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Full Length Research Paper

Irradiation as a quarantine treatment against *Bactrocera invadens,* in *Mangifera indica,* L. in Ghana

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The detection of the African invader fly, *Bactrocera invadens*, in Ghana has led to limitations in the mango export industry. The limitations ranging from increased control costs to rejection of exports has necessitated studies in the area of quarantine treatment. A study was conducted to ascertain the effective dose of gamma irradiation for the control of *B. invadens* in fruits intended for export. Pupae were obtained after incubation of mango fruits collected from various locations. Adults were reared out and 5 – 20 females were placed on fruits in cages and allowed to oviposit. Infested mangoes were then examined to determine larval infestation levels. Late instar (14-21 day old) larvae in fruits were irradiated at 15- 75 Gy to determine an effective dose for the control of *B. invadens.* The mortality of the fly was determined at the various doses to obtain a probit 9 figure of 70 Gy as the effective dose. Confirmatory tests using 3,050 larvae endorsed the effective dose as the probit 9 dose.

Key words: Gamma irradiation, *Bactrocera invadens,* mango fruits, mango industry.

INTRODUCTION

Bactrocera invadens was not known in Africa until its recent detection by Lux et al. (2003) in Kenya and Tanzania in 2003. This pest is known to have originated from Sri Lanka from where it got into Africa (Drew et al., 2005). In a space of one year, it had spread from the East African Countries to West and Central Africa, extending from DR Congo to Senegal (Hanna, 2005). This was detected in Ghana through a survey conducted by Billah et al. (2006). Since then, it has spread throughout the country (Wilson and Cobblah, 2007).

The damage caused by fruit flies stem from the puncturing activity of the female when it is about to lay its eggs. Rot causing bacteria are introduced into the fruit from the intestinal flora of the fly, thereby causing rot of the tissues surrounding the eggs laid. Larvae from the hatched eggs feed on the fleshly portion of the fruit, leading to the creation of galleries which serve as entry points for pathogens. This increases the decay of the fruit, thereby making it unsuitable for human consumption (Billah and Ekesi, 2006). The damage caused is seen as

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direct loss of yield, increased control costs, as well as the loss of export markets, and the cost of the maintenance of treatment and eradication facilities and trade barriers (Bech, 2008).

In Ghana, farmers experience 3-85% losses, exporters 10-15%, processors 2-10% and the total national loss of mango to fruit flies is 65% (Billah, 2008). Irradiation has been used as a quarantine measure as an alternative to methyl bromide when it was banned in the United States of America and other countries (IAEA, 2004). This method of quarantine control has become common for arthropods which are of economic importance. Irradiation has been used as a quarantine measure for sweet potato weevil (*Cylas formicarius elegantulus* [Summers]), oriental fruit moth (*Grapholita molesta* [Busck]), sugarcane borer (*Diatraea saccharalis* [F.]), south-western corn borer (*D. grandiosella* [Dyar]), Mexican rice borer (*Eoreuma loftini* [Dyar]) and Indian meal moth (*Plodia interpunctella* [Hubner]). Thus, irradiation encouraged trade among nations since countries would not want to have the introduction of pests of economic importance into their country. The USA demands that mangoes from affected countries in West Africa, such as Ghana are irradiated as a guarantee that will prevent the introduction of the African invader fly into that country (Bech, 2008).

In Australia, it is a necessity that mangoes from India are irradiated in order to prevent the introduction of the fruit fly. Fruits from the Philippines are to be irradiated before they are sent to the USA (Ignacio, 2008). There is a generic dose of 150 Gy for fruit flies which was proposed for the tephritids in 1986, based on irradiation data for many tephritid fruit fly species and a limited number of other insect pests, and 300 Gy for other insects (ICGFI, 1999; Heather, 2004). It is therefore imperative that doses for specific species of tephritids are established in order to reduce cost of irradiation since the higher the dose the more finance is committed to it and vice versa (Torres and Hallman, 2007). This will lead to increased capacity for treatment facilities by decreasing the required time for treatment (Follet and Armstrong, 2004). Doses below the generic dose have been observed in some fruit flies, some of these include: 100 Gy for *Ceratitis capitata* (Hallman and Torres, 2004); 125 Gy for *Bactrocera dorsalis* and 150 Gy for *B. cucurbitae* (Follet and Armstrong, 2004); 70 Gy for *Anastrepha* spp.; 101 Gy for *Bactrocera jarvisi* and *B. tryoni,* (Hallman and Loaharanu, 2002).

MATERIALS AND METHODS

Fruit collection

Infested Keitt mango fruits were collected from the University farm and the Botanical Garden (W 00° 11. 14^{l} and N 05° 39.63^{l}) of the University of Ghana, Legon, in the Greater Accra Region of Ghana. They were transported to the Zoology Department of the University of Ghana and the Ghana Atomic Energy Commission (GAEC, 00° 13.15^{l} and -05° 40.33^{l}) where experimental studies took place.

Incubation of eggs and larvae in the fruits

Infested fruits were picked from the University of Ghana Farms. These were incubated based on the method used by Utomi (2006). The fruits were kept in screened racks to prevent the access of other insects. The racks measured 0.45 x 0.29 x 0.09 m (length x width x height). Trays were fitted under each rack to collect ready-to- pupate larvae which had bored their way through the fruits. The trays contained moist sterilised soil for the larvae to pupate in them.

Pupae collection

The moist soil containing the pupae was sifted to collect the pupae. The soil was put back into the trays for further collection of subsequent pupae. The collected pupae were counted and put into the jam jars (which had dimensions of bottom and top diameters as 0.195 m and 0.230 m respectively and height of 0.095 m) and kept for sex ratio, percentage emergence and mortality.

Rearing of adults

Emerged adults were counted, and sex ratio determined. They were kept in infestation cages (which had top and bottom diameters of 0.66 and 0.61 m respectively and 0.24 m high). The temperature was between 28 and 30°C. They were fed with a solution of three parts of sugar and one part of yeast. Clean water was provided (soaked in cotton wool) for them to drink. Mortality was recorded on a daily basis.

Infestation level studies

The weight of non-infested fruits was recorded. 5, 10 and 20 males and females of *B. invadens* were put in each cage (in triplicates). A fruit was put in each cage for infestation (in triplicates) for five days. The number of ovipunctures on each fruit per cage was counted. The infested fruits were incubated in plastic containers, which had sterilised soil in them for pupariation. Pupae from each container were counted, incubated in glass containers for adult emergence. Percentage emergence was calculated for each container.

Gamma irradiation of infested fruits

Two hundred and forty insects (males and females in the ratio 1:1) were put into eight infestation cages for infestation (in 3 replicates). Each infestation cage contained a fruit. The fruits were removed after 24 h based on the method used by Follet and Armstrong, (2004) and kept in plastic containers with sterilised soil on racks in the laboratory. The number of larvae in each fruit was estimated based on the infestation levels done as above. Larvae in the fruits were allowed to reach the third instar stage which is the most radiation resistant stage (Balock et al., 1963).

Infested fruits were irradiated at the Radiation Technology Centre (RTC). The larvae in the fruits were irradiated at 15, 25, 35, 45, 50, 60 75 Gy at a dose rate of 193.52 Gyhr^{-1} at a distance of 0.50 m by 0.70 m from a Co^{60} source (type SLL-02) at the Ghana Atomic Energy Commission. Three hundred and forty-one larvae (in the fruits) were irradiated at each dose in three replicates with a control. Fricke dosimeter was used to calibrate the irradiation area and the dose distribution. Pupae collected were kept in Kilner jars with a mesh top for good ventilation for the flies when they emerged. They

Table 1. Development pattern at various infestation levels of *Bactrocera invadens.*

No. Flies	No. of holes/ fruit	No. of eggs/ hole	No. of larvae/fruit	Emergence (%)
5	2.5 ± 0.1[a]	92.6 ± 11.4 [a]	234.7 ± 26.7[a]	83.2± 0.7 [a]
10	3.6 ± 0.3[b]	164.5 ± 15.2 [b]	569.6 ± 43.5 [b]	81.5±1.6[ab]
20	6.3 ± 0.3[c]	176.0 ± 16.6 [b]	1068.7 ± 84.1 [c]	78.0±1.6[b]

Mean separation (Tukey's test): a, b and c are significantly different from each other (p<0.05), in the same column.

were kept in the insectary at the Animal Science Department of the Biotechnology and Nuclear Agriculture Research Institute of Ghana Atomic Energy Commission. Natural mortalities were corrected using Abbott's formula. SPPS 16 was used to analyse the data.

Confirmatory test

A large population of 3,050 late third instar larvae (in infested fruits) were irradiated at 70 Gy at a dose rate of 191.88 Gyh^{-1}. Fricke dosimeter was used to calibrate the irradiation area as well as the dose distribution area. A control group of 345 (larvae in fruit) were not irradiated based on the method used by Follet and Armstrong (2004).

RESULTS AND DISCUSSION

Oviposition behaviour

The adult females (from 10 days old) responded immediately and showed oviposition behaviour when the fruits were put into the infestation cages. The females climbed onto the fruits in the various cages apparently searching for holes before creating one as reported by Ogaugwu (2007). They then spent about five minutes on the fruits until oviposition is completed.

Signs of Infestation

After oviposition, exudates from the holes were visible. This gave the exact position where infestation had taken place. Points of infestation darkened until the eggs hatch after two days. The feeding habits of the larvae caused more exudates to come from the point of infestation and the ovipunctures made the fruits unattractive. As the larvae tunnel throughout, the fruits decayed at a faster rate. The skin of the fruits sunk when pressed, with some larvae occasionally coming out of the fruits. It was observed that the number of larvae increased considerably as the population of the flies was increased. There were significant differences (p>0.05) in the number of larvae per colony of flies shown in Table 1.

Pupariation

Larvae came out of the fruits when they reached the last instar stage. They entered into the soil in the containers to pupate. They pupated a day after entering the soil. The pupae were barrel shaped and had a creamy colour. Pupae had segmented lines as well as two dark projections at one end and the other end smooth.

Adult Emergence

Adults emerged from the collected pupae at a mean age of 8±0.6 (SD) days after pupation with a mean percentage of 80.89 ±5.46 (SD). The development at various infestation levels is shown in Table 1. The number of larvae per fruit was dependent on the number of female flies which have started ovipositing and visited the fruit (Table 1). After the eggs hatched into larvae, the early instars aggregated at the point where they were laid before spreading throughout the fruit as they grow, which confirms the general feeding habit of fruit flies as was reported by Pena et al. (2008). The number of larvae per infestation were significantly different (p<0.05) from each other. Ogaugwu (2007) observed that a female could lay 70 eggs per day on the average. Hence, the significant differences (p<0.05) was due to the number of females that laid eggs in each cage. Percentage emergence was significantly different (p<0.05) from each other among the population. Larger number of larvae in the fruits led to lower percentage of emergence. This was due to larger population feeding on the same fruit. This led to competition among the larvae for food.

Irradiation of infested fruits

After irradiation, fruits were opened (dissected) with a knife to allow the late irradiated instars to get out of the fruits after 24 h in order to pupate in the soil provided in each of 24 containers. All the larvae pupated. There were significant differences (p<0.05) in the number of non-emergence of adults for the various doses applied to the larvae. Irradiation increased the mortalities of the pupae seen in Figure 1, as the doses increased (Ogaugwu, 2007). A higher dose applied led to a high disruption of the cells of the flies and a higher effect of the life process taking place in the larvae. This led to the higher mortalities in the irradiated as compared with the control.

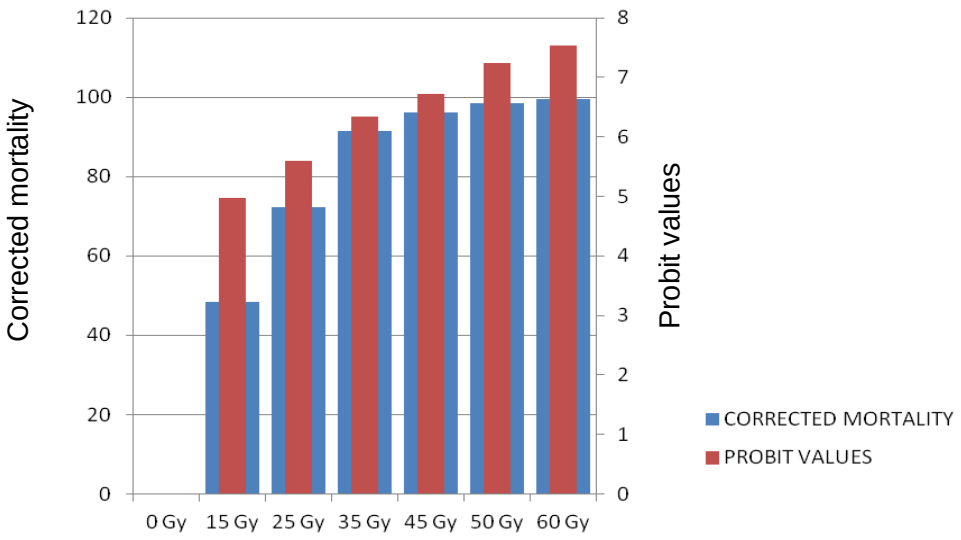

Figure 1. Mortality and the doses of irradiation treatment.

Confirmatory test

The probit 9 figure gave a dose of 68.506 Gy, rounded off to the nearest whole number as 70 Gy. This dose will lead to a mortality of 99.99% mortality of the pupae that where irradiated. All the larvae (3050) that were irradiated at the effective dose of 70 Gy at a dose rate of 191.878 $Gyhr^{-1}$ did not emerge. This confirms the dose as the effective dose for *B. invadens.*

Conclusion

The findings of this study showed that irradiating late larval instars at a dose of 70 Gy will lead to 99.99% of mortality (non-emergence) in *B. invadens.* The results with the large larval population of 3,050 at probit 9 level (99.99% mortality) confirmed that 70 Gy can be used as a quarantine treatment dose against *B. invadens* in *Mangifera indica.*

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Review

Effect of organic bio-stimulators on broiler performance: A review

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Present review article was designed with the aim to see the effect of organic bio-stimulator on the performance of broilers. Due to rapid urbanization and industrialization, the demand for meat, particularly tender chicken poultry meat (broiler) is rapidly increasing day by day. The cost of feeding in poultry production alone accounts for 60 to 70% of the total farm expenditure. This cost can be minimized to some extent by adding some of the growth stimulators in the broiler chicken feed. Result revealed that a large number of growth promoting agents and herbal products are occasionally being used in poultry feed to produce lower feed intake, higher growth rate, disease resistance and higher live weight gain in the poultry. Beside, above biochemical and heamotological attributes including carcass traits is also improved. Further, the ban on the use of synthetic growth promoters in farm animals due to its residual effects on consumers and resistance build up by pathogens or bacteria necessitates the use of natural symbiotic growth promoters.

Key words: Biochemical, broiler, carcass, growth, heamotological.

INTRODUCTION

India is the fifth largest producer of poultry meat in the world after USA, China, Brazil and Mexico (Executive guide, 2006). Poultry meat production increased from 81 thousand tonnes in 1961 to 1900 thousand tonnes in 2005. Poultry meat production increased by 8.7 and 6% per annum during the eighties and nineties (Mehta et al. loc cit.). In a recent study, Fairoze et al. (1995 to 2005) reported a growth rate of 13.9% for poultry meat for the period 1995 to 2005. Chickens are one of the major sources of animal meat. Broiler meat is the cheapest source of animal protein available in the country. Broiler farming is a profitable venture due to continuous increasing demand of the meat in the market. The most flourishing industry with highest growth rate (15% in broiler and 8% in layer sector). It is world's 3rd in egg production and 6th in chicken meat production. Feed is a major component, affecting net return from the poultry business, because about 65% of the total expenditure in term of cash is spent on feed purchase. To ensure more net return and to minimize high expenditure on feed are the main challenges, for which many research strategies have been practiced such as introducing feed supplements and feed additives (Pervez, 1992). Therefore, the aim of this review is to collect the

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information regarding the impact of organic bio-stimulator on feed intake, growth rate, carcass production, cure for diseases haemotological and biochemical attributes of broiler production. The pioneer that worked in this aspect has been reported.

FEED INTAKE

Majdanski (1991) conducted a trial on broiler chickens maintained in cages and on the straw litter fed complete feeds (starter, grower, and finisher) supplemented with 0.6 and 1.0% commercial blends of herbs (Herbagal). Finally, body weight of chickens given herbal additive was slightly higher with better feed conversion efficiency (kg/kg + 11%) in series 2, best results were obtained at 1.0% supplements of herbs to starter feed mixture and 0.6% supplement to grower and finisher. Chickens fed herbal additives had final body weight 63 gm higher and F.C.E. by 6.3% than control. Further, Muhammad et al (2000) observed that the effect of Digestarcom, (an herbal feed additive) on the performance of broiler chicks fed different levels of rapeseed cake was investigated. Fourteen experimental rations containing 7 rapeseed (0, 2, 4, 6, 8, 10 and 12%) × 2 Digestarcom (0 and 150 g per ton feed) levels were formulated and fed to 14 treatment groups, 3 replicates of 10 chicks each. A higher weight gain per bird was observed for all the levels of rapeseed treated with Digestarcom as compared to non-supplemented control group.

The maximum feed intake observed in the group fed 10% rapeseed cake and maximum weight gain was observed at 6% level. More feed was consumed and more weight was gained by broilers fed the ration supplemented with Digestarcom which also exhibited better feed to gain ratio than the non-supplemented control. Thereafter, Wekhe et al. (2007) conducted the experiment to determine the effect of consumption of *Rhizophora racemosa* (Mangrove) incorporated feed on feed intake, body weight gain, and some visceral organs of broiler chicks. Sixty day-old Hubbard broiler chicks were randomly allocated into four treatment groups, A, B, C and D of 15 birds per group and 5 birds per replicate. The dosages of the groups were 0 (control), 10, 20 and 30 g per kilogram of feed, respectively. Results obtained showed that there were no significant differences ($P > 0.05$) among the treatment means with respect to feed consumption/efficiency, and body weight gain.

However, a lineal body weight gain was observed. There was obvious hypertrophy of the bursa of Fabricus of groups B, C, and D, indicative of defensive reaction to *R. racemosa* (foreign body) and increase of antibody production. The observed atrophy of the ovaries and the converse hypertrophy of the testes are analogous to decreased ovarian and increased testicular functions, respectively. Further, Ram et al. (2012) conducted a trial of 35 days on herbal drug (*Livkey* at 50 ml/kg diet) and found the better feed intake than the control one.

GROWTH RATE

Reddy and Rao (1988) reported that acid treatment of decorticated Neem cake and acid plus alkali treatment of all Neem cake improved growth and achieved a significant weight gain and feed efficiency of broilers. Thereafter, Osei et al. (2000) experimented on 288 three-week-old mixed-sex broiler which was randomly divided into three dietary treatment groups and fed diets containing wheat bran 170 g/kg and 0, 100 or 200 g/kg of enzyme preparation (primarily xylanase and pentosanase, plus galactomannase, beta-glucanase, cellulase and pectinase), respectively, for four weeks. Feed and water were supplied *ad libitum*.

The parameters studied included growth rate, feed consumption, feed conversion ratio, carcass evaluation, and the economics of production. The addition of enzymes reduced feed consumption and increased feed conversion efficiency and growth rate ($P < 0.05$). The reduction in feed intake compared with birds on the control diet ranged from approximately 10 to 15%. At seven weeks of age, birds on 100 and 200 g/kg enzyme were respectively 1.9 and 5.8% heavier than their counterparts on the diet with no added enzyme. Birds fed diets containing the highest level of enzyme were 21.1% more efficient in converting feed to body constituents. Carcass dressing percentage increased with added enzyme ($P < 0.05$). Dietary enzyme significantly decreased the total cost of feed per bird and the cost per kg gain. Enzyme added at 200 g/kg diet was the most economical.

Further, Abaza et al. (2003) studied that the effect of some dried medicinal plants, such as *Nigella sativa* seeds (N.S.), German chamomile flower heads (*Matricaria chamomilla Chamomilla recutita*, CH.F), thyme flowers (*Thymus vulgaris*, TH.F) and harmala seeds (*Peganum harmala*, H.S.), as feed supplements as well as that of antibiotics such as zinc bacitracin (ZnB) and virginiamycin (VIR) as growth promoters on the performance of broiler chicks. The N.S., CH.F, TH.F and H.S. were added at levels of 0.25, 0.25, 0.5 and 0.25%, respectively. A total of 240 broiler chicks were randomly divided into 16 treatments with 3 replicates (5 chicks each). ZnB and VIR were added at a level of 20 mg/kg diet. The weight gain, feed intake, feed conversion and blood serum constituents were measured. At the end of the experiment, digestibility values and carcass characteristics were measured. The use of N.S., TH.F, H.S. and CH.F improved the performance of broilers compared to antibiotic-treated and control groups. The use of 0.25% CH.F plus 0.25% N.S. improved the body weight, body weight gain, feed conversion, and carcass traits in broilers. Moreover, it proved to be more economical than the other treatments or control. Das and

Bora (2004) studied the performance of six genetic groups (SC, NZW, SN, NS, SL, NL) of 120 broiler birds feed supplemented with 2ycox, (a herbal anticoccidial growth promoter). Average daily weight gain, feed utilization and survivability percentage were higher ($P > 0.05$) at 0.4% level of zycox supplementation indicating its better efficacy as anticoccidial as well as growth promoter. Emenalom et al. (2005) again observed effect of velvet bean (*Mucuna pruriens*) which was cracked, soaked and cooked meal (CSCM) was included in a broiler mash at 0, 20, 25 and 30%, respectively. None of three dietary levels of seed meal significantly ($P > 0.05$) affected the performance of the birds in terms of feed intake and growth rate. At 30% dietary level, the feed conversion ratio was significantly ($P < 0.05$) decreased as compared to the control. Further Ram et al. (2011 and 2012) conducted a trial of 35 days on broiler bird with herbal drug (Livkey at 50 ml/kg diet) and found the better growth rate than the control one.

CARCASS PRODUCTION

An experiment was conducted under completely randomized design with five replicates and 150 chicks per experimental unit was used to evaluate the effect of growth promoters on feed intake, liveweight gain and feed:weight gain ratio of broilers (Correa et al., 2003). The experimental diets were: I - initial diet (20.2% of crude protein and 2931 kcal of metabolizable energy) from 1 to 20 days of age (ID) and final diet (18.5% of crude protein and 2993 kcal of metabolizable energy) from 21 to 40 days of age (FD); II - ID plus 0.02% of Calsporin 10 probiotic and FD plus 0.02% of Calsporin 10 probiotic; III - ID plus 2.0% of probiotic Estibion and FD plus 0.63% of probiotic Estibion; and IV - ID plus 0.013% of Zinc bacitracin and FD plus 0.013% of Zinc bacitracin. During the initial period, broilers fed on probiotic diets showed lower feed intake and better feed efficiency. However, growth promoters had no effect on the studied traits in the final and total periods. Higher thigh yields were observed for males fed on polyprobiotic diets.

Santos et al. (2004) studied that the performance of broiler chickens, placed to the diets containing different additives. It used 2160 birds distributed in a randomized completely experiment with 6 treatments (T) with 8 repetitions of 45 birds each: T1 without growth promoter, T2 and T3 with antimicrobials, T4, T5 and T6 with probiotics. Feed consumption, body weight gain, food conversion and mortality, and carcass yield, cutting, visceras, factor and cost of production were determined. Significant differences between the treatments were not observed in majority of the treatments.

The birds that received antimicrobial growth promoters showed the best feeding conversion in relation to the birds that received control and probiotic ration. The feeding conversion difference reflected in the production cost. Thereafter, Sirvydis et al. (2004) observed the influence of feed additives of plant origin Digestarom-1317 and Aviance on the growth and meat quality of broiler chickens. Three groups of 100 day-old chicks each (50 males and 50 females) were fed a standard feed mixture (Group I), feed supplemented with Digestarom-1317 at 150 g/tonne feed (Group II) or feed supplemented with Aviance (500 g/tonne feed) until 42 days of age. Liveweight was measured at 1, 7, 21, 35 and 42 days of age, after which the birds were slaughtered for meat quality determination. It was shown that the growth; liveweight gains; carcass composition, yield and weight and meat quality of broilers in the feed additive groups were better than the control group, where the best results were obtained by Group II birds for both males and females.

In conclusion, the use of Digestarom-1317 as a feed additive is more advantageous since it is economical and promotes better growth, liveweight gain, carcass yield and meat quality in broiler chickens. Sarica et al. (2005) again reported that the effects of an antibiotic growth promoter (flavomycin) and two herbal natural feed additives (garlic and thyne) with and without a xylanase-based enzyme complex in wheat-based diets on 112 days-old male broiler chicks raised from 1 to 42 days of age. During the 42 days growth period there were no significant differences in body weight gain, feed intake and feed conversion ratio of the broilers between dietary treatments. Feeding the diet supplemented with the antibiotic plus the enzyme significantly increased hot and cold carcass yield compared to the diets supplemented with thyme, garlic, enzyme and garlic plus enzyme. Total plasma, cholesterol concentration and relative weight of the heart, pancreas, liver, gizzard and spleen were not significantly influenced by dietary treatments.

Im et al. (2007) found the effect of dietary krill meal in one day-old male broiler chicks (Rurs**) were fed basal meal (0.0% krill meal), or diets containing 0.5%, 1.0% and 2.0% krill for 3 weeks. Acute phase response induced a significant reduction in ($P < 0.05$) daily weight gain and feed intake and increases in liver and spleen weight. However, it was not affected by dietary krill meal levels.

Furthermore, Buchanan et al (2008) observed that the use of subtherapeutic levels of Biostrong 505+ and Biostrong 510 as natural growth promoters in broiler chickens. Assessment was based on the performance and carcass quality of broilers fed either a maximum-yield or a least-cost commercial broiler diet. The maximum-yield diet improved broiler performance and carcass quality. Biostrong 505+ can be used to improve feed conversion and breast yield when incorporated into diets devoid of antibiotics. This improvement in feed conversion and breast yield was accentuated when Biostrong 505+ was used in conjunction with a maximum-yield diet. Biostrong 510 improved feed conversion when used in poultry diets containing antibiotics.

CURE FOR DISEASES

Dickens et al. (2000) reported that herbal protecta II extract on an NaCl carrier was evaluated in a 30 min 1 x C simulated chill for its effectiveness of lowering microbial counts on broiler carcasses. Mani et al. (2000) again revealed that the effects of 3 commercial Newcastle disease lentogenic vaccines were studied in broilers during experimental aflatoxicosis. Broilers were fed a diet containing 0.20 ppm of aflatoxin B_1 or aflatoxin B-free diet for 8 weeks. Control birds showed higher immune response after vaccination against Newcastle disease, body weight and feed consumption and feed efficiency than aflatoxin-fed birds. Enlargement of liver, atrophy of bursa and reduced eviscerated yields were observed due to aflatoxicosis in broilers. The aflatoxin-fed birds had reduced packed cell volume, and concentrations of haemoglobin, serum protein and cholesterol and elevated concentration of serum glucose, alanine aminotransferase and aspartate aminotransferase compared with the control group.

