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Review

Review of sheep crossbreeding based on exotic sires and among indigenous breeds in the tropics: An Ethiopian perspective

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The objective of this review paper is to examine whether or not sheep crossbreeding is a feasible option to improve indigenous sheep breeds in developing countries using Ethiopian case as example. The paper reviewed and discussed the history of exotic breed introduction, research, and development efforts in crossbreeding and performance of crossbreds under on-station and on-farm management. Earlier, the choice of breed for crossbreeding overlooked interests and preferences of farmers mainly for physical appearance. More recently the introduction of Awassi sheep considered their preference. Performance evaluation results from the on-station and on-farm (mainly based on Awassi pilot crossbreeding villages) showed that crossbreds often outperformed their local contemporaries. Thus comparisons of pure local sheep and crossbreds among those breeds produced in some areas indicated a good outcome of this type of crossbreeding. However, the performance of crossbred sheep varied by location and depended on management and exotic inheritance levels. For most programs, no comprehensive data were available to do on-farm comparisons of herd productivity and cost-benefits or to evaluate the sustainability of the programs. Regardless of location, farmers participating in crossbreeding often showed keen interest in crossbreeding, mainly due to the fast growth, larger body size of crossbreds resulting in higher market prices as compared to their local sheep breeds. Ram multiplication and dissemination from the government farms were found inefficient. The predominant practice of a ubiquitous dissemination and selling of breeding rams to individual farmer dilute the efforts of crossbreeding and prevents generating the benefits expected from crossbreeding programs. Furthermore, indiscriminate crossbreeding without prior analysis of suitability of crossbreds for a given production environment and without clear breeding objectives presents a potential threat to better adapted indigenous breeds. Crossbreeding programs require strong research and development support from public service and non-governmental institutions for sustainable design, optimization, and implementation in clearly defined production environments.

Key words: Awassi, developing countries, Dorper, on-farm performance, smallholder farmer.

INTRODUCTION

Small ruminant production is an important agricultural activity and has a substantial contribution to smallholder farmers in generating income and securing food in developing countries (Kosgey et al., 2006). Tropical developing countries typically rely on non-specialized multipurpose breeds and extensive production systems and control over breeding animals is often poor. Existing breeds are adapted to the existing environmental situation which is characterized by feed scarcity and disease challenge (Baker et al., 2002; Haile et al., 2002; Gizaw et al., 2008a). However, there is a belief that local breeds are less productive and unlikely to continue sustaining the fast growing demand for food that is created by rapid human population growth, urbanization and income growth. Crossbreeding is considered as one of the options and it is a potentially attractive breed improvement method due to its quick benefits as the result of breed complementarity and heterosis effects (Leymaster, 2002; Hayes et al., 2009). FAO (2007) documented that the transfer of genetic material has been increased dramatically in the recent decades. Sheep are among the most widely distributed livestock species. Consequently, based on a review of Shrestha (2005), the widely practiced breed combination resulted in about 443 composite sheep populations worldwide in 68 countries. Some remarkable results have been achieved in well-designed selective and crossbreeding schemes. One example is the improvement of local Awassi using within-breed selection, crossbreeding and gene introgression resulting in a highly productive and prolific genotype in Israel (Gootwine and Pollott, 2000; Pollott and Gootwine, 2004). Another example is the development of Dorper sheep by crossing Dorset Horn rams with fat-rumped Black Head Persian ewes (de Waal and Combrinck, 2000). Such programs have been favored by resourceful environments and well developed infrastructure and markets (Sölkner et al., 1998). However, in developing countries generally, the adoption of livestock technologies has been low due to the environment and poor resource base of farmers (Iñiguez, 2011). The proportion of exotic and crossbred sheep populations in Ethiopia remains low, only 0.2% (CSA, 2013), indicating that research and development efforts of sheep crossbreeding in Ethiopia did not deliver the anticipated benefit to smallholder farmers so far. However, there is still a growing interest of the government and of farmers in sheep crossbreeding. There is no comprehensive study showing the performance of crossbreeding, both biological and economical, to substantiate the argument on the benefit of sheep crossbreeding for smallholder farmers. This paper reviews sheep crossbreeding efforts particularly in Ethiopia and in other African countries with the aim to support decisions related to the future implementation of crossbreeding strategies.

HISTORY OF EXOTIC BREED INTRODUCTION TO ETHIOPIA

The first introduction of exotic sheep breeds into Ethiopia traced back to 1944 when Merino sheep were introduced from Italy by an American aid organization and were maintained at Entoto (located near Addis Ababa) sheep breeding station (DBHBMC, 2007). Romney, Corriedale, Hampshire, and Rambouillet were introduced from Kenya in 1967 and were kept at the government farm Debre Berhan Sheep Breeding and Multiplication Center (DBSBMC) which was established in 1967 and located at Debre Berhan town, in North Shewa administrative zone of the Amhara region. Another state owned farm, Chilalo Agricultural Development Unit (CADU) was also established in the same year in the former province of Arsi. However, the detection of maedi-visna (respiratory viral disease) in the flock of CADU in 1988-1989 prompted closure of the farm (BoA, 2000). In 1980, Awassi sheep were introduced from Israel and kept at DBSBMC and Amed Guya Sheep Breeding and Multiplication Center (AGSBMC). There were also continuous importations of purebred Awassi sheep totaling 45 (ram and ewe lambs). The two government farms have been engaged in multiplication and distribution of crossbred rams to farmers at a subsidized price. Ram dissemination was banned between the years 2001 and 2009 following the confirmed maedi-visna disease in crossbreds and associated sheep flocks (DBHBMC, 2007). In 2011, about 170 pure Awassi sheep were imported from Israel to recommence crossbreeding in the farms.

Dorper sheep were introduced into the Jijiga area (Somali Region) in the late 1980s. On-station performance of crossbred was very good however there was no on-farm evaluation during that time (Awgichew and Gipson, 2009). All sheep were looted from the ranch during the political instability in 1991 (Awgichew and Gipson, 2009).The Ethiopian Sheep and Goat Productivity Improvement Program (ESGPIP), a USAID-funded 5 year project launched in 2006, operated with the

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goal to sustainably increase sheep and goat productivity in Ethiopia and consequently to enhance economic and food security. A total of 120 Dorper sheep (ewes and rams) were imported again from the Republic of South Africa in 2007 (Awgichew and Gipson, 2009). Regional research institutions also showed interest in Dorper sheep and additional ~250 sheep were imported in 2011, aiming to establish new nucleus flocks.

OVERVIEW OF CROSSBREEDING EFFORTS BASED ON EXOTIC AND INDIGENOUS SIRES

Introduction of Romney, Corriedale, Hampshire, and Rambouillet was targeted to cross them with local sheep breeds aiming to supply wool for the Debre Berhan blanket factory established in 1967. From the year 1969 to 1974, a total of 99 crossbred ewe lambs and ram lambs were distributed to farmers. The breeds were performed well in growth performance under station and farmer situation except Romney breed (DBHBMC, 2007); nevertheless, they were not preferred by farmers due to their physical characteristics (face covered with hair, absence of horn in males and thin tail), fatty nature of wool making it difficult to spin wool in the traditional way, and the suspected poor skin quality. Thus, during the monarchy time, crossbreeding was mainly limited to on-station to supply fattened sheep for the king palaces and occasions. Following the downfall of the monarchy (1974), the crossbreeding efforts were directed to produce and disseminate crossbred rams to smallholder farmers. DBSBMC and AMSBMC distributed more than 4000 crossbred rams of different breeds (Awassi, Corriedale, and Hampshire) to smallholder farmers at subsidized prices between the year 1974 and 2001 (DBHBMC, 2007). Hampshire and Corriedale breeds were initially used while these breeds were gradually replaced by Awassi following the introduction of Awassi in 1980. Awassi breed has been well accepted by Ethiopian farmers due to its similar physical appearance to that of local breeds. In the first four years of Awassi ram distribution, individual smallholder farmers were targeted. Later, from 1979 to 1989, the focus was shifted to farmers organized in cooperatives. However, no performance evaluation was performed in the co-operatives, and animals were looted during the government change in 1991. Consequently, the co-operatives were abolished and dismantled (Emana, 2009) so that the focus was again changed to disseminate rams to individual smallholder farmers. The target has been on disseminating rams with 75% Awassi inheritance to farmers for crossbreeding with their local ewes aimed at replacing the local sheep breed through repeated backcrosses (DBSBMC, 2007).

On-station research results on growth, reproductive performance and carcass performances from CADU farm (Osslon and Beyene, 1990) and Sheno (now Debre Berhan) Agricultural Research Center (Demeke et al., 1995) were reported. A survey by DBARC in the year 1997 to evaluate the on-farm performance of crossbred in North Shewa and South Wollo districts of the Amhara Region exposed the total failure of ram dissemination (Gizaw and Getachew, 2009). It was difficult to find either crossbred rams or offspring from disseminated rams in the surveyed areas. Following that an on-farm evaluation of the performance of Awassi × local crossbred sheep under farmers' management was commenced by DBARC in three villages in the highlands of Ethiopia in 1997. Details of the breeding program followed by DBARC are indicated (Gizaw and Getachew, 2009; Getachew et al., 2013). Similarly, the Awassi × Tikur sheep crossbreeding started in two villages of North Wollo by Sirinka Agricultural Research Center in 2007.

The ESGPIP project was implemented in collaboration with local universities and research centers at 2 nucleuses and 10 Breeding, Evaluation and Distribution (BED) sites, established in different parts of the country since 2007. Their nucleus sites were used to multiply the imported purebred Dorper sheep and provide a continuous supply of pure Dorper sheep to the BED sites, private commercial or cooperative farms and to those individuals who wished to establish their own pure breed producing farms. At BED sites, purebred sires were crossed with indigenous dams to obtain F1 sires for dissemination to farmers. The funding of ESGPIP terminated in 2011 and the activities were handed over to local universities and research centers for further implementation of the crossbreeding program.

Crossbreeding among indigenous breeds has also been practiced at DBARC as an alternative to the use of exotic genotypes for crossbreeding. Indigenous Washera rams were distributed in the highlands of North Shewa, South Wollo, North Wollo, and Gondar areas (ANRS-BoARD, 2004). In 2005, a village-based Farta × Washera sheep crossbreeding program has been started (Mekuriaw et al., 2013) with the aim to increase productivity of medium sized indigenous Farta (Gizaw et al., 2008a) by crossing or introducing male and females of indigenous Washera sheep.

GROWTH PERFORMANCE

On-station performance

Several studies from the on-station evaluation of growth performance of crossbreds using exotic (Awassi and Dorper) sires in different areas of Ethiopia confirmed that the crossbreds are superior to their local counterparts (Demeke et al., 1995; Olsson and Beyene, 1990; Tibbo, 2006; Tsegay et al., 2013; Lakew et al., 2014; Tilahun et al., 2014). The study by Olsson and Beyene (1990) at

CADU showed that birth weight, weaning weight, and annual greasy wool weights improved with increased levels of exotic blood. Evaluation of the effect of 3 genotypes (local Menz, 50% Awassi 50% Menz and 75% Awassi 25% Menz) and 2 levels of feed supplementation (supplemented and non-supplemented) and 2 treatments against internal parasite (treated and non-treated) on the overall productivity of sheep was carried out at DBARC for 10 months (Tibbo et al., 2005). At the beginning of the experiment (yearling age), the 75% Awassi × Menz were superior in live body weight than the indigenous Menz sheep (23.5 vs. 16.6 kg). At the end of the 10 months experimental period, also crossbreds performed better in live body weight, carcass weight, and fleece yield and had higher marginal profit. However, overall marginal profit including skin price showed that the indigenous Menz sheep were more profitable than the crossbreds due to the higher skin price compared to the crossbreds at that time. However, the authors explained that crossbreds already reached market weight of Menz sheep at the beginning of this experiment indicating that keeping those crossbred sheep for longer time can be costly compared to local genotypes. Indigenous Menz sheep and 50% Awassi showed better resistance against parasites compared to 75% Awassi.

A 90 days experiment aiming to compare growth and carcass traits of Hararghea Highland (HH) and Black Head Ogaden (BHO) sheep and their crosses with Dorper (D) sheep with two feeding types (hay *ad libitum* + 150 g day^{-1} concentrate feed and hay *ad libitum* + 300 g day^{-1} concentrate feed) at the age of about 7 months was carried out at Haramaya University (Tsegay et al., 2013). The result revealed that Dorper sired crossbred lambs produced from HH ewes had better growth performance compared to the Dorper sired crossbred lambs produced from BHO ewes. This might be an indication that HH ewes are more suitable for crossing with Dorper for crossbred lamb production than the BO ewes. Crossbreds of Dorper with both BHO and HH showed comparable average daily gains of 69.4 and 63.9 g day^{-1}, respectively, in the 90 days feeding trial. Average daily gains in the 90 days trial for Pure BHO and HH were 40.7 and 39.2 g day^{-1}, respectively, significantly lower than crossbreds. The two locals were not different from each other. Another study at DBARC showed Dorper crossbred lambs produced from improved Menz ewes were better at birth and weaning weight (3.15 and 15.35 kg) than crossbred lambs produced from unimproved Menz (2.87 and 13.86 kg) (Goshme et al., 2014). Weaning, six months and yearling weights of 50% Dorper crossbreds were much higher with values of 14.95, 20.43, and 31.37 kg, respectively compared to the corresponding values for local breed in North Wollo lowland areas of Ethiopia with values of 8.53, 11.92, and 22.38 kg, respectively (Lakew et al., 2014). Feedlot performance of 25 and 50% Dorper crossbred was also superior to the local sheep breeds (Tilahun et al., 2014). Growth performance of Menz sheep and their crosses with either indigenous Bonga or Washera sheep were studied at DBARC (Lemma et al., 2014a). The result revealed that Bonga × Menz showed better growth performance compared to pure Menz and Washera × Menz, whereas the latter two genotypes were not different from each other.

On-farm performance

The first report from an on-farm trial in Ethiopia was by Hassen et al. (2002) who compared 37.5% Awassi crossbreds and local breeds at one of the crossbreeding villages called Serity. Live weights were recorded at birth and then monthly until 210 days. In all the measurements, crossbreds performed better than local breed except for weight on 90 days where both crossbred and local were not significantly different. Results from another village (Chiro) showed that growth of lambs to yearling age increased as the Awassi level increased under farmer's management. Yearling weight of local Wollo, 25 to 50% Awassi and above 50% Awassi was about 22, 26, and 35 kg, respectively (Gizaw and Getachew, 2009). Based on the combined analysis from the three villages (Serity, Negasi-Amba, and Chiro), 37.5% Awassi was recommended for small holder farmers (Gizaw et al., 2014). However, the authors suggested further study in this regard since the data size for high grade crosses is comparatively small.

In 2005, a village-based Farta × Washera sheep crossbreeding program has been started by Andassa Livestock Research Center in Lay Gaint and Farta districts of South Gondar (Mekuriaw et al., 2013) aiming to increase productivity of medium sized Farta (Gizaw et al., 2008a) by crossing or introducing male and female Washera sheep. Improvement program on indigenous Farta sheep using indigenous Washera breed indicated that growth performance of crossbred was better than the pure Farta sheep (Mekuriaw et al., 2013).

REPRODUCTIVE PERFORMANCE AND LAMB SURVIVAL

On-station performance

Mating of related individuals was unavoidable due to small flock size, leading to inbreeding depression. The inbreeding rates per generation derived from the number of breeding males and females were 6.1% at Debre Berhan and 32.5% at Amed Guya (BoA and ARARI, 2001). Based on the data at DBSBMC collected from 25 Awassi rams and 92 different matings between the year 2009 and 2013, number of ewes lambed per ewe mating was on average 37% with a range of 10 to 77.5%.

Demeke et al. (1995) carried out a study at Sheno Agricultural Research Center to evaluate the effect of breeds (pure Menz, Menz × Corriedale and Menz × Awassi) and feed supplement (grazing and grazing + 300 g concentrate supplement day^{-1}) on reproductive performances. The authors reported that age at first lambing and the proportion of ewes lambed was not influenced by genotype and supplementary feeding, whereas body weight at conception was influenced by both factors and the interaction of the two. Crossbreds respond more to supplementary feed than pure Menz breed. Awassi × Menz and Corriedale × Menz crossbred lambs were heavier at birth compared to pure Menz lambs. The overall effect of supplementation was not significant on lamb birth weight. However, breed by feeding analysis showed lambs from supplemented pure Menz and Awassi crosses ewes were heavier at birth than their counterpart lambs born from the non-supplemented ewes. Olsson and Beyene (1990) reported a comparable performance of crossbreds in litter weight and lamb survival whereas in total weaning weight of lambs per ewe lambed were increased with increasing level of exotic genes up to 50%.

On-farm performance

Age at first lambing, lambing interval, ewe post-partum weight, number of lambs born ewe^{-1} year^{-1}, and number of lambs weaned per ewe^{-1} year^{-1} and lamb survival of local and their crosses with Awassi and Corriedale based on the data from the three villages, Serity (Chacha), Negasi-Amba (Menz) and Chiro (Wollo) were studied (Getachew et al., 2013, 2015). A total of 71 farmers and more than 6000 lambing records were used for the analysis. Crossbred ewes had exotic level of 25 to ~50%. The results revealed that crossbreds showed inferior performance for age at first lambing, lambing interval, and number of lambs born ewe^{-1} year^{-1} than local breeds in all locations. However, crossbred and local ewes were similar in number of lambs weaned per ewe per year. Lambing interval of local ewes ranged from 227.1 days in Wollo to 260 days in Menz and of Awassi cross ewes ranged from 249.7 in Wollo to 329.4 days in Menz. Corriedale crosses found only in Wollo site showed comparable reproductive performance with local ewes under farmer management. Crossbreeding increased ewe postpartum weight by 21.5% in Wollo, which was higher compared to 16.4% in Menz and 9.5% in Chacha. Though vary by location crossbred, lambs had better pre-weaning survival than local lamb (Getachew et al., 2015).

SKIN, WOOL AND MILK

Indigenous sheep breeds, particularly those from the Ethiopian highlands, are reputed to have one of the best quality skins for leather products having fine natural qualities of clarity, thickness, flexibility, strength, and compact texture (Mahmud, 1999). It is hypothesized that crossing exotic genotypes with indigenous breeds for improving growth might negatively affect important leather characteristics. Skins from Awassi × Menz crossbred sheep are either sold at lower prices or are rejected completely. Tibbo et al. (2005) found that the market price of skin of the indigenous, Menz, sheep was 3 to 4 times higher than 50 and 75% Awassi. However, skin quality studies (considering percent elongation, tensile strength, size of skin) confirmed that there is no evidence supporting the suspected inferiority of skins from crossbreds up to 50% inheritance of exotic Awassi and Dorper (Getachew et al., 2011; Teklebrhan et al., 2012). Tibbo et al. (2005) reported crossbreeding increased fleece yield of Menz sheep by 147% when upgraded to 50% Awassi-Menz and 218% when upgraded to 75% Awassi–Menz levels.

In one of the crossbreeding villages (Chiro), farmers showed interest to use sheep milk while it is unusual to consume sheep milk in other highland areas. Based on informal interviews with farmers and measurements of milk yield (few observations), in a good season, a ewe can produce in the range of 0.5 to 1 L of milk per day. Further investigation is required to see the possibility of using Awassi crossbreds for milk production.

FARMERS' PERCEPTION ON BREED IMPROVEMENT

Farmers in Ethiopia showed keen interest to adopt and implement breeding programs when they found them working and benefitting them. However, depending on their level of experience and capacity, farmers might support either crossbreeding or pure breeding. Farmers are interested in adoption of sheep crossbreeding due to the fast growth of crossbreds compared to their local sheep breeds in the Awassi × Menz and Farta x Washera crossbreeding attempts in the highlands of the Amhara region (Taye et al., 2011; Teferra et al., 2014). A workshop and field visit of the community based Menz sheep breeding program in the highlands was held with higher officials and different stakeholders engaged in livestock breeding on January 15, 2014. The program has been implemented by DBARC in collaboration with ILRI-ICARDA-BOKU and detailed (Haile et al., 2011). The aim of the workshop was to strengthen the idea of delineating Menz area for pure breeding based on the indigenous Menz breed as the poor feed resource base of the area could not support larger exotic genotypes. Farmers clearly explained that they are benefiting from the community based pure breeding program. Farmers also explained that a previous introduction of larger breeds in the area showed adaptation problems.

Considering the interest of farmers and the performance of sheep in the field the team agreed to support the idea of farmers of strengthening the ongoing community based pure breeding program in such areas.

BENEFITS AND ADOPTION OF SHEEP CROSSBREEDING

A survey based economic evaluation of Awassi x Menz and Awassi x Wollo crossbred sheep was performed and the extent of diffusion of Awassi crosses from three crossbreeding villages in Angolelana Tera, Menz Gera and Legambo districts were studied (Teferra et al., 2014). A total of 208 farmers, 71 starters (got initial breeding ram from research center), 63 co-farmers (start crossbreeding by buying crossbreds from the starters) and 74 non-users (they do not have crossbred sheep in their flocks) were considered in this study. Low input small holder farmers participating in the pilot village crossbreeding program were able to improve their income and livelihood. Similarly, the farm Africa goat development project in Kenya showed that the exotic Toggenburg goat performed well under low input farmers conditions and improved the income of smallholder farmers in Kenya and Tanzania (Peacock et al., 2011). In all locations, crossbreds were superior in market price compared to their corresponding locals at similar age and under similar management. For example the price (in Ethiopian Birr) of crossbred vs. local ram lambs was 319 vs. 171, 362 vs. 177, and 497 vs. 180 in Angolelana Tera, Menz Gera and Legambo, respectively (Teferra et al., 2014). In one of the Awassi crossbreeding villages, farmers started to produce genetic material and they are serving as sources of breeding rams. The positive impact of crossbreeding on their livelihood was mentioned by almost all farmers in the three locations. Increased proportion of crossbreds over time showed the acceptance of crossbreeding (Gizaw et al., 2014; Teferra et al., 2014). Proportion of crossbred sheep in the project participant farmers flocks in Angolelana Tera, Menz Gera, and Legambo districts was 63.6, 42.1, and 64%, respectively. The proportion of crossbreds in flocks of co-farmers was also comparable, with proportions of 47.8, 35.6%, and 54.7 % in respective villages.

GENERAL DISCUSSION

Choice of breed

In developing countries, acceptance of new breeds by farmers is influenced not only by their productive performances but also by non-production traits like beauty and appearance of the animal (Ndumu et al., 2008) and cultural values (Leroy et al., 2015). Traits like coat color, tail type, horn and ear size of sheep can also have significant influence on price in the predominant live animal marketing (Tadesse, 2009). At the beginning, exotic breed introduction targeted wool and meat production that overlooked the preference of farmers for appearance of sheep. Ignoring farmer preference led to low acceptance and resulted in low up-take rates. Introduction of Awassi sheep from Israel considered the preferred physical appearance. This breed is developed for milk and used as a triple purpose for meat, milk and wool (Pollott and Gootwine, 2004; Galal et al., 2008; Gürsoy, 2011). The improved Israeli Awassi is characterized by producing the highest amount of milk, having highest fertility and twinning rate, and heaviest body weight among all Awassi populations (Galal et al., 2008). Promoting a new market for sheep milk in the future in the Ethiopian highlands where traditionally this product is not consumed would be an extra advantage. However, at present looking for meat line Awassi (if any), or selection for meat traits among the crossbred population would be more beneficial due to the expected potential risk of mastitis and other udder problems when using high producing dairy Awassi for crossbreeding in areas where no sheep milking is done.

Breeding ram multiplication and dissemination

Efficient multiplication and dissemination of appropriate genotypes is one of the core elements in a breed improvement program. Until now introduction and maintaining of exotic breeds, as well as multiplication and dissemination of crossbreds are mainly mandated to government farms. Managing animals in government farms characterized by high level of lamb mortality (Getachew et al., 2015) suffered from inbreeding due to small numbers of exotic animals and diseases (e.g. maedi-visna) associated with confinement. In addition, low fertility with natural mating in the farms, lack of infrastructure and logistics (e.g. shortage of mating pens) restricted efficiency of improved ram multiplication. Fertility level observed for Awassi breed in Ethiopia are lower than the fertility of Awassi in the Middle Eastern countries ranging between 60 to 95% (Gursoy et al., 1995; Galal et al., 2008). Ahuya et al. (2005) reported the previous government approach based on multiplication and dissemination of exotic bucks from government farms failed to bring anticipated change. Learning from a previous non-successful Ethiopian dairy goat project (Ayalew et al., 2003), the German Development Cooperation (GIZ) and FARM Africa initiated a community approach which was led by farmers and became more successful in significantly improving the livelihoods of resource poor families in Kenya (Peacock et al., 2011). As mentioned earlier community based breeding ram production has starred in one of the crossbreeding village. It seems more feasible in reducing higher level of mortality associated with confinement and budget limitation under on-station management. However, comparative advantages of ram multiplication at farmers' management need to be assessed. Its sustainability depends on the technical and infrastructural support from government and other institutions.

Efficiency of breeding ram dissemination to the targeted production unit also found unsatisfactory. An informal survey in 1997 in South Wollo and North Shewa to evaluate the on-farm performance of crossbreds exposed that there were no any apparently crossbred sheep found even though a significant number of rams were disseminated to farmers (Tibbo, 2006; Gizaw and Getachew, 2009). Disseminated crossbred rams were castrated, found in farmers having no or few breeding ewes, or sold after castration (Gizaw and Getachew, 2009). Thus the predominant practice of selling crossbred rams to individual farmers does not seem suitable for smallholder situation. Firstly, farmers tempted to sell crossbred rams for their short term need and keeping and managing such a big animal might be difficult for a farmer. Secondly, rams were underutilized due to small flock size. Furthermore, ram dissemination was not focused to specific areas and the effort of crossbreeding was diluted. Instead of selling rams to single farmers, devising schemes involving communal use of breeding rams which have been successfully implemented in the community based sheep breeding programs (Gizaw and Getachew, 2009; Haile et al., 2011; Gizaw et al., 2014) should be considered. Insufficient knowledge of farmers and unreliable external support were also mentioned as limitations leading to low adoption rates of crossbreeding.

Performance of crossbreds

Better growth performance and lamb survival of Awassi and Dorper crossbreds (Gizaw and Getachew, 2009; Tsegay et al., 2013; Tilahun et al., 2014). In the range of inferior to comparable reproductive performances were reported for crossbred ewes (Getachew et al., 2013; Lemma et al., 2014b). The inferiority of Awassi crossbred ewes in age at first lambing and lambing interval are mostly more than compensated by the relative larger size of ewes resulted in better ability of crossbred ewes to raise their lambs to weaning age (Olsson and Beyene, 1990; Tibbo, 2006; Getachew et al., 2013, 2015). The observed performance levels found in this review seem lower than the potential of the breed in its breeding tract (Rihawi et al., 2010; Üsünter 2013). These low performances for both local and crossbred sheep in Ethiopia suggest that output can be increased by improving environmental conditions. Local breeds may sometimes be unprofitable as they have limited genetic capacity to respond to an improved management. The local Awassi managed by Bedouin farmers in the southern dry region of Israel also remain unprofitable and economic assessment showed that traditional extensive sheep farming in low input system based on the local Awassi breed was not positively contributing to family income and nutrition however still regarded as cultural benefit (Valle Zárate et al., 2009). The introduction of improved Awassi (Afec-Awassi) was successful and made these flocks profitable (Gootwine et al., 2009). Similarly, the introduction of different ram breeds in extensive semi-arid regions of Argentina improved carcass yield and conformation with varying performance among sires (Álvarez et al., 2010).

Genotype by environment interaction

Crossbreeds have been benefiting Ethiopian small holder farmers due to their fast growth. Higher price of Awassi crossbreds (about double) compared to locals under farmer management is a clear indication of benefit. Similarly in Kenya, crossbreeding between Dorper and Red Maasai sheep has been used by a majority of farmers in Kajiado district and are playing important role for the livelihood of the people (Liljestrand, 2012). However, presence of genotype by environment interaction on performances of sheep has been well documented in Ethiopia (Demeke et al., 1995; Getachew et al., 2013; Getachew et al., 2015), Kenya (Baker et al., 2002; Zonabend et al., 2014) as well as cattle in tropical countries (Galukande et al., 2013). For example, in Kenya, in the poor environment both local and crossbreds had about the same body weight whereas in the other site (better environment and market oriented farmers) Dorper and crossbreds had superior weight (Zonabend et al., 2014). Those variable research results on the performance of crossbreeding based on location, genotype and management suggested that the importance of differential recommendations for different locations and careful delineation of crossbreeding area.

Safeguarding indigenous genetic resources

Erosion of the diversity of domestic animals due to natural cause and human activity is considered as one of the serious concerns to sustain the production levels and addressing the change in demand of future market (Shrestha and Fahmy, 2005). Due to the massive introduction and multiplication of Awassi and Dorper sheep, large number of crossbred rams might be expected to be disseminated to farmers in wider areas of the country. This might have great potential to contribute to improve productivity of smallholder sheep if planned and utilized appropriately. On the other hand, they present a threat if they are able to dilute the adaptive indigenous genetic resources. Red Maasai sheep in Kenya, which is well known for its maternal traits, disease and drought tolerance are currently at high risk due to the massive introgression of Dorper genes (Muigai et al., 2009) and currently requires breeding program to conserve the breed (Zonabend et al., 2014). Thus, delineating crossbreeding areas and controlling breed introduction should be considered as critical steps to reduce risk of genetic dilution due to indiscriminate crossbreeding. Crossbreeding might focus on sheep populations along the roads, near towns and cities, near market places and buffer zones between two geographically separated areas as those populations are mixed and non-descript. Conservation priorities set for Ethiopian sheep breed considering genetic and non-genetic factors (Gizaw et al., 2008b) need to be consulted for the decision. Crossbreeding among selected indigenous breeds might also be helpful to reduce the effect of genetic dilution expected from indiscriminate crossbreeding by use of relatively higher genetic distant of the exotic breeds.

Choosing appropriate crossbreeding program

Sustainability of crossbreeding program highly depends on the choice of appropriate breeding scheme (Leroy et al., 2015). Once crossbreeding areas are delineated, alternative breeding programs should be evaluated and selected based on their genetic progress, inbreeding level, cost and feasibility under smallholder situation. In addition, access and continuous supply of breeding stock plays crucial role in choosing breeding strategy (Leroy et al., 2015). Combining different merits of the indigenous breeds would also be helpful to develop more productive ewe line which would help to increase productivity in terminal crossbreeding using specialized meat sire breeds. Gizaw et al. (2014) also suggested terminal crossbreeding as an alternative to upgrading as the later takes long time and seem complex under farmers' situation. This would allow to exploit the benefit of all possible heterosis effects while conserving the indigenous breeds (Scholtz and Theunissen, 2010). However, it requires setting up straight breeding programs for the local and exotic breeds to supply the parent populations. The feasibility of terminal crossbreeding might hampered by low levels of organization in small holder environments so that the performance of the exotic sires for terminal crossbreeding as well as its feasibility under small holder situation has to be evaluated before implementation.

Improving efficiency of reproduction

Recent attempt of estrus synchronization and artificial insemination (AI) in station and farmers sheep flocks in Ethiopia provides an opportunity for rapid multiplication of crossbred population. Efficiency of the government farm (DBSBMC) is too low due to poor fertility of the natural mating of Awassi rams to Menz ewes and shortage of mating pens. Estrus synchronization and AI would help to increase the number of crossbred lambs and help in adjusting lambing time to a better season. Furthermore, the use of pure Awassi semen in the farmers flock shortens the upgrading time and reduces complexity associated with upgrading. The success and limitations of the use of AI and other reproductive technologies in tropical countries has been discussed in detail (Galukande et al., 2013).

Farmer participation, institutions, and other operational issues

Adoption of crossbreeding technologies and increased crossbreed population in farmers flock (Taye et al., 2011; Teferra et al., 2014) and continuous introduction of exotic breeds are indicators of the interest of the government to relay on crossbreeding. Gizaw et al. (2013) reported in a review that a total of 26 sheep research projects have been supported by the National Institute of Agricultural Research and in the field of breeding, nutrition, health and marketing and 10 of them were focusing on Dorper (n=9) and Awassi (n=1) crossbreeding. However, there is no national sheep breeding strategy to lead and control the breeding programs (Gizaw et al., 2013) except attempts to develop sheep breeding guidelines in the regional states to some extent. Recent establishment of Livestock and Fishery Minister at national level showed that the focus for livestock development has been increased. Long-term commitment of national government, conducive policies, good development plan, and organized effort of institutions are crucial for success and sustainability of livestock development (Haile et al., 2011; Rege et al., 2011; Wurzinger et al., 2011). However, poor integration among institutions, lack of knowledge about livestock improvement at each level and absence of breeding programs are drawbacks for the success of breeding programs (Sölkner et al., 1998; Kosgey et al., 2006; Gizaw et al., 2013). Crossbreeding programs should be devised at national level considering the agro ecological potential of the areas, level of the community, level of farmers' participation and sociological factors (Sölkner et al., 1998; Philipsson et al., 2011; Peacock et al., 2011; Wurzinger et al., 2011; Leroy et al., 2015).

CONCLUSION

This review paper revealed that growth and reproductive performances of crossbreds greatly vary and are influenced by many factors like management, location, dam breed used for crossing, and breed composition levels. Both under station and farmers management, Awassi based crossbred lambs outperformed their local (Menz and Wollo) contemporaries in some of the North Shewa and Wollo highland areas of the Amhara region, Ethiopia. Dorper crossbreds performed well under station management; however, its wider dissemination should be based on their performance under farmer's management. More complete analyses considering the total inputs and outputs of flocks in the smallholder systems of Ethiopia are required to draw firm conclusions for particular scenarios. Unfortunately such data are not available for most crossbreeding programs in Ethiopia. Gebre et al. (2014) have employed dynamical systems analysis to find whether genetic improvement of body size is profitable in a community based pure breeding system of the Menz area. The authors concluded from the simulations that genetically bigger animals are more profitable under constant management and fattening of young rams is also more profitable compared to the current state. It is likely that results will be similar for fast growing crossbred animals compared to the unimproved Menz sheep if prolificacy and fertility of the crossbreds are not worse compared to purebred Menz. Delineating crossbreeding areas considering breed preference of farmers, climatic conditions and potential for feed, market and health service development should be prerequisite to start crossbreeding.

Efficient and easy crossbreeding systems which maximize ram multiplication in the breeding units and efficient ram dissemination to the production units should also be devised. The appropriate proportion of indigenous and exotic breeds considering the existing management needs to be determined for different areas. Long-term strategies to achieve and maintain the optimal genotypes need to be devised. Experience of implementing community based sheep breeding either based on pure breeding or crossbreeding reveals that the success of any breeding program mainly depends on the full farmers' participation, continuous commitment and integrated effort of institutions. Continuous research and development interventions are crucial to develop and optimize crossbreeding scheme, replicate and scale up the models, establish pedigree and performance recording, determine appropriate level of admixture, and devise appropriate management for each areas. Optimizing the breeding programs considering new products (like milk and wool) created from crossbreeding might be helpful to increase the overall benefit but should be considered in relation to potential market demand.

Conflict of interests

The authors have not declared any conflict of interests.

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REFERENCES

Ahuya CO, Okeyo AM, Peacock C (2005). Developmental challenges and opportunities in the goat industry: The Kenyan experience. Small Rumin. Res. 60:197-206.

Álvarez M, Rodríguez Iglesias RM, García Vinent J, Giorgetti H, Baselga M (2010). Introduction of meat sheep breeds in extensive systems of Patagonia: lamb growth and survival. J. Anim. Sci. 88:1256-1266.

ANRS-BoARD (Amhara National Regional State Bureau of Agriculture and Rural Development) (2004). Sheep improvement breeding strategy and program (Amharic version). Bahirdar, Ethiopia. 53p.

Awgichew K, Gipson A (2009). Overview of Genotype Program Activities Proceedings of mid-term conference of the Ethiopian Sheep and Goat Productivity Improvement Program, Achievement, Challenge and Sustainability. March 13-14, 2009, Hawassa, Ethiopia. pp. 40-52.

Ayalew W, Rischkowsky B, King JM, Bruns E (2003). Cross-breds did not generate more net benefits than indigenous goats in Ethiopian smallholdings. Agric. Syst. 76:1137-1156.

Baker RL, Mugambi JM, Audho JO, Carles AB, Thorpe W (2002). Comparison of Red Maasai and Dorper sheep for resistance to gastro-intestinal nematode parasites, productivity and efficiency in a mid and a semi-arid environment in Kenya In: Proceedings of the 7th World Congress on Genetics Applied to Livestock Production, 18-23 August 2002. Montpellier France 31:639-642.

BoA (Bureau of Agriculture) (2000). Report on the preliminary investigation of sheep disease at sheep ranches and ram distribution sites in North Shoa. Disease Investigation Report. Bahir Dar, Ethiopia.

BoA and ARARI (Bureau of Agriculture, Amhara region and Amhara Regional Agricultural Research Institute) (2001). The control and management of maedi-visna in Debre Berhan and Amed Guya sheep breeding and multiplication centers and their surroundings and the implication of the disease in the country Bahir Dar, Ethiopia.

CSA (Central Statistics Agency) (2013). Agricultural Sample Survey, 2012/13 2005 EC, Volume II: Report on Livestock and livestock characteristics Private peasant holdings) Statistical Bulletin 570. Federal Democratic Republic of Ethiopia, Addis Ababa.

DBSBMC (Debre Berhan Sheep Breeding and Multiplication Center) (2007). Synthesis report of the 39 years activity of the center Debre Berhan, Ethiopia.

De Waal H, Combrinck W (2000). The development of the Dorper, its nutrition and a perspective of the grazing ruminant on veld. Small Rumin. Res. 36:103-117.

Demeke S, Thwaites CJ, Lemma S (1995). Effects of ewe genotype and supplementary feeding on lambing performance of Ethiopian highland sheep. Small Rumin. Res. 15:149-153.

Emana B (2009). Cooperatives : a path to economic and social empowerment in Ethiopia. International Labour Office. Dar es Salaam: ILO, 2009. 1v(CoopAFRICA working paper; no 9).

FAO (2007). The State of the World's Animal Genetic Resources for Food and Agriculture – in brief, edited by Dafydd Pilling and Barbara Rischkowsky. Rome.

Galal S, Gürsoy O, Shaat I (2008). Awassi sheep as a genetic resource and efforts for their genetic improvement - A review. Small Rumin. Res. 79:99-108.

Galukande EH, Mulindwa H, Wurzinger M, Roschinsky R, Mwai O, Sölkner J (2013). Cross-breeding cattle for milk production in the tropics: achievements, challenges and opportunities. Anim. Genet.

