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African Journal of Agricultural Research

Full Length Research Paper

On-farm irrigated maize production in the Somali Gu season

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Domestic production satisfies less than half of Somalia's cereal requirements. In this study, the Somali Agriculture Technical Group (SATG) evaluated different methods of nitrogen application (Broadcast, Hill, or Row) within an improved irrigated maize production system in Somalia's Lower Shebelle riverine region. This improved system consisted of the best management practices (BMPs) recommended by SATG [mineral nitrogen and phosphorus fertilizers, the pesticide Bulldock® (Beta-Cyfluthrin), and an elevated planting population]. The SATG system was also compared with a zero system, which received the same BMPs less mineral nitrogen, and a traditional farming system, which utilized local, unspecified management practices. The research was conducted on eighty-one farms located near the villages of Afgoi and Awdhegle. In the 2014 Gu season, nitrogen application method did not influence grain yields, stover yields or plant heights, but the SATG system (the Broadcast, Hill and Row treatments) was found to have greater grain yields, stover yields and plant heights than both the zero treatment and the traditional system. Significant location by treatment interactions ($p \leq 0.05$) were observed for grain yield. On farms near Afgoi, the grain yield of the improved SATG system (3,530 kg ha^{-1}) was 48% greater than that of the zero treatment and 64% greater than that of the traditional system. Near Awdhegle, these values were 56 and 73%, respectively (SATG = 5,330 kg ha^{-1}). These interactions can likely be attributed to locational differences in farm management and soil properties. Regression analyses demonstrated that when mineral nitrogen was applied, the greatest yields were found at the highest planting populations and earliest planting dates. These data demonstrate that, by utilizing the simple BMPs prescribed by SATG, Somali farmers can dramatically increase maize yields in the Lower Shebelle.

Key words: Maize, nitrogen, on-farm, plant population, planting date, Somalia.

INTRODUCTION

Somalia is one of the poorest countries on the planet. The east African nation has been plagued by civil unrest and harsh environmental conditions, which have led to a perennial state of food insecurity. In January 2017, nearly a quarter of the Somali population could not meet their daily nutritional needs (WFP, 2017). Domestic agricultural production can be a key component of food security. In Somalia, only about half of the population's cereal

Figure 1. A satellite image of the agricultural region illustrating the location of the Afgoi and Awdhegle villages along the Lower Shebelle River in southern Somalia.

requirements are satisfied by domestic production (FAO, 2012). One of the principle cereal crops in the country is maize (*Zea mays*), but Somali maize production has been highly volatile, with total production levels in 2014 nearly identical to those observed in 1980 (FAO, 2017). In order to combat food insecurity and reduce the country's reliance on imported foodstuffs, domestic agricultural production must increase dramatically.

To address this, the Somali Agriculture Technical Group (SATG, www.SATG.org) has been working to develop agricultural best management practices (BMPs) and extension programs in the country. In 2014, SATG utilized an on-farm participatory research approach to compare their recommended BMPs with the traditional farming practices employing the Lower Shebelle region (Figure 1). This region was chosen as the area of interest for the study because it is the heart of irrigated maize production in the country (FAO, 2013).

The BMPs consisted of an increased planting population, mineral fertilizer inputs, and a pesticide application and were selected by SATG because they have repeatedly proven to be important production factors in other areas of the world. For example, in much of the world, the effect of plant population on grain yield has been well established, with yields tending to exhibit a parabolic relationship with plant population (Tetio-Kagho and Gardner, 1988); however, to date, this relationship has not been examined in the Somali context, and as a result, traditional planting populations among Lower Shebelle farmers vary widely. Similarly, though increasing mineral fertilizer use in sub-Saharan Africa has been identified as an essential strategy for increasing food production in the region (Mwangi, 1996), with the effects of nitrogen fertility amendment on maize grain yield being especially well established throughout the world (Binder et al., 2000), the use of mineral fertilizers is still not common practice in the irrigated maize production systems of the Lower Shebelle (FAO, 2018). Separately and in combination, these production techniques have demonstrated important maize yield effects (Asim et al., 2013), but have not been examined in the Somali context.

As such, there were two objectives of the 2014 Gu season research trial: to compare a maize production system incorporating SATG BMPs to the traditional production system currently employed by Lower Shebelle maize farmers; and to examine whether different methods of nitrogen fertilizer application influenced maize yield and growth parameters within the SATG system. An investigation into the most effective method of nitrogen application was necessary because, while the methods of nitrogen application are many and have become increasingly sophisticated in well-developed agricultural contexts (Ma et al., 2004), farmers in developing countries have fewer options for nitrogen delivery and the economic burden of nitrogen fertilizer requires that it be applied judiciously.

MATERIALS AND METHODS

During the 2014 Gu season, a participatory on-farm research trial

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was conducted by SATG in the Lower Shebelle region of Somalia (for details on a similar trial performed in the 2014/15 Deyr season, see Gavin et al., 2018). The Lower Shebelle is the country's principal maize producing region and is characterized by alluvial soils and rainfall-driven seasonality. The area receives approximately 500 mm of rainfall annually and experiences temperatures ranging from 26 to 28°C. The Gu season, which extends from April to June, is the wettest season of the year and serves as the primary maize growing season in Somalia. Normally, farmers can expect anywhere from 200 to 300 mm of rainfall during the Gu season (Muchiri, 2007); however, it should be noted that the 2014 Gu season was especially dry, and at an SATG monitoring station near Afgoi, only 105 mm of rainfall was recorded throughout the entire season (Haji, 2017). Though the maize production systems in the Lower Shebelle are irrigated, seasonal rain failures in the Shebelle river basin can have a major effect on the water level of the Shebelle river and influence a farmer's ability to irrigate.

Soils in the Lower Shebelle region are generally classified as Haplic Vertisols (70%), Fluvisols (11%) or Calcisols (2%) (Jones et al., 2013) in the UN-FAO WRB system (Usterts, Fluvents and, Calcids in U.S. Soil Taxonomy), formed in alluvial sediments deposited over calcareous, unconsolidated and consolidated sedimentary formations (Jones et al., 2013; Gadain et al., 2016). The dominant Haplic Vertisols soil type is characterized by 2:1 clays, smectitic mineralogy, a high cation exchange capacity, and shrink-swell properties.

This trial was unique because of its size and participatory design, which maximizes community involvement and can lead to more effective research and extension results (Macaulay et al., 1999). In the 2014 Gu season, eighty-one farmers participated in the research trial, and each was associated with one of two SATG experiment stations located near the Lower Shebelle villages of Afgoi or Awdhegle (Figure 1). These farmers, forty-one near Afgoi and forty near Awdhegle, worked with SATG-trained advisors to oversee the management, harvest, and data collection of the research plots on their land.

This trial was driven by both research and extension goals and was designed as a multi-location randomized complete block (RCB) experiment in which each participating farmer represented a block nested within either the Afgoi or Awdhegle locations. Each farmer planted five treatments on their land: three SATG treatments, one zero treatment, and one traditional treatment. To achieve this, each participating farmer donated one jibaal (625 m^2) of their land to SATG. This jibaal was subdivided into four 10 m^2 plots, with each subdivision housing one of three SATG treatments or the zero treatment. The traditional treatment was evaluated on farmland adjacent to each SATG jibaal and was managed using each farmers' cultural practices.

The three SATG treatments and the zero treatment were managed using BMPs designed by SATG. These BMPs included mineral fertility inputs, an insecticide application and a relatively high planting population. Supplemental fertility was supplied using two applications of urea, once at planting and once at the V4 growth stage, at a rate of 100 kg ha^{-1} (46 kg N ha^{-1}) each, and a one-time pre-plant application of diammonium phosphate (DAP) at a rate of 200 kg ha^{-1} (36 kg N ha^{-1}, 92 kg P_2O_5 ha^{-1}). The insecticide Bulldock® (Beta-Cyfluthrin) was applied at a rate 5.0 kg ha^{-1} in order to control spotted stem borer (*Chilo partellus*), and a planting population of 53,300 plants ha^{-1}, with a between row plant spacing of 0.75 m and a within row plant spacing of 0.25 m was desired. The zero treatment followed the SATG BMPs but received no urea applications. The traditional treatment received no mineral fertilizers or insecticides and had no specified planting population. All five treatments were planted with the same locally-available, open-pollinated maize variety, "Somtux".

The method of urea application was a factor of interest in this trial, and was represented in three SATG treatments. For these treatments, urea was applied using one of three different techniques: a broadcast application, a hill application and a row application. The broadcast application was performed by evenly applying urea over the entire planting area and then incorporating the urea via a hand hoe; the hill application was performed by applying urea to a small hole that had been dug next to each individual maize plant; and the row application was performed by applying the urea to a trench that had been hand dug along the entire length of each maize row.

For data collection, two 3.0 m^2 subsamples were taken from each plot, and their data were averaged together to provide the treatment data for each farm. The main parameters of interest in this study were grain yield, stover yield, plant height and harvested plant population. Grain and stover yield data were obtained by harvesting the respective plant portions and air drying the material. Grain moisture was obtained using a handheld moisture meter and grain yield data were standardized to 15.5% moisture. Stover moisture contents could not be obtained, which prevented the standardization of stover yields to a specific moisture content. This likely contributed to the abnormally high stover yields observed in this trial. Plant height and harvested plant population measurements were determined at harvest. The precision of grain yield, stover yield and plant height measurements was limited by available technology. Plant height measurements were recorded to the nearest tenth of a centimeter, grain yield to the nearest tenth of a kilogram and stover yield to the nearest half kilogram. The lack of precision in grain and stover yield measurements likely contributed to the relatively high standard deviations observed.

Data analysis was performed using both SAS (SAS, 2016) and R (R Core Team, 2016) statistical software. The SAS software package PROC ANOVA was used to perform an Analysis of Variance (ANOVA) for determining the significance ($p \leq 0.05$) of our independent variables, and Tukey's HSD test was used for mean separation (Table 1). For the ANOVA, the data from one farmer at the Awdhegle location was randomly selected and removed in order to provide a balanced data set across both of the experimental locations. The regression analyses were performed using the entire data set in R.

Although, data on soil properties throughout Somalia and the Lower Shebelle region are limited, topsoil data was compiled [including both novel and legacy (Leenaars et al., 2014) data] from within the study area near Afgoi and Awdhegle, both on selected farms participating in this trial and within a 10 km radius of the experiment stations. This compiled dataset contained pH (1:1 soil/water), electrical conductivity (EC), cation exchange capacity and texture (sand and clay proportions) for eight locations (four at Awdhegle and four at Afgoi).

RESULTS AND DISCUSSION

Analyses of variance

Five different maize management systems were evaluated on eighty-one farms, which were associated with either the village of Afgoi or Awdhegle in the Lower Shebelle, during the 2014 Gu season. Significant treatment by location interactions were observed for grain yield, stover yield and harvested plant population (Table 1). Though these interactions were significant, the interpretation of the data from each of these locations was consistent. The treatment by location interactions that were observed for grain yield and stover yield were the result of differing effect magnitudes between treatments at each location, rather than trend inconsistencies. These differing treatment effect magnitudes were likely

Table 1. The effect of treatment and location on maize production during the 2014 Gu season on farms located near Afgoi and Awdhegle in the Lower Shebelle area of Somalia.

Treatment	Location	Grain yield (kg ha^{-1})	Stover yield (kg ha^{-1})	Harvested plant pop. (ha^{-1})	Plant height (cm)
Broadcast	Afgoi	3500	12100	46920	162
Hill	Afgoi	3640	11600	45800	163
Row	Afgoi	3460	12200	45700	164
Zero	Afgoi	2380	10900	46500	156
Traditional	Afgoi	2150	8930	37700	145
Broadcast	Awdhegle	5390	12600	51800	182
Hill	Awdhegle	5430	13000	52200	187
Row	Awdhegle	5150	12800	51400	182
Zero	Awdhegle	3410	10100	50600	176
Traditional	Awdhegle	3080	7880	32500	169
Treatment averaged across location					
Broadcast	—	4450^{a}	12400 ^{a}	49330^{a}	172 ^{a}
Hill	—	4540^{a}	12300 ^{a}	48980^{a}	175 ^{a}
Row	—	4300^{a}	12500 ^{a}	48520^{a}	173 ^{a}
Zero	—	2900^{b}	10500 ^{b}	48520^{a}	166 ^{b}
Traditional	—	2610^{b}	8400 c	35100^{b}	157 c
Location averaged across treatment					
—	Afgoi	3030^{B}	11100	44490^{B}	158 ^{B}
—	Awdhegle	4490^{A}	11300	47690^{A}	179 ^{A}
Summary statistics					
Tukey's HSD (Treatment)		370	1230	2340	5.1
Tukey's HSD (Location)		168	NS	1060	2.3
R^2		0.77	0.72	0.71	0.76
CV (%)		22.7	25.2	11.7	6.7
Treatment (P>f)		<.0001	<.0001	<.0001	<.0001
Location (P>f)		<.0001	0.8481	0.0012	<.0001
Treatment × Location (P>f)		0.0002	0.0298	<.0001	0.2506

the result of locational soil characteristic differences, which will be discussed in greater detail below, and significant locational differences in harvested plant population.