Thereafter, Das et al. (2001) studied that the efficacy of herbal anticoccidials IHP-250C and herbal immunostimulant Immu-21 (containing *Ocimum tenuiflorum, Withania somnifera, Tirospora cordifolia* and *Phyllanthus emblica* extracts) against experimental *Eimeria tenella* infection in broiler chicks. IHP-250C + Immu-21 provided highest protection against virulent *E. tenella* infection. Total leukocyte count, total serum protein and serum globuline values of Immu-21-treated birds were significantly higher and recommended Immu-21 to be incroperated in broiler rations to augment the immune response against coccidiosis and other infections and increase the economic returns. Christaki et al. (2004) evaluated that the commercial preparation of herbal extracts Apacox (Apa-CT) on the performance of Cobb-500 chicks experimentally infected with 6 x 104 sporulated oocysts of *E. tenella* at 14 days of age. Dietary supplementation with Apacox attained higher body weight gain and feed conversion ratio values than the non-supplemented challenged control group. Bloody diarrhea was absent in Apacox group and mortality in Apacox group was 13.4% whereas in control group 23.4%.

HAEMATOLOGICAL ATTRIBUTES

Akinleye et al. *(*2008) observed that Eight weeks feeding trial was carried out on hematological of one hundred and twenty day old Ross breed broiler chicks maintained on diets containing 1 g/kg biomin - a growth promoter, at both starter and finisher phases. Oral biomin (20 mg/bird) were also given at both phases for three consecutive days. The starter mash fed contained 22% crude protein (cp) and 3,000 kcal metabolizable energy (ME) per kg of feed while the finisher's mash contained 17% cp and 3,020 kcal ME/kg of feed. Control diets without biomin were allowed at both phases. The difference in the haemotological parameters of birds on both treatments were not significant ($P > 0.05$) after eight weeks of feeding. Further, Ozduven et al. (2009) reported to evaluate the effects of organic acid mixture and/or mannanoligosaccharides (MOS) on growth performance, blood parameters and intestinal microbiota in 120 Ross 308 male broiler chicks, over a period of 21 days. Birds were maintained in battery brooders confined in an environmentally controlled experimental room. There were 4 dietary treatments, each consisting of 6 replicates.

Dietary treatments were: (i) basal diet (as a control), (ii) basal diet+MOS 2 kg/ton feed, (iii) basal diet+organic acid mixture 3 kg/ton feed and (iv) basal diet+MOS 2 kg/ton feed+organic acid mixture 3 kg/ton feed. Result revealed that the performance of biochemical attributes was better than the control one. Furthermore, Yalcnkaya et al. (2010) studied the effects of rations containing organic selenium and Vitamin E on live weight, live weight gain, feed consumption, feed efficiency, internal organ weights and blood parameters of broiler chicks. A total of 120, one day old Ross 308 male broiler chicks were used in this study. There were 4 treatment groups each containing 10 chicks of 3 replicates. The control group (K) was fed with basal diet without supplemented organic selenium (Se) and Vitamin E. Treatment groups were fed with 0.6 ppm organic selenium (Sel-Plex) (Se); 150 IU/kg Vitamin E (E) and 0.6 ppm organik selenium (Sel-Plex) +150 IU Vitamin E (Se+E).

The experiment lasted 42 days. There were no significant differences live weight, feed consumption, feed efficiency and relative internal organ weight among the groups ($P > 0.05$). Serum vitamin E levels were higher in vitamin E (E) supplemented group than in other groups ($P < 0.05$).

BIOCHEMICAL ATTRIBUTES

Asma and Nagra (2008) observed the effect of organic (OP) and conventional premix (CP) and found that the Serum alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase activities were within normal physiological range in both treatments. The level of antibody titre against Newcastle disease and infectious bursal disease vaccination due to inclusion of supplements was also statistically similar in both CP and OP group. The hepatic ribonucleic acid (RNA) and Vitamin-C was significantly higher in OP group when compared with CP group. The OP group significantly reduced the intestinal harmful bacteria (*Bacteroides* spp. and *Escherichia coli*). It is suggested that broiler can be reared on organic premix without any adverse effect on their performance. Thereafter Lagana et al. (2007) studied to evaluate the effect of diets supplemented with Vitamins C and E and organic

minerals Zn and Se on serum biochemical parameters in broilers under cyclic heat stress (HS) (25 to 35°C) or termoneutral environment (TN) (21 to 25°C), both with 70% humidity. A 4x2 factorial design was used with four levels of vitamin mineral supplementation (T1 - control diet: 60/30 UI vitamin E in starter and grower diets, respectively; no vitamin C, 80 ppm of inorganic Zn e 0.3 ppm of inorganic Se; T2 - control diet+100 UI vit E and 300 ppm vit C/kg; T3 - control diet+40 ppm of organic Zn and 0.3 ppm of organic Se/kg and T4 - control diet+supplementation levels of T2 and T3) and two environments: cyclic heat stress and termoneutral, from the 14 degrees day of age. The experimental period was from 1 to 35 days of age and noted the reduction in the hemoglobin concentration. In the other hand, types of vitamin and/or mineral supplementation did not affect serum biochemical parameters of broilers under cyclic heat stress.

CONCLUSION

Finally, it is concluded that the enhancement of parameters such as feed intake, growth rate, carcass production, cure for diseases, haemotological and biochemical attributes of broiler production with eco-friendly by using the organic bio-stimulator.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Inoculation of *Herbaspirillum seropedicae* in three corn genotypes under different nitrogen levels

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The biological nitrogen fixation (FBN) associated with maize culture becomes extremely important, even if only part of the need for N by culture may be supplied by this process. The aim of this study was to verify the behavior of three maize hybrids to inoculation of *Herbaspirillum seropedicae* under different nitrogen levels. The test was conducted under controlled conditions of greenhouse and the experimental design was completely randomized design adopted in factorial scheme 3 (hybrid corn) × 2 (plants inoculated and non-inoculated) × 2 (levels nitrogen), with four replicates. Plant height, stem diameter, chlorophyll content, length root, volume of root, content N in shoot and root were determined for 35 days after emergence, as well as the efficiency of use of N. The hybrid BRS 3035 stands out for most of the variables analyzed, producing most of the shoot dry mass, plants with greater height, diameter of stem, root volume and showing the highest rates of N use efficiency. The *H. seropedicae* was inoculated with promoted increase in the volume of root, root length, dry weight of shoot, chlorophyll content, content of N in aboveground and N use efficiency. Inoculation strain Z-94 of *H. seropedicae* plus 80 kg ha^{-1} de N increased the concentration of N in the aerial part of plants of corn by as much as 25% in the evaluated genotypes.

Key words: Inoculants, diazotrophic bacteria, nutrition, biological nitrogen fixation.

INTRODUCTION

In Brazil, corn is cultivated in almost all the national territory, with 90% of its production concentrated in South, Southeast and Central-west regions. The expected harvest for 2012/2013 is nearly 76 million tons of corn, according to the Brazil's state-owned National Food Supply Company-CONAB (CONAB, 2013). This crop is usually influenced by environmental stress problems, among which stands out the low fertility of soils, which frequently show nitrogen (N) deficiency as well. According to Fancelli and Dourado Neto (2008), such deficiency can reduce grain yield by 14 to 80%. The identification, selection and use of corn genotypes more tolerant to N deficiency and more efficient at acquiring this element constitute an important strategy (Reis Junior

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et al., 2008). Therefore, the search for genotypes forming more efficient associations with diazotrophic and growth-promoting bacteria must be considered. It is known that there are interactions between N and those bacteria in the assimilation and use of this nutrient by plants (Reis Junior et al., 2008). Huergo et al. (2008), Cassán et al. (2008) and Donate-Correa et al. (2004) observed for example that diazotrophic bacteria can stimulate plant growth for acting in the process of biological nitrogen fixation (FBN), in the increase of nitrate reductase activity when occurring endophytically and in the production of phytohormones such as auxin, gibberelin and citocianin.

Due to the large area occupied by cereals, nearly five times the area of leguminous plants, the FBN associated with these crops becomes extremely important, even if only part of their N needs can be supplied by FBN (Sala et al., 2007). Regarding FBN contribution, Alves (2007) reports N values up to 67% from FBN, when inoculated with *Herbaspirillum seropedicae* and yield increase of up to 34%, depending on the corn genotype and mineral-N dose.

There are variations between corn genotypes in the response to N fertilization (Alfoldi et al., 1992), and the interactions between corn the diazotrophic bacteria are dependent on plant genotypes and on the microorganisms involved in such associations (García de Salomone and Dobereiner, 1996). Nowadays, among the diazotrophic microorganisms found in associations with cereals and grasses, the species of *Herbaspirillum* constitute one of the most studied groups.

Although the genus *Herbaspirillum* comprise nine species, only four of them can fixate nitrogen: *H. seropedicae*, *Herbaspirillum rubrisubalbicans*, *Herbaspirillum frisingense* and *Herbaspirillum lusitanun*. Most of the inoculation experiments refer to *H. seropedicae*, because of its occurrence in a large number of studied plants (Alves, 2007).

Studies involving *H. seropedicae* are interesting, because, among the characteristics of this species, the capacity to colonize the interior and the aboveground part of plant tissues stands out (Baldani et al., 1997), settling within niches protected from oxygen, and being able to express its potential to FBN in the maximum degree (Kennedy et al., 2004). In Brazil, a few studies have focused on the interactions between corn genotypes, nitrogen fertilization and diazotrophic bacteria. In many cases, the absence of response to diazotrophic bacteria inoculation in grasses has been assigned to the use of inappropriate strains. There is a consensus that the plant genotype is the key factor to obtain the benefits from the FBN, combined with the selection of efficient strains (Reis et al., 2000). Despite many years of research, variable responses are still observed, which justifies and shows the importance of experiments in field and greenhouses.

Therefore, this study aimed to verify the behavior of corn hybrids in response to the inoculation of *H. seropedicae* under different N levels through a test conducted under greenhouse controlled conditions.

MATERIALS AND METHODS

The experiment was performed in a greenhouse, in the Agricultural Science College of the Federal University of Grande Dourados, Dourados-MS, Brazil, from December 2012 to January 2013 (22°12' S; 54°56'W; 452 m). The weather is classified as Cwa (humid climate, warm summers and dry winters) according to Köppen. The soil used, classified an Oxisol with very clayey texture, was collected at the layer of 0 to 20 cm. The results of the soil chemical analysis before the experiment were: pH ($CaCl_2$): 4.15; P: 26 mg dm^{-3}; K: 5.0 $mmol_c$ dm^{-3}; Ca: 9.0 $mmol_c$ dm^{-3}; Mg: 2.0 $mmol_c$ dm^{-3}; Al: 3.3 $mmol_c$ dm^{-3}; H+Al: 41.6 $mmol_c$ dm^{-3}; SB: 115.1 $mmol_c$ dm^{-3}; CEC: 531.1 $mmol_c$ dm^{-3}; and Base Saturation: 21.7%. Granulometric analysis showed 225 g kg^{-1} of sand, 125 g kg^{-1} of silt and 650 g kg^{-1} of clay.

A completely randomized experimental design was adopted in factorial scheme 3 (corn hybrids) × 2 (inoculated and non-inoculated plants) × 2 (levels nitrogen), with four replicates. The experimental units were comprised of 10 dm^{-3} plastic pots, filled with air dried soil, sieved through a 4-mm-grid sieve.

Considering the soil chemical analysis, very fine dolomitic limestone (TNP 100%) was applied 30 days before planting in order to increase base saturation to 50%. Due to the low fertility of the soil, foundation fertilization was applied to grant crop establishment. 200 kg ha^{-1} of P_2O_5 (270 mg dm^{-3}) and 60 kg ha^{-1} of K_2O (51 mg dm^{-3}) was mixed to the soil and applied in the form of single superphosphate and potassium chloride, respectively. Micronutrients were applied according to the crop demand, in the form of solution, using deionized water and salts per annum, according to Epstein and Bloom (2006). The nitrogen fertilization was performed in 80 dose kg ha^{-1} of N (54 mg dm^{-3}) in the form of urea (45%), applied in two times of 40 kg ha^{-1} of N (27 mg dm^{-3}). The first in the sowing and the second, by top-dressing, at 15 days after plant emergence.

Soil moisture was daily controlled, through weighing, to maintain the soil at 60% of the field capacity. Irrigation was performed with deionized water.

Corn seeds of simple hybrid P3646H (Pioneer), triple hybrid BRS3035 (Embrapa) and double hybrid Maximus (Syngenta) were used, being previously inoculated with the Z-94 strain of *H. seropedicae* (inoculant cells concentration around 10^9) in the peat-based formulation, produced by Embrapa Agrobiologia, Seropédica-RJ. The dose was 250 g of peat inoculants for each 10 kg of corn seeds. For inoculation, 60 mL of a sugar solution at 10% (mass/volume) were added to increase inoculant adhesion to seeds. The seeds germinated directly in the pots and the thinning was performed in only one plant in each experimental unit.

Plant height, stem diameter and chlorophyll content in leaves were measured at 35 days after plant emergence. Plant height was obtained by measuring the distance from base to the apical meristem of the stem, with a ruler; stem diameter was obtained using a digital caliper at the height of 2 cm from the base of the stem; and chlorophyll content in leaves (SPAD reading) was measured with a chlorophyll meter (Minolta-Model 502). Plants were then divided into root and shoot parts. All the material was sequentially washed in detergent solution (3 mL L^{-1}), running water, HCl solution (0.1 mol L^{-1}) and deionized water. Root length was determined with a ruler and root volume by the volumetric flask method, in which roots were immersed in a known volume of deionized water in a graduated cylinder, and the volume was obtained by the difference between initial and final volumes in the container. Samples were then packed in paper bags and dried in an oven with forced air circulation at 65ºC for 72 h. After drying, the material was weighed, ground in a Wiley mill and then subjected to

Table 1. Summary of variance analysis for plant height (ALT), stem diameter (DIA), root volume (VR), root length (CR), shoot dry mass (MSPA), root dry mass (MSR), chlorophyll content (CLO), shoot nitrogen content (TNPA), root nitrogen content (TNR) and nitrogen use efficiency (EUN) for three hybrids of corn subjected to different nitrogen levels and inoculated with *Herbaspirillum seropedicae*. Dourados, MS (2013).

Source of variation	GL	Mean square									
		ALT	DIA	VR	CR	MSPA	MSR	CLO	TNPA	TNR	EUN
Hybrid (H)	2	83.01*	7.30*	885.89*	34.39	51.30*	3.94	114.16*	14.77	55.95*	1.36*
Nitrogen (N)	1	485.14*	53.93*	7600.33*	143.52*	224.42*	41.45*	2328.26*	2473.07*	1181.88*	12.26*
Inoculation (I)	1	0.14	7.39	5292.00*	150.52*	33.41*	12.19	75.25*	107.71*	24.73	1.17*
H*N	2	30.54	0.47	294.64	23.39	7.23	0.48	39.07	28.23	13.04	0.79
H*I	2	7.21	11.84*	192.56	18.89	2.55	12.53	2.67	65.63*	4.25	0.06
N*I	1	1.54	3.43	0.33	63.02	4.30	3.49	0.15	157.26*	24.73	0.06
H*N*I	2	47.09	1.93	143.39	141.89	0.77	0.72	3.88	81.53	62.31	0.66
Residue	36	11.16	2.08	84.50	15.32	3.33	5.01	3.36	8.22	10.05	0.12
CV (%)		6.39	8.91	12.41	6.58	24.07	9.18	7.50	13.13	19.35	17.41

* – Significant by Tukey test to 5% probability. CV- coefficient of variation.

Table 2. Effect of nitrogen doses on plant height (ALT), stem diameter (DIA), root volume (VR), root length (CR), shoot dry mass (MSPA), root dry mass (MSR), chlorophyll content (CLO), shoot nitrogen content (TNPA) and root nitrogen content (TNR) of corn plants cultivated in greenhouse and harvested 35 days after sowing. Dourados, MS (2013).

Nitrogen (kg ha^{-1})	ALT (cm)	DIA (mm)	VR (cm^3/plant)	CR (cm)	MSPA (g)	MSR (g)	CLO (SPAD)	TNPA (g kg^{-1})	TNR	EUN (mg g^{-1})
0	49.10^b	15.14^b	61.50^b	57.75^b	5.43^b	23.47^b	17.49^b	14.66^b	11.42^b	1.52^b
80	55.45^a	17.26^a	86.66^a	61.20^a	9.75^a	25.33^a	31.42^a	29.02^a	21.34^a	2.54^a

Means followed by the same letter in the row do not differ statistically between themselves by Tukey test at 5% probability.

sulfur digestion to determine N contents in root and shoot, according to the methodology described in Embrapa (2009). The N use efficiency, ratio between total dry mass produced and the total N accumulation in the plant, was calculated according to Siddiqi and Glass (1981).

The results were subjected to variance analysis and the means compared through Tukey test at 5% of probability, using the statistical program SISVAR (Ferreira, 2000).

RESULTS AND DISCUSSION

Significant effects ($p \leq 0.05$) were observed in the interaction Hybrid (H) × Inoculation (I) for stem diameter and shoot N content, while the interaction Nitrogen (N) × Inoculation (I) influenced for shoot N content. No other interaction had significant effects and the results are presented independently for hybrid, inoculation and nitrogen (Table 1).

The rates N influenced all the studied variables of corn plants (Table 1). There was a significant effect of nitrogen fertilization on plant height (Table 2). The increment in height of plants observed in this study is associated with the elongation of the stem promoted by N due to the fact that, according to Marschner (1995) the application of high doses of N in the early stages of development of cereals promotes increased production of hormones growth promoters (giberilinas and auxins, cytokinins) responsible for the processes of division and cell expansion. Studying the effects of nitrogen application in the culture of corn, Silva et al. (2003) found a positive response at the time of plants with increased doses of nitrogen. This shows that plants properly nourished with N may have higher vegetative development.

The basal stem diameter was influenced positively by increasing the dose of N (Table 2). It is noted that greater stem diameter is directly related to the increase in production, once it operates in soluble solids storage that will be used later to the formation of the grains (Fancelli and Dourado Neto, 2008).

The volume of root, root length and root dry mass increased with the dose of N (Table 2), corroborating the results of Taylor and Arkin (1981) and Glass (1990), which reported a change in growth of roots in soil fertility function. In addition, favors the growth of the root system, the plant, providing conditions for greater absorption of water and nutrients (Rao et al., 1992).

The chlorophyll content in the leaves of corn was higher in fertilized plants with 80 kg ha^{-1} of N (Table 2). Read results of chlorophyll that reinforces nitrogen fertilization improved nutritional nitrogen level in maize, due to the fact that the amount of this pigment correlate positively with N content in the plant (Booij et al., 2000).

Table 3. Behavior of three corn hybrids regarding plant height (AP), stem diameter (DIA), root volume (VR), shoot dry mass (MSPA), chlorophyll content (CLO), root N content (TNR) and N use efficiency (EUN), cultivated in greenhouse and harvested at 35 days after sowing. Dourados, MS (2013).

Hybrid	ALT (cm)	DIA (mm)	VR (cm^3/plant)	MSPA (g)	CLO (SPAD)	TNR (g kg^{-1})	EUN (mg g^{-1})
Maximus	50.40[b]	15.62[b]	67.68[b]	5.91[b]	26.77[a]	18.44[a]	1.79[b]
P3646H	51.62[b]	16.04[ab]	72.31[b]	7.38[b]	25.06[b]	15.91[ab]	1.94[b]
BRS3035	54.81[a]	16.95[a]	82.25[a]	9.47[a]	21.53[c]	14.79[b]	2.36[a]

Means followed by the same letter in the row do not differ statistically between themselves by Tukey test at 5% probability.

Table 4. Effect of inoculation with *Herbaspirillum seropedicae* on root volume (VR), root length (CR), shoot dry mass (MSPA), chlorophyll content (CLO) and shoot N content of corn plants cultivated in greenhouse and harvested at 35 days after sowing. Dourados, MS (2013).

Inoculation	VR (cm^3/plant)	CR (cm)	MSPA (g)	CLO (SPAD)	TNPA (g kg^{-1})	EUN (mg g^{-1})
Without	63.58[b]	57.70[b]	6.75[b]	23.20[b]	20.34[b]	1.87[b]
With	84.58[a]	61.25[a]	8.42[a]	25.71[a]	23.34[a]	2.19[a]
Increase (%)	33.02	61.52	24.74	10.81	14.74	17.11

Means followed by the same letter in the row do not differ statistically between themselves by Tukey test at 5% probability.

In Mello 2012, also found increase in chlorophyll content in the leaves of corn with the doses of N. Kappes et al. (2013) observed that the application of 90 kg ha^{-1} of N in coverage provided greater foliar chlorophyll content in maize plants. The results confirm the role of nitrogen in plant metabolism, directly connected to the biosynthesis of chlorophylls (Andrade et al., 2003), and it is important at the initial stage of plant growth and development, during which the absorption is more intense.

The content of N in aboveground, content of N in root, absorption efficiency and utilization of N by plants of corn was superior at 80 kg ha^{-1} of N (Table 2). Carvalho et al. (2013) evaluating maize cultivars on the efficiency of absorption and utilization of N in contrasting levels of nitrogen consisted of N doses effect on dry matter production of the aboveground N content on the shoot and root, and on the efficiency of use of paragraph in literature several papers corroborating to those found in this study (Carvalho et al., 2013; Kappes et al., 2011, 2013).

The corn hybrids showed significant response ($p \leq 0.05$) to plant height, stem diameter, root volume, shoot dry mass, chlorophyll content, root N content and N use efficiency (Table 1). The hybrid BRS 3035 was statistically higher ($p \leq 0.05$) hybrids P3646H and Maximus for plant height, stem diameter, root volume, dry weight of shoot and N use efficiency (Table 3). Opposite results were found for the other variables. Chlorophyll and root N contents for the hybrid Maximus were higher than those for P3646H and BRS 3035 (Table 3), showing that the former is more efficient in N absorption, while the hybrid BRS 3035 is more efficient in the use of nitrogen. Fernandes et al. (2005), studying six corn cultivars, observed significant differences in N use efficiency by the plants. Reis Junior et al. (2008) also observed differences in dry mass accumulation and N use efficiency between the studied corn hybrids. Araujo et al. (2013) confirms the distinction of response between maize cultivars in terms of dry matter production and N content of increment in the aerial part of plants. Such differences between corn hybrids, regarding N use efficiency, are due to the genetic variations between them (Alfoldi et al., 1992).

Inoculation with the Z-94 strain of *H. seropedicae* influenced root volume, root length, shoot dry mass, chlorophyll content, shoot N content and N use efficiency (Table 1). Plants inoculated with this strain showed an increase on the order of 33.02% in root volume and 61.52% in root length, compared to the control group, non-inoculated (Table 4 and Figure 1). This effect is probably due to the auxin production by the bacteria, which stimulates the growth of secondary roots, thus increasing the specific area for water and nutrients absorption by plants (Radwan et al., 2004). Similar results were found by Quadros (2009), reporting that the root volumes for corn hybrids P32R48 and D2B587, in the treatments inoculated with *Azospirillum*, were approximately 60 and 80% higher than those in non-inoculated treatments. Canellas et al. (2013) found increased root area of corn plants when inoculated with *H. seropedicae* in combination with humic substances.

Shoot dry mass and, the content of N in aboveground, the absorption efficiency of N and N utilization increased in the order of 24.74, 14.74 21.95 and 17.11%, respectively with the inoculation of *H. seropedicae* relative to the control (Table 4), evidencing the beneficial effects of the bacteria in nitrogen assimilation by plants of corn. Corroborating with the results, Alves (2007) verified that inoculation with *Herbaspirillum* spp. contributed with up to 28% of the N absorbed by corn plants. In greenhouse conditions, Guimarães et al. (2007) working

Table 5. Stem diameter (DIA) and shoot nitrogen content (TNPA) of three corn genotypes inoculated and non-inoculated with *Herbaspirillum seropedicae.* Dourados, MS (2013).

Hybrid	DIA (mm)		TNPA (g kg^{-1})	
	Inoculation		Inoculation	
	Without	With	Without	With
Maximus	14.55^{bB}	16.70^{abA}	23.40^{aA}	21.90^{aA}
P3646H	16.61^{aA}	15.46^{bA}	18.72^{bB}	22.84^{aA}
BRS 3035	16.27^{abA}	17.63^{aA}	18.91^{bB}	25.27^{aA}

The lowercase letters separate the inside of the lines and the upper case separates the averages within each column. Same letters do not differ by Tukey test at 5% probability.

Table 6. Shoot nitrogen content (TNPA) in corn plants in response to nitrogen fertilization and inoculation with *Herbaspirillum seropedicae.* Dourados, MS (2013).

Nitrogen	TNPA (g kg^{-1})	
	Inoculation	
	Without	With
0	14.98^{bA}	14.35^{bA}
80 kg ha	25.71^{aB}	32.33^{aA}

The lowercase letters separate the inside of the lines and the upper case separates the averages within each column. Same letters do not differ by Tukey test at 5% probability.

with rice plants inoculated with strain Z-94 of *H. seropedicae*, observed increases of up to 34% in the total nitrogen of the aerial part, in relation to the absolute witness. Similar data were obtained by Dobbelaere et al. (2001), when working with bacteria of the genus *Azospirillum.* These authors report that the highest content of N in plants inoculated is a result both of the FBN, as mechanisms to promote root growth, which can increase the ability of plants to absorb this nutrient. Similar results were also obtained by Ferreira et al. (2010, 2011) and Guimarães et al. (2010) when working with rice plants inoculated with *H. seropedicae*.

Regarding SPAD readings, inoculation increased chlorophyll contents in leaves in 10.81% compared to the control (Table 4), demonstrating the efficiency of this microorganism to increment the contents of chlorophyll in the leaf, and correlating with the increase in the nitrogen content in the plant. According to Chapman and Barreto (1997), this is attributed to the fact that more than 50% of the total nitrogen of the leaves being members of compounds of the chloroplast and chlorophyll from the leaves. Concordant results found in Lima (2010) and Canelas et al. (2013) reported positive effect of inoculation with *Bacillus subtilis* and *H. seropedicae,* respectively, under the chlorophyll content in the leaves of corn, confirming the effect of inoculation with these bacteria in the development of corn and in promoting greater photosynthetic capacity of the plant.