Resour. 52:111-125.
Gebre KT, Wurzinger M, Gizaw S, Haile A, Rischkowsky B, Sölkner J (2014). Effect of genetic improvement of body weight on herd dynamics and profitability of Ethiopian meat sheep: A dynamic simulation model. Small Rumin. Res. 117:15-24.
Getachew T, Gizaw S, Wurzinger M, Abegaz S, Sölkner J (2013). Effect of Crossbreeding Indigenous Sheep with Awassi and Corriedale Sires on Reproductive Performance under Smallholder Production System in Agric. Conspec. Sci. 78:187-191.
Getachew T, Gizaw S, Wurzinger M, Haile A, Rischkowsky B, Okeyo AM, Sölkner J, Mészáros G (2015). Survival analysis of genetic and non-genetic factors influencing ewe longevity and lamb survival of Ethiopian sheep breeds. Livest. Sci. 176:22-32.
Getachew T, Lemma S, Gizaw S, Abebe A (2011). Quality parameters of skin from Menz, Awassi × Menz and Washera sheep breeds for leather production In: Proceedings of the 5th annual regional conference on completed livestock research activities, Amhara Regional Agricultural Research Institute, Bahir Dar, Ethiopia.
Gizaw S, Abegaz S, Rischkowsky B, Haile A, Okeyo AM, Dessie T (2013). Review of sheep research and development projects in Ethiopia. Nairobi, Kenya: International Livestock Research Institute (ILRI).
Gizaw S, Getachew T (2009). The Awassi × Menz Sheep Crossbreeding Project in Ethiopia : Achievements, Challenges and Lessons Learned Proceedings of mid-term conference of the Ethiopian Sheep and Goat Productivity Improvement Program, Achievement, Challenge and Sustainability March 13-14, 2009, Hawassa, Ethiopia. pp. 53-62.
Gizaw S, Komen H, Hanotte O, Van Arendonk JAM (2008a). Indigenous sheep resources of Ethiopia: types, production systems and farmers preferences. AGRI 43:25-39.
Gizaw S, Komen H, Windig,JJ, Hanotte O, Van Arendonk JA (2008b). Conservation priorities for Ethiopian sheep breeds combining threat status, breed merits and contributions to genetic diversity. Genet. Select. Evol. 40:433-447.
Gizaw S, Lemma S, Getachew T (2014). Design of a Village-based Awassi Sheep Crossbreeding Scheme, in: Mekuriaw Z, Zeleke G, Yeheyis, L (Eds), Proceedings of the 6th and 7th Annual Regional Conference on Livestock Completed Research Activities 25-27 January, 2012 and 22-24 January, 2013. Amhara Agric. Res. Institute (ARARI), Bahir Dar, Ethiopia. pp. 138-157.
Gootwine E, Abu Siam M, Reicher S, Albaqain A, Valle Zárate A (2009). Introducing the prolific Afec-Awassi strain to semi extensive Bedouin sheep flocks in Israel as a means to secure their economic success In: Proceedings of the 60th Annual Meeting of the European Association for Animal Production (Barcelona, Spain).
Gootwine E, Pollott GE (2000). Factors affecting milk production in Improved Awassi dairy ewes. Anim. Sci. 71:607-615.
Goshme S, Gizaw S, Bisrat A, Besufekad S, Abebe A (2014). Comparative Growth Performance and Survival Rate of Dorper X Improved Menz, Dorper X Menz type and Dorper X Afar Crossbred Sheep at Debre Berhan Research Center, in: Mekuriaw Z, Zeleke G, Yeheyis L. Proceedings of the 6th and 7th Annual Regional Conference on Livestock Completed Research Activities 25-27 January, 2012 and 22-24 January, 2013, Amhara Agricultural Research Institute (ARARI), Bahir Dar, Ethiopia. pp. 412-419.
Gursoy O, Kirk K, Cebeci Z, Pollot GE (1995). Genetic evaluation of growth performance in Awassi sheep. In: Gabiña D (ed.). Strategies for sheep and goat breeding. Zaragoza: CIHEAM, 1995. pp.193-201. (Cahiers Options Méditerranéennes; n. 11). Meeting of the joint FAO/CIHEAM Network on Sheep and Goats, Subnetwork on Animal Resources, 1995/03/26-28, Sidi-Thabet (Tunisia). Available at: http://om.ciheam.org/om/pdf/c11/96605556.pdf.
Haile A, Tembely S, Anindo D, Mukasa-Mugerwa E, Rege JE, Yami A, Baker R (2002). Effects of breed and dietary protein supplementation on the responses to gastrointestinal nematode infections in Ethiopian sheep. Small. Rumin. Res. 44:247-261.
Haile A, Wurzinger M, Mueller J, Mirkena T, Duguma G, Okeyo M, Sölkner J Rischkowsky B (2011). Guidelines for Setting up Community-based Sheep Breeding Programs in Ethiopia. ICARDA – tools and guidelines No1 Aleppo, Syria, ICARDA.
Hassen Y, Sölkner J, Gizaw S, Baumung R (2002). Performance of crossbred and indigenous sheep under village conditions in the cool highlands of central-northern Ethiopia: growth, birth and body weights. Small Rumin. Res. 43:195-202.
Hayes BJ, Bowman PJ, Chamberlain AJ, Goddard ME (2009). Invited review: Genomic selection in dairy cattle: progress and challenges. J. Dairy Sci. 92:433-443.
Iñiguez L (2011). The challenges of research and development of small ruminant production in dry areas. Small Rumin. Res. 98:12-20.
Kosgey IS, Baker RL, Udo HMJ, Van Arendonk JAM (2006). Successes and failures of small ruminant breeding programmes in the tropics: a review. Small Rumin. Res. 61:13-28.
Lakew M, Haile-Melekot M, Mekuriaw G (2014). Evaluation of Growth Performance of Local and Dorper × Local Crossbred Sheep in Eastern Amhara Region, Ethiopia. Iranian J. Anim. Sci. 4:123-126.
Leroy G, Baumung R, Boettcher P, Scherf B, Hoffmann I (2015). Review: Sustainability of crossbreeding in developing countries; definitely not like crossing a meadow. Animal 10(2):262-73
Lemma S, Gizaw S, Abebe A, Getachew T (2014a). Growth and Reproductive Performance of Menz, Washera x Menz and Bonga x Menz Sheep in the Cool Highlands of Central Ethiopia In Proceedings of the 6th and 7th Annual Regional Conference on Livestock Completed Research Activities 25-27 January, 2012 and 22-24 January, 2013, Mekuriaw Z, Zeleke G, Yeheyis L eds. Amhara Agricultural Research Institute (ARARI), Bahir Dar, Ethiopia). pp. 294-307.
Lemma S, Gizaw S, Getchew T, Abebe A (2014b). On-farm productivity performance of purebred local and Awassi x local crossbred sheep In Proceedings of the 6th and 7th Annual Regional Conference on Livestock Completed Research Activities 25-27 January, 2012 and 22-24 January, 2013, Mekuriaw Z, Zeleke G, Yeheyis L eds. Amhara Agricultural Research Institute (ARARI), Bahir Dar, Ethiopia). pp. 326-342.
Leymaster KA (2002). Fundamental Aspects of Crossbreeding of Sheep: Use of Breed Diversity to Improve Efficiency of Meat Production. Sheep Goat Res. J. 17:50-59.
Liljestrand J (2012). Breeding practices of Red Maasai sheep in Maasai Pastoralist Communities. Master's Thesis. Swedish University of Agricultural Sciences. Uppsala, Sweden.
Mahmud A (1999). Development potential and constraints of hides and skins marketing in Ethiopia In: Merkel RC, Abebe G and Goetsch AL (eds.) The opportunity and challeneges of enhancing goat production in East Africa. Proceedings of a conference held at Debun Univ. pp. 127-139.
Mekuriaw S, Mekuriaw Z, Taye M, Mekuriaw G, Amane A, Bimrew T, Haile A (2013). Growth performance and linear body measurements of Washera, Farta and their crossbreed sheep under farmers management system in Western Highland of Amhara Region. Sci. J. Vet. Adv. 2:132-143.
Muigai AWT, Okeyo AM, Kwallah AK, Mburu D, Hanotte O, Maasai-mutara R, Maasai-olmagogo R, Maasai-kajiado R (2009). Characterization of sheep populations of Kenya using microsatellite markers : Implications for conservation and management of indigenous sheep populations. S. Afr. J. Anim. Sci. 39:93-96.
Ndumu DB, Baumung R, Wurzinger M, Drucker AG, Okeyo AM, Semambo D, Sölkner J (2008). Performance and fitness traits versus phenotypic appearance in the African Ankole Longhorn cattle: A novel approach to identify selection criteria for indigenous breeds. Livest. Sci. 113:234-242.
Olsson A, Beyene T (1990). Performance of indigenous and crossbred sheep in the highlands of Ethiopia in: Proceedings of the Fourth Wld. Cong. Genet. Appl. Livest. Prod. 15:406-409.
Peacock C, Ahuya CO, Ojango JMK, Okeyo AM (2011). Practical crossbreeding for improved livelihoods in developing countries: The FARM Africa goat project. Livest. Sci. 136:38-44.
Philipsson J, Zonabend E, Okeyo AM (2011). Sustainable breeding programmes for tropical low- and medium input farming systems. In: Animal Genetics Training Resource, version 3, 2011. Ojango JM, Malmfors B, Okeyo AM (eds.). International Livestock Research

Institute, Nairobi, kenyaand Swedish University of Agricultural Sciences, Uppsala, Sweden.
Pollott GE, Gootwine E (2004). Reproductive performance and milk production of Assaf sheep in an intensive management system. J. Dairy Sci. 87:690-703.
Rege JEO, Marshall K, Notenbaert A, Ojango JMK, Okeyo AM (2011). Pro-poor animal improvement and breeding - What can science do? Livest. Sci. 136:15-28.
Rihawi S, Iñiguez L, Knaus WF, Zaklouta M, Wurzinger M, Sölkner J, Larbi A, Bomfim MAD (2010). Fattening performance of lambs of different Awassi genotypes, fed under cost-reducing diets and contrasting housing conditions. Small Rumin. Res. 94:38-44.
Scholtz MM, Theunissen A (2010). The use of indigenous cattle in terminal cross-breeding to improve beef cattle production in Sub-Saharan Africa. Anim. Genet. Resour. 46:33-39.
Shrestha JNB (2005). Conserving domestic animal diversity among composite populations. Small Rumin. Res. 56:3-20.
Shrestha JNB, Fahmy MH (2005). Breeding goats for meat production: a review. Small Rumin. Res. 58:93-106.
Sölkner J, Nakimbigwe H, Valle-Zarate A (1998). Analysis of determinants for success and failure of village breeding programmes. Proc. 6th World Congr. Genet. Appl. Livest. Prod. 25:273-280.
Tadesse F (2009). Price and performance analysis of indigenous sheep breed marketing in Menz districts of Ethiopia MSc thesis Haramaya University, Haramaya, Ethiopia.
Taye M, Yitayew A, Mekuriaw S, Bitew A (2011). Adaptability and productivity of Washera rams and its crosses with Farta sheep in South Gonder zone of the Amhara region. O. J. Anim. Feed Res. 1:400-406.
Teferra B, Bizuayehu S, Abebe A (2014). Diffusion and Financial Evaluation of Improved Awassi- Menz Crossbred Sheep in North Shewa and South Wello, Amhara Region, in: Mekuriaw Z, Zeleke G, Yeheyis L. Proceedings of the 6th and 7th Annual Regional Conference on Livestock Completed Research Activities 25-27 January, 2012 and 22-24 January, 2013, Amhara Agricultural Research Institute (ARARI), Bahir Dar, Ethiopia. pp. 158-184.
Teklebrhan T, Urge M, Mekasha Y (2012). Skin/leather quality of indigenous and crossbred (Dorper x Indigenous) F1 sheep. Livest. Res. Rural Dev. 24(4).
Tibbo M (2006). Productivity and health of indigenous sheep breeds and crossbreds in the central Ethiopian highlands. Doctoral Thesis, Swedish University of Agricultural Sciences. Uppsala, Sweden.
Tibbo M, Aragaw K, Philipsson J, Malmfors B, Näsholm A, Ayalew W (2005). Economics of sub-clinical helminthosis control through anthelmintics and supplementation in Menz and Awassi-Menz crossbred sheep in Ethiopia. 56th Annu. Meet. EAAP. pp. 5-8
Tilahun M, Kefelegn K, Abebe G, Goetsch AL (2014). Feed intake, digestibility, weight gain, and slaughter characteristics influenced by genetic percentage of Boer in goats and Dorper in sheep in the central highlands of Ethiopia. Trop. Anim. Health Prod. 46:593-602.
Tsegay T, Yoseph M, Mengistu U (2013). Comparative evaluation of growth and carcass traits of indigenous and crossbred (Dorper×Indigenous) Ethiopian Sheep. Small Rumin. Res. 114:247-252.
Üsünter H (2013). Main Productive Performance of Awassi Sheep in Central Anatolian Region of Turkey. Turkish J. Vet. Anim. Sci. 37:271-276.
Valle Zárate A, Albaqain A, Gootwine E, Herold P, Albaqain R (2009). Socio-economic benefits from Bedouin sheep farming in the Negev. In: Proceedings of the 60th Annual Meeting of the European Association for Animal Production, Barcelona, Spain.
Wurzinger M, Sölkner J, Iñiguez L (2011). Important aspects and limitations in considering community-based breeding programs for low-input smallholder livestock systems. Small Rumin. Res. 98:170-175.
Zonabend E, Mirkena T, Audho JO, Ojango J, Strandberg E, Näsholm A, Malmfors B, Okeyo AM, Philipsson J (2014). Breeding objectives for Red Maasai and Dorper sheep in Kenya - a participatory approach In: Proceedings of the 10th World Congress on Genetics Applied to Livestock Production, August 17-22, 2014. Vancouver, Canada.

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Full Length Research Paper

Persistence of 2,4-D and glyphosate in a *Cerrado* soil, Brazil

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The state of Mato Grosso is the main grain producer in Brazil, and weeds occurrence remains the major obstacle in the production of these grains, also increasing herbicide consumption. The aim of this study was to assess 2, 4-dichlorophenoxyacetic acid and Glyphosate (N-phosphonomethyl-glycine) herbicides mixture persistence in soybeans of Ultisol in the Brazilian *Cerrado*. The study was conducted in a greenhouse with randomized blocks experimental design consisting of 6x5 factorial, six application periods (0, 3, 5, 7, 10 and 14 days before sowing), five herbicide doses (0, 750 , 1500, 2250 and 3000 g ia. ha^{-1}) and four repetitions. Herbicides were sprayed with a knapsack sprayer. Residual effect was assessed through emergence speed index (ESI), plant height, visual phytotoxicity and dry biomass of shoot and root. Results showed that, according to the decrease in the interval between herbicide application and soybean seeding, the residual effect of products was higher. As the dose increased, higher was the plant phytointoxication, influencing in the reduction of emergence speed, shoot dry matter, root dry matter and plant height, and negatively acting on crop development.

Key words: Pesticides, residual effect, sowing, soybean.

INTRODUCTION

Grain production in Brazil was 209 million tons, of which the Brazilian Midwest region accounted for 42% of production. According to Conab (2015), it is the main agricultural region in the country. Weeds occurrence remain a major obstacle in the production of these grains. Among weed management possibilities, the chemical method is still the most popular. According to the National Health Surveillance Agency (Anvisa, 2013), the Brazilian market of pesticides expanded in 190% in the last decade, more than twice the global market (93%).

Growth has primarily been accompanied by herbicides use (Peres, 2009) due to the influence of important crops, such as soybean. Soybean is the main crop responsible for agricultural chain and livestock commercial viability in

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Table 1. Chemical and particle size characterization of an Ultisol sample in the 0-0.20 m depth layer.

pH	P	K	Ca	Mg	H	Al	SB	CTC	V	O.M	sand	silt	clay
$CaCl_2$	mg dm^{-3}		----------Cmol$_c$ dm^{-3}---------						%	g dm^{-3}	--------g kg^{-1}-------		
4.1	2.4	28	0.3	0.2	4.2	1.1	0.6	5.9	9.8	22.7	549	84	367

the Midwest, as it also serves as raw material for the production of animal feed for cattle, swine and poultry (Aprosoja, 2013). The state of Mato Grosso is the largest national producer of grains, and this growth has also been associated with pesticides intensive use, both for weed control and pre-planting desiccation (Mapa, 2010; Conab 2015).

Indiscriminate use of herbicides favors the increase of tolerant individuals in the area and resistant species selection, diminishing efficiency and causing environmental pollution, aspects that demand optimization in pesticides use and distribution (Chiras, 1995; White, 1997; Cunha et al., 2005; Weed Science, 2014; Pacheco, 2012). Glyphosate and 2,4-dichlorophenoxyacetic acid (2,4-D) are officially the most sold in the whole national territory (Ibama, 2009). 2,4-D was the first selective herbicide and is widely used for weeds pre or post-emergence application in various crops (Ibama, 2009).

Glyphosate is a post-emergent herbicide belonging to the substituted glycine chemical group. It is toxic to aquatic organisms and slightly toxic to soil organisms, birds and bees, besides being little bioaccumulative (Rebelo et al., 2010). 2,4-D is a growth regulator which has a similar effect to auxin hormone (Ashton and Crafts, 1973). It belongs to the phenolic compounds family, being salts or esters of high molecular weight and low volatility derived from phenoxyacetic acid (Saad, 1978). Flaws in the control of certain weed species by Glyphosate use has led farmers to use other herbicides, such as 2,4-D, one of the most used in this association, especially in pre-planting desiccation application (Takano et al., 2013). The objective of the present study was to assess 2,4-D and Glyphosate herbicides persistence in an Ultisol of the *Cerrado,* and also to assess their effects on soybeans.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse (temperature between 19-50o C) located at 16°28' South latitude, 50°34' West longitude and altitude of 284 m. The experimental design was of randomized block, consisting of a 6X5 factorial, six application periods before sowing (0, 3, 5, 7, 10 and 14 days before sowing), five 2,4-D doses (0 , 750, 1500, 2250 and 3000 g ia. ha^{-1}) and a constant dose of glyphosate (4000 ia. ha^{-1}), with four repetitions.

Each experimental unit consisted of pots with 5 dm^3 soil capacity and eight TMG 132 cv. (seeded at 5 cm depth) soybean plants. The soil used was an Ultisol (EMBRAPA, 2013) collected in the region of Rondonópolis, MT state, in the depth of 0 to 0.20 m, being subsequently sieved in a 4 mm mesh for insertion in the experimental units. The soil was characterized by chemical and size analysis, according to the methodology of Embrapa (1997) (Table 1). The soil was maintained at 80% field capacity moisture, for the gravimetric method, according to the methodology of Bonfim-Silva et al. (2011).

Herbicide spraying was carried in pre emergency, out with a knapsack sprayer equipped with XR 11002 and syrup consumption corresponding to 200 L ha^{-1}. The persistence of these herbicides in the soil was assessed through emergence speed index (ESI), soybean visual phytotoxicity, with scores ranging from 1 to 5 (where 1 corresponds to no injury and 5 to plant death) (SBCPD 1995), plant height (cm), shoot biomass dry matter - BDM (g) and root biomass dry matter - BDM (g) at 26 days after sowing, at the end of the study. Statistical analysis was conducted in accordance with the polynomial regression model.

RESULTS AND DISCUSSION

Emergence speed index (ESI) was influenced in all assessed dosages, being statistically different (Figure 1). The only emergence speed index which was unaffected by dose was 0, due to herbicides absence (Figure 1a). Increasing doses along the periods showed that the period of 0 days before sowing was the one which reduced emergence the most in all applied doses, being more severe as the dose was increased (Figure 1b).

Similar results were observed by Nascimento and Yamashita (2009), in which species sensitive to auxinic herbicides showed linear decrease in plant emergence as doses were increased. In the periods of 7 and 10 days before sowing (Figure 1a), the interval between spraying and seeding gave higher emergence speed to the maximum dose of 3000, and the dose of 2250 g e. a. ha^{-1} obtained the lowest plant emergence mean. This result may be related to 2,4-D mechanism of action, as it is a herbicide characterized for being similar to auxin. According to Mortensen et al. (2012), 2,4-D acts as a herbicide for dicotyledonous weed species control. However, it also has hormonal action, acting as a synthetic auxin that can be used as plant growth regulator.

The highest herbicide doses (3000 and 2250 g e. a. ha^{-1}) were the most harmful to crop initial development in periods closer to spraying. These results were also found by Silva et al. (2011), where the shorter period between application and sowing (0 days before sowing) showed lower plant emergence. Phytotoxicity visual symptoms found (leaves shriveling and petioles epinasty) were observed in all treatments.

Plant injury effect (Figure 2) over the periods was higher at 0 days before sowing, and intensified as there was an increase of doses (Figure 2a), even in the lowest

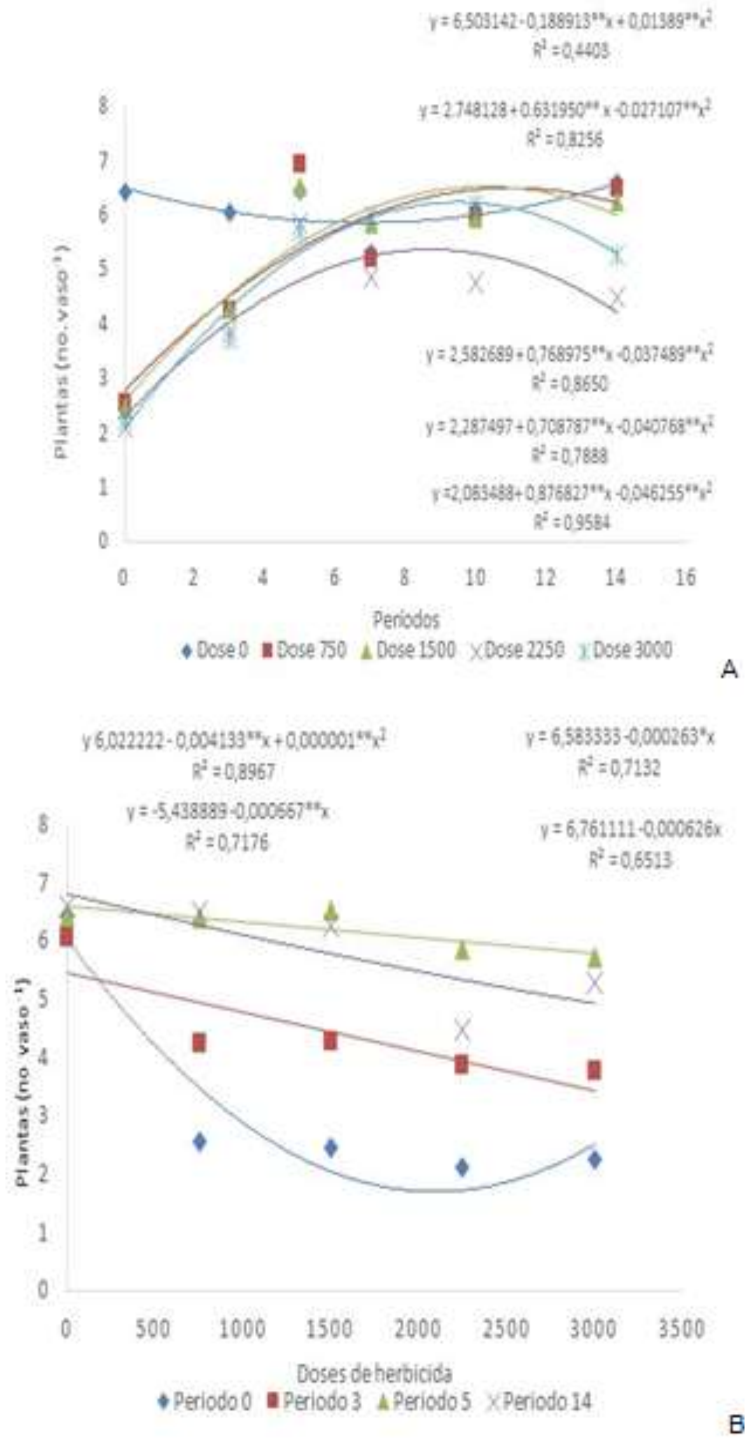


Figure 1. Emergence speed index. Treatments interaction with doses over the periods (a) and treatments interaction in the periods between sowing and spraying, according with the increasing doses (b). ***, **, *: significant a 01, 1 e 5% respectively.

dose used. The effect had a decrease as spraying was distanced from seeding, and at 14 days before sowing, the 3000 g e. a. ha^{-1} dose still was the most harmful to the crop. Phytotoxicity may be related to higher plant exposure from the germination stage. This result corroborates with those found by Silva et al. (2011), in

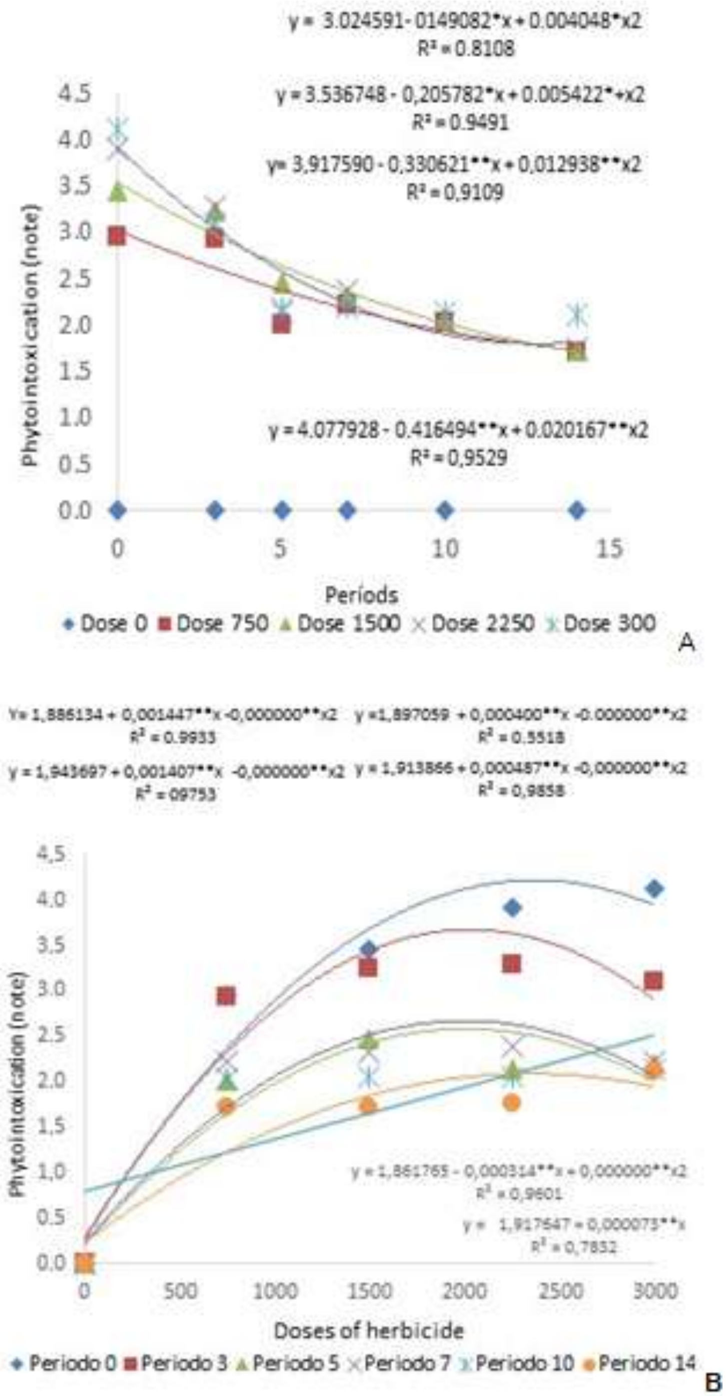
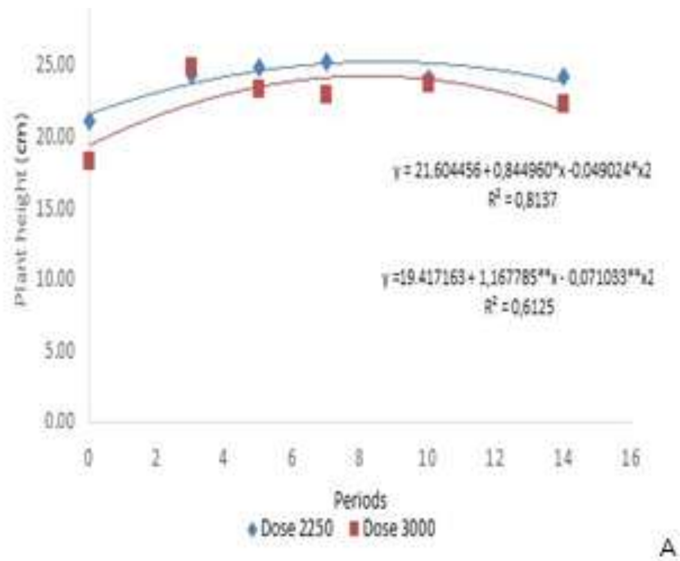


Figure 2. Phytointoxication. Treatments interaction with doses over the periods (a) and treatments interaction in the periods between sowing and spraying, according with the increasing doses (b). ***, **, *: significant a 01, 1 e 5% respectively.

which it was observed that the highest phytotoxicity values were during periods closer to soybean seeding, leading to phytotoxic effects to the crop as a result of residues of this herbicide in the medium texture soil.

Regarding periods, according to each dose, the effect was similar for 0 days before sowing, regardless of the dose used. That is, the shorter the period between spraying and sowing, higher was the appearance of symptoms, as shown in Figure 2b. Injuries increased according with the dose increases at 0 days before



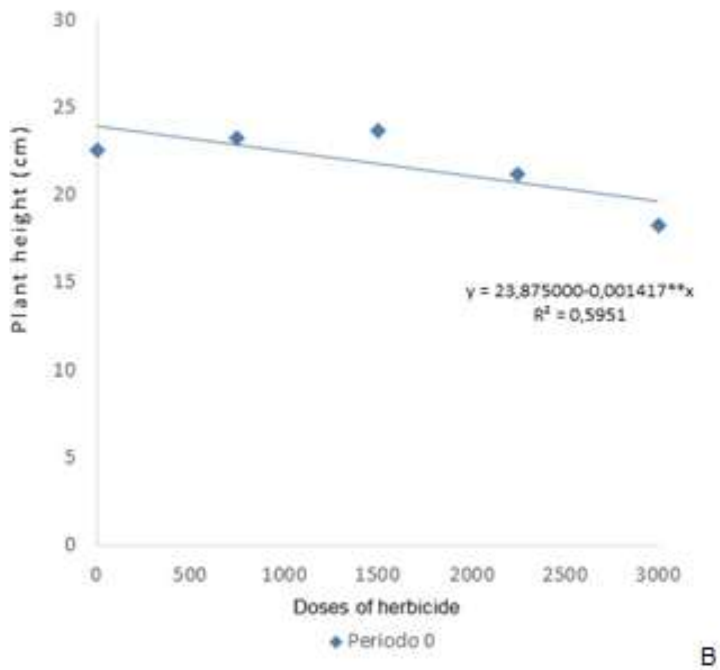


Figure 3. Plant height: Treatments interaction with doses over the periods (a) and treatments interaction in the periods between sowing and spraying, according with the increasing doses (b). ***, **, *: significant a 01, 1 e 5% respectively.

sowing, and as the dose was increased, higher were the symptoms. Similar behavior was observed at 5, 7, 10 and 14 days before sowing. Some studies have reported 2,4-D phytotoxicity in several cultures, alone or mixed with other herbicides, as observed by Nascimento and Yamashita (2009). The authors observed phytotoxicity of species that were sown in a substrate with the mixture of 2,4-D and other herbicides. Similar results were observed in 2,4-D pre-emergence application by Reis (2010), in which there was higher phytotoxicity as the doses increased, causing negative effects on plants.

In the plant height parameter (Figure 3), the only statistically significant treatments were the highest doses (2250 and 3000 e. a. ha^{-1}), and the only application

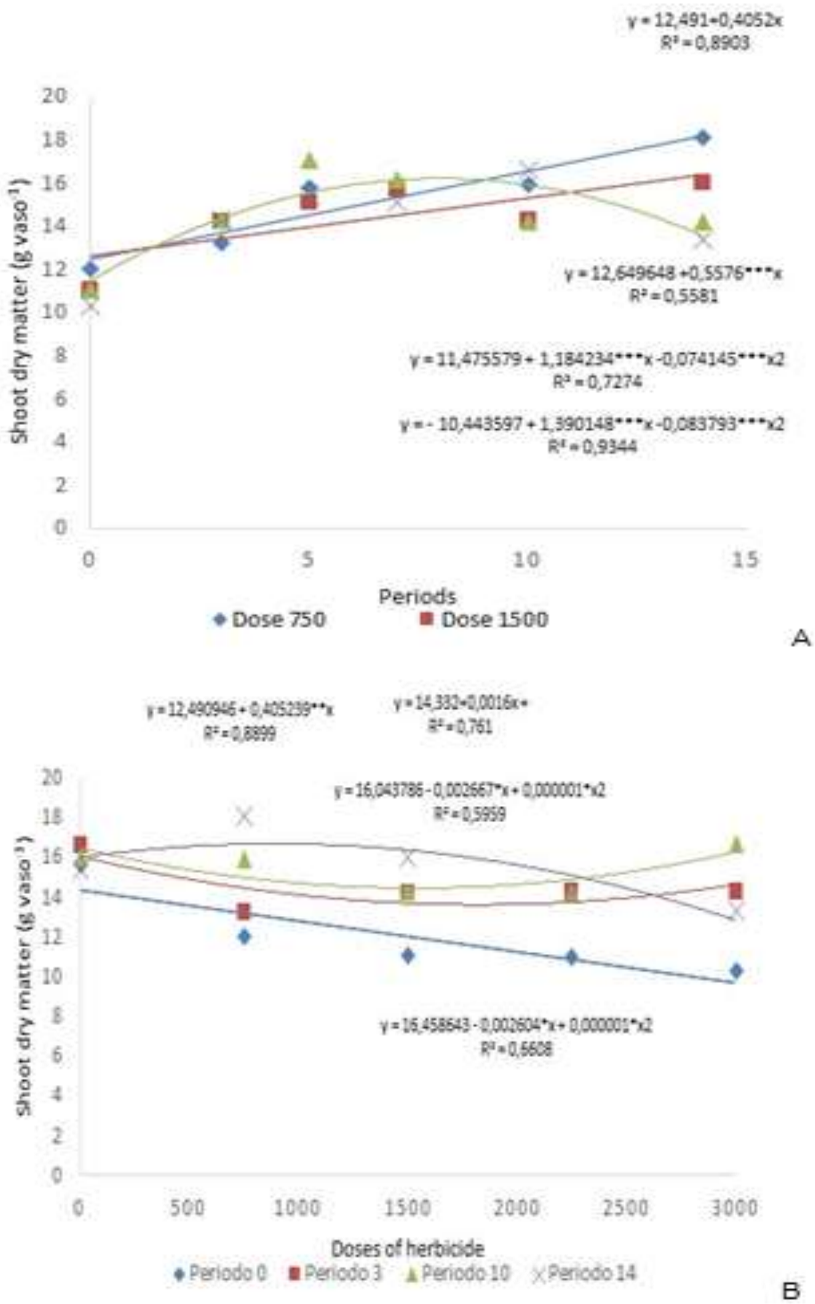


Figure 4. Shoot dry matter. Treatments interaction with doses over the periods (a) and treatments interaction in the periods between sowing and spraying, according with the increasing doses (b). ***, **, *: significant a 01, 1 e 5% respectively.

interval that had lower plant height was at 0 days before sowing. From the following periods (3, 5, 7, 10 and 14 days before sowing), the herbicide mixture did not cause harm to plant height (Figure 3a). Santos et al. (2013) observed plant height decrease in plants that are sensitive to auxinic herbicides, with this variable being inversely proportional to the increase in the dose of the 2,4-D herbicide, in a short interval between product application and seeding. 2,4-D herbicide is of short persistence in the soil, allowing the sowing of crops that are susceptible to it two weeks after application (Rodrigues and Almeida, 2011).

In the study of Silva et al. (2011), all application periods showed reduced plant height. Shoot dry matter (Figure 4) achieved better increases in more distant periods between spraying and seeding (Figure 4a). The 0 dose,

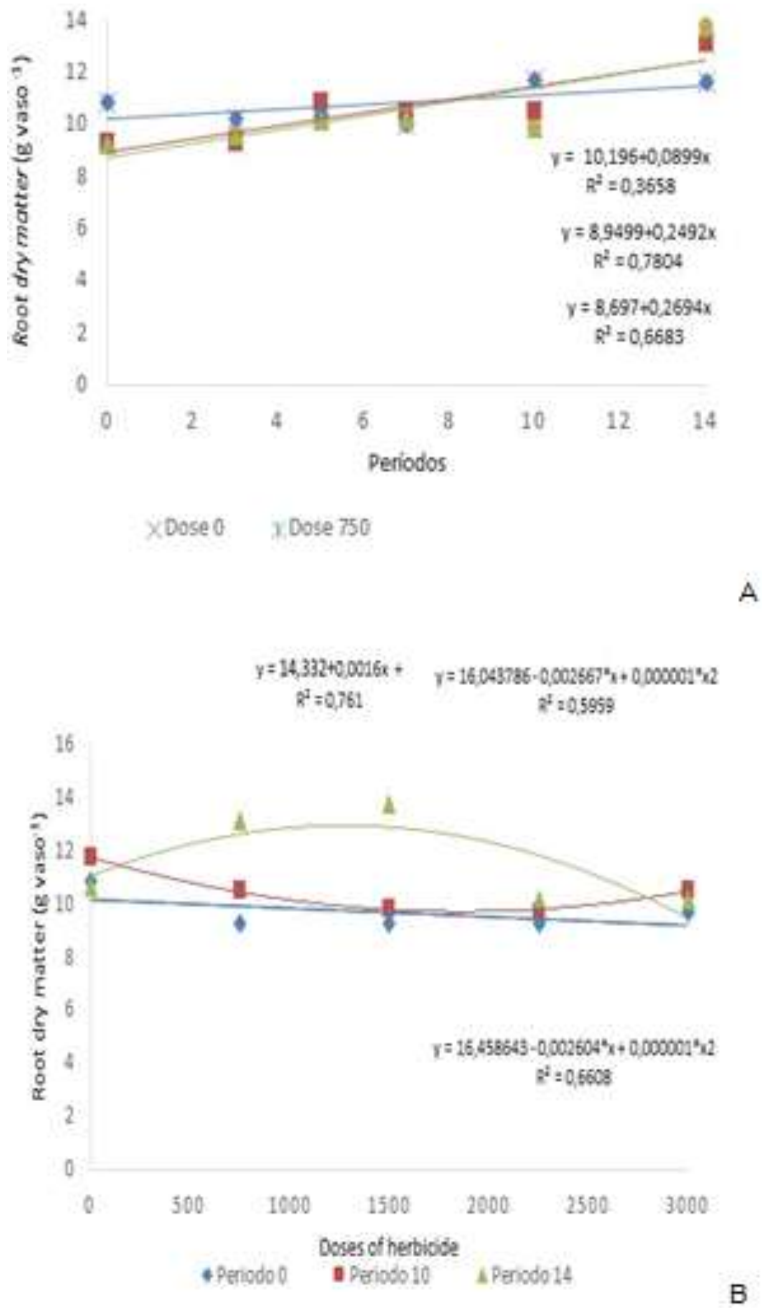


Figure 5. Root dry matter. Treatments interaction with doses over the periods (a) and treatments interaction in the periods between sowing and spraying, according with the increasing doses (b). ***, **, *: significant a 01, 1 e 5% respectively.

without herbicides mixture, performed better, followed by the lowest dose (0.750 g e. a. ha^{-1}). Mixture highest doses (2250 and 3000 g e. a. ha^{-1}) showed better development in the periods of 5 and 10 days before seeding, respectively.

The assessment of doses applied over the days (Figure 4b) showed that the lowest dry matter index occurred at 0 days before sowing. That is, the closer spraying and seeding are, the lower the crop development. The highest shoot dry matter increase occurred at 14 days before sowing. Similar results were obtained by Reis (2010), who observed that 2,4-D doses increase gradually reduced shoot green and dry matter development in corn crops. In the root dry matter parameter (Figure 5), days between each applied dose (Figure 5a) showed that root dry matter was influenced by the distance between seeding and herbicides spraying in both applied doses. The highest number of days between spraying and seeding provided better root development, which is reflected on their weight. Thus, the root system has undergone lower development at shorter herbicide application intervals and increased root growth at 14 days before sowing.

2,4-D and Glyphosate herbicides have deleterious effect on soyben as bioindicator plants, influencing in the reduction of plant dry biomass, height and germination percentage due to their persistence in the soil. Similar results were found by Silva et al. (2011), where dry matter biomass was reduced in all treatments, with the lowest values being observed at 0 days before sowing. There was no statistical difference for 2250 and 3000 doses regarding application periods.

In the doses applied throughout the days (Figure 5b), there was no statistical difference to the doses at 3, 5 and 7 days before sowing. With respect to 0 days before sowing, the lowest herbicide dose could cause low root development. In the interval of 14 days before sowing, there was product dose increase in periods that were more distant from application.

Conclusion

Glyphosate and 2,4-D residual effect is more evident in soybean plants, as the interval between application and sowing decreases. Increased doses act negatively on crop development, providing higher phytotoxic effect and influencing all other parameters. Desiccation with 2,4-D and Glyphosate close to soybean sowing causes phytotoxic effects to the crop and dry biomass reduction due to the residue of this herbicide in the soil

Conflicts of interest

The authors have not declared any conflict of interest.

REFERENCES

Agência Nacional de Vigilância Sanitária (2013). Programa de Análise de Resíduos de Agrotóxicos em Alimentos (PARA). Relatório de Atividades de 2011 e 2012. Brasília: Agência Nacional de Vigilância Sanitária.

Aprosoja (2013). Aprosoja Mato Grosso. Available at: http://www.aprosoja.com.br.

Ash C, Jasny BR, Malakoff DA, Sugden AM (2010). Feeding the future. Science 327:797.

Ashton FM, Crafts AS (1973). Mode of action of herbicides. New York: John Wiley. 504p.

Bonfim-Silva EM, Silva TJA, Cabral EA, Kroth BE, Rezende D (2011). Desenvolvimiento Inicial de Gramíneas Submetidas ao Estresse Hídrico. Rev. Caatinga Mossoró 24(2):180-186.

Carpenter JE (2010). Peer-reviewed surveys indicate positive impact of commercialized GM crops. Nature Biotechnol. 28:319-21.

Chiras DD (1995). New Visions of Life Evolution of a Living Planet. In: Environmental Science: action for a sustainable future. 3. ed. San Francisco: Benjamin Cummings.

Companhia Nacional de Abastecimento (Conab) (2015). Acompanhamento da safra brasileira grãos. ISSN 2318-6852. 1(4) - Safra 2014/15 quarto Levantamento, Brasília. pp. 1-95.

Cunha JPAR, Teixeira MM, Vieira RF, Fernandes HC (2005). Deposição e deriva de calda fungicida aplicada em feijoeiro, em função de bico de pulverização e de volume de calda. Rev. Bras. Engenharia Agríc. Ambiental. Campina Grande, PB. 9(1):133-138.

Empresa Brasileira de Pesquisa Agropecuária, EMBRAPA (2013). Sistema brasileiro de classificação de solos. Brasília 3:353.

Instituto Brasileiro do Meio Ambiente e do Recursos Naturais Renováveis – IBAMA (2009). Available at: http://www.ibama.gov.br/phocadownload/Qualidade_Ambiental/produtos_agrotoxicos_comercializados_brasil_2009.pdf.

Klinkers L (1997). Classics in Environmental Studies: an overview of classic texts in environmental studies. Utrecht: International Books. 10:143-152.

Ministério da Agricultura, Pecuária e Abastecimento (MAPA) (2010). Projeções do Agronegócio: Brasil 2009/ 2010 a 2019/2020. Brasília: MAPA. Available at: http://www.agricultura.gov.br/arq_editor/file/MAIS%20DESTAQUES/Proje%C3%A7%C3%B5es%20Agroneg%C3%B3cio%202009-2010%20a%202019-2020.pdf

Mortensen DA, Egan JF, Maxwell BD, Ryan MR, Smith RG (2012). Navigating a critical juncture for sustainable weed management. Bioscience 62(1):75-84.

Nascimento ER, Yamashita OM (2009). Desenvolvimento inicial de olerícolas cultivadas em solos contaminados com resíduos de 2,4-d + picloram. Semina. Ciências Agrárias Londrina 30(1):47-54.

Pacheco MEL (2012). Os impactos dos agrotóxicos na Segurança Alimentar e Nutricional: Contribuições do Consea. Conselho Nacional de Segurança Alimentar e Nutricional. (CONSEA). 20p.

Rebelo RM, Vasconcelos RA, Buys BDMC, Rezende JA, Moraes KOC, Oliveira RP (2010). Produtos agrotóxicos e afins comercializados em 2009 no Brasil: uma abordagem ambiental, Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis – Brasília: IBAMA,.

Reis TC, Tiago de Souza Santos T, Alexandro Pereira Andrade AP, Neves AF (2010). Efeitos de fitotoxidade do herbicida 2,4-D no milho em aplicações pré e pós-emergência. Rev. Biol. Ciênc. Terra 10(1):1-5.

Saad O (1978). A vez dos herbicidas. 2.ed. São Paulo: Nobel. 267p.

Santos DP, Braga RR, Guimarães FAR, Passos ABRJ, Silva DV, Santos JB, Nery MC (2013). Determinação de espécies bioindicadoras de resíduos de herbicidas auxínicos Rev. Ceres Viçosa 60(3):354-362.

Silva FML, Cavalieri SD, José AR, Ulloa SM, Velini ED (2011). Atividade residual de 2,4-D sobre a emergência de soja em solos com texturas distintas. Rev. Bras. Herb. 10(1):29-36.

Sociedade brasileira da ciência das plantas daninhas (SBCPD) (1995). Procedimentos para instalação, avaliação e análise de experimentos com herbicidas. Londrina: SBCPD.

Takano HK Oliveira Jr RS, Constantin J, Biffe DF, Franchini LHM, Braz GBP, Rios FA, Gheno EA, Gemelli A (2013). Efeito da adição do 2,4-D ao glyphosate para o controle de espécies de plantas daninhas de difícil controle. Revista Bras. Herbicidas 12(1):1-13.

Weed science (2014). Weeds Resistant to EPSP synthase inhibitors (G/9) by species and country. Available at: http://weedscience.org/summary/moa.aspx?MOAID=12

White Jr L (1997). The Historical Roots of Our Ecological Crisis. In: NELISSEN, N.; STRAATEN, J.V. der; KLINKERS, L. (Ed.). Classics in Environmental Studies: an overview of classic texts in environmental studies. Utrecht: International Books. cap. 10:143-152.

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Full Length Research Paper

Degradation of reserves of crambe seed (*Crambe abyssinica*) during germination

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The aim of this study was to determine how lipids, total soluble sugars and starch are degraded during the germination process of crambe seeds (FMS Brilhante Cultivar). The experiment consisted of collecting seeds during every day of the germination test (0, 1, 2, 3, 4, 5, 6 e 7 days) during which were also held the germination counts. After collection, the proportion of seeds and / or seedlings was designed for the determination of water content, and the other part was separated to perform biochemical analysis. The study adopted a completely randomized design with four replications and the means were compared by regression analysis. Crambe seed reserves showed degradation since the metabolic activation with increased germination in that lipid, soluble sugars, and starch are degraded.

Key words: Lipids, total soluble sugars, starch.

INTRODUCTION

The crambe (*Crambe abyssinica* Hochst) belonging to the Brassicaceae family, originated from the Mediterranean region with occurrence in Ethiopia, and being recently introduced in Brazil as an alternative to forage and off-season crop production (Colodetti et al., 2012). The plant has a relatively short cycle of 90 to 120 days, requiring approximately 52 days between sowing and flowering, considerable tolerance to water stress, soil and saline irrigation water and also resistance to low temperatures (Pitol et al., 2010) and productivity of 1428,98 kg ha^{-1} (Brandão et al., 2013). The industrial use of crambe oil is suggested to be a raw material for biodiesel production because their seeds have oil content of up to 38% (Pitol et al., 2010) and features such as kinematic viscosity, density and acid value are suitable for biodiesel production (Silva et al., 2013).

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The crambe oil extracted from the seed may be used as a lubricant in the manufacture of plastic films and in the drug composition. The crambe oil cannot be used for human consumption due to the presence of high erucic acid content, a long chain monounsaturated fatty acid, which causes damage to the heart when present in the human body (Colodetti et al., 2012).

In addition to the purely industrial point of view, it should be considered that the yield obtained in the industries is potentially dependent on the quality and yield of plants. Thus, the germination of this kind becomes important to any industrial process which is intrinsically associated with the mode degradation of its reserves. The main substance stored in crambe seeds are lipids, but carbohydrates and proteins are also found into smaller proportions (Oliva et al., 2012).

During germination, reservations are hydrolyzed and mobilized to embryo growth (Kucera et al., 2005). Lipids and carbohydrates are used as an energy source (Pritchard et al., 2002) and protein to provide amino acids for the formation of new tissues (Ramakrishna, 2007).

Plants need to convert the more lipids stored in a mobile form of carbon, often as sucrose, because they are not able to carry fats cotyledon to other tissues during development of the seedlings (Taiz and Zeiger, 2013). According to Graham (2008) oilseeds metabolize the triacylglycerols stored, converting them to sucrose following germination. The conversion of sucrose to lipid in oil seed germination is initiated by starting with the hydrolysis of stored triglycerides lipids as free fatty acids, and subsequent oxidation of these fatty acids to produce acetyl-CoA. Fatty acids are oxidized in glioxissomo, and acetyl-CoA is metabolized in glioxissomo and cytoplasm to produce succinate, which is transported to mitochondria and converted to fumarate and then malate, and cytosolic malate is converted to glucose via gluconeogenesis and then as sucrose (Taiz and Zeiger, 2013).

Considering the importance of the degradation of reserves during the germination of seeds, the aim of this study was to determine how lipids, total soluble sugars and starch are degraded during the germination process of crambe seeds.

MATERIALS AND METHODS

Installation, experimental design and data analysis

The seeds were purchased from the Mato Grosso do Sul Foundation. The experiment was conducted in the Germination, Seed Dormancy and Plant Physiology Laboratory II, of the Department of Botany, Biosciences Institute, UNESP – Botucatu / SP. The experiment consists of seeds collection during every day of the germination test (0, 1, 2, 3, 4, 5, 6 and 7 days) during which the germination counts were also held. Four replications of 50 seeds were placed for each treatment. After collection, the proportion of seeds and/or seedlings was designed for the determination of water content, and the other part was separated to perform biochemical analyzes.

A completely randomized design was adopted with four replications and the means were compared by regression analysis ($p \leq 0.05$).

Seed moisture content

The water content was determined by the oven method at 105 ± 3°C for 24 h, using three replicates of 4.5± 0.5 g. The results were expressed on a wet basis (Brasil, 2009).

Germination

The germination test was conducted with four replications of 50 seeds for each treatment. The seeds were placed on blotting paper, moistened in water for 2.5 times the mass of dry substrate, packed in transparent plastic boxes (11 x 11 x 3.5 cm). They were then placed on germination B.O.D. in alternating temperature and photoperiod (light - 30°C for 8 h and dark - 20°C for 16 h).

