61.69^{c}	998.91^{e}	
Baixinho × Pecíolo Curto	175.52^{c}	86.64^{b}	10.24^{b}	112.75^{a}	497.83^{f}	
THB	154.00^{d}	71.50^{c}	9.47^{c}	83.75^{b}	438.76^{f}	
	FRL	**FRD**	**FRL/FRD**	**GTP**	**STP**	**DFC**
CP1 × São Mateus	19.69^{d}	11.22^{d}	1.76^{c}	3.24^{c}	2.35^{a}	6.31^{b}
CP1 × 72/12	19.06^{d}	10.90^{d}	1.75^{c}	2.94^{d}	2.03^{b}	6.51^{b}
CP2 × São Mateus	24.27^{c}	11.58^{c}	2.10^{b}	3.27^{c}	2.52^{a}	6.22^{b}
CP3 × São Mateus	21.65^{d}	11.81^{c}	1.83^{c}	3.37^{c}	2.50^{a}	6.43^{b}
CP1 × Maradol	25.77^{c}	14.11^{a}	1.82^{c}	3.58^{b}	2.79^{a}	7.76^{a}
CP2 × Sekati	30.26^{a}	12.88^{b}	2.35^{a}	4.02^{a}	2.86^{a}	7.13^{a}
CP3 × Maradol	27.76^{b}	14.47^{a}	1.92^{c}	3.20^{c}	2.67^{a}	7.27^{a}
CP1 × UENF/Caliman 01	20.41^{d}	11.00^{d}	1.87^{c}	2.85^{d}	2.17^{b}	6.23^{b}
CP3 × Sekati	27.24^{b}	11.94^{c}	2.28^{a}	3.61^{b}	2.50^{a}	5.97^{b}
CP1 × SSAM	19.41^{d}	10.63^{d}	1.82^{c}	2.93^{d}	2.44^{a}	6.64^{b}
Baixinho × Pecíolo Curto	13.27^{e}	8.24^{e}	1.61^{c}	2.56^{e}	1.75^{b}	4.33^{c}
THB	13.96^{e}	7.91^{e}	1.76^{c}	2.59^{e}	1.74^{b}	4.37^{c}
	SS-8	**FIRM-8**	**SS-12**	**FIRM-12**	**YIELD**	
CP1 × São Mateus	10.27^{a}	11.07^{b}	13.75^{a}	10.50^{a}	89.91^{c}	
CP1 × 72/12	10.88^{a}	10.50^{b}	14.25^{a}	9.25^{b}	99.41^{c}	
CP2 × São Mateus	9.09^{b}	12.65^{a}	12.50^{a}	12.67^{a}	110.84^{c}	
CP3 × São Mateus	9.44^{b}	12.45^{a}	11.50^{b}	8.00^{b}	112.50^{c}	
CP1 × Maradol	9.12^{b}	12.08^{a}	13.00^{a}	11.75^{a}	130.22^{b}	
CP2 × Sekati	8.85^{b}	12.26^{a}	9.75^{b}	11.75^{a}	146.67^{a}	
CP3 × Maradol	8.86^{b}	12.33^{a}	9.75^{b}	11.75^{a}	124.54^{b}	
CP1 × UENF/Caliman 01	11.73^{a}	11.55^{b}	12.75^{a}	9.05^{b}	101.06^{c}	
CP3 × Sekati	9.74^{b}	12.58^{a}	11.00^{b}	11.00^{a}	159.34^{a}	
CP1 × SSAM	10.88^{a}	12.14^{a}	14.00^{a}	11.35^{a}	125.42^{b}	
Baixinho × Pecíolo Curto	11.29^{a}	12.12^{a}	12.75^{a}	8.60^{b}	106.97^{c}	
THB	10.29^{a}	13.21^{a}	12.00^{a}	12.67^{a}	71.85^{c}	

[1]PH: Plant height, cm; FFIH: first fruit insertion height, cm; SD: stem diameter, cm; TNMF: total number of marketable fruits; FRM: fruit mass, grams; FRL: fruit length, cm; FRD: fruit diameter, cm; FRL/FRD: fruit length and fruit diameter ratio; GTP: greatest thickness of fruit, cm; STP: smallest thickness of fruit, cm; DFC: diameter of fruit cavity, cm; SS-8: soluble solids at 8 months, ºBrix; FIRM-8: fruit firmness at 8 months, kg cm^{-2}; SS-12: soluble solids at 12 months, ºBrix; FIRM-12: fruit firmness at 12 months, kg cm^{-2}; and YIELD: estimated average yield of marketable fruits in 12 months, t ha^{-1}. [2]Means followed by equal letter in the column are not significantly different by the Scott-Knott test at 5% probability.

was followed by variety THB (TNMF = 83.75), representing group "b". These cultivars belong to the Solo group, which shows high TNMF and low FRM. Groups "c" and "d" comprise more than 80% of the cultivars evaluated, belonging to the Formosa group, with TNMF ranging from 27.36 to 61.69. Papaya cultivars in Brazil are divided into two groups based on the average fruit weight: the Formosa group, weighing from 800 to 1,100 g and the Solo group from 350 to 600 g (Dantas et al., 2002).

FRM varied from 438.76 to 3056.40 g, with more than 80% of the cultivars weighing between 998.91 and 3056.40 g. The small fruit size means of hybrid Baixinho × Pecíolo Curto and the variety THB were not statistically different, and at least for Baixinho × Pecíolo Curto, was somewhat compensated for by a greater number of fruits per plant. FRM has variable classification standards, and the “optimum fruit” will also depend on its shape, which must facilitate packaging and transportation, and ultimately on consumer acceptance.

According to Dias et al. (2011), fruit mass between 800 and 1500 g serves the domestic Brazilian market, while the international markets still require fruit mass around 500 g. Dantas and Lima (2001) reported mean fruit mass from 280 to 850 g in genotypes of the Solo group and 710 to 2200 g in the Formosa group. These results point out the market expectations for commercializing new hybrids in the domestic and international markets.

In Latin America, there is a strong preference in domestic markets for large fruits (Ferreguetti, 2003). Alonso et al. (2009) evaluated papaya hybrids in Cuba and found mean weight with low variability, ranging from 1456.7 to 1682.4 g.

Ferreguetti (2003) observed that the consumer market for Formosa papayas was growing significantly. One example of this is that there is substantial growth in sales of these fruits in Europe, Canada, and the United States, with cultivar Maradol accounting for about 75% of papaya consumption. Therefore, the development of new resistant genotypes with commercial characteristics required by the market is important (Esquivel et al., 2008; Vivas et al., 2013). In this context, CP2 × Sekati, CP2 × Sekati, CP1 × Maradol and CP3 × Maradol hybrids may become interesting, since, in addition to high productivity (Table 2), they use the Sekati or Maradol genotypes as one of the parents, which, according to Vivas et al. (2013) are promising in relation to phoma spot resistance.

The characteristics FRL and FRD ranged from 13.27 to 30.26 cm and 7.91 to 14.47 cm, respectively. Variety THB and hybrid Baixinho × Pecíolo Curto showed the lowest means for FRL (13.96 and 7.91 cm, respectively) and FRD (13.27 and 8.24 cm, respectively), which is typical of Solo papayas.

The FRL/FRD ratio formed three groups, with more than 70% of the hybrids comprising the group with the lowest means, ranging from 1.61 to 1.92. The group with the highest means consists of the two hybrids CP2 × Sekati (2.35) and CP3 × Sekati (2.28), and the group of intermediate means consisted only of the hybrid CP2 × São Mateus. The FRL/FRD ratio is useful as an approximate indication of fruit shape.

GTP and STP ranged from 2.56 to 4.02 and 1.74 to 2.86 cm, respectively, between the evaluated cultivars, with means close to 2.0 cm (Table 2), which is the thickness considered ideal for commercialization (Martins et al., 2006). Oliveira et al. (2010) observed a significant and positive correlation, although low (0.42), between pulp thickness and fruit firmness.

DFC ranged from 4.33 to 7.76 cm, yielding three groups: 'a' group with the highest means formed by the hybrids CP1 × Maradol (8.08 cm), CP3 × Maradol (7.92 cm) and CP2 × Sekati (7.13 cm); 'b' group consisted of 58% of the cultivars evaluated; and 'c' group with the lowest means formed by the variety THB and the hybrid Baixinho × Pecíolo Curto. Fioravanço et al. (1992) and Dias et al. (2011) suggested that DFC is related to fruit quality, since fruits with smaller cavity diameter generally have a greater percentage of their total volume composed of edible pulp and are more resistant to postharvest damage during transport to distant markets.

The cultivars showed SS-8 and SS-12 ranging from 8.85 to 11.73 and 9.75 to 14.25 °Brix, respectively. The Scott-Knott analysis separated two groups of cultivars within the variables SS-8 and SS-12. The cultivars in the high SS-8 group, consisting of CP1 × UEN/Caliman 01, Baixinho × Pecíolo Curto, CP1 × SSAM, CP1 × 72/12, THB, and CP1 × São Mateus, all appeared in the high SS-12 group, as well. The results found in this study are consistent with the characteristics of Solo fruits required by the market, around 11.5 °Brix (Fagundes and Yamanishi, 2001). Variability of soluble solids content in papaya fruits was also verified in the evaluation of different new genotypes obtained by breeding work (Marin et al., 2006; Oliveira et al., 2010; Dias et al., 2011).

FIRM-8 and FIRM-12 varied from 10.50 to 13.21 kgf cm^{-2} and 8.00 to 12.67 kgf cm^{-2}, respectively, and Scott-Knott analysis revealed two groups. The groups of the highest means for the characteristics FIRM-8 and FIRM-12 comprised 75 and 63% of the cultivars with means ranging from 12.08 to 13.21 kgf cm^{-2} and 10.50 to 12.67 kgf cm^{-2}, respectively, indicating that the fruits met a good firmness standard. Evaluating improved papaya genotypes, Viana et al. (2015) found a satisfactory result with maximum firmness of 8.35 kgf cm^{-2}. Less firm fruits require greater care, being less resistant to transportation, storage, and handling damage (Fagundes and Yamanishi, 2001; Morais et al., 2007).

The characteristic YIELD ranged from 71.85 to 159.34 t ha^{-1} and Scott-Knott analysis revealed three groups. Group 'a' with the highest means comprises the hybrids CP3 × Sekati (159.34 t ha^{-1}) and CP2 × Sekati (146.67 t ha^{-1}) which, although categorized in the lowest TNMF group, showed the highest YIELD means, because of large individual fruit size. Group "b" with intermediate means comprises the hybrids CP1 × Maradol (130.22 t ha^{-1}), CP1 × SSAM (125.42 t ha^{-1}), and CP3 × Maradol (124.54 t ha^{-1}). The remaining 58% of the evaluated

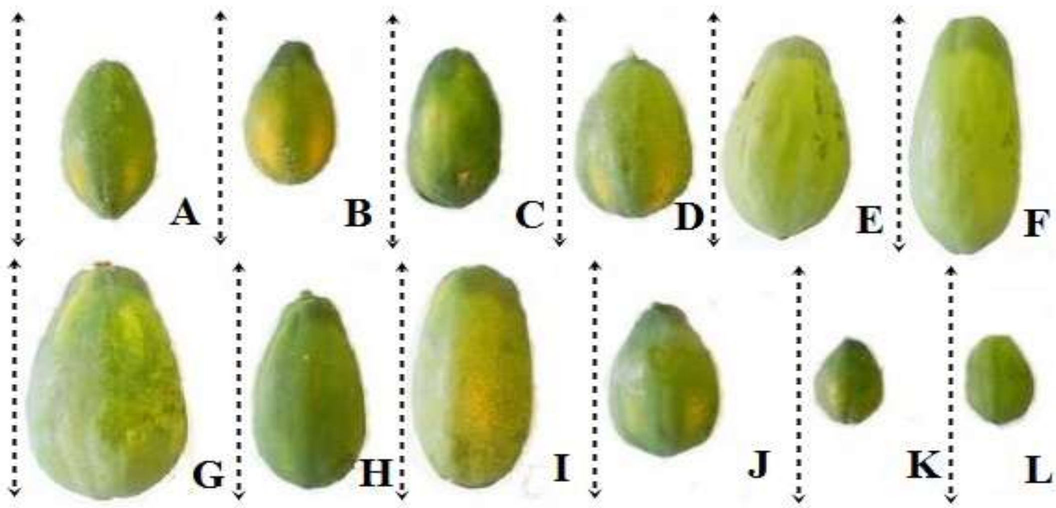

Figure 1. Fruits of 12 cultivars of papaya (*Carica papaya* L.) at 1/4 maturation stage. (A) CP1 × São Mateus; (B) CP1 × 72/12; (C) CP2 × São Mateus; (D) CP3 × São Mateus; (E) CP1 × Maradol; (F) CP2 × Sekati; (G) CP3 × Maradol; (H) CP1 × UENF/Caliman 01; I – CP3 × Sekati; (J) CP1 × SSAM; (K) Baixinho × Pecíolo Curto; (L) THB. *The dotted arrow to the left of the photo represents 40 cm in length.

cultivars belong to group "c", with the lowest YIELD means, ranging from 71.85 to 112.50 t ha^{-1}. Among the hybrids with the highest productivity, CR3 × Sekati shows promise with a fruit weight of around 2 kg, small internal cavity, good pulp thickness and good firmness of the pulp, besides presenting smooth peel fruits with good visual appearance (Figure 1). Because it has large fruits, this hybrid is not suitable for export, but it is a good option for the domestic market to serve the pulp processing market.