Grain Yield

No significant differences between the broadcast, hill and row treatments (henceforth referred to collectively as the SATG treatments) were observed at either location for any parameter of interest (Table 1). In Afgoi, the average grain yield of the three SATG treatments (3,530 kg ha^{-1}) was 48 and 64% greater as compared to that of the zero and traditional treatments, respectively. In Awdhegle, where the average of the SATG treatments was 5,330 kg ha^{-1}, these differences were 56 and 73%, respectively. Across all treatments, grain yield in Awdhegle (4,490 kg ha^{-1}) was 49% higher than that observed in Afgoi. Interestingly, this aligns well with anecdotal evidence suggesting that farmers near Awdhegle are more skilled than those near Afgoi. Though, these data seem to support that belief, underlying chemical and physical soil properties could be major drivers of the locational grain yield differences.

Soils data from the Lower Shebelle region are scarce, but those available demonstrate some potentially important similarities and differences between soils near Afgoi and those near Awdhegle. When examined, pH (mean = 8 ± 0.2), cation exchange capacity (mean = 38 ± 4 cmol kg^{-1}) and clay (50 ± 14%) did not differ significantly between locations (data not shown) and had values consistent with arid region Vertisols dominated by smectitic phyllosilicates (high pH, high CEC and high clay). However, EC and sand proportions did appear to differ significantly between locations. Electrical conductivity values near Afgoi were an order of magnitude higher than EC values near Awdhegle (9.1 ± 6.8 dS m^{-1} and 0.5 ± 0.2 dS m^{-1}, respectively, $p \leq 0.05$, unpaired t-test), and sand percentages were approximately 2-3 times higher near Afgoi (17 ± 6%) as compared to Awdhegle (5 ± 4%) ($p \leq 0.05$, unpaired t-test). Given the very high proportions of clay in these soils (soil textures of silty clay and clay), it is unlikely that differences in sand proportions on the order described here would be large enough to explain the observed differences in yield. However, soil EC can certainly influence crop development (Farooq et al., 2015; Shalhevet et al., 1995), and the strong locational differences in EC may explain at least some of the observed differences in crop growth and yield between the two locations.

Stover yield

Stover yields in the 2014 Gu season must be viewed with skepticism because the stover was air-dried and weight measurements were not standardized to a specific moisture content. That said, major stover yield themes appeared to closely mimic those of grain yield and can be informative. There were no meaningful differences amongst the three SATG treatments in either location, but in both locations, these treatments produced more stover than the zero and traditional treatments (Table 1). In Afgoi, the average stover yield for the three SATG treatments (12,000 kg ha^{-1}) was 10% greater than that of the zero treatment and 34% greater than that of the traditional treatment. In Awdhegle, these differences were 26 and 63%, respectively, with the average stover yield of the three SATG treatments being 12800 kg ha^{-1}. No meaningful difference in overall stover yield between the two locations was observed. This was interesting given the greater harvested plant population observed at Awdhegle, and suggests more moisture laden or poorly dried plants in Afgoi. These treatment differences are unsurprising, and likely result from the increased fertility and plant populations recommended by SATG.

Harvested plant population

Plant populations at harvest did not differ significantly between the three SATG treatments and the zero treatment at either location, though the average harvested plant population of these treatments at Awdhegle (51,500 plants ha^{-1}) was 11% greater than at Afgoi (Table 1). This significant interaction can only be explained by improper thinning. In both locations, the harvested plant population of the three SATG treatments and the zero treatment were higher than those of the traditional treatment. In Afgoi, the average harvested plant population of the SATG and zero treatments(46,200 plants ha^{-1}) was 23% greater than the traditional treatment. In Awdhegle (51,500 plants ha^{-1}), it was 58% greater. Interestingly, unlike the three SATG and zero treatments, the harvested plant population of the traditional treatment in Awdhegle (32,500 plants ha^{-1}) was 14% less than it was in Afgoi. Even though fewer plants were harvested, however, the grain yield of the traditional treatment in Awdhegle (3,080 kg ha^{-1}) was 43% greater than in Afgoi. This further strengthens the above-mentioned argument that the underlying soil characteristics near Awdhegle are more favorable than those near Afgoi and are the primary drivers of locational grain yield differences.

Plant height

No interaction between treatment and location was observed for plant heights, though significant locational and treatment differences were observed (Table 1). Plant heights at Awdhegle, which averaged 179 cm tall across all treatments, were 13% taller than they were at Afgoi. The three SATG treatments were not significantly different from each other, but on average (173 cm), these treatments were 4% taller than plants in the zero treatment and 10% taller than plants in the traditional treatment.

Regression analysis

With eighty-one different observations of each treatment, the size of this experiment also allowed for simple regression analyses to be performed on the relationships that existed between grain yield, plant height, harvested plant population and planting date. When examining these data, it is important to recognize that this study was not explicitly designed to assess these relationships, but because of the dearth of agricultural research in Somalia, it is important to glean knowledge wherever possible.

Planting date

Planting dates in the 2014 Gu season ranged from the 7th of April (day 97 of the year) to the 16th of May (day 136 of the year). A negative relationship between grain yield and planting date was observed for the average of the three SATG treatments, but no relationship was observed between grain yield and planting date for the zero and traditional treatments (Figure 2). This suggests that planting date is not a primary determinate of grain yield in the irrigated maize production systems of the Lower Shebelle during the 2014 Gu season. A more important determinate of grain yield appeared to be adequate fertility, specifically nitrogen and phosphorus

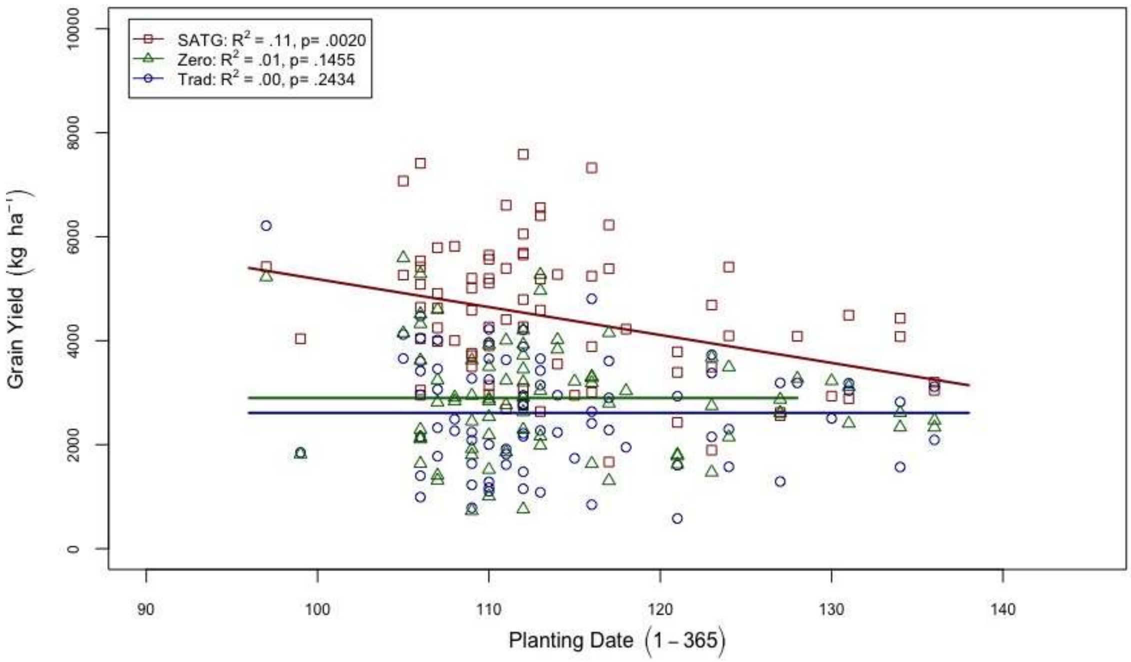

Figure 2. The relationship between planting date (1 = January 1st, 2014) and grain yield under different maize production systems in the 2014 Gu season.

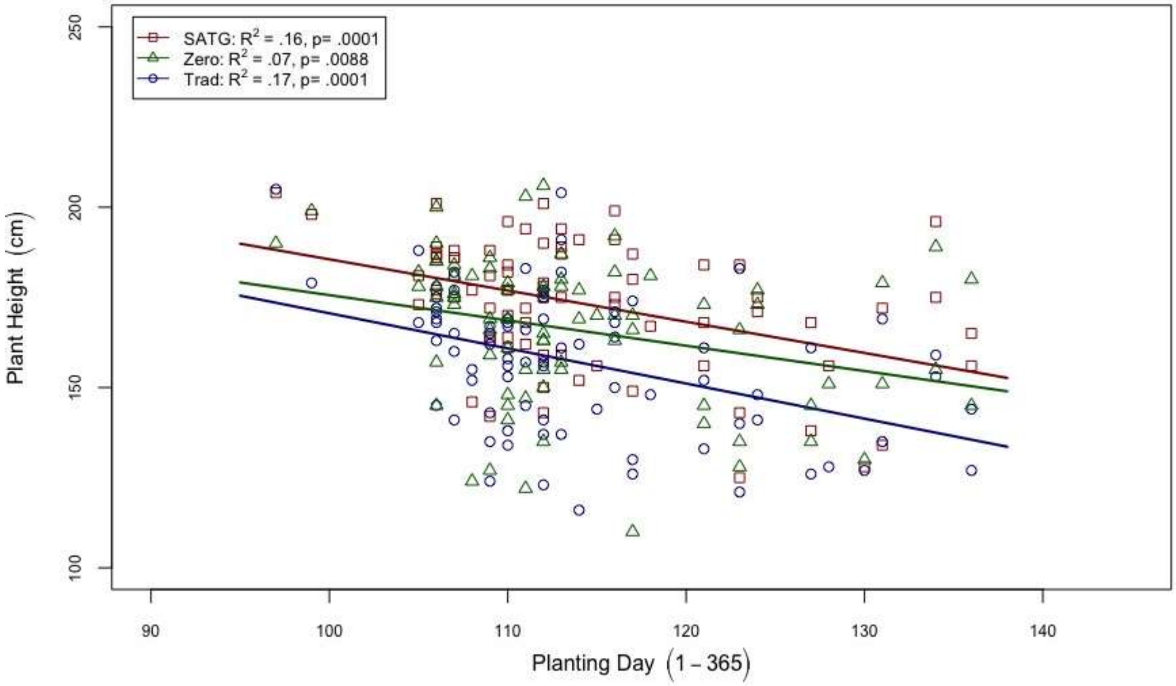

Figure 3. The relationship between planting date (1 = January 1st, 2014) and plant height under different maize production systems in the 2014 Gu season.

availability, but once this fertility was supplied, early planting was advantageous. Planting date also had an effect on plant height (Figure 3). For each production system, later planting dates were associated with shorter

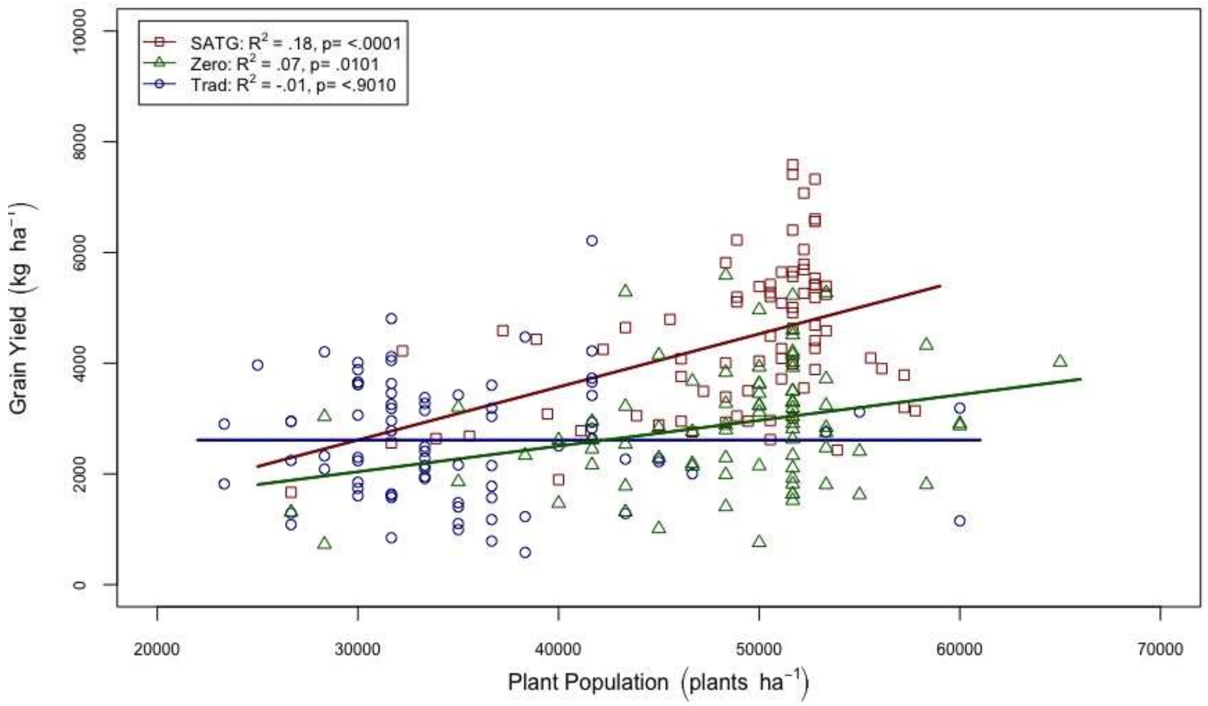

Figure 4. The relationship between harvested plant population and grain yield under different maize production systems in the 2014 Gu season.

plants.