Total soluble sugars

The extraction of total soluble sugars was carried out according to the methodology of Garcia et al. (2006) with minor modifications. The seeds were pulverized with a pestle and mortar in N_2 liquid. To obtain the alcoholic extract, 100 mg of the pulverized seeds were homogenized in 1 mL of 80% ethanol (v/v) and incubated for 15 min at 80°C. The homogenate was centrifuged at 12,000 *g* for 15 min at room temperature. At the end of centrifugation, the supernatant was removed and reserved. This procedure was performed three times for the complete removal of total soluble sugars, combining the supernatants from the three extractions at the end. Then, the final volume was adjusted to 3 ml with deionized water resulting in the alcoholic extract. These extract was stored in separate microtubes at -20°C until determination. The pellets were stored at -20°C for further starch extraction.

For the determination of total soluble sugars, the methodologies used were taken from Morris (1948) and Yemm and Willis (1954). The anthrone reagent was prepared dissolving 0.1 g of anthrone in 45 mL of sulfuric acid 95% (v/v). The reaction mixture consisted of 50 µL of alcoholic extract + 950 µL deionized water (final volume 1000 µL), kept in an ice bath, and 2000 µL of cold anthrone solution was added. The reaction mixture was incubated for 3 min at 100°C. After cooling, the total sugar content was determined by taking the absorbance at 620 nm using glucose as a standard and was expressed as mg per mg of dry weight.

Starch

For extraction of starch, the study followed the the method described by Clegg (1956) with minor modifications. The pellet, derived from the alcoholic extract, was homogenized in 500 µL of deionized water, in an ice bath. To the homogenate was added 650 µL of 52% perchloric acid (v/v) and kept in an ice bath for 15 min (shaken every 5 min). Then, 2000µL of deionized water was added and centrifuged at 12,000 *g* for 15 min at 4°C.

At the end of centrifugation, the supernatant was removed and reserved. This procedure was performed again, however the mixture containing pellet + deionized water + 52% perchloric acid (v/v) was kept in an ice bath for 30 min (shaken every 5 min) and centrifuged at 12,000 *g* for 15 min at 4°C. The two supernatants were combined at the end. These extract was stored in separate microtubes at -20°C until determination of starch content. The determination of starch content was performed like the total sugar determination.

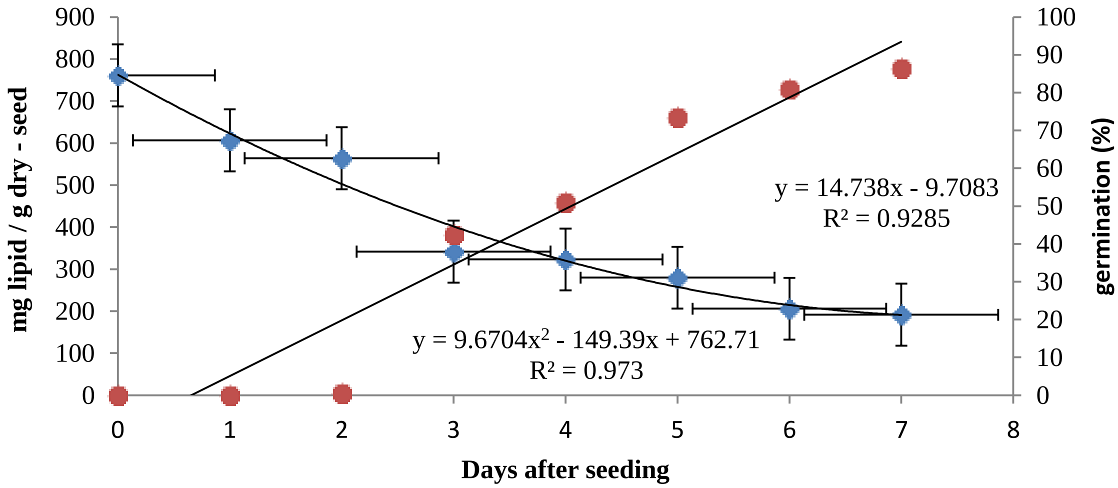

Figure 1. Degradation of lipids during germination crambe seeds ($p \leq 0.05$).

Total lipid

The quantification of total lipids was performed according to the method of Manirakiza et al. (2001) and Ambalkar et al. (2011). To obtain the extract, about 1.0 to 2.0 grams of sample soaked in liquid nitrogen was weighed. In these samples, 100 ml of hexane was added, arranged in flat-bottom flasks and placed in fatty material extract in 3 cycles of 8 h. Subsequently, the material was filter while hot, and brought to a rotary evaporator for separation of solvent and lipids. Lipids were removed from the flasks with the aid of a Pasteur pipette, transferred to glass jars with lid, and subsequently weighed.

RESULTS AND DISCUSSION

The results of germination of crambe seed, obtained during the 7-day evaluation and the degradation reservations are presented in tables. It can be seen that seed germination process started on the third day of evaluation.

The data relating to lipid content in the seeds set to a decreasing quadratic regression equation, indicating that there has been rapid consumption of reserves during the germination period from the first day after the metabolic activation (Figure 1).

In *Cucumis sativus* L. the degradation of lipids started on the 2nd day after germination, leaving only 3% of total initial six days. This rapid degradation begins with the emergence of the radicle and ends with the complete expansion of the cotyledons (Matsui et al., 1999). However, according Suda & Giorgini (2000) this pattern is unusual when compared to other oil seeds, in which the lipid content remains unchanged during the initial period of germination, diminishing. It was observed in *Arabidopsis thaliana* (L.) that the degradation of lipid reserves is inhibited in the presence of soluble sugars such as glucose and sucrose, and in general stemmed starch metabolism (To et al., 2002).

The total soluble sugar seeds also exhibit marked reduction during the initial stage of germination, during imbibition (Day 1), indicating their use in breathing, with subsequent stabilization of cotyledons between 3 and 6 days (Figure 2) and decrease in the seventh day. Borges et al. (2002) and Buckeridge and Dietrich (1996) verified the consumption of sucrose and raffinose during germination and *Sesbania marginata, Platymiscium pubescens*, respectively, considering the first two reservations of soluble sugars to be used. The data relating to starch content in the seed set to a downward quadratic regression equation indicates the intake and mobilization of reserves during the germination period (Figure 3). According to Magalhaes et al. (2010), starch provides glucose to be used both as air for breath, to generate electricity, and to compose physical structures for embryo growth during germination phase.

In summary, crambe seed germination lipids, soluble sugars and starch are degraded quickly providing energy for the development of the embryo during germination, which is completed at 7 days after metabolic activation, when observing the emission of the primary root. Thus, while lipid reserves are considered slow in degradation, crambe seed process starts from the moment the seeds are placed in contact with water and the metabolism is activated.

Conclusion

Crambe seed reserves had degradation since the metabolic activation with increased germination in that lipid, soluble sugars and starch are degraded.

Conflicts of interest

The authors have not declared any conflict of interest.

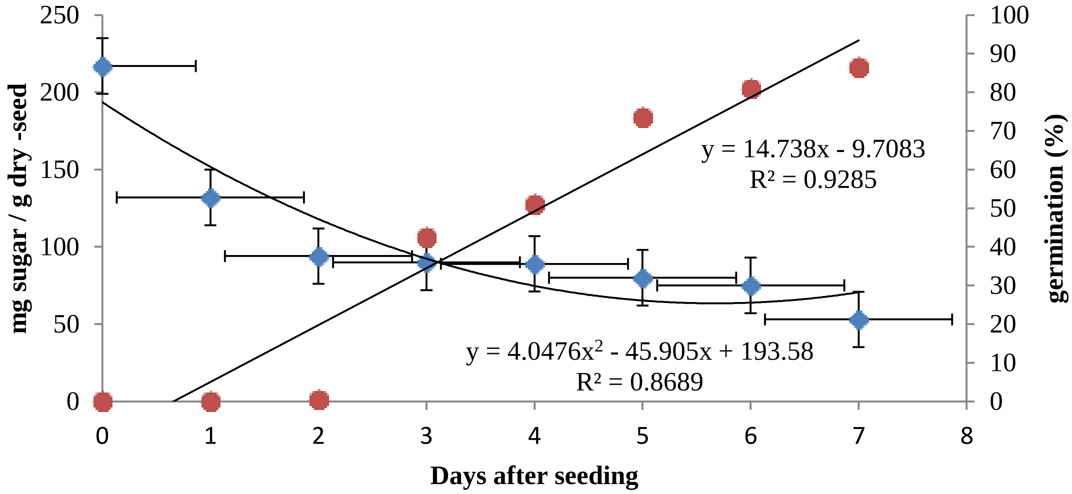

Figure 2. Degradation of total soluble sugar during germination crambe seeds (p≤0.05).

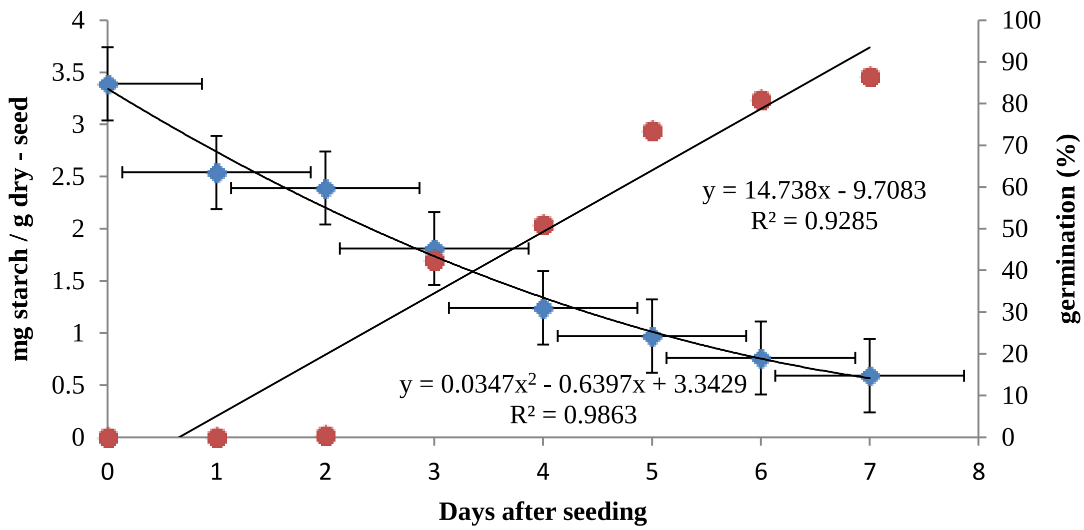

Figure 3. Degradation of starch during germination crambe seeds (p≤0.05).

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REFERENCES

Ambalkar VU, Sapkal VS, Talib M, Khandelwal SA (2011). Soxhlet extraction of Neem seed (*Azadirachta indica A.* Juss) using hexane as a solvent. Int. J. Chem. Anal. Sci. 2:2.

Borges EEL, Borges RCG, Soares CPB, Perez SCJGA (2002). Crescimento e mobilização de carboidrato em embrião de sementes de fedegoso (Senna macranthera Irwin et Barneby) durante a germinação. Cerne. Lavras 8(1):69-76.

Brandão FJB, Silva ARB, Silva MAP, Sperotto FCS (2013). Desempenho operacional e produtividade agrícola do crambe nos preparos convencional e reduzido de solo. Enciclopédia Biosfera. Centro Científico Conhecer - Goiânia 9:17.

Brasil (2009). Ministério da Agricultura, Pecuária e Abastecimento. Regras para análise de sementes. 399p.

Buckeridge MS, Dietrich SMC (1996). Mobilization of the raffinose family oligosaccharides and galactomannan in germinating seeds of Sesbania marginata Benth (Leguminosae Faboideae). Plant Sci. Amsterdan 117:33-43.

Clegg KM (1956). The application of anthrone reagente to the estimation of starch in cereals. J. Sci. Food Agric. 7(1):40-44.

Colodetti TV, Martins LD, Rodrigues WN, Brinate SVB, Tomaz MA (2012). Crambe: Aspectos Gerais da Produção Agrícola. Enciclopédia Biosfera, Centro Cientifico Conhecer, Goiânia 8(14):258-269.

Garcia IS, Souza A, Barbedo CJ, Dietrich SMCE, Figueiredo-Ribeiro RCL (2006). Changes in soluble carbohydrates during storage of *Caesalpinia echinata* Lam. (Brazilwood) seeds, an endangered leguminous tree from the brazilian atlantic forest. Braz. J. Biol. 66(2B):739-745.

Graham IA (2008). Seed storage oil mobilization. Ann. Rev. Plant Biol. 59:115-42.

Kucera B, Cohn MA, Leubner-Metzger G (2005). Plant hormone interactions during seed dormancy release and germination. Seed Sci. Res. Wallingford 15:281-307.

Magalhaes SR, Borges EEL, Berger APA (2010). Mobilização de reservas no eixo embrionário e nos cotilédones de sementes de *Schizolobium parahyba* (vell.) *S. f. blake* durante a germinação. Ciência Florestal Santa Maria 20(4):589-595.

Manirakiza P, Covaci A, Schepens P (2001). Comparative study on total lipid determination using Soxhlet, Roese-Gottlieb, Bligh & Dyer, and modified Bligh & Dyer extraction methods. J. Food Composit. Anal. 14(1):93-100.

Matsui K, Hijiya K, Tabuchi Y, Kajiwara T (1999). Cucumber Cotyledon Lipoxygenase during Postgerminative Growth. Its Expression and Action on Lipid Bodies. Plant Physiol. 119:1279-1287.

Morris DL (1948). Quantitative determination of carbohydrates with dreywood's anthrone reagent. Science 107(2775):254-255.

Oliva ACE, Biaggioni MAM, Cavariani C (2012). Efeito imediato do método de secagem na qualidade de sementes de crambe. Energ. Agric. Botucatu. 27(3):16-30.

Pitol C, Broch DL, Roscoe R (2010). Tecnologia e produção: crambe 2010. Fundação MS, Maracajú. Fundação MS. 60p.

Pritchard SL, Charlton WL, Baker A, Graham IA (2002). Germination and storage reserve mobilization are regulated independently in *Arabidopsis*. Plant J. 31(5):639-647.

Ramakrishna V (2007). Mobilization of albumins and globulins during germination of Indian bean (Dolichos lablab L. var. lignosus) seeds. Acta Bot. Croat. 66(2):135-142.

Silva MAP, Biaggioni MAM, Sperotto FCS, Bezerra PHS, Brandão FJB (2013). Qualidade do oleo bruto de grãos de crambe (*Crambe abyssinica* Hochst) sob diferentes métodos de secagem. Energ. Agric. Botucatu. 28(3):193-199.

Taiz L, Zeiger E (2013). Fisiologia Vegetal. 5. Ed. Porto Alegre. Artmed.

To JPC, Reiter W, Gibson SI (2002). Mobilization of seed storage lipid by Arabidopsis seedlings is retarded in the presence of exogenous sugars. BMC Plant Biol. 2:4-15.

Yemm EW, Willis AJ (1954). The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. 57(3):508-514.

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Full Length Research Paper

Water use efficiency of sunflower genotypes under drip irrigation

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This investigation was conducted to determine the productivity and water use efficiency (WUE) for new sunflower genotypes obtained from selfing and induced mutation. Ten sunflower genotypes were evaluated under drip irrigation using two treatments of irrigation (100 and 75% from water requirement of sunflower). Statistical analysis showed that there were significant differences among genotypes, as well as between irrigation treatments and the interaction between them. Results indicate that decreasing the amount of irrigation water from 1500 to 1130 (mm/ha) significantly reduced all studied traits. Mutation ($M_{2,1}$-63) surpassed all the other genotypes in seed yield and WUE. Lines which gave the highest yield of the seed have WUE under drought conditions higher than WUE under normal irrigation. The lowest depression in seed yield due to drought conditions compared to the seed yield under normal irrigation has been registered for Line 20, Line $M_{2,1}$-63, and Sakha 53 genotypes (11, 18, and 16%, respectively), but the highest depression recorded for Line 48, Line $M_{2,3}$-63, and Line $M_{2,4}$-63 (49, 46, and 43%, respectively). The genotypes (Line 20, Line $M_{2,1}$-63 and Sakha 53) are more tolerant to drought than others and we can used its in breeding program to develop sunflower hybrids suitable for cultivation under drought condition.

Key words: Sunflower genotypes, water use efficiency, mutation, inbred lines.

INTRODUCTION

Available water is a limited factor for the expansion of agriculture in the desert which occupied about 93% of the total area in Egypt. The development of new genotypes of crops having the ability to be tolerant to drought is the useful way to expand in the desert cultivation using modern irrigation systems.

Sunflower (*Helianthus annuus* L.) is one of the four most important oil crops in the world (Demir et al., 2006). Because of its moderate cultivation requirements and high oil quality, its acreage has increased in both developed and developing countries (Skoric, 1992). In Egypt, great emphasis must be given towards this crop to decrease the gap in oil production. Sunflower oil is highly demanded not only for human consumption, but also for chemical and cosmetic industries. In respect of total yield produced, water requirements of sunflower are relatively high as compared to most crops. Despite its high water use, the crop has the ability to withstand short periods of

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Table 1. The genotypes evaluated in this investigation.

Genotype	Source
Line 20	S_5 (selfing of Maiak variety)
Line 48	S_5 (selfing of Maiak variety)
Line 61	S_5 (selfing of Maiak variety)
Line 63	S_5 (selfing of Maiak variety)
$M_{2,1}$-63	Mutation from Line 63
$M_{2,2}$-63	Mutation from Line 63
$M_{2,3}$-63	Mutation from Line 63
$M_{2,4}$-63	Mutation from Line 63
Maiak	Parent of above genotypes
Sakha 53	Commercial variety (as a check variety)

severe soil water deficit of up to 15 atmosphere tensions. Long periods of severe soil water deficit, particularly, at water-sensitive growth stages cause significant reduction in seed yield (Beyazgul et al., 2000) by limiting evapotranspiration (ET) through stomata closure, reduced assimilation of carbon and decreased biomass production (Demir et al., 2006).

It has been shown that, sometimes, periods of reduced growth may trigger physiological processes that actually increase yield (Smith et al., 2002). Severe water deficits during the early vegetative growth result in reduced plant height, but may increase root depth. Adequate water during the late vegetative period is required for proper bud development. The flowering period is the most sensitive to water deficits which cause considerable yield decrease since fewer flower come to full development (Beyazgul et al., 2000; Ali and Shui, 2009). Seed formation is the next most sensitive period to water deficit, causing severe reduction in both yield and oil content (Doorenbos and Kassam, 1979). Although sunflower is known to be a drought tolerant crop or grown under dry land conditions, substantial yield increases can be achieved by supplementary irrigation, which is one of the most effective strategies to mitigate the effects of dry spells in crop production (Fox and Rockstrom, 2000; Xiao et al., 2007).

The objective of this study was to determine the productivity and water use efficiency (WUE) for new sunflower genotypes obtained from selfing and induce mutation.

MATERIALS AND METHODS

This work started in 2009 at the Farm of Plant Production Department, El-Kufra City, Garyonus University, Libya by planting the open pollinated cultivar "Maiak" (*H. annuus* L.) on 20th March, 2009. A total of 500 plants were grown in a non-replicated experiment in rows set of 60 cm apart with 20 cm between plants within a row. Just before flowering, the 100 plant were selected, selfed, and tagged. After harvest, the best (S_1) heads were saved and regrown in a non-replicated experiment on 1st July, 2009 to obtain S_2 achene. Selfing and selection were repeated twice in 2010 and 2011 until the S_5-generation. Four inbred lines (S_5) were thus recovered from the open pollinated cultivar "Maiak", namely (Line 20, Line 48, Line 61, and Line 63). In addition, four new mutants, namely, $M_{2,1}$-63, $M_{2,2}$-63, $M_{2,3}$-63, and $M_{2,4}$-63 were obtained from treating the seeds of inbred line-63 (S_3) with gamma rays (Co^{60} source) at three doses, namely, 70, 100, and 150 Gy and the seeds were planted in the field a day after irradiation to obtained M_1-63. The phenotypical observations and biometrical measurements were made during the vegetation period. Part of the plants, produced from treatment with gamma rays were isolated in paper bags and self-pollinated in order to obtain M_2. The four inbred lines, four new mutant forms along with the base population Maiak, and a check cultivar Sakha-53 were evaluated under drip irrigation using two treatments of irrigation (100 and 70% from water requirement of sunflower). The experiment was conducted in Randomized Complete Block Design using split-plot arrangement with three replications at EL Wady EL Assiuty Farm, Faculty of Agriculture, Assiut University where the soil is sandy calcareous. Drip irrigation was used in this farm. Irrigation has been placed in the main plot and genotypes in the sub plot. Date of planting was on 13th June, 2012. The experiment unit was one row, 5 m long, and set 50 cm apart with 25 cm distance between plants within a row. Days to 50% flowering were recorded on plot mean basis. At harvest, ten random plants/plot from each genotype were collected to record the following traits: plant height (cm), head diameter (cm), achene yield/plant (g), and weight of 100 seed (g).

Seed oil content was determined by Soxhlet apparatus using petroleum ether (40 to 60°C) as a solvent according to AOAC (1995). Seed yield (kg/ha) calculated from all plants in row then WUE value was calculated according to the equation:

$$WUE(kglmm) = -\frac{Seed\ yield\ \ (kg/ha\ )}{Applied\ \ water\,(mm/ha)}$$

Genotypes and irrigation treatments

Ten genotypes of sunflower were evaluated under two treatments of irrigation: (1) I_1 = Application of 100% from water requirements (1500 mm/ha)/season; (2) I_2 = Application of 75% from water requirement (1130 mm/ha)/season (Table 1).

All other agronomic practices were done according to the recommendations for growing sunflower production in Egypt under sandy soil. Statistical analysis was done according to Gomez and Gomez (1984) for all of the studied traits.

Table 2. Studied traits for sunflower genotypes under normal and drought conditions.

Genotypes	Plant height (cm)			Head diameter (cm)			Achene yield (g/plant)			100-Achene weight (g)		
	Irrigation		Mean	Irrigation		Mean	Irrigation		Mean	Irrigation		Mean
	1_1	I_2		1_1	I_2		1_1	I_2		1_1	I_2	
Line 20	184.0	179.0	181.5	17.7	16.3	17.0	41.0	35.3	38.2	7.3	6.7	7.0
Line 48	139.0	130.7	134.8	16.3	13.3	14.8	42.3	31.0	36.7	7.9	6.6	7.3
Line 61	171.7	160.0	165.8	17.3	14.7	16.0	43.0	35.3	39.2	7.5	7.1	7.4
Line 63	100.0	75.0	87.5	7.0	5.7	6.3	9.7	7.3	8.5	3.0	2.7	2.9
$M_{2,1}$-63	160.0	158.3	159.2	18.0	15.7	16.8	44.7	36.3	40.5	6.2	5.7	6.0
$M_{2,2}$- 63	153.0	117.7	135.3	13.3	11.7	12.5	18.7	14.0	16.3	4.4	5.2	4.8
$M_{2,3}$-63	156.0	144.3	150.2	15.3	11.3	13.3	35.7	19.3	27.5	5.9	4.2	5.1
$M_{2,4}$-63	145.0	115.0	130.0	13.3	11.0	12.2	39.7	22.7	31.2	6.7	4.5	5.6
Sakha53	157.3	161.3	159.3	16.7	15.7	16.2	36.0	30.0	33.0	7.2	6.5	6.9
Maiak	168.3	161.7	165.0	17.7	16.3	17.6	35.0	33.3	34.2	7.5	6.8	7.2
Mean	153.4	140.3	-	15.3	13.2	-	34.6	26.5	-	6.4	5.6	-
$L.S.D_{0.05}$												
G	-	-	4.8	-	-	1.1	-	-	1.6	-	-	0.2
G×I	-	-	6.8	-	-	n.s	-	-	2.3	-	-	0.3

Genotype	Seed yield (kg/ha)			WUE (Kg/mm)			Oil (%)		
	Irrigation		Mean	Irrigation		Mean	Irrigation		Mean
	I_1	I_2		I_1	I_2		I_1	I_2	
Line 20	1721.7	1540.0	1630.8	1.15	1.35	1.25	42.3	34.3	38.3
Line 48	1779.7	908.0	1343.8	1.19	0.80	0.99	30.3	38.3	34.3
Line 61	1813.0	1148.0	1480.5	1.21	1.01	1.11	30.7	39.7	35.2
Line 63	405.7	310.3	358.0	0.27	0.27	0.27	45.3	44.0	44.7
M_1-63	1820.7	1488.0	1654.3	1.22	1.34	1.27	32.7	37.7	35.2
M_2- 63	784.0	588.0	686.0	0.52	0.52	0.52	39.7	36.7	38.2
M_3-63	1497.7	812.0	1154.8	1.00	0.71	0.86	45.3	30.0	37.7
M_4-63	1665.7	951.7	1308.7	1.11	0.83	0.97	39.7	40.7	40.2
Sakha53	1512.0	1267.3	1389.7	1.03	1.11	1.07	34.3	33.0	33.7
Maiak	1552.7	1228.0	1390.3	1.04	1.08	1.06	41.3	40.3	40.8
Mean	1455.3	1024.1		0.97	0.91		38.2	37.5	-
$L.S.D_{0.05}$									
G	-	-	125	-	-	0.08	-	-	0.8
G × I	-	-	177	-	-	0.11	-	-	1.2

RESULTS AND DISCUSSION

Statistical analysis showed that there were significant differences among genotypes, as well as between irrigation treatments and the interaction between them.

The results in Table 2 indicate that decreasing the amount of irrigation water from 1500 to 1130 mm/ha significantly reduced all studied traits. It seems evident that subjecting sunflower plants to water deficit, through reducing the amount of irrigation water reduced all growth parameters, probably due to impairing photosynthetic process which could have been decreased by the drastic decrease of leaf relative water content. In this concern, Khalilv and Yarnia (2007) reported that drought stress conditions increased stomatal resistance as a result of relative closing of stomata; consequently, this condition increases the total resistance of the given plant against its H_2O movement in comparison to CO_2. Afkari (2010) showed that the application of water deficit stress decreased significantly plant height, seed number per head, leaf water potential, leaf area index, leaf relative water content, stomatal resistance, and harvest index.

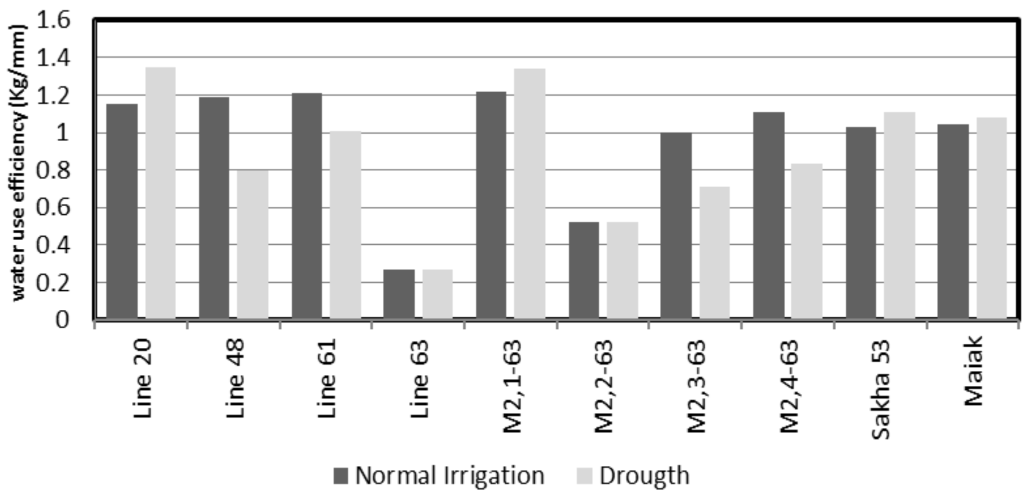

Figure 1. Water use efficiency for sunflower genotypes under drought and normal conditions.

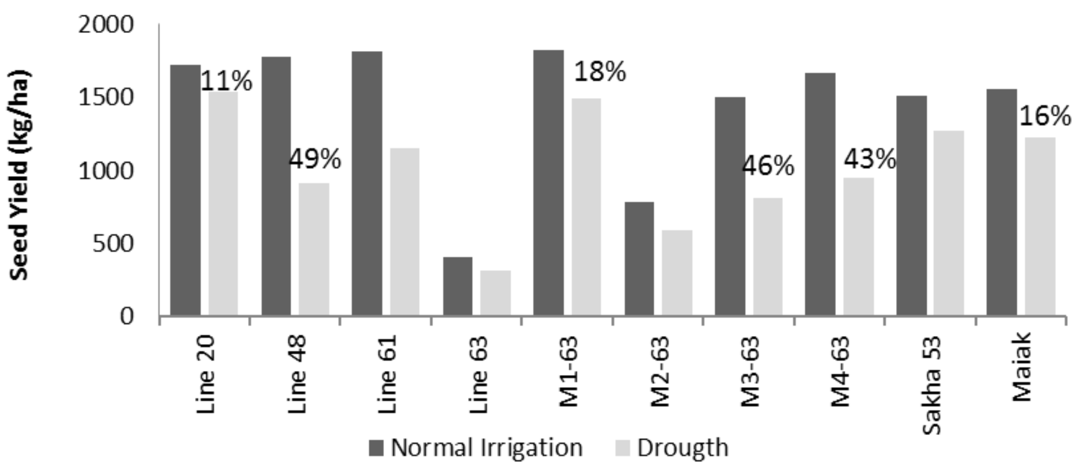

Figure 2. Seed yield (kg/ha) and percentage of yield shortage due to drought for sunflower genotypes under drought and normal conditions.

These results are in agreement with those obtained by Mehrpouyan et al. (2010), Kassab et al. (2012), and Öner et al. (2014).

The present data in Table 2 clearly show differences among the sunflower genotypes under study with respect to all studied traits. In this regard, Mutation ($M_{2,1}$-63) surpassed all the other genotypes in seed yield and WUE. The conspicuous differences among the genotypes suggest the presence of genetic differences and this illustrates the use of selfing and inducing the mutations in the creation of new recombination differ significantly from its parents. The results obtained by Encheva et al. (2008) and Alahdadi et al. (2011) confirm our findings.

The interaction between irrigation treatments and genotypes was significant in all studied traits except for head diameter. It is evident from Table 2 and Figures 1 and 2 that the lines which gave the highest yield of the seed (Line $M_{2,1}$-63 and Line 20) have WUE under drought conditions higher than WUE under normal irrigation. This observation was also in commercial variety (Sakha 53). The lowest depression in seed yield (Figure 2) due to drought conditions as compared to the seed yield under normal irrigation has been registered for Line 20, Line $M_{2,1}$-63, and Sakha 53 genotypes (11, 18, and 16%, respectively), but the highest depression recorded for Line 48, Line $M_{2,3}$-63 and Line $M_{2,4}$-63 (49, 46, and 43%, respectively). So, the genotypes (Line 20, Line $M_{2,1}$-63, and Sakha 53) are more tolerant to drought than others and it can be used in breeding program to develop sunflower hybrids suitable for cultivation under drought condition. Kassab et al. (2012) found that variety Sakha 53 gave WUE under drought condition higher than its under normal irrigation.

Conclusion

Self-pollination and mutagenesis are effective ways to get the new genetic differences in sunflower. Genotypes Line 20, Line $M_{2,1}$-63, and Sakha 53 are more tolerant to drought than others and it can be used in breeding program to develop sunflower hybrids suitable for cultivation under drought condition.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Afkari AB (2010). The Effect of water deficit on characteristics physiological-chemical of sunflower (*Helianthus annuus L*.) Varieties. Adv. Environ. Biol. 4(1):24-30.

Alahdadi I, Oraki H, Khajani FP (2011). Effect of water stress on yield and yield components of sunflower hybrids. Afr. J. Biotechnol. 10(34):6504-6509.

Ali Md, Shui LT (2009). Potential evapotranspiration model for Muda Irrigation Project, Malaysia. Water Resour. Manag. 23:57-69.

AOAC (1995). Association of Official Analytical Chemists. Official methods of Analysis16 Ed. AOAC International, Washington, D.C., USA

Beyazgul M, Kayam Y, Engelsman F (2000). Estimation methods for crop water requirements in the Gediz Basin of western Turkey. J. Hydrol. 229:19-26.

Demir AO, Goksoy AT, Buyukcangaz H, Turan ZM, Koksal ES (2006). Deficit irrigation of sunflower (*Helianthus annuus* L.) in a sub-humid climate. Irrigation Sci. 24:279-289.

Doorenbos J, Kassam AH (1979).Yield response to water Irrigation and drainage paper 33. In: Landon JR (Ed.) Booker tropical soil manual, Long man Inc., New York, U.S.A.

Encheva J, Christov M, Shindrova P (2008). Developing Mutant Sunflower Line (*Helianthus annuus* L.) by Combined used of Classical Method with Induce Mutagenesis and Embryo Culture Method. Bulg. J. Agric. Sci. 14(4):397-404)

Fox P, Rockström J (2000). Water-harvesting for supplementary irrigation of cereal crops to overcome intra-seasonal dry-spells in the Sahel. Phys. Chem. Earth, Part B: Hydrol. Oceans Atmos. 25(3):289-296.

Gomez KA, Gomez AA (1984).Statistical Procedures for Agricultural Research.2nd Edn.,John Wiely and Sons Inc., New York. pp. 95-109.

Kassab OM, Abo Ellil AA, Abo El-Kheir MS (2012). Water Use Efficiency And Productivity Of Two Sunflower Cultivars As Influenced By Three Rates Of Drip Irrigation Water. J. Appl. Sci. Res. 8(7):3524-3529.

Khalilv B, Yarnia M (2007). The effects of water deficit stress on some of the physiological characters of sunflower in the different density of planting. J. Agric. Sci. Islamic Azad Uni. Tabriz Branch 1(2):37-52.

Mehrpouyan M, Nazari-Golshan A, Say zadea S (2010). Effect of irrigation stop at different growth stages on some agronomic traits of sunflower (*Helianlhus annuus* L.) under three plant densities in Takestan Region, Iran. Plant Ecophysiol. 2:137-144.

Öner C, Klaus-Peter G, Frank E, Frank-Michael C, Mustafa AK (2014). Determination of the relationship between water use efficiency, carbon isotope discrimination and proline in sunflower genotypes under drought stress. Aust. J. Crop Sci. 8(2):232-242.

Skoric D (1992). Achievements and future directions of sunflower breeding. Field Crops Res. 30:231-270.

Smith M, Kivumbi D, Heng LK (2002). Use of the FAO-CROPWAT model in deficit irrigation studies. Deficit irrigation practices, FAO Water Reports No.22, Rome.

Xiao G, Zhang Q, Xiong Y, Lin M, Wang J (2007). Integrating rainwater harvesting with supplemental irrigation into rain-fed spring wheat farming. Soil Till. Res. 93:429-437.

academicJournals

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Full Length Research Paper

Herd mortality and cattle off-take rates among small-holder producers in the North West Province of South Africa

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This study was done to determine the influence of herd mortality on off-take rates through face to face interviews of 308 smallholder cattle producers from Dr RSM District Municipality, North West. Most deaths were caused by diseases (50%) and drought (34%). Producer's gender had no influence on herd performance and off-take even though extension ward with higher proportion of women had higher mortality and lower off-take rates. The sale of steers decreased significantly (P<0.05) as the death of adult animals as well as that of the overall herd increased. Herd off-take also declined as mortality of suckling calves as well as that for overall herd, increased. Herd mortality logically reduces stock and therefore suppresses sales. It is recommended that herd mortality should be monitored through animal recording to improve the competitiveness of small-holder production systems.

Key words: Adult mortality rates, causes of death, cattle producers.

INTRODUCTION

Recent population census revealed that cattle production especially small herds comprising 1 to 10 head of cattle, plays an important role in rural livelihoods (Statistics South Africa, 2013). Small-holder cattle producers in South Africa own 40% of the national herd (RMRDT, 2008), but are less productive than commercial producers. Even though the low competitiveness of this sector has been partially attributed to low off-take rates (Tapson, 1990; Scholtz and Bester, 2010), risk factors contributing to this poor performance have not been identified. According to Swai et al. (2010), tick-borne diseases were the main cause of deaths in small-holder dairy cattle in Tanzania. It is well known that high mortality of young stock is an indicator of low productivity among small-holder producers in Africa (ILCA, 1982). Diseases causing deaths among young calves are often attributable to poor management (Mansour et al., 2014; Wudu et al., 2008). Scholtz and Bester, (2010) also estimate high herd mortality amongst South Africa's small-holder cattle producers, which Meissner et al. (2013) cite as liable for poor productivity and low off-take rates. The calculation of herd mortality requires elaborate

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Table 1. Distribution of farmers according to extension ward and gender.

Extension ward	Number farmers	Age head	Years of experience	Herd size	% Herd mortality	% Herd sales
	M/F	M/F	M/F	M/F	M/F	M/F
Taung North	41/36	63.7/53.9	19.7/15.2	16.1/15.6	16.6/9.6	25.6/20.1
Mean	77	59.2[a]	17.7[a]	15.9[a]	13.4[a]	23.1[a]
Ganyesa	105/30	55.1/60.7	23.0/21.1	45.1/31.2	7.6/ 9.0	13.7/14.6
Mean	135	56.3[b]	22.6[b]	42.7[b]	7.9[b]	13.9[b]
Morokweng	86/10	48.6/63.1	20.9/24.9	50.0/26.5	8.6/10.2	10.9/4.4
Mean	96	50.8[b]	21.2[ab]	46.5[b]	8.9[b]	9.9[b]
Total	232/76	54.3/58.4	20.9/20.9	42.6[a]/24.1[b]	9.4/9.5	14.6/15.0
	308	56.8	20.9	35.3	10.0	15.0
SE	-	0.97	1.02	1.99	1.18	0.96

Values with different superscripts differ significantly (P <0.05).

data (Woodbury et al., 2005; Swai et al., 2010). However, crude death, which is the total number of animals found dead in a specified population during a specified period divided by the average number of animals in that population in that specified period, are the most commonly used form of measuring mortality (Putt et al., 1988). The North West Province is prone to drought, which tends to be severe in the western areas. Previous studies found no significant shifts in cattle sales during droughts in West Africa (Fafchamps et al., 1998) and Kenya (McPeak, 2004). It was hypothesize that cattle producers would dispose other categories of animals in favour of females. The purpose of this study was to determine the relationship between crude herd mortality and off-take rates.

MATERIALS AND METHODS

This study was conducted in the Dr Ruth Segomotsi Mompati (RSM) District Municipality of the North West Province. A random sample of 308 was selected from a list of 1700 cattle farmers from the District Department of Agriculture and Rural Development. Three wards of Taung North (25%), Morokweng (31%), and Ganyesa (44%) were used as strata to draw proportionate samples. A questionnaire aimed at capturing cross-sectional data on factors affecting cattle off-take for 2011 breeding season was developed and administered through face to face interviews with respondents. The instrument containing 73 questions ranging from demographic data to production data including mortality and sales was administered between May and July, 2012. The interviews lasted for 45 min with each respondents. Crude herd mortality was calculated using the following equation: number of deaths during 2011/average of opening and closing herd sizes in 2011 × 100. Calculations for mortality were first made according to animal categories within the herd to distinguish suckling calves (pre-wean) from weaners and adult animals. Off-take rate was calculated as number of sales during 2011/average of opening and closing herd sizes during 2011 × 100. For this purpose, animals were categorized as bull, ox, cow, heifer, bullock, and steer.

Data were analyzed using IBM SPSS statistics 22 (2013). Descriptive statistics were computed using frequencies and means to determine patterns between variables. Herd sizes were classified according to categories suggested by Tapson (1990) where herds of less than 10 were regarded as small. The GLM multivariate analysis was performed to test effect of farming area and farm level variables on herd mortality and off-take rates. Means were separated using least significant differences (LSD) tests. Correlation analysis was performed to measure associations between herd mortality and herd off-take.

RESULTS AND DISCUSSION

The majority (76%) of respondents were males, 82% of whom lived at home. The age of household heads ranged from 24 to 86 with an average of 57 years. The majority (46%) of respondents belonged to middle age of between 45 and 65 although seniors (>65) constituted a significant proportion of the population (34%). These households owned herds ranging from one to 169 with an average of 35 head of cattle. The average calving rate was 55% ranging from 5.6 to 100% whilst herd mortality ranged from 0.5 to 94.8% with an average of 10%. Herd off-take rates ranged from 2.7 to 66.7% with an average of 15%. The herd dynamics in the study area were atypical of the lower levels reported in other studies of South African small-holder cattle (Tapson, 1990; RMDT, 2008; Scholtz and Bester, 2010).

Table 1 shows that female farmers owned significantly smaller herds than men (P<0.05). However, except tendencies for women experiencing lower calving rates (P<0.09), no significant differences were observed between men and women regarding herd performance. However, farmers from the Taung North extension ward had the highest proportion of female farmers who owned smaller herds than other farmers (P<0.05). Previous studies have shown that there is a strong correlation

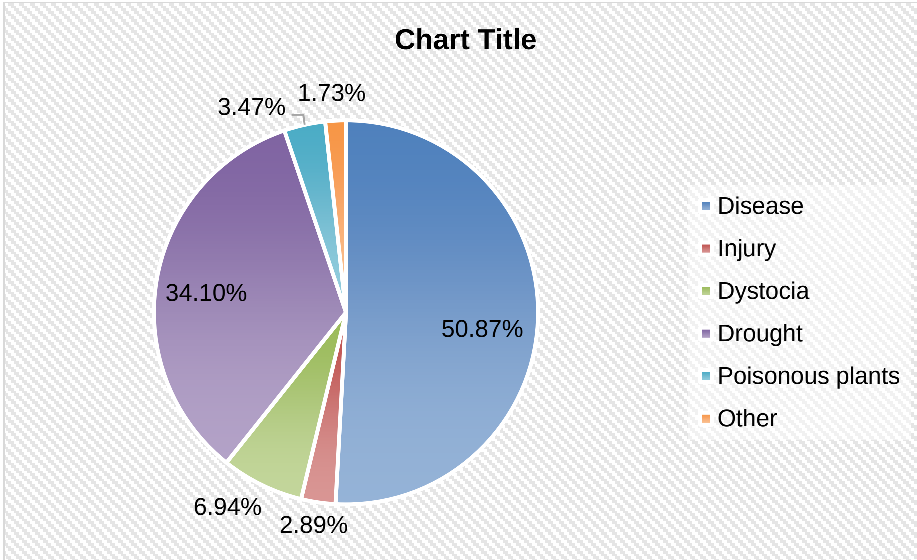

Figure 1. Distribution of herds according **to c**auses of deaths among small-holder herds in Dr Ruth Segomotsi Mompati District Municipality (N=173).

Table 2. Average crude herd mortality rates by herd size.

Herd category	Pre wean (N=101)	Weaner (N=94)	Adult (N=176)	Herd (N=214)
1-10(N=40)	2.51	3.14[a]	12.23[a]	18.16[a]
11-30(N=117)	1.99	1.99[ab]	4.84[b]	8.63[b]
31-70(N=99)	2.16	1.72[b]	4.75[b]	8.12[b]
>70(N=52)	2.02	1.01[b]	3.08[b]	6.12 [b]
SE	0.30	0.30	0.77	1.06

Values with different superscripts differ significantly (P<0.05).

between women's economic opportunities and access to affordable, safe food (EIU, 2012; MuGeDe, 2014). The predominance of female farmers in Taung North suggests that cattle production plays an important role in meeting household needs. Although not significantly different from the Morokweng ward, farmers from Taung North were less experienced than those from Ganyesa (P<0.05). Furthermore, these farmers experienced higher herd mortality but sold the highest proportion of stock than other farmers (P<0.05). This apparent low herd performance in the Taung North extension ward is a matter of concern, because it affects female headed households with relatively limited resources.

Most (41%) of the respondents utilized exotic cattle breeds and non-descript types (33%) in their herds, while only 26% utilized indigenous breeds. Approximately 70% of the herds experienced 10% average mortality during 2011 ranging from 0.5 to 94.8%. Figure 1 shows that most deaths were caused by diseases (50%) followed by drought (34%). The study area has experienced severe drought since 2008, which may explain the high incidence of drought related deaths among adult animals as well as high mortality among small herds. The incidences of dystocia related death could also be attributed to the high adoption rate of exotic breeds, which produce large framed calves.

Previous studies showed that most deaths occur among young animals (Swai et al., 2010) and tends to decline for yearlings and rise afterwards (Fiore et al., 2010). This trend is confirmed in our study showing higher mortality rates for pre-wean calves, a decline for weaners and a rise for adults (Table 2). Small herds had significantly higher crude herd mortality rates (18.16%) than other herd size categories and mortality rates improved significantly with larger herd size categories(P<0.05). Weaner mortality rates of small herds differed significantly (P<0.05) with those for all herd size categories except medium size (11-30). Small herds (1-10) had significantly higher mortality rates for both old animals and total herd than other herd size categories (P<0.05).