In contrast, even though the hybrid Baixinho × Pecíolo Curto and the variety THB had the highest TNMF means, they were grouped with the cultivars of the lowest YIELD means. This result is due to the low FRM means of both cultivars, which characterize them as belonging to the Solo group. However, the hybrid Baixinho × Pecíolo Curto should be studied further because it has important characteristics to be explored, such as fruits with mass around 0.5 kg and smaller diameter of the internal cavity of all hybrids evaluated (Figure 2), good soluble solids content, characteristics sought for *in natura* consumption, internal and external market.

The YIELD of the 12 cultivars evaluated was very satisfactory when compared with other hybrids with similar fruit sizes such as Tainung 01. This cultivar showed, in response to irrigation depths and soil covers, yield varying from 138.1 to 175.7 ton ha^{-1}, with each plant producing, on average, 55.6 fruits throughout the cycle (Gomes Filho et al., 2008).

Marin et al. (2003) stated that the growers' preference is for hermaphroditic plants with pear-shaped and/or elongated fruits, small fruit cavity and greater pulp thickness. This set of characteristics gives greater commercial value to the fruit in the market. Photographs of fruit phenotypes of the twelve evaluated cultivars are provided in Figures 1 and 2.

The shape of the ovarian cavity depends on the carpel formation. Ruggiero (1988) discussed that a small cavity is preferred, as it provides a greater amount of pulp and the seeds are easy to remove. An example among the hybrids we evaluated is Baixinho × Pecíolo Curto, with a very small ovarian cavity (Figure 2).

Overall, the most new hybrids studied have phenotypic characteristics that are acceptable to the domestic and international consumer market of papaya. The results of this study indicate that we can use the papaya cultivars as alternatives with potential to meet the demands of both consumers and producers. Further research is indicated to check the resistance to diseases that affect the crop.

Conclusions

Among the hybrids evaluated, characteristics of fruit production and fruit quality of interest were found suggesting that they should undergo value for cultivation and use testing for future release as commercial hybrids.

The hybrid CP3 × Sekati was shown to be promising because of the highest estimated average yield of marketable fruits in 12 months, which is directly related to production and sustainability of the papaya crop. It is also

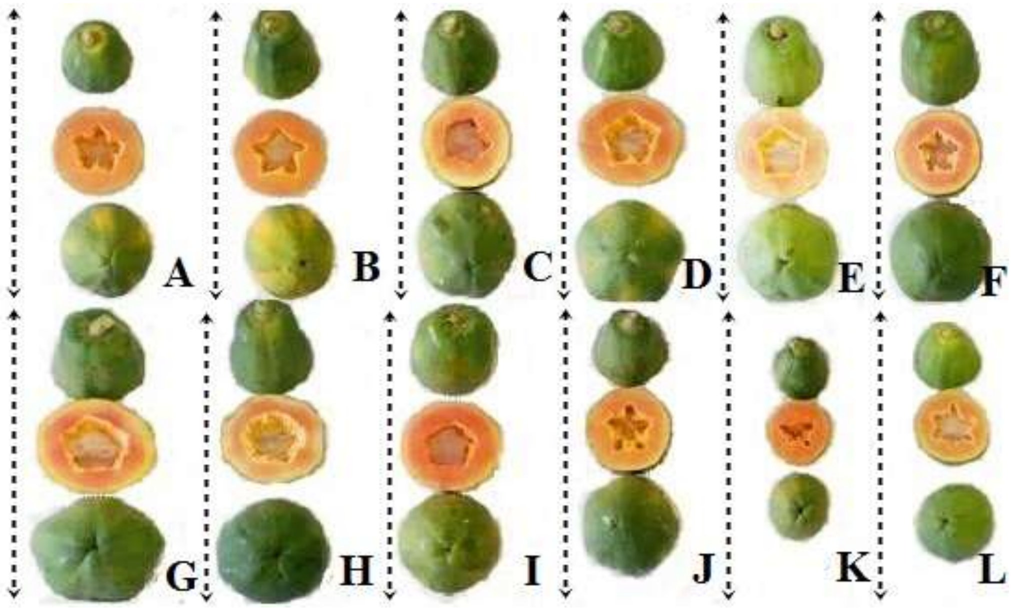

Figure 2. Fruits representation, in cut, of the 3/4 maturation stage of the 11 papaya hybrids (*Carica papaya* L.). (A) CP1 × São Mateus; (B) CP1 × 72/12; (C) CP2 × São Mateus; (D) CP3 × São Mateus; (E) CP1 × Maradol; (F) CP2 × Sekati; (G) CP3 × Maradol; (H) CP1 × UENF/Caliman 01; I – CP3 × Sekati; (J) CP1 × SSAM; (K) Baixinho × Pecíolo Curto; (L) THB. *The dotted arrow to the left of the photo represents 40 cm in length.

indicated that the hybrid Baixinho × Pecíolo Curto, that presents fruits with quality acceptable for the internal and external market. Another four hybrids merit attention in new studies, because they present fruits around 1 kg and good content of soluble solids: CP1 × UENF/Caliman 01, CP1 × 72/12, CP1 × SSAM, and CP1 × São Mateus.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Patterns of pre-weaning piglet mortality and economic losses in field condition

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The study was purposively taken up with the objective to find out the patterns of pre-weaning piglet mortality and economic losses in field condition. The study was purposively taken up in Gorkhaland Territorial Administration area where pig farming is a common practice. The study shows that overall pre-weaning piglet mortality was 15.62% where it was slightly higher in exotic than indigenous breed. Highest pre-weaning mortality was recorded among 0 to 15 days age group piglets mainly during winter months and in third parity of dam. The major reasons of pre-weaning piglet mortality found in the study area were chilling, piglet anemia and scouring. Overall economic losses due to pre-weaning piglet mortality were around $18696 during the last 3 years in which it was around $5453 in indigenous breed and around $13243 in exotic breeds. Economic losses due to pre-weaning piglet mortality was recorded highest for scouring, followed by chilling and low birth weight in indigenous breed, whereas it was highest for piglet anemia followed by chilling and scouring in exotic breed. Therefore, proper healthcare programme and management practices must be undertaken to avoid these huge economic losses under field condition.

Key words: Piglet mortality, indigenous breed, exotic breed, piglet anemia.

INTRODUCTION

Pig production, particularly in the tropical Indian condition, has high potentials for optimum profit making. The profitability of swine industry largely depends on the survival of piglets/litters up to weaning besides other closely related factors such as litter size and weight of piglets at birth. The overall mortality as well as morbidity of pigs depends on pre-weaning care, management, litter size, weight of litter, age, season and effective health care. Causes of mortality and morbidity may be multi factorial, including lack of awareness among the farmers and pig breeders regarding management practices, disease prevention and control measures, and above all,

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a high incidence of fatal diseases (Mondal et al., 2012). Several studies had attributed neonatal mortality in pigs to be of multifactor causes including diseases; other factors were low viability, chilling, maternal overlay and poor management practices (Hrupka et al., 1998; John, 2004; Damron, 2009). Frazer (1990) defined neonatal mortality in pig as death that occurs in piglets within few days of life. In agreement, Hughes (1993) noted that 50% of all pre-weaning death occurs within the first three days of life and that 90% of all were within one week of parturition. Accordingly various researchers recorded neonatal mortality, such as Pathiraja et al. (1987) who noted it to be as high as 50%; Kumar et al. (1990) reported 28.14%; Grissom et al. (1990) reported between 12.2 and 24.2%; Boe (1994) reported 14.4%; Vaillancourt et al. (1994) reported between 10 and 15%; Varley (1995) reported 13% among cross breed; Tuchscherer et al. (2000) reported between 10 and 20%; Nandakumar et al. (2004) reported 31.36% among indigenous breed and 10.49% among crossed-bred; Wabacha et al. (2004) reported 18.70%; Dutta and Rahman (2006) reported 30.62%; Kliebenstein et al. (2007) reported 26.40%; Li et al. (2010) reported 23 and 27% and Pedersen et al. (2011) had reported 19%.

Further, the mortality pattern and occurrence of different diseases and disorders may also vary with different genetic groups of pigs (Gupta et al., 2001; Nandakumar et al., 2004). Not all the factors associated with mortality can be controlled, but understanding them will assist the farmers and producers in minimizing death loss (Holyoake et al., 1995). Retrospective study on mortality may play a role in forecasting the future occurrence of disease in a particular geographical area (Basumatary et al., 2010). Although a few studies have been conducted in a scattered way on mortality incidence of piglet in organized swine farm under tropical condition but mortality pattern needs to be documented in field condition. Therefore, the present study was undertaken to document the pre-weaning piglet mortality patterns and economic losses in field condition.

MATERIALS AND METHODS

Study location

The study was carried out in backyard pig farms located within Gorkhaland Territorial Administration (GTA) area. GTA is a semi-autonomous administrative body for Darjeeling hills in West Bengal, India. GTA replaced Darjeeling Gorkha Hill Council which was formed in 1988 and administered Darjeeling hills for 23 years (Anonymous, 2011; Dutta, 2011). GTA presently has three hill Sub-divisions Darjeeling, Kalimpong, Kurseong and some areas of Siliguri Sub-division under its authority. It has an area of 3,149 square kilometers. Annual mean maximum and minimum temperature at administrative headquarter of GTA (that is, Darjeeling town) is 14.9 and 8.9°C, respectively. Average rainfall is 2831.9 mm and average numbers of rainy days are 106 days (Anonymous, 2012). The altitude of Darjeeling town is 2134 m above sea level.

Study design

An ex post facto study was designed to analyze the patterns of pre-weaning piglet mortality and economic losses in field condition. A cross sectional field survey on backyard pig farms was conducted by stratified purposive sampling methods. The respondents selected for the study were those who maintain a breeding stock of at least 2 sows. One community block each was randomly selected from 3 sub-divisions to collect data for recording the parameters such as sex, age, season, dam, causes of mortality and economic losses due to mortality. From each community block, 2 villages were purposely selected on the basis of large numbers of pig breeders. From each village, 5 pig breeder of indigenous and exotic breed were selected randomly for data collection through simple random sampling, thus forming a sample size of 60 respondents' compound, 30 indigenous pig breeders and 30 exotic pig breeders. Data presented in the study were collected through personal interview schedule from the respondents for last 3 years from 2012 to 2014. Since backyard farmers did not keep record of their farm; data were collected from the respondents on recall basis. Pre-weaning mortality was calculated from the percent ratio of the piglet dead pre-weaning to piglet born alive. The pig mortality was again divided into five seasons (that is, Spring = March to April, Summer = May to July, Rainy = June to August, Autumn = September to November and Winter = December to February). The parity of dam was determined as first (Pty-1), second (Pty-2), third (Pty-3), fourth (Pty-4) and fifth onwards (Pty-5).

Data analysis

Data was coded and entered into excel spreadsheets and simple statistical analysis such as frequency, percentage, chi-square test was performed using SPSS 20.0 software.

RESULTS AND DISCUSSION

The study was conducted to record the pre-weaning piglet mortality only in the study area. Though still born mortality was also recorded. Stillborn mortality was 78 in indigenous breed (Male 37, Female 41) and 434 in exotic breed (Male 195, Female 239) during the last 3 years. The rate of stillborn is reported to be greatest in high parity sows (Li et al., 2010), probably due to dystocia caused by fatness or poor uterine muscle tone (Kirkden et al., 2013).