There was positive interaction between hybrids (H) and inoculation (I) with *H. seropedicae* on the stem diameter and shoot N content. It is observed that the hybrid P3646H showed higher stem diameter, differing statistically ($p<0.05$) from the hybrids BRS 3035 and Maximus, while the latter was superior to the others in the shoot N accumulation, when not inoculated with the bacteria. With inoculation, the corn hybrid BRS 3035 was superior to the others for both the increase in stem diameter and shoot N accumulation, even with no statistical difference ($p\leq0.05$) between hybrids for shoot N content (Table 5). The stem diameter of the hybrid P3646H and the shoot N content of hybrids BRS 3035 and Maximus were not affected by the inoculation with *H. seropedicae*, with significant difference ($p\leq0.05$) only for stem diameter of the hybrid Maximus and shoot N content of hybrids P3646H and BRS 3035 (Table 5).

There was positive interaction between inoculation with *H. seropedicae* and the N levels only for shoot N content. It is observed that the inoculation with the Z-94 strain of *H. seropedicae* plus 80 kg ha^{-1} of N caused an increase in the shoot N content of corn plants in the order of 25. 74% relative to the control composted with 80 kg ha^{-1} (Table 6). Regarding the inoculated and non-fertilized control, the increase was greater than 100%. It should be pointed out that commonly a greater contribution of inoculation, associated with nitrogen fertilization, is verified. According to Baldani et al. (1996), the inoculation of *Herbaspirillum* combined with small doses of N is proven to be more efficient for the system

plant/bacteria when compared to the isolated use of bacteria. This is due to the fact that the wealth of organic compounds excreted, deposited and/or exudates in the rhizosphere by plant in the presence of small doses N produces intense activities and microbial interactions that allow these bacteria to effect settlement, namely, provides signals to the micro-organisms. Dobbelaere et al. (2002) found that the effect of *Azospirillum brasilense* strain Sp inoculation of 245 and *Azospirillum irakense* KBC1 strain was higher when associated to doses of nitrogen. In inoculation experiments conducted under greenhouse conditions, using the strains M130 (*Burkholderia* sp.), ZAE94 (*H. seropedicae*) and M209 (*Burkholderia* sp.), it was observed that there was a contribution ranging from 11 to 20% in the accumulated N in the dry mass of rice plants (Baldani et al., 2000). Dalla Santa et al. (2004), in corn experiments, using the strains RAM-7 and RAM-5 of *Azospirillum* sp., observed that the use of these strains was able to reduce by 40% the amount of nitrogen fertilization recommended.

Conclusions

The hybrid BRS 3035 stands out for most of the variables analyzed, producing most of the shoot dry mass, plants with greater height, diameter of stem, root volume and showing the highest rates of N use efficiency.

The inoculation with the Z-94 strain of *H. seropedicae* promoted increase in root volume, root length, shoot dry mass, chlorophyll content, shoot N content and N use efficiency.

The inoculation with the Z-94 strain of *H. seropedicae* plus 80 kg ha^{-1} of N increased the N content in the shoot of corn plants by up to 25% in the analyzed genotypes.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Fertility capability classification and GIS mapping of soils in Agbarho town, Delta State, Nigeria

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The rapid increase in population growth in Nigeria has led to increased pressure on available land for agricultural uses, resulting in land degradation and subsequent soil infertility. This situation has in turn resulted in the desperate need of farmers to improve their yields and profits. The fertilizers utilized for this purpose are usually not compatible with the soil needs which are known to vary over space. The need therefore arises for the classification of soils based on their nutrient deficiencies. This study attempts a Fertility Capability Classification of the soils of Agbarho town, an area located within the Niger Delta region of Nigeria. A mapping of this classification was carried out with the use of Geographic Information System. A total of 60 soil samples from a depth of 0-20 cm were collected and analyzed for macronutrients, organic matter, pH, cat ion exchangeable capacity and particle size composition. It was revealed that four out of the nine villages within the study area had major fertility constraints characterizing them as predominantly sandy soils possessing low ability of retaining nutrients such as potassium, calcium and magnesium. It is therefore recommended that a more detailed classification and mapping of the entire Niger Delta area be carried out.

Key words: Agriculture, soil infertility, fertility capability classification, fertility mapping, fertilizers, Niger Delta region.

INTRODUCTION

Soil is a vital resource to the sustenance of human and animal existence on earth. As a renewable resource, however, the period of renewal is long spanning hundreds of years, often loosing the topmost layer at a rate that far exceeds the capacity of its natural process of regeneration. Within the last decade, inventories of the soils' productive capacity indicate severe degradation on more than 10% of the earth's vegetative land as a result of soil erosion, atmospheric pollution, excessive tillage, overgrazing, land clearing and desertification (Wood et al., 2000).

The relevance of agriculture to the sustenance of life cannot be overemphasized. This is especially so for a country like Nigeria with its large population and consequent pressure on the use of land for agriculture. Aside from the challenge of the availability of the land for agriculture, the issue of soil fertility is also a problem. This is especially so, in areas where the parent material is inherently deficient in basic nutrients. This is the case in the Niger Delta region of Nigeria, which is underlain basically by sedimentary rocks. In this case, the soils are mainly fertilized by the overlying decomposing leaves from trees. As a result of large scale deforestation, due mainly to urbanization, resulting in a major proportion of

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the soil being laid bare, the above situation is no longer the case. Consequent on the above, any improvement on the fertility of the soil, at this juncture is therefore largely dependent on inputs such as fertilizers, farming systems or soil management methods adopted.

In confirming this situation further, Agboola and Akinnfesi (1991) noted that the soils of the humid tropics are known to generally possess a low fertility status as a result of high mean temperatures and high rainfall intensities. It was also noted that the soils of the humid tropics are known to suffer multiple deficiencies of nutrients and by implication having low productivity. The major constraints of tropical soils by implication are chemical and not physical.

Against this backdrop, a need therefore arises for the evaluation and testing of specific soils from a general to a more localized setting. This is with the purpose of effectively determining the appropriate fertilizer to be utilized in any of such areas, as this will aid in improving the soil fertility of the area in question. The goal of soil testing or evaluation has to do with helping farmers to increase production and profits dependability through the proper use of fertilizers and soil amendments. The proper use and right proportion of soil amendments and fertilizers is greatly influenced by the nature of the soil in terms of the nutrient content of the soil. Enwezor (1985) however noted that it was futile to expect to be able to predict productivity accurately from soil available nutrient content alone.

In recent times, a variety of attempts have been made to further counter the problem of soil infertility. One major attempt in this direction is the advent of the Fertility Capability Classification (FCC). The Fertility Capability Classification is the first technical soil classification that categorizes soils according to their fertility constraints in a qualitative manner. This classification was developed in response to a perceived under utilization of soil survey information. It was discovered that the upper few centimeters of the soil are excluded from soil taxonomic consideration. In actual fact, properties in the top soil have been more significant to growth than subsoil properties. The FCC aids in the identification of the fertility status of the soils through the discovery of its fertility constraints (chemical and physical). This is with the aim of proffering appropriate soil management techniques which are compatible with the soil in question. Several studies have used FCC to group soils in smaller regions as the basis for further technology transfer and research (Avilan 1979). It has however been noted that in all these studies, not all the needs of the users were satisfied by the FCC resulting in slight modifications being made. This is understandably so since specific needs vary from user to user and location to location. The main aim of the FCC is however to group soils that are homogenous enough in properties that soil management decisions are the same in kind within groups and different between groups. The ultimate test of the FCC appears to be in an evaluation of the use of the system in the delivery of soil management information to the user. The FCC has great potentials for producing results which provide a less sophisticated comprehension of soil terminology and information relevant to the area. This is extremely necessary as this is expected to be useful to the local farmers.

It is against this background therefore, that this study was carried out on Fertility Capability Classification and mapping of soils in Agbarho town, Niger Delta region of Nigeria. This is with the aim of determining the extent of applicability of the classification to the environment in question and also to further determine the relevance and importance of the various criteria involved in the classification.

Study area

Agbarho town is located between longitudes 5° 50' and 5° 59'E and latitudes 5° 30' and 5° 35'N. It has an area of about 700 sq Km. The town comprises basically of the following villages namely Orhokpokpo, Oguname, Uvwiama/Uvwiamuge, Orherhe, Ikweghwu, Ughwrughelli, Ekwerhe and Ekrerahwe. The area enjoys the Tropical Equatorial climate with an average annual temperature of 30°C and 3130 mm of rainfall, while relative humidity is 80% - 90% (Efe, 2007). The area is underlain by three stratigraphic units starting with the unconsolidated coastal plain sands (Benin) formation at the top, followed by an intervening unit of sandstone and shale called Agbada formation and bottom unit shale known as the Akata formation, representing continental, paralic and marine depositional environments respectively (Short and Stauble, 1967). Sedimentary rock formations form both the surficial and subsurface geology of the area (Odemerho, 2007). The relief of the area is generally a sloping, gently undulating plain. The vegetation is characterized by the Lowland Tropical Rainforest. This forest is characterized by distinguishable crown layers with emergent upper, middle and lower storey's giving the forest an irregular structure. The forest is endowed with trees such as *Triplochiton scleroxylon, Milicia excelsa* etc. The soils consist of well – drained sandy loam over coarse sandy clay loam subsoil. Agriculture dominates the economic life of the people of the study area. The bush fallow, rotational bush fallow and the compound farming, which can all be categorized as traditional farming practices are all practiced within the area. The main food crops grown include cassava, yam, maize and plantain. The major cash crops are oil palm and rubber (FEPA, 2001).

MATERIALS AND METHODS

Data for the purpose of this study were obtained basically through soil survey and laboratory analysis. The results of the laboratory

Table 1. Mean values of chemical and physical properties of the soils of Agbarho town.

Soil property	Location							
	Orhokpokpor	Uvwiamuge	Ekrerahwe	Orherhe	Ughwrughelli	Ikweghwu	Ekwerhe	Oguname
Sodium (cmol/kg)	0.29	1.15	0.18	0.29	0.38	0.24	0.25	0.23
Potassium (cmol/kg)	0.14	0.13	0.17	0.20	0.19	0.19	0.18	0.17
Calcium (cmol/kg)	2.82	2.81	2.98	2.49	2.95	2.78	2.91	2.87
Magnesium (cmol/kg)	0.48	0.52	0.44	0.54	0.49	1.14	0.52	0.56
Av. P(mg/kg)	1.74	2.27	2.56	5.11	5.86	5.25	3.22	3.94
Organic Carbon (%)	3.25	0.96	0.85	3.91	0.56	2.70	0.94	2.08
Organic Matter (%)	5.60	2.21	1.65	4.68	0.97	4.64	1.62	3.58
Nitrogen (%)	0.33	0.10	0.21	0.27	0.06	0.27	0.10	0.21
pH	2.92	4.26	3.87	2.57	3.69	3.85	4.00	3.88
CEC (cmol/kg)	3.70	3.80	3.83	4.08	4.80	3.73	4.90	3.61
Sand (%)	87.7	77.8	75.1	87.1	87.5	88.9	86.4	80.4
Silt (%)	8.5	37.1	21.5	9.53	5.92	5.3	8.3	13.6
Clay (%)	5.0	5.3	3.33	3.3	4.2	5.8	5.3	4.8

Source: Author's field work (2005).

analysis can be seen in Table 1.

Soil sampling

Soil samples were obtained from the farmers' fields in each of the villages making up the town. The soil samples were taken at predetermined depths of 0-10cm and 10-20cm. The approach of sampling from predetermined depths was adopted in order to ensure comparability between samples collected from different sample quadrats (Aweto, 1978). In all, 30 farms were sampled throughout the town. A quadrat of 15m by 15m (representative of each farm) was delineated within each farm from which the soil samplings were carried out. Replicate soil samples were collected from five points within each farmland and mixed to form a composite sample, representative of each farmland. The purpose of this procedure is to minimize the influence of any local non-uniformity of the soil (Tisdale et al., 1985). Thirty soil samples each were taken respectively from depths of 0-10cm and 10-20cm, resulting in a total of 60 samples in all. The samples were collected in polythene bags, labeled and air dried in preparation for the laboratory analysis.

Laboratory analysis

The soil samples were subsequently analyzed in the laboratory, for particle size composition using the hydrometer method (Bouyoucos, 1951). The exchangeable acidity was determined through the titration method (Mclean, 1965). The Cation Exchangeable Capacity was determined by the addition of the exchangeable bases and the exchangeable acidity.

The soil pH was determined in 1:1 soil: water solution using the pH meter. The organic matter content was determined by the Walkley - Black combustion method (Walkley and Black, 1934). The soil samples were leached using 1 m neutral ammonium acetate, and the extracts were used for determining exchangeable calcium, potassium and sodium through flame photometry, while exchangeable magnesium was determined by atomic absorption spectrophotometer. The available phospohorus was determined using the Olsen (1954) method.

RESULTS AND DISCUSSION

The soils of the study area were classified based on the Fertility Capability Classification. A mapping based on this classification was subsequently carried out with the aid of the Geographic Information System using the Idrisi GIS software. This was done to demonstrate the possibility of classification as well as visualizing the results on a map. The FCC, according to Sanchez et al. (1982), consists of categorical levels. They include three basic criteria and levels for classification which include: Type (top soil texture), Substrata (subsoil texture) and Modifiers. The class designations therefore, for each of these three levels are combined to form an FCC unit. Where more than one criterion is listed for each modifier, only one is meant to be met. The criterion listed first is usually the most desirable one and ought to be used, if data is available. Subsequent criteria are presented for use where data is limited. The modifiers are designated in the lower case letters. Not all the modifiers however were utilized in this study. It was observed that some of the criteria had the possibility of being inferred from any of the others. Aside from this, some of the other modifiers were discovered to be inapplicable to the study area. The "l" modifier was not used as it applied to only clay soil types. This was rendered inapplicable to the extremely sandy soils of the study area. The slope modifier was also deemed not necessary because the study area is generally low lying since it is located in the Niger Delta where much of the land is rather low lying and close to the sea level as can be seen in the section on the description of the study area. The vertisol modifier was found to be inapplicable to the study area as well, since the soils of the area had been discovered through the soil analysis, to be sandy and not clayey as this modifier assumes. The (') gravel and (d) dry modifiers were also

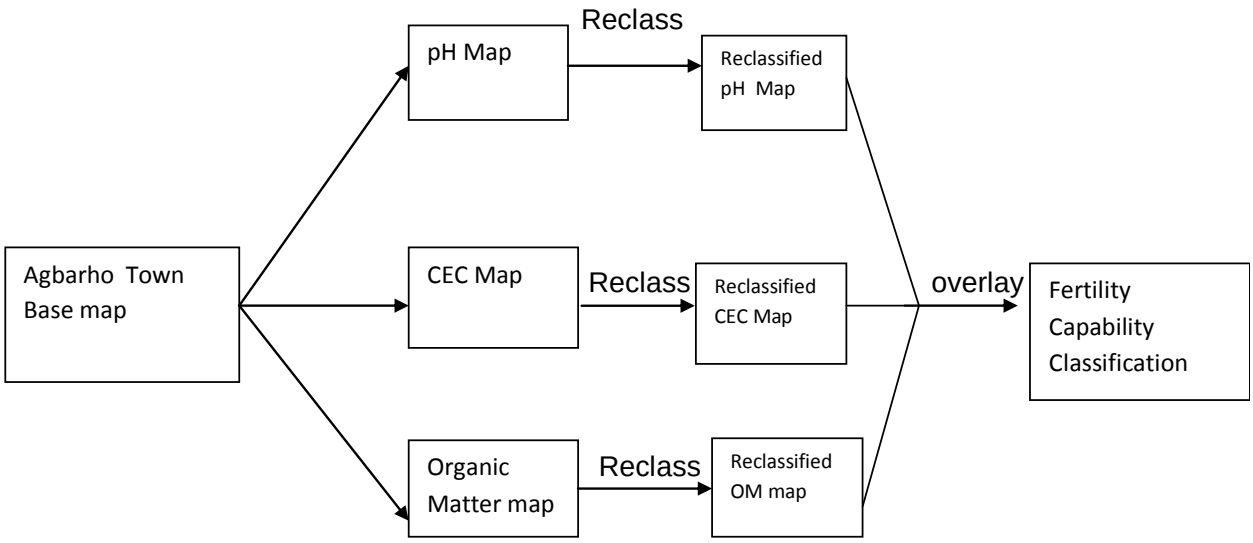

Figure 1. Cartographic model of the operations and creation of the fertility capability classification mapping of the study area.

found to be irrelevant to the study area due to the sandy and muddy nature of the soil as a result of its location in the Niger Delta and hence very high ground water level. Some other modifiers were inferred from other modifiers. The basic reaction modifier, for instance, was inferred from the pH modifier with the criterion of having a pH of > 7.3.

In addition to all these other modifiers, the modifiers on pH, CEC and Potassium were also considered. The criteria for the aforementioned modifiers included the following: pH modifier / (h) modifier = pH in 1: 1 H_20 between 5.0 and 6.0, (e) modifier = (low cation exchange capacity) applies only to the top layer or surface 20 cm whichever is shallower: CEC < 4 meq /100g soil and (k) (low k reserves) : < 0.20 meq/100 g. This was done in conjunction with the categorization of the Type level based on the top soil. The study area was therefore mapped basically on the basis of the aforementioned criteria.

The mapping was carried out with aid of the GIS software (Idrisi GIS). This further facilitated an efficient fertility capability classification of the soils of the study area. The cartographic model of the analysis resulting in the fertility mapping can be seen in Figure 1. The cartographic model gives a step by step breakdown of the operations carried out in the process of the mapping. Maps were generated for the pH, Potassium and CEC modifiers. This was done through the process of reclassification based on the criteria contained in the modifiers. The overlay operation was subsequently carried out on all the maps resulting therefore in the production of the final map.

The resultant map indicated the various capability classifications of all the villages within the study area. The map revealed that Uvwiama, Uvwiamuge, Orhokpokopo and Oguname possessed major fertility constraints as seen in Figure 2. These villages were classified as Sehk. This classification implies that the aforementioned villages were characterized by the following: S = Sandy topsoils, e = low ability to retain nutrients against leaching of nutrient elements mainly Potassium, Calcium and Magnesium, h = low to medium acidity and k = low ability to supply potassium.

Based on the foregoing, it is being recommended that heavy applications of these nutrients and of Nitrogen fertilizers be split to avoid potential danger of over liming. These areas therefore require liming for Aluminium-sensitive crops such as cotton and alfalfa, and good latex flow in rubber. It should be noted at this point that rubber is a major cash crop of the study area and as such the relevance of this information cannot be overemphasized. Manganese toxicity may occur on some of these soils. Availability of potassium should be monitored and the use of potassium fertilizers required frequently. It should be noted that potential k-mg-ca imbalances are likely to occur. The above description and classification of the soils suggest an explanation for the observation of the planting of cassava on all the farms surveyed in the area. Cassava possesses a number of merits which could be the reason for its wide acceptability amongst the majority of local farmers within the study area and indeed the Niger Delta region of Nigeria. Apart from cassava being a staple crop for the average Nigerian, it has been found to possess the potential of adapting to marginal conditions and degraded soils. It has the advantage of possessing the ability of not being easily affected by pests and diseases. It also does not require any specialized cultivation techniques and requires no input from the harvested crop for the next season's planting material except for its stem cuttings.

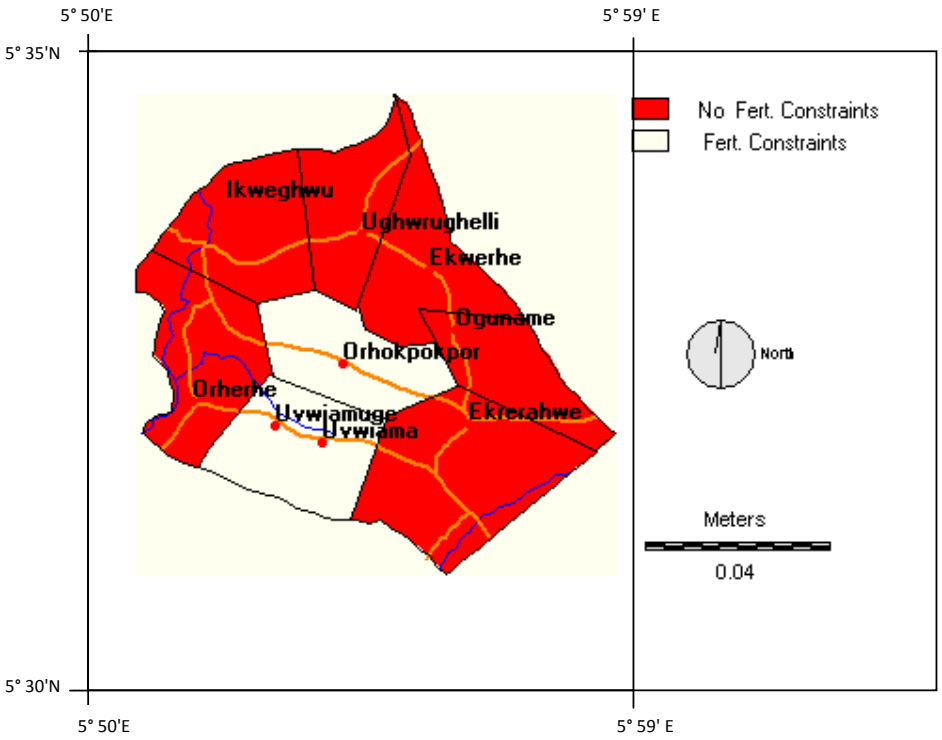

Figure 2. Fertility Capability Classification of Agbarho Town.

Conclusion

With the increasing population and decreasing availability of land for the practice of agriculture, the survival of the earth has become critical. The countries which are more likely to be affected, are the developing countries and by implication the local populace. Critical issues require radical steps. One of which, in this case, is the applicability of the Fertility Capability Classification. Several classifications have been carried out in the past, however not many have been able to effectively connect with the indigenous smallholder farmer. This classification demonstrates the possibility of the FCC of being carried out in a localized small area as against larger areas such as whole countries and continents. It has also demonstrated the ability of determining the specifics as it pertains to the nutrient deficiencies of the soils in question. The results arrived at, is expected to pave a way forward in the formulation of fertilizers containing the deficient nutrients in the right proportions. This is as against what is presently the case where fertilizers are applied to the soils without first of all determining the needs and deficiencies of the soil. In this information driven age, it is expected that computer based resources and tools are utilized for the purpose of arriving at more accurate results within a shorter time frame, as has been demonstrated with the use of GIS in this study. The GIS served as a veritable tool in effectively carrying out this classification with the major advantage of being able to visualize the nutrient deficiencies of the soil in a spatial dimension. It is indeed pertinent that studies of this type be improved upon and carried out in other localized areas. This study is of great importance and necessity, if the focus of the general populace is to be changed from oil exploration to agriculture for the purpose of sustenance and sustainability of the people.

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Full Length Research Paper

Wheat cultivars under bulk density levels in Cerrado Rhodic Hapludox, Central Brazil

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The Brazilian Cerrado presents potential to expand its wheat cultivation, but soil compaction is one of the factors that may limit production. The objective of this study was to evaluate production characteristics and chlorophyll index of two wheat cultivars (*Triticum aestivum* L.) under bulk density levels. The experiment was conducted in a greenhouse, with Rhodic Hapludox collected at 0.00-0.20 m depth. The experimental design was entirely randomized and the treatments were arranged in a 5x2 factorial scheme, corresponding to five bulk densities levels (1.0; 1.2; 1.4; 1.6 and 1.8 Mg m^{-3}) and two wheat cultivars (BRS Guamirim and IAC 350) with four replications. The experimental plot was composed of one poly (vinyl chloride) pot of 0.1913 m internal diameter and 0.20 m height. Variables evaluated were: spike number, dry mass of spikes, aerial part, roots and chlorophyll index. There was no significant interaction between wheat cultivars and bulk density levels. The increasing bulk density reduced production and chlorophyll index of the wheat cultivars BRS Guamirim and IAC 350. The IAC 350 cultivar presented better spike production and higher chlorophyll index, regardless the bulk density levels.

Key words: Soil compaction, Soil management, *Triticum aestivum* L., Chlorophyll index, spike number.

INTRODUCTION

Brazil's greatest wheat production is located in the southern region of the county, which is responsible for more than 90% of the national production (Conab, 2012). However, the central-western region is also a very good alternative for the wheat production expansion under both non- and irrigated conditions (Coelho et al., 2010). Wheat flour is the main raw material used for food preparation, such as bread, biscuits, cakes and pastries. Therefore, improvement of wheat production potential via soil management practices is one of the main research challenges facing the worldwide growing demand for food. In this sense, concerns on soil compaction are important, as soils may have appropriate nutrient contents, but because of physical conditions, efficiency of nutrient absorption by plants may be affected, resulting in yield decrease (Bonelli et al., 2011; Cabral et al., 2012).

The soil compaction results in a rearrangement of particles and aggregates due to pressure application on

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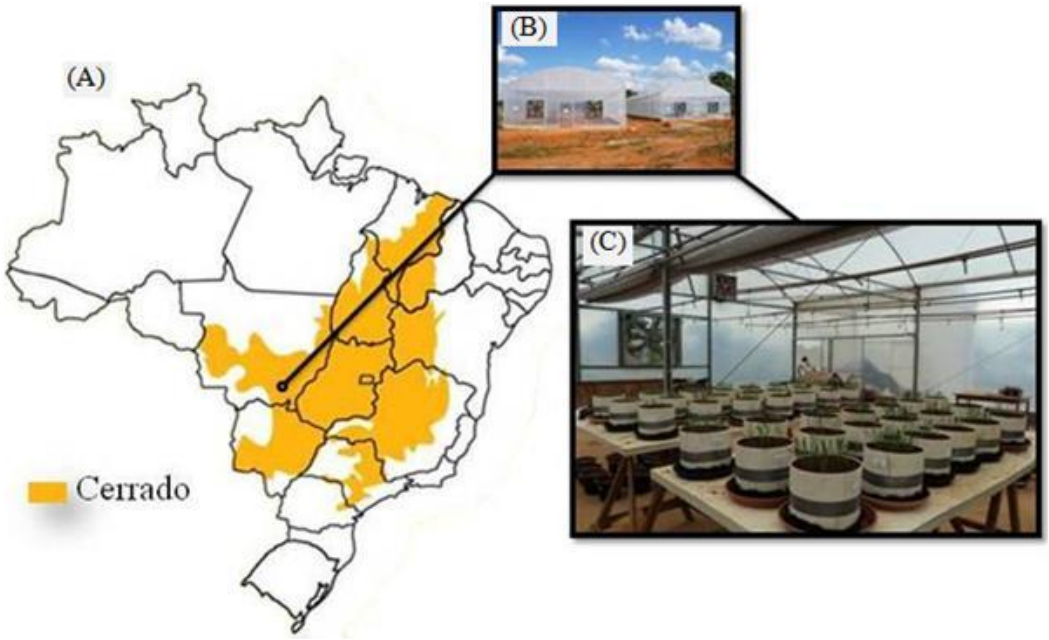

Figure 1. Representation of the experiment geographic location in the Brazilian Cerrado, which geographic coordinates are 16°27'49" S and 50°34'47" W, in Rondonópolis, Mato Grosso State, Brazil (A), Detail of the greenhouse (B) and experiment overview (C).

soil surface by intense traffic of machinery andimplements used in soil management practices (Hakansson and Lipiec, 2000; Reichert et al., 2009). A direct compaction consequence is the elimination of existing gases within macropores, reducing porous space and increasing density. This results in a mechanical barrier to root growth, impairing water and nutrient absorption (Batey, 2009), especially of nitrogen, which is absorbed by mass flow. Also, it negatively influences gas exchange in the soil-plant system, reducing yield.