The effect of drought and other sources of income and

Table 3. Relationship between crude mortality rates and off-take rates.

Parameter	Pre weanmortality	Adultmortality	Herdmortality	Steeroff-take
Adult mortality	0.242**	-	-	-
Herd mortality	0.595**	0.881**	-	-
Steer sale	-0.036	-0.123*	-0.131*	-
Herd off-take	-0.150**	-0.103	-0.147*	0.265**

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

asset shocks has been widely reported (Kinsey et al., 1998; Fafchamps et al., 1998; McPeak, 2004). Some of these studies found no significant shifts in cattle sales during droughts in West Africa (Fafchamps et al., 1998) and Kenya (McPeak, 2004). Contrary to expectation, our study did not find a significant correlation between herd mortality and the sale of cows. However, the sale of steers decreased significantly (P<0.05) as the death of adult animals as well as that of the overall herd increased (Table 3). Furthermore, herd off-take also declined as mortality of suckling calves as well as that for overall herd, increased. These results imply the loss of calves reduces the number of available steers for the markets. Similarly, the death of old animals especially cows reduces the number of calves to be raised for the market and thus affecting the overall herd off-take, such that fewer animals become available for sale when herds experience high mortality. Some studies reported high off-take rates associated with the adoption of animal health practices (Muma et al., 2009; Hüttner et al., 2001) where stock is culled to acquire remedies. In our study, 90% of respondents reported that they always vaccinate their animals for notifiable diseases and dose animals for parasites. It can therefore be inferred that the adoption of animal health practices is in place, which suggests that cattle producers in the study area use disease incidences as a herd management tool. These results imply that herds with high mortality rates have limited stock to sell.

Conclusions

This study has provided a descriptive analysis of crude herd mortality rates in the study area. Even though the Taung North extension ward has a high proportion of female farmers and smaller herds, gender did not show significant influence on both herd mortality and off-take. The results confirm previous findings showing curvilinear pattern where mortality rates plummet for weaner calves. Diseases are the main causes of deaths in the study area followed by drought. It was also concluded that the considerable mortality incidences resulting from dystocia is a sign of a high adoption of exotic breeds, which are liable for large-framed calves.

Larger herds of more than 10 head of cattle had significantly lower mortality rates than smaller ones. It was therefore concluded that larger herds are more efficient than small herds in terms of mortality rates across all animal age categories. Finally, it is evident that herd mortality influences herd off-take rates. The inclusion of herd mortality data in animal recording systems may enhance the monitoring of small-holder production systems and thus improve competitiveness. The high mortality rates for old animals should receive focused extension efforts to improve disease management and implement a drought management strategy.

Conflict of interests

The authors have not declared any conflict of interests.

REFERENCES

EIU (2012). Eat better? Let women do the work. Available at: http://www.economist.com/blogs/graphicdetail/2012/07/global-food-security

Fafchamps M, Udry C, Czukas K (1998). Drought and saving in West Africa: Are livestock a buffer stock? J. Dev. Econ. (55):273-305.

Fiore G, Hofherr J, Natale F, Stifter E, Costanzi C (2010). On-farm mortality in cattle: Analysis of on-farm mortality data for cattle for retrospective and prospective epidemiological surveillance. JRC Scientific and technical reports. Trento, Italy.

Hüttner K, Leidl K, Pfeiffer DU, Kasambara D, Jere FBD (2001). The effect of a community-based animal health service programme on livestock mortality, off-take and selected husbandry applications: A field study in Northern Malawi. Livest. Prod. Sci. 72(3):263-278.

ILCA (1982). Evaluation of the productivities of Djallonke sheep and N'Dama cattle at the Centre de Recherches Zootechniques, Kolda, Senegal. Research report no.3. Addis Ababa, Ethiopia.

Kinsey B, Burger k, Gunning JW (1998). Coping with drought in Zimbabwe: Survey evidence of responses of rural household to risk. World dev. 26(1):89-110

McPeak J (2004). Contrasting income shocks with asset shocks: Livestock sales in northern Kenya. Oxf. Econ. Pap. (56):263-284.

Mansour AEM, Abdelgadir AE, El Zubeir IEM (2014). Major causes and risk factors associated with calf mortality in dairy farms in Khartoum State, Sudan. J. Vet. Med. Anim. Health 6(5):145-153.

Meissner HH, Scholtz MM, Engelbrecht FA (2013). Sustainability of the South African livestock sector towards 2050 Part2: Challenges, changes and required implementation. S. Afr. J. Anim. Sci. 43(3):298-319.

MuGeDe SM (2014). The role of women in agriculture. Available at: http://www.wfo-oma.com/women-in-agriculture/articles/the-role-of-rural-women-in-agriculture.html

Muma JB, Munyeme M, Samui KL, Siamudaala V, Olaya J, Mwacalimba K, Skjerve E (2009). Mortality and commercial off-take rates in adult traditional cattle of Zambia. Trop. Anim. Health Prod. 41(41):783-789.

Putt SNH, Shaw APM, Woods AJ, Tylor N, James AD (1988). Veterinary epidemiology an economics in Africa. A manual for use in the design and appraisal of livestock heath policy. ILCA.

RMRDT (2008). Research and development plan for the large stock and small stock meat industries in South Africa.

Scholtz MM, Bester J (2010). Off-take and production statistics in the different South African cattle sectors: Results of a structured survey. Appl. Anim. Husb. Rural Dev. 3:19-23

Statistics South Africa (2013). Census 2011 Agricultural households. Report no. 03-11-01. Pretoria.

Swai ES, Karimuribo ED, Kambarage DM (2010). Risk factors for small-holder dairy cattle mortality in Tanzania. J. S. Afr. Vet. Assoc. 81(4):241-246.

Tapson DR (1990). A socio-economic analysis of small-holder cattle producers in KwaZulu. PhD Thesis, Vista University, Pretoria.

Woodbury MR, Berezowski J, Haigh J (2005). A retrospective study of the causes of morbidity and mortality in farmed elk (*Cervus elaphus*). Can. Vet. J. 46(12):1108-1121.

Wudu T, Kelay B, Mekonnen HM, Tesfu K (2008). Calf morbidity and mortality in smallholder dairy farms in Ada'a Liben district of Oromia, Ethiopia. Trop. Anim. Health Prod. 40(5):369-376.

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Abscisic acid content in roots and root characteristics of alfalfa under deficit irrigation

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The effect of drought stress on Abscisic acid (ABA) on roots and root characteristics of three alfalfa varieties (Aohan, Zhongmu No.1 and Suntory) was studied. The study was conducted in greenhouse of the Chinese Academy of Agricultural Sciences (CAAS), Beijing, China from September 2014 to May 2015. Alfalfa varieties were exposed to four irrigation levels of water-holding capacity *viz* 100% (w1) (control); 85% (w2); 70% (w3) and 55% (w4), which were considered as deficit irrigation treatments. The results showed that ABA was significantly affected by deficit irrigation treatments during different plant growth stages. With the exception of 90 and 105 days after transplanting, ABA increased after transplanting up to harvest in all treatments. Increasing deficit irrigation showed significant increase in ABA content in roots of alfalfa. The minimum content of ABA was obtained from w1 (44 ng/g.FW) and then increased to 56.6, 64.6 and 94.4 ng/g.FW for w2, w3 and w4 respectively on 105 days after transplanting. Moreover, the results showed that ABA content was differently affected among different varieties of alfalfa under different water stress levels. The maximum ABA content was 83.2, 61.7 and 49.9 ng/g.FW obtained with Aohan, Suntory and Zhongmu No.1 varieties respectively, at 105 days after transplanting. Highest water stress w4 (55%) reduced root length by 20.92%, lateral roots by 20.71%, root fresh biomass by 43.79% and root dry biomass by 37.96%. The root to shoot ratio was 1.9 times higher in water stressed plants compared with the control, indicating that water stress in alfalfa is affected more shoot growth than root one.

Key words: alfalfa, deficit irrigation, Abscisic acid (ABA), root characteristics.

INTRODUCTION

In recent years, drought has becomes a leading threat to agriculture in the world (Loss and Siddique 1994; Grando and Ceccarelli 1995). It is one of the main environmental stresses that limits plant growth and production (Lawlor and Cornic, 2002; Martínez et al., 2003; Tadina et al., 2007; Ren et al., 2007; Wu et al., 2009). Drought affects many *Medicago* species (Chebouti et al., 2001; Bouizgaren et al., 2011). Plants can avoid drought by having a root system adapted to drought (Morgan et al., 1986; Liu et al., 2005).

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Roots are the first part of a plant to sense water stress and its growth affects the answer to drought cues (Shimazaki et al., 2005). Root characteristics are defined as root length, root laterals number and root diameter are very important for good plant stand (Nanjo et al., 1999). Effect of water stress on root growth was studied in different crops such as wheat (Pritchard et al., 1991), maize (Wu and Cosgrove, 2000; Shimazaki et al., 2005) and barley (Sahnoune et al., 2004). One adaptation by plants to drought is the change in root to shoot ratio (Turner, 1997). Drought decreases growth of amaranth (*Amaranthus* spp.) in both root and shoot, but with less effect on root growth (Liu and Stützel, 2004). Lateral roots are one of the root system components which play an important role in water absorption. The development of lateral roots and growth are affected by complex interactions of different factors such as, environment, development and hormones (Casimiro et al., 2003; López-Bucio et al., 2003; Malamy, 2005). One important factor that affects the growth and development of roots to tolerate the environment such as water, drought, salt, cold, light, and temperature is ABA hormone (Signora et al., 2001; Sharp and LeNoble, 2002). ABA hormone is a key hormone that confers tolerance to environmental stresses. Plants increased ABA content when placed under water stress which effectively closes stomata to protect plants from adverse effects of drought (Schroeder et al., 2001; Rock et al., 2010; Mori and Murata., 2011) and to enhance drought resistance in crops (Schroeder et al., 2001; Wang et al., 2005; Shinozaki and Yamaguchi-Shinozaki, 2007). This study was conducted to evaluate the effects of deficit irrigation on abscisic acid content in roots and root characteristics of alfalfa.

MATERIALS AND METHODS

Plant material and destructive samples

The experiment was conducted in the green house of Animal Science Institute, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China from September 2014 to May 2015.The treatments comprised of three varieties of alfalfa *(Medicago sativa* L.) *viz* Aohan, Zhongmu No.1 and Suntory. Seeds were germinated first on wet filter paper in petri-dishes. Four plants seeding were transplanted to each pots-culture (25 x 30 cm) with10 kg clay soil (pH 7.7%, organic matter 9.2 g kg^{-1}, total P 0.7 g kg^{-1}, total K 19 g kg^{-1}, bulk density 1.3 g cm^{-3} and field capacity 30%). The varieties were evaluated under four irrigation levels of water holding capacity *viz*: w1 (100%), w2 (85%), w3 (70%) and w4 (55%). The full water holding capacity w1 was considered as normal irrigation, while the other treatments w2, w3 and w4 were considered as deficit irrigation. Irrigation water was application (manually) commenced from the first day of transplanting and continued for the whole growing period of alfalfa (195 days).

Pot moisture contents measured daily by using HH2 moisture meter version 4.0 (Delta- T Devices Ltd. UK) to maintained the percentage of water-holding capacity (100, 85, 70 and 55%). Samples of roots were collected every 15 days and immediately frozen in liquid nitrogen and then stored at -80 ^{0}C for further ABA extraction and analysis. Samples of roots were carefully removed from the pot and washed several times in water to obtain the complete roots. ABA was then extracted and purified according to Dobrev and Kaminek (2002) method and analyzed as described by Albacete et al. (2008). One homogenized gram of fresh root weight was placed in 5 ml of cold mixture of methanol, water and formic acid at pH 2.5 and was separated by centrifuging (20 000 g for 15 min after overnight extraction at -20°C. The root material was then subjected to generate more extract in 5 ml of the same solution for 30 min. Supernatants were filtered through Sep-Pak Plus†C18 (®Waters, Milford, MA, USA) to remove plant pigments and interfering lipids and evaporated to dryness. Residues were dissolved in 5 ml of methanol/water solution (1 to 4 ratio) using an ultrasonic bath and filtered by nylon membrane Millex filters (Ø 0.22 μm) (®Millipore, Bedford, MA, USA) then put in tubes adding extraction solution adjusting to 1.5 ml. Analyses were carried out on an HPLC/MS system consisting of an Agilent 1100 Series HPLC (Agilent Technologies, Santa Clara, CA, USA). Equipped with autosampler and connected to an Agilent Ion Trap XCT Plus mass spectrometer using ESI (an electrospray interface). It was filtered with Millex filters and injection, 100 μl of each fraction. For quantification of ABA calibration curves were constructed for component analyzed using internal standards: $[^{2}H_{6}]$cis,trans-abscisic acid (©Olchemin Ltd, Olomouc). Root length (cm) and lateral roots number/plant were measured by using Scan Maker i800 plus (MICROTEK, shanghai, China). Fresh root biomass (g) was weighted with digital balance directly after sample of root collection and then dried at oven 60°C until a constant weight for determining dry biomass of roots. Root to shoot ratio were calculated by dividing fresh root weight by the fresh shoot weight.

Experimental design and statistical analysis

The experiment was laid out in complete randomized design (CRD) with four replications. Treatments were three varieties of alfalfa and four irrigation level of water holding capacity. Data were calculated and arranged as means and analysis using one way analysis of variance (ANOVA) followed by Duncan's multiple range test ($P < 0.05$).

RESULTS

Deficit irrigation and Abscisic acid (ABA) content

The effects of deficit irrigation treatments on the content of ABA in roots were shown in Figure 1. The results show that the content of ABA was affected by deficit irrigation at different plant growth stages. Increasing deficit irrigation showed a significant increase in ABA content. ABA content gradually increased with time under all deficit irrigation treatments until 75 days after transplanting. From 75 days after transplanting, ABA content hastily decreased under w1, w2 and w3 for 30 days until it reached the lowest ABA content at 105 days after transplanting. From 105 days after transplanting ABA content tended to increase until harvest. Moreover, under w4, ABA content increased from 30 to 75 days after transplanting, and then declined until 90 days and remained to 105 days after transplanting, after that tended to increase until harvest. The minimum ABA content was obtained under w1 (44 ng/g.FW) and then increased to 56.6, 64.6 and 94.4 ng/g FW for w2, w3 and w4 at 105 days after transplanting, respectively.

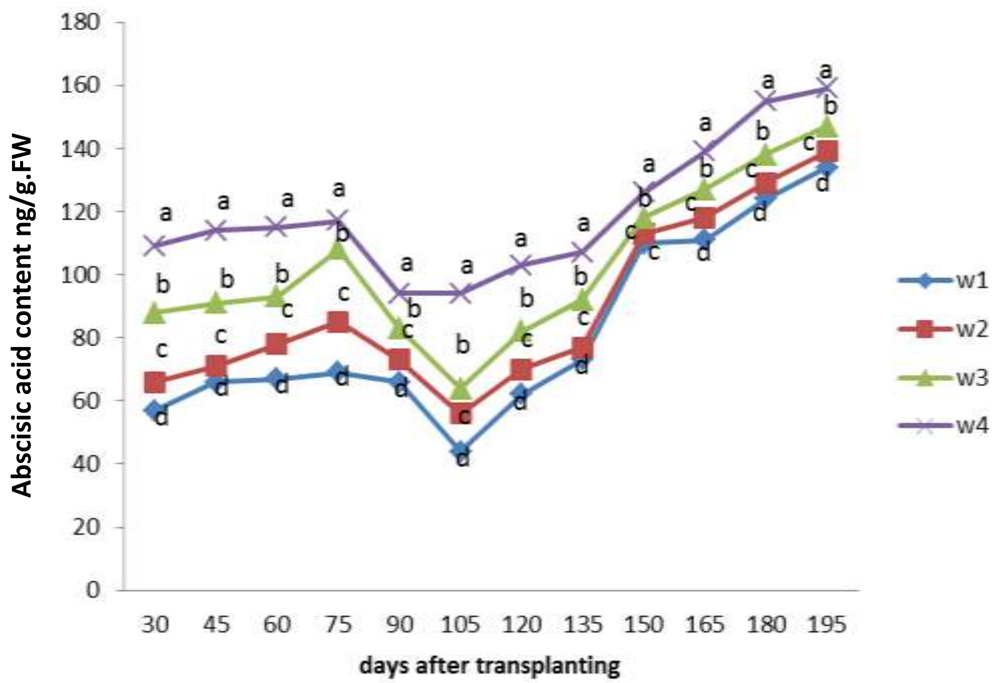

Figure 1. Effect of deficit irrigation on Abscisic acid content in roots of alfalfa during different days after transplanting. w1, 100% of water holding capacity; w2, 85% of water holding capacity; w3, 70% of water holding capacity and w4, 55% of water holding capacity. In each days separately, the same letter are not significantly different (P < 0.05).

Abscisic acid (ABA) content and varietal response

The effect of alfalfa varieties on ABA content are presented in Figure 2. ABA content was affected by different varieties of alfalfa. ABA content progressively increased with time in all varieties up to 75 days after transplanting. ABA content under Zhongmu No.1 and Suntory decreased rapidly after 75 days after transplanting until reached its lowest activity level at 105 days after transplanting while Aohan decreased gradually. After that all the three varieties started to increase in ABA content and continued with that trend until harvest.

The minimum ABA content was 83.2, 61.7 and 49.9 ng/g .FW which was obtained by Aohan, Suntory and Zhongmu No.1 at 105 days after transplanting respectively. However, Zhongmu No.1 presented the lowest ABA content in all times, except for 180 and 195 days after transplanting in which was higher than Suntory while Aohan recorded the highest ABA content compared to the other two varieties.

Deficit irrigation and root characteristics

The effect of deficit irrigation treatments on root length, number of root laterals, fresh and dry weights of roots and root to shoots ratio are shown in Table 1. The results reveal that root length, number of root laterals, fresh and dry weights of roots were significantly reduced by increasing the deficit irrigation ($P<0.05$). The lowest reduction in root length was 9.3% followed by 12.7 and 20.9%. The least reduction in number of root laterals was 14.5% followed by 15.7 and 20.7%. Whereas the lowest reduction in root fresh weight was 9.9% followed by 11.1 and 43.8%. However, the least reduction in root dry weight was 7.3% followed by 21.7 and 38% was obtained by w2, w3 and w4 respectively, compared with control plants. Root to shoot ratio was significantly increased by increasing the deficit irrigation ($P<0.05$). The maximum increase in root to shoot ratio was 94.3%, followed by 78.6 and 30% in w4, w3 and w2 respectively compared to control treatment.

Root diameter was not significantly increased by increasing deficit irrigation. Irrigation of alfalfa plants by 55% of water holding capacity (W4) resulted in highest reduction in root diameter (5.3%) in comparison to the control plants (100% of water holding capacity).

Alfalfa varieties and root characteristics

The effect of alfalfa varieties on root length, number of root laterals, root diameter, fresh and dry weights of roots are shown in Table 2. Aohan resulted in significantly different root length, number of root laterals and dry root

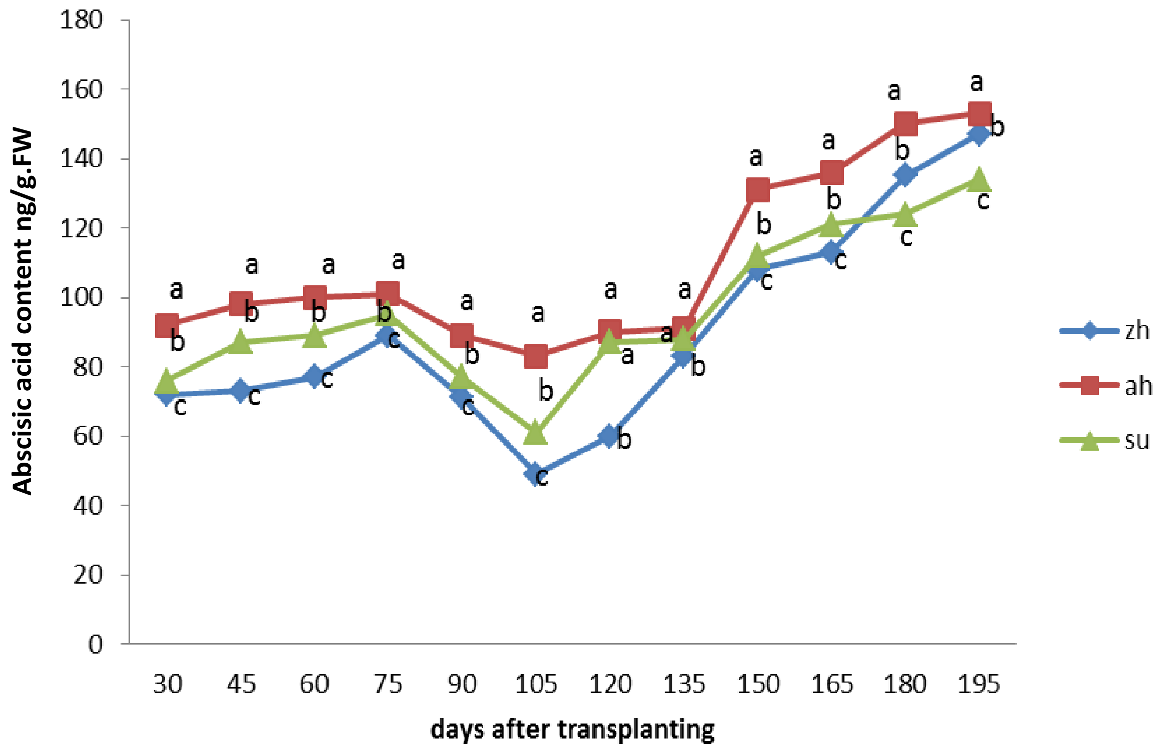

Figure 2. Effect of three varieties of alfalfa on abscisic acid content, during different days after transplanting in roots of alfalfa. zh, Zhongmu No.1; ah, Aohan and su, Suntory. In each days separately, the same letter are not significantly different (P < 0.05).

Table 1. Effect of deficit irrigation on root characteristics and root to shoot ratio.

WHC%	Root length (cm)	No. of root lateral	Root diameter (cm)	Root fresh weight (g)	Root dry weight (g)	Root/shoot ratio
w1	457.23[a]	1452.16[a]	0.38[a]	87.91[a]	21.00[a]	0.70[b]
w2	418.45[b]	1241.52[b]	0.38[a]	79.18[b]	19.47[a]	0.91[ab]
w3	399.20[c]	1224.93[b]	0.36[a]	78.15[b]	16.45[b]	1.25[a]
w4	361.59[d]	1151.38[c]	0.36[a]	49.42[c]	13.03[c]	1.36[a]
SE ±	17.18	41.49	0.01	5.51	0.80	0.04

WHC%, water holding capacity; w1, 100% (control); w2, 85%; w3, 70% w4, 55%. The values in each column followed by the same letters are not significantly different (P < 0.05).

Table 2. Effect of alfalfa varieties on root characteristics.

Varieties	Root length (cm)	No. of root lateral	Root diameter (cm)	Root fresh weight (g)	Root dry weight (g)	Root/shoot ratio
Zh	428.21[b]	1202.92[b]	0.38[a]	81.18[a]	19.01[a]	1.06[a]
Ah	357.92[c]	1145.67[c]	0.38[a]	67.69[b]	15.37[c]	1.05[a]
Su	441.23[a]	1453.90[a]	0.35[a]	72.12[b]	18.08[b]	1.06[a]
SE ±	14.88	35.93	0.01	4.77	0.69	0.036

zh, Zhongmu No.1; ah, Aohan and su, Suntory. The values in each column followed by the same letters are not significantly different (P < 0.05).

biomass, while the minimum root diameter was observed in Suntory. Moreover, Zhongmu No.1 presented higher root fresh and dry weights compared with other two varieties. The highest root laterals number was observed in Suntory while no significant difference was observed in root to shoot ratio among all varieties.

DISCUSSION

Abscisic acid (ABA) is a hormone that indicates to endure environmental conditions such as drought and high salinity. Our study indicated that increasing deficit irrigation significantly increased ABA content. These results are in agreement with those reported by Brodribb and McAdam (2011) and McAdam and Brodribb (2012) on ferns (*Pteridium esculentum* and D. antarctica) and a lycophyte (*Selaginella kraussiana*). Outlaw (2003) reported that during drought stress ABA concentration was increased up to 30- fold.

Deficit irrigation reduced root length. This result is similar to that reported by Benlaribi et al. (1990); Ali Dib and Monneveux (1992) who attributed the reduction in root length and root fresh and dry weight to the reduction of turgor pressure in wheat. Similar results were observed in Albizzia seedlings (Nanjo et al., 1999), Erythrina seedlings (Nativ et al, 1999), *Eucalyptus microtheca* seedlings (Marron et al., 2002), and *Populus* species (Nautiyal et al., 2002). Moreover, Sacks et al. (1997) and Rao et al. (1993) found that under deficit irrigation, root growth of wheat and maize was not significantly reduced. The results showed that fresh and dry weights of roots were significantly reduced by increasing the deficit irrigation. Similar results were reported by Manivannan et al. (2007) and Lowlor and Cornic (2002) in pearl millet. Nicholas (1998) observed that in Avocado cultivars, water stress reduced the fibrous roots biomass. Under mild and severe water stress conditions, Populus species decreased root dry weight (Wullschleger et al., 2005). Similar results were observed in sugar beet (Pan et al., 2002). Furthermore, Amina et al. (2014) reported that drought reduced significantly the vegetative mass of the shoots and roots of wheat. Liu and Stützel (2004) observed that the effect of water stress was less in root growth than shoot one.

Our results showed that root to shoot ratio was significantly increased by increasing the deficit irrigation. Other studies demonstrated that under water-stress conditions, root-to-shoot ratio was increased to facilitate water absorption (Morgan 1984; Nicholas, 1998). Also Sharp and LeNoble (2002) and Manivannan et al. (2007) related this increase in water absorption to ABA content in roots and shoots.

Conclusion

Our results demonstrate that ABA was significantly affected by deficit irrigation treatments and Alfalfa varieties. Increasing deficit irrigation showed significant increase in roots ABA content. Moreover, root length, laterals number, fresh and dry weights of roots were significantly reduced by increasing the deficit irrigation. The results showed that the root to shoot ratio was significantly increased by increasing the deficit irrigation, indicating that water stress in alfalfa crop affected more shoot growth than roots. Aohan presented the lowest root length, number of root laterals, dry root biomass and less root to shoot ratio. In contrast this variety had the highest ABA content.

Conflict of interests

The authors have not declared any conflict of interests.

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REFERENCES

Albacete A, Ghanem M E, Martinez-Andujar C, Acosta M, Sanchez-Bravo J, Martinez V, Lutts S, Dodd IC, Perez-Alfocea F (2008). Hormonal changes in relation to biomass partitioning and shoot growth impairment in salinized tomato (*Solanum lycopersicum* L.) plants. J. Exp. Bot. 59:4119-4131.

Ali Dib T, Monneveux P (1992). Adaptation à la sécheresse et notion d'idéotype chez le blé dur. Caractères Morphologiques d'enracinement. Agronomie 12:371-379.

Amina L, Ahmed A, Youcef H, Samira S (2014). Study of the Mechanisms involved In the tolerance of Durum Wheat (*Triticum durum* desf.) to Drought to Improve productivity. Turk. J. Agric. Nat. Sci. 1:682- 687.

Benlaribi M, Monneveux P, Grignac P (1990). Etude des caractères d'enracinement et de leur rôle dans l'adaptation au déficit hydrique chez le blé dur (*Triticum durum* Desf.). Agronomie 10:305-322.

Bouizgaren A, Farissi M, Khalida R, Ghoulam C, Barakate M, Al Feddy MN (2011). Assessment of summer drought tolerance variability in Mediterranean alfalfa (*Medicago sativa* L.) cultivars under Moroccan fields conditions. Arch. Agro. Soil Sci. 1-14.

Brodribb TJ, McAdam SAM (2011). Passive origins of stomatal control in vascular plants. Science 331:582-585.

Casimiro IT, Beeckman N, Graham R, Bhalerao H, Zhang P, Casero G, Sandberg M J, Bennett (2003). Dissecting Arabidopsis lateral root development. Trends Plant Sci. 8:165-17.

Chebouti A, Mefti M, Abdelguerfi A (2001). Effet du stress hydrique sur le rendement en gousses et en grains chez trois espèces de luzernes annuelles: Medicago aculeteata, Medicago orbicularis Medicago truncatula. Cah. Opt. Médit. 45:163-166.

Dobrev PI, Kaminek M (2002). Fast and efficient separation of cytokinins from auxin and abscisic acid and their purification using mixed-mode solid-phase extraction. J. Chromatogr. A. 950:21-29.

Grando S, Ceccarelli S (1995). Seminal root morphology and coleoptile length in wild (*Hordeum vulgare* ssp. spontaneum) and cultivated (*Hordeum vulgare* ssp. vulgare) barley. Euphytica 86:73-80.

Liu F, Stützel H (2004). Biomass partitioning, specific leaf area and water use efficiency of vegetable amaranth (*Amaranthus* spp.) in response to water stress. Sci. Hortic. 102:15–27.

Liu F, Christian R, Shahanzari JA, Andersen MN, Jacobsen EE (2005). ABA regulated stomata control and photosynthetic water use efficiency of potato (*Solanum tuberosum* L.) during progressive soil drying. Plant Sci. 168:831-836.

López-Bucio J, Cruz-Ramírez A, Herrera-Estrella L (2003). The role of nutrient availability in regulating root architecture. Curr. Opin. Plant Biol. 6:280-287.

Loss SP, Siddique KHM (1994). Morphological and physiological traits associated with wheat yield increases in Mediterranean environments. Adv. Agron. 52:229-276.

Lowlor DW, Cornic G (2002). Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant. Cell Environ. 25:275-294.

Malamy JE (2005). Intrinsic and environmental response pathways that regulate root system architecture. Plant Cell Environ. 28:67-77.

Manivannan P, Jaleel CA, Kishorekumar A, Sankar B, Somasundaram R, Sridharan R, Panneerselvam R (2007). Drought stress induced changes in the biochemical parameters and photosynthetic pigments of cotton (*Gossypium hirsutum* L.). Indian J. Appl. Pure Biol. 52:369-372.

Marron N, Delay D, Petit JM, Dreyer E, Kahlem G, Delmotte FM, Brignolas F (2002). Physiological traits of two *Populus* × *euramericana* clones, Luisa avanzo and dorskamp, during water stress and re watering cycle. Tree Physiol. 22:849-858.

Martínez JP, Ledent JF, Bajji M, Kinet JM, Lutts S (2003). Effect of water stress on growth, Na+ and K+ accumulation and water use efficiency in relation to osmotic adjustment in two populations of *Atriplex halimus* L. Plant Growth Regul. 41: 63-73.

McAdam SAM, Brodribb TJ (2012). Fern and lycophyte guard cells do not respond to endogenous abscisic acid. Plant Cell 24:1510-1521.

Morgan JM (1984). Osmoregulation and water stress in higher plants, Annu. Rev. Plant Physiol. 35:299-319.

Morgan JM, Hare RA, Fletcher RJ (1986). Genetic variation in osmoregulation in bread and durum wheats and its relationship to grain yield in a range of field environments. Aust. J. Agric. Res. 37:449-457.

Mori IC, Murata Y (2011). ABA signaling in stomatal guard cells: lessons from *Commelina* and *Vicia*. J. Plant Res. 124:477-487.

Nanjo T, Kobayashi M, Yoshiba Y, Sanada Y, Wada K, Tsukaya H, Kakubari Y, Yamaguchi Shinozaki K, Shinozaki K (1999). Biological functions of proline in morphogenesis and osmotolerance revealed in antisense transgenic Arabidopsis thaliana. Plant J. 18:185-193.

Nativ R, Ephrath JE, Berliner PR, Saranga Y (1999). Drought resistance and water use efficiency in *Acacia saligna*. Aust. J. Bot. 47:577–586.

Nautiyal PC, Ravindra V, Joshi YC (2002). Dry matter partitioning and water use efficiency under water deficit during various growth stages in groundnut. Indian J. Plant Physiol. 7:135-139.

Nicholas S (1998). Plant resistance to environmental stress. Curr. Opin. Biotechnol. 9:214-219.

Outlaw WH (2003). Integration of cellular and physiological functions of guard cells. Crit. Rev. Plant Sci. 22:503-529.

Pan XY, Wang YF, Wang GK, Cao RD, Wang J (2002). Relationship between growth redundancy and size inequality in spring wheat populations mulched with clear plastic film. Acta Phytoecol. Sin. 26:177-184.

Pritchard J, Wyn Jones RG, Tomos AD (1991). Turgor, growth and rheological gradients of wheat roots following osmotic stress. J. Exp. Botany 42:1043-1049.

Rao RCN, Williams JH, Wadia KDR, Hubikk KT, Fraquhar GD (1993). Crop growth, water use efficiency and carbon isotope discrimination in groundnut genotypes under end season drought conditions. Ann. Appl. Biol. 122:357-367.

Ren J, Dai WR, Xuan ZY, Yao YA, Korpelainen H, Li CY (2007). The effect of drought and enhanced UV-B radiation on the growth and physiological traits of two contrasting poplar species. For. Ecol. Manage. 239:112-119.

Rock CD, Sakata Y, Quatrano RS (2010). Stress signaling I: the role of abscisic acid (ABA). In: Pareek A, Sopory SA, Bohner HJ, Govindjee (eds) Abiotic stress adaptation in plants. Springer Dordrecht pp. 33-73.

Sacks MM, Silk WK, Burman P (1997). Effect of water stress on cortical cell division rates within the apical meristem of primary roots of maize. Plant Physiol. 114:519-527.

Sahnoune M, Adda A, Soualem S, Kaid-Harche M, Merah O (2004). Early water deficit effect on seminal root barley. C.R. Bilogies III. Agron. 327:389-398.

Schroeder JI, Allen GJ, Hugouvieux V, Kwak JM, Waner D (2001). Guard cell signal transduction. Annu. Rev. Plant Physiol. Plant Mol. Biol. 52:627-658.

Sharp RE, LeNoble ME (2002). ABA, ethylene and the control of shoot and root growth under water stress. J. Exp. Bot. 53: 33-37.

Shimazaki Y, Ookawa T, Hirazawa T (2005). The root tip and accelerating region supress elongation of the decelerating region without any effects on cell turgor in primary roots of maize under water stress. Plant Physiol. 139:458-465.

Shinozaki K, Yamaguchi-Shinozaki K (2007). Gene networks involved in drought stress response and tolerance. J. Exp. Bot. 58:221-227.

Signora L, De Smet I, Foyer CH, Zhang H (2001). ABA plays a central role in mediating the regulatory effects of nitrate on root branching in Arabidopsis. Plant J. 28:655-662.

Tadina N, Germ M, Kreft I, Breznik B, Gaberščik A (2007). Effects of water deficit and selenium on common buckwheat (*Fagopyrum esculentum* Moench.) plants. Photosynthetica 45:472-476.

Turner NC (1997). Further progress in crop water relations. Adv. Agron. 58:293-338.

Wang Y, Ying J, Kuzma M, Chalifoux M, Sample A, McArthur C, Uchacz T, Sarvas C, Wan J, Dennis DT, McCourt P, Huang Y (2005). Molecular tailoring of farnesylation for plant drought tolerance and yield protection. Plant J. 43:413-24.

Wu TY, Feng DL, Bai ZY, Yang XJ, Liu XJ, Zheng YP (2009). Advances of research on drought-resistant mechanism of wheat. Agric. Res. Arid Areas 27:97-100.

Wu Y, Cosgrove Dj (2000). Adaptation of roots to low water potentials by changes in cell wall extensibility and cell wall proteins. J. Exp. Bot. 51:1543-1553

Wullschleger SD, Yin TM, DiFazio SP, Tschaplinski TJ, Gunter LE, Davis MF, Tuskan GA (2005). Phenotypic variation in growth and biomass distribution for two advanced-generation pedigrees of hybrid poplar. Can. J. For. Res. 35:1779-1789.

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Full Length Research Paper

Microbiological features of dystroferric and dystrophic red oxisols under sugar cane crops subject to different management procedures

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The Quirinopolis microregion (QMR) is located in the Southwestern Goiás State, and represents the recent expansions of sugar cane crops in the state. The maintenance of the agricultural and forest ecosystem productivity depends largely on the organic matter transformation process and therefore on the microbial biomass. The goal of this study was to evaluate the effects of the different management methods used in sugar cane cultivation on soil microbiological parameters and on indices derived from dystroferric (DfRO) and dystrophic (DRO) red oxisols in the region of Quirinopolis, Goiás. Eight sampling sites were selected in this study within areas occupied by DfRO and DRO, and two reference sites with semidecidual forest vegetation. The microbial biomass of the soil planted with sugar cane responded to changes caused by the two different soil management types (fertilized and not fertilized), so it may be considered a potential soil quality bioindicator. The soil profiles subject to crop succession before planting sugar cane favored the maintenance of the soil microorganism community in regards to other managements. DfRO profiles previously planted with soybean showed the best physical and chemical condition for a dynamic biomass balance. Profiles with vinasse showed no improvement in the development of soil microorganisms nor better physical and chemical conditions.

Key words: Soil quality, bioindicators, organic C, fertilization, soybean crop, vinasse.

INTRODUCTION

Sugarcane (*Saccharum* spp.) expansion in the Brazilian agricultural scenario has been driven by technologies that

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contribute to soil conservation, reduction of production cost and higher productivity (Schultz et al., 2010). The recent agriculture expansion has been driven towards the West Central (Brazilian savanna) since the late 1990s, particularly to the states of Mato Grosso do Sul (MS) and Goiás (GO). Both states have landscapes characterized by large - flat areas and weather conditions, especially, due to lower slopes and to the Oxisol domain. Therefore, the Brazilian savanna is considered to have a greater agricultural potential for sugar cane (among others) cropping.

The Quirinopolis microregion (QMR) is located in the Southwestern region of the state of Goiás, and represents this recent expansion process of the sugar cane, having a high aggregation of large mills for sugar and bioethanol production. Sugar cane cultivation at the QMR began in 2004. The first crop was harvested in the 2006/2007 season growing in areas under dystroferric red oxisol (DfRO) (EMBRAPA, 2006), previously intended for grain production (corn and soybean), and dystrophic red oxisol (DRO), previously intended for extensive cattle raising .

The soil is an open system that concentrates plant organic matter (carbon or C) and the products of organic C transformations. The vegetation is the main factor responsible for depositing organic matter into the soil (Santos et al., 2008). Vegetation type and environmental conditions are factors that determine quantity and quality of the organic matter being deposited. Thus, vegetation influences soil heterogeneity and decomposition rates of the material deposited on the soil surface (Moreira & Siqueira, 2002; Scherer-Lorenzen et al, 2007). Continued use of the soil, a natural resource, through anthropic activities, such as sugar cane (*Saccharum* spp*.)* planting may alter environmental structure. Organic C conservation is essential for nutrient recycling, water and energy flow balance, C storage, gas emissions and maintenance of animal and vegetal diversity (Mendes et al., 2009).

The maintenance of agricultural and forest ecosystem productivity depends mostly on the transformation of organic C and, consequently, on the microbial biomass (Gama-Rodrigues and Gama-Rodrigues, 2008). Therefore, the interest in elements of the soil biological function under natural and agricultural systems has grown (Matsuoka et al., 2003). The soil microbial communities are responsible for the decomposition of organic compounds, through nutrient cycling and soil energy flow. Microbial biomass and activity in the soil have been identified as the characteristics most sensible to soil quality changes caused by changes in soil use and management practices (Trannin et al., 2007). For this reason, the microbial community may be an important bioindicator in assessing soil quality, and consequently, the quality of an agroecosystem.

The microbial biomass is the living fraction of soil organic C, and comprises bacteria, fungi, actinomycetes, protozoa and algae. It is an important bioindicator, once it operates in the natural decomposition processes, interacting with nutrient dynamics and regeneration of stability of the aggregates (Franzluebbers et al., 1999; Da Silva et al., 2010). The microbial biomass is influenced by seasonal humidity and temperature variation, soil management, cultivation and by plant residues. The microbial biomass represents a small portion of the active fractions of organic matter (De Luca, 1998; Gama-Rodrigues et al., 2005), comprising only 2 to 5% of the soil organic C. Nevertheless, microbial biomass is more sensible than organic C and total N contents to measure changes in organic matter caused by farming practices (Gama-Rodrigues, 1999).

In this context, the use of microbiological attributes (for example, microbial biomass and derived indexes), has been proposed to assess soil quality, in terms of different management practices (Doran and Parkin, 1994). Thus, studies regarding soil biodynamics in different sugar cane cropping systems may lead to different forms of more sustainable management, mitigating environmental consequences of negative impacts. Therefore, the objective of this study was to monitor microbiological indices to evaluate land-use change in sugarcane cropping on dystroferric and dystrophic red oxisols, in the region of Quirinopolis, Goiás.

MATERIALS AND METHODS

Study area

The study was carried out in Southwest Goiás (GO), at the Quirinópolis microregion (QMR), where the sugar cane expansion is recent. The QMR has rapidly reached prominence as the new sugar cane expansion center (Borges, 2011), replacing grain and pasture areas (Figure 1). The soils of the QMR rest on Cretaceous sandstones and basalts of the Paraná Sedimentary Basin, which sustains a rather smooth relief consisting of plateaus leveled at altitudes ranging from 400 to 1000 m. These plateaus are covered by Oxisols that range from medium to clayey texture, and vary from dystrophic to dystroferric as related to base saturation (Embrapa, 2006). The flat areas were preferred for the sugar cane expansion due to its characteristics, which favor intensive mechanization and soil management for planting. The climate at the QMR is tropical wet and dry (Aw) with two disparate seasons and significant annual variations in regards to humidity, precipitation and temperature, according to climate typology established by Koeppen (1918). The QMR has frequent rainfalls from October to March and a dry winter from June to September, with transitions from wet and dry periods and total average annual precipitation of 1700 mm, average temperature of 24°C and a temperature range of ca. 15°C during the year.

Sampling design

Eight sampling sites were selected in dystroferric red oxisol (DfRO) and Dystrophic red Oxisol (DRO) areas, and two reference profiles, with native vegetation (semidecidual forest). Semidecidual forests

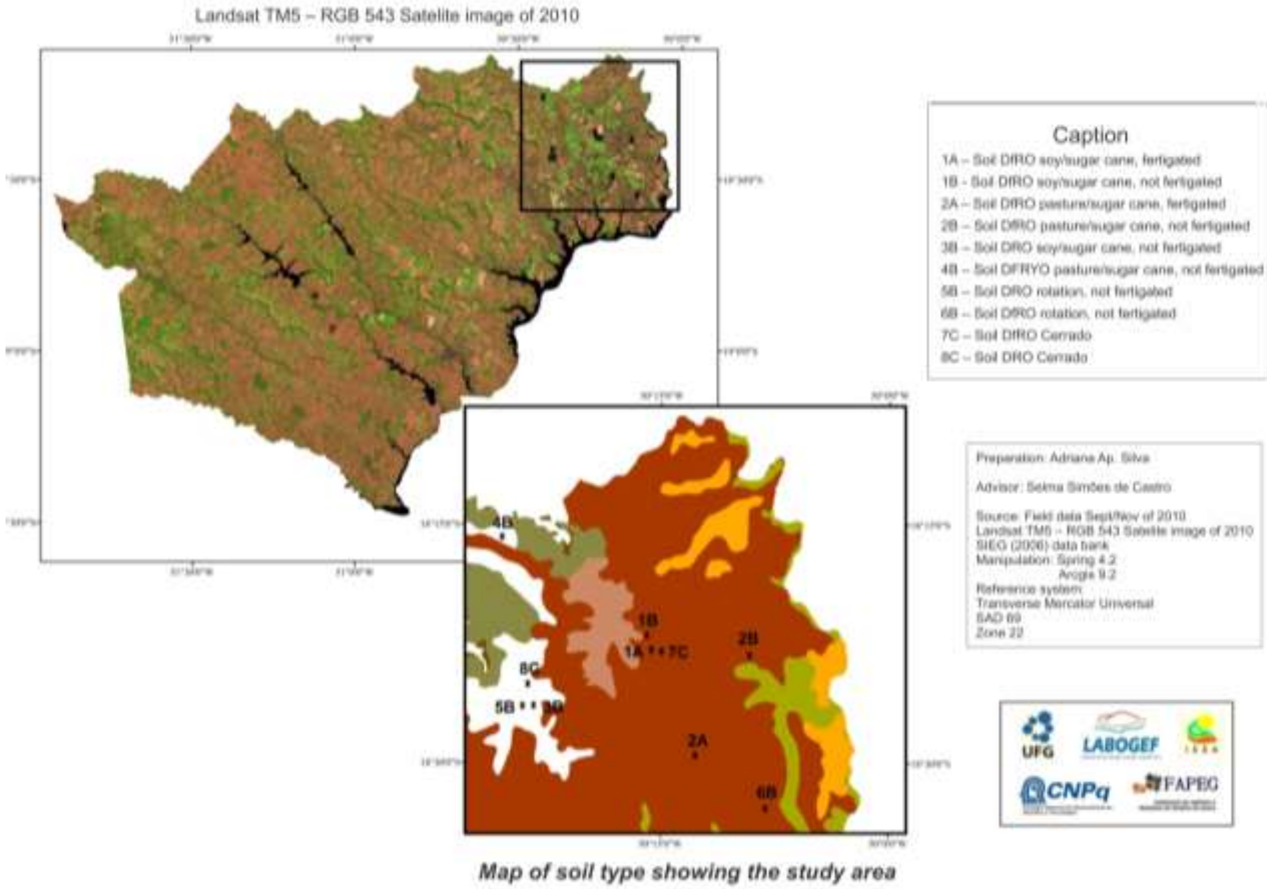

Figure 1. Map of the Quirinopolis micro-region, Goiás with the location of the studied soil profiles. DFRO means dystroferric red oxisol, DRO means dystrofic red oxisol and DFRYO means dystroferric red-yellow oxisol. Source: Silva (2012).

occupied most of the southern QMR before 1980 (Figure 1). The cultivated soil profiles were described following recommendations made by Santos et al. (2005) and were chosen in regards to management history. All sugarcanes are plantations in 4th cutting of the 1st productive cycle. The same sugar cane cultivar (SP81-3250) was planted. Planting and harvesting of sugarcane were performed by machines, and the crop was harvested without prior burning (June, 2008, May, 2009 and May, 2010). Soil preparation (plowing and harrowing) was the same at all sites, varying to fertigation with vinasse only. Detailed description, land use and management of each site are present in Table 1.