The average pre-weaning piglet mortality was 15.62%. The study further shows that mortality in exotic breeds was slightly higher (15.81%) than indigenous breed (15.26%). Mortality in female piglet was found higher in both indigenous and exotic breeds but chi-square test shows no significant difference in piglet mortality between male and female piglet among indigenous and exotic breeds. The chi-square test also shows no significant difference in piglet mortality between indigenous and exotic breeds (Table 1).

Mortality was found highest (6.94%) among piglets in the age group of 0 to 15 days. Piglet mortality reduced

Table 1. Sex-wise piglet mortality in indigenous and exotic breed in different years.

Year	Indigenous						Exotic						Total Mortality		
	No of birth			No. of death (%)			No of birth			No. of death (%)			No of birth	No of death	Mortality (%)
	M	F	Total	M	F	Total	M	F	Total	M	F	Total			
2012	153	205	358	22 (14.38)	28 (13.66)	50 (13.97)	371	344	715	54 (14.56)	59(17.15)	113 (15.80)	1073	163	15.19
2013	165	162	327	28 (16.97)	31 (19.14)	59 (18.04)	343	296	639	59 (17.20)	67 (22.64)	126 (19.72)	966	185	19.15
2014	204	186	390	19 (9.31)	36 (19.35)	55 (14.10)	356	384	740	41 (11.52)	51(13.28)	92 (12.43)	1130	147	13.01
Total	**522**	**553**	**1075**	**69 (13.22)**	**95 (17.18)**	**164 (15.26)**	**1070**	**1024**	**2094**	**154 (14.39)**	**177 (17.29)**	**331 (15.81)**	**3169**	**495**	**15.62**
χ^2	--	--	--	2.154^{NS}		--	--	--	--	0.232^{NS}		--	--	--	--
	--	--	--					0.350^{NS}					--	--	--

Figures in parenthesis indicate percentage; M= Male, F= Female, NS= Non significant.

Table 2. Age and sex-wise piglet mortality in indigenous and exotic breed.

Age (days)	Indigenous			Exotic			Total Mortality	
	No. of death (%)			No. of death (%)			No of death	Mortality (%)
	M	F	Total	M	F	Total		
0-15	27 (5.17)	46 (8.32)	73 (6.79)	64 (5.98)	83 (8.11)	147 (7.02)	220	6.94
16-30	15 (2.87)	28 (5.06)	43 (4.00)	43 (4.00)	47 (4.59)	90 (4.30)	133	4.20
31-45	10 (1.92)	17 (3.07)	27 (2.51)	26 (2.43)	32 (3.13)	58 (2.77)	85	2.68
45 up to weaning	17(3.26)	4 (0.72)	21 (1.95)	21 (1.96)	15 (1.47)	36 (1.72)	57	1.80
χ^2	1.893^{NS}		--	0.646^{NS}		--	--	--
			0.000^{NS}				--	--

Figures in parenthesis indicate percentage; M= Male, F= Female, NS= Non significant.

the age of the piglet increased. Mortality in female piglet was found higher in both indigenous and exotic breeds but chi-square test showed no significant difference in piglet mortality between male and female piglet among indigenous and exotic breeds due to age factors. The chi-square test also showed no significant difference in piglet mortality between indigenous and exotic breeds due to age factors (Table 2).

Highest mortality (33.16%) was recorded in winter seasons followed by rainy (19.57%) and spring seasons (10.46%). Similarly, mortality of both indigenous and exotic breed of piglet was recorded highest in winter followed by rainy and spring seasons. Mortality of female piglets was found higher in both indigenous and exotic breeds. Chi-square test shows highly significant difference in piglet mortality between male and female piglet in exotic breeds due to the effect of seasons but shows no significant difference in piglet mortality between male and female piglet in indigenous breeds due to the effect of seasons. The chi-square test also shows significant difference in piglet mortality between indigenous and exotic piglets due to the effect of seasons (Table 3). Kabuga and Annor (1991) also reported that the pre-weaning piglet mortality was highest at cold and rainy months.

Table 4 reveals that highest (17.96%) mortality was recorded in Pty-3 followed by Pty-1 (17.59%)

Table 3. Piglet mortality in indigenous and exotic breed according to season of death.

Season	Indigenous						Exotic						Total Mortality		
	No. of birth			No. of death (%)			No of birth			No. of death (%)			No of birth	No of death	Mortality (%)
	M	F	Total	M	F	Total	M	F	Total	M	F	Total			
Spring	124	128	252	12 (9.68)	17 (13.28)	29 (11.51)	241	253	494	11 (4.56)	38 (15.02)	49 (9.92)	746	78	10.46
Summer	96	85	181	3 (3.13)	8 (9.41)	11 (6.08)	227	209	436	7 (3.08)	22 (10.53)	29 (6.65)	617	40	6.483
Rainy	116	123	239	18 (15.52)	26 (21.14)	44 (18.41)	204	201	405	46 (22.55)	36 (17.91)	82 (20.25)	644	126	19.57
Autumn	84	120	204	7 (8.33)	15 (12.5)	22 (10.78)	209	158	367	12 (5.74)	21 (13.29)	33 (8.99)	571	55	9.632
Winter	102	97	199	29 (28.43)	29 (29.9)	58 (29.15)	189	203	392	78 (41.27)	60 (29.56)	138 (35.2)	591	196	33.16
χ^2	--	--	--	2.453^{NS}		--	--	--	--	14.015**		--	--	--	--
	--	--	--					23.546**					--	--	--

Figures in parenthesis indicate percentage; M= male, F= female, NS= Non Significant, **p<0.01

Table 4. Piglet mortality in indigenous and exotic breed according to parity of dam.

Parity	Indigenous						Exotic						Total Mortality		
	No. of birth			No. of death (%)			No of birth			Mortality (%)			No of birth	No of death	Mortality (%)
	M	F	Total	M	F	Total	M	F	Total	M	F	Total			
Pty-1	74	89	163	9 (12.16)	15 (16.85)	24 (14.72)	102	116	218	15 (14.70)	24 (20.69)	39 (19.72)	381	67	17.59
Pty-2	89	101	190	8 (8.99)	12 (11.88)	20 (10.53)	179	162	341	12 (6.70)	25 (15.43)	37 (11.44)	531	59	11.11
Pty-3	136	124	260	22 (16.18)	24 (19.35)	46 (17.69)	249	215	464	24 (9.64)	36 (16.74)	60 (18.10)	724	130	17.96
Pty-4	121	137	258	18 (14.88)	27 (19.71)	45 (17.44)	324	276	600	27 (9.78)	53 (19.20)	80 (15.67)	858	139	16.2
Pty-5	102	102	204	12 (11.76)	17 (16.67)	29 (14.22)	216	255	471	17 (7.87)	39 (15.29)	56 (15.07)	675	100	14.81
χ^2	--	--	--	0.168^{NS}		--	--	--	--	0.706^{NS}		--	--	--	--
	--	--	--					0.618^{NS}					--	--	--

Figures in parenthesis indicate percentage; M= male, F= female, NS= Non Significant

and Pty-4 (16.2%). Lowest mortality was recorded in Pty-2. The study shows no trends of piglet mortality due to parity of dam as found in earlier studies. Mortality in female piglet was found higher in both indigenous and exotic breeds but chi-square test shows no significant difference in piglet mortality between male and female piglet among indigenous and exotic breeds due to parity of dam. The chi-square test also shows no significant difference in piglet mortality between indigenous and exotic breeds due to parity of dam. The study contradicts the study of Daza et al. (1999) and Li et al. (2010) who had reported that piglet mortality rate increased by parity order.

This study has previously stated that the average pre-weaning piglet mortality was 15.62%. Further the study revealed that chilling (2.87%), piglet anemia (2.71%) and scouring (2.62%) were the major reasons for pre-weaning piglet mortality in the study area. The death due to scouring, individual low birth weight, starvation and gaining access to colostrums were comparatively higher in indigenous breeds of piglets than exotic piglets whereas death due to maternal overlay, piglet anemia were comparatively higher in exotic breeds of piglets than indigenous piglets (Table5).

Overall economic loss due to pre-weaning piglet

Table 5. Pre-weaning piglet mortality in indigenous and exotic breed according to causes.

Causes	Indigenous			Exotic			Overall mortality
	M	F	Total	M	F	Total	
Maternal over lay	2 (0.38)	6 (1.08)	8 (0.74)	11 (1.03)	22 (2.15)	33 (1.58)	41 (1.29)
Scouring	15 (2.87)	19 (3.44)	34 (3.16)	18 (1.68)	31 (3.03)	49 (2.34)	83 (2.62)
Hypoglycemia	6 (1.15)	8 (1.45)	14 (1.30)	12 (1.12)	15 (1.46)	27 (1.29)	41 (1.29)
Individual low birth weight	10 (1.92)	16 (2.89)	26 (2.42)	21 (1.96)	15 (1.46)	36 (1.72)	62 (1.96)
Piglet anemia	8 (1.53)	16 (2.89)	24 (2.23)	34 (3.18)	28 (2.73)	62 (2.96)	86 (2.71)
Cannibalism	0	0	0	9 (0.84)	3 (0.29)	12 (0.57)	12 (0.38)
Starvation and gaining access to colostrums	9 (1.72)	15 (2.71)	24 (2.23)	17 (1.59)	19 (1.86)	36 (1.72)	60 (1.89)
Chilling	17 (3.26)	12 (2.17)	29 (2.70)	26 (2.43)	36 (3.52)	62 (2.96)	91 (2.87)
Unknown causes	2 (0.38)	3 (0.54)	5 (0.47)	6 (0.56)	8 (0.78)	14 (0.67)	19 (0.6)

Figures in parenthesis indicate percentage; M= male, F= female.

Table 6. Economic losses due to piglet mortality in filed condition.

Causes of Mortality	Indigenous							Exotic							Total economic losses ($)
	Male			Female			Economic loss ($)	Male			Female			Economi c loss ($)	
	2012	2013	2014	2012	2013	2014		2012	2013	201 4	2012	201 3	2014		
Maternal over lay	1	0	1	2	3	1	246	2	5	4	6	8	8	1285	1531
Scouring	5	6	4	7	5	7	1099	12	3	3	9	13	9	1863	2962
Hypoglycemia	2	1	3	2	3	3	455	2	6	4	6	4	5	1053	1508
Individual low birth weight	2	6	2	6	6	4	837	7	9	5	3	5	7	1428	2265
Piglet anemia	6	1	1	2	6	8	756	15	12	7	14	8	6	2378	3134
Cannibalism	0	0	0	0	0	0	0	4	3	2	1	0	2	478	478
Starvation and gaining access to colostrums	2	4	3	4	5	6	782	4	7	6	9	6	4	1393	2175
Chilling	3	9	5	4	3	5	982	8	12	6	11	16	9	2393	3375
Unknown causes	1	1	0	1	0	2	161	0	2	4	0	7	1	566	727
Total economic losses	**5318**							**12837**							**18155**

mortality was around $18155 among the respondents during last 3 years. Economic losses due to pre-weaning piglet mortality in indigenous breed were around $5318 whereas the economic losses in exotic breed were around $12837 during last 3 years (Table 6). Economic losses due to pre-weaning piglet mortality was recorded highest for scouring followed by chilling and low birth weight in indigenous breed whereas it was highest for piglet anemia followed by chilling and scouring in exotic breed.