Soil compaction issues became extremely worrying due to intensification of agricultural mechanization with the indiscriminate use of heavy machines under conditions of high soil moisture (Roboredo et al., 2010). Secco et al. (2009), who studied the compaction effect on no-till Rhodic Hapludox in the Brazilian southern region, verified that high bulk density values (1.62 and 1.54 Mg m^{-3}) promoted decreases in wheat yields that ranged from 18.35 to 34.05%.

Currently, information on the bulk density levels that may restrict crop yields in the Brazilian Cerrado is scarce, especially on wheat. In this context, basic researches need to be developed with the aim to evaluate: bulk density levels that may compromise the wheat production potential; and also, whether there are adaptation differences to the bulk density levels among the wheat cultivars recommended for Cerrado soil and climate conditions. Results from this study may be used as a basis for future field researches on soil compaction for wheat crops.

The objective of this study was to evaluate the effect of Rhodic Hapludox bulk density levels on production characteristics and chlorophyll index of two wheat cultivars (BRS Guamirim and IAC 350) grown in a greenhouse in the Cerrado of Mato Grosso State, Brazil.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse located in Rondonópolis, Mato Grosso State, Brazil, with geographic coordinates of 16°27'49" S and 50°34'47" W, Figure 1, from December 2011 to January 2012. The used soil collected from 0.00-0.20 m depth, was classified as Rhodic Hapludox (Santos et al., 2006); with the following chemical and textural characteristics: pH 4.1 ($CaCl_2$); 2.4 mg dm^{-3} P; 28 mg dm^{-3} K; 0.3 $cmol_c$ dm^{-3} Ca; 0.2 $cmol_c$ dm^{-3} Mg; 4.2 $cmol_c$ dm^{-3} H; 1.1 $cmol_c$ dm^{-3} Al; 5.9 $cmol_c$ dm^{-3} CEC; base saturation of 9.8 (V%); 22.7 g dm^{-3} OM; 549 g kg^{-1} sand; 84 g kg^{-1} silt; and 367 g kg^{-1} clay (Claessen et al., 1997).

For experiment implementation in the greenhouse, the soil sample was sieved on a 4 mm mesh (Silva et al., 2006) to remove root fragments or particles larger than that diameter that may exist in the soil. Soil acidity was corrected with incorporation of dolomitic limestone (80.3% relative power of total neutralization) into soil samples of 8 dm^3, increasing the base saturation to 60%. After liming, soil samples were moistened at 80% water retention capacity and placed in plastic bags for 30 days (soil incubation period with limestone).

After incubation with limestone for soil acidity correction, basic fertilization was performed with incorporation of solid granular fertilizer, using 100 mg dm^{-3} N, 300 mg dm^{-3} P_2O_5, and 150 mg dm^{-3} K_2O; fertilizer sources were urea, superphosphate and potassium chloride respectively. The pot entire soil volume was homogeneously fertilized, ensuring the same fertilization level for all

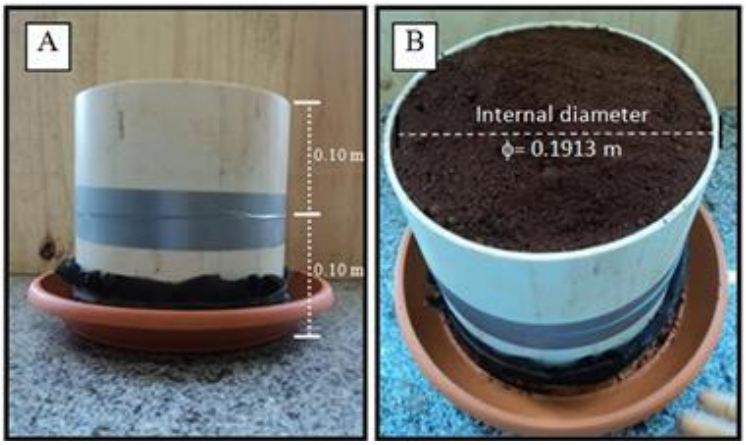

Figure 2. Experimental unit made of poly (vinyl chloride) and respective dimensions: height (A) and diameter (B).

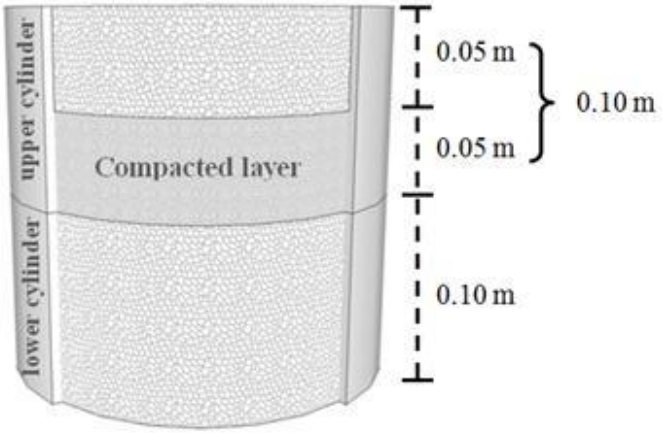

Figure 3. Graphical representation of the experimental unit, showing the position of the 0.05 m compacted layer.

layers. Fertilization, with respective fertilizer sources was performed based on a study by Pietro-Souza et al. (2013), who cultivated wheat in Rhodic Hapludox collected from the same area of this study.

The experimental unit was represented by one poly (vinyl chloride) pot of 0.1913 m internal diameter and 0.20 m height, comprising 5.748 dm^3. Each pot was composed of two cylinders of 0.10 m height, which were joined by a duct tape; the anti-aphid screen was used to close the pot bases which were affixed with a rubber ring, Figure 2.

Bulk density was used as a parameter to represent soil compaction levels (Grossman and Reinsch, 2002). For all bulk density levels, the compacted layer thickness was 0.05 m, comprising 1.44 dm^3 of the total pot volume, which was 5.748 dm^3, Figure 3. Dry soil masses varied according to the bulk density level of each treatment. Therefore, Equation 1 was used to determine the dry soil mass to compose the 0.05 m compacted layer in the pots with internal diameter of 0.1913 m (volume of compacted layer = 1.44 dm^3). Dry soil masses were 1.44; 1.73; 2.02; 2.30 and 2.59 kg for the respective bulk densities 1.0; 1.2; 1.6 and 1.8 Mg m^{-3}.

$$Bd = \frac{Dm}{V} \quad (1)$$

Where:
Bd - Bulk density (Mg m^{-3})
Dm -nDry soil mass (Mg)
V - Cylinder volume corresponding to the 0.05 m layer (m^3).

According to the Proctor test (Abnt, 1986) 16% moisture was adopted as compaction optimum moisture for the intermediate bulk density level which was 1.4 Mg m^{-3}. The mean moisture of 16% is used in the laboratory of soil physics where compaction tests, as pilot tests, were performed for the study. To achieve the bulk density levels of 1.0; 1.2; 1.6 and 1.8 Mg m^{-3} for 16% soil moisture, the compaction energy applied by the press varied, so that curve construction for determination of the ideal moisture for each bulk density was not necessary.

Moisture of the soil reserved for this experiment was determined by the gravimetric method (Claessen et al., 1997) using Equation 2. The mean moisture value found for soil samples was 16%.

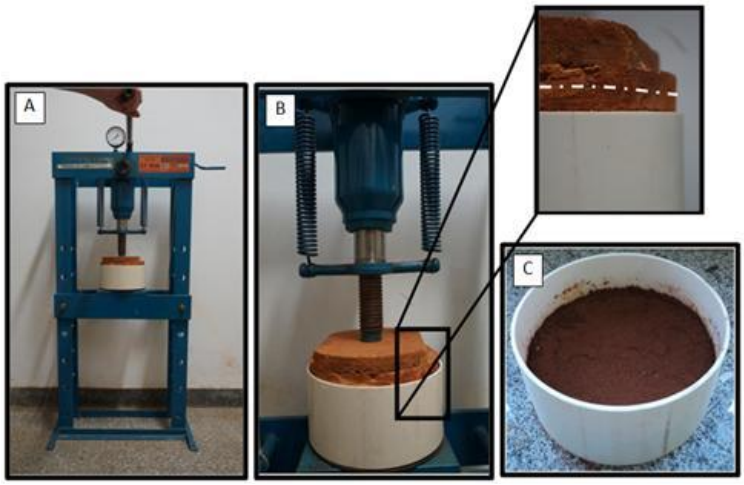

Figure 4. Procedure of soil compaction with the aim of a hydraulic press (A); Detail of the wooden mold used during the compaction process (B); Compacted layer of 0.05 m thickness (C).

Figure 5. Implementation of the experimental unit: Filling the lower cylinder with soil at the density of 1.0 Mg m-3 (A); Joining both cylinders with duct tape, showing the compacted layer (B); Filling the remaining volume of the upper cylinder above the compacted layer at the density of 1.0 Mg m-3 (C).

Determination of the wet soil mass that would be compacted from the dry soil mass corresponding to each treatment (bulk density) was performed according to Equation 3, as well as determination of the sample water content.

$$\theta m\ Sm = \frac{Wm - Dm}{Dm} \quad (2)$$

$$Wm = (Dm.(1 + Sm)) \quad (3)$$

Where:
Wm - Wet soil mass (g)
Dm - Dry soil mass (g)
Sm - Soil moisture-based mass (g g^{-1})

For pot implementation (experimental units), half of the upper cylinder was filled with moist soil (16%) according to the predetermined masses from Equation 1.

The compacted layers were performed with the aim of a hydraulic press with pressure capacity of 15 tons (BOVENAU® brand, model P15ST). Thickness of the compacted layer for all experimental units was 0.05 m that corresponded to 1.44 dm^3 volume. A marked wooden mold was used to indicate the moment the compacted layer achieved 0.05 m thickness, indicating when to stop applying pressure, Figure 4.

The lower cylinder was completely filled with non-compacted soil (density of 1.0 Mg m^{-3}). The upper one was filled with the previously compacted layer and placed on top of the lower cylinder. Both cylinders were joined with duct tape. The upper one was then completed with soil at the density of 1.0 Mg m^{-3} above the compacted layer (1.44 dm^3), Figure 5. Plastic trays were used at the pot bottoms to aim at the irrigation by capillarity.

The experimental design was entirely randomized and treatments were arranged in a 5x2 factorial scheme, with five bulk density levels (1.0; 1.2; 1.4; 1.6 and 1.8 Mg m^{-3}); two wheat cultivars (BRS Guamirim and IAC 350) and four replications. Twenty seeds were sown per pot. At 12 days after sowing, nitrogen was applied, using

Table 1. Mean values of spike number and dry mass for BRS Guamirim and IAC 350 wheat cultivars, regardless bulk density.

Variables	BRS Guamirim	IAC 350
Spikes (number pot^{-1})	10.7 ^{b}	13.1 ^{a}
Dry mass of spikes (g pot^{-1})	3.19 ^{b}	4.58 ^{a}

Means followed by different letters in the line differ from each other by the Tukey test at 5% probability level.

urea as the source at the dose of 100 mg dm^{-3}. Seedling thinning was performed at 15 days after sowing, remaining 5 wheat plants per pot which were cultivated for 54 days.

Controlled surface irrigation were carried out until plant establishment (20 days after sowing), then soil moisture was maintained according to the proposed methodology by Silva et al. (2006). After the cultivation period (54 days), spike number and dry mass of spikes, aerial part and roots were evaluated. Also, SPAD readings were performed for determination of the chlorophyll index using the chlorophyll meter Minolta SPAD-502, which was fixed at the middle third of two leaves after the flag leaf (leaves +1 and +2). The mean of 10 readings was considered the SPAD value for each experimental unit. Spike number from each pot was evaluated before harvest. The aerial part was then cut close to the soil surface and leaf mass was separated from spikes. Roots were also collected and washed on a 4-mm sieve. All collected material was placed in paper bags, dried in an airtight circulation heater at 65 °C for 72 h and weighed.

Data were subjected to variance analysis (F test); when significant mean of the wheat cultivars were compared by the Tukey test, while the bulk density levels were submitted to regression analysis, both at 5% probability ($p<0.05$), using SISVAR 5.3 software (Ferreira, 2008).

RESULTS AND DISCUSSION

Number and dry mass of spikes presented isolated effects for wheat cultivars and bulk density shown in Table 1 and Figure 6. Regardless of the bulk density levels, the IAC 350 cultivar, when compared with BRS Guamirim, had higher number and production of spikes, Table 1.

Considering that a crop production potential is linked to nutrient absorption, this result indicates that IAC 350 has greater capacity to absorb nutrients regardless of the studied bulk density levels. Also, this result may be assigned to the cultivar intrinsic genetic factors, since there was no interaction among bulk density and wheat cultivars. Similarly, Fageria et al. (1995), when studying the response of rice genotypes to soil fertility, reported that absorption and appropriate nutrient use by rice plants are subjected to physiological processes inherent to the studied cultivars.

For bulk density, number and dry mass of spikes were described by regression quadratic models shown in Figures 6A and 6B, regardless of the wheat cultivars. The bulk density level that promoted the highest spike number was 1.12 Mg m$^{-3,}$ Figure 6A. Regarding the spike dry mass, the best bulk density level was 1.16 Mg m^{-3}, Figure 6B, achieving yield of 4.86 g pot^{-1} that represents a 60% increase when compared with the maximum bulk density level (1.8 Mg m^{-3}).

Bonfim-Silva et al. (2011) observed a decrease in the structural development and yield of wheat plants when cultivated under the bulk density of 1.30 Mg m^{-3}, which is a higher value than that found in this study (1.16 Mg m^{-3}). This indicates that, in this study, the wheat cultivars were more sensitive to the increasing bulk density. Similar results were obtained by Secco et al. (2009), who verified, under field conditions, a grain yield decrease of wheat and maize plants cultivated in Rhodic Hapludox.

However, Cabral et al. (2012), when studying tropical forages (*Brachiaria brizantha* 'Piatã' and *Panicum maximum* 'Mombaça'), observed higher concentrations of nitrogen and phosphorus in leaves from plants cultivated under intermediate soil compaction levels of 1.28 and 1.40 Mg m^{-3}, respectively. This indicates that a moderate compaction increment often increases soil contact with roots, which contributes to a better nutrient absorption. Nevertheless, according to Bonini et al. (2011), over compaction directly affects crop yield. These authors evaluated the compaction effect on Rhodic Hapludox in Southern Brazil under field conditions, and observed that the compaction level applied by five steamroller passes resulted in a 23% decrease in wheat yield. Regarding the dry mass of aerial part, there was an isolated effect only for bulk density. This variable was adjusted to the regression quadratic model, presenting its maximum yield at 1.05 Mg m^{-3} Figure 7, regardless of the wheat cultivars. Such low value indicates greater root susceptibility to compaction, as there was lower water and nutrient absorption from that level, resulting in minor production of dry mass of aerial part (Collares et al., 2008). Merotto Jr. and Mundstock (1999) also described a decrease, according to soil compaction, in the wheat dry mass of aerial part. On the other hand, Bonelli et al. (2011), when studying four bulk density levels (1.0; 1.2; 1.4 and 1.6 Mg m^{-3}) verified that, for *P. maximum* 'Mombaça', the dry mass of aerial part presented high susceptibility to increasing bulk density, as also indicated by the resulting linear model found for this variable in this study.

The dry mass of roots of the wheat cultivars BRS Guamirim and IAC 350 was adjusted to the regression linear model; it decreased with increasing bulk density, Figure 8. The comparison between compaction absence (1.0 Mg m^{-3}) and highest bulk density level (1.8 Mg m^{-3}) showed a 64.19% decrease in its production.

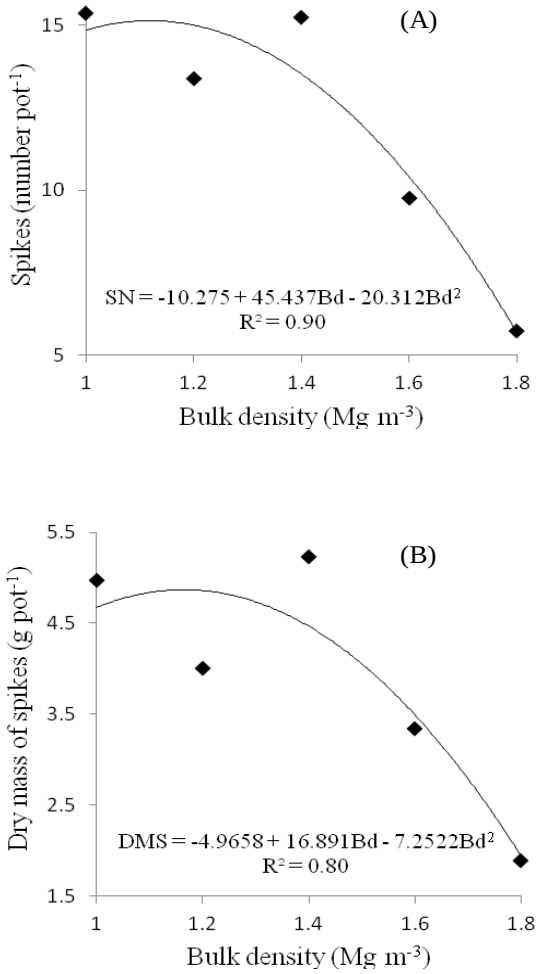

Figure 6. Number (A) and dry mass (B) of wheat spikes (cultivars BRS Guamirim and IAC 350) according to bulk density levels.

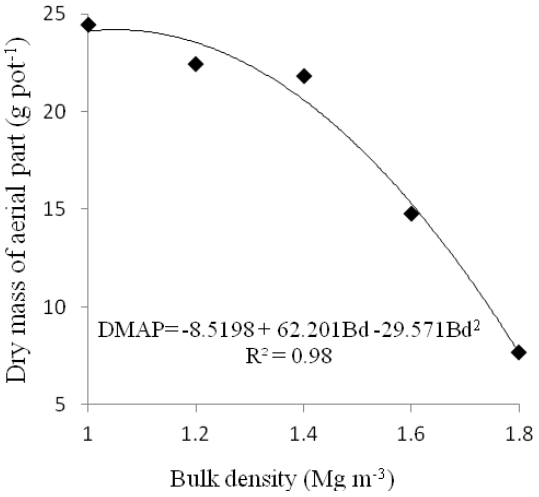

Figure 7. Dry mass of aerial part of wheat plants (cultivars BRS Guamirim and IAC 350) according to bulk density levels.

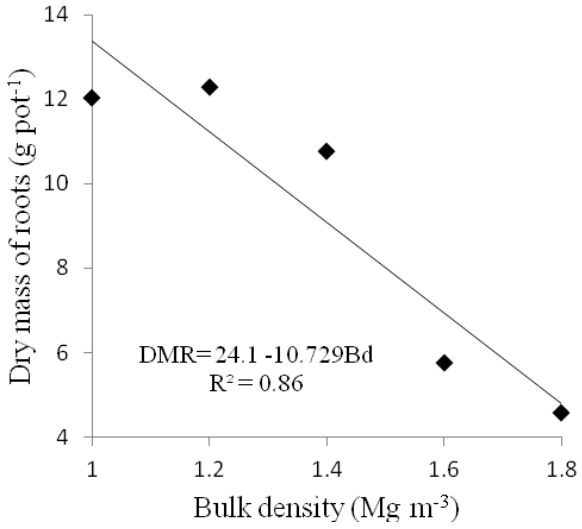

Figure 8. Dry mass of roots of wheat plants (cultivars BRS Guamirim and IAC 350) according to bulk density levels.

Table 2. Mean of SPAD readings (chlorophyll index) for BRS Guamirim and IAC 350 wheat cultivars, regardless bulk density.

Variable	BRS Guamirim	IAC 350
SPAD Reading	44.61[b]	48.45[a]

Means followed by different letters differ from each other by the Tukey test at 5% probability level.

These results corroborate that described by Bergamin et al. (2010), who concluded that soil compaction negatively influences the maize root system.

We observed that the compacted layer promoted a concentration of roots next to the soil surface due to the imposed restriction to root growth. This result is linked to that previously described for dry mass of aerial part, which decreased from the bulk density of 1.05 Mg m^{-3}. Regarding the chlorophyll index (evaluated by SPAD readings), there were isolated effects for wheat cultivars and bulk density. The IAC 350 cultivar presented higher chlorophyll index, in comparison with BRS Guamirim (Table 2), regardless of bulk density.

The chlorophyll index response to the bulk density levels was adjusted to a linear regression model, with a value of 50.9 obtained from the lower compaction level (1.0 Mg m^{-3}), Figure 9. Such value was similar to that observed by Espindula et al. (2009) for wheat plants as maximum leaf chlorophyll index corresponded to the SPAD reading of 50.

The chlorophyll index decrease with increasing bulk density may have been promoted by lower nitrogen absorption by plants, since the dry mass of roots also decreased as bulk density levels increased, Figure 8. The compaction process increases bulk density and reduce soil total porosity that will directly affect water dynamics in the soil, thus, nitrogen absorption occurs mainly, by mass flow. The reduction in nitrogen absorption under high bulk density levels was also observed by Cabral et al. (2012). According to Teixeira et al. (2010), wheat yield is positively correlated with leaf chlorophyll index; therefore, our results indicate that high bulk density levels may compromise the wheat grain production in the Cerrado of Mato Grosso State.

Conclusions

High bulk density reduced the production characteristics and chlorophyll index of the wheat cultivars BRS Guamirim and IAC 350 cultivated in the Cerrado Rhodic Hapludox; it may be considered as an evaluation parameter of soil physical quality in production systems. The IAC 350 cultivar presented the best spike production and chlorophyll index, regardless of the bulk density levels, so it is considered a promising cultivar for cultivation in Rhodic Hapludox, Central Brazil.

The highest chlorophyll index observed for the wheat

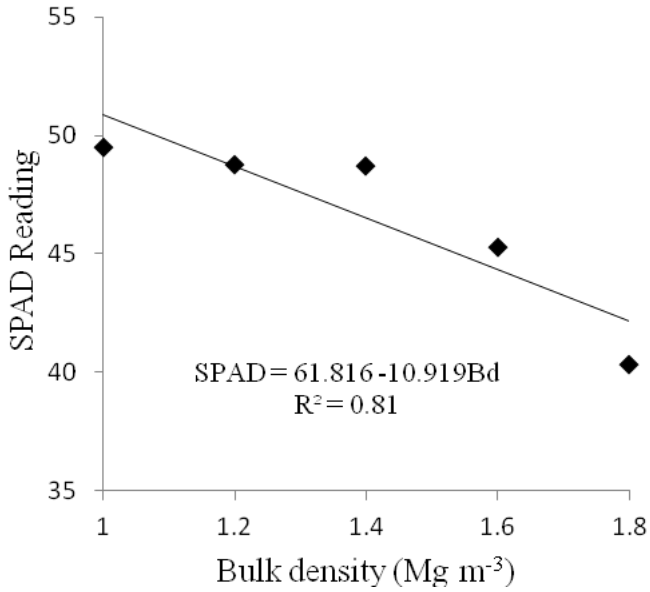

Figure 9. SPAD reading (chlorophyll index) for wheat plants (cultivars BRS Guamirim and IAC 350) according to bulk density levels.

cultivars BRS Guamirim and IAC 350 corresponded to the bulk density of 1.0 Mg m^{-3}.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Dissipation and residue behavior of oxine-copper on litchi field application

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An analytical method was established for oxine-copper residues in litchi, litchi pulp and soil. The residue levels and dissipation rates of oxine-copper was detected by high performance liquid chromatography- photodiode array (HPLC-PDA). At three fortification levels of 0.10, 0.50 and 2.00 mg/kg in litchi, litchi pulp and soil, recoveries were in the range 80.1 to 103.5%, with relative standard deviations (RSD) of 1.52 to 12.07%. The limit of quantification (LOQ) of method was 0.01 mg/kg for litchi, litchi pulp and soil. The half-lives of oxine-copper in litchi and soil were 9.12 and 7.02 day, respectively. The final residue levels of oxine-copper in litchi, litchi pulp and soil were lower than 1.99 mg/kg at harvest.

Key words: Oxine-copper, pesticide residue, dissipation, litchi, soil.

INTRODUCTION

Litchi is one of the subtropical fruits in China. The planting area of litchi is about 300000 hm^2, mainly distributed in Guangdong, Guangxi and Fujian provinces, which has become the main economic source of local farmers (Zhang and Lu, 2011). The main diseases of litchi are downy mildew disease, anthracnose and ulcer disease. As litchi production in China is being affected by fungus-caused plant diseases, many kinds of pesticide were applied to protect the litchi from diseases, pests and fungi.Oxine-copper with CAS number of [10380-28-6] is a new type of pesticide, which is in harmony with the environment and is used in a variety of fruit trees, vegetables, tobacco, and other crops (Kobayashi et al., 1989; Renault et al., 1965). The maximum residue limits established for oxine-copper in fruits is 2 mg/kg. However, in China to our knowledge, there is no regulation on the maximum residue limits (MRL) in litchi, and no work has been done to determine the oxine-copper residues and to estimate the dissipation behavior of oxine-copper residue in litchi.

Until now, little was known about the analytical methods of oxine-copper in various materials. At present, the oxine-copper residue analysis is mainly performed via high performance liquid chromatography (HPLC), such as liquid–liquid extraction method in other crops (Zhou et al., 2008). In this work, a simple HPLC-PDA method was established to detect the residue of oxine-copper in litchi, litchi pulp and soil. A field study was done to investigate the dissipation and residue of oxine-copper in litchi and soil.

MATERIALS AND METHODS

Oxine-copper standard material (purity = 99.0%) was supplied from Dr. Ehrenstorfer GmbH, Germany. Acetonitrile was of HPLC grade (Shanghai ANPEL Scientific instrument Co., Ltd). Sodium chloride

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Table 1. Diagram of the field experimental plot.