Harvested plant population

A significant positive relationship between harvested plant population and grain yield was observed for both the average of the three SATG treatments and the zero treatment, but no significant relationship was found for the traditional treatment (Figure 4). This is interesting because it suggests that simply increasing the planting population in traditionally managed maize systems will not have an appreciable effect on grain yield in the Lower Shebelle. It also suggests that the low planting populations currently employed by farmers in the region are appropriate for their fertility limitations, and not the result of poor management strategies. When adequate fertility was supplied, however, the positive relationships exhibited by the three SATG and zero treatments suggest that increasing planting populations resulted in higher grain yields. Though the regressions of both the average of the three SATG treatments and the zero treatment were found to be significant and positive, the slope of the average of the three SATG treatments (0.096) was 108% greater than that of the zero treatment. This suggests that the addition of DAP allowed the system to capture some of the yield benefits that come with an increased plant population, but that these benefits require greater nitrogen fertility to be fully realized.

The relationship between harvested plant population and plant height mirrored that of grain yield and harvested plant population (Figure 5). No relationship was observed for the traditional treatment, where plant growth was likely limited by fertility, but positive relationships were observed for the average of the three SATG treatments and the zero treatment, with the average of the three SATG treatments exhibiting the greatest response to increased plant population. This is in line with previous research, which demonstrated a relationship between planting population and plant height (Tetio-Kagho and Gardner, 1988). When plant height and grain yield data were analyzed, a significant positive relationship was observed for the average of the three SATG treatments, the zero treatment, and the traditional treatment, indicating that taller plants yield more grain regardless of treatment (Figure 6).

Conclusion

In this on-farm study, the implementation of SATG BMPs resulted in an irrigated maize grain yield (4,428 kg ha^{-1}) that was 70% greater than that of the traditional farming system employed in the Lower Shebelle region, with larger grain yields being observed at higher harvested plant populations and at earlier planting dates. This work

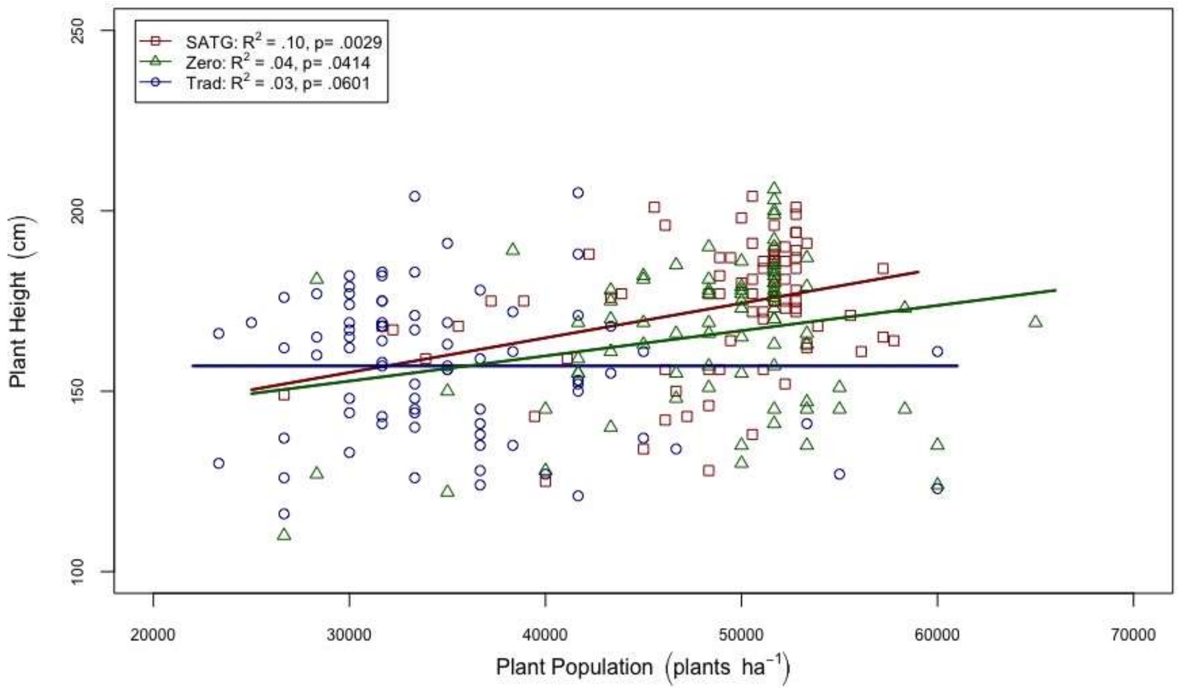

Figure 5. The relationship between harvested plant population and plant height under different maize production systems in the 2014 Gu season.

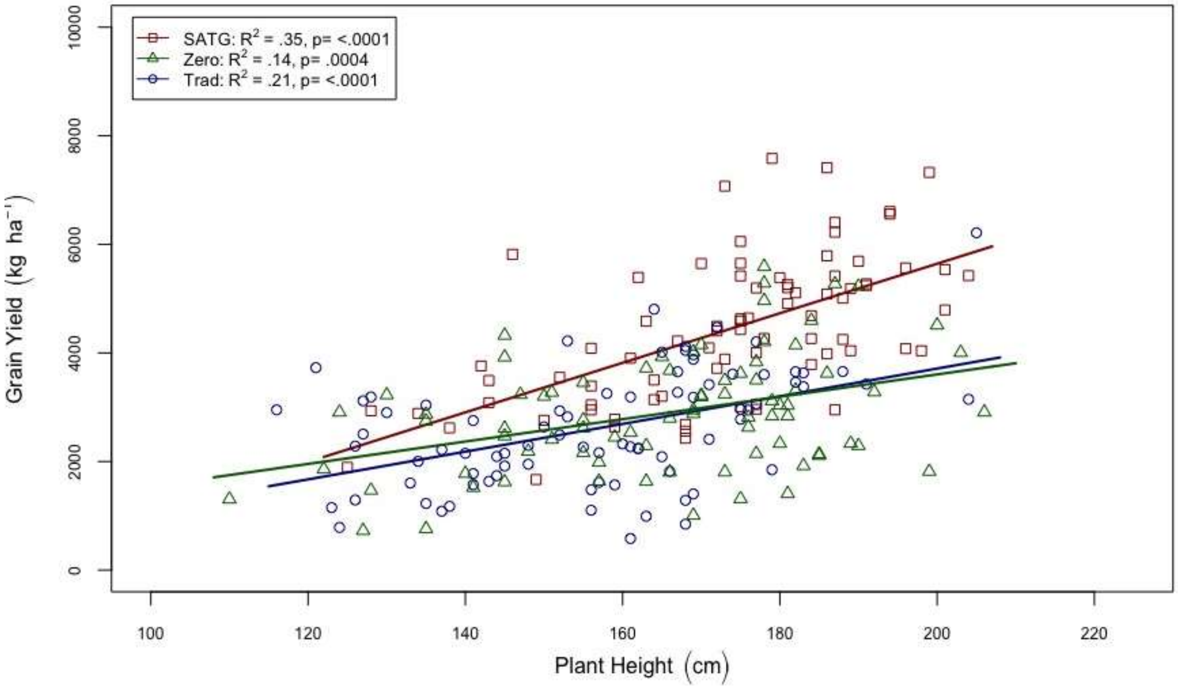

Figure 6. The relationship between plant height and grain yield under different maize production systems in the 2014 Gu season.

method of nitrogen application (broadcast, hill, or row) did not influence grain yield. Though limited in scope, this work constituted one of the first controlled agronomic research trials undertaken in Somalia in more than two decades and was unique in that the research was performed by Somalis, under the supervision of Somalis, and on the farms of Somalis. Future research should focus on better understanding the underlying soil characteristics of the region, performing a true plant population study and testing other maize varieties.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Single nucleotide polymorphism (SNP)-based genetic diversity in a set of Burkina Faso cowpea germplasm

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The potential of cowpea to address food security in Burkina Faso in particular is well established as it is a nutritious, cash and cover crop. However, there is limited information on existing germplasm diversity in Burkina Faso. This study was designed to gather some information on the genetic diversity in a set of cowpea lines introduced from different breeding programs. The diversity was therefore assessed using 181 single nucleotide polymorphism (SNP) markers on 50 cowpea lines. Leaf samples of young plants were collected using LGC genomics genotyping platform protocols for DNA extraction and genotyping. Data were then analyzed using 3 software for pair-wise distance, phylogenetic pattern by UPGMA and for the descriptive statistics determination. The phylogenetic pattern of this germplasm revealed seven clusters. The lines were almost grouped based on their geographical origin, and the breeding background. Thus, materials which originated from Burkina Faso were clustered in the same group while those from IITA/Nigeria were also almost all clustered in the same group. The genetic distance was low (≤0.29) suggesting a narrow genetic base in the cowpea germplasm used in this study. SNPs were efficient in the study of the diversity and a core collection of 20 lines was generated for further use in the breeding program.

Key words: Cowpea, single nucleotide polymorphisms (SNPs), genetic diversity, germplasm, Burkina Faso.

INTRODUCTION

Despite considerable phenotypic diversity that exists in cultivated cowpea germplasm, there is limited genetic efforts on rapid delivery of varieties with a specific range of production and quality traits. However, most of the breeding programs tend to cross and re-cross cultivars with similar yield potentials and other traits and many of variability in cowpea breeding programs (Pasquet, 1999, 2000). Breeding programs must focus most of their these cultivars are related to some degree. This leads to reduced genetic variability among cultivars that are released and among advanced breeding lines in the program, and in most cases the released varieties and the advanced lines are used as parents in new breeding cycles (Fang et al., 2007). The lack of diversity is a special concern because cowpea appears to have lower inherent genetic diversity than other cultivated crops as a result of a hypothesized single domestication event (Pasquet, 1999, 2000).

Markers based on single nucleotide polymorphisms (SNPs) have rapidly gained the center stage of molecular genetics during the recent years due to their abundance in the genomes and their amenability for high-throughput detection formats and platforms (Mammadov et al., 2012). Among these platforms is the LGC genomics' Kompetitive Allele Specific PCR (KASP) combined with the SNP line platforms in United Kingdom. SNP markers are increasingly being used for a large number of genetic studies including genetic diversities. Such studies have been reported in pea (Deulvot et al., 2010), cowpea (Huynh et al., 2013; Egbadzor et al., 2014), and cassava (Thompson, 2013). SNPs provide the simplest form of molecular markers as a single nucleotide base is the smallest unit of inheritance, and therefore, they can provide a large number of markers to be used in diversities or in marker assisted breeding. SNPs are co-dominant markers and they are most often linked to genes, and thus, they are the most attractive genetic markers in genetic studies (Jiang, 2013). The use of these markers could therefore help group germplasm which will also help breeders make informed choice of parents for breeding purposes. SNP markers therefore help in decision making when the variability within the germplasm is known.

Available breeding materials should be well known and described in any breeding program for any crop for better exploitation of the potential variability. The description of the variability among breeding materials can be done by morphological, biochemical, and molecular characterization. There exist important cowpea genetic materials in the cowpea breeding program in Burkina Faso. However, no in-depth investigation has been made to establish the variability using molecular markers. Therefore, the objective of this study was to molecularly assess the genetic diversity in the set of cowpea germplasm using SNP markers.

MATERIALS AND METHODS

Cowpea genotypes

Fifty cowpea genotypes were used for the genetic diversity study using SNP markers. The origin and seed coat color of the 50 cowpea genotypes used in the study have been described in Table 1.

SNP genotyping

Leaf samples of 2-weeks old plants were collected in a 96-wells plate and sent to LGC genomics in the United Kingdom for DNA extraction and SNP genotyping. The KASP technology as described by Thompson (2013) was used for the genotyping at LGC genomics. The DNA was extracted using LGC genomics internal protocol described. One hundred and eighty-one SNP markers selected from the Generation Challenge Programme (GCP) platform were used. After excluding the SNPs that were not informative enough (more than 10% missing data), a total of 170 markers and 47 cowpea lines were used for further analysis.

Analysis of genetic diversity

Pair-wise genetic distances between genotypes were measured with the software GGT 2.0 (Van Berloo, 2008) based on the allele-sharing method (Bowcock et al., 1994). The simple matching algorithm considers both presence and absence of markers in calculating degrees of similarity. Phylogenetic relationships dendrogram were generated based on the genetic-distance matrix using the un-weighted pair group method (UPGMA) with the software MEGA 6.0 (Tamura et al., 2013). Descriptive statistics like polymorphism information content (PIC) value, major allele frequency (MAF), and expected heterozygosity (He) were calculated for all the SNPs using PowerMarker 3.25 software (Lui and Muse, 2005). A core collection of genotypes was generated from GGT2.0 software based on the maximum diversity sum.