Conclusion

The study shows that pre-weaning piglet mortality was a major problem among the pig farmers in the study area. As we know that all the factors associated with mortality cannot be controlled, but understanding them and taking proper healthcare, feeding and management practices will assist the farmers and producers in minimizing death loss. Therefore, proper healthcare programme and management practices must be undertaken in advance to avoid these huge economic losses under field condition. The extension workers in the study area also need to enrich knowledge of the pig breeders with scientific pig farming practices comprising breeding, feeding, healthcare and management practices so that pre-weaning mortality of the piglet can be reduced. Policy makers further need to take initiative to provide healthcare services to their doorstep without much time lag.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Performance of maize hybrids from a partial diallel in association with Azospirillum

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One of the most prominent strategies to increase maize grain yield with a higher benefit/cost ratio and a lower environmental impact is the inoculation of plant growth-promoting bacteria. Among other factors, the success of the interaction plant-microorganism depends on genetic traits, therefore, selection of plant genotypes compatible with this association is extremely important to the viability of this technology. This article presents an innovative study that investigates the interactions between *Azospirillum brasilense* Ab-V5 and 27 genotypes of maize, including 24 experimental hybrids from a partial diallel (denotated H_{ij} as a result of the crosses among the parental inbred lines L_i and the tester breeding lines T_j), the variety ST0509 from UEL and the commercial hybrids DKB390 and DKB390H from Monsanto. The plots consisted of treatments with or without inoculation in three replicates and the 27 maize genotypes were randomly distributed in the sub-plots. The inbred lines L_2, L_3, L_6, L_{11}, T_2 and T_3 present the highest general combining ability, producing the best hybrid combinations. The additive effects of genes are more important than the non-additive effects for all traits evaluated. The most promising experimental hybrids are $H_{2\ 3'}$, $H_{3\ 2'}$, $H_{11\ 2'}$, $H_{11\ 3'}$ and $H_{12\ 3'}$. Significant effect for inoculum was not verified when performed at the seedling stage in the experimental conditions of this study.

Key words: *Zea mays* L., *Azospirillum brasilense*, inoculation, biological nitrogen fixation, combining ability.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal crops for mankind due to the type and quantity of reserve substances of its grains, being used for human food and animal feed, consumed *in natura* and in industrial forms (Pereira et al., 2009). This grass presents high productive potential as well as high demand for nutrients, especially

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Table 1. Incomplete partial diallel formed by simple hybrids (H) derived from the crosses among 12 elite breeding lines (L_1 and L_{12}) and three tester lines (T_1, T_2 and T_3).

Lines	$T_{1`}$	$T_{2`}$	$T_{3`}$
L_1	-	$H_{1\ 2'}$	$H_{1\ 3'}$
L_2	$H_{2\ 1'}$	-	$H_{2\ 3'}$
L_3	$H_{3\ 1'}$	$H_{3\ 2'}$	-
L_4	$H_{4\ 1'}$	$H_{4\ 2'}$	-
L_5	-	$H_{5\ 2'}$	$H_{5\ 3'}$
L_6	$H_{6\ 1'}$	-	$H_{6\ 3'}$
L_7	$H_{7\ 1'}$	$H_{7\ 2'}$	-
L_8	$H_{8\ 1'}$	-	$H_{8\ 3'}$
L^9	$H_{9\ 1'}$	$H_{9\ 2'}$	-
L_{10}	$H_{10\ 1'}$	-	$H_{10\ 3'}$
L_{11}	-	$H_{11\ 2'}$	$H_{11\ 3'}$
L_{12}	$H_{12\ 1'}$	-	$H_{12\ 3'}$

(N), which directly influences grain yield components such as photosynthesis rate, ear size, mass, sanity, number and protein content of grains (Dechorgnat et al., 2011).

Despite the benefits of the use of nitrogen fertilizer, it represents up to 40% of the total cost of maize production, due to the facts that it is largely required to reach high yields and that the use efficiency of this nutrient by the crop is low (Rambo et al., 2007). In addition to the high cost of this input, it presents risks of environmental pollution associated with leaching, denitrification and volatilization (Vitousek et al., 2009), which may lead to acidification of soils, eutrophication and increase of greenhouse gases in the atmosphere (Galloway et al., 2008). Therefore, the importance of developing strategies to increase the nitrogen use efficiency (NUE) of crops and consequently decrease the economic and environmental impact on agricultural systems is evident.

One of the strategies to increase yield, with the best benefit/cost ratio considering the environmental impact, is the use of inoculation of plant growth-promoting bacteria (diazotrophic PGPB), representing a technology of low cost and simple implementation. The mechanisms of plant growth-promotion manifested by diazotrophic PGPB encompass direct processes such as biological nitrogen fixation (BNF), production of plant growth regulators, nutrient mineralization, inorganic phosphate solubilisation and increased uptake by roots; as well as indirect effects including biological control of phytopathogens, production of siderophores and induction of systemic resistance in plants (Oliveira et al., 2014).

A great number of studies have shown that plant growth-promoting bacteria (PGPB), including Azospirillum, are able to promote growth and increase yield of numerous plant species (Fallik and Okon, 1996), such as wheat, rice, maize and sorghum, where the average increase in productivity was around 20 to 30 % (Kennedy et al., 2004, Morrissey et al., 2004, Andreotti et al., 2008).

Commercial inoculants formulated with the diazotrophic plant growth-promoting bacteria (PGPB) *Azospirillum brasilense* are available for use in Brazil; however, its application is not yet adopted as a routine practice for partial substitution of synthetic nitrogen fertilizers. Inconsistencies in the performance of the inoculation with diazotrophic PGPB in field studies are a major obstacle to its wide spread, resulting mainly from limitations in the process of plant colonization. Among the factors that hinder the establishment of the inoculated microorganisms are: the use of low quality formulations and/or improper practices during transport, storage and field application, and the occurrence of unfavorable edaphoclimatic conditions for the maintenance of a high population size of the inoculated bacteria within the plant (Bashan et al., 2014).

In addition, specific molecular interactions between the associative pair are crucial for plant colonization by PGPB, which depends on genetic factors (Drogue et al., 2012; Jha et al., 2013). In this regard, the identification of highly compatible plant genotypes for association with PGPB may enhance plant colonization, enabling a higher level of expression of genes related to the compatibility of the interaction and consequently maximizing growth promotion (Meneses et al., 2011; Alquéres et al., 2013; Beauregard et al., 2013).

Therefore, the selection of genotypes favorable to this association is a field of research to be explored in order to consolidate the inoculation technology with diazotrophic PGPB as a viable alternative to synthetic nitrogen fertilizers for maize production. In this context, diallel analysis is an essential tool to identify superior parents for hybrid or cultivar development related to several traits of interest (Patel et al., 1998). Thus, the objectives of this work were to determine, using partial diallel crossing, the general and specific combining ability of twelve elite inbred lines of maize with three tester lines and to verify their possible interactions with *A. brasilense* strain Ab-V5.

MATERIALS AND METHODS

The experimental hybrids used in these experiments were developed by the Maize Breeding Programme at the Department of Biology from the State University of Londrina (UEL), derived from partial diallel crosses among three tester lines (T_1, T_2 and T_3) and twelve elite breeding lines (L_1 to L_{12}) obtained from the synthetic cultivars (improved varieties) ST06 and ST20, respectively (Table 1).

A total of 27 genotypes were evaluated: 24 experimental hybrids from the partial diallel, the variety ST0509 developed at UEL and the commercial hybrids DKB390 and DKB390H from Monsanto. The commercial hybrids were used as a performance standard for the comparison of the experimental hybrids. The variety was used for inoculation purposes, to test whether its rustic genotype would favor the association with the rhizobacteria.

The experiments were conducted at the State University of Londrina, located in the Northern region of the State of Paraná (23° 19'19 ''S and 51° 12'04'' W, 580 m of altitude) during the first and second growing season of 2011/2012, in a randomized block design with treatments arranged in split-plots with or without inoculation, with three replicates. Each plot with or without inoculum contained one representative of each genotype in a row of 4 m containing 30 plants per row, with 0.8 m between rows and 0.2 m between plants within the row. Soil preparation for sowing was done by harrowing and applying 300 kg ha^{-1} of the formulated 08-28-16 (N-P-K). Weed was controlled by manual weeding and pest control (for example, *Spodoptera frugiperda*) was carried out according to technical recommendations for the crop.

The inoculum was prepared with *A. brasilense* strain Ab-V5 from isolated colonies grown in the solid medium Dygs (2 g glucose, 1.5 g peptone, 2 g yeast extract, 0.5 g K_2HPO_4, 0.5 $MgSO_4$, 1 L distilled water, pH 6.0) and further multiplied in the liquid medium M15 for 48 h on orbital shaker at 30 ± 2 °C. The cell concentration of the bacterial culture was estimated by reading its absorbance in a spectrophotometer at 560 nm and diluting it in water to a final concentration of 3×10^7 cells mL^{-1}. The inoculation was performed on the seventh day after the seedlings emergence (V2), in the afternoon (after 16 h), using a portable spray to apply a dose of 30 mL per meter of culture directed at the seedlings.

The characteristics evaluated were: grain yield (GY, t ha^{-1}); ear length (EL, cm); ear diameter (ED, cm); cob diameter (CD, cm); number of grain rows per ear (RE); percentage of damaged ear (% DAE); percentage of diseased ear (% DIE); days to male flowering (DF); plant height (PH, cm) and ear height (EH, cm). Grain yield was estimated based on the mass of grains harvested in each experimental subplot, with moisture corrected to 13.5 % and an ideal stand of 20 plants per row, and it was extrapolated to tons per hectare. Corrections of grain weight to ideal stand (STi) were performed using the covariance methodology, modified by Miranda Filho (Vencovsky and Barriga, 1992).

Individualized and combined analysis of variance was made for the first and second harvest for the evaluation of hybrids. The individual analyses of variance were performed with the effects of genotypes decomposed on effects of controls (C), experimental hybrids (Hy) and the contrast C vs Hy. The degrees of freedom of the experimental hybrids were decomposed using diallel analysis, according to the model proposed by Griffing (1956): $Y_{ij} = m + \hat{g}_i + \hat{g}_j + \hat{s}_{ij} + \bar{e}_{ij}$, where: Y_{ij} is the mean value of the hybrid combination of the inbred line L_i with the tester line T_j; *m* is the overall mean of the experimental hybrids; $\hat{g}_i$ and $\hat{g}_j$ are the effects of the general combining ability (GCA) of the i-th inbred line L_i and the j-th inbred line T_j, respectively; $\hat{s}_{ij}$ is the effect of the specific combining ability (SCA) for crosses among the genitors i and j; and $\bar{e}_{ij}$ is the average experimental error.

The analyses of the diallels, for the first and second harvest, and their respective decomposition were made following the methodology proposed by Filho and Vencovsky (1995). For the analysis of variance of the diallel and the estimates of $\hat{g}_i$, $\hat{g}_j$ and $\hat{s}_{ij}$, the matrix algebra model was used: $Y = X\beta + \varepsilon$ where: Y is the vector of observed data for experimental hybrids; X is the matrix of constants related to the parameters *m*, $\hat{g}_i$, $\hat{g}_j$, and $\hat{s}_{ij}$; β is the vector of the parameters m, $\hat{g}_i$, $\hat{g}_j$, and $\hat{s}_{ij}$ and ε is the vector representing the error associated with the values ($\bar{e}_{ij}$). The program used to perform the analysis of variance was the Statistical Analysis System (SAS/STAT® software) and the groupings of means from the treatments of each experiment were done by the Scott-Knott test, at a significance level of 5 % of probability, using the program GENES (CRUZ, 2013).

RESULTS AND DISCUSSION

The data indicates significant effect for the majority of the traits investigated regarding growing season (harvest), except for percentage of diseased ear (Table 2). The second harvest presented a reduction of 3.45 t ha^{-1}, ears 3.6 cm smaller in length and 0.5 cm in diameter, cobs 0.5 cm smaller in diameter, 3 less grain rows per ear, 7.2% more damaged ears, 1.1% less diseased ears, 1 extra day to male flowering, and plant and ear height was 52 and 39 cm lower, respectively (Table 3). These findings are in accordance with the literature, since the climatic conditions of the second harvest are generally less favorable to the development of the plants compared to the spring-summer period (first harvest), mainly due to the decrease in light intensity and rainfall (Magalhaes et al., 2007).

The effect of inoculation was not significant for any of the traits evaluated, neither for the interactions inoculum x harvest and inoculum x cultivar x harvest (Table 2). Although the recommendation for most of the commercial inoculants based on *Azospirillum* is an application to the seeds before planting (Soja, 2011), in this study, the introduction of the inoculant was performed via spraying on V2 seedlings in order to avoid contact of the bacteria with chemicals commonly used in seed treatment, what would possibly reduce its efficiency.

These results indicate that the procedure of spraying the inoculant at the seedling stage in this study was probably not able to successfully carry the bacteria due to unfavorable environmental factors that affects the colonization and establishment of their population, such as extreme temperatures, water stress and competition with native bacteria (Figure 1) (IAPAR, 2012). Optimization of this methodology should be sought to elude climatic influence on bacterial survival on the soil and plant colonization.