The profiles were analyzed in open trenches (near 2 × 1.5 × 2.5 m) where morphological descriptions regarding the identification of compressed horizons were performed. Samples were gathered and described in May, 2010 (dry season; profiles 1A, 1B, 2A, 2B and 7C) and September, 2010 (rainy season; 3B, 4B, 5B, 6B and 8C). The samples were carried out in each profile at five depths and three replicates of soil samples, with approximately 1.5 kg each. Soil samples were homogenized and divided into two parts: 1 kg for physical and chemical characterization and 0.5 kg to determine biotic variables. Soil samples for biological studies were gathered in sterile containers and stored in a cold chamber (4°C) until analysis.

Soil samples for physical and chemical characterization were stored in gas-permeable plastic bags. These soil samples were air-dried and subsequently sieved through 2.0 mm mesh opening. The following variables were determined: pH in $CaCl_2$ (ratio of 1:2.5 for soil and solution – v/v); P and K (extraction with Mehlich^{-1}, P determination by colorimetry and K by flame photometry); Ca and Mg (extracted with 1 mol L^{-1} KCl, determined by atomic absorption spectrophotometry and titration, respectively); organic C (determined using sodium dichromate and colorimetry); potential acidity (H + Al/potentiometer); cation exchange capacity (CEC); base saturation (V%). All variables were determined according to Embrapa (1997). The microorganisms (fungi and bacteria) were quantified and underwent microbial biomass analyzes, following Moreira and Siqueira (2002). Fungi and bacteria were quantified using the pour plate technique (Filho and Oliveira, 2007).

Microbial biomass C (MB-C) was determined using the microwave irradiation technique followed by oxidation with potassium dichromate ($K_2Cr_2O_7$ 0.066 mol L^{-1}) and titration with $(NH_4)_2Fe(SO_4)_2$ 0.033 mol L^{-1}. The MB-C was estimated (mg kg^{-1} of soil MB-C) using the following equation: MB-C (mg kg^{-1}) = CF × k_c, where CBM is the microbial biomass C; CF represents the flow of C obtained from the difference between the C quantity (mg kg^{-1}) in the extract of the irradiated sample and in the non-irradiated sample, and kc is the correction factor (Mendonça and Matos, 2005). Basal respiration was also determined (CO_2-C) through sample incubation with CO_2 trapping in NaOH 1 mol L^{-1} solution for seven days, as

Table 1. Characterization of the studied soil profiles.

Profile	Elements of the physical environment				Use and management	
	Soil	Geology	Declivity	Altitude (m)	Land use before cane/vegetation	Fertigation
1A	DfRO	Basalt	0 to 3%	576	Soybean	Fertigated
1B				540	Soybean	Not Fertigated
2A				503	Pasture	Fertigated
2B				460	Pasture	Not Fertigated
6B				458	Succession: pasture/soybean/sugar cane	Not Fertigated
7C				545	Natural vegetation	-
3B	DRO	Sandstone	0 to 3%	558	Soybean	Not Fertigated
4B			3 to 6%	595	Pasture	Not Fertigated
5B			0 to 3%	633	Succession: pasture/soybean/sugar cane	Not Fertigated
8C			3 to 6%	589	Natural vegetation	-

Source: Silva (2012)

proposed by Jenkinson and Powlson (1976).

The metabolic quotient (qCO_2) was obtained following the Anderson and Domsch (1990) protocol after measurement of MB-C and CO_2-C. The qCO_2 was calculated by the ratio CO_2-C/MB-C. In turn, microbial quotient (*q*Mic) was obtained by the ratio of MB-C and organic C. The parameters regarding microbial biomass, derived indices and the physical and chemical variables were submitted to multivariate analysis (MANOVA) by the principal component analysis (PCA), using the software PAST 2.17c (Hammer et al., 2001). PCA was carried out for each horizon, containing the biological and chemical parameters {Chemical variables: H+Al (potential acidity); CEC (cation exchange capacity); OM (organic C); K (potassium); Ca (Calcium); pH (hydrogen potential); V (percent base saturation); Mg (magnesium); P (phosphorus); Microbiological variables: CO2q: Carbon dioxide quotient (metabolic quotient); C-CO2: basal respiration: MICq: microbial quotient; MBS-C: microbial biomass; Bacteria (Bac-UFC); Fungi (F-UFC)} and the different dystroferric and dystrophic red oxisol profiles with the different management types.

RESULTS AND DISCUSSION

The DRO and DfRO profiles and its different management types had different locations in horizon A. Profiles 7C (native vegetation) and 1B (soybean as prior management and currently with sugar cane not fertigated) were grouped with most physical and chemical variables (Figure 2). Such results indicate that profile 1B favors a better balance of soil chemical components, compared to the other profiles evaluated.

The fertigated profiles 1A (soybean as prior management type) and 2A (with pasture as prior management type) were grouped with H + Al and P (Figure 2). This result indicates that the use of vinasse directly influences soil dynamics, and may alter soil chemical parameters in horizon A. The native vegetation (for DfRO and DRO) was grouped with most biological parameters related to soil microbial biomass in horizon A (Figure 3). Therefore, horizon 1A favors biological community maintenance. These results support previous findings that report a greater diversity of processes in natural ecosystems reducing soil imbalance factors (Roscoe et al., 2006).

Profiles 6B and 4B had an overall tendency similar to natural environments (Figure 3). Thus, better conditions for the development of the soil microbial community were observed in horizon A. The maintenance of plant residues on the soil surface and the absence of soil disturbance enabled better conditions for soil biological activity (Lisboa et al., 2012). On the other hand, the fertigated profiles (1A and 2A) did not have grouped biological attributes, indicating that stressful conditions for biological soil communities exist in horizon A (Figure 2).

The chemical variables of profile 7C (Horizon AB; DfRO) are grouped with the chemical variables of the native vegetation (Figure 3). This result may be due to the increased nutrient recycling in the natural system, consequent of the higher input of organic substrates into the soil of horizon 7C. Organic C is a key nutrient source for plants, providing elements such as N, P, K, Ca, Mg and micronutrients (Mielniczuk, 2008; Salton et al., 2011). The profile among the ones with different managements most similar to the native vegetation was profile 1B.

The biological attributes (Figure 3) of horizon AB are grouped within the native vegetation profiles (7C and 8C), along the principal component axes. Probably, the lack of anthropogenic interference coupled with the accumulation of leaf litter on the soil surface led to this result. Profiles 6B (succession pasture/soybean/cane, not fertigated, DfRO) and 5B (succession pasture/soybean/cane, not fertigated, DRO) had the best conditions among the soils that underwent management, indicating the occurrence of an organic C of better quality among the profiles.

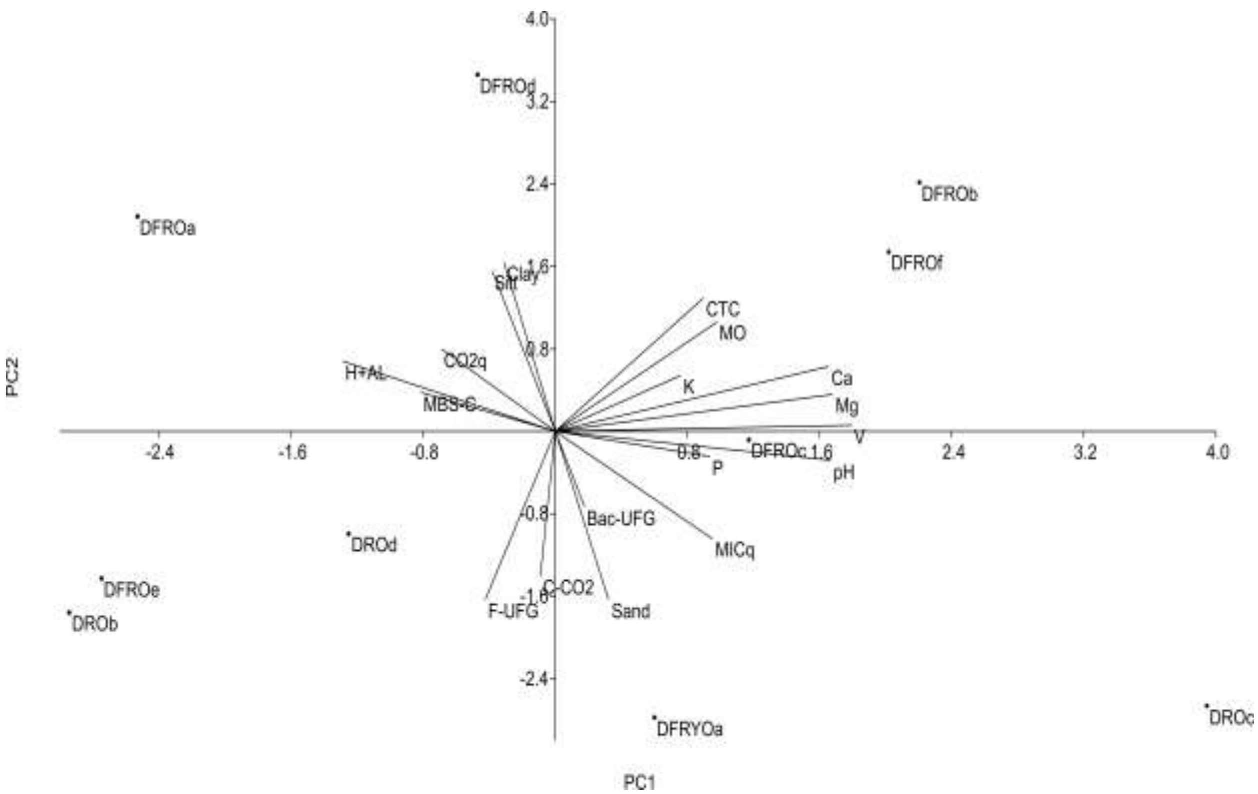

Figure 2. Principal component analysis of the biological, physical and chemical attributes of the dystrophic (DRO) and dystroferric (DfRO) red Oxisols, with different managements in horizon A, Quirinópolis, Goiás. DfROa: 1A – soybean/sugarcane, fertigated; DfROb: 1B – soybean/sugarcane, not fertigated; DfROc: 2A – pasture/sugarcane, fertigated; DfROd: 2B – pasture/sugar cane, not fertigated; DfROe: 6B – Rotation pasture/soybean/sugarcane, not fertigated; DfROf: semideciduous forest; DFRYOa: 3B – soybean/sugarcane, not fertigated; DROb: 4B – pasture/sugarcane, not fertigated; DROc: 5B – Rotation pasture/soybean/sugarcane, not fertigated; DROd: 8C – semideciduous forest. Physical Variables: sand, silt and clay; Chemical variables: H+Al (potential acidity); CEC (cation exchange capacity); OM (organic C); K (potassium); Ca (Calcium); pH (hydrogen potential); V (percent base saturation); Mg (magnesium); P (phosphorus); PC1 and PC2: 71%. Microbiological variables: CO_2q: Carbon dioxide quotient (metabolic quotient); C-CO_2: basal respiration: MICq: microbial quotient; MBS-C: microbial biomass; Bacteria (Bac-UFC); Fungi (F-UFC) (PC1 and PC2: 74%).

The physical and chemical characteristics of horizon BA (Figure 4) are grouped with profile 7C (native vegetation, DfRO) and 1B (soybean/sugar cane, not fertigated) along the principal component axes. Chemical characteristics such as pH and available N, P and K are used as soil quality indicators. The elements N, P and S, are nutrients that comprise the organic C. Thus, these nutrients are available to the plants and are directly influenced by the mineralization process (Vezzani et al., 2008).

Organic C is not an endless source of N, P and S. The annual mineralized quantity of each of these nutrients must be restored to the soil, either through biological fixation (as for N), or fertilization (for N, P and S). Thus, the OM is a reservoir for these elements and crucial for biological cycling, enabling the soil to store and promote cycling of elements to the atmosphere (Duxbury et al., 1989; Pôrto et al, 2009).

Profile 1B has a better condition in establishing organic C and, consequently, for nutrient cycling, although not consisting of native vegetation due to prior soil management using fertilization and other techniques. The biological attributes in the PCA of horizon BA were once again grouped with profile 8C (DfRO) and 7C (DRO), both with native vegetation (Figure 4). Profile 4B (previously managed with pasture/sugar cane not fertigated) has a high organic C content, as shown by the high correlation between MICq and B-BMC. This profile consists of horizon BA and has pasture as the previous soil use. Pasture soil management does not revolve the soil, thus conserving soil structure, and has the aggressive and abundant grass root system. Pasture soil management confers such characteristics to horizon AB, which in turn support results found in other studies (Marchiori-Júnior, 1998; Matsuoka et al., 2003).

Profile 3B (DRO with soybean/sugar cane, not fertigated) was highly correlated with CO_2q (Figure 4)

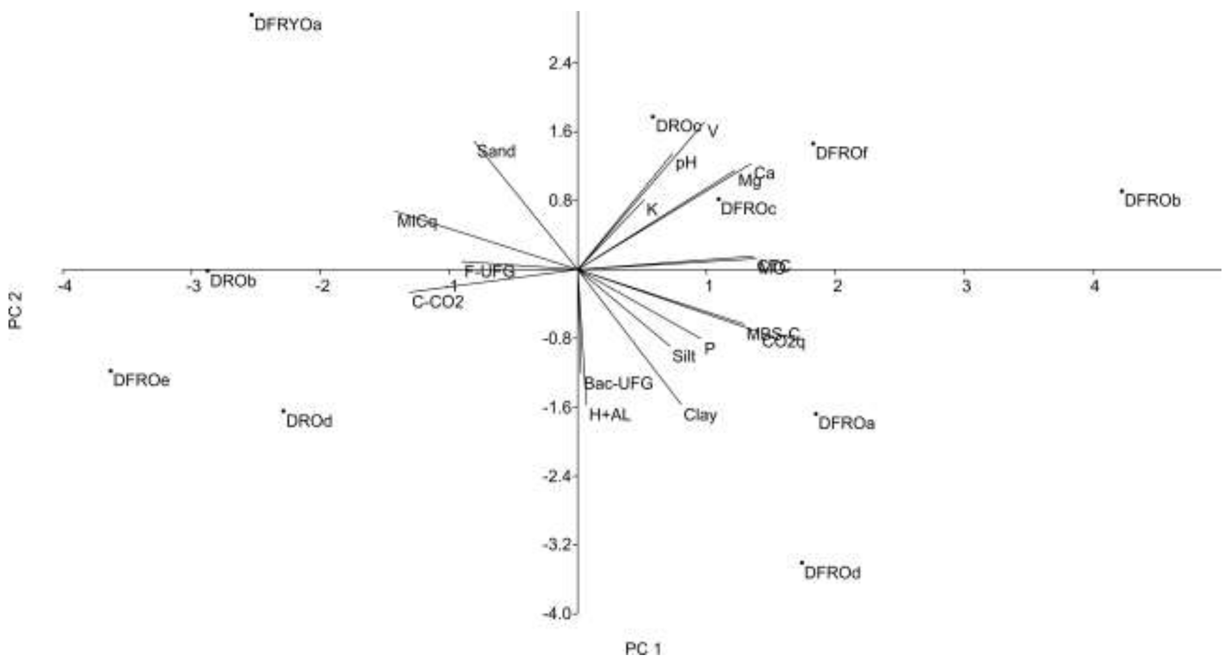

Figure 3. Principal component analysis of the physical and chemical attributes of the dystrophic (DRO) and dystroferric (DfRO) red oxisols, with different managements in Horizon AB, Quirinópolis, Goiás. DfROa: 1A – soybean/sugarcane, fertigated; DfROb: 1B – soybean/sugarcane, not fertigated; DfROc: 2A – pasture/sugarcane, fertigated; DfROd: 2B – pasture/sugarcane, not fertigated; DfROe: 6B – Rotation pasture/soybean/sugarcane, not fertigated; DfROf: semideciduous forest; DFRYOa: 3B – soybean/sugarcane, not fertigated; DROb: 4B – pasture/sugarcane, not fertigated; DROc: 5B – Rotation pasture/soybean/sugarcane, not fertigated; DROd: 8C – semideciduous forest. Physical Variables: sand, silt and clay; Chemical variables: H+Al (potential acidity); CEC (cation exchange capacity); OM (organic C); K (potassium); Ca (Calcium); pH (hydrogen potential); V (percent base saturation); Mg (magnesium); P (phosphorus); PC1 and PC2: 65%. Microbiological variables: CO_2q: Carbon dioxide quotient (metabolic quotient); C-CO_2: basal respiration: MICq: microbial quotient; MBS-C: microbial biomass; Bacteria (Bac-UFC); Fungi (F-UFC) (PC1 and PC2: 78%).

indicating an environment with stressful conditions for soil microbiota. The CO_2q is considered important to assess the effects of environmental conditions on microbial soil communities (Anderson and Domsch, 1993; Zhang et al., 2008). Higher CO_2q values under stressful conditions (for example, excess or lack of nutrients in the soil, altering the physical and chemical soil structures) have implications in more carbon used in biomass maintenance.

The chemical attributes of horizon Bw_1 were grouped with profile 7C (native vegetation, DfRO), 1B (soybean/sugar cane, not fertigated) and 2B (pasture/sugar cane not fertigated) (Figure 5). The microbiota is fostered in different soil management systems, due to plant residue composition (that is, residues from different plant species) and soil preparation methods. This leads to differences in the microbial activity, in the nitrogen immobilization-mineralization relationship and residue decomposition rates (Resck et al., 2008).

The number and location of the organic residue input sites affects the active pool of organic C (Hernández and Hernandez and Hernandez, 2002). This explains why profiles 1B (previously with soybean) and 2B (previously with pasture) have higher concentrations of elements, that is, two previous management types presenting an active cycling of nutrients in the same horizon.

In horizon Bw_1, P was highly correlated with profile 2A (pasture/fertigated sugar cane, DfRO) and 3B (soybean/sugar cane not fertigated, DRO) (Figure 5). Cambuim and Cordeiro (1986), studying the use of vinasse as a fertilizer and soil conditioner, observed a meaningless phosphorous accumulation, while potassium, calcium and magnesium contents occurred almost in direct proportion to the vinasse dose. This high correlation between phosphorus and the soil profiles (Cambuim and Cordeiro, 1986) is not due to vinasse, but to previous soil managements, also explaining why profile 3B was also highly correlated with phosphorus in this study.

The variables representing biological attributes were grouped with profile 8C (native vegetation, DRO) (Figure 5). Profiles 4B (pasture/sugar cane, DRO), 5B (succession/pasture/soybean/sugarcane/not fertigated,

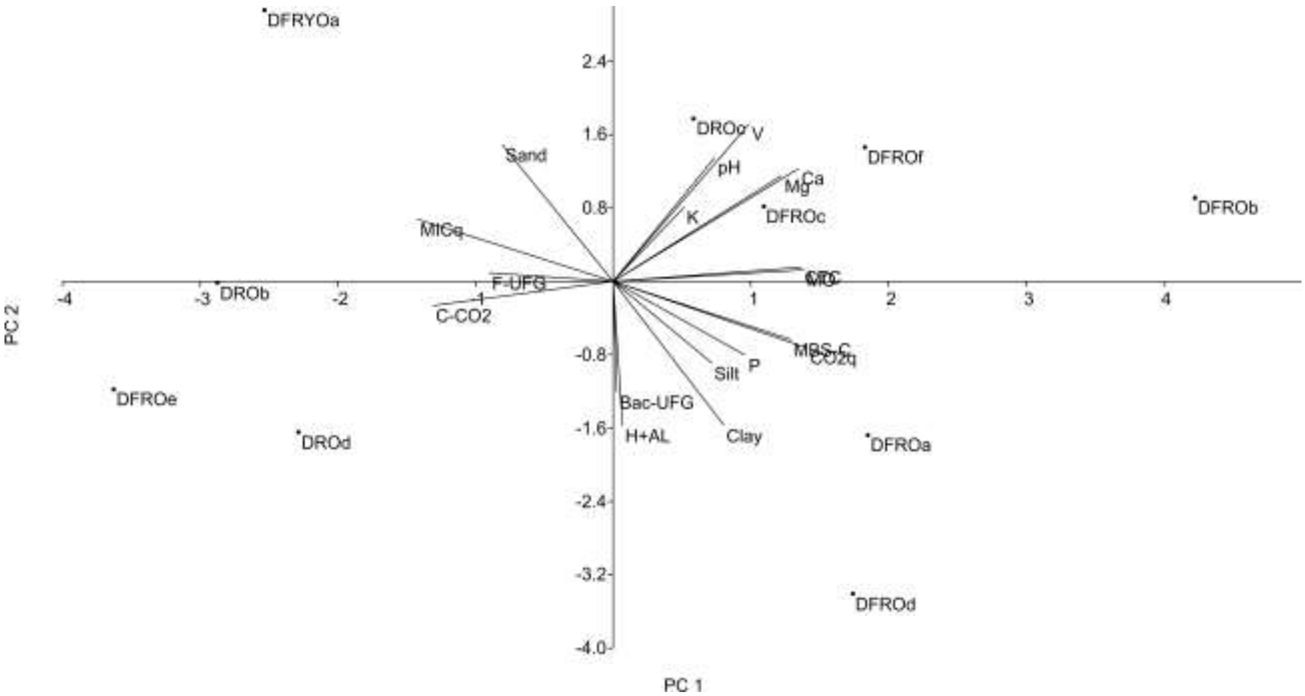

Figure 4. Principal component analysis of the physical and chemical attributes of the dystrophic (DRO) and dystroferric (DfRO) red oxisols, with different managements in Horizon BA, Quirinópolis, Goiás. DfROa: 1A – soybean/sugarcane, fertigated; DfROb: 1B – soybean/sugarcane, not fertigated; DfROc: 2A – pasture/sugarcane, fertigated; DfROd: 2B – pasture/sugarcane, not fertigated; DfROe: 6B – Rotation pasture/soybean/sugarcane, not fertigated; DfROf: semideciduous forest; DFRYOa: 3B – soybean/sugarcane, not fertigated; DROb: 4B – pasture/sugarcane, not fertigated; DROc: 5B – Rotation pasture/soybean/sugarcane, not fertigated; DROd: 8C – semideciduous forest. Physical Variables: sand, silt and clay; Chemical variables: H+Al (potential acidity); CEC (cation exchange capacity); OM (organic C); K (potassium); Ca (Calcium); pH (hydrogen potential); V (percent base saturation); Mg (magnesium); P (phosphorus); PC1 and PC2: 74.34%. Microbiological variables: CO_2q: Carbon dioxide quotient (metabolic quotient); C-CO_2: basal respiration: MICq: microbial quotient; MBS-C: microbial biomass; Bacteria (Bac-UFC); Fungi (F-UFC) (PC1 and PC2: 81.99%).

DRO) and 6B (succession/pasture/soybean/sugarcane/not fertigated, DfRO) are similar to the native vegetation in horizon Bw_1, as indicated by cluster of the microbial biomass parameters and microbial quotient in the soil profiles. These results support the statement that a greater balance and diversity of factors would occur in natural environments, possibly mitigating negative impacts in the soil (Júnior, 2012).

On the other hand, profile 3B (soybean/sugar cane not fertigated, DRO) was grouped with the metabolic quotient (CO_2q), indicating that profile 3B provides stressful environmental conditions for soil biological communities on horizon Bw_1 (Figure 5). In horizon Bw_2, the variables CEC, Ca, V, Mg, K and pH were grouped with profiles 1B (soybean/sugarcane not fertigated, DfRO) and 2B (pasture/sugarcane not fertigated, DfRO), and were positioned near the native vegetation (DfRO, 7C). Clay and silt were grouped in profile 7C (Figure 6). These results show that profiles 1B, 2B and 7C have a higher cationic exchange capacity, favoring the chemical, physical and biological soil relationships. Consequently, these systems favor a better balance of chemical and physical soil components.

Phosphorus and sand were more correlated with profile 2A (Pasture/sugarcane fertigated, DfRO) (Figure 6). These results show that, as observed by Cambuim and Cordeiro (1986), this high correlation of phosphorus with profile 2A, is not a consequence of phosphorus input from the neighboring population. In contrast, such correlation was due to the soil preparation methods and input of residues and other elements from managements prior to the sugar cane. Organic C was grouped with profile 6B (succession pasture/soybean/sugarcane not fertigated, DfRO) (Figure 6). The proximity between OM and profile 6B suggests that a dynamic soil management (as in succession) leads to more complex biotic interactions, and a greater likelihood of emerging important soil regulation properties and approaching natural conditions (Beare et al., 1995).

The biological attributes of horizon Bw_2 were grouped with profile 4B (pasture/sugarcane not fertigated, DRO) and profile 1B (soybean/sugarcane not fertigated, DfRO)

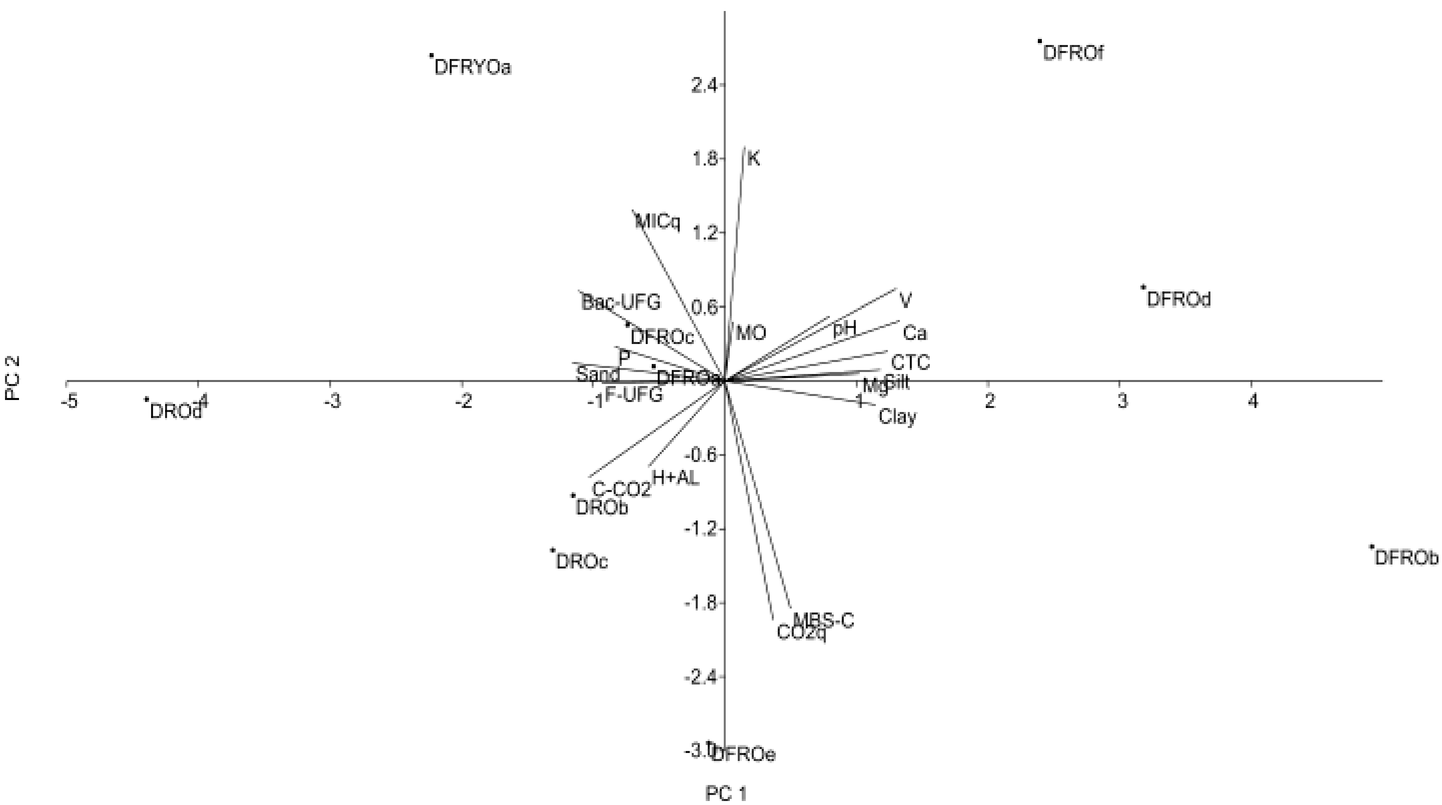

Figure 5. Principal component analysis of the physical and chemical attributes of the dystrophic (DRO) and dystroferric (DfRO) red oxisols, with different managements in Horizon Bw_1, Quirinópolis, Goiás. DfROa: 1A – soybean/sugarcane, fertigated; DfROb: 1B – soybean/sugarcane, not fertigated; DfROc: 2A – pasture/sugarcane, fertigated; DfROd: 2B – pasture/sugarcane, not fertigated; DfROe: 6B – Rotation pasture/soybean/sugarcane, not fertigated; DfROf: Semideciduous forest; DFRYOa: 3B – soybean/sugarcane, not fertigated; DROb: 4B – pasture/sugarcane, not fertigated; DROc: 5B – Rotation pasture/soybean/sugarcane, not fertigated; DROd: 8C – semideciduous forest. Physical Variables: sand, silt and clay; Chemical variables: H+Al (potential acidity); CEC (cation exchange capacity); OM (organic C); K (potassium); Ca (Calcium); pH (hydrogen potential); V (percent base saturation); Mg (magnesium); P (phosphorus); PC1 and PC2: 67.67%. Microbiological variables: CO_2q: Carbon dioxide quotient (metabolic quotient); $C\text{-}CO_2$: basal respiration: MICq: microbial quotient; MBS-C: microbial biomass; Bacteria (Bac-UFC); Fungi (F-UFC) (PC1 and PC2: 82.26%).

(Figure 6). Organic C preservation tends to be larger in pasture soil, because soil revolving is minimal. Carbon input is higher in pasture soil than in cultivated areas (Lathewell and Bouldin, 1981; Carneiro et al, 2008). According to Silveira et al. (2005), legumes are crucial as nutrient suppliers, once this plant group promptly provides nutrient for succeeding cultures due to the rapid residue decomposition. The previous management with soybean or pasture leads to an organic C increase and, consequently, an increase of microorganisms (Silveira et al, 2005; Carneiro et al, 2008). Profiles 4B and 1B exhibited a similar characteristic for horizon Bw_2 due to prior soil management.

The variables basal respiration ($C\text{-}CO_2$) and metabolic quotient (CO_2q) were grouped with profile 8C (native vegetation, DRO) (Figure 6). This correlation may have occurred due to the large amount of roots or to the deposition of organic substrates in horizon Bw_2 (Silva et al*.,* 2010).

Conclusions

The microbial biomass of the soil may be a bioindicator used to assess soil quality, once it responds to the changes caused by the two different management types (fertigated and not fertigated). The profiles undergoing culture succession before sugar cane planting favor the soil microbial community in regards to other management types. The profile previously planted with soybean in the DfRO, had the best physical and chemical conditions for the dynamic balance of the soil biomass.

The profiles with vinasse showed no improvement regarding soil microorganism development or physical and chemical conditions. Therefore, previous soil use was important to identify soil quality conditions. Better

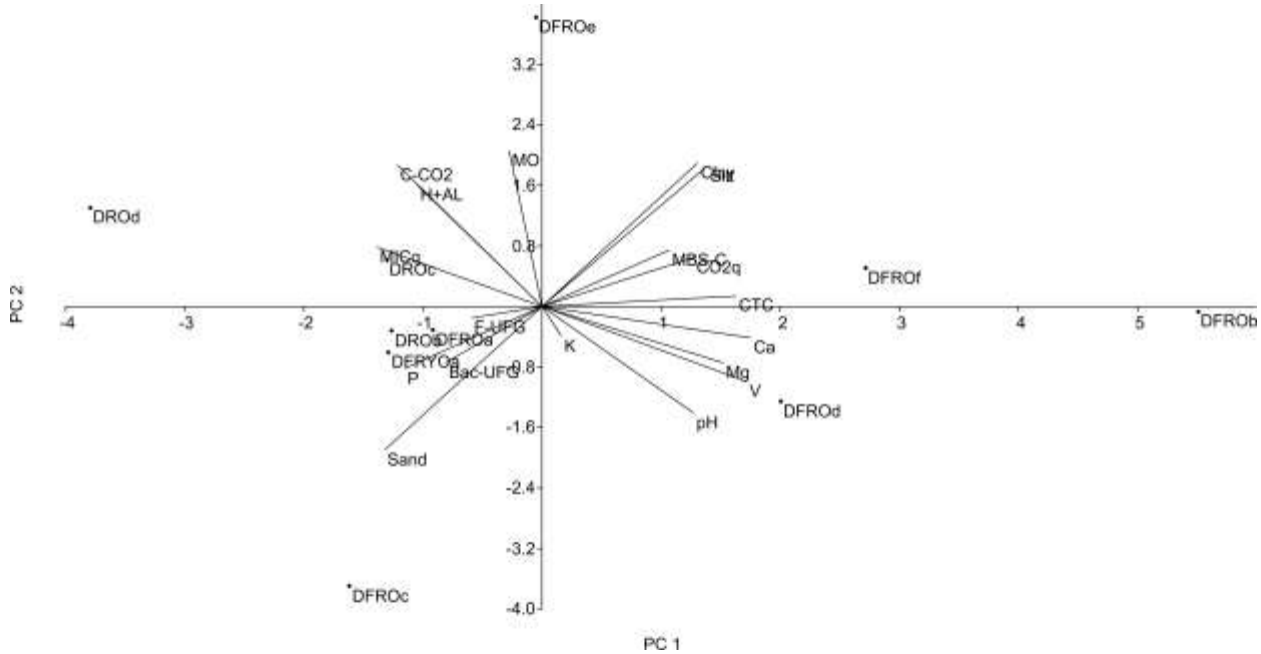

Figure 6. Principal component analysis of the physical and chemical attributes of the dystrophic (DRO) and dystroferric (DfRO) red oxisols, with different managements in Horizon Bw_2, Quirinópolis, Goiás. DfROa: 1A – soybean/sugarcane, fertigated; DfROb: 1B – soybean/sugarcane, not fertigated; DfROc: 2A – pasture/sugarcane, fertigated; DfROd: 2B – pasture/sugarcane, not fertigated; DfROe: 6B – Rotation pasture/soybean/sugarcane, not fertigated; DfROf: Semideciduous forest; DFRYOa: 3B – soybean/sugarcane, not fertigated; DROb: 4B – pasture/sugarcane, not fertigated; DROc: 5B – Rotation pasture/soybean/sugar cane, not fertigated; DROd: 8C – Semideciduous forest. Physical Variables: sand, silt and clay; Chemical variables: H+Al (potential acidity); CEC (cation exchange capacity); OM (organic C); K (potassium); Ca (Calcium); pH (hydrogen potential); V (percent base saturation); Mg (magnesium); P (phosphorus); PC1 and PC2: 72.34%). Microbiological variables: CO_2q: Carbon dioxide quotient (metabolic quotient); C-CO_2: basal respiration: MICq: microbial quotient; MBS-C: microbial biomass; Bacteria (Bac-UFC); Fungi (F-UFC). (PC1 and PC2: 78.20%).

conditions were found for soil that underwent a succession and were planted with crops prior to sugar cane.

Conflict of interests

The authors have not declared any conflict of interest

REFERENCES

Anderson JPE, Domsch KH (1993). The metabolic quotient (qCO2) as a specific activity parameter to asses the effects of environmental conditions, such as pH, on the microbial biomass of forest soils. Soil Biol. Biochem. 25:393-395.

Anderson TH, Domsch KH (1990). Application of ecophysiological quotients (qCO2and qD) on microbial biomasses from soils of different cropping histories. Soil Biol. Biochem. 22:251-255.

Beare MH, Coleman DC, Crossley DA, Hendrix PF, Odum EP (1955). A Hierarchical approach to evaluating the significance of soil biodiversity to biogeochemical cycling. Plant soil 170:5-22.

Borges VMS (2011). Formação de uma nova centralidade do setor sucroenergético no Cerrado: o caso de Quirinópolis, Estado de Goiás. 2011. 333 f. Tese (Doutorado) Instituto de Estudos Socioambientais, Universidade Federal de Goiás, Goiânia, Cambuim FA, Cordeiro D (1986) A Ação da vinhaça sobre pH, acidez total, acumulação e liviviação de nutrientes em solo arenoso. STB 4(4):27-33.

Carneiro MAC, Assis PCR, Melo LBC, Pereira HS, Paulino HB, Neto ANS (2008). Atributos Bioquímicos em dois solos de Cerrado sob diferentes sistemas de manejo e uso. Pesquisa Agropecuária tropical 38(4):276-283.

Da Silva RR, Silva MLN, Cardoso EL, Moreira FMS, Curi N, Alovisi AMT (2010). Biomassa e atividade microbiana em solo sob diferentes sistemas de manejo na região fisiográfica Campos das Vertentes – MG. Rev. Bras. Cienc. Solo 34:1585-1592.

De Luca TH (1998). Relationship of 0,5 M K2SO4 extractable anthronereactive carbon to indices of microbial activity in forest soils. Soil Biol. Biochem. 30:1293-1299.

Doran JW, Parkin TB (1994). Defining and assessing soil quality. In: Doran JW, Oleman DC, Bezdicek DF, Stewart BA. (eds). Defining soil quality for a sustainable environment. Madison, Soil Sci. Soc. Am. pp. 107-124..

Duxbury JM, Smith MS, Doran JW, Jordan C, Szott L, Vance E (1989). Soil organic matter as a source and a sink of plant nutrients. In: Dynamics of soil organic matter in tropical ecosystems. Coleman DC, Oades JM, et al. (eds.). Honolulu: NIFTAL Project. pp. 33-67.

EMBRAPA (1997). Manual de métodos de análise de solos. Centro Nacional de Pesquisas de Solos, Rio de Janeiro, Brasil, 2ª Ed. 212p.

EMBRAPA (2006). Sistema Brasileiro de Classificação de solos. Centro Nacional de Pesquisas de Solos, Rio de Janeiro, Brasil, 2ª Ed.

Filho GNS, Oliveira VL (2007). Microbiologia: Manual de aulas práticas. Editora UFSC, Florianópolis, Brasil. 160p.

Franzluebbers AJ, Haney RL, Hons FM (1999) Relationships of chloroform fumigation-incubation to soil organic matter pools. Soil Biol. Biochem. 31:395-405.
Gama-Rodrigues EF, Gama-Rodrigues AC (2008). Biomassa microbiana e ciclagem de nutrientes. In: Fundamentos da matéria orgânica do solo: ecossistemas tropicais e subtropicais. Santos GA, Silva LS, Canellas LP, Camargo FAO (eds.). Metrópole, Porto Alegre, Brasil. pp. 7-16.
Gama-Rodrigues EF (1999). Biomassa microbiana e ciclagem de nutrientes. In: Fundamentos da matéria orgânica do solo: ecossistemas tropicais e subtropicais. Santos GA, Camargo FAO. (eds.). Gênesis, Porto Alegre, Brasil. pp. 227-244.
Gama-Rodrigues EF, Barros NF, Gama-Rodrigues AC, Santos GA (2005). Nitrogênio, carbono e atividade da biomassa microbiana do solo em plantações de eucalipto. Rev. Bras. Cienc. Solo 9:893-901.
Hammer Ø, Harper DAT, Ryan PD (2001). PAST: Paleontological statistics software package for education and data analysis. Palaeontol. Electronica 4(1):9.
Hernandez-Hernandez RM, Hernandez DL (2002). Microbial biomass, mineral nitrogen and carbon content in Savana soil aggregates under conventional and no-tillage. Soil Biol. Biochem. 34(11):1563-1570.
Jenkinson DS, Powlson DS (1976). The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. Soil Biol. Biochem. 8:209-213.
Junior PP (2012). Bioindicadores de qualidade do solo em cultivos de cana-de-açúcar sob diferentes manejos. Dissertação (Mestrado – Agronomia) – Universidade Estadual de Mato Grosso do Sul – UEMS, 95 f.
Kõppen W (1918). Klassifications dês klimate nach temperatur, niedershlag und jahrelauf. Petermanns Geogr. Mitt. Goth 64:193-203.
Lathewell DJ, Bouldin DR (1981). Soil organic matter and soil nitrogen behaviour inoxidizing, thiosulfate producing heterotrophs in soil. Soil Biol. Biochem. 20(4):577-578.
Lisboa BB, Vargas LK, Silveira AO, Martins AF, Selbach PA (2012). Indicadores Microbianos de Qualidade do Solo em Diferentes Sistemas de Manejo. Rev. Bras. Cienc. Solo 36:45-55.
Marchior-Junior M (1998). Carbono, nitrogênio, biomassa microbiana e atividade enzimática num solo sob mata natural ou cultivado com pastagem ou algodoeiro 1998. 89 f. Dissertação (Mestrado em Agronomia – Produção Vegetal) – Universidade Estadual de São Paulo, Jaboticabal).
Matsuoka M, Mendes IC, Loureiro MF (2003). Biomassa microbiana e atividade enzimática em solos sob vegetação nativa e sistemas agrícolas anuais e perenes na região de Primavera do Leste/MT. Rev. Bras. Cienc. Solo 27:425-433.
Mendes IC, Hungria M, Reis-Junior FB, Fernandes MF, Chaer GM, Mercante FM, Zilli JE (2009). Bioindicadores para avaliação da qualidade dos solos tropicais: utopia ou realidade? Embrapa Cerrados, Planaltina, Brasil, Documentos. 246 p.
Mendonça ES, Matos ES (2005). Matéria orgânica do solo: métodos de análises. UFV ,Viçosa, Brasil. 107p.
Mielniczuk J (2008). Matéria orgânica e a sustentabilidade de sistemas agrícolas. In: Fundamentos da matéria orgânica do solo: ecossistemas tropicais e subtropicais. Santos GA, Silva LS, Canellas LP, Camargo FAO. (eds.). Metropole, Porto Alegre, Brasil pp. 01-07.
Moreira FMS, Siqueira JO (2002). Microbiologia e bioquímica do solo. UFLA, Lavras, Brasil. 626p.
Pôrto ML, Alves JC, Diniz AA, Souza AP, Santos D (2009). Indicadores biológicos de qualidade do solo em diferentes sistemas de uso no Brejo Paraibano. Ciência Agrotecnica 33:1011-1017.
Roscoe R, Mercante FM, Mendes IC, Reis Junior FB, Santos JCF, Hungria M (2006). Biomassa microbiana do solo: fração mais ativa da matéria orgânica. In: Dinâmica da 20 matéria orgânica do solo em sistemas conservacionistas: modelagem matemática e métodos auxiliares. Roscoe R, Mercante FM, Salton JC. (eds.). Embrapa Agropecuária Oeste, Dourados, Brasil. pp. 163-198.
Salton JC, Mielniczuk J, Bayer C, Fabricio AC, Macedo MCM, Broch DL (2011). Carbono no solo e em frações da matéria orgânica do solo sob sistemas de integração lavoura-pecuária em Mato Grosso do Sul. Pesqui. Agropecu. Bras. 46:1349-1356.
Santos GA, Silva LS, Canellas LP, Camargo FAO (2008). Fundamentos da matéria orgânica do solo: ecossistemas tropicais e subtropicais. Porto Alegre: Metrópole pp. 7-16.
Santos RD , Lemos RC , Santos HG, Ker JC, Anjos LHC (2005). Manual de descrição e coleta de solo no campo. EMBRAPA, Viçosa, Brasil, 5ª Ed.
Scherer-Lorenzen M, Bonilla JL, Potvin C (2007). Tree species richness affects litter production and decomposition rates in a tropical biodiversity experiment. Oikos 116(12):2108-2124.
Schultz N, Lima E, Pereira MG, Zonta E (2010). Efeito residual da adubação na cana-planta e da adubação nitrogenada e potássica na cana-soca colhidas com e sem a queima de palhada. Rev. Bras. Cienc. Solo 34:811-820.
Silva RR, Silva MLN, Cardoso EL, Moreira FMS, Curi N, Alovisi AMT (2010). Biomassa e atividade microbiana em solo sob diferentes sistemas de manejo na região fisiográfica Campos das Vertentes, MG. Rev. Bras. Cienc. Solo 34:1585-1592.
Silveira PM, Pereira AJB, Kliemann HJ (2005). Adubação nitrogenada do feijoeiro cultivado sob plantio direto em sucessão de culturas. Pesqui. Agropecu. Bras. 40:377-381.
Trannin ICB, Siqueira JO, Moreira FMS (2007). Características biológicas do solo indicadoras de qualidade após dois anos de aplicação de biossólido industrial e cultivo de milho. Rev. Bras. Cienc. Solo 3:1173-1184.
Vezzani FM, Conceição PC, Mello NA, Dieckow J (2008). Matéria Orgânica e Qualidade do Solo. In: Fundamentos da matéria orgânica do solo: ecossistemas tropicais e subtropicais. Santos GA, Silva LS, Canellas LP, Camargo FAO (eds.). Metropole. Porto Alegre, Brasil, pp. 01-07.
Zhang Y, Zhang H, Su Z, Zhang C (2008). Soil microbial characteristics under long-term heavy metal stress: A case study in Zhangshi wastewater irrigation area, Shenyang. Pedosphere 18(1):1-10.