RESULTS

Descriptive statistics

The summary statistics for major allele frequencies (MAF), expected heterozygosity (H_e), and polymorphic information content (PIC) is presented in Table 2. A low expected heterozygosity (0.08) was observed with the SNP marker (1_0992) that has the high major allele frequency (0.96). The mean of the expected heterozygosity was 0.41 and that of the major allele frequency was 0.68. The allele frequencies of all the SNP markers were greater than their corresponding expected heterozygosity values. The allele frequencies of all the markers were below 0.95 except 1_0992 (0.96), indicating the polymorphic nature of the SNP markers used. The PIC values ranged from 0.08 (1_0992) to 0.38 with an average of 0.32. Out of the 177 SNPs, 170 were useful representing 96.04% of the total. One hundred and three SNPs were the most informative markers with a PIC value greater than the mean which represents 60.59% of the useful SNPs. Out of the 103 SNPs seven have a PIC of 0.38, 40 a PIC of 0.37, 26 a PIC of 0.36, 13 a PIC of 0.35, nine a PIC 0.34, and eight a PIC of 0.33. The seven most informative markers were 1_0126,

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Table 1. Cowpea genetic materials used for the genotyping.

S/N	Genotypes	Origin	Seed color
1	KVx404-8-1	Burkina Faso	White
2	Kaya local	Burkina Faso	White
3	KVX525	Burkina Faso	White
4	F8/SR	Burkina Faso	White
5	KVX421-2J	Burkina Faso	Brown
6	Djouroum local	Burkina Faso	White
7	KVx780-3	Burkina Faso	White
8	KVx780-6	Burkina Faso	White
9	KVX396-4-5-2D	Burkina Faso	White
10	KVX771-10	Burkina Faso	White
11	KN1	Burkina Faso	Brown
12	KVx780-1	Burkina Faso	White
13	Pobe local	Burkina Faso	White
14	KVX61-1	Burkina Faso	White
15	Moussa Local	Burkina Faso	White
16	KVX414-22-2	Burkina Faso	White
17	Donsin local	Burkina Faso	White
18	KVx780-4	Burkina Faso	White
19	BulkF7/SR	Burkina Faso	White
20	KVX775-33-2	Burkina Faso	White
21	Komsare	Burkina Faso	Cream
22	KVX30-309-6G	Burkina Faso	White
23	KVX745-11P	Burkina Faso	White
24	KVX442-3-25	Burkina Faso	White
25	Gorom Local	Burkina Faso	Brown
26	Apagbaala	Ghana	White
27	IT96D-610	IITA/Nigeria	White
28	IT95K-1479	IITA/Nigeria	White
29	IT00K-901-6	IITA/Nigeria	White
30	IT84S-2246	IITA/Nigeria	White
31	IT99K-499-39	IITA/Nigeria	White
32	IT98K-205-8	IITA/Nigeria	White
33	IT98K-317-2	IITA/Nigeria	White
34	IT95M-190	IITA/Nigeria	White
35	IT99K-573-2-1	IITA/Nigeria	White
36	IT93K-693-2	IITA/Nigeria	Brown
37	IT98K-1111-1	IITA/Nigeria	White
38	IT93K-503-1	IITA/Nigeria	White
39	IT84S-2049	IITA/Nigeria	White
40	IT97K-207-15	IITA/Nigeria	White
41	TN88-63	Niger	White
42	Bambey-21	Senegal	White
43	Mouride	Senegal	White
44	Melakh	Senegal	White
45	58-57	Senegal	White
46	UC-524B	UCR-USA	White
47	UCR-P-24	UCR-USA	White
48	CB46	UCR-USA	White
49	CB27	UCR-USA	White
50	Iron Clay	UCR-USA	White

1_0351, 1_0362, 1_0594, 1_1130, 1_1367, and 1_1393.

Core collection of cowpea germplasm

Twenty cowpea genotypes forming a core collection is presented in Table 3. This collection comprises 15 improved varieties from Burkina Faso, 3 advanced breeding lines from International Institute of Tropical Agriculture (IITA) in Ibadan – Nigeria, 1 line each from Niger and Senegal.

Phylogenetic relationships between cowpea lines

The cowpea lines were grouped into 7 clusters based on genetic distance based on the allele sharing similarity. The cluster analysis showed that lines are generally grouped together according to their geographical origin and traditional genetic background (Figure 1). Cluster VII and IV can be considered as outliers as they contained only one line (Mouride, IT86D-610). Cluster I consisted of 16 genotypes, Cluster II had 6 lines, Cluster III had 14 lines, Cluster V contains 7 lines, and Cluster VI has 2 lines. United States and Burkina Faso landraces respectively fell into Clusters II (US) and V (BF_2Loc) while the improved varieties were all in Cluster III (BF_1). The genetic materials from IITA fell into 2 main Clusters I ($IITA_1$) and VI ($IITA_3$) with slight mixture of some improved varieties from Burkina, Senegal, and Ghana.

DISCUSSION

In the present study, one hundred and seventy SNP markers were used to genotype forty-seven cowpea lines. The results showed a good level of polymorphism but a moderate level of diversity based on the average polymorphic information content values (0.32). Almost all of the 47 lines shared a very narrow genetic distance (≤0.29) which is consistent with the results reported by Li et al. (2001). Moreover, the markers enabled the grouping of lines based on their similarity. Likewise, the SNP markers were able to associate more or less the cluster to the geographical origin of the line. Breeding programs generally work within restricted pools of genetic variation (Huynh et al., 2013) and might be the cause of this narrow genetic diversity observed in this study. A number of authors have come to the conclusion that cowpea lacks significant variability (Pasquet, 1999, 2000; Fang et al., 2007). Narrow genetic base has also been observed within different lines from breeding programs (Li et al., 2001). The materials from IITA collection have been widely used by different breeding programs in different countries. This can explain the relatedness between some cowpea improved varieties from Burkina Faso (KVx745-11P, KN1, KVx780-6, and KVx61-1).

Table 2. Summary statistics of genetic variation using 170 SNP markers among 47 cowpea lines.

Marker	MAF	Avail	He	PIC
1_0126	0.50	0.94	0.50	0.38
1_0351	0.50	0.98	0.50	0.38
1_0362	0.50	0.98	0.50	0.38
1_0594	0.50	0.94	0.50	0.38
1_1130	0.50	0.94	0.50	0.38
1_1367	0.50	0.98	0.50	0.38
1_1393	0.50	0.94	0.50	0.38
1_0531	0.51	1.00	0.50	0.37
1_0605	0.51	1.00	0.50	0.37
1_0123	0.51	0.96	0.50	0.37
1_0771	0.51	0.96	0.50	0.37
1_1467	0.51	0.96	0.50	0.37
1_0183	0.52	0.98	0.50	0.37
1_1007	0.52	0.98	0.50	0.37
1_0001	0.52	0.94	0.50	0.37
1_0982	0.52	0.94	0.50	0.37
1_1141	0.52	0.94	0.50	0.37
1_0905	0.53	1.00	0.50	0.37
1_0604	0.53	0.96	0.50	0.37
1_0425	0.54	0.98	0.50	0.37
1_0565	0.54	0.98	0.50	0.37
1_1072	0.54	0.98	0.50	0.37
1_0081	0.55	0.94	0.50	0.37
1_0146	0.55	0.94	0.50	0.37
1_0153	0.55	0.94	0.50	0.37
1_0056	0.55	1.00	0.49	0.37
1_1103	0.56	0.96	0.49	0.37
1_0058	0.57	0.98	0.49	0.37
1_0062	0.57	0.98	0.49	0.37
1_0525	0.57	0.98	0.49	0.37
1_0690	0.57	0.98	0.49	0.37
1_1021	0.57	0.94	0.49	0.37
1_1371	0.57	1.00	0.49	0.37
1_0136	0.58	0.96	0.49	0.37
1_0923	0.58	0.96	0.49	0.37
1_0993	0.58	0.96	0.49	0.37
1_1038	0.58	0.96	0.49	0.37
1_0259	0.59	0.98	0.48	0.37
1_1117	0.59	0.98	0.48	0.37
1_1189	0.59	0.98	0.48	0.37
1_0987	0.59	0.94	0.48	0.37
1_0127	0.60	1.00	0.48	0.37
1_0388	0.60	1.00	0.48	0.37
1_0449	0.60	1.00	0.48	0.37
1_0401	0.60	0.96	0.48	0.36
1_0752	0.60	0.96	0.48	0.36
1_0806	0.60	0.96	0.48	0.36
1_1135	0.60	0.96	0.48	0.36
1_0052	0.61	0.98	0.48	0.36
1_0377	0.61	0.98	0.48	0.36

Table 2. Contd.

1_0397	0.61	0.98	0.48	0.36
1_0657	0.61	0.98	0.48	0.36
1_0670	0.61	0.98	0.48	0.36
1_0437	0.61	0.94	0.47	0.36
1_1360	0.61	0.94	0.47	0.36
1_0025	0.62	1.00	0.47	0.36
1_0945	0.62	1.00	0.47	0.36
1_1512	0.62	1.00	0.47	0.36
1_0917	0.62	0.96	0.47	0.36
1_0567	0.63	0.98	0.47	0.36
1_0652	0.63	0.98	0.47	0.36
1_0706	0.63	0.98	0.47	0.36
1_1214	0.57	0.98	0.49	0.37
1_1246	0.57	0.98	0.49	0.37
1_1431	0.57	0.98	0.49	0.37
1_1129	0.63	0.98	0.47	0.36
1_1370	0.63	0.98	0.47	0.36
1_0256	0.64	0.94	0.46	0.36
1_0319	0.64	0.94	0.46	0.36
1_1151	0.64	0.94	0.46	0.36
1_0699	0.64	0.96	0.46	0.35
1_0290	0.65	0.98	0.45	0.35
1_0823	0.65	0.98	0.45	0.35
1_0246	0.66	0.94	0.45	0.35
1_0317	0.66	0.94	0.45	0.35
1_0757	0.66	0.94	0.45	0.35
1_0482	0.66	1.00	0.45	0.35
1_0730	0.66	1.00	0.45	0.35
1_1271	0.66	1.00	0.45	0.35
1_0033	0.67	0.96	0.44	0.35
1_0065	0.67	0.96	0.44	0.35
1_0306	0.67	0.96	0.44	0.35
1_0649	0.67	0.96	0.44	0.35
1_0438	0.67	0.98	0.44	0.34
1_0473	0.67	0.98	0.44	0.34
1_0834	0.67	0.98	0.44	0.34
1_1037	0.67	0.98	0.44	0.34
1_1042	0.67	0.98	0.44	0.34
1_1062	0.67	0.98	0.44	0.34
1_1520	0.68	1.00	0.43	0.34
1_0322	0.68	0.94	0.43	0.34
1_0911	0.69	0.96	0.43	0.34
1_0111	0.70	0.98	0.42	0.33
1_0157	0.70	0.98	0.42	0.33
1_0370	0.70	0.98	0.42	0.33
1_0937	0.63	0.98	0.47	0.36
1_0977	0.63	0.98	0.47	0.36
1_1096	0.63	0.98	0.47	0.36
1_0022	0.70	0.91	0.42	0.33
1_0746	0.70	0.91	0.42	0.33
1_0807	0.70	1.00	0.42	0.33
1_0647	0.71	0.96	0.41	0.33

Table 2. Contd.

1_0709	0.71	0.96	0.41	0.33
1_0392	0.72	0.98	0.41	0.32
1_0755	0.72	0.98	0.41	0.32
1_0853	0.72	0.98	0.41	0.32
1_0242	0.72	1.00	0.40	0.32
1_0957	0.72	1.00	0.40	0.32
1_0142	0.73	0.96	0.39	0.31
1_0775	0.73	0.96	0.39	0.31
1_0983	0.73	0.96	0.39	0.31
1_0107	0.74	0.98	0.39	0.31
1_0330	0.74	0.98	0.39	0.31
1_0529	0.74	0.98	0.39	0.31
1_0679	0.74	0.98	0.39	0.31
1_1281	0.74	0.98	0.39	0.31
1_0060	0.74	1.00	0.38	0.31
1_0238	0.76	0.96	0.37	0.30
1_0451	0.76	0.96	0.37	0.30
1_0583	0.76	0.96	0.37	0.30
1_0053	0.76	0.98	0.36	0.30
1_0323	0.76	0.98	0.36	0.30
1_0740	0.76	0.98	0.36	0.30
1_0876	0.76	0.98	0.36	0.30
1_1087	0.76	0.98	0.36	0.30
1_1170	0.76	0.98	0.36	0.30
1_0128	0.77	1.00	0.36	0.29
1_0663	0.77	1.00	0.36	0.29
1_0082	0.77	0.91	0.36	0.29
1_0105	0.77	0.94	0.35	0.29
1_1333	0.77	0.94	0.35	0.29
1_0171	0.78	0.96	0.35	0.29
1_1073	0.78	0.96	0.35	0.29
1_1157	0.78	0.96	0.35	0.29
1_0139	0.78	0.98	0.34	0.28
1_0510	0.78	0.98	0.34	0.28
1_0718	0.78	0.98	0.34	0.28
1_0889	0.78	0.98	0.34	0.28
1_1255	0.78	0.98	0.34	0.28
1_0514	0.79	1.00	0.33	0.28
1_1517	0.80	0.96	0.32	0.27
1_0773	0.80	0.98	0.31	0.27
1_0801	0.80	0.98	0.31	0.27
1_1121	0.80	0.98	0.31	0.27
1_0280	0.81	1.00	0.31	0.26
1_0691	0.81	0.91	0.30	0.26
1_0014	0.83	0.98	0.29	0.25
1_0436	0.83	0.98	0.29	0.25
1_0519	0.83	0.98	0.29	0.25
1_0625	0.83	0.98	0.29	0.25
1_0866	0.83	0.98	0.29	0.25
1_1092	0.83	0.98	0.29	0.25
1_0074	0.83	1.00	0.28	0.24
1_0262	0.83	1.00	0.28	0.24

Table 2. Contd.