Santos (2011) tested the efficiency of some inoculation methods: seedling spraying, via peat and liquid path in the seed, concluding they were all successful as vehicles, especially peat and liquid under seed. Thus, this methodology, as well as the inoculation in the plantation furrows or in the soil has demonstrated efficacy even though further studies are necessary for the fine adjustment of dose, volume applied by area and time of application (Fukami et al., 2016; Morais et al., 2016).

However, there was a significant difference for percentage of damaged ear with a decrease of 2.83% in this trait for inoculated plants cultivated in the first growing season (Table 4). Although the factors that determine associative efficiency between *Azospirillum*

Table 2. Mean squares based on treatment totals, significance levels of F test, means of inoculated and non-inoculated plots, general means and the coefficients of variation for grain yield (GY, t ha_{-1}), ear length (EL, cm), ear diameter (ED, cm), cob diameter (CD), number of grain rows per ear (RE), percentage of damaged ear (% DAE), percentage of diseased ear (% DIE), days to male flowering (FL), plant height (PH, cm) and ear height (EH, cm), evaluated in Londrina in the first and second harvest of 2011/2012.

Source of variation	DF	GY	EL	ED	CD	RE	% DAE	% DIE	FL	PH	EH
Block/Harvest	4	4.2102*	0.6991	0.1023*	0.0161*	1.6815	356.68*	419.40*	0.8781	414.01*	204.95*
Harvest (Ha)	1	968.05*	1060.6*	17.700*	4.5986*	598.62*	4148.1*	90.798	61.797*	217342*	125450*
Inoculum	1	0.5262	2.8900	0.0378	0.0474	6.7600	294.94	2.6039	8.5069	29.642	115.68
Inoculum x Ha	1	0.4170	1.5211	0.1304	0.0465	0.2612	69.843	82.318	1.4267	307.03	27.040
Error (a)	4	1.8918	2.8381	0.0411	0.0699	2.2128	67.739	174.98	5.3210	616.63	591.02
Cultivar	26	6.3059*	6.8935*	0.2237*	0.3033*	9.0875*	140.96	187.00*	17.117*	1199.6*	766.93*
Control (C)	2	37.749*	5.0544*	0.7811*	0.5426*	15.453*	326.78*	382.87*	56.694*	995.68*	181.88*
Exp Hybrid (Hy)	23	3.8400*	7.2386*	0.1828*	0.2957*	8.8638*	127.10	169.64	8.1476*	1223.4*	842.55*
GCA-L	11	3.6721*	8.4926*	0.2651*	0.5032*	9.8843*	73.225	134.53	14.657*	2113.3*	1377.0*
GCA-T	2	20.194*	23.075*	0.2905*	0.2891*	39.428*	111.72	28.323	2.4345	1687.0*	1580.2*
SCA	10	0.7534	2.6886*	0.0711*	0.0694*	1.6259*	189.45	236.52*	2.1296	151.73*	107.09*
C vs Hy	1	0.1332	2.6322	0.0493	0.0000	1.5022	88.024	194.60	144.26*	1060.3*	197.78*
Cultivar x Ha	26	1.8685*	1.9827*	0.0367*	0.0195*	0.8947	133.71	276.20*	3.0310*	189.76*	83.103*
Control x Ha	2	11.584*	4.9478*	0.0033*	0.0100	0.6711	134.62	210.11	3.5833	425.92*	102.45
Hy x Ha	23	1.1044	1.8059	0.0407	0.0211*	0.9063	137.73	263.72*	1.5697	126.24*	78.142*
GCA-L x Ha	11	1,0659	2.3496*	0.0310*	0.0213	1.0466	120.17	220.92*	1.0645	214.56*	103.77*
GCA-T x Ha	2	3.0627*	4.7056*	0.1226*	0.0746*	0.9234	345.20*	737.41*	6.7446*	16.193	108.90
SCA x Ha	10	0.7560	0.6218	0.0343	0.0094	0.7690	115.63	216.07*	1.0977	51.102	43.845
(C vs Hy) x Ha	1	0.0108	0.1168	0.0117	0.0016	1.0756	39.269	695.23*	35.537*	1178.6*	158.52*
Inuculum x Cultivar	26	0.4992	1.0115	0.0255	0.0140	0.9918	115.28	109.79	1.2986	75.310	50.376
Inoculum x Culti x Ha	26	0.8949	0.8662	0.0327	0.0236	0.6469	103.58	84.471	1.8402	98.306	54.953
Error (b)	208	0.7565	1.1494	0.0242	0.0120	0.7882	104.77	109.24	1.8239	75.319	40.508
Inoculated	-	6.66	15.96	4.71	2.89	15.50	13.77	24.18	65.98	163.05	93.02
Non-inoculated	-	6.58	15.77	4.69	2.87	15.21	15.68	24.35	66.30	162.45	91.82
General Mean	-	6.62	15.90	4.70	2.90	15.40	14.70	24.30	66.10	162.80	92.40
CV% (a)	-	8.5	4.3	1.8	3.7	3.9	22.8	22.3	1.4	6.2	10.7
CV% (b)	-	13.1	6.8	3.3	3.8	5.8	69.5	43.1	2.0	5.3	6.9

*Significance level of 5 %, = Variance analysis with dada transformed to arc sine of (% DAE or DIE /100)^{0,5}.

and maize are unknown, several studies demonstrate significant increases in grain yield components in response to inoculation, even though a large number of trials are required to eliminate spatiotemporal variations that may mask such effects (Díaz-Zorita et al., 2015).

The absence of significance for the interaction between *A. brasilense* and the different maize genotypes used in the present study indicates the

Table 3. Means of experimental hybrids (H_{ij}), resulting from the crosses of the inbred lines $L_i x T_j$, and genotype controls for grain yield (GY, in t ha^{-1}), ear length (EL, cm), ear diameter (ED, cm), cob diameter (CD, cm), umber of grain rows per ear (RE), percentage of damaged ear (% DAE), percentage of diseased ear (% DIE), days to flowering (FL), plant height (PH, cm) and ear height (EH cm), evaluated in Londrina in the first and second harvest of 2011/2012.

Cultivars	GY		EL		ED			CD		RE		% DAE		% DIE		FL		PH		EH	
	Harvest		Harvest		Harvest			Harvest		Harvest		Harvest		Harvest		Harvest		Harvest		Harvest	
	1st	2nd	1st	2nd	1st		2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
$H_{1\,2'}$	8.53^{b}	4.34^{b}	17.60^{a}	12.8^{b}	5.0^{a}	4.6^{b}	3.2^{a}	2.9^{a}	15.1^{c}	13.4^{b}	1.0^{b}	22.9^{a}	15.0^{b}	24.1^{a}	64^{c}	65^{c}	186^{c}	128^{c}	102^{d}	64^{c}	-
$H_{1\,3'}$	8.08^{b}	5.03^{a}	18.10^{a}	14.3^{a}	4.9^{a}	4.5^{c}	3.1^{b}	2.8^{b}	15.5^{c}	13.6^{b}	3.2^{b}	10.6^{a}	22.5^{b}	13.9^{a}	65^{c}	66^{c}	189^{c}	131^{c}	107^{d}	65^{c}	-
$H_{2\,1'}$	7.89^{b}	4.43^{b}	16.60^{b}	13.5^{b}	5.0^{a}	4.4^{c}	3.0^{c}	2.7^{c}	18.6^{a}	14.9^{a}	7.5^{a}	9.2^{a}	27.1^{b}	18.4^{a}	65^{c}	66^{c}	185^{c}	135^{c}	111^{c}	74^{b}	-
$H_{2\,3'}$	8.84^{b}	5.65^{a}	16.90^{b}	13.8^{b}	5.0^{a}	4.5^{b}	3.0^{c}	2.7^{c}	16.5^{b}	13.5^{b}	9.5^{a}	12.3^{a}	23.4^{b}	13.0^{a}	64^{c}	66^{c}	190^{c}	141^{b}	124^{b}	78^{b}	-
$H_{3\,1'}$	8.56^{b}	4.80^{a}	17.73^{a}	15.0^{a}	4.9^{b}	4.5^{c}	3.1^{b}	2.9^{b}	17.9^{a}	15.1^{a}	10.8^{a}	15.5^{a}	13.2^{b}	28.3^{a}	65^{c}	67^{b}	202^{b}	143^{b}	111^{c}	70^{c}	-
$H_{3\,2'}$	9.89^{b}	5.35^{a}	18.83^{a}	14.9^{a}	5.0^{a}	4.6^{b}	3.1^{b}	3.0^{a}	17.1^{a}	14.7^{a}	2.4^{b}	11.3^{a}	7.7^{b}	14.2^{a}	67^{b}	66^{c}	191^{c}	140^{b}	105^{d}	68^{c}	-
$H_{4\,1'}$	6.95^{c}	4.17^{b}	15.80^{b}	12.6^{b}	5.0^{a}	4.5^{b}	3.2^{a}	2.9^{b}	17.8^{a}	15.0^{a}	5.5^{b}	11.6^{a}	13.6^{b}	16.0^{a}	65^{c}	66^{c}	166^{d}	123^{d}	94e	62^{c}	-
$H_{4\,2'}$	8.46^{b}	3.75^{b}	17.73^{a}	13.0^{b}	5.0^{a}	4.4^{c}	3.1^{b}	2.8^{b}	16.4^{b}	13.4^{b}	8.8^{a}	18.9^{a}	24.0^{b}	27.8^{a}	65^{c}	66^{c}	162^{d}	119^{d}	94e	61^{c}	-
$H_{5\,2'}$	8.33^{b}	4.30^{b}	17.73^{a}	12.6^{b}	5.1^{a}	4.6^{b}	3.3^{a}	2.9^{a}	16.9^{a}	13.5^{b}	4.4^{b}	22.3^{a}	19.2^{b}	19.4^{a}	67^{b}	68^{b}	190^{c}	133^{c}	116^{c}	77^{b}	-
$H_{5\,3'}$	8.50^{b}	5.32^{a}	18.50^{a}	15.6^{a}	4.9^{b}	4.6^{b}	3.0^{c}	2.8^{b}	16.5^{b}	14.3^{a}	4.9^{b}	5.9^{a}	19.1^{b}	12.1^{a}	67^{b}	68^{b}	201^{b}	153^{a}	128^{b}	92^{a}	-
$H_{6\,1'}$	7.94^{b}	4.95^{a}	16.87^{b}	14.2^{a}	5.1^{a}	4.5^{b}	3.2^{a}	2.8^{b}	18.4^{a}	15.5^{a}	2.8^{b}	11.0^{a}	18.8^{b}	17.6^{a}	67^{b}	67^{b}	191^{c}	141^{b}	114^{c}	77^{b}	-
$H_{6\,3'}$	9.41^{b}	5.17^{a}	18.17^{a}	15.3^{a}	5.0^{a}	4.5^{b}	3.0^{c}	2.8^{b}	17.1^{a}	14.3^{a}	7.5^{a}	10.7^{a}	16.1^{b}	18.9^{a}	67^{b}	67^{b}	213^{a}	153^{a}	134^{a}	90^{a}	-
$H_{7\,1'}$	7.82^{b}	4.50^{b}	17.77^{a}	13.1^{b}	4.9^{a}	4.4^{c}	3.1^{b}	2.7^{c}	16.5^{b}	14.0^{b}	4.7^{b}	17.4^{a}	19.7^{b}	20.5^{a}	65^{c}	68^{b}	191^{c}	137^{b}	121^{b}	73^{b}	-
$H_{7\,2'}$	8.68^{b}	5.07^{a}	18.00^{a}	13.1^{b}	4.7^{c}	4.4^{c}	2.8^{d}	2.6^{c}	15.1^{c}	12.7^{c}	4.7^{b}	13.6^{a}	14.6^{b}	17.9^{a}	67^{b}	67^{c}	193^{c}	137^{b}	120^{b}	73^{b}	-
$H_{8\,1'}$	7.95^{b}	4.20^{b}	18.10^{a}	14.1^{a}	4.8^{b}	4.1^{d}	2.8^{d}	2.5^{d}	17.3^{a}	14.7^{a}	9.7^{a}	19.8^{a}	21.5^{b}	26.1^{a}	65^{c}	67^{b}	200^{b}	142^{b}	108^{d}	71^{c}	-
$H_{8\,3'}$	8.86^{b}	4.80^{a}	19.03^{a}	14.6^{a}	4.7^{c}	4.3^{c}	2.7^{d}	2.6^{d}	15.4^{c}	13.7^{b}	9.5^{a}	15.3^{a}	44.1^{a}	12.5^{a}	65^{c}	66^{c}	203^{b}	149^{a}	116^{c}	70^{c}	-
$H_{9\,1'}$	6.74^{c}	4.33^{b}	16.37^{b}	13.5^{b}	4.9^{b}	4.4^{c}	3.1^{b}	2.9^{b}	17.8^{a}	14.7^{a}	9.8^{a}	19.4^{a}	17.8^{b}	18.3^{a}	66^{b}	68^{b}	173^{d}	125^{d}	100e	66^{c}	-
$H_{9\,2'}$	8.67^{b}	5.64^{a}	18.13^{a}	14.8^{a}	5.0^{a}	4.8^{a}	3.2^{a}	3.0^{a}	17.6^{a}	14.4^{a}	6.6^{b}	5.7^{a}	22.3^{b}	13.1^{a}	65^{c}	66^{c}	169^{d}	131^{c}	105^{d}	68^{c}	-
$H_{10\,1'}$	7.46^{c}	4.60^{b}	16.87^{b}	13.7^{b}	5.1^{a}	4.4^{c}	3.0^{c}	2.8^{b}	17.6^{a}	14.6^{a}	13.1^{a}	10.4^{a}	31.1^{a}	15.1^{a}	65^{c}	66^{c}	171^{d}	132^{c}	106^{d}	74^{b}	-
$H_{10\,3'}$	8.30^{b}	5.29^{a}	17.67^{a}	14.1^{a}	4.9^{b}	4.4^{c}	2.7^{d}	2.7^{c}	15.7^{c}	12.9^{c}	5.0^{b}	9.2^{a}	36.2^{a}	15.6^{a}	65^{c}	66^{c}	182^{c}	138^{b}	114^{c}	79^{b}	-
$H_{11\,2'}$	8.90^{b}	5.78^{a}	18.30^{a}	14.6^{a}	5.1^{a}	4.7^{a}	3.2^{a}	3.0^{a}	16.2^{b}	14.3^{a}	3.8^{b}	12.8^{a}	17.9^{b}	18.9^{a}	64^{c}	65^{c}	183^{c}	141^{b}	107^{d}	76^{b}	-
$H_{11\,3'}$	9.18^{b}	5.51^{a}	18.83^{a}	15.2^{a}	4.9^{b}	4.4^{c}	2.8^{d}	2.7^{c}	16.1^{b}	12.7^{c}	8.7^{a}	7.7^{a}	16.0^{b}	17.2^{a}	64^{c}	66^{c}	188^{c}	142^{b}	119^{c}	77^{b}	-
$H_{12\,1'}$	8.10^{b}	4.92^{a}	17.37^{b}	14.2^{a}	4.7^{c}	4.3^{d}	2.8^{d}	2.5^{d}	16.8^{a}	13.8^{b}	6.6^{b}	9.1^{a}	11.5^{b}	22.1^{a}	64^{c}	66^{c}	185^{c}	30c	109^{c}	65^{c}	-