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Full Length Research Paper

Construction and assessment of a hydraulic weighing lysimeter

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Hydraulic weighing lysimeters have been showing good results in evapotranspiration studies. Thus, the aim of this study was to construct, calibrate, and assess a small (0.7 m^3) and low cost hydraulic weighing lysimeter. The lysimeter consists of two containers. The inner container has a circular area of 1 m^2 and volume of 0.7 m^3, while the outer container is constructed with concrete slabs. The inner container is supported by three hydraulic load cells, arranged in the shape of an equilateral triangle. For calibration, standard weights with 1 kg were added and subsequently removed. Calibration results indicated high linearity between mass changes and their readings, with determination coefficient of 0.999. For pressure readings automation, it is necessary to correct temperature-related errors. Wind causes mechanical oscillations in the lysimeter, with subsequent pressure data errors that need to be corrected for proper evaporation measurements, especially in smaller time scales. Comparison between measured ETo and ETo estimated by Penman-Monteith method obtained a coefficient of determination of 0.56.

Key words: Evapotranspiration, calibration, hydraulic load cell, pressure gauge.

INTRODUCTION

Evapotranspiration is an environmental parameter required for crop implantation and management. It expresses the total water amount lost in a system by transpiration and evaporation of water from the soil. Depending on the conditions in which it is obtained, evapotranspiration may represent the site water demand or indicate the water amount that should be returned to the soil to meet crop needs. In regions that have a well-defined dry season, such as the Brazilian Cerrado, for example, there are periods when rainfall is insufficient to meet crop needs. In this case, knowledge of evapotranspiration for irrigation management purposes is indispensable (Valipour and Eslamian, 2014; Valipor, 2014a, 2015a; Khoshravesh et al., 2015).

Many devices and methods can be used to determine evapotranspiration. Among them, lysimeters have been used to directly obtain this variable with extreme reliability. Aboukhaled et al. (1982) considered the weighing lysimeter the best equipment for accurate measurement of evapotranspiration, serving as standard

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methodology to calibrate equations that estimate this variable.

One of the difficulties to use the lysimeter comes from its high cost of construction and installation. In general, it is recommended that the lysimeter surface area should be large enough to maximize the sample area and minimize the effect of the space between the lysimeter and the surrounding soil. Aboukhaled et al. (1982) recommended a minimum size of 4 m^2, while Sarnie and Villele (1970) recommended an area of 2 m^2. Nevertheless, the construction of lysimeters with areas of less than 2 m^2 has become common recently, with relative success. As an example, studies by Lima et al. (2013), Mariano et al. (2015), and Silva (2005), with respective areas of 1.32, 1.52, and 1.038 m^2, can be cited. Wherley et al. (2009) constructed and assessed in field a lysimeter with 0.05 m^2 and total volume of 15 L. Besides reducing construction costs, smaller lysimeters facilitate installation, operation, and maintenance in field.

However, there is concern that smaller lysimeters are subject to a number of problems and limitations among which stand out (Dugas and Bland, 1989; Allen et al., 2011): smaller population of sampled plants; the so-called “bloom effect” where the area of exposed plant canopy exceeds the assumed effective area of the lysimeter; influence of lysimeter’s wall on the thermal regime of the soil and of canopy environment; smaller accuracy and resolution in mass variation measurement.

Dugas and Bland (1989) compared measurements from three lysimeters with surface areas of 0.18, 0.75, and 3.0 m^2. They concluded that no consistent effect of lysimeter size on accuracy was found for the crop tested (sorghum and wheat), although the measurements were made in low temporal resolution (5 to 29 days). Grimmond et al. (1992) tested over short time periods two weighing mini-lysimeters (<0.2 m^2) and compared with evaporative flux measurements obtained using eddy correlation instrumentation from an extensive homogeneous surface. The authors concluded that the mini-lysimeters provided relatively accurate and reliable measurements of latent heat flux. In addition, according the authors, the mini-lysimeters developed can be used for continuous automatic monitoring of evapotranspiration at the resolution of an hour.

Therefore, given the importance of automation and properly measuring ETo, this study aimed to construct, calibrate, and assess a hydraulic weighing lysimeter with 1 m^2 surface, in order to monitor evapotranspiration.

MATERIALS AND METHODS

The experiment was conducted at the Federal University of Mato Grosso, Rondonopolis, MT State Campus, with the following geographical coordinates: 16°28”15’ S, 54°38”08’W and altitude of 284 m.

A hydraulic weighing lysimeter containing two cylindrical containers was constructed. The inner container was constructed with iron plates with 4 mm thickness, 1 m^2 area, and volume of 0.7 m^3. The outer container was constructed with 16 concrete plates with 2 cm thickness, in order to support the surrounding soil. The lysimeter setup consisted of the following components: hydraulic weighing system, drainage system, reading system, and automation system.

For the hydraulic weighing system, three hydraulic load cells were constructed with self-extinguishing hoses made of butyl propylene reinforced nylon, with the following dimensions: 850 mm length and 101.60 mm inner diameter. Hoses had their extremities closed by pressure, using two pairs of galvanized pipes with 0.2 m length. Pipes were transversely drilled to fix screws. A metal connector was coupled in the central part of each of the load cells, in order to couple a polystyrene flexible tube. Flexible tubes of the three load cells were connected through a shutoff valve which, in turn, had an outlet tube connected to the pressure gauge.

Three support bases for the load cells were constructed on the bottom of the outer container. Cells were arranged in the container in the shape of an equilateral triangle, with an angle of 120 degrees between the sides. Bases were made of concrete, with rectangular shape and dimensions of 0.60 × 0.15 × 0.20 m. Bases were carefully leveled to support the hydraulic load cells, so that sealing tubes did not have contact with the soil or the concrete base. Subsequently, a 20 mm thick polyvinyl polychloride film was put on each load cell, in order to isolate the bottom of the inner container from contact with sealing tubes.

For the drainage system, a polyvinyl polychloride tube with 0.2 m diameter and 2.0 m depth was buried alongside the outer container. Inside the inner container, three polyvinyl polychloride tubes with 75 mm diameter, containing a porous clay capsule, were vertically inserted. Porous capsules were connected to the tube receiving the drained water via polyethylene tubes. Accounting of the amount of drained water was made by a folding system used in automatic rain gauges.

The reading system was formed by two independent components: mercury manometer and hydrostatic pressure sensor. The pressure gauge reading mechanism was based on a scale in millimeters. The hydrostatic pressure sensor used was the (PX26-001DV-Omega) model with (-0.007 to +0.007 mV) range, connected to the CR-1000 model (Campbell Scientific, Inc., Logan, USA) data logger.

Inner container filling was first conducted with a 0.1 m layer of gravel. The other layers, added at every 0.1 m, were filled with soil, respecting the order found in the original layers during their removal from the field. For each layer completed, the lysimeter soil received water to approach its natural density. Lysimeter implementation process was completed after coupling an access tube in the center of the lysimeter, in order to conduct soil moisture reading through the humidity probe. In Figures 1 and 2, there is a representation of the hydraulic weighing lysimeter constructive part, with some of its main components.

Load cells optimum volume calculation

In order to determine the optimal water amount for load cells, recommendations by Silva et al. (2003) were followed. This step was carried out in laboratory. Load cells filling was conducted with 13 L of distilled water at rest. Through a control shutoff valve linked to the load cells, equal volumes of 50 ml were released in each step, whose pressure values were observed in the mercury manometer and in the data logger. The procedure was repeated until reading differences were stable and proportional. Recorded values were subsequently correlated with the accumulated withdrawal volume. The optimal water volume for load cells corresponds to the minimum point of the regression curve fitting between manometer readings variation and accumulated water amount.

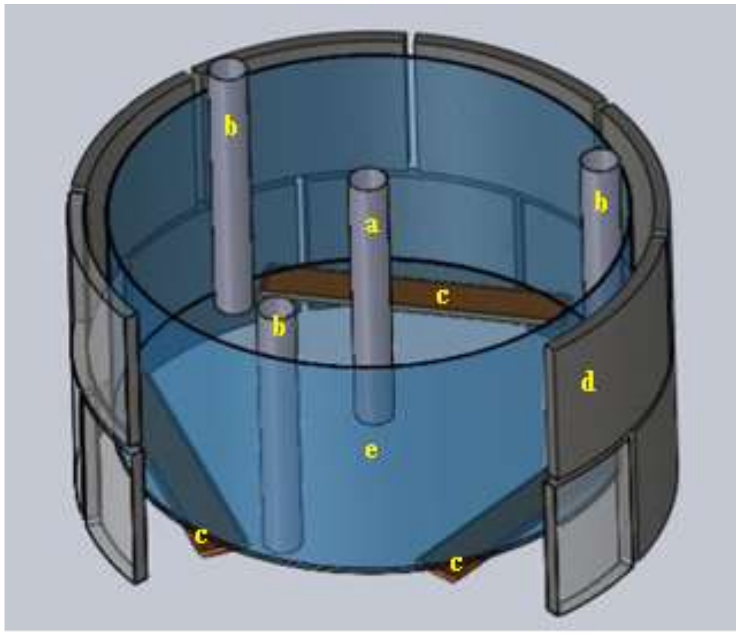

Figure 1. Lysimeter General Representation: (a) humidity probe access tube; (b) drainage pipes; (c) load cells; (d) casing and (e) inner container.

Figure 2. Details of the structure and mounting of the hydraulic lysimeter. (A): hydraulic load cells arranged in an equilateral triangle format and attached to each other by a metal connector. (B): arrangement of hydraulic load cells supported on concrete foundations in the field; (C): mounting the lysimeter in the field, with layer of gravel and the tubes to the drainage system. (D): tipping bucket rain gauge adapted for measuring the water drained.

Lysimeter calibration

This step was carried in field, after the installation of lysimeter. Calibration was divided into two stages. The first step consisted of lysimeter central calibration, gradually adding 1 kg weights until the total weight of 100 kg was reached. Each individual weight was equivalent to a 1 mm water blade. Subsequently, added weights were gradually removed, one by one. At every weight addition or withdrawal, 1 min was waited, in order to stabilize the reading system. In the second step, loads were applied on each load cell and in the vertices of the equilateral triangle formed by load cells. In this step, 36 weights of 1 kg were added and subsequently removed. As in the central calibration, addition and removal of weights in the latter stage occurred gradually, respecting the time required for system stabilization. The purpose of this last step was to verify lysimeter stability.

Temperature effects on data quality

Evapotranspiration data quality in hydraulic lysimeters may be affected by environmental factors that act in the several components of the lysimeter system. Temperature is a major factor, as it influences fluid expansion and contraction in the load cell, in the fluid transmission system and in the reading system. Errors may still occur even in thermal insulation, in an attempt to minimize thermal fluctuation effects (Wangati, 1965).

Thus, in order to correct temperature effects acting on the whole system, pressure transducer readings were correlated with temperature values inside the weather shelter. The analysis was conducted in four days in a row. During this period, the lysimeter surface was sealed to avoid water loss by evapotranspiration, and therefore, pressure variation recorded in the data logger was related to temperature variation.

The measured data of evapotranspiration were compared with simulated data by the Penman-Monteith model. The Penman-Monteith model is usually used as reference in evapotranspiration simulation studies. Thus, it is noteworthy that the construction of the lysimeter is also useful for further evaluation of the various models available to simulate evapotranspiration or even for building empirical models. Examples of studies comparing different models of evaporation can be found in Valipour (2012, 2014b, c, d, 2015b).

RESULTS AND DISCUSSION

Optimum fluid volume in the load cells

Pressure variation registered in the pressure gauge showed tendency to constant readings, with fluid withdrawal in volumes equal of 50 ml. Initially, differences measured in the pressure gauge were higher, gradually decreasing as the contact area between the load cell and the base became practically invariable with subsequent extractions. Taking into account that the optimal water volume for load cells corresponds to the minimum point of the regression curve fitting between manometer readings variation and accumulated water amount, then:

$$\Delta L = 0.0000003Va^2 - 0.0038Va + 12,33 \quad (1)$$

where ΔL is the readings variation (mm) and Va is the accumulated volume (ml).

Volume was obtained by the derivative of the following function:

$$\frac{dy}{dx} = 0.0000006Va - 0.0038$$

By solving Equation 2, after the tangent is defined as equal zero (minimum point), the optimum volume of 6333 ml is found.

A similar result was observed by Silva (2005), while using hydraulic weighing lysimeters to determine evapotranspiration and crop coefficients of passion fruit, in which the result reduced hydraulic load cells pressure along with successive 50 ml water decreases. According to Silva (2005), inadequate fluid volume within the load cell does not allow a constant contact area, interfering with pressure responses that are transmitted to the reading system.

Calibration and stability coefficient

In Figure 3, lysimeter central calibration results are shown. It is noted that both mercury manometer and pressure transducer had linear responses between the added or removed weight and the corresponding pressure. In both cases, fitting quality was excellent (R^2>0.999). It was also noted that small hysteresis effect occurred in both measurement systems. Similar results were found by Lima et al. (2013), while calibrating load cells and hydraulic load cells.

Results of calibrations conducted in each load cell and in the vertices are shown in Tables 1 and 2. In general, it was observed that determination coefficients were all above 0.99 for both individual cells and vertices, in both measurement methods. This indicates that the lysimeter is stable, which is extremely important to obtain reliable data. It is important to highlight that equipment balance is due to the fact that the three hydraulic load cells were arranged to form an equilateral triangle. Another important factor is that the water amount used in hydraulic load cells should not make the contact area between the inner container base and the cells cause inconsistent estimates, as mentioned by Silva (2005).

Calibration coefficients (*k*) obtained in the mercury manometer ranged from 3.577 to 4.011 mm with the addition and removal of 1 kg weights (Table 3). For the transducer, this coefficient ranged from 0.982 to 1.169 kg for 1 kg weights added or removed. The central calibration coefficient (*k*), which is the standard used in lysimeters, was of 3.434 mm kg^{-1} for the mercury manometer and of 1.017 kg for the electronic pressure transducer. That is, each 1 kg of added or removed weight in the lysimeter corresponds to 1.017 mm of evapotranspired depth.

Lysimeter stability was determined by the mean of the calibration coefficients of the three hydraulic load cells

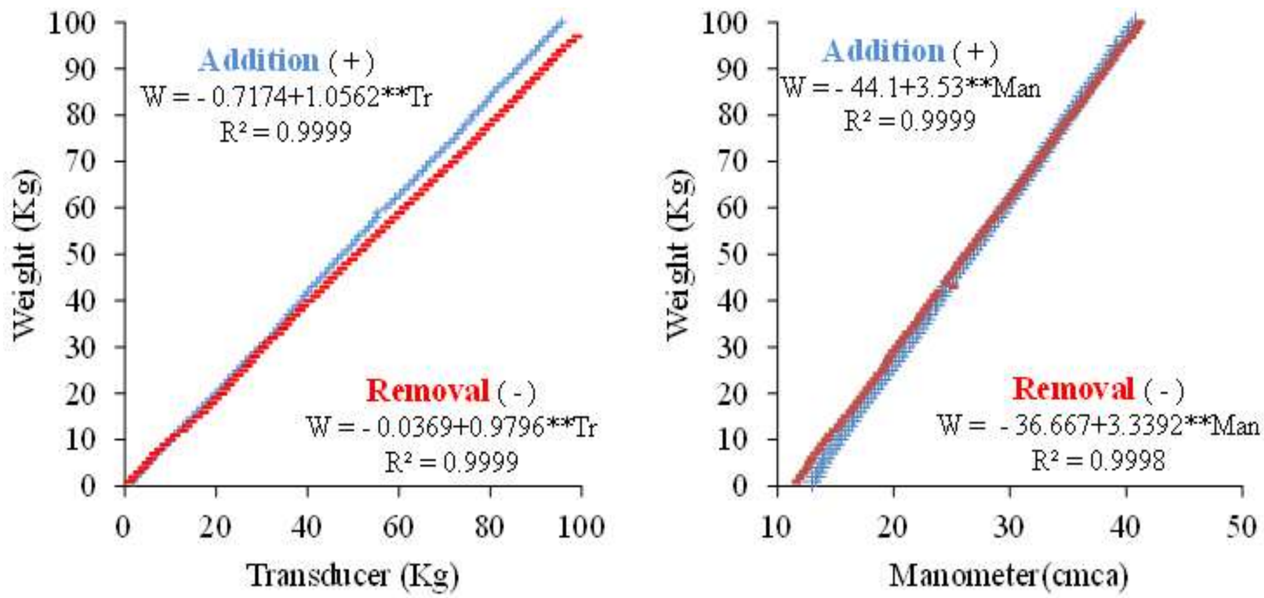

Figure 3. Lysimeter central calibration regression analysis. Readings observed in the transducer (Tr) and in the mercury manometer (Man) were related to the addition (+) and removal (-) of 1 kg weights (M). **Significant at 1% probability.

Table 1. Relationship between weight variation (kg) and pressure (mm) in the mercury manometer and in the pressure transducer, with point load in each of the three hydraulic load cells.

System of measurement	Load cell	Addition		Removal	
		Model**	R^2	Model**	R^2
Manometer	1	Y=30.072+3.522x	0.998	Y=-25.469+3.617x	0.996
Transducer		Y=-23.706+1.061x	0.997	Y=-14.74+0.9021x	0.996
Manometer	2	Y=-30.083+3.712x	0.997	Y=-32.723+3.811x	0.996
Transducer		Y=-17.524+1.150x	0.999	Y=-19.847+1.189x	0.998
Manometer	3	Y=-34.255+3.731x	0.998	Y=-36.188+3.521x	0.997
Transducer		Y=-24.239+1.024x	0.998	Y=-26.586+1.042x	0.997

**Significant at 1% probability.

Table 2. Relationship between weight variation (kg) and pressure (mm) in the mercury manometer and in the pressure transducer, with point load in each of the three vertices of hydraulic load.

System of measurement	Vertex	Addition		Removal	
		Model**	R2	Model**	R^2
Manometer	1	Y= -2.355+3.744x	0.996	Y=-21.213+3.606x	0.995
Transducer		Y=-7.7445+1.098	0.998	Y=-6.540+1.049x	0.996
Manometer	2	Y=-20.628+3.636x	0.999	Y=-20.902+3.519x	0.995
Transducer		Y=-6.051+1.057x	0.999	Y=-5.977+1.034x	0.997
Manometer	3	Y=-23.960+4.4x	0.995	Y=-14.331+3.622x	0.998
Transducer		Y=-3.666+1.124x	0.997	Y=-2.338+1.023x	0.992

**Significant at 1% probability.

Table 3. Calibration coefficients (k) and mean error, with point load in the centers and vertices of hydraulic cells and in the lysimeter center, in mm kg^{-1}.

Posição	Calibration coefficient (*k*)	Calibration coefficient (*k*)
	Manometer	Transducer
cell 1	3.569	0.982
cell 2	3.761	1.169
cell 3	3.626	1.033
vertex 1	3.675	1.074
vertex 2	3.577	1.046
vertex 3	4.011	1.073
Mean	3.703	1.063
Lysimeter center	3.434	1.017
Mean positional error (%)	7.83	4.50

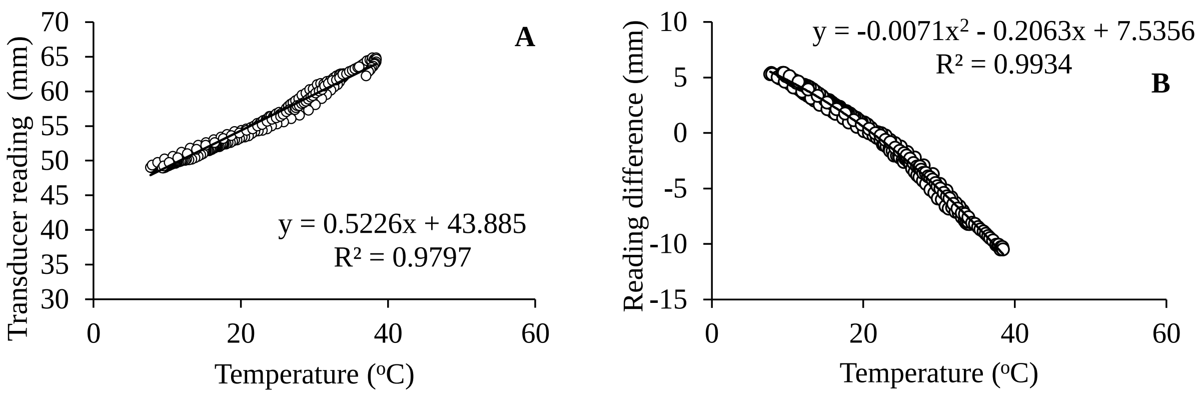

Figure 4. Relationship between pressure data generated by the transducer and the temperature obtained in the data logger (A); differences between original data and standard data, estimated at 20°C. Timescale of 10 min.

and of the three vertices compared to the lysimeter central calibration coefficient. Mean positional errors of 7.83 and 4.50% were observed for the mercury manometer and the transducer, respectively (Table 3). This result confirmed that the lysimeter is stable, especially when used in an automated manner, that is, by readings generated by the transducer in the data logger. Compared to other studies, hydraulic weighing lysimeters constructed by Santos et al. (2008), showed mean errors of up to 3.93 and 1.73%, respectively, using mercury manometer.

It is noteworthy that Black et al. (1968), quoted by Silva et al. (2003), stated that the tolerable error limit is 10%, as tank inclination influences fluid deformity in hydraulic cells, causing lysimeter reading errors. Thus, the mean positional error of 7.83% obtained by pressure gauge reading was lower than the 10% limit.

Temperature influence on lysimeter reading

It is possible to observe correlation between the transducer reading in the data logger and the temperature (Figure 4A). These data were obtained during the four days in which lysimeter surface was sealed to prevent water loss. A linear equation was fitted to the data from this relationship, in order to subsequently obtain a correction factor. The value regarded as reading standard was observed substituting the "x" value by 20°C in the linear equation. The 20°C value was established in accordance to the recommendations of the Instituto Nacional de Metrologia (2011). Figure 4B represents the differences between the amounts recorded over the four days and the standard value. Having with the equation fitted to the data in Figure 4B, temperature values associated with the pressure values generated by the transducer during the period were substituted (Figure 4A). The result represents the value to be corrected to the original data. In Figure 5, original data and corrected data are shown.

It is observed that the correction factor obtained by the methodology was efficient to linearize lysimeter readings under temperature effect, minimizing reading errors.

This correction procedure is important because there

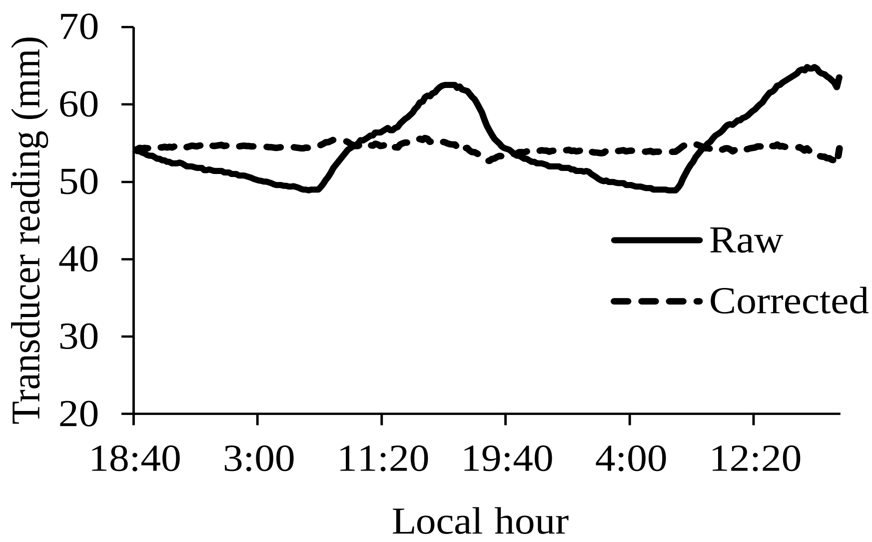

Figure 5. Uncorrected and corrected pressure transducer response (converted to mm) in a timescale of 10 min.

are basically two types of errors related to pressure transducer. One related to the sensor itself and the other related to external factors. So, for example, ambient temperature variations can affect the output signal of the sensor, even in a condition in which there was no variation of the applied pressure. These measurement errors are usually corrected by a compensation to the output signal of the sensor. However, there are additional variations throughout the hydraulic lysimeter measurement system that are transmitted to the pressure transducer. The main error is due to thermal expansion of the water column along the hydraulic system. The magnitude of this expansion is difficult to quantify exactly. However, Wangati (1965) calculated that the fluctuation of the water column due to temperature would be 0.66 mm. Of course, this value is different depending on the weather conditions of the region.

Evapotranspiration assessment

Correlation between measured and estimated evapotranspiration by Penman-Monteith method is as shown in Figure 6. The determination coefficient was of 0.5683, which is a mean value. However, similar results were found by other authors. For example, Medeiros (2002) and Mendonca et al. (2003) obtained determination coefficient values of 0.56 and 0.58, respectively, comparing lysimetric measurements on a daily scale by the Penman-Monteith method.

Among the possible additional sources of errors in lysimetric measurements, wind has an important contribution. It was observed that variations in the measured data occurred over time (Figure 7A). These variations are correlated to the wind speed values throughout the day (Figure 7B). However, wind is a very difficult variable to model and to predict. In addition, it is also very difficult to eliminate its effects on the system. One option would be to smooth the data using means on larger timescales, such as 1 h for example (Figure 8).

Weighing lysimeter reading errors are common (Schrader et al., 2013), especially in mechanical vibrations caused by the wind in high temporal resolution data (Vaughan and Ayars, 2009). Schrader et al. (2013) and Vaughan and Ayars (2009) discussed methods to reduce these errors, such as the application of filters to remove inconsistent data. They also suggested the statistical processing of collected data, in order to smooth noises.

Pressure transducer response during the day varies according to lysimeter water loss dynamics (Figure 9A), in which three variation stages stand out. Initially, it is noted that variation is very small during the night. During this period, there would only be water decrease by evaporative process. Subsequently, there is sharp, near-linear fall, indicating intense water loss to the atmosphere. Again, there is apparent pressure variation stabilization with the beginning of the nocturnal period (Figure 9A).

The tendency described earlier was observed for four sequential days in which there was no water replacement in the soil (Figure 9B). In this case, soil water variation represents the actual evaporation process. It is noted that radiation during those days did not decrease sharply, demonstrating that there was no energy availability reduction. It was observed that the period of increased pressure depletion apparently decreases with each passing day, which is typical of water reduction in the soil.

Finally, it is noteworthy that the lysimeter constructed in

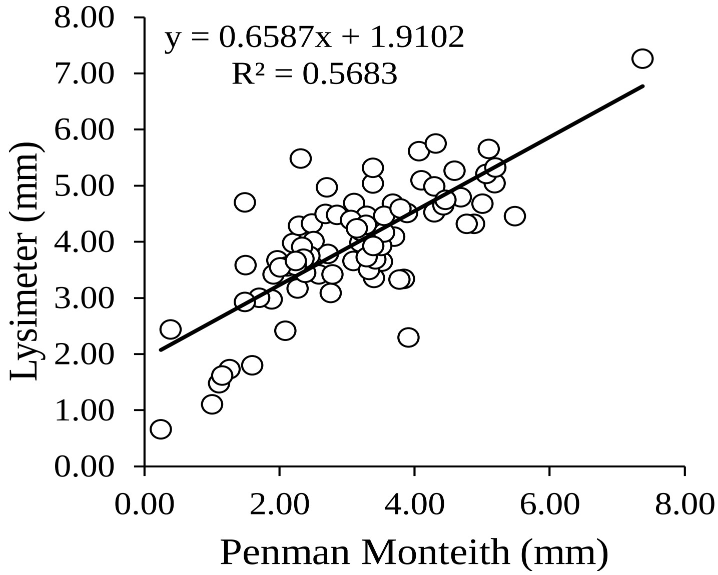

Figure 6. Correlation between ETo daily values measured by the lysimeter and estimated by Penman-Monteith (mm) for the period from 08.01.2013 to 10.31.2013, in the southern region of Mato Grosso, Brazil.

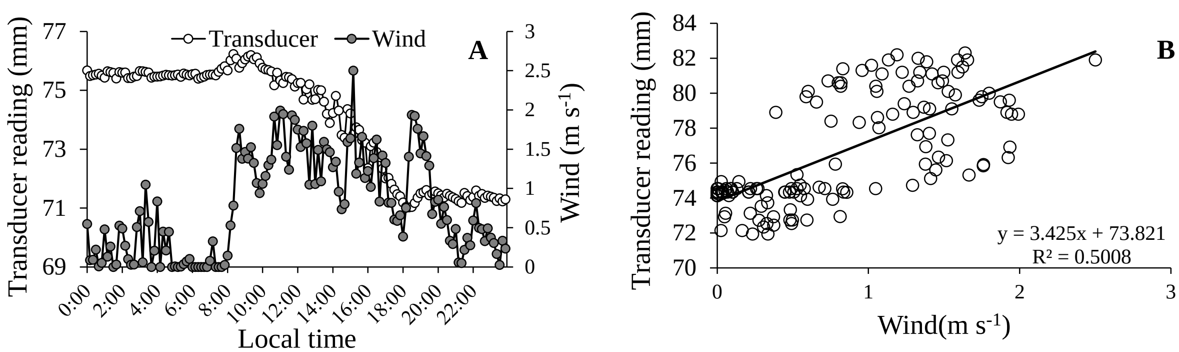

Figure 7. Pressure transducer response (converted to mm) and wind velocity (m s^{-1}) over the day in a timescale of 10 minutes (A); correlation between pressure transducer response (mm) and wind velocity (m s^{-1}) (B).

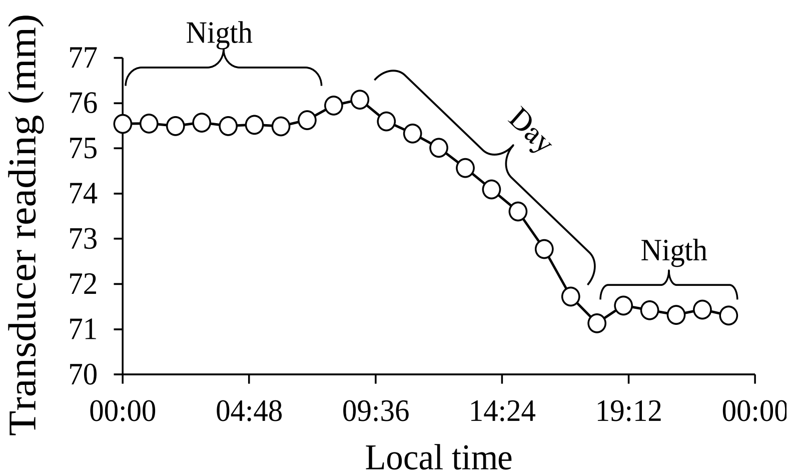

Figure 8. Pressure transducer response time means (converted to mm).

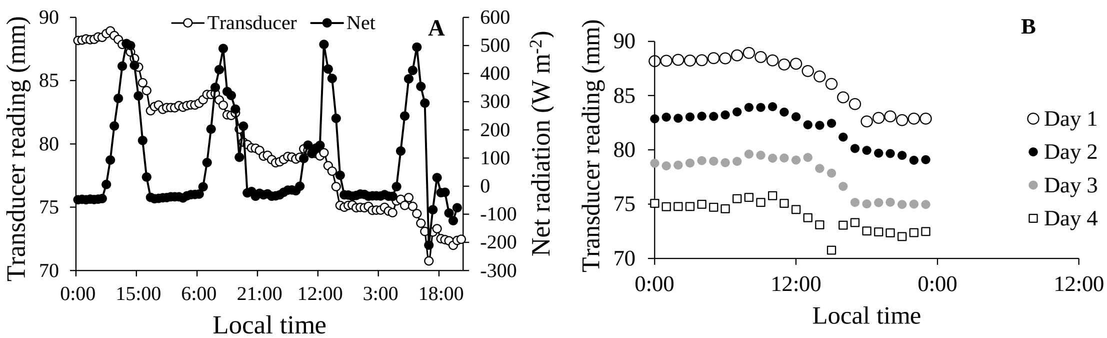

Figure 9. Pressure transducer response (converted to mm) and net radiation (W m^{-2}) time means variation in four sequential days (A); comparison between pressure transducer response (mm) in four sequential days.

this study has relatively small dimensions if compared to those usually described in the literature. The surface area was of 1.0 m^2, with total volume of 0.7 m^3, facilitating lysimeter use not only in the scientific research area, but also in farms or sectors related to the environment.

Conclusions

The hydraulic weighing lysimeter showed highly significant and accurate calibration responses for both central and localized calibrations. Therefore, the construction methodologies described in this study can be used as methodological reference, especially with regard to the disposal of hydraulic load cells in the form of an equilateral triangle.

Lysimetric readings automation with the pressure transducer is efficient and accurate. However, it is necessary to correct reading errors caused by temperature. In addition, mechanical oscillations caused by the wind are important data error sources, especially if smaller timescales are used.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Aboukhaled A, Alfaro JF, Smith M (1982). Lysimeters. Rome: FAO (FAO Irrigation and Drainage Paper. 39:68.

Allen RG, Pereira LS, Howell TA, Jensen ME (2011). Evapotranspiration information reporting: I. Factors governing measurement accuracy. Agric. For. Meteorol. 98(6):899-920.

Black TA, Thurtell GW, Tanner CB (1968). Hidraulic load-cell lysimeters, construction, calibration and test. Soil Sci. Soc. Am. Proc. 32:623-629.

Dugas WA, Bland WL (1989). The accuracy of evaporation measurements from small lysimeters. Agric. For. Meteorol. 46(1-2):119-129.

Grimmond CSB, Isard SA, Belding MJ (1992). Development and evaluation of continuously weighing mini-lysimeters. Agric. For. Meteorol. 62(3-4):205-218.

Instituto Nacional de Metrologia - Inmetro (2011). Orientação para a acreditação de laboratórios na área de volume, (Documento de caráter informativo), DOQ-CGCRE-027 – 2011. P 24.

Khoshravesh M, Sefidkouhi MAG, Valipour M (2015). Estimation of reference evapotranspiration using multivariate fractional polynomial, Bayesian regression, and robust regression models in three arid environments. Appl. Water Sci. pp. 1-12.

Lima NS, Montenegro SMGL, Albuquerque Filho JAC, Silva EFF, Montenegro AAA, Nascimento AHC (2013). Desempenho de um lisímetro de pesagem hidráulica com sensor de pressão hidrostática e sistema manométrico. Irriga.18(3):540-557.

Mariano DC, Faria RT, Freitas PSL, Lena BP, Johann AL (2015). Construction and calibration of a bar weighing lysimeter. Acta Sci. Agron. 37(3):271-278.

Medeiros AT (2002). Estimativa da evapotranspiração de referência partir da equação de Penman-Monteith, de medidas lisimétricas e de equações empíricas em Paraibapa, CE. Piracicaba, SP. Thesis - Escola Superior "Luiz de Queiroz", Universidade de São Paulo. P 103.

Mendonca JC, Souza EF, Bernardo S, Dias GP, Grippa S (2003). Comparação entre métodos de estimativa da evapotranspiração de referência (ETo), na região norte fluminense, RJ. Rev. Bras. Engenharia Agric. Ambiental 7(2):275-279.

Santos FX, Rodrigues JJV, Montenegro AAA, Moura RF (2008). Desempenho de lisímetro de pesagem hidráulica de baixo custo no semi-árido nordestino. Engenharia Agric. 28(1):115-124.

Schrader F, Durner W, Fankb J, Gebler S, Pütz T, Hannes M, Wollschläger U (2013). Estimating precipitation and actual evapotranspiration from precision lysimeter measurements. Proc. Environ. Sci. 19:543-552.

Silva TJA (2005). Evapotranspiração e coeficiente de cultivo de maracujazeiros determinados pelo método de balanço de radiação e lisimetria de pesagem hidráulica. São Paulo, SP. Thesis - Escola Superior "Luiz de Queiroz", Universidade de São Paulo. P 99.

Silva TJA, Montenegro AA, Rodrigues JJV, Bonfim EMS (2003). Aplicação de lisímetro de pesagem hidráulica na determinação na determinação da evapotranspiração de referência em Petrolina – PE. Engenharia Agric. 23(3):511-520.

Valipour M (2012). Ability of Box-Jenkins Models to Estimate of Reference Potential Evapotranspiration (A Case Study: Mehrabad Synoptic Station, Tehran, Iran). J. Agric. Vet. Sci. 1(5):1-11.

Valipour M (2014a). Handbook of hydrologic engineering problems. OMICS Group eBooks, Foster City.

Valipour M (2014b). Assessment of different equations to estimate potential evapotranspiration versus FAO Penman Monteith method. Acta Adv. Agric. Sci. 2(11):14-27.

Valipour M (2014c). Analysis of potential evapotranspiration using limited weather data. Appl. Water Sci. pp. 1-11.

Valipour M (2014d). Application of new mass transfer formulae for computation of evapotranspiration. J. Appl. Water Eng. Res. 2(1): 33-46.

Valipour M (2015a). Calibration of mass transfer-based models to predict reference crop evapotranspiration. Appl. Water Sci. pp. 1-11
Valipour M (2015b). Evaluation of radiation methods to study potential evapotranspiration of 31 provinces. Meteorol. Atmos. Phys. 127(3):289-303.
Valipour M, Eslamian S (2014). Analysis of potential evapotranspiration using 11 modified temperature-based models. Int. J. Hydrol. Sci. Technol. 4(3):192-207.
Vaughan P, Ayars J (2009). Noise Reduction Methods for Weighing Lysimeters. J. Irrig. Drainage Eng. 135(2):235-240.
Wangati FJ (1965). A note on temperature variation in the muguga lysimeter system. Agric. Meteorol. 2:43-54.
Wherley B, Sinclair TR, Dukes M, Miller G (2009). Design, construction, and field evaluation of a lysimeter system for determining turfgrass water use. Proc. Fla. State Hortic. Soc. 122:373-377.

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Full Length Research Paper

Identification of new descriptors for differentiation of soybean genotypes by Gower algorithm

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The descriptors used to differentiate new soybean cultivars which ensure their protection, has become insufficient. This makes evident the need to expand the list of that already used. The morphological traits that may be used as descriptors can be quantitative or qualitative; however, in the evaluation of their efficiency, they are not always analyzed simultaneously. Thus, this study aimed to identify new morphological characteristics for purposes of discrimination of soybean cultivars with the aid of Gower algorithm, which allows evaluation of quantitative and qualitative characters simultaneously. Ten soybean cultivars were used in two experiments, one in the summer and another in the winter. They were conducted in a greenhouse. The experiments were carried out in an experimental randomized complete block design with four replications. Each experimental unit consisted of one pot with two plants. Five quantitative and seventeen qualitative characteristics were evaluated and the discrimination analysis was performed by Gower algorithm. It was concluded that there are differences among cultivars for all traits analyzed in both sowing dates, except for the opening of the petioles of the unifoliolate leaves. The use of Gower algorithm was efficient in the evaluation of discriminatory capacity of quantitative and qualitative characteristics simultaneously, demonstrating that they are useful as soybean descriptors.

Key words: *Glycine max* (L.), law on plant variety protection, productivity, qualitative variables.

INTRODUCTION

The expansion and establishment of agricultural frontiers was only possible due to the development of cultivars with high yield, broad adaptation to different environmental conditions, and resistant to pests and diseases (EMBRAPA, 2011). In Brazil, the protection of intellectual property rights on the varieties is performed

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by obtaining the protection certificate. To get this, it is necessary to prove the new plant variety is distinct, uniform, and stable. The distinction is made by a minimum margin of descriptors, specific to each species (Neto et al., 2005).

Currently, 38 descriptors are used among the mandatory and additional to prove the distinction of soybean cultivars required for protection. However, these are still insufficient, mainly due to the narrow genetic base of this specie in Brazil (Boldt et al., 2007; Nogueira et al., 2008). There is therefore a clear need for studies aimed at identifying new descriptors for soybeans in order to expand the list of already used in the differentiation of soybean cultivars.

There are reports relating to effects of genetic differences for the hypocotyl length (Knittle and Burris, 1979; Nóbrega and Vieira, 1995). Costa et al. (1999) also reported that the variation in hypocotyl length is assigned to genetic differences among cultivars. In order to separate seeds that have the same characters, Dorchester (1945) used as auxiliary trait, the base shape of unifoliate leaf (acuminate, straight and auriculate), concluding that the shape of the leaf has well-defined characteristics, although there is variation within the same cultivar.

Nogueira et al. (2008) identified the hypocotyl and epicotyl length as being useful in soybean genotypes distinction. To evaluate the significance of a variable to descriptor, several statistical methods can be used. However, most of these take into account only quantitative variables, although the joint analysis of quantitative and qualitative variables potentially raises the chances of new varieties distinction. A technique that allows the simultaneous analysis of quantitative and qualitative data was proposed by Gower (1971) by means of an algorithm that estimates the similarity between two individuals using continuous data and discrete distributions.

The technique proposed by Gower (1971) allows that values of distance matrix stood between 0 and 1. Having the distance matrix is performed in a single cluster analysis for the different variables. This grouping has in principle to join the accesses in groups, so that there is uniformity within and heterogeneous between groups (Gonçalves et al., 2009).

Although this analysis have been proposed more than 40 years ago. Only in recent years, it has been the most frequently used to quantify the genetic dissimilarity in *Brassica napus* L. (Rodriguez et al., 2005), *Triticum aestivum* L. (Vieira et al., 2007), *Solanum lycopersicum* (Gonçalves et al., 2008), and *Capsicum* spp. (Moura et al., 2010) for example. However, their use in the identification of morphological descriptors was not reported. This may be due to lack of knowledge of statistical techniques that allow this approach and the lack of free computer programs that can analyze such a procedure (Rocha et al., 2010).

Thus, this study aimed to identify and evaluate new morphological traits for purposes of differentiation of soybean cultivars using Gower algorithm.

MATERIALS AND METHODS

The experiment was carried out in soybean breeding program, in greenhouse in the Department of Plant Science at the Federal University of Viçosa, Minas Gerais, Brazil. Two experiments were performed, one in the summer (December) and another in the winter (May).

To express a possible influence of environmental variations in the discrimination capacity of the genotypes through of the evaluated characteristics, the control of temperature or brightness were not performed, since by stability criterion, the discrimination ability of a descriptor can not be influenced by environmental variations.

The treatments consisted of 10 soybean cultivars: BRS Carnaúba, BRS Candeia, BRS 278 RR, BRS 271 RR, BRS Tracajá, UFVTN 105 AP, TMG 401, BRSMG 68 (Vencedora), TMG 801, and FMT Tucunaré, which were arranged in a randomized block design with four replications and each experimental unit consists of a pot with two plants.

In total, 22 characteristics were evaluated, which were not used as soybean descriptors, being five qualitative [base shape of unifoliate leaf (BSUL), phyllotaxy of the first pair of trifoliate leaves (PFTL), depression in the pod (DV), hypocotyl color (HC), and size of pod apiculus (SPA)] and seventeen quantitative traits [hypocotyl length (HL), plant height (PH), epicotyl length (EL), length of the first internode (LFI), petiole length of the first trifoliate leaf (PLFTL), petiole length of the unifoliate leaf (PLUL), rachis length of the first trifoliate leaf (RLFTL), angle formed by the insertion of petiole of the unifoliate leaf (APUL), opening angle of the petioles of the unifoliolate leaves (OAPUL), stem diameter (SD), being these measured only once when the plants were in stage V3 (Fehr and Caviness, 1977).