1_1039	0.84	0.91	0.27	0.24
1_0067	0.85	0.98	0.26	0.22
1_0703	0.85	0.98	0.26	0.22
1_0878	0.85	0.98	0.26	0.22
1_0432	0.87	0.96	0.23	0.20
1_0420	0.87	0.98	0.23	0.20
1_0588	0.87	0.98	0.23	0.20
1_0754	0.87	1.00	0.22	0.20
1_1492	0.87	1.00	0.22	0.20
1_0732	0.88	0.91	0.21	0.18
1_0678	0.89	0.98	0.19	0.17
1_1249	0.91	0.96	0.16	0.15
1_0421	0.91	0.98	0.16	0.15
1_0539	0.91	0.98	0.16	0.15
1_1217	0.93	0.98	0.12	0.11
1_0992	0.96	0.98	0.08	0.08
Mean	**0.68**	**0.97**	**0.41**	**0.32**

MAF: major allele frequency; Avail: allele availability; He: Expected Heterozygosity; PIC: polymorphic information content.

Table 3. Core collection of cowpea germplasm.

Genotypes	Origin
MOURIDE	Senegal
KVX525	Burkina Faso
KVX396-4-5-2D	Burkina Faso
KVX780-3	Burkina Faso
KVX780-4	Burkina Faso
IRON CLAY	IITA/Nigeria
KVX30-309-6G	Burkina Faso
KVX61-1	Burkina Faso
TN88-63	Niger
KVX404-8-1	Burkina Faso
KVX780-6	Burkina Faso
IT98K-317-2	IITA/Nigeria
F8_SR	Burkina Faso
BULKF7_SR	Burkina Faso
KVX771-10	Burkina Faso
KVX775-33-2	Burkina Faso
KVX421-2J	Burkina Faso
KOMSARE	Burkina Faso
IT99K-499-39	IITA/Nigeria
KVX414-22-2	Burkina Faso

Looking at also the pedigree of Melakh (IS86-292 x IT83S-742-13) (Diouf and Hilu, 2005), it becomes easy to understand why this line fell into the cluster of IITA lines because of its relatedness among line from the IITA breeding program. Huynh et al. (2013) provided some useful assumptions that tend to explain the reduction of the genetic distance among cowpea wild types, landraces, and improved germplasm within African germplasm accessions and among African and Non-African germplasm accessions. These authors concluded that the small genetic differentiation observed between the African and non-African collections indicated that the entire genetic diversity in the African germplasm might already have spread over cowpea-growing regions in the world as a whole although not completely within any single region. Nevertheless, the clustering of these 47 lines into 7 distinct groups gives important insights that can improve the efficiency of germplasm used in cowpea for breeding purposes. With the exception of the materials from Senegal (Bambey in Cluster II, Mouride in Cluster VII, 58-57 in Cluster III, and Melakh in Cluster I), from Niger (TN88-63 in Cluster III), and from Ghana (Apaagbala in Cluster I) that were not grouped according to their geographical origin, the rest were clustered based on their country of origin. That could be helpful for new ways of genetic improvement of cowpea by exchanging material from different countries to broaden the genetic base of the crop. In contrast with these findings, a numbers of genetic diversity studies conducted on cowpea have reported absence of correlation between geographical origin of the accessions and their clustering pattern (Asare et al., 2010; Egbadzor et al., 2014). This was also observed in a genetic diversity study in maize using SSR markers (Oppong, 2013). In this study, the genotypes were clustering following a regional basis of maize cultivation in Ghana. The differences shown between landraces and the improved varieties from

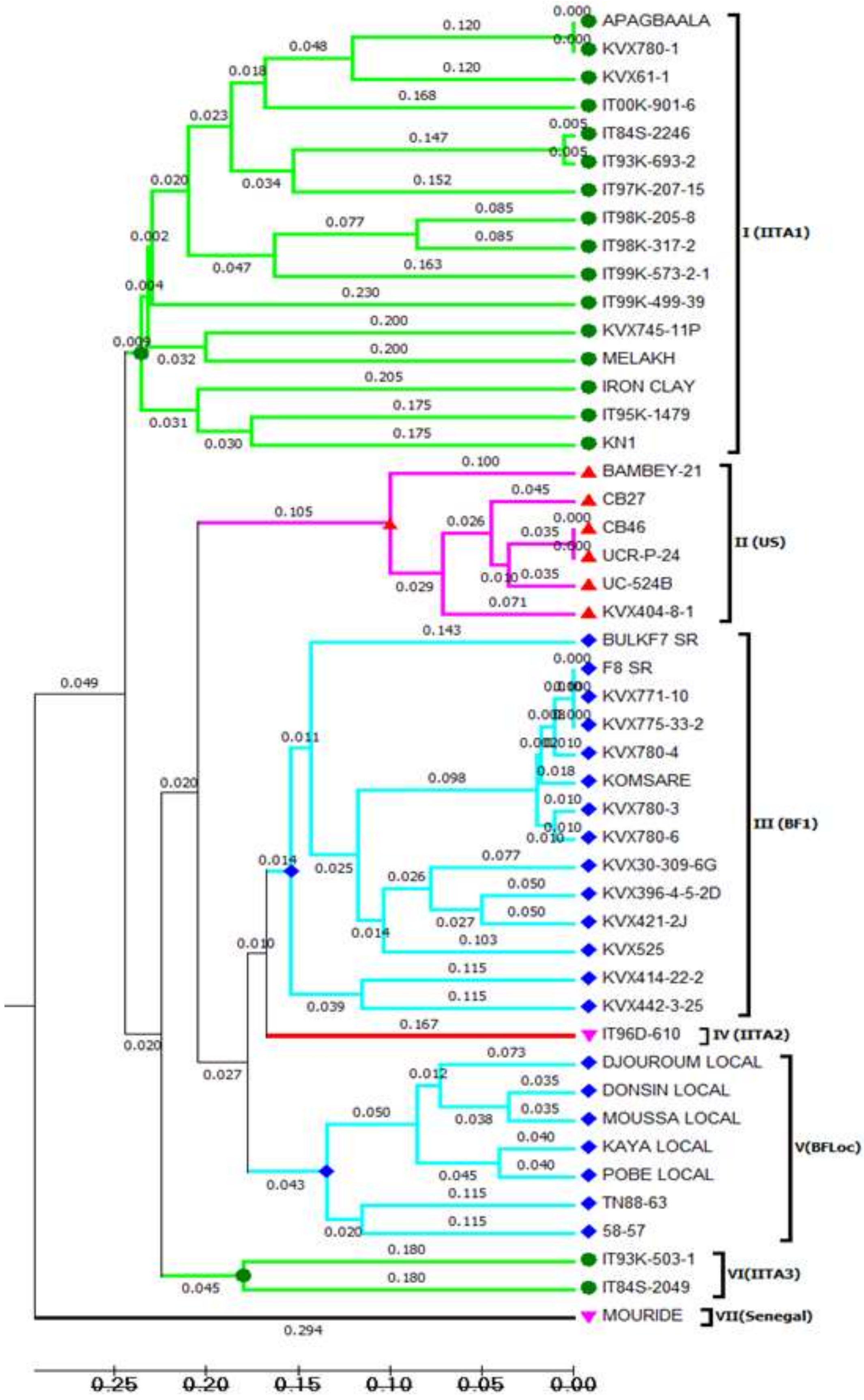

Figure 1. UPGMA dendrogram of 47 cowpea genotypes constructed using 170 SNP markers.

Burkina Faso may also be useful as a little diversity still exists among the local germplasm for new variety development. SNP markers have demonstrated their capacity in assessing genetic diversity in cowpea (Huynh et al., 2013; Egbadzor et al., 2014). Varshney et al. (2007) reported on the robustness of SNP markers. As compared to SSR markers, SNPs are more robust as they are able to detect slight changes in the genome and discriminate genotypes. This assumption is confirmed by the findings from a genetic diversity study on sweet cherry (*Prunus avium* L.) (Marti et al., 2012). In this study, SNP markers were able to discriminate mutants from their original parents than SSR markers. In addition, SNP markers confirmed parentage and also determined relationships of the accessions in a manner consistent with their pedigree relationships. The latter statement confirmed our findings. Lines like Melakh from Senegal, KVx745-11P from Burkina Faso was grouped with the IITA accessions because of the large contribution in their genome of materials from IITA.

Extension of gene pool is important for crop improvement (Varshney et al., 2007). As such a core collection of 20 lines was proposed from this study based on the maximum diversity among them. Several genetic diversity studies have been conducted in cowpea (Pamella and Gepts, 1992; Vaillancourt and Weeden, 1992; Fotso et al., 1994; Coulibaly et al., 2002; Ba et al., 2004). Despite of the presence of little diversity within the collection used for this study and the core collection, the separation of the broader germplasm of cowpea landraces into gene pools as done by Huynh et al. (2013) could be useful for expanding the genetic diversity within breeding materials and could lead to development of more efficient strategies and genetic gain within future breeding programs.

Conclusion

The present study was undertaken to determine the genetic variability in a set of germplasm used by INERA Cowpea Breeding Program in Burkina Faso using SNP markers. The germplasm used has some moderate variability with narrow genetic base. These results were comparable to previous studies that have also reported the narrow genetic base of cowpea.

The phylogenetic patterns and clustering of relatively similar individuals into groups provide important information on the germplasm used for cowpea improvement. The materials were grouped based on the geographic origin and the genotypic background. Materials from United State/University of California Riverside clustered together. Likewise, materials from IITA/Nigeria, Burkina Faso clustered in country base.

SNP markers were able to group the genotypes in a way that they could be used to link the genotype clusters and their pedigree. A panel of 20 genotypes representing the maximum variability of the germplasm used in the study was generated based on the maximum diversity sum. This panel constituted a collection that could be together with the information on the clustering of great importance for further plant breeding to develop superior varieties of cowpea.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Productivity of Irish potato varieties under increasing nitrogen fertilizer application rates in Eastern Rwanda

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Irish potatoes are an important crop only promoted in cool and high moist conditions of Rwanda. This study explored the productivity of Irish potatoes varieties under increasing nitrogen fertilizer applications in drier agro-climatic conditions of the eastern Rwanda. Potato seeds mass-selected from locally-grown varieties in the region surrounding Kibungo town (-2.160897°, 30.543591°) were planted under rain-fed conditions, during the March to June agricultural season of 2015, in the three experimental farms of the University of Kibungo at Karenge, Mugesera and Rwamagana, respectively located in Kibungo town, 30 km West and 50 km North of Kibungo town. The most performing three varieties were tested again in 2016A season (October to January, 2016) at Kibungo and Rwamagana. For each season, and at each farm, four nitrogen application rates (0, 60, 120, and 180 kg N ha^{-1}) were tested. Phosphate and potash were supplied in sufficient amounts of 150 kg P_2O_5 ha^{-1} and 60 kg K_20 ha^{-1}, respectively. No fertilizers were applied on the control treatment. A split plot design and three replicates were used with varieties in main plots and nitrogen in sub-plots. Plant growth rate, shoot counts, tuber calibration, and total and market potato tuber yields were monitored. Four varieties yielded 10 tons ha^{-1} or more of total potato tuber yields in 2015B season. Three of them, namely Kirundo, Gasore, and Peko varieties, were re-tested in 2016. Over the two seasons, Kirundo variety stood out with 12.8 and 10.5 tons ha^{-1} of total and marketable tuber yields, respectively. All the varieties significantly responded to nitrogen fertilizer. However, Kirundo variety, respectively yielded 22 tons and 17 tons ha^{-1} of total and market potato tuber yields under 120 kg N ha^{-1} during the 2016A season. Irish potato can therefore be grown and produce substantial yield in eastern Rwanda, provided that appropriate nitrogen fertilization and seed quality are available.

Key words: Irish potatoes, varieties, nitrogen, yields, fertilizer, rates.

INTRODUCTION

The Crop Intensification Program (CIP), initiated in September, 2007 across Rwanda, has focused on six priority crops namely Maize, Rice, Banana, Beans, Cassava, and Coffee in the Eastern Province (MINAGRI,

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2013). The selection of these crops was based on the agro-ecological zones established in the mid-seventies (Delepierre, 1974) and continuously referred to (Verdoodt and van Ranst, 2003; MINAGRI, 2010) with no update with regard to climate change impact and adaptation strategies. Consequently, under CIP, Irish potatoes have been promoted in northern and western regions of Rwanda while the eastern region has been considered marginal for this crop (REMA, 2009). In that regard, it was assumed that Irish potatoes required cooler and higher moisture conditions to achieve optimum yields. In terms of drought susceptibility, the risks increase from West to East of the province. This drought susceptibility also varies with the season (MIDMAR, 2014), from very low to moderate in Season A (October to January) and moderate to high in Season B (March to June). Despite these conditions, at community level, Irish potatoes are grown in eastern Province in both seasons. However, with no financial support of Government programs, yields have remained very low, from 3.5 to 6.5 tons ha^{-1}, compared to 12 to 15 tons ha^{-1} for the regions under the Government assistance (MINAGRI, 2014).