Cultivars	GY		EL		ED		CD		NR		% D^{A}E		% DIE		FL		PH			EH	
	Harvest		Harvest		Harvest		Harvest		Harvest		Harvest		Harvest		Harvest		Harvest			Harvest	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd		1st	2nd
$H_{12\,3'}$	8.34^{b}	5.66^{a}	18.13^{a}	15.4^{a}	4.7^{c}	4.3d	2.7d	2.6d	15.6^{c}	13.4^{b}	5.8^{b}	10.1^{a}	47.3^{a}	18.9^{a}	65^{c}	66^{c}	194^{c}	140^{b}	113^{c}	75^{b}	-
DKB390	8.25^{b}	5.07^{a}	17.13^{b}	13.6^{b}	5.1^{a}	4.6^{b}	3.2^{a}	2.9^{b}	17.1^{a}	13.7^{b}	3.5^{b}	13.7^{a}	18.3^{b}	16.6^{a}	67^{b}	67^{b}	191^{c}	138^{b}	109^{c}	72^{b}	-
DKB390H	11.08^{a}	5.49^{a}	18.73^{a}	14.0^{a}	5.1^{a}	4.7^{a}	3.1^{b}	2.9^{b}	17.7^{a}	14.6^{a}	0.6^{b}	9.5^{a}	2.9^{b}	16.6^{a}	67^{b}	66^{c}	191^{c}	131^{c}	117^{c}	73^{b}	-
ST0509	5.59d	3.89^{b}	16.23^{b}	14.0^{a}	4.7^{c}	4.2d	2.7d	2.6d	15.2^{c}	12.7^{c}	12.7^{a}	16.5^{a}	17.8^{b}	26.3^{a}	72^{a}	69^{a}	216^{a}	140^{b}	123^{b}	74^{b}	-
Mean of hybrids	8.35	4.90	17.71	14.08	4.46	4.93	3.00	2.78	16.72	14.05	6.50	13.03	21.65	18.33	65.35	66.46	187.34	136.83	111.57	72.71	-
Mean of Control	8.30	4.82	17.36	13.87	4.50	4.96	2.99	2.80	16.69	13.67	5.57	13.23	13.00	19.83	68.53	67.33	199.16	136.33	116.28	73.00	-

Means followed by the same letter belong to the same group by the Scott-Knott test at a significance level of 5%.

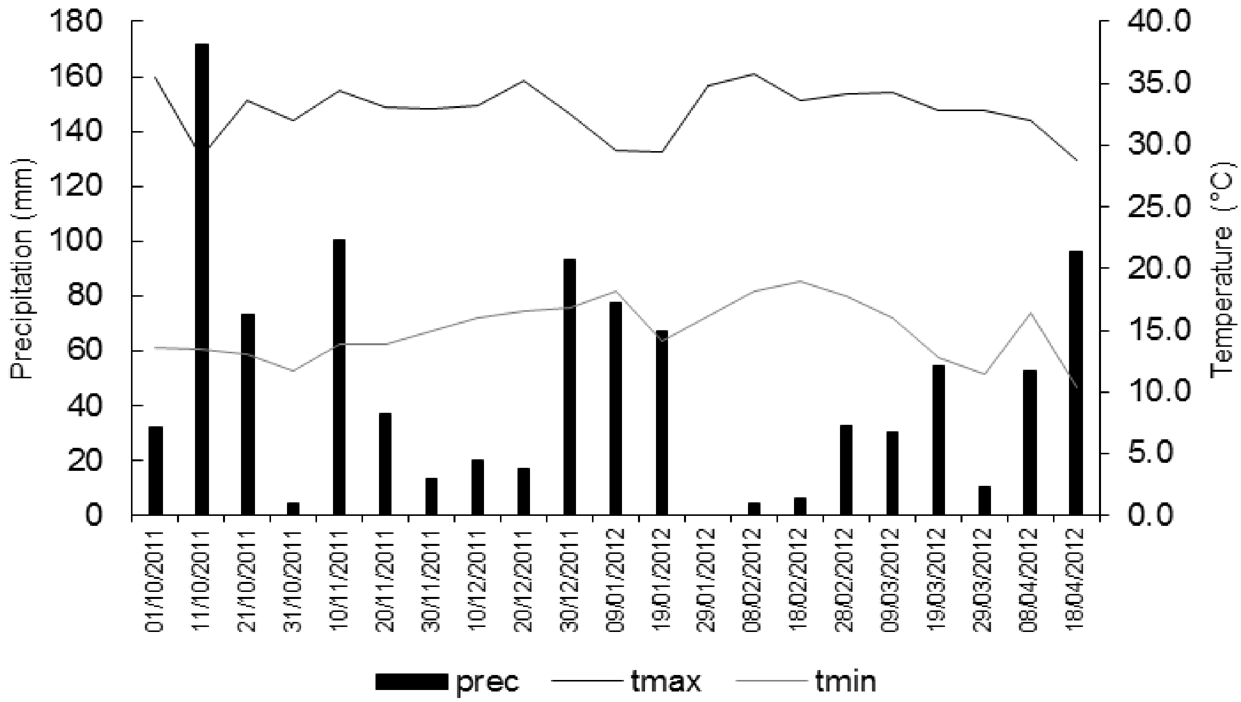

Figure 1. Maximum (Max) and minimum (Min) temperatures and precipitation (mm) in Londrina, from October 2011 to April 2012.
Source: Agronomic institute of Paraná- Technical Report Nº77. July/2012 (IAPAR, 2012).

Table 4. Levels of significance from F (significance level of 5 %) test and means of plots inoculated and non-inoculated for grain yield, ear length, ear diameter, cob diameter, ear rows number, percentage of damaged ear, percentage of diseased ear, days to flowering, plant height and ear height, evaluated in Londrina in the first and second harvest of 2011/2012.

Traits	1st Harvest			2nd Harvest		
	F	Inoculated	Non-inoculated	F	Inoculated	Non-inoculated
Grain yield (t ha^{-1})	ns	8.35	8.34	ns	4.96	4.81
Ear length (cm)	ns	17.7	17.65	ns	14.22	13.89
Ear diameter (cm)	*	4.93	4.95	ns	4.5	4.44
Cob diameter (cm)	ns	3	3	ns	2.79	2.74
N° of grain rows per ear	ns	16.83	16.6	ns	14.17	13.83
Percentage of damaged ear (%)	*	9.73	12.56	ns	17.81	18.79
Percentage of diseased ear (%)	ns	25.21	24.38	ns	23.14	24.33
Days to flowering	ns	65.48	65.93	ns	66.48	66.67
Plant height (cm)	ns	187.98	189.32	ns	138.13	135.57
Ear height (cm)	ns	112.4	111.8	ns	73.63	73.85

need to furthering this approach since up to the present moment there is no knowledge built up on compatibility factors associated with the plant genotype that can be applied in genetic improvement programmes. However, it is evident that the proposed method of including interaction with PGPB as a desired trait in maize breeding programmes has great potential to select more suitable genotypes to finally consolidate this technology. Furthermore, these findings suggest that this approach could be useful for selecting elite cultivars more adapted to different growing seasons.

Regarding cultivars, percentage of damaged ear was the only variable with no significant effect, proving the heterogeneity of the evaluated genotypes. By decomposing

Table 5. Estimates of general combining ability (GCA) of the inbred lines from the synthetic ST20 (g_L) and the tester lines (g_T) originated from the synthetic ST06 for grain yield (GY, in t ha^{-1}), ear length (EL, in cm), ear diameter (ED, cm), cob diameter (CD), umber of grain rows per ear (RE), percentage of damaged ear (% DAE), percentage of diseased ear (% DIE), days to flowering (DF), plant height (PH, in cm) and ear height (EH, in cm), evaluated in Londrina in the first and second harvest of 2011/2012.