Buffer							
Buffer	CK1	Buffer	Z1-HS3	Buffer	Z1-HS4	Buffer	
	CK2		Z2-HS3		Z2-HS4		
	CK3		Z3-HS3		Z3-HS4		
Buffer							
Buffer	X1	Buffer	Z1-LS3	Buffer	Z1-LS4	Buffer	
	X2		Z2-LS3		Z2-LS4		
	X3		Z3-LS3		Z3-LS4		
Buffer							

CK—Blank plot, X—Dissipation plot, Z—Final residue plot, H—high concentration of final residue plot, L—low concentration of final residue plot, S3—spray three time, S4—spray four time.

was purchased from Chengdu area of the industrial development zone xindu mulan. Sodium dodecyl sulfate and sodium dihydrogen phosphate were purchased from Tianjin Bodi Chemical Limited by Share Ltd. Anhydrous magnesium sulfate was heated at 500°C in muffle furnace for 5 h, allowing to be cooled to room temperature before use. Primary secondary amine (PSA) was purchased from Agilent Technologies.

Oxine-copper stock standard solutions of 40 mg/L were prepared in acetonitrile and stored at -20°C. Working standard solutions were prepared by dilution of the corresponding stock standard solution with acetonitrile and stored at -20°C.

Field experiment design

The field trials include the dissipation and final residue study. The supervised field trials were carried out in Guangxi and Guangdong during two consecutive years (2012 and 2013). The area of experiment plot was 30 m^2 (5 m × 6 m) and each treatment was designed with three replicated plots. A buffer area was maintained between the plots (Wang et al., 2012). The experiment was designed according to “Guide line for Pesticide Residue Trials issued’’ by the Institute of the Control of Agrochemicals, Ministry of Agriculture (ICAMA), People’s Republic of China (Table 1).

Residue dynamic experiments

The rate of application in dissipation experiments was 502.5 $g.a.i.hm^{-2}$ (1.5 times of the recommended dosage) with one time spray. Representative litchi and soil samples were collected in 0 (2 h after application), 1, 3, 7, 14, 21 and 30 days after spraying of the pesticide. All samples were stored at -20°C until analyzed.

Final residue experiments

The final residue field experiment was applied at two dosage levels, 335.0 $g.a.i.hm^{-2}$ (recommended dosage) and 502.5 $g.a.i.hm^{-2}$ (1.5 times of recommended dosage). Each dosage level was designed to spray three and four times. Representative litchi and soil samples were collected at Pre-Harvest Interval (PHI) of 14 and 21 days from each plot. All samples were stored at -20°C until analyzed.

Analytical methods

Sample preparation

Litchi: The whole litchi after removing the seeds was divided into litchi and litchi pulp. Each matrix of these samples was mixed in a blender separately, and then stored in a deep freezer at-20℃. Soil: Soil samples were dried in the shade at room temperature and sieved through 40-mesh sieves.

Sample extraction

Litchi and litchi pulp: Five grams of litchi or litchi pulp samples were weighed into a 100 ml FEP centrifuge tube and added into 4 ml acetonitrile and 2 ml water. The mixture was shaken vigorously for 3 min with vortex mixer. Then 2 g sodium chloride was added, the sample was extracted in an ultrasonic bath for 4 min. The extracts were then centrifuged for 5 min at 4500 rpm. An aliquot of 1.5 ml of the upper layer was placed into a 2.0 ml micro-centrifuge vial containing 25 mg PSA and 150 mg MgSO4. The sample was again vortexed for 2 min and then centrifuged for 5 min at 4500 rpm with a microcentrifuge. The upper extract was filtered through a 0.25 mm pore membrane filter and transferred into a 1.5 ml glass autosampler vial for HPLC analysis.

Soil: Ten grams of soil samples were weighed into a 100 ml FEP centrifuge tube, 40 mL acetonitrile and 4 mL 2 mol/L sodium hydroxide were added. The mixture was shaken vigorously for 30 min on a shaker. The extracts were filtered with a filter paper and then again extraction with another 40 mL acetonitrile. The filtrate was transferred to a 250 mL separator funnel containing 5 g sodium chloride. The sample solution was then extracted by liquid–liquid partition with 40 mL petroleum ether first and then 2 × 20 mL acetonitrile. The dichloromethane layers were combined, dehydrated with anhydrous sodium sulfate, filtered through a funnel and evaporated to near dryness with a vacuum rotary evaporator at 40°C. The residue was redessolved in 2 mL acetonitrile and was filtered through a 0.25 mm pore membrane filter for HPLC analysis.

HPLC condition

HPLC was performed using a Waters e2695 series liquid chromatography system (Waters Corporation, USA) with a PDA detector. A EclipseXDB-C18 column, 4.6 mm × 260 mm × 5 µm (Agilent), was operated at a flow rate of 0.4 mL min^{-1}. The isocratic elution condition employed a mobile phase of acetonitrile and aqueous phosphate buffer (v/v 40:60). The injection volume was 20 µL and detection wavelength was 250 nm.

Statistical analysis

The dissipation process follows the first-order kinetic reaction. The

Table 2. Average recovery and repeatability of oxine-copper in litchi, litchi pulp and soil (n=5).

Sample	Fortified level (mg/kg)	Recovery (percent)	RSD (percent)	LOQ (mg/kg)
Litchi	0.10	87.0	3.67	0.01
	0.50	87.6	12.07	0.01
	2.00	80.2	2.71	0.01
Litchi pulp	0.10	103.5	12.05	0.01
	0.50	95.1	5.98	0.01
	2.00	81.0	6.53	0.01
Soil	0.10	92.6	1.52	0.01
	0.50	90.1	4.09	0.01
	2.00	80.1	3.69	0.01

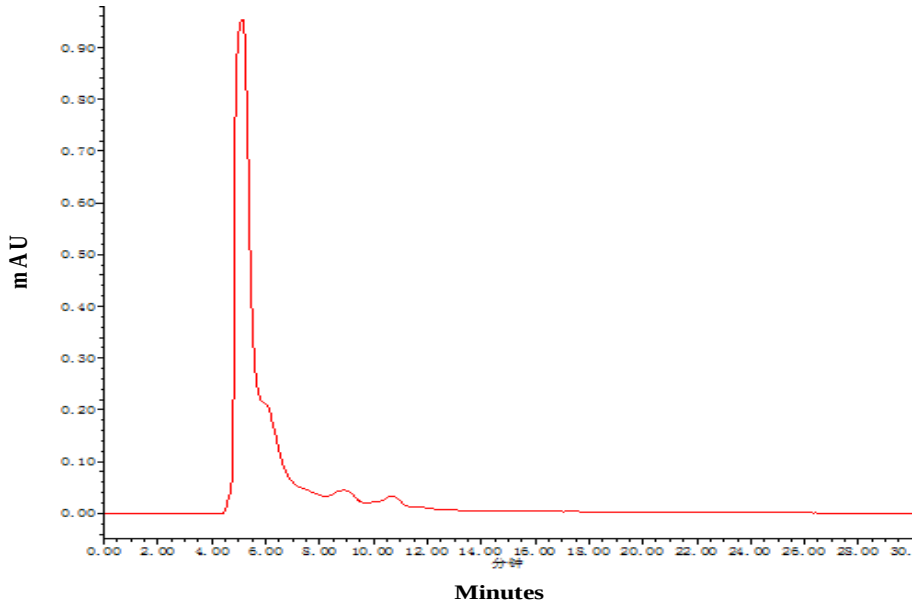

Figure 1. The blank sample chromatograms of Litchi.

degradation rate constant and half-life were calculated using the first-order rate equation: $C_t = C_o\ e^{-kt}$, where C_t represents the concentration of the pesticide residue at the time of t, C_o represents the initial concentration after application and k is the dissipation degradation rate constant ($days^{-1}$). The half-life ($t_{1/2}$) was calculated from the k value for each experiment ($t_{1/2} = \ln 2/k$) (Pan et al., 2012).

RESULTS AND DISCUSSION

Method validation

Quantification of standard solution during validation was done using a calibration curve based on matrix-matched standards. Linearity was studied in the range 0.05 to 5.00 mg/L with five calibration points (0.05, 0.10, 0.50, 1.00 and 5.00 mg/L). Linear calibration graphs were constructed by plotting analytic concentrations against peak areas. Linearity values of standard liquid, calculated as determined at ion coefficients (R^2) were 0.9996. The fortified recovery experiment was studied at three concentration levels (0.10, 0.50 and 2.00 mg/kg). Five samples of each concentration were processed. The recoveries ranged 80.1 to 103.5% with relative standard deviation (RSD) of 1.52 to 12.07%. Hence, the methods can be adopted. The limit of quality (LOQ) of oxine-copper in litchi and soil were 0.01 mg/kg at a signal-to-noise (S/N) ratio of 10, as shown in Table 2 (NY/T788-2004; Xu et al., 1993). The typical HPLC chromatograms of the blank and spiked samples are shown in Figures 1 and 2.

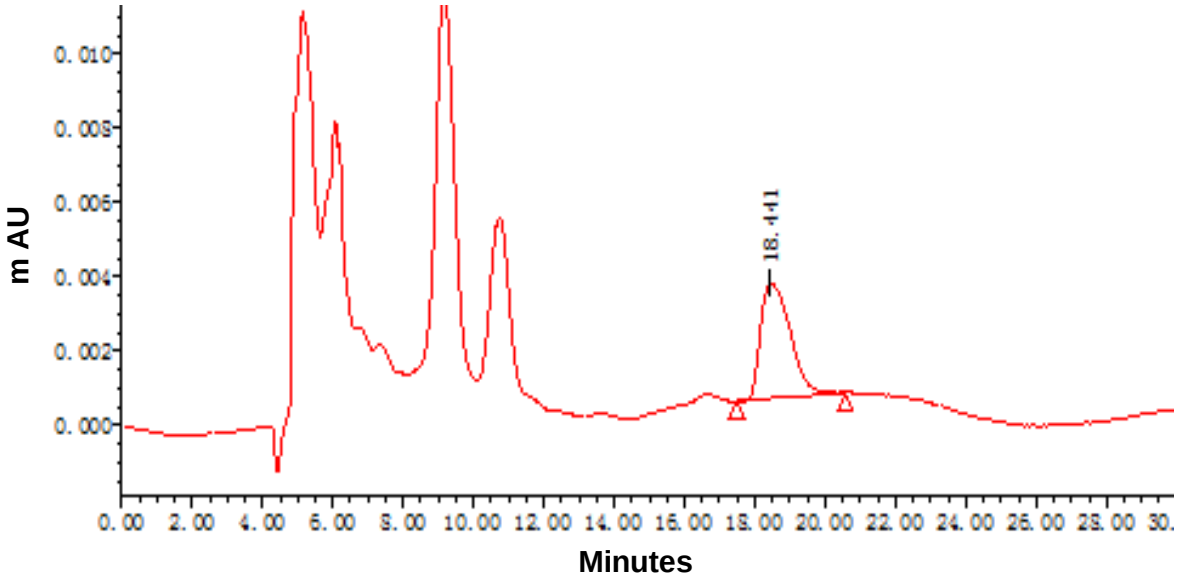

Figure 2. 0.5 mg/kg oxine-copper add litchi sample chromatograms.

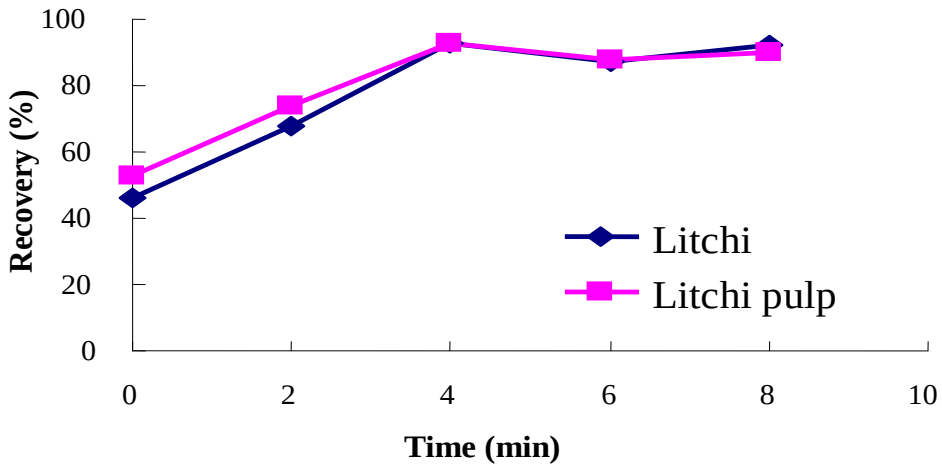

Figure 3. Effect of ultrasonic time on the recoveries. Spike level: 0.5 mg/kg (n = 5).

Optimization of the extraction process

Due to original liquid-liquid distribution extraction, the fortified recoveries were below 60%. Therefore, ultrasonic extraction was added to the study to investigate the extraction effectiveness of litchi and the spiking recovery was improved obviously. The litchi and litchi pulp extracted with acetonitrile experiments were carried out at different ultrasonic time (0, 2, 4, 6 and 8 min). The result can be seen in Figure 3. The recoveries–time curve revealed that the recoveries could be accepted within 4 min, longer ultrasonic time would not affect the extraction efficiency much. Therefore, 4 min was selected as the ultrasonic time.

Dissipation of oxine-copper in litchi and soil

Dissipation of oxine-copper in litchi and soil was listed in Table 3. The residues of samples at different intervals were detected after the application of pesticide. Dissipation curve of oxine-copper in litchi and soil can be seen in Figure 4. As expected, a gradual and continuous decrease of the pesticide residues in the litchi and soil was observed. In the supervised field trials, 90% of the initial residue in litchi had dissipated after 30 day, while 21 day in soil with the half-lives of oxine-copper in litchi and soil were 9.12 and 7.02 day, respectively. Dissipation of oxine-copper in soil was faster than that in litchi. It suggested that the behavior of pesticide in soil is

Table 3. The half-life and other statistical parameters for oxine-copper in litchi and soil.

Year	Experiment site	Sample	Regression equation	Determination Coefficient (R^2)	Degradation Constant (day^{-1})	Half-life (days)
2012	Guangxi	Litchi	$y = 1.9624e^{-0.0682x}$	0.8545	0.0682	10.2
		Soil	$y = 1.3886e^{-0.1199x}$	0.9682	0.1199	5.8
	Guangdong	Litchi	$y = 0.7463e^{-0.0784x}$	0.7736	0.0784	8.8
		Soil	$y = 0.8556e^{-0.0841x}$	0.8058	0.0841	8.2
2013	Guangxi	Litchi	$y = 0.7261e^{-0.0813x}$	0.7707	0.0813	8.5
		Soil	$y = 0.8329e^{-0.0898x}$	08297	0.0898	7.7
	Guangdong	Litchi	$y = 0.8175e^{-0.0769x}$	0.7450	0.0769	9.0
		Soil	$y = 1.4598e^{-0.1083x}$	0.9812	0.1083	6.4

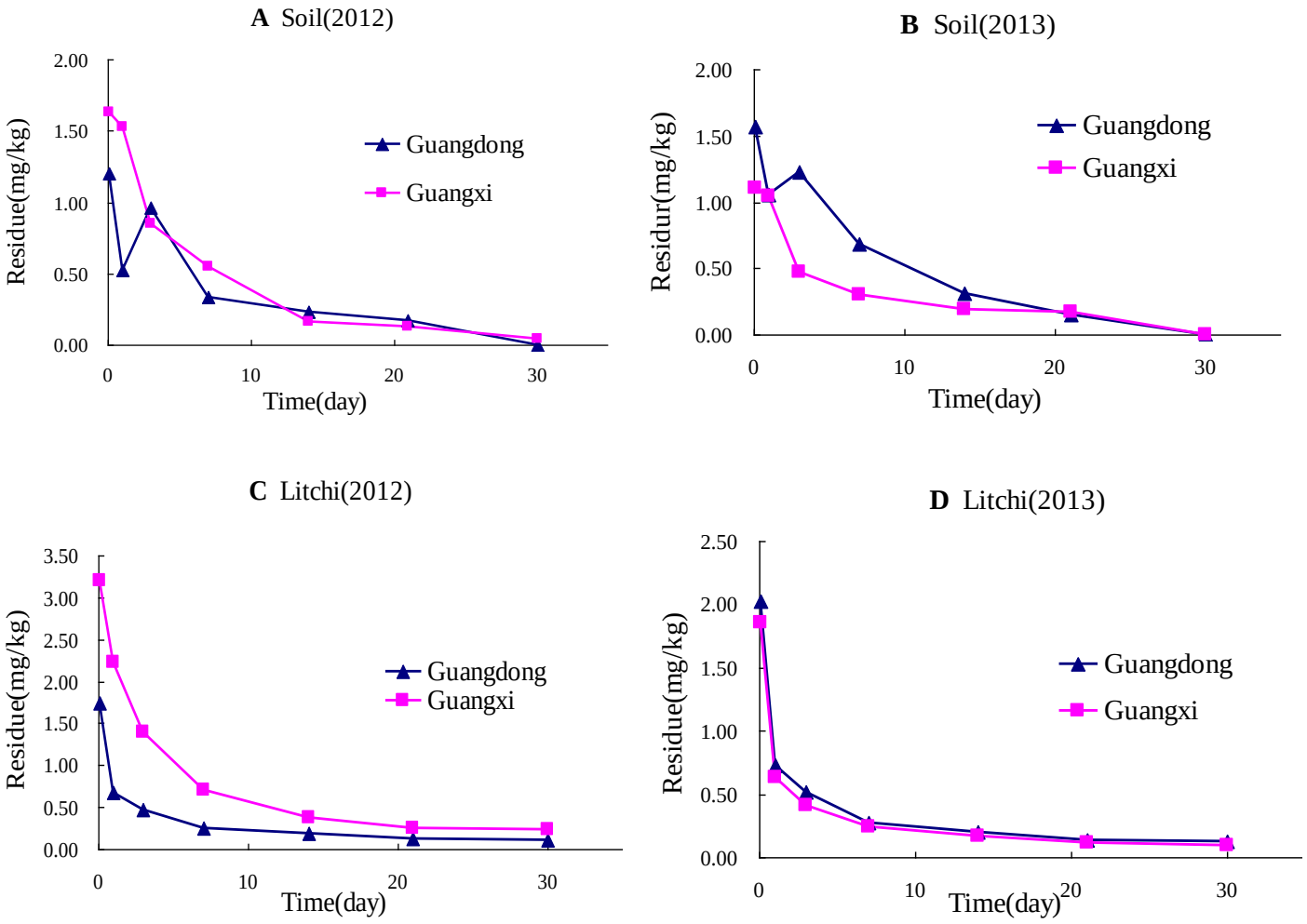

Figure 4. Dissipation curve of oxine-copper in litchi and soil.

governed by a variety of complex physical, chemical and biological processes, including properties of the soil, volatilization, chemical and biological degradation, plants uptake, surface run-off and leaching (Liu et al., 2010). Initial deposits of oxine-copper in litchi and soil differed among the two experimental sites. Dissipation of oxine-

Table 4. Final residues of oxine-copper in litchi, litchi pulp and soil.

Year	Experiment pre-harvest site	Dosage (g.a.i.hm^{-2})	Number of times sprayed	Intervals	Residue (mg/kg)		
					Litchi pulp	Litchi	Soil
2012	Guangxi	335.0	3	14	0.19	0.33	0.03
				21	0.17	0.42	0.03
			4		0.20	0.10	0.06
				21	0.20	0.55	0.05
		502.5	3		0.22	1.77	0.08
				21	0.20	1.69	0.06
			4		0.28	1.96	0.10
				21	0.25	1.80	0.08
	Guangdong	335.0	3	14	0.17	0.34	0.12
				21	<0.01	0.25	0.15
			4	21	0.18	1.02	0.17
					<0.01	0.61	0.15
		502.5	3	14	0.20	1.67	0.12
				21	<0.01	1.48	0.11
			4		0.24	1.92	0.19
				21	<0.01	1.84	0.18
2013	Guangxi	335.0	3	14	0.04	0.09	0.16
				21	0.04	0.09	0.14
			4		0.32	0.20	0.16
				21	0.12	0.09	0.16
		502.5	3	14	0.67	0.51	0.14
				21	0.43	0.47	0.17
			4		0.82	0.33	0.17
				21	0.35	0.51	0.16
	Guangdong	335.0	3	14	<0.01	0.35	0.05
				21	<0.01	0.39	0.04
			4		<0.01	1.02	0.11
				21	<0.01	0.55	0.10
		502.5	3	14	<0.01	1.84	0.08
				21	<0.01	1.77	0.09
			4		<0.01	1.91	0.15
				21	<0.01	1.99	0.12

copper was different in Guangxi and Guangdong in 2012 and 2013; it might be affected by some physical and chemical factors, the rain, growth dilution factor, soil characteristics and microorganisms.

Final residues of oxine-copper in litchi and soil

The final residue of oxine-copper in litchi and soil collected at harvest time were shown in Table 4. As the data shown, the residue of oxine-copper in litchi samples ranged from 0.09 to 1.99 mg/kg after 14 and 21 day of last application; the residue of oxine-copper in litchi pulp samples ranged from 0.04 to 0.82 mg/kg after 14 and 21 day of last application; the residue of oxine-copper in soil samples ranged from 0.03 to 0.19 mg/kg after 14 and 21 day of last application. The final residue levels of oxine-copper in litchi, litchi pulp and soil were lower than 2.0 mg/kg at harvest, which suggested the use of this fungicide to be safe to both human and environment.

The final residue results showed no oxine-copper residues were detected in litchi pulp samples of Guangdong in 2013. In litchi, only trace amount of oxine-copper residues were detected of Guangxi in 2013, compared with Guangxi in 2012. The phenomenon is most likely to be affected by the environment factors like wind, temperature, relative humidity, rain and growth dilution factor. According to test record, there was higher rainfall and relative humidity of Guangxi in 2013 than the same period last year. There is no MRL for oxine-copper in litchi, and 2 mg/kg in apple and cucumber in China. This work would be helpful for the Chinese government to establish MRL of oxine-copper in litchi and to provide guidance on the safe and proper use of this pesticide, and the recommended MRL of oxine-copper in litchi should be 2 mg/kg.

Conclusion

A relatively simple and fast HPLC-PDA method for the oxine-copper residue analysis was developed in this work. The LOQ of this method were 0.01 mg/kg for litchi, litchi pulp and soil. The dissipation rates of oxine-copper residue in litchi and soil under field condition were investigated for the safe and proper use of this pesticide. The results show that when oxine-copper was used under the experiment design, the final residue levels of oxine-copper in litchi, litchi pulp and soil were lower than 2.0 mg/kg at harvest, which suggested the use of this fungicide to be safe to both human and environment. The work can be a reference for the MRL establishment and safe use of oxine-copper. It is recommended that oxine-copper (33.5%, SC) can be applied in litchi at a dosage of 502.5 g a. i.hm^{-2} with pre-harvest interval (PHI) of 14 day, and maximum application below four times (Wu et al., 2012; Kang et al., 2013). The recommended MRL of 2 mg/kg in litchi was safe.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Response of selected sorghum (*Sorghum bicolor* L. Moench) germplasm to aluminium stress

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Sorghum (*Sorghum bicolor* L. (Moench) is an important food security crop in sub-Saharan Africa. Its production on acid soils is constrained by aluminium (Al) stress, which primarily interferes with root growth. Sorghum cultivation is widespread in Kenya, but there is limited knowledge on response of the Kenyan sorghum cultivars to aluminium stress. The aim of the study was to identify and morphologically characterise aluminium tolerant sorghum accessions. The root growth of three hundred and eighty nine sorghum accessions from local or international sources was assessed under 148 µM Al in soaked paper towels, and 99 of these were selected and further tested in solution. Ten selected accessions were grown out in the field, on un-limed (0 t/ha) or limed (4 t/ha) acid (pH 4.3) soils with high (27%) Al saturation, and their growth and grain yield was assessed. Although the Al stress significantly (P ≤ 0.05) reduced root growth in most of the accessions, there were ten accessions; MCSRP5, MCSR 124, MCSR106, *ICSR110*, Real60, IS41764, MCSR15, IESV93042-SW, MCSRM45 and MCSRM79f, that retained relatively high root growth and were classified as tolerant. The stress significantly (P ≤ 0.05) reduced seedling root and shoot dry matter in the Al-sensitive accessions. Plant growth and yield on un-limed soil was very poor, and liming increased grain yield by an average 35%. Most of Kenya sorghums were sensitive to Al stress, but a few tolerant accessions were identified that could be used for further breeding for improved grain yield in high aluminium soils.

Key words: Aluminium tolerance, grain yield, liming, root growth sorghum.

INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is a staple cereal crop in many parts of Africa and Asia, especially in sub-humid and semi-arid agro-ecologies (Simpson and Conner-Ogorzaly, 2001). Despite its importance, sorghum grain yield in sub-Saharan Africa is low (2 t/ha) and has been declining over the years (Wortmann et al.,

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2006) mainly because of poor agronomy, or abiotic and biotic stresses. Many of the soils used for sorghum cultivation in the tropics are acidic (pH<5.5). Soil acidity is common in the tropics and subtropics because of the nature of the parent rocks and the high degree of weathering and base leaching that has occurred (Johnson, 1988). The greater proportion of potentially arable land worldwide is acidic (Von Uexküll and Mutert, 1995), and in Kenya acid soils cover up to 13 % of the arable land (Kanyanjua et al., 2002).

Although Aluminium (Al) is one of the most abundant mineral elements in soil, it occurs in insoluble or non-toxic oxide and hydroxide compounds under neutral or basic pH. However, the compounds become more soluble under acidic (pH<5.5) conditions and release a variety of Al species, especially the trivalent aluminium ion (Al^{3+}) and soluble hydroxides. The Al^{3+} is toxic to plants, and occurs both in solution and at the cation exchange sites, where it can be easily exchanged with other soluble cations. Acid soils in Kenya have between 8 and 61% Al saturation (Obura et al., 2010). Most plants are adversely affected if the soil contains more than 20% aluminium saturation.

The primary effect of Al stress is stunting of the roots (Rengel, 1996). The resulting restricted root system is inefficient in water and mineral absorption, making the plant more susceptible to water stress or mineral nutrient deficiency. The combined limitation on water and mineral nutrient absorption leads to poor plant development and low crop yield. However, aluminium tolerant plants maintain high root growth and plant vigour under Al through the exclusion of Al from the root symplasm or tolerance to high Al^{3+} concentration in the symplasm (Kochian, 1995). The exclusion of Al from the root is achieved by releasing Al-chelating ligands such as organic acids. The organic acid exudates, secreted in significant amount by the tolerant genotypes, form Al-carboxylate complexes that are not taken up by plant roots. Al-tolerant sorghum genotypes have been shown to secrete relatively large quantities of citric, malic and transaconitic acids (Goncales et al., 2005; Magalhaes et al., 2007).