Scattered research reports have shown that the applications of 30 tons of manure and 50 kg ha^{-1} of each of N, P_2O_5 and K_2O actually recommended on Irish potatoes did not result in optimum tuber yields for Cruzza potato variety grown in two suitable agro-ecological zones (Turamyenyirijuru, 2013). In fact, harvested total potato tuber yields varied from 13.8 tons ha^{-1} in Nyaruguru, southwest Rwanda, to 17 tons ha^{-1} in Kinigi, northern Rwanda. On the contrary, nitrogen application rate in the amount of 140 kg N ha^{-1} on Kinigi potato variety resulted in yields varying from 31 to 37.7 tons ha^{-1} of tubers (Nyiransabimana, 2011) in Busogo (northern Rwanda) while more than 42 tons ha^{-1} were harvested from the application of 150 kg N ha^{-1} on the same Kinigi potato variety in Kinigi area, same region (Fashaho et al., 2013). Therefore, potato yields remain dependent on the weather conditions, even when it is grown in recommended regions.

Otherwise, previous findings on potatoes crop have pointed out that nitrogen management was an important challenge, economically and environmentally (Zebarth et al., 2007; Karemangingo et al., 2007). Several studies indicated that, depending on the varieties, potatoes usually required more than 100 kg N ha^{-1} to yield 30 tons ha^{-1} or more of tubers under rain-fed conditions (Zebarth et al., 2007; Barascu et al., 2015; Getie et al., 2015). It appeared therefore that a better control of nitrogen fertilization of potatoes was needed before any conclusion can be made on the potential of the eastern Rwanda for Irish potatoes production. This study therefore aimed at evaluating the yield potential of the most commonly grown Irish potatoes varieties in eastern Rwanda under increasing nitrogen fertilizer application rates with the view of promoting the production of Irish potatoes in this region.

MATERIALS AND METHODS

Site characteristics

This study was conducted in the Eastern Province of Rwanda for two agricultural seasons: from March to June, 2015 (or 2015B season) and from October to January, 2016 (or 2016A season). It was undertaken on three experimental farms of the University of Kibungo: at Kibungo (-2.160897°, 30.543591°), near the main Campus of the University (1680m asl), at the Mugesera Lake shore (1350m asl), about 30 km West of Kibungo, and at Rwamagana town (1528m asl), 50 km North of Kibungo and 40 km East of the Kigali City. The rainfall pattern of the study area follows a bimodal type with the average precipitation amount of 986.7 mm per annum, a major peak in April (B Season) and a small one in October – November (A Season). The mean minimum, maximum and average temperatures are 13.5, 27.2 and 19.5°C in Kibungo (www.weatherspark.com/kibungo) and 13.8, 27.8, 22.6 in Rwamagana, (www.weatherspark.com/rwamagana), respectively. No such information is available for the Mugesera farm; but the conditions are very similar. The soils of the region are mainly Ferralsols (Oxisols) depleted in clay and organic matter as a result of continuous cultivation and water erosion (Nzeyimana et al., 2014). The Karenge experimental farm is located on a 5% slope loam soil, the Mugesera site on a 5% slope sandy loam soil, and the Rwamagana site is on a 2% slope sandy-clay loam soil.

Treatments and experimental design

Genetic materials

Healthy potato plants were selected and harvested from different farmers' fields of the region in the 2015A season. The harvested potato varieties included Gasore, Kruzza and Mabondo varieties for Kibungo site, Kruzza, Makara, and Peko for the Mugesera site, and Gasore and Mabondo for Rwamagana site. A third variety for this Rwamagana site was the Kirundo potato variety graciously supplied by Rwanda Agricultural Board (RAB) from their Office of Musanze (North Province). Harvested plants could not supply sufficient potato seeds for all sites. Therefore, although six varieties were harvested and tested, only three varieties were tested at each site. No fungicide treatment was applied on the seeds. The study was continued in the 2016A season with the best performing potato varieties from 2015.

Nitrogen fertilization

Four nitrogen fertilizer application rates were supplied from 0 to 180 kg N ha^{-1} with a 60 kg N increment from a blend (17-17-17) and urea (46-0-0). Phosphorus and potassium were applied from the same blend (17-17-17) and triple superphosphate (0-46-0) to supply 150 kg P_2O_5 ha^{-1} and 60 kg K_2O ha^{-1} over all the experimental units, but the control. These amounts of N, P_2O_5 and K_2O were applied on all the potato varieties at each site.

Experimental design

A split plot design and three replicates were used. The potato varieties were tested in the main plots while the effects of nitrogen fertilizer application rates were tested in the sub-plots. Each sub-plot was 3.6 m wide × 4.0 m long; the plant spacing was 90 cm between rows and 40 cm within each row. Fertilizers were manually band-applied in the trench below the seeds and slightly covered by the soil before planting. Hilling and weed control operations were also manually done. Dithane M 45 was applied a couple of times in

2015 to control fungi development on leaves at Kibungo and Rwamagana.

Evaluation of the effects of treatments

Data collection was done from the two central rows of each plot for growth parameters. Collected data included plant emergence counts, plant growth rate through measuring plant height at different dates after planting, number of plants and shoots at harvest. For yield evaluation, the whole plot was considered for tuber calibration as follows: small size tubers (lower than 10cm of circumference), medium size tubers (from 10 cm to 15 cm of circumference), big size tubers (above 15 cm of circumference), tuber quality (rough and rot tubers, hollow heart tubers), and total and market potato tuber yields. Statistical analyses were performed using NCSS software package (Hintze, 2004) and mean yields compared using Duncan's multiple range test (DMRT). All statistical analyses were performed site by site and by season/year. The test signification was considered at 5% probability level.

RESULTS AND DISCUSSION

Comparative performance of different potato varieties

During the 2016 season, Kirundo and Peko varieties had no significant difference in sprout rates (respectively 94.2 and 89.2%) 30 days after planting, and no significant difference in growth rate, 60 days after planting (respectively 53.4 and 59.7cm plant height). All the potatoes varieties had equal shoot numbers with an average of 4.3 shoots by planted tuber as monitored at harvest. No significantly different effects were detected with regard to nitrogen fertilizer application rates on the sprout and growth rates as monitored 30 and 60 days after planting. With regard to the tuber numbers by size grade, the results on the yield performance of potato varieties over the two seasons (2015B and 2016A) indicated significant differences between the three varieties tested in Kibungo in 2015 ($P < 0.05$) with regard to the size of the tubers. Although the number of small size tubers was high for Kruzza, this variety stood out with 173,800 market potato tubers against 127,600 and 112,000 tubers for Gasore and Mabondo, respectively. At Rwamagana, Kirundo and Mabondo varieties yielded equal market potato tuber numbers (154,700 and 131,800 tubers, respectively), but significantly higher ($P < 0.01$) than Gasore potato variety (77,600 tubers). At Mugesera, all the three varieties yielded equal numbers of tubers for each tuber size grade. In 2016, Kirundo potato variety yielded as equal numbers of market potato tubers as Peko; the two varieties yielded twice and thrice as much as Gasore (58,700 tubers), respectively.

The results related to the yields are presented in Table 1 by grade, total and market tuber by site. Kirundo potato variety at Rwamagana and Peko variety at Mugesera significantly yielded much higher than the two other varieties tested at the same time in each site in 2015. All varieties that yielded 10 tons ha^{-1} of market potato tubers in 2015 were selected for subsequent tests in 2016 season. The results obtained in 2016 confirmed the higher performance of Kirundo variety in comparison to the other varieties in the two sites, Kibungo and Rwamagana. It yielded more than 10 tons/ha of total and/or market potato tubers, particularly due to medium and big size potato tuber yields. Peko variety reasonably sustained a high yield although lower than Kirundo. It is important to note that locally-selected varieties could have lost their production potential over the years and this could explain their lower yield performance when compared to Kirundo potato variety.

Overall, however, all the varieties yielded much lower than their potential (above 30 tons/ha) (MINAGRI, 2010) under rain-fed conditions (Getie et al., 2015; Fashaho et al., 2013). They are however in the range of national average yields of 10.0 to 12.5 tons potato tubers ha^{-1} (MINAGRI, 2011; RAB, 2014). Most importantly, compared to promoted crops for Eastern Province such as rice and maize, Irish potatoes appear potentially very competitive for both yields (NISR, 2016) and profitability for the growers (GoR, 2013).

Comparative effects of nitrogen fertilizer application rates on potato yields and yield components

With regard to the plant potato sprout rates, no significant differences were observed between the different rates of nitrogen fertilizer. Significant differences detected with regard to plant growth rate as measured by the plant height 30 and 60 days after planting only indicated higher growth for all nitrogen application rates than the control, regardless of the season /year.

With regard to the number of tubers per grade, the numbers of medium and big tubers were always higher with higher application rates of nitrogen fertilizer, regardless of the season/year. Total tuber numbers were significantly equal from 120 kg N ha^{-1} (228,200 tubers) and 180 kg N ha^{-1} (232,100 tubers) but higher than from 60 kg N ha^{-1} (181,700 tubers) and the control (135,500 tubers) in 2015 at Kibungo site. Similar trends were observed for the other sites. The same was also true for the numbers of market potato tubers. The impact of bad weather conditions in 2016 explains the increase of small and rough tubers and the decrease of the numbers of market tubers comparatively to total tuber numbers. In this respect, at Kibungo, the numbers of market potato tubers represented 51.7 and 47.9% of total potato tuber numbers under 120 kg and 180 kg N ha^{-1}, respectively. At Rwamagana, the market potato tuber numbers represented 68.4 and 59.9% of total potato tuber numbers under similar N application rates, respectively. In the two sites, the N application rate in the amount of 120 kg ha^{-1} constantly yielded higher or equal tuber numbers than 180 kg N ha^{-1}. This latter and 60 kg N ha^{-1} yielded significantly equal numbers of tubers.

Table 1. Yield performance of different potato varieties by grade, total and market tubers by site and by season × year.

Season	Farm /Site	Potato variety	Small tuber (Tons/ha)	Medium size (Tons/ha)	Big size tuber (Tons/ha)	Rot tubers (Tons /ha)	Total yields (Tons/ha)	Market yields (Tons /ha)
2015B	Kibungo	Gasore	0.54^{a}	3.9^{a}	8.1^{a}	0.36^{a}	10.6^{a}	9.8^{a}
		Kruzza	1.00^{b}	4.8^{a}	5.7^{a}	0.06^{a}	11.7^{a}	10.6^{a}
		Mabondo	0.43^{a}	4.1^{a}	2.3^{b}	0.12^{a}	6.5^{a}	5.9^{a}
	Rwamagana	Gasore	0.74^{ab}	2.0^{a}	1.1^{a}	0.46^{a}	4.3^{a}	3.1^{a}
		Kirundo	0.11^{a}	3.5^{b}	7.0^{b}	1.04^{a}	11.6^{b}	10.5^{b}
		Mabondo	1.2^{b}	4.2^{c}	1.0^{a}	0.72^{a}	7.0^{a}	5.2^{a}
	Mugesera	Kruzza	1.7^{a}	1.9^{a}	2.0^{a}	-	5.6^{a}	3.9^{a}
		Makara	1.9^{a}	2.0^{a}	1.7^{a}	-	5.5^{a}	3.6^{a}
		Peko	2.9^{b}	4.6^{b}	2.8^{b}	-	10.5^{b}	8.5^{b}
2016A	Kibungo	Gasore	2.3^{a}	1.9^{a}	1.7^{a}	0.5^{a}	6.4^{a}	3.6^{a}
		Kirundo	2.0^{a}	4.2^{b}	6.5^{b}	1.5^{b}	14.1^{b}	10.6^{b}
		Peko	1.9^{a}	3.3^{b}	4.7^{c}	0.8^{a}	10.6^{c}	8.0^{c}
	Rwamagana	Kirundo	1.6^{a}	4.0^{a}	5.4^{a}	-	11.0^{a}	9.4^{a}
		Mabondo	1.9^{a}	4.1^{a}	3.0^{b}	-	9.0^{b}	7.1^{b}
		Peko	1.1^{b}	3.0^{a}	2.9^{b}	-	7.0^{c}	5.9^{b}

Yield levels suffixed with different letters are significantly different by site and season-year.

Table 2. Mean yield responses of different potato varieties to increasing nitrogen fertilizer application rates in 2015.