Estimates	GY		EL		ED		CD		RE		% DAE		% DIE		DF		PH		EH	
	Harvest		Harvest		Harvest		Harvest		Harvest		Harvest		Harvest		Harvest		Harvest		Harvest	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Means	8.40	4.91	17.8	14.1	4.9	4.5	3	2.8	17	14.1	6.4	13.1	21.8	18.3	65.4	66.4	187.3	136.9	111.6	72.7
Estimates of the GCA of the lines ($\hat{g}_L$) from the synthetic ST20																				
g_{L1}	-0.47	-0.43	-0.2	-0.7	0.1	0.0	0.2	0.1	-0.9	-0.2	-3.9	4.2	-5.2	1.8	-0.7	-0.3	-0.8	-9.0	-8.5	-9.3
g_{L2}	0.21	0.13	-0.9	-0.6	0.1	0.0	0.0	-0.1	0.7	0.1	1.3	-1.3	3.1	-2.1	-0.6	-0.8	-1.8	0.0	4.5	2.0
g_{L3}	0.96	0.35	0.7	1.3	0.0	0.1	0.0	0.2	0.5	0.7	0.5	-1.2	-8.9	1.4	0.7	0.2	11.9	7.4	-0.1	-1.8
g_{L4}	-0.56	-0.77	-0.8	-0.9	0.0	0.0	0.1	0.0	0.1	0.0	1.1	0.7	-0.6	2.0	-0.5	-0.2	-20.3	-12.9	-14.1	-9.1
g_{L5}	-0.37	-0.30	0.0	-0.2	0.1	0.1	0.2	0.1	0.5	0.3	-1.3	1.6	-4.8	-1.5	1.5	1.4	7.4	4.6	8.3	10.6
g_{L6}	0.52	0.15	-0.1	0.5	0.2	0.1	0.1	0.1	0.9	0.7	-2.0	-1.2	-4.6	0.5	1.6	0.5	12.8	8.7	10.6	10.0
g_{L7}	-0.02	0.06	0.3	-0.6	-0.2	-0.1	-0.1	-0.2	-1.2	-0.9	-1.4	0.9	-2.2	-0.7	0.7	0.7	7.2	2.9	12.1	2.3
g_{L8}	0.25	-0.41	0.9	0.1	-0.1	-0.2	-0.2	-0.2	-0.5	0.1	2.5	5.4	10.7	1.5	0.1	0.0	12.0	7.2	-1.3	-3.5
g_{L9}	-0.57	0.26	-0.3	0.4	0.0	0.2	0.1	0.1	0.7	0.3	2.1	-2.1	0.7	-4.2	0.0	0.4	-13.0	-6.1	-5.4	-3.5
g_{L10}	-0.28	0.04	-0.4	-0.4	0.1	0.0	-0.1	0.0	-0.2	-0.4	1.9	-2.3	11.6	-2.4	-0.3	-0.6	-12.6	-3.3	-3.2	3.1
g_{L11}	0.26	0.53	0.5	0.7	0.1	0.0	0.0	0.1	-0.1	-0.2	0.3	-2.3	-7.0	0.8	-1.5	-0.5	-2.7	3.4	-0.4	2.8
g_{L12}	0.06	0.38	0.1	0.5	-0.2	-0.1	-0.2	-0.2	-0.6	-0.5	-0.9	-2.4	7.3	2.8	-0.9	-0.8	0.0	-3.0	-2.6	-3.5
Estimates of the GCA of the lines originated from the synthetic ST06 used as testers ($\hat{g}_T$)																				
g_{T1}	-0.75	-0.39	-0.7	-0.3	0.0	-0.1	0.1	0.0	0.9	0.7	0.9	1.0	-4.3	2.1	-0.1	0.5	-2.0	-2.8	-3.5	-2.1
g_{T2}	0.49	0.02	0.3	-0.4	0.1	0.1	0.1	0.1	-0.3	-0.2	-1.5	2.0	-0.5	1.1	0.1	-0.4	-3.8	-2.7	-3.3	-1.9
g_{T3}	0.26	0.38	0.4	0.7	-0.1	0.0	-0.1	0.0	-0.6	-0.4	0.6	-3.1	4.9	-3.2	0.0	-0.1	5.8	5.5	6.8	4.0

the effects of cultivar in the joint analysis, a significant effect of control (C) was observed for all traits evaluated and for the experimental hybrids (Hy), except for the percentage of damaged and diseased ear (Table 2). Thus, there are experimental hybrids with different agronomic performances, allowing for genetic selection among the genotypes. For the contrast control versus hybrids (C vs Hy), the overall mean of these groups of genotypes differed statistically for male flowering and plant and ear height; moreover, in the first harvest the hybrids showed a higher percentage of diseased ear compared to the control group (Table 3). However, interestingly, no significant difference was found for grain yield between experimental and control hybrids.

As for the interaction of the control group and harvest (C x Ha), the joint analysis indicated significant differences for grain yield, ear length, ear diameter and plant height (Table 2), while experimental hybrids versus harvest (Hy x Ha) only showed significant values for cob diameter, percentage of diseased ear, and plant and ear height, demonstrating that these genotypes did not present a differentiated behavior between the different periods of cultivation, which means they suffered less with the unfavaroble conditions of the second harvest, showing a more stable performance. From the data gathered, we can assume that the hybrids with the best average yield between the 1st and the 2nd harvest are those that should be selected for grain yield, ear length, ear diameter, number of grain rows per ear, percentage of damaged ear and male flowering.

The decomposition of the experimental hybrids from the partial diallel reveals significant effects for the general combining ability of the inbred lines (GCA-L) and the tester lines (GCA-T) for the majority of the characteristics analyzed, except for percentage of damaged and diseased ear to GCA-L and percentage of damaged and diseased ear and male flowering to GCA-T (Table 5). The specific combining ability was significant for ear length and diameter, cob diameter, number of grain rows per ear, percentage of diseased ear and plant and ear height.

The absence of significance for the other traits indicates that the parents do not present an appreciable degree of gene complementation in relation to the frequencies of the alleles in the loci of dominance (Vencovsky and Barriga, 1992). Experimental hybrids and period of cultivation (harvest) interaction showed significant GCA-L data for ear length and diameter, percentage of diseased ear, plant and ear height, and for GCA-T in almost all traits except number of grain rows per ear and plant and ear height.

In general, the inbred lines L_2, L_3, L_6, L_{11}, L_{12} and testers T_2 and T_3 showed the best estimates of general combining ability for grain yield and other characteristics, producing the best hybrid combinations (Table 5). High estimates of GCA are associated with genotypes with high frequency of favorable alleles for agronomic traits of interest (Vencovsky, 1987). As can be seen from Table 2, the mean squares for general combining ability were, in general, higher than those of specific combining ability, indicating predominance of the additive effects of genes, which is in agreement with results obtained by Simon et al. (2004) and Júnior et al. (2006). Additionally, the greater contribution of effects of dominance to grain yield, found in this work, corroborates studies made by Simon et al. (2004) and Júnior et al. (2006).

Among the 24 experimental hybrids evaluated in the first harvest (Table 3), 13 did not differ statistically from the commercial hybrid DKB390 (control) for grain yield and showed similar performance for the other traits, especially the experimental hybrids $H_{3\ 2'}$, $H_{6\ 3'}$ and $H_{11\ 2'}$. In the second harvest, 15 of the experimental hybrids did not differ statistically from the controls, and from this total, nine experimental hybrids showed a higher average grain yield than DKB390 and five surpassed its transgenic version DKB390H, which shows the excellent performance of the genetic material generated by this particular maize breeding programme that aims at outstanding varieties.

In general, the most promising hybrids in the second harvest were $H_{2\ 3'}$, $H_{3\ 2'}$, $H_{9\ 2'}$, $H_{11\ 2'}$ and $H_{11\ 3'}$ e $H_{12\ 3'}$, showing the highest means of the traits of interest and the smallest oscillations between the two growing seasons. Furthermore, 50% of the experimental hybrids out-yielded the commercial hybrid DKB390 when cultivated in conditions of high abiotic stress (2nd harvest) (data not shown).

Conclusions

From the research that has been carried out, it is possible to conclude that:

(1) The most promising experimental hybrids are $H_{2\ 3'}$, $H_{32'}$, $H_{11\ 2'}$, $H_{11\ 3'}$ and $H_{12\ 3'}$ and that

(2) The additive effects of genes are more important than the non-additive effects for all the traits evaluated.

Regarding the association with the diazotrophic bacteria.

(3) It is possible that the direct inoculation of *A. brasilense* on maize seedlings was not successful enough to allow significant effects of inoculum in the experimental conditions of this study.

Further research should be conducted to optimize the inoculation method in order to guarantee the evaluation for detection of maize genotypes more prone to PGPB colonization and its introduction in maize breeding programmes.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Microbial diversity as a soil quality indicator in agroecosystems in Brazilian Savannas

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The importance of sustainable use of natural resources, especially of soil and water, has been a subject of increasing relevance. The increase of human activity in ecosystems has great impact on the dynamics of soil organisms. The comparison between cultivated systems and native areas without anthropic interference can be used as soil quality index. Microorganisms are ideal indicators because they are very sensitive to changes and show variations in their community when subjected to stressful environments. The objective of the present study was to evaluate the quality of soil microbial abudance as an index of soil quality in agroecosystems Integrated Sustainable Agroecological Production, Agroforestry System and Isolation of springs in Brazilian Savanas. The experiment was conducted in the areas of the Vitória settlement, in the region of São Patrício Valley, Goianésia, Goiás, in an area of native "cerrado". The climate is classified as seasonal tropical (Aw), being characterized by two well defined seasons (dry and rainy), as well as with the occurrence of drought periods during the rainy season. The experimental design adopted was a 3 × 2 × 2 block factorial randomized with three replications, where factor 1 was represented by the systems used: Sustainable Integrated Agroecological Production (SIAP), Agroforestry System (AS) and springs isolation (SI), factor 2 was represented by the soil depth, 0-5 cm and 5-10 cm, and factor 3 was the installation time of the systems: 5 years and recently installed. Soil samples were collected at random in the rhizospheric soil in each plot. There was a greater number of fungal colonies in the AS system with 5 years of implantation, but did not differ with soil depth. There was a higher number of bacteria colonies in the SIAP system after 5 years. At the depth of 0 to 5 cm, the SIAP system had higher microbial abundance, but it was higher at 5 to 10 in AS system.

Key words: Cerrado Brazilian, agroecology, environment, natural resources.

INTRODUCTION

The importance of sustainable use of natural resources, especially of soil and water, has been a subject of

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increasing relevance. The increase of human activity in ecosystems has great impact on the dynamics of soil organisms (Araújo et al., 2007).

According to Doran et al. (1994), soil quality can be conceptualized as the ability of soil to perform various functions, within the limits of land use and ecosystem, to sustain biological productivity, maintain or improve environmental quality and contribute to plant, animal and human health.

The comparison between cultivated systems and native areas without anthropic interference can be used as soil quality index. Microorganisms are ideal indicators because they are very sensitive to changes and show variations in their community when subjected to stressful environments (Moreira and Siqueira, 2006). The establishment of soil quality indeces can be used as a criterion for the evaluation of environmental impacts on ecosystems (D’Andréa et al., 2002).

Aim of sustainable agro-systems in agricultural production is to stop the damage of the ecosystem's ability to recover from anthropogenic interference (Altieri, 1998); degraded areas can recover their productive capacity if managed with practices that aim to maintain the sustainability and preservation. Areas for permanent preservation, such as hillside areas and springs of water bodies, should be maintained without human cultivation and influence (Corrêa et al., 1996). However, permanent preservation areas already degraded must be recovered, and the soil microbial quality index can be used as a parameter of evaluation of this recovery.

Agroecology is a set of general principles applicable to sustainable farming systems. It can be described as a science that aims to study agroecosystems that seek to copy natural ecosystems, thus producing lower rates of environmental impact (Altieri, 1998).

According to Klink and Machado (2005), the “cerrado”, Brazilian Savanas, productive areas are mostly occupied by degraded pastures, mainly because the producer does not treat these areas as crops, do not apply appropriate soil management to maintain the fertility of soils. An alternative for the management of pastures within sustainable systems is the use of plant species diversification, through the maintenance of polyculture of pasture species. The grass and legume consortium, together with the installation of native and exotic tree species, promotes better conditions for the development of soil microbiota diversity, such as agroforestry system (SA) (Soares et al., 2010).

The Sustainable Integrated Agroecological Production System (SIAP) is a small-scale irrigated cultivation system, conducted in circular beds formed around a system of production of small animals such as birds or fish. The purpose of this system is to meet local needs by developing a model of family farming also based on the cultivation of several different species of vegetables (Mendonca et al., 2010). This system is widely used by settled communities in the St. Patrick's Valley region.

Agroforestry Systems (AS) are ways of use and management of natural resources, in which perennial woody species (trees, shrubs and palms) are used in association with agricultural crops and/or grazing animals, in the same area, simultaneously or in a temporal sequence, resulting in a biological diversity promoted by the presence of different plant and animal species which explore diverse niches within the system, integrated with the application of management practices compatible with traditional cultural techniques of farmers (Carvalho et al., 2004).

Among the microbiological indicators of soil quality, microbial diversity (MD) stands out. Soil biomass is the measure of CO_2 production resulting from the metabolic activity of macro and microorganisms (Doran et al., 1994; Azevedo and Melo, 1998; Da Silveira and Dos Santos Freitas, 2007).

The activity of these organisms in the soil is considered a positive attribute for soil quality and is used as an indicator because it is more generic and encompasses the activity of communities and consortia of micro-organisms present, showing better reproducibility (Rice et al., 1996).

Agroecology is defined as a new productive paradigm, being agroecology linked to sustainability and sustain-ability which is key to the maintenance of productive processes over time. This type of management should not be exclusive of extensive production systems. Family agriculture, when well structured, leaves the subsistence level and becomes responsible for the maintenance of products, such as vegetables that cannot be produced by large-scale monoculture systems. This project carried out through the partnership of “Gente do Cerrado” Association with the Evangelical Faculty of Goianésia has installed agroforestry systems in parcels of a Settlement region of the São Patrício Valley.