The measurements made from pods were: length (PL), diameter (PD), thickness (PT), thickness of bandaging (TBP), and curvature (PC), which are measured in ten pods of three seeds from each experimental unit. Finally, the width (HW) and the length of the heel (HL) obtained in a seed pod each were evaluated. With the information collected, an analysis of variance was performed to verify the existence of genetic variability among the materials evaluated for the traits studied.

The ability of joint discrimination of traits that have proven useful in distinguishing cultivars was estimated by genetic distance matrix obtained by Gower algorithm (1971), expressed as:

$$S_{ijk} = \frac{\sum_{k=1}^{p} Wijk .Sijk}{\sum_{k=1}^{p} Wijk}$$

where k is the number of variables (k = 1, 2, ..., p); i and j are two individuals representing the access; Wijk is the weight given to ijk comparison, assigning value 1 for valid comparisons and 0 for invalid comparisons (when the variable value is missing in one or both individuals); Sijk is the variable contribution k in the similarity between i and j individuals, with values between 0 and 1. For a qualitative variable (nominal), if the value of the variable k is the same for both individuals i and j, then Sijk = 1, otherwise it is equal to 0; for a quantitative variable (continuous) Sijk = 1 - | xik - xjk | / Rk where xik and xjk are values to variable k for individuals i and j, respectively, and Rk is the range (maximum minus minimum), the variable k in the sample. The division by Rk eliminates the differences between the variables scales, producing a value within the range [0, 1] and equal weights.

The graphical representation of genetic distances was obtained by Unweighted Pair-Group Method Using an Arithmetic Average (UPGMA) method, the cut-off point in dendrogram was obtained by Mojema method. The data were analyzed by the program genes: Biometrics (Cruz, 2013).

RESULTS AND DISCUSSION

A necessary condition for a trait to be useful in differentiation of the cultivars is the existence of genetic variability. For most quantitative variables, there were significant differences by the F test, except for the AAFU characteristic (Table 1). In addition to distinctness, stability can be observed by the uniformity of variability in two sowing dates.

Genetic variability among soybean cultivars for the characteristics hypocotyl length, epicotyl length, petiole length of unifoliate leaf, petiole length of the first trifoliate leaf, rachis length of terminal leaflet in the first trifoliate leaf and shape of unifoliate leaf had reported itself by Nogueira et al. (2008), demonstrating that they can be useful as additional soybean descriptors.

Table 1 also presented the genotypic determination coefficient (H^2) for quantitative traits evaluated in two sowing dates. Large genetic influence was found for most traits at different times, with values ranging between 0.716 and 0.939, showing little environmental effect, however, the variables OAPUL, APUL, and HL showed minimum values of 4.5, 55.8, and 64.9%, respectively, showing significant environmental effect.

The coefficients of variation of all variables showed values of low to high magnitude, ranging from 2.94% to PT trait in the experiment conducted in the summer up 24.04% for RLFTL also in the summer (Table 1). High values of coefficient of variation were also obtained by Nogueira et al. (2008) for RLFTL. The high CV values can be associated with non-homogenization of traits during the cultivars development process.

In Figures 1, 2 and 3, the discrimination capacity of the traits are measured by the amount of cultivars that are isolated below the cutting line, so that the more genotypes are isolated, the greater the possibility of using characteristics as descriptors.

Results for discriminatory ability of the traits, quantitative, and qualitative, considered together are as shown in Figure 1, in which the groups formed by the cultivars in summer and winter conditions are demonstrated.

Excluding the trait OAPUL, which did not show variability among genotypes analyzed, the remaining 21 morphological traits did not allow a complete distinction of all varieties. However, in the summer, sowing was possible to differentiate the varieties to each other, except for the BRS 271 RR, BRS, and BRS Tracajá Carnauba which formed a distinct group of the others and BRS 278 RR and BRS MG 68 (Vencedora), which were also together in one group (Figure 1A).

In winter sowing, the traits provided a distinction of 60% of the studied varieties. However, it is not enough to make differentiation of BRS Carnaúba of the BRS 271 RR and of BRS Candeia of the BRS 278 RR (Figure 1B). The efficiency of the use of quantitative and qualitative morphological characteristics together for discriminating cultivars by Gower algorithm becomes clear when compared with their effectiveness in distinguishing materials with the results which were obtained when they were used only quantitative or qualitative variables.

In summer sowing, the use of variables together provided a distinction of 50% of all cultivars (Figure 1A), while only the use of quantitative variables (Figure 2A) did not provide the differentiation of any cultivar individually, forming only two major groups. In turn, the use of only qualitative variables (Figure 3A) allowed the differentiation of 20% of the evaluated materials.

In winter, the simultaneous use of quantitative and qualitative variables resulted in the differentiation of 60% of the cultivars (Figure 1B), while when none of the quality characteristics (Figure 2B) was being considered, there was no distinction of individual cultivar, with only the formation of three groups of similar cultivars to each other. The use of qualitative variables only (Figure 3B), allowed the distinction of 40% of the cultivars.

These results indicate the possibility of using the Gower algorithm in soybean genotypes distinction when quantitative and qualitative traits are evaluated together, resulting in a more efficient discrimination since a greater number of descriptors will be considered.

It should be noted that cultivars that were not distinguished in the discrimination analysis of the two sowing periods (Figure 1) does not imply the futility of variables to differentiate cultivars, because the number of adopted traits was limited. Thus, by including traits already used as descriptors with the new identified, can improve the efficiency of the discriminatory capacity.

Since it was not used in this analysis, none of the 38 descriptors already used in soybean analysis, differentiation of 60% of 10 cultivars strengthens the possibility of using traits as descriptors. Since the analysis of a larger quantity of cultivars possibly would result in higher levels of discrimination. With regard to lack of consistency in the classification of cultivars between the different sowing dates, a likely explanation is the lack of homogeneity of cultivars for the traits evaluated that have not yet been considered in the process of selection and breeding of the species. The same effect was observed by Nogueira et al. (2008) who studied the efficiency of discriminant analysis in the differentiation of soybean cultivars for new descriptors.

According to Vieira et al. (2009), the morphological traits evaluated at the seedling stage and that evaluated

Table 1. Mean square of the genotypes (MSG), coefficients of variation (CV%) and genotypic determination coefficient (H2) of quantitative characteristics; hypocotyl length (HC), plant height (PH), epicotyl length (EL), length of the first internode (LFI), petiole length of the unifoliate leaf (PLUL) petiole length of the first trifoliate leaf (PLTL), rachis length of the first trifoliate leaf (RLFTL) angle formed by the insertion of petiole of the unifoliate leaf (APUL), opening angle of the petioles of the unifoliolate leaves (OAPUL), stem diameter (SD), pod length (PL), pod diameter (PD), pod thickness (TP), thickness of the pod bandaging (TPB), pod curvature (PC), heel width (HW) and the heel length (HL) in soybean cultivars in the summer sowing (December) and winter (May) in Viçosa-MG.

Character	Summer					Winter				
	QMG		H^2	Average	CV%	QMG		H^2	Average	CV%
HC	4.807	**	86.5	3.506	11.845	6.161	**	88.5	3.564	11.761
PH	339.215	**	91.6	25.691	10.455	166.657	**	83.2	25.691	11.217
EL	7.103	**	85.5	5.130	14.122	14.126	**	89.4	4.882	12.917
LFI	9.439	**	79.6	5.117	14.199	7.702	**	76.2	4.693	15.458
PLUL	268.396	**	92.3	13.768	17.668	320.244	**	94.5	14.503	14.276
PLTL	7.450	**	78.4	8.216	8.211	12.325	**	81.8	7.7752	10.188
RLFTL	59.330	**	82.3	7.790	21.798	42.423	**	74.2	7.125	24.041
APUL	1184.62	**	79.0	67.075	13.064	533.149	**	55.8	52.843	15.200
OAPUL	318.129		4.5	121.58	17.199	316.718		04.9	144.13	9.299
SD	7.969	**	77.0	6.830	11.035	11.398	**	86.3	5.806	11.669
PL	1.002	**	84.4	4.889	4.360	1.151	**	91.9	5.074	3.077
PD	3.254	**	77.5	6.532	6.886	3.446	**	87.1	6.647	5.112
TP	2.324	**	79.2	3.984	9.475	2.257	**	85.9	9.737	2.944
TPB	1.900	**	72.3	7.695	5.009	2.937	**	90.1	7.825	3.497
HW	0.435	**	82.4	1.549	9.244	0.269	**	64.9	1.481	10.736
HL	0.438	**	71.6	3.553	6.008	1.505	**	83.1	3.576	7.412
PC	23.564	**	89.5	3.973	20.992	29.808	**	93.9	3.997	16.938

**,*Significant at 1 and 5% probability by F test, respectively.

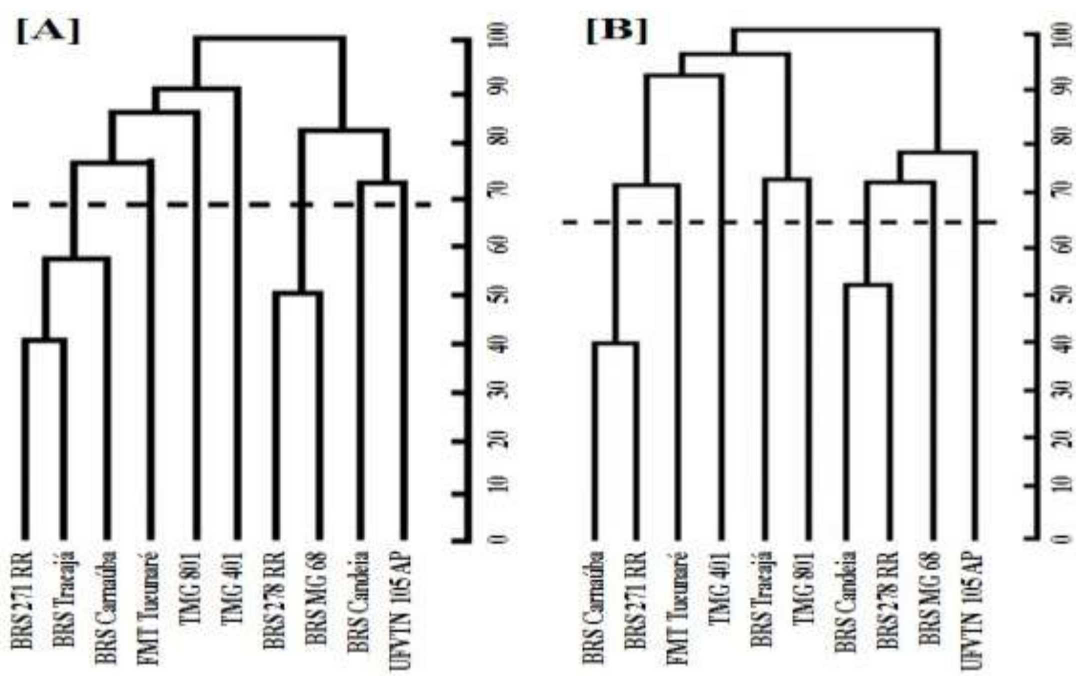

Figure 1. Dendogram of the similarity pattern between 10 soybean cultivars based on qualitative and quantitative traits, obtained by the UPGMA method through Gower algorithm in the summer sowing [A] and Winter [B].

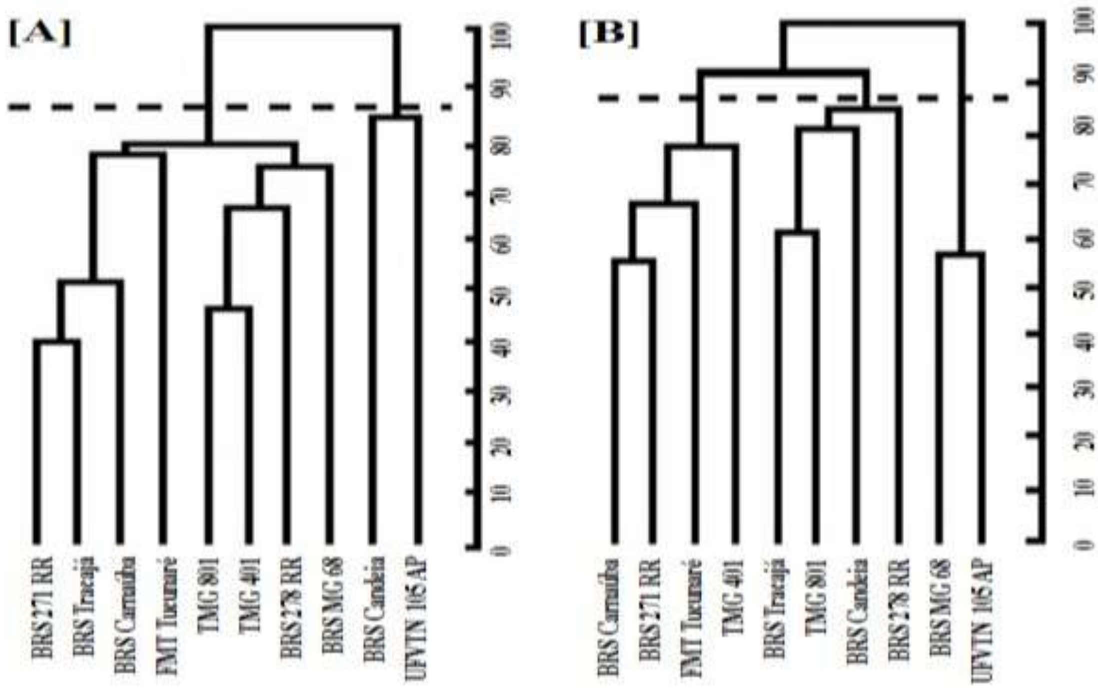

Figure 2. Dendogram of the similarity pattern between 10 soybean cultivars based only on quantitative characteristics, obtained by the UPGMA method through Gower algorithm in the summer sowing [A] and Winter [B].

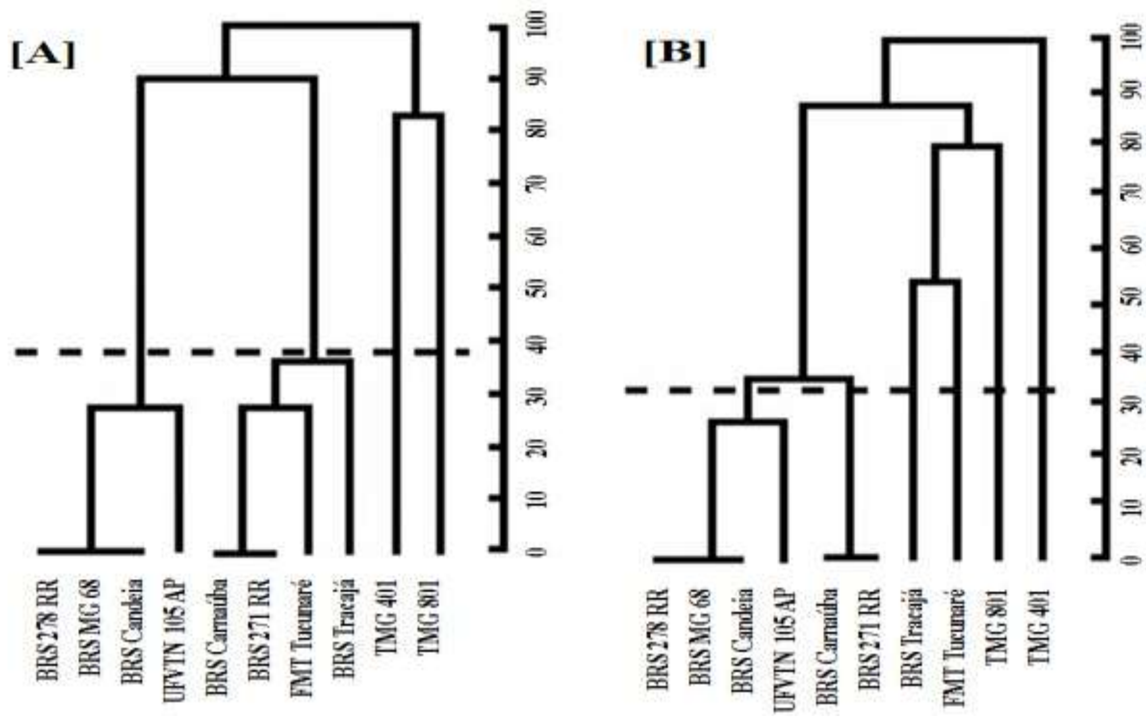

Figure 3. Dendogram of the similarity pattern between 10 soybean cultivars based on the qualitative characteristics obtained by the UPGMA method through Gower algorithm in summer seeding [A] and Winter [B].

in the seeds should be preferred, as they allow the rapid achievement of a result, thus, there is no need to wait for adult plants. The BRS Candeia, BRS Tracajá, UFVTN 105 AP, TMG 401, BRSMG 68, GMT 801 and FMT Tucunaré showed morphological traits in seeds and seedlings that were helpful to their separation from other cultivars.

Some qualitative morphological descriptors were specific to a small number of varieties, as the opposite phyllotaxy of the first couple of trifoliate leaves present only in cultivar TMG 801 and FMT Tucunaré; auriculate form of the base of unifoliate leaf in TMG 801 and 401 cultivars; pod depression, which was visible only in the cultivar TMG 401 RR and pod prominent apiculus in UFVTN 105 AP. The quantitative descriptors in turn were also able to differentiate the genotypes as it provided a refinement distinction in two sowing dates (Figures 2 and 3).

Vieira et al. (2009), Nogueira et al. (2008), and Boldt et al. (2007) also suggested new morphological descriptors based on the evaluation of seedlings and pod, for soybeans, since the 38 descriptors recommended by the SNPC have not been sufficient.

The assessed morphological descriptors allowed the characterization and distinction of most cultivars, being useful for the distinctness, uniformity and stability (DUS testing).

Conclusions

The evaluated soybean cultivars presented genetic variability to the characteristics basis shape of unifoliate leaf, phyllotaxy of the first pair of trifoliate leaves, pod depression, hypocotyl length, plant height, epicotyl length, length of the first internode, length of the petiole of first trifoliate leaf, length of the petiole of the leaf unifoliate, length of the rachis of the first trifoliate leaf, angle formed by the insertion of petioles of unifoliate leaf, pod length, pod diameter, pod thickness, thickness of bandaging pod, pod curvature, heel width end, and the hilum length .

The use of Gower algorithm was efficient in evaluation of the discriminatory capacity of quantitative and qualitative characteristics simultaneously in soybean.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Boldt AS, Sediyama T, Nogueira APO, Matsuo E, Teixeira RC (2007). Influência do tamanho de semente na caracterização de descritores adicionais de soja. In: reunião de pesquisa de soja da região central do brasil, Campo Grande. Anais. Londrina: Embrapa Soja 1:120-122.

Costa JA, Pires, JLF, Thomas AL, Alberton M (1999). Comprimento e índice de expansão radial do hipocótilo de cultivares de soja. Ciência Rural Santa Maria 29(4):609-612.

Cruz CD (2013). GENES - a software package for analysis in experimental statistics and quantitative genetics. Acta Sci. 35(3):271-276.

Dorchester CS (1945). Seed and seedling characters in certain varieties of soybeans. J. Am. Soc. Agron. Madison 37(1):223-232.

EMBRAPA (2011). Sistema de produção 15: tecnologias de produção de soja – região central do Brasil 212 e 2013. Londrina: EMBRAPA SOJA. 261 pp.

Gonçalves LSA, Rodrigues R, Amaral AT, Karasawa M, Sudré CP (2008). Comparison of multivariate statistical algorithms to cluster tomato heirloom accessions. Genet. Mol. Res. 7(4):1289-1297.

Gonçalves LSA, Rodrigues R, Amaral AT, Karasawa M, Sudré CP (2009). Heirloom tomato gene bank: assessing genetic divergence based on morphological, agronomic and molecular data using a Ward-modified location model. Genet. Mol. Res. 8(1):364-374.

Gower JC (1971). A general coefficient of similarity and some of its properties. Biometrics 27(2):857-874.

Knittle KH, Burris JS (1979). Soybean hypocotyl growth under field conditions. Crop Sci.. Madison. 19(1):37-41.

Moura MCCL, Gonçalves LSA, Sudré CP, Rodrigues R, Amaral AT, Pereira TNS (2010). Algoritmo de Gower na estimativa da divergência genética em germoplasma de pimenta. Hortic. Bras. 28(2):155-161.

Neto MOV, Borém A, Portugal RS (2005). Leis de proteção de genótipos. In: Borém, A. (Ed.). Melhoramento de Espécies Cultivadas. Viçosa: UFV. pp. 931-960.

Nóbrega LHP, Vieira RD (1995). Avaliação e classificação de cultivares de soja quanto ao comprimento do hipocótilo, sob condições de laboratório e de casa de vegetação. Revista Brasileira de Sementes, Brasília 17(2):160-164.

Nogueira APO, Sediyama T, Cruz CD, Reis MS, Pereira DG, Jangarelli M (2008). Novas características para diferenciação de cultivares de soja pela análise discriminante. Ciência Rural, Santa Maria. 38(9):2427-2433.

Rodríguez VM, Cartea ME, Padilla G, Velasco P, Ordás A (2005). The nabicol: a horticultural crop in northwestern Spain. Euphytica 142(3):237-246.

Vieira EA, Carvalho FIF, Bertan I, Kopp MM, Zimmer PD, Benin G, Silva JAG, Hartwig I, Malone G, Oliveira AC (2007). Association between genetic distances in wheat (*Triticum aestivum* L.) as estimated by AFLP and morphological markers. Genet. Mol. Biol. 30(2):392-399.

Vieira ESN, Pinho EVRV, Carvalho MGG, Silva PA (2009). Caracterização de cultivares de soja por descritores morfológicos e marcadores bioquímicos de proteínas e isoenzimas. Revista Brasileira de Sementes 31(1):086-094.

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Effects of field attack by *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) on the morphology and nutritional quality fresh fruit of *Citrus sinensis* L.

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The sweet orange, *Citrus sinensis* L. is an important fruit crop in Nigeria and its production is seriously constrained at maturity by attack of the fruit fly, *Ceratitis capitata* which results in severe yield loss. Effects of attack by *C. capitata* on fruit morphology and nutritional characteristics of *C. sinensis* from selected sites in southwest Nigeria were investigated. Twenty five mother citrus trees with >45 cm diameter at breast height (DBH) were randomly selected at two plantation sites: Ede (Osun state) and Olodo (Ogun state) in southwestern Nigeria. Ten mature fruits, plucked randomly from four cardinal sections of each tree were classified based on levels of infestation. Effects of attack on fruit morphometrics were assessed by standard measurements while the effects on fruit quality were determined by proximate and mineral analyses. Results indicated that attack by *C. capitata* was characterized by oviposition punctures which predisposed the fruit to secondary infection by *Penicillium notatum*. Fruits were attacked at any point on the surface but significantly more at about 2 cm from the fruit stalk scar. Multiple attacks did not follow a regular pattern. Fruit attack by *C. capitata* significantly decreased moisture content of fruit by 79% and mineral content; calcium, phosphorous, copper, zinc and iron while percentage dry matter, crude protein and crude fiber decreased significantly (P<0.50) with increased infestation. Fruit infestation also caused significant decrease in the vitamin C content by about 50% but increased saccharose, maltose and glucose content of attacked fruits by about 10%.

Key words: *Citrus sinensis,* multiple infestations, *Ceratitis capitata,* fruit morphometrics, *Penicillium notatum*

INTRODUCTION

Sweet orange (*Citrus sinensis* L.) is the most widely grown fruit crop in Nigeria and throughout the world (Morton, 1987; Yang et al., 2000) for its richness in essential minerals and vitamins (Olaniyan, 2001; Onibon

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Table 1. Agro-ecological characteristics of the survey sites.

Location	Lat. °E	Long. °N	Altitude (m)	Rainfall (mm)	Temperature (°C)		Relative humidity (%)
					Min.	Max.	
Ede, Osun State	4.25	7.43	300	2000	22	27	50-80
Olodo, Ogun State	3.37	7.20	200	2500	21	27	60-80

et al., 2007; Janati et al., 2012; Ekpete et al., 2013). However, production of citrus fruits is constrained at fruiting by pests; causing partial or total fruit loss (Parra et al., 2004). The Mediterranean fruit fly, *Ceratitis capitata* is a major fruit insect pest of sweet orange and the symptoms of its attack include different kinds of morphological distortions on the fruit surface which could predispose the mature fruit to infections by pathogens (Weems, 1981; Helle, 1989). Christenson and Foote (1960) had reported that the genus *Ceratitis* originated in Africa. It is well known that *C. capitata* has the most extensive geographical distribution among the pests of citrus. However, due to the devastating effects of this pest as an invasive species, citrus fruit consuming countries have imposed strict quarantine regulations on importation from endemic countries (Okwu and Emelike, 2006).

Citruses are attacked by a complex of many pests and diseases which often demand expenditures in the order of 160 million US dollars to initiate and effect control in order to avoid what could develop into total loss (Parra et al., 2004). These species which constitute the harmful entomofauna of this crop include the fruit flies, scale insects and mite species among others (Parra et al., 2004).

The fruit flies constitute a complex group of dipteran insects known to be characteristically polyphagous; attacking different kinds of crops. The extent of damage by the pest depends on the host crop, the agro-ecological characteristics of the location where the host crop is established as well as the fruit fly species; thus it becomes important to identify, classify and characterize the damage caused by *C. capitata* to *C. sinensis* in the southwest Nigeria.

This paper reports the effects of infestation by this insect pest on some morphological characteristics and nutritional quality of fresh fruits of sweet orange attacked by this insect pest and its implication for secondary infection by fungal pathogens.

MATERIALS AND METHODS

The study was carried out in two years on two farmers' fields in two states of southwestern Nigeria. Fresh fruits of citrus were sampled from mixed plantations of citrus and lemon from two sites located in Ede (Osun state) and Olodo (Ogun State). The samples were sorted and assessed for attack by *C. capitata.* Field collected samples of the insect were identified at the Insect Reference Collection and Identification Centre of the Department of Crop Protection and Environmental Biology (CPEB), University of Ibadan, Nigeria and by comparison with published samples (Alford, 1984). Detailed observation and fruit classification were carried out at the Entomology Research Laboratory, Department of CPEB, UI, Ibadan, Nigeria. Biochemical analyses for effects of attack on the nutritional characteristics of the samples were conducted at the Central Laboratory, Institute of Agricultural Research and Training, Moor Plantation, Ibadan, Nigeria.

Agro-ecological characteristics of the study sites and confirmation of pest's identity

The agro-ecological characteristics of the two survey sites are presented in Table 1. The first site: Ede is located in Osun State, Nigeria; with a bimodal annual rainfall of 2000 mm which peaks in June and September. The field was a mixed orchard of C. *sinensis* and C. *limon.* The stands of *C. sinensis* were distributed at 15 stands/50 m^2. The second site: Olodo village is located in Ogun State; also with a bimodal annual rainfall of 2500 mm which peaks in June and September. This field was also a mixed orchard with *C. sinensis* as the predominant species occurring at 18 stands/50 m^2. The agro-ecological characteristics of these sites were conducive to field infestation of Citrus fruits by *C. capitata*.

Pest sampling

Fifty mother citrus trees with ≥ 75 cm Girth at Breast Height (GBH) were randomly selected per study site. Five fruits were carefully harvested randomly per tree and thereafter sorted, examined and classified based on morpho-physical characteristics of damage on the attacked fruit into four groups (Table 1). Each fruit was particularly examined for oviposition puncture that was characteristic of female *C. capitata* on the outer surface of the fruit epicarp.

Characterization and classification of severity of field infestation by *C. capitata* on *C. sinensis*

Ten fruits were randomly selected from each class of attack (Table 2) and examined for the following morpho-physical parameters.

1. Fresh fruit weight (g) using a Chemical balance
2. Fresh fruit diameter (cm) via a transverse cut through the mid section of fruit
3. Fresh fruit diameter through a longitudinal section across the two fruit scars
4. Distance of oviposition puncture to the fruit stalk

The fruits were further examined to detect qualitative alteration of colour, secondary attack by other arthropods, opportunistic infections, surface damages and internal morphological changes. Field infestation of *C. sinensis* by *C. capitata* was classified based on the percentage of fruits attacked per tree (Table 3).

Table 2. Classification of field infestation of citrus fruit by *Ceratitis capitata.*

Classification	Description
Unattacked	No observable oviposition puncture and therefore used as the control
Single attack	One point of oviposition puncture
Double attack	Two points of oviposition punctures
Multiple attack	More than two oviposition punctures

Table 3. Classification of severity of field infestation by *C. capitata* in sampled trees of *C.sinensis.*

Class of infestation	Description of severity of field infestation
Unattacked	0% infestation
Mild infestation	1-5% of fresh fruit samples with at least one. Oviposition puncture/larval exit hole
Severe infestation	6-25% of randomly selected fresh fruits with at least one oviposition mark/larval exit hole
Very severe infestation	> 25% of randomly harvested fresh fruit with at least one oviposition mark/larval exit hole

Table 4. Morpho-physical characteristics of infested and uninfected fruits of *citrus sinensis* by *C. capitata.*

Class of infestation	Fresh weight of citrus			Description of physical distortion
	Weight (g)	Length (cm)	Width (cm)	
Unattacked	170.5[a]	7.2[a]	7.26[a]	None
Single attack	198.5[b]	7.31[a]	7.32[a]	Fruit Surface with one exit hole, surface hard; discolored brown
Double attack	198.5[b]	7.36[a]	7.35[a]	Surface around exit widens, becomes hard and appeared/ compressed; about 3 .5mm radius (coloured brown and dry)
Multiple attack	194[b]	7.4[a]	7.45[a]	Fruit surface with three or more larval exit holes; up to 4mm radius around exit hole; appeared compressed, discoloured brown, hard and dry; sells rottenness

Means followed by same letter in the same column are not significantly different (Tukey HSD, P>0.05); site.

Isolation and identification of field pathogens associated with oviposition puncture of *C. capitata* on *C. sinensis*

Ten diseased samples of fresh fruits of citrus collected from the field were used for this study in the laboratory. All samples were initially surface sterilized with sodium hypochlorite to remove external infections and thereafter rinsed several times with distilled water before blotting to dry on filter paper. A flamed wire loop was used to pick a little part of the discolored fruit at the oviposition puncture area created by *C. capitata.* All the scooped samples including an uninfested were streaked and thereafter plated on potato dextrose agar (PDA) in sterilized plastic Petri-dishes and incubated at room temperature control in three replications. The set up was observed for outgrowth of pathogens on the scooped skins for 48 to 72 h.

The emerged pathogens were sub-cultured in fresh PDA to obtain a pure culture of each pathogen sample. Each preparation was flamed gently on a Methylated spirit lamp to remove air bubbles before examination under microscope at increased magnification.

Effects of attack by *C. capitata* on proximate and other metabolites of citrus fruits

Samples were randomly selected from each class of infestation from each site in three replicates and analyzed for proximate, mineral and vitamin components at the Institute of Agricultural Research and Training, Moor Plantation, Ibadan using the official methods of AOAC (1990).

Statistical analysis

All the data were analyzed using descriptive statistics and analysis of variance and where significant, the means were separated using Tukey HSD test at 5% level of probability (SAS, 1990).

RESULTS AND DISCUSSION

Morpho-physical damage to infested fresh fruits of *C. sinensis* by *C. capitata*

The Morpho-physical characteristics of fruits of *C. sinensis* attacked by *C. capitata* and the subsequent distortions caused by the feeding activities of the developing larva inside the hesperidium are presented in Table 4. Infestations by *C. capitata* caused significant increase in the fresh fruit size of *C. sinensis*. Although the

Table 5. Morphometrics of fruits of *C. sinensis* attacked by *C. capitata* in two locations in southwest Nigeria.

Degree of attack	Fruit length (cm)		Fruit diameter (cm)		Fruit weight (g)	
	Ede	Olodo	Ede	Olodo	Ede	Olodo
Control	22.7[a]	24.3[a]	7.3[a]	7.7[a]	178.8[a]	219.0[a]
Single	23.2[a]	22.7[a]	7.7[a]	7.2[a]	213.8[a]	189.0[a]
Double	26.8[a]	23.7[a]	7.5[a]	7.6[a]	196.0[a]	206.0[a]
Multiple	23.3[a]	23.2[a]	7.4[a]	7.4[a]	188.0[a]	194.0[a]

Means followed by same letter in the same column are not significantly different (Tukey HSD, P > 0.05); site A = Ede (Osun state); site B = Olodo (Ogun state).

Table 6. Percentage field infestation of fruits of *Citrus sinensis* by *Ceratitis capitata* at two sites in the Southwest Nigeria.

Classification of attack	Mean percentage of fruit attacked/ site	
	Ede	Olodo
No attack	72 ± 4.32[a]	74 ± 2.83[a]
Single point attack	14 ± 1.63[b]	16 ± 1.63[b]
Double point attack	8 ± 1.63[b]	6 ± 2.83[b]
Multiple point attack	6 ± 2.31[b]	4 ± 1.63[b]

Means followed by same letter in the same column are not significantly different (Tukey HSD, P > 0.05); site.

fruit length and width were not significantly affected by different levels of attack by the pest; yet, attack by this pest caused different levels of severity of physical distortions on the surface of attacked fresh fruits with increasing number of attacks per fruit. For example, the surface of fruits attacked with one exit hole just hardens and gets discoloured while those with two exit holes or more had the fruit surface discoloured at about 3.5 mm radius around the exit hole which widens and become brown and dry, hardened and appeared compressed. Similarly, the fruit surface with three or more larval exit holes which occurred usually up to around 4.0 mm radius around the exit hole; appeared compressed, discoloured brown, hard and dry with foul smell of rottenness (Table 4).

Thus, field attack by *C. capitata* caused a characteristic water-soaked area with rancid odour oozing out of the infested fruit. Although there were visible physical distortions of the original spherical shape on the surface of the citrus fruit, there was no significant (P>0.05) difference between the diameter of attacked fruits and the control (Table 5). Each fly-exit hole on the fruit surface was 1.3 to 2.4 mm in diameter and the area around the puncture appeared hard and dehydrated.

There was however a significant increase (P<0.05) in fruit weight with increase in the number of oviposition punctures compared to the control (Table 5). Although, another very important fruit fly, *Bactrocera dorsalis* appears to compete and displace this pest in some orchards in Nigeria (Sapkota et al., 2010); *C. capitata* is a very important pest of agriculture that attacks several fruit crops like citrus, mango and apples (White and Elson-Harris, 1992). Damage to fruits is done first by the adult; which deposits its eggs by piercing the fruit with the ovipositor and also by the feeding larvae within the damaged fruit. These inflict heavy losses on the fruit yield of the citrus tree. This pest is economically important as it causes direct loss of yield and increased control costs (Bateman, 1972; Dowell and Wange, 1986; Okwu and Emelike, 2006).

It also leads to a great reduction and loss of export markets and/or the cost of constructing and maintaining fruit treatment and eradication facilities (Okwu and Emelike, 2006; Khamis et al., 2012). The fruit fly is considered a quarantine pest in many countries and this affects the export value of citrus fruits. The perception of farmers interviewed in this study agreed with Dowell and Wange (1986) that field infestation by *C. capitata* causes a lot of setbacks to citrus yield.

Severity of field infestation of *C. sinensis* by *C. capitata*

Field infestation of fruits of *C. sinensis* by *C. capitata* varied significantly (Table 3). Severity of fruit attack at the two sites ranged from 26 to 28% in the field. Of these, 14 to 16% were single point attack; 6 to 8% of the fruits had double points attack while 4 to 6% had multiple points attack (Table 6). Crown infestations of all mother Citrus trees at the two sites also varied significantly (Table 7). Whereas, 72 to 74% of the sampled citrus trees were

Table 7. Citrus crown infestation by *C. capitata* in two locations in the southwest Nigeria.

Class of infestation	Percent crown infestation/site	
	Ede	Olodo
Unattacked	0	0
Mild infestation	72	74
Severe infestation	16	18
Very severe infestation	12	8

Table 8. Effects of field infestation by *C. capitata* on the proximate composition of attacked and unattacked fresh fruits of *citrus sinensis* from two sites in the southwest Nigeria.

Class of infestation	Dry matter (mg)		Crude protein (mg/100 g)		Fibre (mg/100 g)		Vit.C (mg/100 g)		M.C. (%)	
	Ede	Olodo	Ede	Olodo	Ede	Olodo	Ede	Olodo	Ede	Olodo
Unattacked (Control)	20.00[a]	20.37[a]	0.41[a]	0.46[a]	0.84[a]	0.85[a]	48.47[a]	46.67[a]	79.00[a]	79.23[a]
Single	18.57[b]	18.81[b]	0.33[b]	0.35[b]	0.67[b]	0.68[b]	37.44[b]	33.52[b]	64.13[b]	61.27[b]
Double	16.50[c]	16.40[c]	0.22[c]	0.24[c]	0.44[c]	0.45[c]	23.95[c]	25.83[c]	40.57[c]	47.80[c]
Multiple	13.25[d]	13.30[d]	0.13[d]	0.11[d]	0.21[d]	0.28[d]	18.18[d]	20.29[c]	27.70[d]	29.7[d]
$LSD_{(0.05)}$	0.75	0.85	0.06	0.05	0.06	0.06	3.38	6.17	1.60	1.15

Mean followed by the same letter in the same column are not significantly different (P>0.05).

classed as mild infestation, 16 to 18% of the trees had severe infestations while 8 to 12% had very severe infestations. Damage to fruits of citrus and other host plants by fruit fly species is directly related to severity of field infestation (Nasiruddin et al., 2002) and so; it is particularly important to distinguish between attacks of different Tephritid species. Further characterization of the nature and specificity of physical damage to citrus by *C. capitata* is underway; and this would help field characterization of damage by specific fruit fly species to different host crops.

Morphophysiology and microbial spoilage of citrus fruit at oviposition punctures created by *C. capitata*

Morphological assessment of the surface around each point of attack up to 3.5 cm radius showed a somewhat compressed epicarp with a characteristic dehydrated and hardened surface. The original spherical shape of the orange fruit was distorted. The 3 to 5 mm surface on the fruit; around the exit hole turned brownish to black with a characteristic foul odour; indicating decay and collapse of fruit cells within the hesperidium as the deposited eggs begin to hatch and the larvae feed. This indicates that the factors of damage is the larvae developing inside the fruit and the associated pathogens that attack as consequence of attack by the flies (Okwu and Emelike, 2006; Khamis et al., 2012).

Initially, the oviposition puncture on the fruit surface was difficult to spot by an untrained and unaided eye but this point later became visible as a needle point with a characteristic black dot which spreads with time on the fruit surface. After pathogenicity test, the 'black points' on the oviposition puncture/exit hole were confirmed to be colonies of *Aspergillus* sp. and *Penicillium* sp. The extra cellular digestive activities of the pathogens degraded the nutritional components of the attacked fruits; thus the surface around the infected puncture/exit hole became hardened with time. The degree of surface distortion was directly related to the number of exit holes appearing as dent patches. It is believed that the holes created at oviposition had provided opening for opportunistic infection by pathogens particularly *Penicillium notatum.* Characteristically, the openings became rusty, hard and the spoilage spread from the locus.

Attacked fruits also had a characteristic rancid smell which resulted from the feeding activities of the larvae and the decay organisms encountered. From this study also, it is evident that the growth, development and feeding activities of the larvae of *C. capitata* caused extensive damage to the juicy endocarp. This was characterized by an offensive odour while the juice also tasted bitter. The deterioration and shrinking of the infected fruit is believed to have been increased by the fungus *P. notatum* encountered.

Effects of infestation by *C. capitata* on the nutritional composition of citrus

Infestation caused very significant depletion of the nutritional content of the attacked fruits especially in the carbohydrate and minerals (Tables 8 to 10). Similarly,

Table 9. Effects of field infestation by *C.capitata* on different types of carbohydrate in fresh fruits of *C. sinensis.*

Class of infestation	Saccharose (mg)		Lactose (mg)		Maltose (mg)		Glucose (mg)	
	Ede	Olodo	Ede	Olodo	Ede	Olodo	Ede	Olodo
Unattacked (Control)	6.26[a]	9.36[ab]	12.00[a]	9.98[a]	4.03[b]	7.50[a]	3.82[b]	5.93[ab]
Single	12.53[b]	7.91[b]	11.21[a]	8.70[a]	8.33[a]	5.21 [b]	7.00[a]	5.06[ab]
Double	12.61 [b]	1O.79[a]	4.82[b]	6.82[ab]	9.28[a]	7.20[a]	8.02[a]	6.01[a]
Multiple	6.31[a]	6.67[bc]	4.70[b]	5.13[b]	2.96[b]	4.27[b]	2.34[b]	3.55[b]
$LSD_{(0.05)}$	1.86	1.85	2.67	2.38	1.78	1.48	1.84	2.44

Mean followed by the same letter in the same column are not significantly different (P>0.05).

Table 10. Effects of field infestation by *C. capitata* on the mineral composition of fresh fruits of C. *sinensis.*

Class of infestation	Calcium		Phosphorus (mg)		Copper		Zinc		Iron	
	Ede	Olodo	Ede	Olodo	Ede	Olodo	Ede	Olodo	Ede	Olodo
Unattacked (Control)	67.60	69.33	51.63	49.05	0.007	0.003	0.65	0.67	0.09	0.07
Single	40.95	42.89	31.27	31.18	0	0	0.44	0.45	0.05	0.02
Double	21.28	20.84	16.89	16.32	0	0	0.23	0.21	0	0
Multiple	9.47	0.65	9.52	9.48	0	0	0.11	0.11	0	0

infestation caused significant reduction in the crude protein from 0.33 to 0.11 mg. This suggests that field infestation is serious and efforts should be directed at effective control of this pest. Observations on the distance of first point of attack indicated by the oviposition puncture created by *C. capitata* from the fruit stalk did not show a particular trend or pattern in relation to the point of second and third attacks respectively. The preference for the distance between the first point of oviposition and the fruit scar is worthy of note.

However, carbohydrate content of attacked fruits showed a peculiar trend which was different from the other parameters and metabolites under study (Table 9). From the result, the Saccharose, Lactose, Maltose and Glucose values increased in value with single and double point attack per fruit except for Lactose which significantly dropped to 4.82% (P<0.5). There was no significant difference between the values of Saccharose, Maltose and Glucose in the single and double point attack per fruit. The quantitative sugar values recorded for the multiple point attacks were not significant compared to the control except for lactose (P<0.05). The trend observed for the sugars could have been as a result of *C. capitata* attack on the oranges which resulted in deterioration of the fruit. Similarly, the level of disaccharide sugars reduced due to their conversion to monosaccharide. The drop in quantitative values generally observed at the multiple point attack stage could have been due to the stress caused by the feeding activities of the larva of *Ceratitis* growing inside the fruit.

The result of the microbiological investigation of fungal growth on the rind of the sweet orange confirmed earlier studies by Adegoke (2000) which identified *P. notatum* as an invading and colonizing organism around the slit created by *C. capitata* at oviposition. The *Penicillium* derives its metabolites from the orange fruit. This could have been responsible for accelerated sweet orange fruit damage. It may also be partly responsible for the significant drop of the quantitative values of the sugar observed on the fruits with multiple point attack in this study.

This is because the damage to the rind and endocarp was severe and larval/microbial activities increased at the multiple attack stage. The vitamin C content of unattacked sweet orange (control) in this study agreed with earlier reports by Morley (1987) which suggested that *C. capitata* attack and activities on the sweet orange fruit caused decreased values of vitamin C progressively as the severity of attack increased. The fruit dry matter content decreased significantly (P<0.05) with increased attack. This may be attributed to the growth and development activities of the larvae of *C. capitata* on the sweet oranges (Tables 8, 9 and 10).

The saccharose content of attacked and unattacked citrus fruits reduced significantly with infestation at the two sites. Although, the differences between the saccharose content of fruits from the two sites with single attack and those with double attack were not significant (P>0.05), yet the saccharose content of fruits with single and double attack were reduced by as much as 50% compared to unttacked fruits.

Whereas there was no significant difference between lactose and maltose content of single and non attacked fruits from both sites; the difference between the double and multiple attacked fruit were significantly different (P<0.05) compared to the non attacked fruits (Table 9). At Ede, infestation caused a reduction of lactose content of attacked citrus fruit from about 12.0 to 4.7 mg; and at Olodo site, from about 10.0 mg to about 5.0 mg which translated to more than 50% loss. Similarly infestation caused a reduction of the maltose and glucose by more than 50%.

The most abundant mineral element in citrus fruit was calcium (67.6-69 mg) followed by Phosphorus (49.0-51.0 mg). Infestation caused significant reduction of the calcium from about 38% at single attack to about 86 to 99% when the fruit suffered multiple attacks. Similarly, infestation reduced the phosphorus content by about 39% at single attack to about 86% when the fruit suffered multiple attacks. Infestation has a significantly high impact on the trace elements of copper, zinc and iron in which attacked fruits were depleted to zero levels.