Although lime is conventionally applied to amend soil acidity and related stresses, the practice increases farming cost. Large quantities of lime (2 to 10 t/ha) are required to ameliorate the acidity and enhance growth of crops (Kisinyo et al., 2013). Moreover, sub-soil acidity is not effectively corrected by surface liming (Ernani et al., 2004) unless lime is applied in large quantities and mixed into the deeper soil layers. Therefore, the use of Al-tolerant crop cultivars in addition to lime application could greatly enhance yields in soils that have high percentage of exchangeable aluminium.

Sorghum has a significant genotypic variation in relation to tolerance to Al stress (Caniato et al., 2007) that can be exploited to develop varieties with superior tolerance. However, although significant sorghum cultivation in Kenya occurs on acid soils of western Kenya (Obura, 2008; Kisinyo, 2011), there has been limited selection and breeding for Al tolerant sorghum for this region. Moreover, the amount of yield loss occasioned by Al toxicity in Kenya is not known. The objectives of this study were to determine the level of tolerance in selected Kenyan sorghum lines and to identify Al tolerant accessions, under laboratory and field conditions with specific reference to seedling root growth and grain yield.

MATERIALS AND METHODS

Three hundred and eighty nine sorghum accessions comprising of Kenyan landraces, commercial varieties, breeding lines, recombinant inbred lines (RILs) and Al tolerant and sensitive standard lines, hereinafter termed accessions, were pre-screened for tolerance to Al stress using moistened paper-towels. The sorghum seeds were surface sterilized in 1% sodium hypochlorite for 8 min, rinsed with sterile distilled water, germinated and grown at 26°C for 5 days between sterilized paper towels that were moistened with 10 ml treatment solution (pH 4.0) at two levels of Al stress; 0.82 mM Al or without Al (control). The cellulose fibres in the paper bind Al^{3+} and thus reducing the effective concentration. Earlier studies had shown that 0.82 mM Al^{3+} in filter paper tests is equivalent to 148 µM Al in free solution (Tamas et al., 2006). The root length was measured and root tolerance index (RTI) was calculated as follows:

$$\mathrm{RTI} = \frac{\text{Root length in aluminium}}{\text{Root length in control}} \quad (1)$$

The RTI was used to group the accessions into tolerant or sensitive categories. After the pre-screening, a representative sample of 99 accessions (Table 1) that had been rated as tolerant, sensitive or intermediate were selected and subjected to Al stress in aerated nutrient solution (Magnavaca et al., 1987). Sterilized sorghum seeds were pre-germinated in the dark for 72 h at 25°C between sheets of sterilized paper towels that were moistened with sterile distilled water. Healthy seedlings with the similar root size and form were grown in the nutrient solution without Al for 24 h to equilibrate. The initial length of the main root (IRL) was measured and recorded. Thereafter the seedlings were transferred individually into the growth vials that were placed in holding plastic rafts and transferred to trays containing eight litres of nutrient solution without (control) Al or with 148 or 222 µM Al (Caniato et al., 2007). The seedlings were grown in a plant growth chamber with gentle, continuous aeration for 120 h at 28°C with 17/7 photoperiod and light intensity of 200 µmol $m^{-2}s^{-1}$. The set up was replicated five times. The length of the main root with branches in the control (RLB_c) and in the Al treatment (RLB_{Al}) was measured and recorded. The shoot and root dry weight (68°C for 48 h) of five representative sorghum accessions were determined and recorded.

The data was used to calculate seedling growth indices: net root length (NRL), percentage of response (% response), relative net root length (RNRL) and percentage of reduction in root branching (% RRB) (Magalhaes et al., 2004), thus;

$$\mathrm{NRL} = \mathrm{FRL} - \mathrm{IRL} \quad (2)$$

Where FRL is the final root length in both Al treated and control plants and IRL is the initial root length. The response (%) was measured as:

Table 1. Origin of selected sorghum germplasm used in the study.

Sorghum Line	Source	Classification	Sorghum accession	Source	Classification
MCSR P5	Oyugis	Landrace*			
MCSR124	MUSRT	RIL	MCSRF-6	Kilifi	landrace
MCSR106	MUSRT	RIL	MCSR N140c	Isebania	Landrace
ICSR110	ICRISAT	Al standard	MCSRN79	Kisii	Landrace
Real60	ICRISAT	Al standard	MCSRN102	Karungu	Landrace
IS41764	ICRISAT	Al standard	MCSRN20	Mosocho	Landrace
MCSR15	MUSRT	RIL	MCSRI17	Kilibasi	Landrace
IESV93042-SW	ICRISAT	Breeding line	MCSR N77	Ndhiwa	Landrace
MCSRM45	Koyonzo	Landrace	MCSRN140	Isebania	Landrace
MCSRM79f	Nangeni	Landrace	MCSRM69e	Nangeni	Landrace
MCSRN24	Mabera	Landrace	MCSRM42b	Nangeni	Landrace
MCSRM41	Nangeni	Landrace	MCSR 60	MUSRT	RIL
MCSR M65b	Nangeni	Landrace	MCSRJ3b	Makueni	Landrace
Macia	ICRISAT	Released Var.	MCSRM19	Nangeni	Landrace
ICSB609	ICRISAT	Standard	MCSRT71	ICRISAT	Landrace
MCSRN140b	Isebania	Landrace	MCSR140d	Isebania	Landrace
MCSRM42d	Nangeni	Landrace	MCSRF9b	Kilifi	Landrace
MCSRI6d	Kilibasi	Landrace	MCSRG1a2	Ukunda	Landrace
MCSRN60	Kanyamua	Landrace	MCSRM63c	Nangeni	Landrace
PGRC/E216740	ICRISAT	Breeding line	MCSRQ4	Malaba	Landrace
MCSRM62	Nangeni	Landrace	MCSR N74	Ndhiwa	Landrace
MCSRN81	Kisii	Landrace	MCSRM33a	Nangeni	Landrace
MCSRM5	Nangeni	Landrace	*P20SP*	KARI	Breeding line
MCSRM23	Nangeni	Landrace	MCSRN85	Ndhiwa	Landrace
MCSRP3	Oyugis	Landrace	MCSRN68	Mabera	Landrace
KAK7540	KARI	Breeding line	*ICSB608*	ICRISAT	Standard
MCSRL3b	Busia	Landrace	MCSRN72	Ndhiwa	Landrace
MCSRN21	Mabera	Landrace	MCSRM33b	Nangeni	Landrace
MCSRQ3	Malaba	Landrace	MCSRN88a	Ndhiwa	Landrace
MCSRN83	Ndhiwa	Landrace	MCSRK5b	Eldoret	Landrace
MCSRI6	Kilibasi	Landrace	*ICSB613*	ICRISAT	Standard
MCSRM68b	Nangeni	Landrace	MCSRN35	Mabera	Landrace
MCSRH2a	Ukunda	Landrace	MCSRN2	Mabera	Landrace
MCSRS66	Sega	Landrace	MCSRN13	Mabera	Landrace
MCSRL3	Busia	Landrace	MCSRK5e	Eldoret	Landrace
MCSRM47g	Nangeni	Landrace	MCSRM21	Nangeni	Landrace
MCSRF9d	Kilifi	Landrace	MCSRM73e	Nangeni	Landrace
MCSRL6	Sega	Landrace	Serena x Esuti	KARI	Breeding line
MCSRS65	Bumala B	Landrace	MCSR N157a	Karungu	Landrace
MCSRM69	Nangeni	Landrace	MCSRM3	Nangeni	Landrace
MCSRM33	Nangeni	Landrace	MCSRN84	Ndhiwa	Landrace
MCSRN103	Karungu	Landrace	ICSV112	ICRISAT	Breeding line
MCSRF-1	Kilifi	Landrace	Pato	ICRISAT	Released var
Seredo	Kenya seed	Cultivar	MCSRG2	Ukunda	Landrace
MCSR N51	Migori	Landrace	MCSRM44	Nangeni	Landrace
MCSRN57	Mabera	Landrace	MCSRL5	Sega	Landrace
MCSRN61	Mosocho	Landrace	MCSRN120	Karungu	Landrace
MCSRI19	Kilibasi	Landrace	Hakika	ICRISAT	Released var
MCSRT94	ICRISAT	Breeding line	MCSR N88	Ndhiwa	Landrace
MCSRN88c	Ndhiwa	Landrace	MCSRM45b	Koyonzo	Landrace

* The Landraces were purified through three cycles of selfing before they were tested. RIL – recombinant inbred line. The commercial varieties, standards and breeding lines are written in italics.

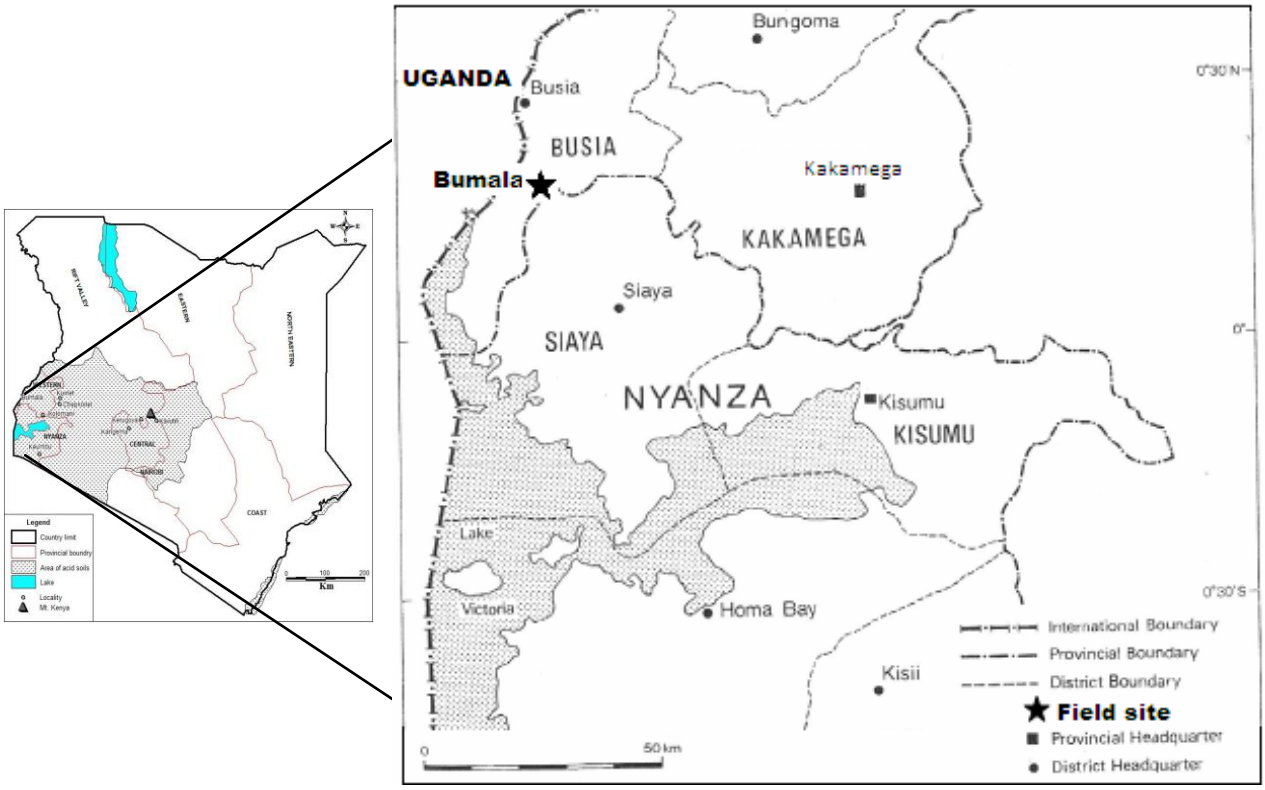

Figure 1. Map of Kenya showing field sites used in this study. Bumala site was used as a testing site and falls within regions with acidic soils, as characterized by Kanyanjua et al. (2002).

$$\% \text{ Response} = \frac{FRL_C - FRL_{Al}}{FRL_C} \times 100 \quad (3)$$

Where FRL_C is final root length in control and FRL_{Al} is the final root length in Al. RNRL was calculated as:

$$RNRL = \frac{NRL_{Al}}{NRL_C} \text{ x } 100 \quad (4)$$

Where NRL_{Al} is net root length in Al, and NRL_C is net root length in control

$$\% \text{ RRB} = \frac{RLBC - RLBAl}{RLBC} \times 100 \quad (5)$$

Where % RRB is the percent reduction in root branching, RLB_C is the length of root with branches in control, and RLB_{Al} is length of root with branches in aluminium.

The percentage response to Al and RNRL were used to classify the sorghum lines as tolerant (≤30% response to Al; RNRL > 70%) or susceptible (>70% response; RNRL < 30%) as defined by Caniato et al. (2007).

A sample of five of the accessions: MCSRP5 (Al-tolerant popular landrace); ICSR110 (Al- tolerant standard check); MCSR15 (Al-tolerant RIL); Seredo (Al-sensitive commercial variety) and MCSRL5 (Al-sensitive popular landrace) were used to evaluate the effect of Al on root and shoot dry weight. To show root injury caused by Al stress the root tips of some lines were visualized and photographed using a microscope (Leica DMLB) fitted with a Leica DC 300 digital camera.

Ten accessions; *ICSR110*, Real60 and *IS41764* (Al-tolerant check), and MCSRM45 (Al-tolerant popular high yielding landrace); *Macia* (moderately tolerant released variety); *Seredo* (Al-sensitive commercial variety), MCSRM33, MCSRL5 and MCSRN61 (Al sensitive, high yielding popular landraces), and *ICSV112* (Al-sensitive breeding line) were evaluated in the field at Bumala in Busia, Western Kenya (Figure 1) for response to Al stress on basis of vegetative growth and grain yield. Bumala is located at N 00°19' E 034°12', at an altitude of 1294 m. The soil at the test site is well drained firm, acidic (pH 4.3), nitisol, with high (> 27%) Al saturation percentage (Obura, 2008; Kisinyo, 2011).

The accessions were grown out in plots in the field with or without lime in a split plot design. Lime (21% Calcium oxide) was applied and mixed with the top soil in one block 60 days before planting at a rate equivalent to 4 t/ha. The plots were ploughed to a fine tilt. The seeds were hand sowed at a spacing of 60 cm between rows and 20 cm within rows in plots measuring 2 × 3 m, which translated into 83,333 plants per hectare. Both blocks received uniform application of 75 kg/ha of diammonium phosphate (DAP) at sowing. The number of leaves and leaf area per plant were assessed at 50% flowering. The length and width of individual leaves per plant were measured using a meter ruler and then leaf area was calculated using the following formula (Stickler et al., 1961):

$$\text{Leaf area} = (\text{leaf length} \times \text{leaf width}) \times 0.75 \quad (6)$$

Grain yield and thousand-seed weight were assessed and recorded after harvest. All the data were subjected to analysis of variance

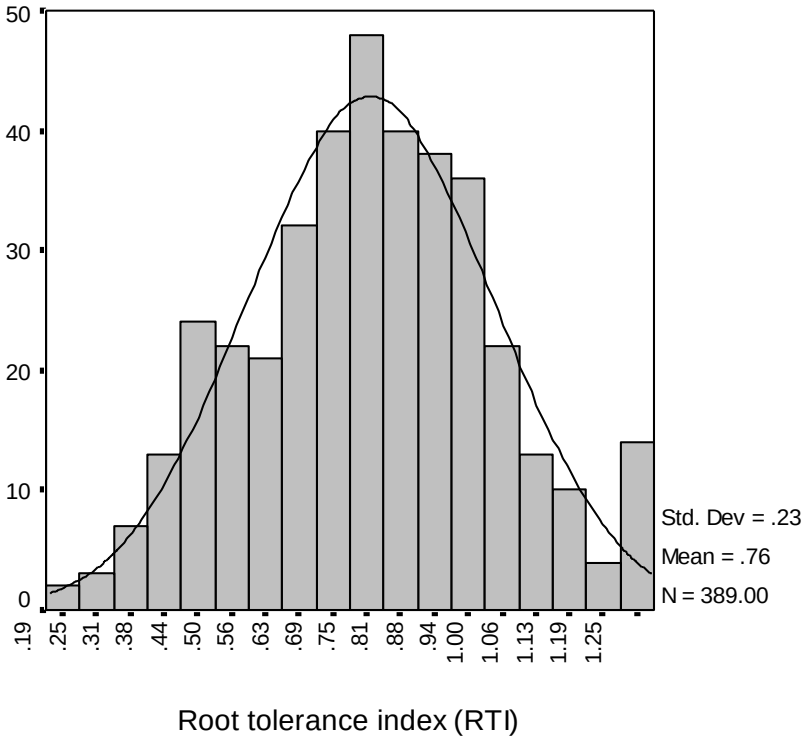

Figure 2. Frequency distribution of root tolerance indices of 389 Kenyan local sorghum accessions. Sterilized seeds were germinated and grown at 26oC for 5 days between paper towels moistened with nutrient solution containing 148 µM Al or without Al.

(ANOVA) using SPSS®. Differences were adopted as significant at $P \leq 0.05$. Means were separated using Tukey's 'honestly significant difference' (HSD) test. The indices data were subjected to square root transformation before statistical analysis.

RESULTS

It was possible to grade the 389 sorghum accessions for aluminium tolerance using the RTIs of filter-paper grown seedlings. Fifty percent of the accessions had RTI of more than 0.75, whereas the other half had RTI of less than 0.75 (Figure 2). Some of the resistant accessions had better root growth (RTI>1.0) when grown under the 148 µM than under control.

In the nutrient solution, the net root length of most sorghum accessions was significantly ($P \leq 0.05$) reduced by the 148 µM Al stress (Table 2). Percent response to Al corresponds to Al-induced reduction in root growth. Only 10 accessions; MCSRP5, MCSR124, MCSR106, *ICSR110*, *Real60*, *IS41764*, MCSR15, *IESV93042-SW*, MCSRM45 and MCSRM79f, had less than 30% root growth reduction in response to Al (RNRL > 70%), and were therefore classified as tolerant to Al stress. Twenty-five accessions expressed root growth reduction ranging between 35 and 50% (RNRL- 50 to 65%), and were classified as moderately tolerant. Sixty-four accessions had between 51 to 82% root growth reduction (RNRL- 18 to 49%) and were classified as sensitive to Al stress. The accessions that expressed more the 70% reduction in root growth (RNRL ≤30%) were classified as highly sensitive; they included MCSRG2, MCSRM44, MCSRL5, MCSRN120, *Hakika,* MCSRN88 and MCSRM45b.

A relative effect of Al stress on root growth in representative sensitive and tolerant sorghum accessions is presented in Figure 3. The root growth in sensitive accessions was severely reduced by the stress, whereas that of tolerant accessions was only minimally affected. Figure 4 shows the appearance of root tips under bright field microscope examination. Although the root tip morphology of the Al-resistant accessions was fairly normal, those of Al-sensitive accessions developed surface lesions after 120 h of exposure to 148 µM Al.

Some accessions, such as MCSR124, MCSR15, MCSR 17, MCSR60, MCSRJ3b, MCSRI19, *ICSV112*, *Pato* and MCSRM45b had significantly longer roots than the rest of the accessions when grown without Al stress. However, only two accessions from this group; MCSR124 and MCSR15, maintained high root growth under the Al stress. There was a significant ($P \leq 0.05$) variation in root branching both among the different sorghum accessions grown without the Al stress, and among those subjected to the 148 µM of Al stress (Table 2). The root branching was significantly reduced by the stress, with most accessions having a percent relative root branching

Table 2. Effect of aluminium stress on seedling root growth in some selected sorghum plants.

Sorghum accession	NRL-Al	%Resp	RNRL	%RRB	Sorghum accession	NRL-Al	%Resp	RNRL	%RRB
MCSRP5	3.68$^{a-d\dagger}$	4.0^{p}	96^{a}	59^{a-d}	MCSRT71	2.90^{a-m}	52.8^{a-k}	47^{b-l}	80^{a-c}
MCSR124	6.08^{a}	7.0^{op}	93^{a}	48^{b-d}	MCSR140d	2.04^{f-o}	52.9^{a-k}	47^{b-l}	68^{a-c}
MCSR106	4.20^{ab}	10.0^{op}	90^{ab}	56^{a-d}	MCSRF9b	2.13^{e-o}	53.7^{a-k}	46^{b-l}	80^{a-c}
ICSR110 **^{S}**	4.23^{a}	10.2^{op}	90^{ab}	51^{a-d}	MCSRG1a2	2.05^{f-o}	53.7^{a-k}	46^{b-l}	94^{ab}
Real60 **^{S}**	3.64^{a-e}	14.55^{n-p}	85^{abc}	58^{a-d}	MCSRM63c	2.88^{a-m}	53.8^{a-k}	46^{b-l}	76^{a-c}
IS41764 **^{S}**	3.66^{a-e}	18.3^{m-p}	82^{abc}	67^{a-c}	MCSRQ4	2.45^{b-o}	54.2^{a-k}	46^{b-l}	92^{ab}
MCSR15	5.28^{a}	25.0^{l-p}	75^{a-d}	45^{b-d}	MCSRN74	2.18^{d-o}	54.5^{a-k}	46^{b-l}	95^{ab}
IESV93042 -SW	3.43^{a-f}	25.5^{l-p}	75^{a-d}	50^{a-d}	MCSRM33a	2.76^{a-n}	54.6^{ak}	45^{c-l}	96^{ab}
MCSRM45 **^{S}**	3.79^{abc}	26.3^{k-p}	74^{a-e}	64^{a-d}	*P20SP*	2.49^{b-o}	54.7^{a-k}	45^{c-l}	68^{a-c}
MCSRM79f	2.92^{a-l}	28.6^{j-p}	71^{a-f}	96^{a-b}	MCSRN85	2.25^{c-o}	54.7^{a-k}	45^{c-l}	75^{a-c}
MCSRN24	3.03^{a-l}	35.0^{h-o}	65^{a-g}	62^{a-d}	MCSRN68	1.93^{f-o}	54.8^{a-k}	45^{c-l}	88^{a-c}
MCSR M41	2.72^{a-n}	35.2^{h-o}	65^{a-g}	87^{a-c}	*ICSB608*	2.23^{c-o}	55.2^{a-k}	45^{c-l}	100^{a}
MCSR M65b	2.49^{b-o}	37.1^{g-o}	63^{a-h}	78^{a-c}	MCSRN72	1.57^{j-o}	55.5^{a-k}	45^{c-l}	70^{a-c}
Macia **^{S}**	3.26^{a-h}	37.7^{g-o}	62^{a-h}	99^{ab}	MCSRM33b	1.92^{f-o}	55.9^{a-k}	44^{d-l}	99^{a}
ICSB609	3.36^{a-g}	38.7^{e-o}	61^{a-h}	70^{a-c}	MCSRN88a	1.86^{f-o}	56.7^{a-k}	43^{d-l}	94^{ab}
MCSRN140b	3.15^{a-j}	39.9^{d-o}	60^{a-i}	60^{a-d}	MCSRK5b	2.32^{b-o}	56.8^{a-k}	43^{d-l}	90^{ab}
MCSRM42d	3.72^{a-d}	40.2^{d-o}	60^{a-i}	84^{a-c}	*ICSB613*	2.56^{b-o}	57.0^{a-k}	43^{d-l}	95^{ab}
MCSRI6d	2.28^{b-o}	40.8^{d-o}	59^{b-j}	68^{a-c}	MCSRN35	2.49^{b-o}	57.0^{a-k}	43^{d-l}	95^{ab}
MCSRN60	2.80^{a-n}	41.7^{c-o}	58^{b-j}	70^{a-c}	MCSR N2	1.78^{h-o}	57.1^{a-k}	43^{d-l}	95^{ab}
PGRC/E216740	3.01^{a-l}	42.5^{c-o}	58^{b-j}	86^{a-c}	MCSRN13	1.97^{f-o}	57.6^{a-k}	42^{d-l}	93^{ab}
MCSRM62	2.80^{a-n}	42.7^{c-o}	57^{b-k}	70^{a-c}	MCSRK5e	2.27^{b-o}	57.7^{a-k}	42^{d-l}	75^{a-c}
MCSRN81	3.34^{a-g}	42.8^{c-o}	57^{b-k}	44^{b-d}	MCSRM21	2.18^{d-o}	58.0^{a-k}	42^{d-l}	81^{a-c}
MCSRM5	2.49^{b-o}	43.4^{b-o}	57^{b-k}	76^{a-c}	MCSRM73e	1.73^{h-o}	58.5^{a-j}	41^{e-l}	67^{a-c}
MCSRM23	2.49^{b-o}	43.6^{b-0}	56^{b-k}	80^{a-c}	MCSRL6	2.05^{f-o}	58.6^{a-j}	41^{e-l}	46^{cd}
MCSRP3	3.19^{a-i}	44.7^{b-o}	55^{b-k}	77^{a-c}	MCSRS65	2.17^{d-o}	59.4^{a-j}	41^{e-l}	62^{a-d}
KAK7540	2.41^{b-o}	45.6^{b-o}	54^{b-k}	52^{a-d}	MCSRM69	1.79^{g-o}	59.5^{a-j}	41^{e-l}	58^{a-c}
MCSRL3b	2.29^{b-o}	46.1^{b-o}	54^{b-k}	71^{a-c}	**MCSRM33** **^{S}**	2.31^{b-o}	59.6^{a-j}	40^{e-l}	82^{a-c}
MCSRN21	2.43^{b-o}	46.5^{a-o}	53^{b-k}	93^{ab}	MCSRN103	1.93^{f-o}	59.8^{a-j}	40^{e-l}	77^{a-c}
MCSRQ3	2.91^{a-m}	47.1^{a-o}	53^{b-k}	92^{ab}	MCSRF-1	1.73^{h-o}	61.0^{a-i}	39^{g-l}	100^{a}
MCSRN83	2.70^{a-n}	47.6^{a-o}	52^{b-k}	89^{a-c}	***Seredo*** **^{S}**	1.54^{k-o}	61.2^{a-i}	39^{g-l}	57^{a-d}
MCSRI6	2.69^{a-n}	47.9^{a-o}	52^{b-k}	70^{a-c}	MCSRN51	1.48^{l-o}	61.3^{a-i}	39^{g-l}	59^{a-d}
MCSRM68b	2.89^{a-m}	48.3^{a-o}	52^{b-k}	88^{a-c}	MCSRN57	1.72^{h-o}	61.9^{a-i}	38^{g-l}	90^{ab}
MCSRH2a	2.89^{a-m}	49.3^{a-o}	51^{b-l}	57^{a-d}	**MCSRN61** **^{S}**	1.97^{f-o}	62.5^{a-i}	38^{g-l}	60^{a-d}
MCSRS66	1.84^{g-o}	50.4^{a-o}	50^{b-l}	68^{a-c}	MCSRI19	2.63^{b-o}	62.5^{a-i}	38^{g-l}	82^{a-c}
MCSRL3	2.14^{d-o}	50.4^{a-n}	50^{b-l}	74^{a-c}	MCSRT94	1.92^{f-o}	62.5^{a-i}	37^{g-l}	90^{ab}
MCSRM47g	2.41^{b-o}	51.0^{a-m}	49^{b-l}	95^{ab}	MCSR N88c	1.86^{f-o}	63.1^{a-h}	37^{g-l}	66^{a-c}
MCSRF9d	2.88^{a-m}	51.5^{a-m}	49^{b-l}	97^{a}	SerenaxEsuti	1.94^{f-o}	63.6^{a-g}	36^{g-l}	88^{a-c}
MCSRF-6	2.99^{a-l}	51.5^{a-m}	48^{b-l}	100^{a}	MCSRN157a	1.63^{i-o}	64.4^{a-g}	36^{g-l}	68^{a-c}
MCSR N140c	2.07^{f-o}	51.6^{a-m}	48^{b-l}	76^{a-c}	MCSRM3	1.75^{h-o}	64.6^{a-g}	35^{g-l}	81^{a-c}
MCSRN79	2.44^{b-o}	51.6^{a-m}	48^{b-l}	75^{a-c}	MCSRN84	1.68^{h-o}	64.8^{a-g}	35^{g-l}	75^{a-c}
MCSRN102	2.17^{d-o}	51.7^{a-m}	48^{b-l}	70^{a-c}	***ICSV112*** **^{S}**	2.52^{b-o}	65.6^{a-f}	34^{g-l}	85^{a-c}
MCSRN20	2.62^{b-o}	51.8^{a-m}	48^{b-l}	94^{ab}	*Pato*	2.51^{b-o}	67.1^{a-e}	33^{h-l}	84^{a-c}
MCSRI17	3.25^{a-h}	52.1^{a-m}	48^{b-l}	65^{a-c}	MCSRG2	1.56^{j-o}	70.1^{a-d}	30^{i-l}	42^{cd}
MCSRN77	2.71^{a-n}	52.1^{a-m}	48^{b-l}	95^{ab}	MCSRM44	1.56^{j-o}	70.4^{a-d}	30^{i-l}	89^{a-c}
MCSR N140	1.83^{g-o}	52.2^{a-l}	48^{b-l}	83^{a-c}	**MCSRL5** **^{S}**	1.50^{j-o}	73.0^{abc}	27^{j-l}	90^{ab}
MCSRM69e	2.16^{d-o}	52.2^{a-k}	48^{b-l}	88^{a-c}	MCSRN120	1.33^{mno}	74.1^{abc}	26^{j-l}	71^{a-c}
MCSRM42b	2.13^{e-o}	52.2^{a-k}	48^{b-l}	77^{a-c}	*Hakika*	1.07^{o}	74.2^{abc}	26^{j-l}	89^{a-c}
MCSR60	3.83^{abc}	52.0^{a-k}	48^{b-l}	70^{a-c}	MCSRN88	1.26^{no}	77.2^{a}	23^{kl}	100^{a}
MCSRJ3b	3.08^{a-k}	52.6^{a-k}	47^{b-l}	82^{a-c}	MCSRM45b	1.20^{no}	82.0^{a}	18^{l}	95^{ab}
MCSRM19	2.78^{a-n}	52.7^{a-k}	47^{b-l}	92^{ab}					

† Means sharing letters within the columns are not significantly different at P ≤ 0.05. Means were separated using Tukey's HSD test. NRL-Al = net root length in aluminium, % resp = percentage response to 148 µM Al, RNRL = relative net root length, RLB148 = length of branched root in 148 µM Al, and % RRB = percentage reduction in root branching. Accessions written in bold with superscript letter 'S' were selected for field experiments.