It is necessary to quantify the benefit of the installation of these systems using as a parameter, the soil microbial quality index comparing them with native “cerrado” vegetation areas.

The objective of this study was to evaluate the quality of soil microbial activity as an index of soil quality in Integrated Sustainable Agroecological Production, Agroforestry System, and springs in Brazilian Savannas.

MATERIALS AND METHODS

The experiment was conducted in the areas of the Vitória settlement, in the region of the São Patrício Valley, in Goianésia, Goiás, in an area of native “cerrado”. The climate is classified according to Koppen (1931), as seasonal tropical (Aw), being characterized by two well defined seasons (dry and rainy), as well as the occurrence of drought periods during the rainy season.

The experimental design adopted was a 3 × 2 × 2 block factorial randomized with three replications, where factor 1 was represented by the systems used: Sustainable Integrated Agroecological Production (SIAP), Agroforestry System (AS), and springs isolation (SI), factor 2 was represented by the soil depth of 0 to 5 cm and 5 to 10 cm, and factor 3 was the installation time of the systems: 5

Table 1. Mean CFU values of fungi in each agroecosystem.

Systems	Mean
AS	6.50000[a]
SIAP	3.33333[b]
SI	2.33333[b]

CV% = 42.47. *Sustainable Integrated Agroecological Production (SIAP); Agroforestry System (AS); Springs Isolation (SI).

Table 2. Mean CFU values of fungi per soil depth.

Depth	Mean
0 - 5	3.94444[a]
5 - 10	4.16667[a]

CV% = 42.47

Table 3. Mean CFU values of fungi per time of system implantation.

Season	Mean
Newly	2.94444[b]
5 years	5.16667[a]

CV% = 42.47

years and recently installed.

Samples were collected at random. Each repetition was composed of the rhizospheric soil in each plot. The rhizospheric soil was collected at a depth of 0 to 5 cm and 5 to 10 cm, the composite samples were homogenized and stored under refrigeration.

The number of fungi and bacteria was determined by quantification of colony forming units (CFU) using the method of inoculation of diluted suspensions of soils in Potato-Dextrose-Agar (PDA) culture medium, with four replicates per dilution. From the collected samples, 1.0 g of soil was removed and diluted in Erlenmeyer, adding 10.0 mL of distilled water, the same procedure being carried out until the dilution of 10^4. During preparation of the culture medium, 1.0 g of antibiotic was placed and the number of fungal colonies and number of bacterial colonies were counted.

Petri dishes with inoculated media were incubated at room temperature (±35°C) by counting the colonies of fungi and bacterial colonies which was performed 5 days after incubation. The data for statistical treatment was obtained through the program Assistat (Silva, 2016).

RESULTS AND DISCUSSION

The colony forming units of fungi and bacteria were quantified with the objective of identifying systems, depth and periods of time that present greater microbial diversity. Tables 1 to 6 show the CFUs of fungi and Tables 7 to 11 refer to the CFUs of bacteria. Table 1 shows the average of CFUs of fungi by agroecological system. Results showed that the Agroforestry System was superior to the other agroecosystems, so that system presented higher amount of fungi in soil (Altieri, 2002), since plants residues are decomposed in soil and serve as energy source for soil microorganisms, such as fungi.

Table 2 presents the average values of fungal CFU in relation to soil depth. Values were not significantly different. In the work of Angelini et al. (2012), it was observed that in the 0 to 5 cm layer there was a significant effect when associated with crops and vegetation cover, thus it is possible to explain the highest values in the AS and layer of 5 to 10 for crop species.

Table 3 shows the average of fungal CFU in relation with the time of system implantation, demonstrating that the systems that were implanted 5 years ago were superior to the value found in the newly implanted systems. Thus, the longer the systems, the greater the amount of fungi present in the soil. Factors as environmental conditions favor the increase of fungi.

In relation to the effect of the interaction between agroecological systems and soil depth, the values found do not present significant statistical differences. Although, not significantly different, the higher value of the agroforestry system can be was explained by Facci (2008) who stated that due to the diversity of trees, it has an accumulation of plant residues on the surface, having an accumulation of organic matter, becoming a favorable environment and source of energy for fungi.

Table 4 presents the interaction averages of the agroecological systems in relation to the time of implantation. The results obtained showed that the newly installed systems were statistically similar, and that in the time of 5 years, the Agroforestry System presented a superior value in relation to the SIAP systems and SI. However, the Agroforestry System presented lower value in relation to the amount of fungi presented in the newly implanted system. Angeline et al. (2012), stated that areas with crops up to the 10 cm layer have a significant value of fungi, thus, the forest that contributes to the greatest amount of organic matter in the soil over time, due to plant residues, makes it an ideal environment.

Table 5 shows the interaction effect between soil depth and time of implantation. Results obtained show that the depth of 0 to 5 cm in the system installed at 5 years had higher fungi CFUs as compared to other soil depth and recent system, and can be explained by a higher accumulation of organic matter on the soil surface. At the depth of 0 to 5 cm, there was a lower value in the newly installed system, showing less amount of fungi colonies. After counting bacterial colonies, the following results were obtained. Table 6 shows the mean values in each cropping system. The AS and SIAP did not differ between both systems, but were significantly higher than SI. The same result was obtained by Moreira et al. (2010), who verified high diazotrophic density in agriculture and agroforestry.

Table 4. Interaction effect between agroecosystems and time of system implantation on fungi CFUs.

System	Time	
	Newly installed	5 years
AS	6.8333[aB]	6.1667[aA]
SIAP	2.3333[aA]	4.3333[bA]
SI	2.6667[aA]	2.0000[bA]

Lowercase letters for columns; uppercase letters for lines; CV% = 42.47. *Sustainable Integrated Agroecological Production (SIAP); Agroforestry System (AS); Springs Isolation (SI).

Table 5. Interaction effect between soil depth and time of system implantation on fungi CFUs.

Depth	Time	
	Newly installed	5 years
0 – 5 cm	2.1111[aB]	5.7778[aA]
5 – 10 cm	3.7778[aA]	4.5556[bA]

Lowercase letters for columns; uppercase letters for lines. CV% = 42.47.

Table 6. Mean CFU values of soil bacteria in each agroecosystem.

System	Mean
AS	28.92[a]
SIAP	31.92[a]
SI	15.17[b]

CV% = 17.21; *Sustainable Integrated Agroecological Production (SIAP); Agroforestry System (AS); Springs Isolation (SI).

Table 7. Mean CFU values of soil bacteria in different soil.

Depth	Mean
0 – 5 cm	27.83[a]
5 – 10 cm	22.83[b]

CV% = 17.21.

As shown in Table 7, the average values of the depths were found, in which, in the range of 0-5 cm, higher values were found, thus, in this range, there was a greater amount of bacteria. In the work of Pereira (2015), even with the presence of fungi in all the layers, the deeper layers presented a decrease in the diversity of bacteria.

Table 8 presents the average of the systems implementation times, in which, in systems installed for a longer period of time, there was a greater amount of bacteria in the soil. This is explained as follows, in the course of time, with favorable environment and energy source, the bacteria carries out its dissemination.

Table 8. Mean CFU values of soil bacteria as affected by time of implantation of agroecological systems.

Time	Mean
Newly	16.61[b]
5 years	34.06[a]

CV% = 17.21.

Table 9. Interaction effect between agroecological systems and soil depth on CFU values of soil bacteria.

System	Depth	
	0 – 5 cm	5 – 10 cm
AS	24.17[bB]	33.67[aA]
SIAP	45.33[aA]	18.50[bB]
SI	14.00[cA]	16.33[bA]

Lowercase letters for columns; uppercase letters for lines; CV% = 17.21; *Sustainable Integrated Agroecological Production (SIAP); Agroforestry System (AS); Springs Isolation (SI).

Table 9 presents the interaction effect of agro-ecosystems and soil depths. The results demonstrated that SIAP had higher bacteria colomies than lower depth and other cropping systems. At 5 to 10 cm depth, the AS had the highest CFU value than other systems. Table 10 presents the interaction effect between agroecological systems and time of their installation. Results showed that in the newly installed AS bacteria, abundance was superior in relation to the other newly installed systems, but in the systems implanted 5 years ago, the SIAP system had greater abundance than other systems. All systems had significantly higher bacteria abundance for those implanted 5 years ago as compared to recent systems.

Table 11 presents the interaction effect of soil depth and implantation time. In the 5-year-old system, the amount of bacteria colonies in the 0 to 5 cm layer was higher than at 5 - 10 cm depth, which is different from the newly installed systems. At both depths, the 5-year period had more bacteria colonies than the newly installed systems.

Conclusions

There was a higher number of fungal colonies in the Agroforestry System oldest system, but did not vary with soil depth. There was a higher number of bacteria colonies in the Sustainable Integrated Agroecological

Table 10. Interaction effect between agroecological systems and implantation time on CFU values of soil bacteria.

System	Time	
	Newly installed	5 years
AS	25.83^{aB}	32.00^{bA}
SIAP	17.00^{bB}	46.83^{aA}
SI	7.00^{cB}	23.33^{cA}

Lowercase letters for columns; uppercase letters for lines; CV% = 17.21; *Sustainable Integrated Agroecological Production (SIAP); Agroforestry System (AS); Springs Isolation (SI).

Table 11. Interaction effect between soil depth and implantation time on CFU values of soil bacteria.

Depth	Time	
	Newly installed	5 years
0 – 5 cm	16.78^{aB}	38.89^{aA}
5 – 10 cm	16.44^{aB}	29.22^{bA}

Lowercase letters for columns; uppercase letters for lines; CV% = 17.21.

Production system after 5 years. At the depth of 0 to 5 cm, the Sustainable Integrated Agroecological Production system had high microbial abundance, but at 5 to 10 cm, the Agroforestry System showed higher number of bacteria colonies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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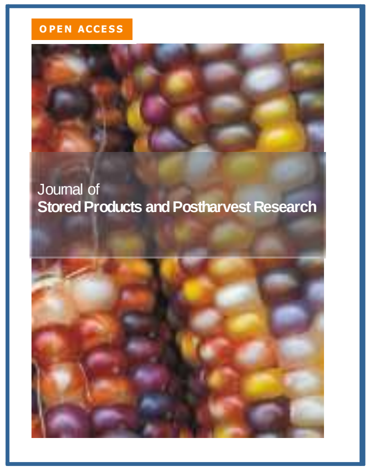

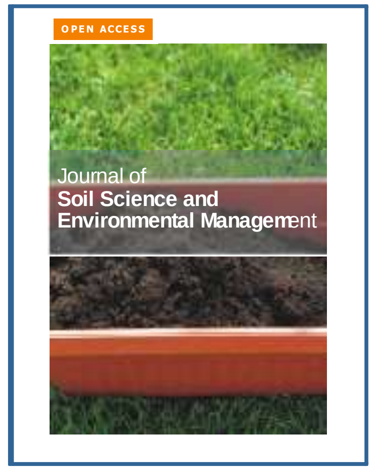

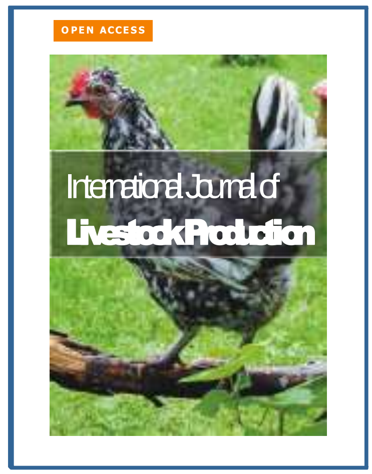

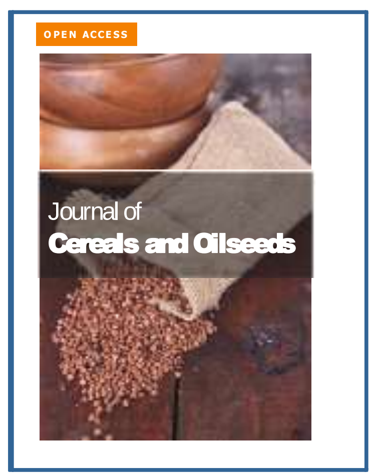

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