Farm	Nitrogen application rates (kg N /ha)	Small tuber (Tons/ha)	Medium size (Tons /ha)	Big size tuber (Tons /ha)	Rough and rot (Tons /ha)	Total yields (Tons/ha)	Market yields (Tons /ha)
Kibungo	0	0.42^{a}	3.1^{a}	4.2^{a}	0.10^{a}	6.7^{a}	6.2^{a}
	60	0.92^{b}	3.8^{a}	6.1^{a}	0.07^{a}	9.4^{b}	8.5^{b}
	120	0.49^{a}	4.8^{b}	5.1^{a}	0.16^{a}	10.8^{b}	9.9^{b}
	180	0.80^{ab}	5.5^{b}	6.2^{a}	0.38^{a}	11.5^{b}	10.3^{b}
Rwamagana	0	0.32^{a}	2.3^{a}	1.9^{a}	0.40^{a}	4.8^{a}	4.2^{a}
	60	0.56^{a}	3.7^{b}	2.8^{b}	0.56^{a}	7.6^{b}	6.5^{b}
	120	1.07^{b}	3.6^{b}	3.8^{c}	0.97^{b}	9.4^{b}	7.4^{b}
	180	0.73^{ab}	3.3^{b}	3.7^{c}	1.02^{b}	8.7^{b}	7.0^{b}
Mugesera	0	1.7^{a}	1.7^{a}	1.6^{a}	-	4.7^{a}	3.1^{a}
	60	1.9^{ab}	2.7^{b}	2.3^{ab}	-	7.0^{b}	5.0^{b}
	120	2.8^{c}	4.0^{c}	2.6^{b}	-	9.5^{c}	6.6^{c}
	180	2.4^{bc}	3.0^{b}	1.9^{ab}	-	7.7^{b}	5.2^{b}

Yield levels suffixed with different letters are significantly different by site and season-year.

With regard to potato tuber yields, mean yield responses of different potato varieties to nitrogen fertilizer application rates are presented in Table 2 for 2015 and Table 3 for 2016 for all sites. In 2015, there were significant differences ($P < 0.05$) between the mean responses of potato varieties to nitrogen fertilizer application rates by site.

The same was true in the 2016 at Kibungo ($P< 0.05$) and Rwamagana ($P<0.001$) for each of total and market potato tuber yields. Nitrogen application rates have significantly increased total and market yields at all sites comparatively to the control. However, the three N rates had no significant effects on yield levels in 2015 while in 2016 the application rate in the amount of 120 kg N ha^{-1} significantly yielded higher than any other N rate. Under this N amount, harvested total and market yields were

Table 3. Mean yield responses of different potato varieties to increasing nitrogen fertilizer application rates in 2016.

Farm	Nitrogen application rates (kg N /ha)	Small tuber (Tons/ha)	Medium size (Tons /ha)	Big size tuber (Tons /ha)	Rough and rot (Tons /ha)	Total yields (Tons/ha)	Market yields (Tons /ha)
Kibungo	0	1.2^a	2.1^a	3.3^a	0.7^a	7.3^a	5.4^a
	60	1.9^{ab}	2.5^a	4.3^{ab}	0.8^a	9.5^a	6.8^a
	120	2.8^b	4.6^b	5.4^b	0.9^a	13.6^b	10.0^b
	180	2.4^b	3.3^{ab}	4.1^{ab}	1.2^a	11.1^{ab}	7.4^a
Rwamagana	0	1.5^a	2.9^a	2.5^a	-	6.9^a	5.4^a
	60	1.5^a	3.5^a	2.7^a	-	7.7^a	6.2^a
	120	1.9^a	5.2^b	6.9^b	-	14.0^b	12.1^b
	180	1.2^a	3.2^a	3.1^a	-	7.5^a	6.3^a

Yield levels suffixed with different letters are significantly different by site and season-year.

13.6 and 10.0 tons ha^{-1} at Kibungo and 14.0 and 12.1 tons ha^{-1} of potato tubers at Rwamagana, respectively. Big size tuber yields (>15 cm circumference) represented 63 and 57% of market yields at the two respective sites under 120 kg N ha^{-1}; the balances compose medium size potato tubers or seed potatoes.

The nitrogen application rate in the amount of 120 kg ha^{-1} has constantly yielded equal to or higher than the amounts of 60 kg and 180 kg N ha^{-1}. Over all sites, varieties, and seasons, the application of 120 kg N ha^{-1} has resulted in average yields of 11 tons and 8.8 tons ha^{-1} of total and market potato tuber yields, respectively. This represents 1.87 times yield increase when compared to the control. Similar potato tuber yields were also harvested from suitable agro-ecological zones for Irish potatoes (Turamyenyirijuru, 2013; Fashaho et al., 2013; Nyiransabimana, 2011). Worldwide, under non-irrigated conditions, Irish potatoes nitrogen requirement usually varies from 100 kg ha^{-1} for early maturing varieties to more than 200 kg N ha^{-1} for late maturing ones and for more than 30 tons ha^{-1} of market tuber yields (Getie et al., 2015; Barascu et al., 2015; Zebarth et al., 2007). The eastern Rwanda can therefore grow Irish potatoes and expect as good yields as elsewhere in Rwanda, and probably more under irrigation.

Comparative potato variety responses to nitrogen fertilizer application rates

Over the two seasons of the study, significant interaction effects were detected between potato varieties and N application rates at Kibungo site with regard to total and market potato tuber yields. The same also happened at Mugesera in 2015. No such interaction effects were observed in Rwamagana over the two agricultural seasons. In 2015 at Kibungo, Mabondo and Kruzza varieties, respectively reached their maximum yields with 60 kg and 120 kg N ha^{-1} while no maximum potato tuber yield was achieved for Gasore variety on this site (Figure 1). Although no significant interaction effects were observed in Rwamagana, there is a constant trend for potato varieties to specifically respond to increasing nitrogen application rates, with Gasore and Kirundo yielding highest at around 120 kg N ha^{-1} while the highest yield for Mabondo was at 60 kg N ha^{-1}. At the Mugesera site, the Peko variety yielded the maximum with 120 kg N ha^{-1} while the yields of the other two varieties were highest with 60 kg N ha^{-1} (Figure 2). In 2016, the Kirundo potato variety continuously stood out with 22 tons ha^{-1} of total potato tuber yields from the application of 120 kg N ha^{-1} (Figure 3). Market potato tuber yield was slightly higher than 17 tons ha^{-1} under the same amount of nitrogen fertilizer.

Potato varieties differently responded to nitrogen fertilizer application rates. Previous findings on the responses of different potato varieties to nitrogen fertilizer application rates indicated optimum amounts varying from 100 to 200 kg N ha^{-1} for yields varying from 27 to 47 tons ha^{-1} (Shunka et al., 2017 and Barascu et al., 2015). Otherwise, upon 130 to 190 kg N ha^{-1} yields varying from 20 to 25 tons ha^{-1} potato tubers were harvested (Manorama et al., 2012). Rens et al. (2015) found peak potato marketable yields with 112 kg ha^{-1} applied at emergence time. Kirundo yield around 20 tons ha^{-1} under the application of 120 kg N ha^{-1} is within these ranges. Higher N rates resulted in higher potato yields in Zimbabwe (Mutubuki et al., 2015) and Ethiopia (Zewide et al., 2012). But, the absence of interaction effects between potato varieties and nitrogen fertilizer application rates has also been documented (Rykbost and Charlton, 2000). Therefore, the nitrogen fertilizer application in the

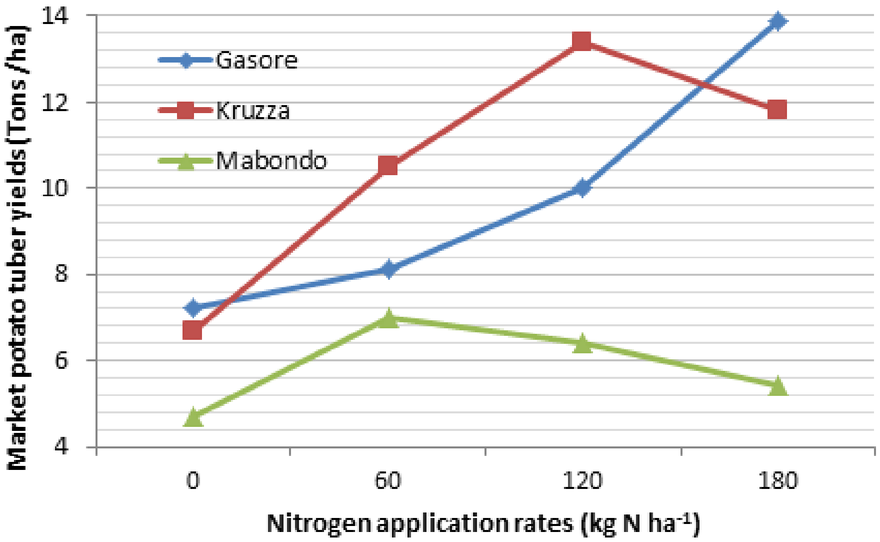

Figure 1. Comparative responses of different potato varieties to nitrogen application rates as measured by market potato tuber yields at Kibungo in 2015.

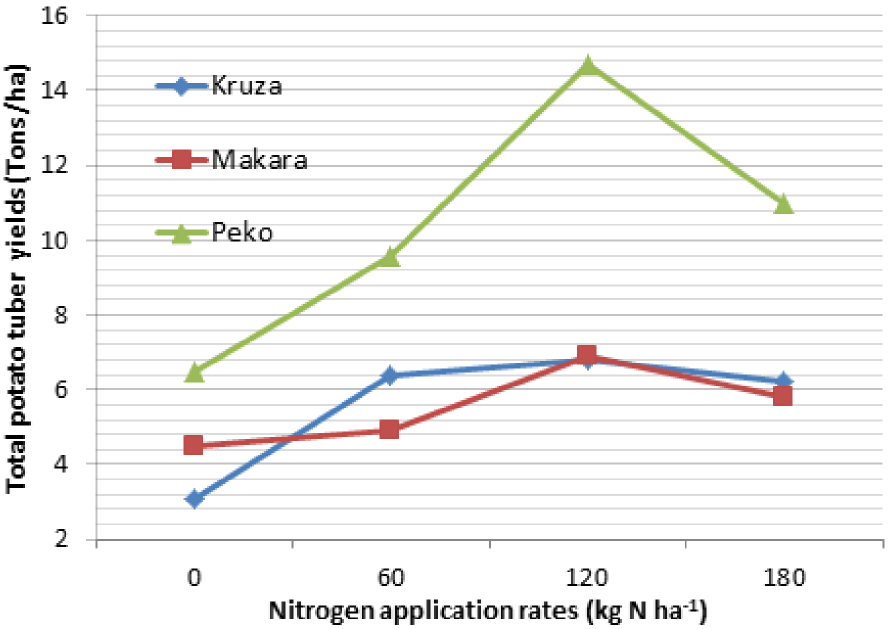

Figure 2. Comparative responses of different potato varieties to nitrogen application rates as measured by total potato tuber yields at Mugesera in 2016.

amount of 120 kg N ha^{-1} is within the range of many findings worldwide.

Conclusion

This factorial experiment explored the productivity of Irish potatoes in the agri-climatic conditions of the Eastern Province of Rwanda, comparing potato tuber yields and yield components of mass-selected, locally-grown potato varieties under increasing nitrogen fertilizer application rates. The results have indicated that Irish potato varieties can be grown and yield as much as they do in regions assumed more suitable for the crop in Rwanda. Yields

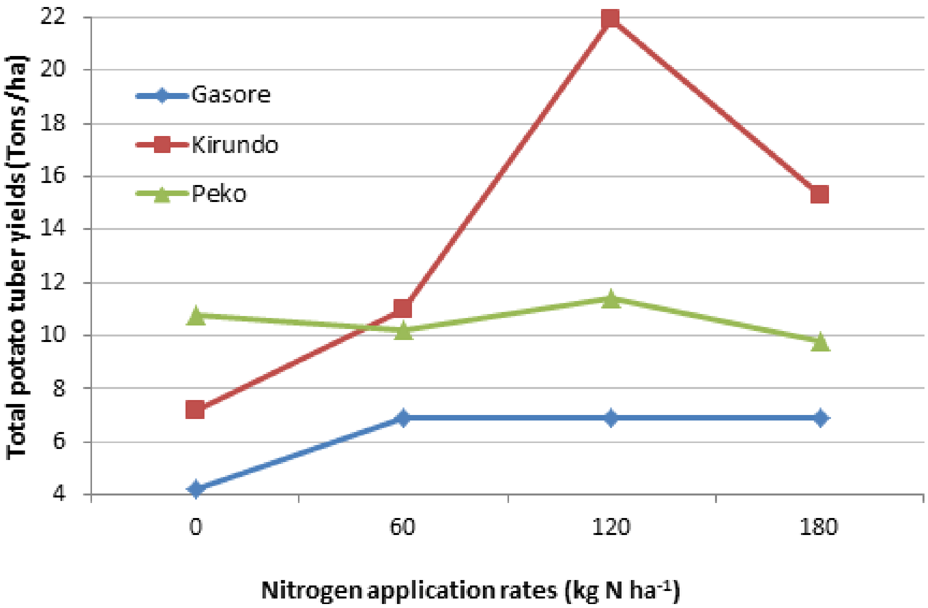

Figure 3. Comparative responses of different potato varieties to nitrogen application rates as measured by total potato tuber yields at Kibungo in 2016

from 10 tons to more than 17 tons ha^{-1} of market potato tubers were harvested. Irish potato varieties have responded differently to nitrogen fertilization. However, nitrogen application rate in the amount of 120 kg N ha^{-1} has constantly resulted in highest yields, particularly with Kirundo variety. Therefore, 120 kg N ha^{-1} should be recommended for expected yields above 20 tons ha^{-1} of market potato tubers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest

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Nutritional quality of tomatoes as a function of nitrogen sources and doses

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Vegetable consumers are increasingly demanding high quality products. Among factors that can influence the nutritional quality of tomato fruits, mineral nutrition stands out. The objective of this study was to evaluate the effect of nitrogen sources and doses on the nutritional quality of tomato fruits. The experiment was carried out in pots in an experimental area at the Universidade Federal de Viçosa - Paranaíba Campus, in Rio Paranaíba (Minas Gerais State, Brazil). The commercial hybrid Dominador was cultivated with four plants per pot. The treatments consisted of nitrogen fertilizer doses with 50 and 200 mg dm^{-3} of N, combined with four sources (urea, ammonium sulfate, ammonium nitrate and calcium nitrate), in a randomized block design with four repetitions. A (4 x 2) + 1 (four sources combined with two doses of N, plus one treatment without the application of N) factorial scheme was used. We evaluated the increase in ºBrix and titratable acidity values with the increase of the N dose applied. Urea and the ammonium nitrate resulted in higher pH values in the tomato fruits. The potassium, lycopene and total carotenoid contents in the tomatoes did not present significant differences in relation to the sources and doses used. The sources and doses of nitrogen fertilizers affected the nutritional quality of the tomato fruits, influencing parameters such as ºBrix, pH, titratable acidity and sodium content.