Figure 3. Effect of aluminium stress on root growth and morphology of selected sorghum accessions; (A) Al-sensitive (MCSRM44) and (B) Al-tolerant (MCSRP5) after screening in solution culture. (C) Al-sensitive accession (MCSRN88) depicting stunted roots with brown colouration after screening using the paper towel method. The seedlings to the left are the controls.

reduction of >50% (Table 2). However, some accessions, such as MCSR124, MCSR15, *IESV93042-SW*, MCSRN81, MCSRL6 and MCSRG2 had ≤50% relative reduction in root branching, whereas in some, root branches were initiated but failed to elongate. The roots of MCSRF-6, *ICSB608*, MCSRF-1 and MCSRN88, did not branch at all under the Al stress.

Aluminium stress at 148 µM significantly ($P \leq 0.05$) reduced root and shoot dry weight in MCSRL5, *Seredo* and MCSRP5, but not in *ICSR110* and MCSR15 (Figure 5a and b). MCSR15 and MCSRP5 had the highest root and shoot dry weight, respectively, at 148 µM, whereas MCSRL5 and *Seredo* had the lowest root and shoot dry weight, respectively. At 222 µM Al, all the accessions had a significant reduction in root and shoot dry weight ($P \leq 0.05$).

Results on the effect of soil liming on plant growth in the field are presented in Table 3 and Figure 6. There were differences in vigour between sorghum plants grown in the limed and un-limed field plots at the early vegetative stages with the crop in the limed plots showing higher vigour than those in the un-limed plots (Figure 6). Lime application did not cause a significant change in leaf area per plant in any of the sorghum accessions (Table 3). ICSV112 and MCSRM33 had the highest and the lowest total leaf area per plant, respectively, in un-limed

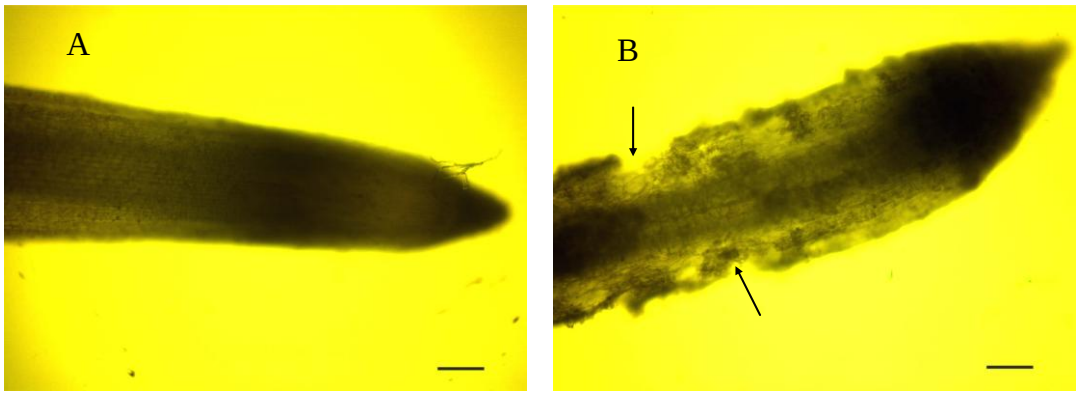

Figure 4. Bright field micrographs showing root tips of (A) Al-tolerant (ICSR110) and (B) Al-sensitive (MCSRL5) sorghum accessions subjected to 148 µM Al for 120 hours. The arrows point at lesions caused by aluminium stress. Scale bars = 200 µm.

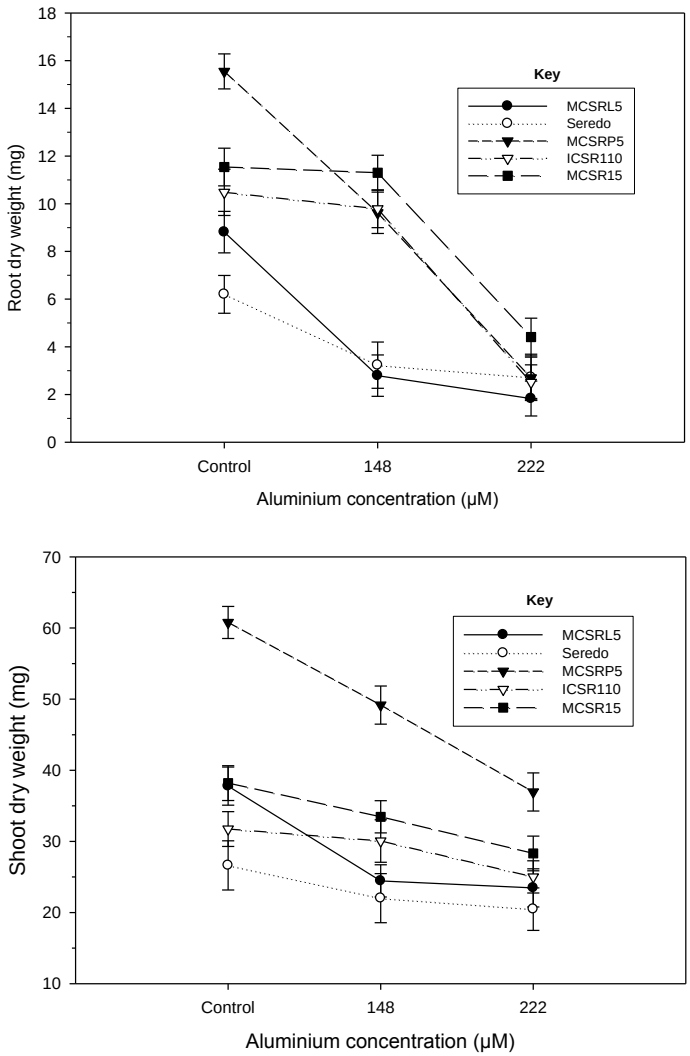

Figure 5. Effect of aluminium stress on (a) root and (b) shoot dry weight (mg) of selected sorghum accessions. Data were subjected to one-way ANOVA and means were separated using Tukey's HSD test. The vertical bars represent standard error.

Table 3. Effect of liming (4 t/ha) on total leaf area and number of leaves per plant of selected sorghum accessions.

Sorghum line	Leaf area per plant (cm^2)		Number of leaves per plant		C
	-Lime	+Lime	-Lime	+Lime	
Macia	$1935^{b-d\dagger}$	2073^{a-d}	7.8^{d-g}	9.5^{a}	MT
Real60	1972^{b-d}	1996^{b-d}	8.0^{c-g}	9.5^{a}	T
MCSRM45	2075^{a-d}	2845^{ab}	7.1^{fg}	7.6^{e-g}	T
MCSRL5	2109^{a-d}	2561^{abc}	6.8^{g}	8.6^{a-e}	HS
Seredo	1858^{cd}	2150^{a-d}	7.6^{e-g}	8.4^{b-f}	S
ICSR110	2032^{b-d}	2279^{abc}	8.1^{b-g}	8.8^{a-e}	T
IS41764	2294^{abc}	2989^{a}	8.3^{b-g}	9.0^{ab}	T
MCSRM33	1267^{d}	1996^{b-d}	6.8^{g}	7.5^{e-g}	S
ICSV112	2642^{abc}	2766^{ab}	8.2^{b-g}	8.4^{b-f}	S
MCSRN61	1952^{b-d}	2679^{ab}	7.8^{d-g}	8.4^{b-f}	S
Mean	2013	2433	7.7	8.6	

† Values with similar letters within the column and row of the same attribute are not significantly different at P ≤ 0.05. The means were separated using Tukey's HSD test. S.E. 273 and 0.46 for total leaf area and number of leaves respectively. C= Classification based on solution culture assay for response to Al stress; HS = highly sensitive, MT = moderately tolerant, S = sensitive, T – tolerant.

Figure 6. Twenty six days old sorghum growing on limed (A) and non-limed (B) plots at Bumala site.

soil. *IS41764* had the highest, whereas MCSRM33 and Real60 had the lowest total leaf area per plant in the limed soil. The number of leaves per plant was significantly higher in limed soil than in non-limed soil in *Macia, Real60* and MCSRL5 (P ≤ 0.05), whereas lime application had no significant effect on number of leaves in the rest of the accessions. In non-limed soil, MCSRL5 and MCSRM33 had the least number of leaves per plant whereas *IS41764* had the highest number of leaves per plant.

In non-limed soils, MCSRM33 had the lowest grain yield per plant (21.2 g – equivalent to 1767 kg/ha), while *Real60* had the highest grain yield per plant (47.9 g – equivalent to 3916 kg/ha) (Table 4). In limed soils, *ICSR110* had the lowest grain yield (33.9 g – equivalent to 2825 kg/ha), while *ICSV112* had the highest grain yield

Table 4. Effect of liming (4 t/ha) on 1000 seed weight (g) and grain yield per plant in some selected sorghum accessions.

Sorghum line	1000 seed weight (g)		Total grain yield (g) per plant		I	C
	-Lime	+Lime	-Lime	+Lime		
Macia	22.8^{de}†	29.4^{bc}	24.1^{ij} (2008)	39.9^{c-j} (3325)	40	MT
Real60	20.1^{fg}	28.8^{bc}	47.9^{b-l} (3916)	64.3^{a-d} (5358)	26	T
MCSRM45	20.1^{fg}	23.9^{d}	42.1^{b-j} (3508)	65.9^{abc} (5492)	36	T
MCSRL5	23.3^{d}	28.1^{bc}	45.9^{b-j} (3825)	61.9^{a-e} (5158)	26	HS
Seredo	23.4^{d}	34.3^{a}	38.6^{d-j} (3217)	56.3^{a-f} (4692)	31	S
ICSR110	17.2^{h}	18.8^{gh}	25.7^{h-j}(2142)	33.9^{f-j} (2825)	24	T
IS41764	20.3^{efg}	24.9^{d}	42.3^{b-j}(3525)	61.0^{a-e}(5083)	31	T
MCSRM33	12.8^{i}	20.4^{efg}	21.2^{j} (1767)	36.9^{e-j}(3075)	43	S
ICSV112	19.6^{fgh}	34.5^{a}	44.0^{b-j} (3667)	81.4^{a} (6783)	46	S
MCSRN61	21.5^{ef}	28.6^{bc}	37.8^{e-j} (3150)	66.2^{ab} (5517)	43	S
Mean	20.1	27.2	37.0 (3083)	56.8 (4733)	35	

† Values with similar letters within the column and row of the same attribute are not significantly different at P ≤ 0.05. The means were separated using Tukey's HSD test. S.E 0.8 and 7.6 for 1000 seed weight and total grain yield respectively. The values given in brackets are equivalent to grain yield in kg/ha. I = percent increase in grain yield. C= Classification based on solution culture assay for response to Al stress; HS = highly sensitive, MT = moderately tolerant, S = sensitive, T – tolerant.

(81.4 g – equivalent to 6783 kg/ha).

Lime application caused a significant increase in total grain yield per plant in *ICSV112* and MCSRN61 (P ≤ 0.05). The increase in grain yield ranged from 24 to 46%, where *ICSR110* and *ICSV112* had the lowest and highest increase in grain yield, respectively. An average of 35% increase in overall grain yield was registered as a result of lime application. Similarly, the application of lime significantly increased the 1000 seed weight in all the sorghum accessions, except *ICSR 110* (P ≤ 0.05; Table 4).

DISCUSSION

Differential response to Al stress was observed at 148 µM Al concentration, where only 10% of the 389 accessions were tolerant. At 222 µM Al root growth was severely restricted in all the sorghum accessions, which showed that this concentration was too high to be used to differentiate sorghum response to Al stress. Therefore, screening for Al resistance in sorghum should be carried out at 148 µM Al concentration. Aluminium concentrations at 148 µM and 222 µM correspond to 27 µM and 39 µM free Al ions (Al^{3+}) (Magalhaes et al., 2004). These concentrations have previously been reported to reduce root growth in sorghum (Caniato et al., 2007). In this study, some of the accessions had inherently long roots in nutrient solution without Al. A few of these accessions were tolerant to Al stress, whereas most of them were sensitive. These accessions can be crossed with the sorghums that had short roots but tolerant to Al stress. A combination of long roots and Al tolerance are good attributes for enhanced acquisition of nutrients and moisture in acid soils with high levels of Al consequently improving growth, drought tolerance and grain production in such soils.

The most Al sensitive accessions used in this study which included MCSRG2, MCSRM44, MCSRL5, MCSRN120, *Hakika,* MCSRN88 and MCSRM45b had stubby roots with brown colouration at the 148 µM Al concentration. The root tips had surface lesions due to injury caused by Al stress. Similar observations on root injury due to Al stress have been previously reported (Mossor-Pietraszewska et al., 1997). Root stunting is a consequence of Al-induced inhibition of root elongation, which is the most evident symptom of Al toxicity (Matsumoto, 2000). Aluminium stress has been reported to reduce cell wall extensibility in wheat roots and that this Al-induced change in the cell wall contributes to the inhibition of root growth (Ma et al., 2004). In addition, Al-induced inhibition of K+ uptake by blocking the responsible channels would interfere with turgor driven cell elongation (Liu and Luan, 2001).

Aluminium stress significantly reduced root branching in most sorghum accessions; where ninety five percent of the accessions had ≤50% reduction in root branching. The most sensitive accessions did not develop any lateral roots, while in some, the root branches were initiated but failed to elongate, which is in line with previous reports (Roy et al., 1988). Differential elongation of root branches in response to aluminium stress was also reported in maize (Bushamuka and Zobel, 1998) and apparently is a common reaction of plant root systems to the stress.

Aluminium stress significantly reduced root and shoot dry matter especially in the Al-sensitive sorghum accessions. The Al tolerant accessions had higher average root and shoot dry matter than the susceptible

accessions. Similar results have been reported in barley (Foy, 1996). Aluminium has been reported to interfere with uptake, transport and utilization of nutrients, especially Ca, Mg, P, N and K and reduce accumulation of dry matter (Nichol and Oliveira, 1995). Larger root systems are known to have a greater capacity for absorbing water and minerals, as they are able to explore a larger rhizosphere (Osmont et al., 2007).

The sorghum accessions grown on acid non-limed soil had lower above ground growth and yield compared to that grown in limed soil. Some sorghum accessions that were Al-sensitive in solution culture were also severely affected by the stress in the field. Application of lime significantly increased total leaf area and number of leaves per plant. High leaf area is important in interception of photosynthetic active radiation, which translates to enhanced rates of photosynthesis and consequently high biomass accumulation. It has been reported that high levels of Al inhibited leaf growth in soybean (Zhang et al., 2007). The significant increase in growth and production in the limed soil can be attributed to increased root growth and establishment which translates to improved access to water and nutrients. Liming the acid soil raised soil pH, as reported by Kisinyo (2011), and because the solubility of Al is highly pH dependent, this could result in concentrations of exchangeable Al being lowered to negligible levels that did not limit sorghum growth.

Soil chemical factors that limit root growth in acid soils, such as aluminium diminish crop production through a rapid inhibition of root growth that translates to a reduction in vigour and crop yields (Kochian et al., 2005). Plants grown in soils with high levels of aluminium have reduced root systems and exhibit a variety of nutrient-deficiency symptoms, with a consequent decrease in yield. Decreased above ground plant growth in soil with high percentage of Al saturation has been reported (Miller et al., 2009). This was accompanied by reduced uptake of P and N in the acidic soil. An Al-tolerant maize line had increased levels of mineral nutrients in roots and shoots compared with a sensitive inbred line when grown in an Al-treated-nutrient solution (Giannakoula et al., 2008). Genotypic variation in nutrient uptake in the presence of toxic levels of aluminium has also been reported in sorghum (Baligar et al., 1993), where the Al-tolerant genotypes had higher nutrient uptake efficiency than the Al-sensitive genotypes.

An overall 35% reduction in sorghum grain yield was realized in non-limed soil, with the Al-sensitive accessions having higher reductions than the Al-tolerant accessions. In this regard, some researchers (Gallardo et al., 1999) reported 50 and 30% reduction of grain yield in Al sensitive and resistant cultivars of barley respectively, when they were grown in soil that contained high levels of exchangeable Al.

The Al tolerant standard check *ICSR110* registered low grain yields in non-limed soil but had the lowest response to lime application. Similar results have been reported in maize (*Zea maize*), where 'Cateto', one of the most Al-tolerant Brazilian lines has been shown to be a low yielder and has been used as a source of genes for Al tolerance in maize breeding programmes (Ouma et al., 2013). The Al sensitive lines MCSR L5 and *ICSV112* had relatively higher yields but had low and moderate response to lime respectively. The yield of these accessions could be improved in acid Al-toxic soils by crossing with *ICSR 110* which had better root growth under Al stress conditions. *Real60* and MCSRM45 registered high yields and were also tolerant to Al stress in solution culture and therefore in addition to *ICSR110* are potential sources for Al tolerance genes in sorghum breeding programmes.

Conclusions

Al toxicity significantly reduced development and elongation of main roots and root branches in aluminium sensitive sorghum accessions. Only 10% of the sorghum accessions used in the study were tolerant Al stress reduced root and shoot dry weight as well as the plant growth and grain production under field conditions. Therefore, there is a need to disseminate the Al-tolerant lines to the sorghum farmers for cultivation in areas where soil acidity and aluminium stress are known to occur. Future sorghum breeding programmes should include the identified superior sorghum accessions as donors of aluminium tolerance genes to the locally adapted sorghums cultivated in acid soils with high levels of Al.

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Conflict of Interests

The author(s) have not declared any conflict of interests.

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Agronomic, morphological, anatomical and physiological characteristics of tolerant and non-tolerant drought maize varieties

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The agronomic, morphological, anatomical and physiological characteristics of native and improved varieties of rain fed drought tolerant maize (DTV) and non-tolerant varieties (NDTV) was compared. Correlation analysis of each variety in terms of production characteristics and other variables was done. This was done in order to observe if some of the latter could be used as selection indices of productive plants. The study was conducted under irrigated conditions in Zacatecas, Mexico (2280 musl; 15.8°C mean temperature; 448 mm of annual rainfall average). The average of 10 fully competitive plants of each variety was evaluated. There were no differences between the DTV and NDTV as a group, but they differed when considering characteristics of each individual variety. It suggests that to find differences between these varieties, they should be studied individually. The indices for selecting productive plants in the early DTV native are: C-5: NGC, NRC, NL and W5G for both grain and stubble; in the DTV semi early native: C-7: high values of: NKR and low: EH, DMF, DS and PH for grain, also high values of: W5G, V5G, NSP and NPBT for stubble; in the DTV native semi early C-23, high values of: W5G and a low one of PL for grain, also high values of: W5G, V5G and EH and low of: ASY, NRC and ILE, for stubble.

Key words: *Zea mays* L., native varieties, rain fed, grain yield, stubble yield.

INTRODUCTION

More than 80 years ago, research on drought tolerance in maize had been conducted (Jenkins and Richey, 1931; Jenkins, 1932; Sayre, 1932; Moreno et al., 2001). In Mexico, more than 50 years ago, Moncada (1957) determined the drought tolerance of a group of corn varieties in Northeastern Mexico; Palacios (1959) selected in Chapingo, Mexico State, a drought resistant maize line for its behavior of “latency”. This topic was thoroughly studied in this country from 1960 to 1990 (Luna and Gutiérrez, 1998). Currently, several researchers

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Table 1. Genetic material used in the present study, place of origin, donor and growth cycle.

Variety	Community and municipality	Donor	Cycle
C-19 NDTV	Col. R. Jaramillo, Ojocaliente	Didn´t give his name	Early
C-5 DTV	Col. G. Ortega, Sombrerete	Humberto Salazar	Early
V-209	Cieneguillas, Zacatecas	INIFAP and UAA-UAZ	Early
VS-202	Cieneguillas, Zacatecas	INIFAP and UAA-UAZ	Early
C-18 NDTV	Malpaso, Jerez	Didn´t give his name	Semi early
C-7 DTV	Zaragoza, Sombrerete	Martín Salazar	Semi early
C-23 DTV	La Florida, Valparaíso	J. Guadalupe Alba	Semi early
VS-201 DTV	Cieneguillas, Zacatecas	INIFAP and UAA-UAZ	Semi early

DTV = Drought tolerant variety; NDTV = Not drought tolerant variety; Early = 90-100 days to physiological maturity; Semi early = 100-120 days to physiological maturity (Veríssimo-Correa, 2008).

are doing work in Mexico to form varieties of maize tolerant to drought (Reyes-Mendez et al., 2007; Dávila, 2011; CIMMYT, 2012); however, they do it in tropical or subtropical regions, where recorded rainfall exceeds 500 mm during the growing season of maize, and with average temperatures exceeding 21°C. Very little research has been done to develop drought tolerant *varieties for rain fed crops in arid and semi-arid* temperate region of Central north of Mexico, where rainfall is generally 250 to 400 mm during the growing season of 100 to 120 days, with average temperatures ranging between 17.5 and 20.5°C (Medina et al., 1998).

The National Institute for Forest, Agricultural and Livestock Researching (INIFAP) formed the open-pollination varieties (OP), CAFIME and VS-201, released in 1957 and 1963, respectively, (Gámez-Vázquez et al., 1996), for dry land sowing in the arid and semi-arid temperate region of North central México. Several years later, it was determined that these varieties have drought tolerance characteristics (Gutiérrez et al., 1988). In 1975, other OP four varieties were released by the INIFAP also for rain fed crops in that region, and in 1991, another one was released.

In the arid and semi-arid temperate region of North central Mexico, 750,000 ha of rain fed maize are sown annually on average (SAGARPA, 2012); but for more than 20 years, there has not been maize breeding for this sowing system.

Kakani et al. (2003) indicate that plants under stress through generations can lead to tolerant varieties through changes in the morphology and anatomy. This increases the ability to capture and conserve water and other resources, thereby increasing the tolerance to adverse factor that causes stress. In the above region, rain fed maize with drought stress has been cultivated for 800 years (Velasco, 1896; Rodríguez, 1977). Based on this, the Autonomous University of Zacatecas, Mexico, iniciated a Project in the month of January 2005, with the collection of native corn varieties in rain fed locations in the state of Zacatecas plateau (1900 to 2250 musl); the localities have representative ecological characteristics of arid and semi-arid temperate region of North central Mexico.

The varieties were tested for two years under the drought-irrigation scheme (Muñoz and Rodriguez, 1988) in a village of this region; based on the results, the following collections were selected: C-5, C-7 and C-23. This is because they showed greater drought tolerance than all the rest (Loera, 2008), according to their productivity under drought and drought susceptibility index, indicated by Fisher and Maurer (1978).

The aim of this work is to observe if these varieties can be distinguished from non-drought tolerant varieties, based on some agronomic, morphological, anatomical and physiological characteristics, as indicated by Williams et al. (1967), Muñoz (2003) and Kakani et al. (2003); in addition to that, these features could be used as selection index in breeding programs of rain fed maize in the Central northern Mexico.