Conclusion

This study had shown the oviposition activities of *C. capitata* and the subsequent development of the larvae caused extensive damage to the juicy endocarp and this reduced the quality of attacked fruits, making the juice to taste bitter and created site for and aggravate opportunistic infection by pathogens on the fruits especially by *P. notatum.* It also created rusty openings which become hard, spreading the spoilage from the locus and accelerating fruit deterioration and the shrinking of infected fruit.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Adegoke GO (2000). Understanding Food Microbiology. Shalom Prints, Ibadan, Nigeria.

Alford DV (1984). Flies. A colour Atlas of fruit pests. Wolfe Publishing Limited, London. pp. 111-112.

AOAC (Association of Analytical Chemists) (1990). Official Methods of Analysis. 15th Edition AOAC Inc., Arlingon, Virginia, U.S.A.

Bateman MA (1972). The Ecology of Fruit Flies. Annu. Rev. Entomol. 17:493-494.

Christenson LD, Foote RH (1960). The biology of fruit flies. Annu. Rev. Entomol. pp. 5-173.

Dowell RV, Wange LK (1986). Process analysis and failure avoidance in fruit fly programs. pp. 43-65. In: Mangel et al. (eds). Pest Control: Operations and Systems Analysis in Fruit Fly Management. NATO Advanced Science Institutes Series G. Ecological Sciences Springer-Verlag, New York. 11:468.

Ekpete OA, Edori OS, Fubara EP (2013). Proximate and mineral composition of some nigerian fruits. Br. J. Appl. Sci. Technol. 3(4):1447-1454.

Janati SSF, Beheshti HR, Feizy J, Fahim NK (2012). Chemical composition of lemon (*citrus limon*) and peels its considerations as animal food. GIDA 37(5):267-271

Khamis FM, Masiga DK, Mohamed SA, Salifu D, de Meyer M (2012). Taxonomic identity of the invasive fruit fly pest, *Bactrocera invadens*: Concordance in morphometry and DNA barcoding. PLoS One 7(9):44862.

Nasiruddin M, Alam SN, Khorsheduzzaman M, Jasmine HS, Karim ANMR, Rajotte E (2002). Management of cucurbit fruit fly, *Bactrocera cucurbitae*, in bitter gourd by using pheromone and indigenous bait traps and its effect on year-round incidence of fruit fly [Online]. Available on: http//www.oired.vt.edu/ipmcrsp/communications/ammrepts/annrepoz/Bangladesh/bang-topic 10 pdf.

Okwu, DE, Emelike IN (2006). Evaluation of the phytonutrients and vitamin content of citrus fruits. Int. J. Mol. Med. Adv. Sci. (2):1-6.

Onibon VO, Abulude FO, Lawal LO (2007). Nutritional and anti-nutritional composition of some Nigerian fruits. J. Food Technol. 5(2):120-122.

SAS (1990). SAS/STAT SOFTWARE; Changes and Enhancement through release 6.12 1996 SAS Institute Cay, North Carolina, USA. 1028p

Sapkota R, Dahal KC, Thapa RB (2010). Damage assessment and management of cucurbit fruit flies in spring-summer squash. J. Entomol. Nematol. 2(1):7-12.

Weems Jr JN (1981). The Mediterranean fruit fly, *Ceratitis capitata* Wiedemann. Florida Department of Agriculture and Consumer Services Division of Plant Industry. pp. 1-4.

Yang ZN, Ingelbrecht IL, Louzada E, Skaria M, Mirkov TE (2000). *Agrobacterium*-Mediated Transformation of the Commercially Important Grapefruit Cultivar Rio Red (*Citrus paradisi* Macf.). Plant Cell Rep. 19(12):1203-1211.

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Partial diallel analysis among maize lines for characteristics related to the tassel and the productivity

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Diallel crosses are important to the prediction of the best combinations among different heterotic groups of maize lines. The work objective was to estimate the combining ability among inbred lines from two heterotic groups to predict the best combinations in traits related to tassel and grain yield, conducted in five environments in the southern region. The inbred lines of the two heterotic groups are from the company KSP seeds. The tests were conducted based on crosses with partial diallel, using 15 female parents and male parents 8, 2011/2012 agricultural harvest in five environments in randomized blocks design with three replications. The variables analyzed were tassel length (TL), distance from the last node to the first branch of the tassel (DLN), distance from the flag leaf to the first branch of the tassel (DFL), number of tassel branches (NB), mass thousand grains (MTG) and grains yield (GY). The data were submitted the individual and joint variance analysis, after were realized analyze partial diallel analysis. The additive effects were more important for group II, already to the group I the non-additive effects were more pronounced, for the traits tassel and of yield. The GCA (group I) of lines inbred 15; 4 and 3-4 (group II) are favorable for increasing the grains yield. The crosses promising tassel traits been: 1-3, 2-4, 3-3, 4-4, 7-3, 8-3, 9-3, 10-3, 10-4, 11-4, 12-4, 14-3 and 15-4. The crosses promising increasing of grains yield been: 1-1, 1-4, 2-3, 3-1, 3-4, 4-3, 4-6, 4-7, 5-3, 5-4, 6-3, 6-4, 7-4, 8-4, 9-4, 10-5, 11-3, 12-3, 14-4, 15-2, 15-5 e 15-8.

Key words: Combinatorial capacity, genic effect, diallel crosses, grain yield.

INTRODUCTION

Corn is one of the most cultivated cereal in the world; mainly due to its diversity of applications, both in food and feed, the crop promotes several agro-industrial complex, with great social and economic importance (Ku et al., 2010). According to the survey from Conab (2015) for the corn crop 2014/2015, Brazil presented a

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production of over 78 million tons, being the states of Mato Grosso (23.1%) and Paraná (18.52%) accounted for more than 40% of the national production of corn.

The success of a genetic breeding program is conditioned by the efficient choice of parents, which when crossed produce superior hybrids. In this sense, the diallel crosses have great importance in predicting the best heterotic combinations (Paterniani and Viegas, 1987; Hallauer et al., 2010; Cruz et al., 2012).

In order to predict the behavior of the parents, the diallel crosses are employed in the study of genetic parameters, in the combining ability and in the gene actions that are involved in the character expression. Thereby, providing crucial information to meet the maximum heterotic expression in combinations (Hallauer et al., 2010). The use of techniques, such as diallels, which are intended to assist breeders in identifying the parents/lines with higher combining ability in different heterotic groups (partial diallel) are of great importance for the development of elite hybrids, with competitiveness in the market.

The main models of diallel analysis employed are the balanced diallels, where all the possible combinations are performed, the peculiarity of this model is the limitation of working with a large number of parents. The partial diallels involve two heterotic groups of parents, allowing to maximize the information about the study groups with a lower number of crossings, yet the reciprocal effects are generally not estimated, allowing loss of additional information. Circulating diallels are represented in the design by the same number of crossings, but less than p-1, reducing the number of total combinations, however losses of information occur regarding certain hybrid combinations, for being absent. Incomplete diallels are represented by a variable number of crossings, this design is a consequence of combinations losses. In the unbalanced diallels are estimated all hybrid combinations and also the other generations are represented, but in variable frequency due to the unequal number of replicates per treatment (Cruz et al., 2012).

To Paterniani and Viegas (1987), the genetic breeding programs have been working with the joint selection of characters and directing attention to plant architecture for increased photosynthetic efficiency and use of photoassimilates. These changes, whether spontaneous or not, have provided changes in the morphological characteristics, especially of the tassel (Neto and Miranda-Filho, 2001). Apical structures have priority in the use of available resources, especially when subjected to conditions of stress (Sangoi et al., 2006). It were also identified, by techniques of biometric analysis in diallel crosses, associations of the tassel characters with characters such as ear height, ear placement, prolificacy, leaf angle, which in turn relate directly with mass of corn kernels (Mickelson et al., 2002; Jung et al., 2007; Andrade and Miranda-Filho, 2008).

Thus, the morphological characteristics of the tassel have been gaining more attention from plant breeders in the prediction of crossings of inbred lines for tassel size reduction, due to the increased energy expenditure and the morphological changes associated with other phenotypic characteristics, as well as the gene actions that are involved in the expression of the characters (Neto et al., 1997; Sangoi et al., 2002; Westgate et al., 2003; Edwards, 2011). Allied with these reasons, this study aimed to estimate the combining ability of maize inbred lines belonging to two heterotic groups by partial diallel analysis, to predict the best combinations, in the characters related to the tassel and grain yield, and identification of predominant gene effects for the characters in five environments.

MATERIALS AND METHODS

The inbred lines of the two heterotic groups used in the diallel crosses are from the breeding program of the company KSP Seeds Ltd., based in the city of Pato Branco - PR. The tests were conducted based on crossings with partial diallel scheme, using 15 (female lineages) and eight (male lineages) from homozygous lines (S_9). After performing artificial crosses, the F_1 seeds were obtained, which were sown in the 2011/2012 harvest.

The sowing of F_1 hybrids was conducted in the 2011/2012 harvest in five separate areas of the three states of southern Brazil. In Rio Grande do Sul, the test was conducted in the city of Frederico Westphalen, with the coordinates 27°23'80" South latitude and 53°25'26 " West longitude, with 480 m of altitude in the trial site, the region is located in the north of the state and has as main climate features average annual temperature of 18.8°C and average temperature of the coldest month of 13.3°C, with sub-temperate, sub-humid type of climate. In Santa Catarina, the test was conducted in the municipality of Itapiranga, situated in the far west of Santa Catarina, with the coordinates 27°10'10 "South latitude and 53°42'44" West longitude, with 206 m of altitude, and temperatures in the coldest month of 13 and 30°C in the hottest month, with average monthly rainfall of 150 mm. In the state of Paraná, the tests were conducted at three sites. In Pato Branco, with the coordinates 26°13'44" South latitude and 52°40'15" West longitude, with 760 m of altitude in the trial place, with average temperature of the hottest month above 22°C, and of the coldest month, 18°C, with humid subtropical climate. Ampére with the coordinates 25°54'65" South latitude and 53°25'39" West longitude with 580 m above sea level in the place of the test, with minimum temperature of 13°C and maximum of 29°C, and average monthly rainfall of 170 mm. Clevelândia with the coordinates 26°21'52" South latitude and 52°28'22" West longitude, with 860 m of altitude at the site of the test, minimum temperature of 11°C and maximum of 27°C, monthly average rainfall of 170 mm.

In the five environments, the partial diallel was conducted in experimental design of complete blocks with treatments at random, with three replications per location. The experimental unit consisted of two rows of five meters in length, spaced 0.70 m. The sowing lines were marked with no-till system seeder, thus, hybrid seeds from the crosses were sown by hand. Sowing was performed according to the agricultural zoning of each site (September 20 in Frederico Westphalen, September 22 in Itapiranga, September 25 in Pato Branco, September 27 in Clevelândia and September 29 in Ampére). The soil management and cultivation were the same for the five sites, respecting the phenological stages and the requirement of the crop. After emergence and initial establishment of maize, it was held the manual adjustment of the population of the plots to 42 plants per experimental unit, equivalent to 60.000 plants ha^{-1}.

Table 1. Joint partial diallel analysis resulting from the crossing of two heterotic groups with 15 lines of Group I and 8 lines of Group II, evaluated at five environments in the southern region. Frederico Westphalen-RS. 2015.

SV	Mean square						
	DF	DFL	DLN	TL	NB	TGW	GY
Crossing (C)	119	557.26**	1238.25**	9115.97**	625.39**	279617.05**	293738328.97**
G.C.A. I	14	346.33**	723.64**	5771.39**	326.21**	184592.99**	188655708.23**
G.C.A. II	7	2415.01**	5638.75**	40388.46**	3005.97**	1225917.91**	1360391542.52**
S.C.A.	98	454.69**	997.44**	7360.01**	498.09**	225598.99**	232560616.68**
Environment (E)	4	1.58^{ns}	19.41**	56.39**	10.71^{ns}	15716.27**	125541311.89**
C x E	476	1.17^{ns}	3.32^{ns}	8.10^{ns}	2.83^{ns}	856.38^{ns}	5077896.23^{ns}
GCA I x E	56	1.28^{ns}	3.18^{ns}	6.93^{ns}	2.98^{ns}	578.18^{ns}	3446717.79^{ns}
GCA II x E	28	1.70**	7.99^{ns}	15.72^{ns}	3.25^{ns}	2744.78**	18703010.06**
SAC x E	392	1.11^{ns}	3.01^{ns}	7.77^{ns}	2.78^{ns}	761.24^{ns}	4337699.30 ^{ns}
Waste	1200	1.08	6.73	11.42	5.58	1155.74	6947083.83
Overall Average		14.94	22.23	60.41	15.67	333.14	11142.86

DFL: Distance from the flag leaf to the insertion of the first branch of the tassel. DLN: Length of the last node to the first branch of the tassel. TL: Tassel length. NB: Number of tassel branches. TGW: thousand grain weight. GY: Grain yield. ** Significant at 1% probability of error. ^{ns} Non-significant

The evaluated traits were tassel length (TL) in centimeters, distance from the last node of the stem to the first branch of the tassel (DLN) in centimeters, distance from the flag leaf to the first branch of the tassel (DFL) measured in the stem at the height of collar of the flag leaf to the insertion of the first branch in the tassel in centimeters, number of branches on the main stem of the tassel (NB), in units, thousand grain weight (TGW) measured by manual count of eight repetitions of 100 seeds, in grams and grain yield (GY), accomplished by manual harvesting of all experimental units and expanded to Kg ha^{-1}.

The data were submitted to individual variance analysis to verify the homogeneity of variances. Then, it was carried out joint analysis of variance to verify the significance of all sources of variation. Subsequently, the joint partial diallel analysis was performed, where, having interaction for combinatorial x environmental capacity, estimates were disrupted by environment, in the absence of interaction, estimates were snared by the average of environments. The analyzes were developed in the computer program Genes (Cruz, 2013).

For the diallel analysis, it was followed by the model IV of Griffing (1956), adapted by Geraldi and Miranda-Filho (1988) for partial diallel, involving two groups of parents. For the joint diallel study, it was followed the statistical model, where;

$$Y_{ij} = \mu + g_i + g_j + s_{ij} + A_k + GA_{ik} + GA_{jk} + SA_{ijk} + \bar{e}_{ij}$$

RESULTS AND DISCUSSION

Analysis of variance showed significant effects by F test for DFL character for the hybrid and hybrid x environment variation sources, the TL character revealed significance for environment and hybrid x environment. The characters DLN, NB, TGW and GY showed significance for all variation sources of hybrid, environment and hybrid x environment, respectively. In all traits evaluated, the F test showed significance for hybrid x environment interaction. The presence of significance for interaction is often, due to the narrow genetic base of simple hybrids (Pinto et al., 2007; Oliboni et al., 2013).

The joint diallel analysis showed significance for the source of variation of crossing for the characters tassel and yield. In the dismemberment of the source of variation, crossing, general combining ability (GCA I and II) and specific combining ability (SCA), it were found significant effects for all combining abilities (Table 1). This characteristic indicates that the set of lineages contribute differently to the crossings in which they are involved and also that the performance of hybrids differed to the expected based on the effects of the general capacity of parents (Oliboni et al., 2013).

The group II of lineages showed the highest estimates of GCA mean squares for the characters tassel and grain yield, indicating that there was a predominance of additive gene effects in all lineages of group II, being their estimates higher than the group I of lineages and the SCA estimates (Table 1). Estimates of the mean squares of SCA were higher than the GCA of the group I of lineages, indicating that the non-additive effects are most important for the variation in this set of hybrids, similar to the results of Machado et al. (2009). In addition, the significant effects of specific combining ability (SCA) reported that there were different degrees of complementarity between the two heterotic groups for the assessed traits (Pinto et al., 2007).

The joint diallel analysis reveals significant interaction effects for GCA II x environments for distance of the flag leaf to the first branch of the tassel (DFL), TGW and yield, this effect contributes to the results of Rodrigues et al. (2009), which state that the significance of the interaction indicates that the lineages contribute differently in the expression of the characters in distinct environments. The number of tassel branches, distance from the last node to the first branch and the length of the tassel do not interfere with the environment for both

Table 2. Estimates of the average general combining ability for group I and II of lines evaluated at five environments in the southern region. Frederico Westphalen-RS. 2015.

Group I	DFL	DLN	TL	NB	TGW	GY
1	0.938	0.722	2.420	0.425	14.752	545.655
2	-1.296	-1.436	-5.052	-1.085	-29.031	-943.148
3	0.542	0.997	2.425	0.717	14.941	391.494
4	2.577	4.216	10.863	3.139	56.637	1618.84
5	0.922	0.975	2.107	0.669	16.461	635.227
6	0.578	0.964	2.558	0.889	11.529	403.860
7	-1.327	-1.881	-4.957	-1.219	-29.799	-877.159
8	-1.283	-1.829	-5.057	-1.386	-30.811	-989.409
9	-1.341	-2.010	-5.334	-1.447	-28.944	-826.219
10	-1.251	-2.007	-5.393	-1.454	-25.820	-1091.592
11	-1.260	-1.569	-4.780	-1.189	-30.119	-1065.149
12	-1.333	-2.124	-5.535	-1.275	-26.825	-776.867
13	0.504	0.848	2.512	0.344	16.067	531.717
14	-1.201	-1.807	-4.616	-0.822	-31.213	-882.808
15	4.231	5.940	17.841	3.694	102.175	3325.557
Group II						
1	-	-1.679	-4.483	-1.198	-	-
2	-	-3.219	-8.571	-2.536	-	-
3	-	7.567	19.16	5.774	-	-
4	-	8.511	23.707	5.972	-	-
5	-	-1.820	-4.508	-1.361	-	-
6	-	-3.026	-8.332	-2.199	-	-
7	-	-3.072	-8.382	-2.071	-	-
8	-	-3.262	-8.591	-2.382	-	-

-- Characters with interaction GCA II x environment, results shown in Tables 3 and 4. *DLN: distance from the last node to the first branch of the tassel, TL: tassel length, NB: number of branches and for the group I to the characters distance from the flag leaf to the first branch of the tassel (DFL), thousand grain weight (TGW) and grain yield (GY).

heterotic Groups I and II, which gives opportunity to interpretations in a single environment. The SCA x E interaction revealed no significant effects for the characters tassel and yield, in this way there is no need to explore specific combinations for each location (Oliboni et al., 2013).

With respect to the estimates of general ability (Group I) for the characters distance from the flag leaf to the first branch of the tassel (DFL) and distance from the last node to the first branch of the tassel (DLN), the lines 2, 7, 8, 9, 10, 11, 12 and 14 have negative estimates (Table 2). Thus, the intersections of these lines would contribute to reduction of these tassel characters, according to Sangoi et al. (2002), minor amounts of nutrients and photoassimilates would be required by the hybrids, and besides, it would reduce the production of auxin, resulting in reducing the inhibitory effect on the growth and development of the female structure.

Regarding the assessment of the effects of GCA (Group I) for the tassel length (TL), the lines 12 and 2 had negative estimates (Table 2), which are favorable from an agricultural point of view. On the other hand, lines 1, 3, 4, 5, 6, 13 and 15 showed positive magnitude, indicating the presence of additive alleles for expression of longer length of tassel, a characteristic that is little desired in the production of simple hybrids due to the higher energy demand by the reproductive structure, and because the apical meristem has priority on the partition of photoassimilates, thus, crossings that result in hybrids with smaller tassel are sought (Sangoi et al., 2006).

In the evaluation of the effects of GCA (Group I) to the number of tassel branches (NB), the lines 2, 7, 8, 9, 10, 11, 12 and 14 had negative estimates (Table 2), a factor which enables use in breeding for the production of simple hybrids with fewer branches. According to Neto et al. (1997), this character has taken attention of breeders in breeding programs, showing that the reduced size of the tassel is favorable for the increase of grain production, mainly to the adverse conditions of stress. The current hybrids have already suffered major reductions in the number of tassel branches, which is directly related to lower production of pollen per area. According to Westgate et al. (2003), the hybrids can further reduce the pollen production by tassel, providing more energy to the cob and boosted grain yield.

For Group II, lines 1, 2, 5, 6, 7 and 8 have negative estimates for the number of branches. Edwards (2011) reports that the selection for reducing the number of branches, the lower leaf angle and synchrony between male and female flowering, contribute to the increase in grain yield. In the same context, Câmara et al. (2007) reported that the breeding programs for tolerance to abiotic stresses regard for character selection purposes as the number of branches, the period between the male and female flowering, prolificacy and delayed senescence of leaves (stay green).

With respect to thousand grain weight (TGW), lines 1, 3, 4, 5, 6, 13 and 15 (Group I) showed positive effects of GCA (Table 2), being the magnitude of the lines 4 (56.637) and 15 (102.175) highlighted among the others, which leads to increased TGW and, consequently, grain yield, because of the close relationship between the two characters, where, getting favorable magnitudes for one of the characters, possibly the magnitudes will be favorable to the other character (Lopes et al., 2007; Khayatnezhad et al., 2010). In the grain yield analysis (Group I), lines 1, 3, 4, 5, 6, 13 and 15 showed favorable assumptions for the trait (Table 2). It may be emphasized that the same lines that showed positive effects on the increase in the TGW showed it for the grain yield.

In assessing the DFL to the lines of group II, in relation to the five environments (Table 3), with the exception of lines 3 and 4, all other have shown negative effects, being lines 2, 6, 7 and 8 with more pronounced negative effects for all environments, which contributes to lower DFL, characteristic that is desired in the generation of progenies.

Regarding TGW characters and yield (Group II), estimates of CGA of lines 3 and 4 are positive for both characters. The crossing of these lines with the lines of the 4 and 15 of group I would add frequencies favorable for greater mass and grain yield, but to do so, the analysis of the specific interactions of the SAC is required to verify the complementarity of the genetic effects that are acting.

WIth respect to the SAC (*si*) for the tassel length (Table 4), the promising specific combinations for reduction in tassel size, are between the pairs 1-3, 2-4, 3-3, 4-4, 7-3, 8-3, 9-3, 10-3, 11-4, 12-4, 14-3 and 15-4, since the estimates *si* are high and negative, and the lines 10 and 12 (Group I) also showed negative effects for GCA. Negative estimates should be observed due to the fact that modern hybrids, developed with high grain yield, present smaller tassel, thus reducing the effect of apical dominance in stress conditions, which favors the best grain filling (Sangoi et al., 2006).

As to the evaluation of the SAC for the distance of the flag leaf to the insertion of the first branch of the tassel (Table 4), promising specific crossings to reduce the character are among the lines 1-3, 2-4, 3-3, 4- 4, 7-3, 8-3, 9-3, 10-3, 10-4, 11-4, 12-4, 14-3, 15-1 and 15-4, for having negative estimates of *si*. The lines 2, 7, 8, 9, 10, 11 and 12 (Group I) also revealed adverse effects for *gi*. Combinations of *si* are far superior to the effects of *gi*, predicting the predominance of genes of non-additive effect on the character of expression. The other pairs of specific crosses had low values and close to those observed for *gi*, which refers to hybrids presenting the performance as expected in the *gi*, (Cruz et al., 2012).

With respect to SAC estimates for the distance of the last node to the first branch of the tassel (DLN), the promising crossings are between the pairs of lines 1-3, 2-4, 3-3, 4-4, 7-3, 8-3, 9-3, 10-3, 10-4, 11-4, 12-4, 14-3, 15-1 and 15-4 (Table 4). In group II, the lines 3 and 4 showed favorable combinations with a greater number of lineages. The magnitude of the estimates *si* are again above the *gi* estimates, pointing out that the non-additive gene effects are more pronounced.

Regarding the number of branches (NB) to the estimates of the SAC, the most promising crossings to obtain hybrids with fewer branches are revealed between the pairs of lines 1-3, 2-4, 3-3, 4-4, 7-3, 8-3, 9-3, 10-3, 10-4, 11-4, 12-4, 14-3 and 15-4 (Table 5), it should also be considered that lines 2, 7, 8, 9, 10, 11, 12, and 15 (Group I) have negative effects for the GCA. Based on the superiority of estimates *si* over *gi*, it is assumed that non-additive gene actions occur in controlling the number of branches in the tassel.

For the component of thousand grain weight (TGW) yield, promising crosses are for the pairs of lines 1-1, 1-4, 2-3, 3-3, 3-4, 4-3, 4-6, 4-7, 5-3, 5-4, 6-3, 6-4, 7-4, 8-4, 9-4, 10-5, 11-3, 12-3, 13-3, 13-4, 14-4, 15-2, 15-5 and 15-8 (Table 5), being its estimates positive and also considering the effects of GCA (Group I) of the lines 15 and 4 with the highest positive estimates. Comparison with estimates *gi* indicate superiority of the estimates *si* with non-additive effects in the character control. The lines 3 and 4 used as a parent are responsible for most of the positive effects to increase TGW for demonstrating combining ability with a large number of lineages of group I. Lineages that present these magnitudes of combining ability are important for genetic breeding programs of hybrids, in which are high the specific complementarity actions with ample heterotic group.

With regard to the estimates of specific combining ability for grain yield (GY), promising specific crossings for increasing yield are among the combinations 1-1, 1-4, 2-2, 2-3, 2-6, 2-7, 2-8, 3-1, 3-4, 4-3, 4-6, 4-7, 5-3, 5-4, 6-3, 6-4, 7-2, 7-4, 7-6, 7-7, 7-8, 8-2, 8-4, 8-6, 8-7, 8-8, 9-2, 9-4, 9-6, 9-7, 9-8, 10-2, 10-5, 10-6, 10-7, 10-8, 11-2, 11-3, 11-6, 11-7, 11-8, 12-2, 12-3, 12-6, 12-7, 12-8, 13-3, 13-4, 14-2, 14-4, 14-6, 14-7, 14-8, 15-2, 15-3, 15-5 and 15-8, in which are assigned the crossings 1-1, 1-4, 2-3, 3-1, 3-4, 4-3, 4-6, 4-7, 5-3, 5-4, 6-3, 6-4, 7-4, 8-4, 9-4, 10-5, 11-3, 12-3, 14-4, 15-2, 15-5 and 15-8 as the most promising in the exploration of dominance effects. The most pronounced SAC estimates indicate that parents may generate hybrids with higher heterosis (Oliboni et al., 2013),

Table 3. Estimates of the general combining ability of individual for the interaction GCA II x environment evaluated at five environments of the southern region. Frederico Westphalen-RS. 2015.

Group II*	DFL					
	Ampére	**Clevelândia**	**Itapiranga**	**Pato Branco**	**Frederico**	**Mean**
1	-0.939	-1.208	-1.068	-0.882	-0.745	-0.968
2	-2.128	-2.156	-2.180	-2.156	-2.156	-2.155
3	4.646	5.181	4.897	4.233	4.507	4.693
4	5.713	5.666	5.628	5.966	5.944	5.783
5	-1.305	-0.978	-0.980	-1.286	-1.393	-1.188
6	-2.068	-2.045	-2.113	-2.030	-2.141	-2.080
7	-2.009	-2.245	-2.120	-2.038	-1.986	-2.079
8	-1.909	-2.215	-2.065	-1.808	-2.030	-2.005
SD (GCA II)	0.132	0.175	0.174	0.092	0.134	
SD (GCA II - GCA II')	0.200	0.264	0.263	0.139	0.203	
Group II**			**TGW**			
1	-26.084	-26.327	-22.001	-25.821	-25.293	-25.105
2	-39.254	-53.755	-45.241	-46.923	-46.431	-46.321
3	104.544	123.528	103.722	119.049	106.213	111.411
4	108.906	149.110	118.267	122.258	126.648	125.038
5	-18.267	-30.276	-19.671	-17.484	-23.051	-21.750
6	-42.826	-54.393	-45.960	-50.187	-45.167	-47.707
7	-44.066	-53.162	-42.019	-50.007	-45.540	-46.959
8	-42.953	-54.726	-47.097	-50.886	-47.379	-48.608
SD (GCA II)	4.984	4.493	4.833	5.514	3.685	
SD (GCA II - GCA II')	7.534	6.793	7.306	8.337	5.571	
Group II***			**GY**			
1	-862.121	-1083.438	-826.827	-680.536	-638.13	-818.21
2	-1460.28	-2138.494	-1457.879	-1539.36	-927.29	-1504.66
3	3589.077	4947.278	3301.854	4207.595	2305.70	3670.30
4	4134.745	5818.478	4460.024	3861.601	2825.03	4219.97
5	-758.349	-1266.472	-813.054	-992.234	-537.01	-873.42
6	-1650.842	-2128.144	-1562.557	-1668.461	-968.28	-1595.65
7	-1559.994	-2055.389	-1457.727	-1749.267	-1069.72	-1578.42
8	-1432.237	-2093.819	-1643.834	-1439.337	-990.31	-1519.91
SD (GCA II)	178.276	283.078	245.234	694.923	142.96	
SD (GCA II - GCA II')	269.528	427.973	370.759	1050.624	216.14	

DFL: distance from the flag leaf to the first branch of the tassel, TGW: thousand grain weight and GY: grain yield. *Results of the interaction for general combining ability for the length of the flag leaf to the first branch of the tassel of group II of lines, in the five environments. **Results of the interaction for general combining ability for the thousand grain weight of group II of lines, in the five environments. ***Results of the interaction for general combining ability for grain yield in group II of lines, in the five environments.

being SAC manifested by virtue of the non-additive effects and differences in allelic frequencies of the parents for the loci involved in character expression (Valério et al., 2009; Hallauer et al., 2010).

The lines 4 and 15, as female parents, and 3 and 4, as male parentes, have shown favorable estimates and combining ability with a larger number of lines of the other heterotic group. The female parents 4 and 15 and the male parents 3 and 4 presented high estimates for both general capacity and for specific capacity combination.

Conclusions

The additive effects were more important for the Group II

Table 4. Average specific combining ability from the crossings of two heterotic groups (group I, fifteen, and group II, eight strains), evaluated at five environments in the southern region. Frederico Westphalen-RS. 2015.

Group I / Group II	TL							
	1	2	3	4	5	6	7	8
1	49.199	-6.434	-34.166	21.610	-10.498	-6.674	-6.623	-6.414
2	-3.050	1.038	33.570	-31.240	-3.025	0.799	0.849	1.058
3	51.285	-6.439	-34.170	19.550	-10.502	-6.678	-6.628	-6.419
4	-18.965	-14.877	18.136	-47.156	-18.941	48.682	47.978	-14.857
5	-10.209	-6.121	23.584	21.700	-10.184	-6.360	-6.310	-6.101
6	-10.660	-6.572	25.109	22.883	-10.635	-6.811	-6.761	-6.552
7	-3.145	0.943	-26.788	29.687	-3.120	0.704	0.754	0.963
8	-3.045	1.043	-26.688	28.987	-3.020	0.804	0.854	1.063
9	-2.768	1.320	-26.412	27.052	-2.743	1.081	1.131	1.340
10	-2.709	1.379	-26.352	-30.899	54.852	1.140	1.190	1.399
11	-3.322	0.766	35.478	-31.513	-3.298	0.526	0.577	0.786
12	-2.567	1.521	30.190	-30.757	-2.542	1.282	1.332	1.541
13	-10.614	-6.526	25.564	22.150	-10.589	-6.765	-6.715	-6.506
14	-3.486	0.602	-27.130	32.079	-3.462	0.362	0.413	0.622
15	-25.943	38.357	10.075	-54.133	37.705	-22.094	-22.044	38.077
Group I / Group II				**DFL**				
1	14.167	-1.891	-8.739	5.293	-2.857	-1.966	-1.966	-2.040
2	-0.843	0.344	7.987	-7.595	-0.623	0.268	0.268	0.194
3	12.162	-1.495	-8.343	4.923	-2.462	-1.570	-1.570	-1.645
4	-4.716	-3.530	4.256	-11.468	-4.496	11.816	11.817	-3.679
5	-3.061	-1.874	7.679	6.020	-2.841	-1.950	-1.950	-2.024
6	-2.718	-1.531	6.021	5.619	-2.498	-1.606	-1.606	-1.681
7	-0.812	0.374	-6.473	6.681	-0.592	0.299	0.299	0.225
8	-0.856	0.330	-6.518	6.990	-0.636	0.255	0.255	0.181
9	-0.798	0.388	-6.459	6.583	-0.578	0.313	0.313	0.239
10	-0.889	0.298	-6.550	-7.640	14.187	0.223	0.223	0.148
11	-0.879	0.308	8.237	-7.630	-0.659	0.232	0.232	0.158
12	-0.807	0.380	7.732	-7.558	-0.587	0.305	0.305	0.230
13	-2.643	-1.456	5.852	5.339	-2.423	-1.532	-1.532	-1.606
14	-0.939	0.248	-6.600	7.565	-0.719	0.173	0.173	0.098
15	-6.370	9.106	1.919	-13.121	7.783	-5.259	-5.259	11.201
Group I / Group II				**DLN**				
1	17.936	-2.135	-12.921	7.356	-3.534	-2.328	-2.282	-2.092
2	-1.516	0.024	14.804	-11.707	-1.376	-0.170	-0.124	0.066
3	18.728	-2.410	-13.196	8.214	-3.809	-2.603	-2.557	-2.368
4	-7.168	-5.628	6.878	-17.359	-7.028	18.268	17.624	-5.586
5	-3.927	-2.387	10.358	7.203	-3.787	-2.581	-2.535	-2.345
6	-3.916	-2.376	9.159	8.336	-3.776	-2.570	-2.524	-2.334
7	-1.072	0.468	-10.318	10.748	-0.931	0.275	0.321	0.510
8	-1.123	0.417	-10.369	11.108	-0.983	0.223	0.269	0.459
9	-0.943	0.597	-10.189	9.844	-0.802	0.404	0.450	0.639
10	-0.946	0.594	-10.192	-11.137	20.197	0.401	0.447	0.636
11	-1.383	0.157	13.871	-11.574	-1.243	-0.037	0.009	0.199
12	-0.829	0.711	9.992	-11.020	-0.688	0.518	0.564	0.753
13	-3.801	-2.261	8.996	7.808	-3.660	-2.454	-2.408	-2.219
14	-1.146	0.394	-10.392	11.264	-1.005	0.201	0.247	0.437
15	-8.893	13.836	3.518	-19.084	12.425	-7.546	-7.500	13.245

TL: tassel length, DFL: distance from the flag leaf to the insertion of the first branch of the tassel and DLN: distance from the last node to the insertion of the first branch of the tassel.

Table 5. Specific combining ability from the crossing of two heterotic groups (group I, fifteen, and group II, eight strains), evaluated at five environments in the southern region. Frederico Westphalen-RS. 2015.

Group I / Group II	NB							
	1	2	3	4	5	6	7	8
1	12.876	-1.153	-9.463	4.483	-2.328	-1.490	-1.618	-1.307
2	-0.981	0.357	9.479	-8.151	-0.818	0.020	-0.108	0.203
3	12.841	-1.445	-9.754	6.268	-2.620	-1.782	-1.909	-1.598
4	-5.205	-3.867	5.178	-12.375	-5.042	11.774	13.558	-4.021
5	-2.735	-1.397	9.976	1.873	-2.572	-1.734	-1.861	-1.550
6	-2.955	-1.617	7.073	6.097	-2.792	-1.954	-2.082	-1.770
7	-0.847	0.491	-7.818	8.339	-0.684	0.154	0.027	0.338
8	-0.680	0.658	-7.651	7.171	-0.517	0.321	0.193	0.505
9	-0.619	0.719	-7.590	6.744	-0.456	0.382	0.255	0.566
10	-0.612	0.726	-7.584	-7.782	14.030	0.389	0.261	0.572
11	-0.877	0.461	8.751	-8.047	-0.714	0.124	-0.004	0.307
12	-0.791	0.547	8.149	-7.961	-0.628	0.210	0.082	0.393
13	-2.410	-1.073	4.751	5.153	-2.248	-1.410	-1.537	-1.226
14	-1.244	0.094	-8.215	11.119	-1.081	-0.243	-0.371	-0.059
15	-5.760	6.500	4.719	-12.930	8.470	-4.759	-4.887	8.647
Group I / Group II	**TGW**							
1	259.918	-38.073	-195.805	146.512	-62.644	-36.687	-37.435	-35.786
2	-15.506	5.710	172.865	-165.649	-18.861	7.096	6.348	7.997
3	289.365	-38.261	-195.994	118.196	-62.833	-36.876	-37.624	-35.974
4	-101.173	-79.957	103.266	-251.316	-104.529	250.454	260.926	-77.670
5	-60.997	-39.781	156.879	123.287	-64.353	-38.396	-39.144	-37.494
6	-56.066	-34.85	143.540	107.037	-59.421	-33.464	-34.212	-32.563
7	-14.737	6.478	-151.254	153.860	-18.093	7.864	7.116	8.766
8	-13.726	7.490	-150.242	146.779	-17.081	8.876	8.128	9.777
9	-15.592	5.624	-152.109	159.844	-18.948	7.009	6.261	7.911
10	-18.717	2.499	-155.233	-168.859	328.501	3.885	3.137	4.787
11	-14.417	6.799	165.245	-164.560	-17.773	8.184	7.436	9.086
12	-17.712	3.504	188.308	-167.855	-21.067	4.890	4.142	5.791
13	-60.604	-39.388	162.189	115.615	-63.959	-38.002	-38.750	-37.101
14	-13.324	7.892	-149.84	143.964	-16.679	9.278	8.530	10.179
15	-146.712	224.315	58.186	-296.855	217.740	-124.11	-124.858	192.294
Group I / Group II	**GY**							
1	9010.057	-1295.755	-6470.716	4390.669	-1926.992	-1204.757	-1221.996	-1280.507
2	-493.401	193.049	5510.975	-5531.586	-438.187	284.046	266.807	208.296
3	8727.939	-1141.593	-6316.555	3747.819	-1772.830	-1050.596	-1067.835	-1126.346
4	-3055.393	-2368.942	3413.272	-8093.578	-3000.179	7608.582	7849.934	-2353.695
5	-2071.776	-1385.326	5448.563	4001.079	-2016.563	-1294.328	-1311.568	-1370.079
6	-1840.410	-1153.959	4999.943	3061.498	-1785.196	-1062.962	-1080.201	-1138.712
7	-559.389	127.060	-5047.901	5423.223	-504.176	218.057	200.818	142.307
8	-447.139	239.310	-4935.651	4637.473	-391.926	330.307	313.068	254.557
9	-610.330	76.119	-5098.842	5779.808	-555.117	167.117	149.877	91.366
10	-344.956	341.493	-4833.468	-5383.142	9015.590	432.491	415.251	356.740
11	-371.400	315.049	4656.972	-5409.586	-316.187	406.047	388.807	330.296
12	-659.682	26.767	6674.944	-5697.867	-604.469	117.765	100.526	42.015
13	-1968.267	-1281.817	5337.785	3490.800	-1913.054	-1190.819	-1208.058	-1266.569
14	-553.740	132.709	-5042.252	5383.680	-498.527	223.706	206.467	147.956
15	-4762.106	7175.834	1702.929	-9800.292	6707.818	-3984.658	-4001.898	6962.373

NB: number of tassel branches, TGW: thousand grain weight and GY: grain yield.

of lines, for group I of lines, the non-additive effects were more important in the range of all characters. The improvement of hybrids for lower tassel size can be performed simultaneously, since the estimates of general combining ability remain constant in the lines of the two heterotic groups for all characters of the tassel. For the characters tassel lenght, distance from the last node and of the flag leaf to the first branch of the tassel, promising crosses are 1-3, 2-4, 3-3, 4-4, 7-3, 8-3, 9-3, 10-3, 10-4, 11-4, 12-4, 14-3 and 15-4.

The promising crosses to increase yield are 1-1, 1-4, 2-3, 3-1, 3-4, 4-3, 4-6, 4-7, 5-3, 5-4, 6-3, 6-4, 7-4, 8-4, 9-4, 10-5, 11-3, 12-3, 14-4, 15-2, 15-5 and 15-8. Among the most promising crosses to SAC, at least two parents have high GCA.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Andrade JAC, Miranda Filho JB (2008). Quantitative variation in the tropical maize population, ESALQ-PB1. Scientia Agricola, Piracicaba 65(2):174-182.

Câmara TMM, Bento DAV, Alves GF, Santos MF, Moreira JUV, Júnior CLS (2007). Parâmetros genéticos de caracteres relacionados à tolerância à deficiência hídrica em milho tropical. Bragantia, Campinas 66(4):595-603.

Conab (2015). Companhia Nacional de Abastecimento. Acompanhamento da safra brasileira de grãos.v.2, n.7 – Sétimo Levantemento. Disponível em: http://www.conab.gov.br/OlalaCMS/uploads/arquivos/15_04_10_09_22_05_boletim_graos_abril_2015.pdf.

Cruz CD (2013). Genes - a software package for analysis in experimental statistics and quantitative genetics. Acta Scientiarum Agron. 35(3):271-276.

Cruz CD, Regazzi AJ, Carneiro PCS (2012). Modelos biométricos aplicados ao melhoramento genético. 4ª ed.Viçosa: UFV. 514 pp.

Edwards J (2011). Changes in plant morphology in response to recurrent selection in the Iowa Stiff stalk synthetic maize population. Crop Sci. 51(6):2352-2361.

Geraldi IO, Miranda Filho JB (1988). Adapted models for the analysis of combining abilty of varieties in partial diallel crosses. Rev. Bras. Genét. 11(2):419-30.

Griffing B (1956). Concept of general and specific combining ability in relation to diallel crossing systems. Aust. J. Biol. Sci. Collingwood 9:463-493.

Hallauer AR, Carena JM, Miranda Filho JB (2010). Quantitative genetics in maize breeding. New York: Springer, 500 pp.

Jung MS, Vieira EA, Silva GO, Brancker A, Nodari RO (2007). Capacidade de combinação por meio de análise multivariada para caracteres fenotípicos em maracujazeiro-doce. Pesqui. Agropecuária Bras. Brasília 42(5):689-694.

Khayatnezhad M, Gholamin R, Somarin SJ, Mahamoodabad RZ (2010). Correlation coefficient analysis between grain yield and its components in corn (*Zea mays* L.) hybrids. Am-Euras J. Agric. Environ. Sci. 9(1):105-108.

Ku LX, Zhao WM, Zhanq J, Wu LC, Wang CL, Wang PA, Zhang WQ, Chen YH (2010). Quantitative trait loci mapping of leaf angle and leaf orientation value in maize (*Zea mays* L.). Theor. Appl. Genet. 121(5):951-959.

Lopes SJ, Lúcio AD, Storck L, Damo HP, Brum B Santos VJ (2007). Relações de causa e efeito em espigas de milho relacionadas aos tipos de híbridos. Ciência Rural 37(6):1536-1542.

Machado JC, Souza JC, Ramalho MAP, Lima JL (2009). Stability of combining ability effects in maize hybrids**.** Scientia Agricola 66(4):494-498.

Mickelson SM, Stuber CS, Senior L, Kaeppler SM (2002). Quantitative trait loci controlling leaf and tassel traits in a B73 x Mo17 Population of Maize. Crop Sci. 42(6):1902-1909.

Neto ALF, Miranda-Filho JB (2001). Genetic correlation between traits in the ESALQ-PB1 maize population divergently selected for tassel size andear height. Sci. Agricola Piracicaba 58(1):119-123.

Neto AR, Nass LL, Miranda Filho JB (1997). Potential of twenty germplasms to improve Brazilian maize architecture. Brazilian J. Genet. Ribeirão Preto 20(4).

Oliboni R, Faria MV, Neumann M, Resende JTV, Battistelli GM, Tegoni RG, Oliboni DF (2013). Análise dialélica na avaliação do potencial de híbridos de milho para a geração de populações-base para obtenção de linhagens. Semina: Ciências Agrárias, Londrina 34(1):7-18.

Paterniani E, Viegas GP (1987). Melhoramento e Produção de Milho. 795p.

Pinto RJB, Kvitschal MV, Scapim CA, Fracaro M, Bignotto LS, Neto ILS (2007). Análise dialélica parcial de linhagens de milho-pipoca. Rev. Bras. de Milho e Sorgo 6(3):325-337.

Rodrigues F, Von Pinho RG, Albuquerque CJB, Faria Filho EM, Goulart JC (2009). Capacidade de combinação entre linhagens de milho visando a produção de milho verde. Bragantia 68(1):75-84.

Sangoi L, Almeida ML, Silva PRPF, Argenta G (2002). Bases morfofisiológicas para maior tolerância dos híbridos modernos de milho a altas densidade de plantas. Bragantia, Campinas 61(2):101-110.

Sangoi L, Guidolin AF, Coimbra JLM, Silva PRF (2006). Resposta de híbridos de milho cultivados em diferentes épocas à população de plantas e ao despendoamento. Ciência Rural, Santa Maria 36(5):1367-1373.

Valério IP, Carvalho FIF, Oliveira AC, Lorencetti C, Souza VQ, Silva JAG, Harwing I, Schmidt AM, Bertan I, Ribeiro G (2009). Estabilidade da produção e da capacidade de combinação de diferentes populações de aveia. Semina: Ciências Agrárias, Londrina 30(2):331-346.

Westgate ME, Lizaso J, Batchelor W (2003). Quantitative relationships between pollen shed density and grain yield in maize. Crop Sci. 43:934-942.

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