MATERIALS AND METHODS

Genetic material

The studies were made with varieties shown in Table 1. In order to obtain more precise information, varieties were divided into two groups: Early and semi early, according to the classification of Verissimo-Correa (2008). In average of two tests conducted in 2005 and 2006 in Cieneguillas, Zacatecas, Mexico, with drought in the soil during the phenological stages of vegetative growth, flowering and grain filling, Loera (2008) found that the average grain yield of drought tolerant varieties with early vegetative cycle (DTV) (C-5, VS-202, V-209) was statistically higher than the non drought tolerant (NDTV) (C-19) variety by 40% and in yield stubble by 41%. The semi early cycle growth of DTV (C-7, C-23, VS-201) outperformed NDTV (C-18) by 37% in grain and by 41% in stubble yield. On the average of experiments treated under rain fed conditions in the year 2008 (425 mm of rainfall during the growing season) and 2009 (280 mm of rainfall during the growing season), in Valparaiso, Zacatecas, Mexico (1900 musl and 20.5 ^{o}C average temperature in the growing season), Luna et al. (2010) determined that the DTV with early vegetative cycle outperformed the NDTV in grain yield by 80% and in stubble yield by 23%; the VTS of semi early vegetative cycle outperformed grain and stover in NDTV by 28% and 10%, respectively. Varieties: C-5, C-7, C-18, C-19 and C-23 are native, while V-209, VS-201 and VS-202 are open pollinated varieties improved by INIFAP (Gámez-Vázquez et al., 1996).

Morphological and agronomic characteristics

Area of study

The research was carried out at the experimental field of the Agronomy Academic Unit of the Autonomous University of Zacatecas, Mexico, located at 22° 31' 28" N latitude, 102° 41' 10" W longitude and at 2280 musl. The climate is BS_1 (h_1)w(w) of the dry group. The average annual temperature is 15.8°C and precipitation of 448 mm; but between 60 and 70% is recorded during the dry growing season (July to September) (Medina et al., 1998). The soil used was loamy, has a depth of 0.8 to 1.0 m, 1.5% organic matter, pH 7.2 and has less than 4% slope (Zelaya, 2002).

Management of the seeding plot

Each variety occupied two rows of 5 x 0.75 m, with 16 plants each. According to the Experimental Zacatecas Field recommendation (Medina et al., 1998), the plants were sown on moist soil in 12 April, 2007; the management of the plot consisted of: fallow, tracking, tracing the grooves, manual seeding, fertilization with 80-40-00 dose, two spuds, watering enough to keep the soil moist and a manual weeding. There were no problems with pests or diseases.

Measured variables

In 10 fully competitive plants of each variety, data were taken: GW= Grain weight in g; SW= Stover weight in g; DMF= Days from sowing to male flowering average; DS= Days from seeding to half silking; ASY= Floral asynchrony; GWCD= Grain weight per cob/DMF in g/day; SWD= Stubble weight/DMF in g/day; SP= Silking period in days; MFP= Male flowering period in days; EH= Ear height in cm (from the soil surface to the lower ear position); PH= Plant height in cm (from the soil surface to the tip of the tassel); NL= Number of main stem leaves; NSP= Number of shoots per plant; IDE= Diameter of the middle part of the ear internode in mm; ILE= Internode lenght of ear in cm; PL= Peduncle lenght in cm; TL= Tassel lenght in cm; NTPB= Number of tassel primary branches; DPM= Days from seeding to physiological maturity; GFP= Grain filling period in days (DPM – DMF); CL= Cob lenght in cm; DC= Diameter of the middle part of cob in mm; NRC= Number of rows of the cob; NKR= Number of kernels per row; NGC= Number of grains per cob (NRC x NKR); CD= Corncob diameter in the middle part in mm; CW= Corncob weight in g; W5G= Weight of 50 grains in g; V5G= Volume of 50 grains in cc; GD= Grain density (W5G/V5G); LG= Lenght of grain (DC-CD)/2. Variables were measured as indicated by the International Union for the Protection of New Varieties of Plants (UPOV) (Carballo, 2010). The results are presented as average of 10 plants.

Anatomical feature

Stomatal density

This work was were conducted in the greenhouse and plant pathology laboratory of the Academic Unit of Agronomy of the Autonomous University of Zacatecas. The stomatal density was measured as indicated by Briones and Delgado (1988) and Gutierrez and Luna (1992). For the study, developed seedling leaves in styrofoam cups with capacity of 500 mL in volume were used. To each beaker was added 115 g of a sterilized substrate composed of loamy soil mixed with 10% organic matter. The seed was sown in 3 cm depth. To each beaker was added 50 mL of water. Samples were taken from epidermal tissue of 10 plants (repetitions) of each variety; both at the beam and the underside, by a thin film of exactoden previously applied with a brush. Each sample taken from leaves was placed on a slide for stomata counting under a microscope of 40 magnifications.

Physiological characteristics

Seed germination at different osmotic pressures

Seeds of approximately the same size and age of each variety were germinated in Petri dishes at 0, 5, 10 and 15 atm osmotic pressure, to simulate drought stress (Michel and Kaufmann, 1973; Martínez, et al., 1999). A completely randomized design with five replications was used. The experimental unit was a Petri dish with 20 seeds per treatment. The seeds were placed between filter paper dusted with fungicide to prevent fungal damage. To each box was added 15 mL of sucrose solution in distilled water according to the treatment, except the 0 atm, consisting only of distilled water. The Petri dishes were put in an oven at 25°C. Five days after starting the experiment, the germinated seeds were counted.

Resilience of seedlings subjected to PWP

For this test, an experimental design in randomized complete blocks with five replications was used; the experimental unit was 10 styrofoam cups of 500 mL volume capacity, with one seedling in each. To each beaker was added 120 g of a sterilized substrate composed of loamy soil mixed with 10% organic matter. The seed was sown in 3 cm depth. To each beaker was added 50 mL of wáter; 30 days later were added again 50 mL of water to each beaker, to subsequently obtain the percentage of plantlets recovered, as indicated by Williams et al. (1967), Palmeros (1985) and Rojas (2003).

Transpiration index

For this test, the same methodology noted in the previous paragraph was used, respectively in the use of glasses, substrate, sowing, etc. Weights of vessels were taken from the first day after adding water to each glass. With these data, by substracting the initial weight from the everyday weight, the transpiration rate of each variety was determined (Gutiérrez and Luna, 1992).

Statistical analysis

In order to observe whether there was difference between drought tolerant varieties and non-tolerant ones, the variables measured in each study were subjected to analysis of variance; those variables that showed statistical significance, the mean test was done with the method of averages of Tukey ($p<0.05$). Also a simple correlation analysis between variables for each variety was carried out, in order to observe if some of them can be used as selection indices in breeding programs of regional drought tolerant varieties.

RESULTS AND DISCUSSION

Early varieties

Agronomic and morphological characteristics

In 12 of the 31 measured quantitative variables, analysis of variance found no statistical difference ($p<0.05$)

Table 2. Means comparison of the measured variables in rain fed maize varieties of Early Vegetative Cycle. UAA-UAZ. 2007.

Variety	SW	SWD	DMF	PH	PL	T L	NTPB	NSP	DPM
C - 5 DTV	512	6.19^{a}	82.9^{ab}	241^{a}	27.7^{ab}	37.5^{ab}	12.2^{c}	1.50^{a}	127^{a}
V-209 NDTV	480^{ab}	5.73^{ab}	83.9^{a}	216^{b}	23.2^{c}	40.6^{a}	18.9^{a}	1.00^{b}	123^{b}
VS-202 DTV	365^{c}	4.61^{b}	79.2^{bc}	217^{b}	23.6^{bc}	35.8^{b}	16.1^{ab}	0.10^{c}	119^{c}
C – 19 NDTV	387^{bc}	5.05^{ab}	77.0^{c}	242^{a}	28.2^{a}	37.0^{ab}	14.8^{bc}	0.10^{c}	126^{ab}
DSH (0.05)	99	1.21	4.3	20	4.4	3.7	3.2	0.45	3
GFP	**CL**	**DC**	**NRC**	**NGC**	**W5G**	**V5G**	**GD**	**CD**	**CW**
44.7^{ab}	17.6^{a}	43.0^{ab}	13.0^{b}	416^{ab}	21.5^{a}	14.1^{a}	1.52^{b}	24.9^{a}	21.6^{a}
38.8^{c}	14.6^{b}	41.6^{b}	14.9^{ab}	480^{a}	17.5^{b}	10.8^{b}	1.62^{a}	21.8^{bc}	13.8^{b}
40.1^{bc}	15.3^{ab}	45.0^{a}	13.0^{b}	377^{b}	21.6^{a}	13.4^{a}	1.61^{ab}	24.3^{ab}	17.8^{ab}
49.0^{a}	13.7^{b}	42.6^{ab}	16.0^{a}	483^{a}	17.2^{b}	10.3^{b}	1.69^{a}	20.1^{c}	12.9^{b}
5.2	2.7	3.3	2.1	86	2.4	1.8	0.12	2.6	5.4

SW = Stover weight, SWD = Stubble weight per day to male flowering, DMF = Days to male flowering, PH = Plant height, PL= Peduncle lenght, TL = Tassel lenght, NTPB = Number of tassel primary branches, NSP = Number of shoots per plant, DPM = Days to physiological maturity, GFP = Grain filling period, CL = Cob lenght, DC = Diameter of cob, NRC = Number of rows per cob, NGC = Number of grains per cob, W5G = Weight of 50 grains, V5G = Volume of 50 grains, GD = Grain density, CD = Corncob diameter, CW = Corncob weight. DSH = Honest significant difference. Values with the same letter in the same column are not statistically different ($p < 0.05$).

Table 3. Correlation coefficients greater than 0.39 between grain weight per day (gwd) and other variables of rainfed maize varieties early vegetative cycle. UAA -UAZ. 2007.

Variety	EH	NL	NTPB	ILE	TL	CL	NRC	NKR	NGC	W5G	V5G	CW	DC	SP
C - 5 DTV		0.63		-0.46			0.65	0.56	0.87	0.65	0.63	-0.40		
V-209 DTV	-0.44		0.68										0.46	
VS-202 DTV					-0.59				-0.70					-0.67
C – 19 NDTV						0.43								

DMF	ASY	GFP	GD	DPM
-0.43		0.56	-0.42	0.51
-0.42	-0.79	-0.4		

EH = Ear Height, NL = Number of main stem leaves, NTPB = Number of tassel primary branches, ILE= Internode lenght of ear, TL = Tassel lenght, CL = Cob lenght, NRC = Number of rows per cob, NKR = Number of kernels per row, NGC = Number of grains per cob, W5G = Weight of 50 grains, V5G= Volume of 50 grains, CW = Corncob weight, CD = Corncob diameter, SP = Silking period, DS = Days to silking, DMF = Days to male flowering, ASY = Floral asynchrony, GFP = Grain filling period, GD = Grain density, DPM = Days to physiological maturity.

the analysis of variance (Table 2). In all the variables there is at least one value of DTV statistically equal to NDTV. There was no trend of similarity or difference between the values of at least 2 DTV that distinguishes them from the NDTV.

Correlation coefficients greater than 0.39 between the measured variables and weights of grain per day (Table 3) and stover (Table 4) showed no difference between the group of DTV and the NDTV, except cob lenght with grain weight per day. This suggests that it is not easy to differentiate a group of DTV early vegetative cycle from NDTV through agronomic and morphological characteristics as assessed here. As noted by some authors (Bartels and Salamini, 2001; Bruce et al., 2002; Muñoz, 2003; Mahajan and Tuteja, 2005), drought tolerance in maize is complex; assuming that is governed by several pairs of genes and mechanisms of tolerance, it may vary from one variety to another. Comparing only the DTV native C-5 with the NDTV C-19 (Table 4), it shows that C-5 had values greater than C-19 in features: SW, NSP, CL, W5G, V5G, CD and CW, and minor: NRC and GD.

Nilsen and Orcutt (1996) indicate that mechanisms of drought tolerance in plants can be improved by selection; so, if somebody would like to use the native variety of early growing season, C-5 is classified as drought tolerant (Loera, 2008). In a regional breeding program, some outstanding rates to be taken into account when selecting plants with high grain yield are: high values in NGC, NRC, W5G, V5G and NL (Table 3); for stover

Table 4. Correlation coefficients greater than 0.39 between stubble weight per day (swd) and other variables of rainfed maize varieties early vegetative cycle. UAA-UAZ. 2007.

Variety	NL	LE	IDE	TL	NTPB	CL	NRC	NKR	NGC	W5G	V5G	CW	CD	SP DS
C - 5 DTV		0.64	-0.45	0.50				0.76	0.56	0.92	-0.73	-0.53	-0.47	-0.50
V-209 DTV						0.74			0.49	0.46				
VS-202 DTV			-0.49			-0.45				-0.67	-0.65			
C – 19 NDTV			0.56	-0.64	-0.40	0.50				-0.73				
DMF		ASY	GFP	DPM										
-0.43			0.52	0.55										
			-0.79	-0.40										

NL = Number of main stem leaves, ILE = Internode lenght of the ear, IDE = Internode diameter of the ear, TL = Tassel lenght, NTPB = Number of primary branches in tassel, CL = Cob lenght, NRC = Number of rows per cob, NKR = Number of kernels per row, NGC = Number of grains per cob, W5G = Weight of 50 grains, V5G = Volume of 50 grains, CW = Corncob weight, CD = Corncob diameter, SP = Silking period, DS = Days to silking, DMF = Days to male flowering, ASY = Floral asynchrony, GFP = Grain filling period, DPM = Days to physiological maturity.

Table 5. Stomatal density (per mm^2) of studied varieties early vegetative cycle.

Variety	Stomatal density		
	Upper	Underside	Sum
C – 5	74^d	123^c	197^c
VS – 202	161^a	266^a	427^a
V – 209	87^c	112^d	199^c
C – 19	108^b	168^b	276^b
DSH (0.05)	5	5	12

Values with the same letter in the same column are not statistically different ($p < 0.05$).

yield, high values: NGC, NRC and NL (Table 4).

Anatomical feature

The stomatal density did not differ in the DTV group early growing cycle from the NDTV (Table 5); but stomatal density of native DTV C-5 was significantly lower than the NDTV. It should be noted, that DTV C-5 had a lower stomatal density in the beam than the DTV V-209, but higher in the back. This coincides with Roth et al. (1986) and Bunce (2010), in the sense that stomatal density may vary from one variety to another, both in the beam and the underside.

Physiological characteristics

As in the above features, the physiological characteristics did not differ between DTV group of early growing cycle and NDTV (Table 6). The NDTV had a germination percentage of seeds with high osmotic pressures as high as the DTV C-5.

There was no difference in the recovery of seedlings subjected to Permanent Withering Point (PWP), or the Transpiration Index of seedlings; although in this last variable, NDTV had a numerically lower value. The failure to observe any difference between the DTV group of early vegetative cycle and NDTV, based on the variables evaluated in this study, suggests that studies should be done for each variety, comparing it with an already known variety which is susceptible to drought.

Semi early varieties

Agronomic and morphological characteristics

Unlike what was observed in the early growing season varieties, in the semi early vegetative cycle varieties, only in seven of the 31 quantitative variables measured was no statistical difference ($p<0.05$) between the varieties (Table 1); the range of values in these variables were: GW, 147-170 g; GWD, 1.63-1.89 mg; IDE, 18.2-19.8 mm; SP, 8.0-8.9; MFP, 11.5-12.6; NGC, 396-436; LG, 9.0-10.9 mm.

Like in the early growing season varieties, in the semi early vegetative cycle, drought tolerant varieties (DTV) did not differ as a group from non-drought tolerant variety (NDTV) in variables that showed significance ($p<0.05$) with the analysis of variance (Table 7); in all the variables there was at least a value of DTV, which is statistically equal to the NDTV. Comparing the DTV native C-7 with NDTV C-18 (Table 7), C-7 showed values higher than C-18 in characteristics: NL, NTPB, GFP, GD and CD, and minor: TL, CL and NKR. The DTV native C-23 had values greater than C-18 in: SWD, PH, NSP, ASY, CL, W5G and V5G, and minors: NRC and CW. Correlation coefficients greater than 0.39 between the measured variables and weights of grain per day (Table 8) and stubble (Table 9) showed no significant difference between the DTV group and the NDTV, except in the number of leaves and cob lenght with grain weight per day; also the cob lenght and

Table 6. Germination of seeds to different osmotic pressures (%), recovery of seedlings subjected to PWP (RSSD) (%) and rate of seedlings perspiration (mm) of studied varieties early vegetative cycle.

Variety	Osmotic pressure (atm)				RSSD	Transpiration rate
	0	5	10	15		
C – 5	100	89^{b}	60^{ab}	29^{b}	1^{a}	4.14^{ab}
VS – 202	100	83^{c}	36^{c}	2^{c}	1^{a}	4.52^{a}
V – 209	100	97^{a}	75^{a}	53^{a}	1^{a}	4.07^{ab}
C – 19	100	83^{c}	55^{b}	22^{b}	2^{a}	3.37^{c}
DSH (0.05)	5	15	16	2	0.50	

Values with the same letter in the same column are not statistically different ($p < 0.05$).

Table 7. Means Comparison of the measured variables in rain fed maize varieties, semi early vegetative cycle. UAA-UAZ. 2007.

Variety	SWD	DMF	EH	PH	NL	IDE	PL	TL	NTPB	NSP	DPM	ASY	GFP	CL
C - 7 VTS	5.69^{b}	90.5^{a}	113^{b}	237^{b}	14.6^{a}	15.3^{b}	21.4^{b}	40.0^{b}	24.3^{a}	0.21^{b}	134^{a}	1.25^{b}	43.9^{a}	16.9^{b}
C - 23 VTS	8.52^{a}	88.2^{ab}	132^{a}	258^{a}	13.2^{b}	18.4^{a}	26.6^{a}	42.6^{ab}	18.5^{b}	1.50^{a}	128^{b}	2.45^{a}	39.4^{b}	18.9^{a}
VS-201 VTS	5.81^{b}	87.0^{b}	119^{ab}	228^{b}	11.9^{c}	16.5^{ab}	23.0^{b}	37.9^{b}	21.1^{ab}	0.10^{b}	123^{c}	0.05^{c}	36.1^{b}	17.4^{ab}
C – 18 VNTS	5.13^{b}	89.8^{ab}	118^{ab}	237^{b}	12.3^{bc}	17.8^{ab}	24.2^{ab}	46.2^{a}	17.8^{b}	0.23^{b}	129^{ab}	0.35^{bc}	39.6^{b}	17.3^{ab}
DSH (0.05)	1.65	3.3	18	13	1.0	2.4	3.2	4.5	3.4	0.32	3	1.05		1.8

DC	NRC	NKR	W5G	V5G	GD	CD	CW
46.9^{ab}	13.4^{a}	30.4^{b}	21.4^{b}	13.2^{b}	1.63^{a}	29.0^{ab}	26.5^{a}
44.5^{b}	11.0^{b}	36.0^{a}	25.1^{a}	17.3^{a}	1.46^{b}	23.0^{c}	19.4^{b}
49.2^{a}	14.4^{a}	30.3^{b}	22.9^{ab}	14.8^{b}	1.53^{ab}	30.8^{a}	29.9^{a}
45.7^{ab}	13.2^{a}	32.6^{a}	21.5^{b}	14.6^{b}	1.48^{b}	26.0^{bc}	25.4^{a}
4.4	1.9	3.5	2.9	2.1	0.13	3.6	5.2

SWD = Stubble weight per day to male flowering, DMF = Days to male flowering, EH = Ear height, PH= Plant height, NL = Number of main stem leaves, IDE = Internode diameter of the ear, PL = Peduncle lenght, TL= Tassel lenght, NTPB= Number of tassel primary branches, NSP= Number of shoots per plant, DPM = Days to physiological maturity, ASY = Floral asynchrony, GFP = Grain filling period, CL = Cob lenght, DC = Diameter of cob, NRC = Number of rows per cob, NKR = Number of grains per row, W5G = Weight of 50 grains, V5G = Volume of 50 grains, GD = Grain density, CD = Corncob diameter, CW = Corncob weight. DSH = Honest significant difference. Values with the same letter in the same column are not statistically different ($p < 0.05$).

Table 8. Correlation coefficients greater than 0.39 between grain weight per day (gwd) and other variables of rain fed maize varieties Semi early vegetative cycle. UAA-UAZ. 2007.

Variety	EH	PH	NL	TL	PL	NSP	CL	NRC	NKR	NGS	W5G	CW	SP	MFP
C - 7 DTV	-0.62	-0.54		-0.54	-0.47	0.40			0.85	0.51		0.40		
V-23 DTV				-0.41	-0.47			-0.43			0.54		0.40	
VS-201 DTV					0.48				0.55	0.70	-0.52	-0.59		-0.85
C – 18 NDTV	0.59		0.47				0.79	0.58	0.69	0.68	0.58		0.41	
DS	**DMF**	**ASY**	**GFP**											
-0.57	-0.59													
		-0.52	0.61											

EH = Ear height, PH = Plant height, NL = Number of main stem leaves, TL = Tassel lenght, PL = Peduncle lenght, NSP = Number of shoots per plant, CL = Cob lenght, NRC = Number of rows per cob, NKR = Number of kernels per row, NGC = Number of grains per cob, W5G = Weigh of 50 grains, CW = Corncob weight, SP= Silking period, MFP= Male flowering period, DS = Days to silking, DMF = Days to male flowering, ASY = Floral asynchrony, GFP = Grain filling period.

days to male flowering, with stubble weight per day. Similar with the varieties of early growing season, in the semi early vegetative cycle, it is not easy to differentiate the DTV group from the NDTV, through agronomic and

Table 9. Correlation coefficients greater than 0.39 between the weight of stover per day (wsd) and other variables of rain fed maize varieties semi early vegetative cycle. UAA -UAZ. 2007.

Variety	EH	PH	ILE	PL	NTPB	NSP	CL	DC	NRC	NKR	NGC	W5G	V5G	CW
C - 7 DTV		-0.54	-0.50	-0.70	0.55	0.59						0.71	0.70	0.41
C-23 DTV	0.50		-0.54					-0.40	-0.59			0.58	0.51	
VS-201 DTV				0.62	-0.55				0.56	0.40	0.73	-0.67	-0.46	-0.59
C – 18 NDTV					-0.45		0.44							
CD	**SP**	**MFP**	**DS**	**DMF**	**ASY**	**GFP**	**GD**							
						0.40								
	-0.40				-0.74									
-0.41		0.80	-0.54			0.82	-0.43							
	0.51		0.53	0.51										

EH = Ear height, PH = Plant height, ILE = Internode lenght of the ear, PL = Peduncle lenght, NTPB = Number of primary branches of tassel, NSP = Number of shoots per plant, CL = Cob lenght, DC = Diameter of cob, NRC = Number of rows per cob, NKR= Number of kernels per row, NGC= Number of grains per cob, W5G= Weight of 50 grains, V5G= Volume of 50 grains, CW= Corncob weight, CD = Corncob diameter, SP= Silking period, MFP= Male flowering period, DS = Days to silking, DMF = Days to male flowering, ASY = Floral asynchrony, GFP = Grain filling period, GD = Grain density.

Table 10. Stomatal density (per mm^2) of studied varieties semi early vegetative cycle.

Variety	Stomatal density		
	Upper	Underside	Sum
C – 7	71^c	98^d	169^c
C – 23	101^a	153^a	254^a
VS – 201	94^b	144^b	238^b
C – 18	74^c	104^c	178^c
DSH (0.05)	5	5	12

Values with the same letter in the same column are not statistically different ($p < 0.05$).

and morphological characteristics as valued in this work. Anyway, if somebody would want to use the native variety of semi early vegetative cycle, C-7 is classified as drought tolerant (Loera, 2008). In a regional breeding program, some outstanding rates that should be taken into account when selecting plants with high grain yield are: high values of: NKR and low: EH, DMF, DS and PH (Table 8); for stover yield, high value: W5G, V5G, NSP and NTPB, also low values of PL (Table 9); for selection in the native variety C-23, for grain yield should be considered high values of W5G and low PL; for stover yield, high values of: W5G, V5G and EH, also low values: ASY, NRC and ILE.

Anatomical feature

The stomatal density did not differentiate the DTV group of semi early vegetative cycle from the NDTV (Table 10); however, the NDTV C-18 stomatal density was greater than the DTV C-7 and significantly lower than the native DTV C-23, both at the beam and back. The NDTV (C-18) had a number of stomata per square millimeter equal to the DTV C-7 in the beam and in total, and is greater than C-7 on the underside. As in the early vegetative cycle varieties, these results agree with those reported by Roth et al., (1986) and Bunce (2010), in the sense that the stomatal density may vary from one variety to another, both at the beam and back.

Physiological characteristics

Again, these features were not different between the DTV group semi early vegetative cycle and NDTV (Table 11). The NDTV had a germination rate of seeds with high osmotic pressures as high as some of the DTV; it also showed the same recovery rate of seedlings subjected to PWP and the same transpiration index of seedlings. As noted for the early growing season varieties, in the semi early vegetative cycle ones, studies must be done for each drought tolerant variety, comparing it with an already known variety which is susceptible to drought, in order to know the characteristics that differentiate them

Table 11. Germination of seeds to different osmotic pressures (%), recovery seedlings subjected to drought (RSSD) (%) and rate of transpiration (mm) of the studied varieties with semi early vegetative cycle.

Variety	Osmotic pressure (atm)				RSSD	Transpiration rate
	0	5	10	15		
C – 7	100	86[a]	50[a]	27[a]	2[a]	4.62[a]
C – 23	100	90[a]	36[a]	3[b]	1[a]	4.43[ab]
VS – 201	100	89[a]	46[a]	4[b]	1[a]	4.04[b]
C – 18	100	90[a]	38[a]	18[ab]	2[a]	4.07[b]
DSH (0.05)	5	15	16	2	0.50	

Values with the same letter in the same column are not statistically different ($p < 0.05$).

and mechanisms that allow the respective drought tolerance.

Conclusions

The drought tolerant varieties (DTV), both early vegetative cycle and semi early ones, did not differ as a group from the non drought tolerant (NDTV) used as control, in the agronomic, morphological, anatomical and physiological features assessed in this study. There were differences only when comparing the drought tolerant varieties individually from the non drought tolerant ones.

Some features that could be considered to select plants with high grain yield and stubble in the DTV native early growing season C-5 are: NGC, NRC, W5G and NL. In the variety C-7, for grain yield: high values of NKR and low values of EH, DMF, DS and PH; for stover yield, high values: W5G, V5G, NSP and NTPB; in variety C-23, for grain yield: high values of W5G, and low values of PL; for stover yield, high values of: W5G, V5G and EH, also low values of: ASY, NRC and ILE.

The DTV early C-5, showed a greater stomatal density than the NDTV C-19. The DTV semi early C-7 had a lower stomatal density than the NDTV C-18 on the underside, and also a higher transpiration rate.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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