Key words: Mineral nutrition, *Solanum lycopersicum*, nutritional value.

INTRODUCTION

In vegetable production, demand for high quality products has steadily increased (Iglesias et al., 2015). Both organoleptic and functional properties are required, with the latter considered a source for preventing specific diseases (Lahoz et al., 2016). In this way, tomato consumption is considered a way to improve health, because of the ingestion of diverse compounds (Dorais et al., 2008; Adalid et al., 2010), such as antioxidants, which help to eliminate free radicals, thus reducing cellular damage (Ding et al., 2016).

Other properties of tomato fruits, such as soluble solids concentration, acidity content, sugars and organic acids

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are used to evaluate the nutritional state of the fruits (Scibisz et al., 2011; Ding et al., 2016). Thus, their physico-chemical constituents may influence nutritional and sensorial properties, conferring different attributes to the tomatoes and promoting greater or lesser acceptance of the fruits by both consumers and industry (Rosa et al., 2011).

The soluble solids content confers sweetness to the tomato fruit (Baldwin et al., 2008). The pH determines the organic acids content in the fruits (Ayvaz et al., 2016), which also contributes to the peculiar acidic flavor of the tomato and is a product security parameter (Anthon et al., 2011). Both parameters influence fruit acceptability by consumers (Baldwin et al., 2008). Titratable acidity also is an important characteristic for determining the nutritional quality of tomatoes (Anthon and Barrett, 2012).

The presence of minerals in the tomato fruits is highly relevant for human consumption, since mineral consumption aids in the intake of antioxidant compounds and fibers and contributes to adequate intake of certain minerals (Hernández-Suárez et al., 2007; Erba et al., 2013), such as potassium (K) and sodium (Na).

Tomato fruits are rich in carotenoids, which are required for human consumption. Lycopene is the principal carotenoid present in tomatoes, characterized by having beneficial health properties (Eh and Teoh, 2012). In addition to presenting high nutritional value (Adalid et al., 2010), lycopene can help prevent some types of cancer, such as prostate and lung cancer, in addition to cardiovascular diseases (Dillingham and Rao, 2009; Ford and Erdman, 2012).

Various factors may influence the nutritional quality of tomato fruits. Among them are mineral nutrition, with nitrogen (N) being one of the most required nutrients by the tomato plant, contributing to growth, plant development and crop reproduction (Ferreira et al., 2010; Mehmood et al., 2012; Kumar et al., 2013; Kuscu et al., 2014), in addition to influencing characteristics that confer quality to the fruits (Amans et al., 2011).

Adjusting nitrogen fertilization is very complex, because of both the doses applied and the sources used. The availability of N in the soil depends on various factors, among them the processes of nitrification, leaching, volatilization and denitrification, which is responsible for the loss of this nutrient. In addition to this, the various forms of N, nitrate (NO_3^-), ammonium (NH_4^+) and amide (NH_2), differ in terms of costs, leaching potential, soil acidification, volatilization and plant absorption (Marouelli et al., 2014), making it difficult to choose the best source for a given crop condition. As such, the objective was to evaluate the effects of nitrogen sources and doses on the nutritional quality of the tomato fruit.

MATERIALS AND METHODS

The experiment was conducted in an experimental area at the Universidade Federal de Viçosa - Rio Paranaíba Campus, in Rio Paranaíba (Minas Gerais State, Brazil) (19°12'53"S and 46°13'56"W, altitude 1140 m) in the period from September to December 2015, corresponding to the spring planting. The soil used is classified as Red-Yellow Latosol of very clayey texture, with the following chemical attributes: pH (water) = 5.5; P (Mehlich-1) = 18.4 mg dm^{-3}; S = 11.6 mg dm^{-3}; Ca^{2+}, Mg^{2+}, K^+, H+Al and CTC potential = 30; 9; 1.6; 53 and 93.6 $mmol_c$ dm^{-3}; organic material = 2.9 dag kg^{-1}; B, Cu, Fe, Mn and Zn = 0.3; 1.1; 37; 9.3 and 2.9 mg dm^{-3}.

The commercial hybrid Dominador was cultivated in pots of 150 dm^3 (87 cm diameter and 43 cm height), with seedlings transplanted in the central area, with a total of four seedlings per pot arranged in a zigzag. The pots were used to impede N leaching, since they were not bored through and remained covered with canvas. Each plant was tutored with bamboo and led to the 4th raceme, without thinning the fruits. The first bunch was removed, with the intention of redirecting photoassimilates for other plant organs (Guimarães et al., 2009). Other cultural practices such as weeding, thinning, mooring, irrigation, pest and disease management and weeding were done according to the recommendations for this crop (Silva and Vale, 2007).

Fertilizer was distributed manually in the experimental pots. The nitrogen fertilizer doses were 50 and 200 mg dm^{-3} of N, equivalent to 100 and 400 kg ha^{-1} of N when considering the layer from 0 to 20 cm. The doses of N were combined with four sources (urea, ammonium sulphate, ammonium nitrate and calcium nitrate). The doses were calculated based on the total contents of N in the sources and distributed in four coverage according to the emission of the bunchs.

One mg dm^{-3} of boron and copper and 3 mg dm^{-3} of zinc were applied to the planting throughout the volume of the soil in the pot. In a central groove 8 cm in depth, 300 mg dm^{-3} of P was deposited. The total dose of K_2O was 240 mg dm^{-3}, where 90 mg dm^{-3} was applied to the transplanting of the seedlings and the remainder was distributed in four coverings along with the N.

The treatments were distributed in a (4 x 2) + 1 factorial scheme (two doses of N combined with four sources, plus one treatment without the application of N) in a randomized block design with four repetitions. For the analysis, two fruits per plot were collected, totaling eight fruits per treatment at 82 days after transplanting.

For the analyses, the fruits were crushed and passed through a 230 mm sieve to determine soluble solids contents, with the values expressed in °Brix, measured in portable digital refractometer (PAL-1) and the pH of the pulp was measured with the help of a countertop pHmeter (MS Tecnopon Instrumentos mPA-210P) (AOAC, 1997), totaling three repetitions for both variables.

Titratable acidity (TA) was determined in accordance with the method described in AOAC (1997). A sample of 20 g of pulp was taken and diluted in 50 mL of distilled water. This mixture was titrated with standardized solution of NaOH at 0.05 M, with phenolphthalein as an indicator (pH 8.1). TA was expressed as a % of citric acid, by the following formula:

$$AT = \frac{VxNxE}{10xM}$$

Where, V = volume of the NaOH solution used to reach pH 8.1 (mL); N = normality of the NaOH solution; E = gram-equivalent of the predominant acid (64.02 for citric acid); 10 = constant; M = mass of sample used (g).

The K and Na contents of the fruits were determined. The tomatoes were washed in deionized water and dried in an incubator with forced air ventilation at 70ºC for 72 h. Afterward, the samples were crushed in Wiley type mill equipped with a sieve of 1.27 mm and the nutrients analyzed after mineralization by nitric-perchloric digestion. Thus, K and Na were measured by flame emission photometry according to the methodology of Malavolta et al. (1997).

The lycopene and total carotenoids content were determined based on the methodology proposed by Rodriguez-Amaya (2001), obtained by spectrophotometric analyses. After the fruits were crushed in a blender, 5 g samples of the pulp were taken and 40 mL of acetone was added (P.A.). The mixture was agitated for 1 h using the MMS Multi Shaker at 200 rpm. Afterward, the solution was vacuum filtered with the help of a Kitasato flask wrapped in aluminum foil, in order to avoid photo-oxidation of the pigment. Each sample was washed three times with acetone, aiming for total extraction of the pigments. Forty five millilitre of petroleum ether was added to the funnel of separation. After filtering, the lower phase was discarded and the samples were washed to remove all of the acetone. The solution of the pigments was transferred to a 100 mL volumetric flask, with the volume completed with petroleum ether. The spectrophotometer reading was done at a wavelength of 470 nm.

The lycopene content was obtained by the following formula (Carvalho et al., 2005):

$$ug/g = \frac{(AxVx1.000.000)}{(A_{1cm}^{1\%}xMx100)}$$

Where, *A* = absorbance of the solution at the wavelength of 470 nm; *V* = final volume of the solution; 1,000,000 = constant; $A_{1cm}^{1\%}$ = the extinction coefficient or the absorptivity coefficient (3450) and *M* = sample mass taken for analyses; 100 = constant.

The total carotenoid concentration (*Ct*) was calculated from the following formula (Rosa et al., 2011):

$$Concentration\ Ct\ (\mu g/g) = \frac{(AbsxDil.xVol.x10,000)}{(2,592xma)} \div 100$$

Where, *Abs* = absorbance of the solution at the wavelength of 470 nm; *Dil.* = dilution of the extract; *Vol.* = volume of the volumetric flask used (mL); 10,000 = constant; 2,592 = extinction coefficient; *ma* = sample mass (g).

The data obtained were subjected to analysis of variance (ANOVA), with the source means compared by the Tukey test and the doses compared by the F test, both at 5%. Additional comparisons of the control and factorial mean were done by means of contrasts tested by the t test. The program R was used for the statistical analyses.

RESULTS AND DISCUSSION

The 200 mg dm^{-3} dose provided higher mean ºBrix values (6.14) for all sources of N evaluated, conferring higher sugar content to the fruits (Table 1). In a similar way, Kuscu et al. (2014) observed the increase of the soluble solids content with the dose of N applied to evaluate the response of three levels of irrigation and four doses of N (0, 60, 120 and 180 kg ha^{-1}) in the yield and quality of tomato fruits in two years of crops. This result may be explained by the higher photosynthetic rate with increasing doses of N, which causes increased production of photosynthates, which may be stored as reducing sugars (Wang et al., 2007). Contrary results were reported however, in a manner that the soluble solids content increased with a reduced supply of N (Bénard et al., 2009). On the other hand, when applying increasing doses of N (0, 80, 160, 240, 320, 400 kg ha^{-1}), no change was observed in the ºBrix value, which was maintained at an average of 4.6 (Marouelli et al., 2014).

For the 50 mg dm^{-3} dose, the highest fruit pH values were found with the urea and ammonium nitrate applications. For the 200 mg dm^{-3} dose, only the ammonium nitrate presented the highest value (Table 1). This result may be associated to the large accumulation of mineral solutes in the tomato fruit pulp, because of the presence of NH_4^+, resulting in the consumption of organic acids in the assimilation of N (Porto, 2013). Regarding the dose, only with the use of the urea did the 50 mg dm^{-3} dose provide higher pH.

The 200 mg dm^{-3} dose provided higher values of titratable acidity relative to the 50 and 0 mg dm^{-3} doses. With regard to the sources, for the 50 mg dm^{-3} dose, the calcium nitrate together with the urea presented the highest values, while the same occurred for the 200 mg dm^{-3} dose when the ammonium nitrate was used (Table 1). It is worth mentioning that the values presented in this work were lower than those in the Brazilian literature. This however demonstrates that the crop conditions used, as well as the hybrid chosen, led to fruits with low titratable acidity.

In a similar way, Kuscu et al. (2014) observed a significant increase of titratable acidity with the applied N dose. This increase in the N dose provided an increase in both the titratable acidity as well as that of the soluble solids content (Wang et al., 2007). Different results however were evidenced when evaluating the impact of the reduction of the N doses on the yield and quality of the tomato fruits, reducing titratable acidity by 10% (Bénard et al., 2009).

In this experiment there was no significant difference for the K content in the fruits, in relation to the sources and doses of N (Table 1). The same occurs when evaluating the influence of the proportion of NO_3^-:NH_4^+ in the contents of macro and micronutrients in the fruits, where no significant differences were observed (Borgognone et al., 2013). It is worth mentioning that various factors may influence the mineral composition of tomato fruits such as the hybrid used, water availability, climatic conditions and cultivation method (Hernández-Suárez et al., 2007). Different results however were found by Hernández-Suárez et al. (2007) who determined the influence of the mineral composition and analyzed the influence of crops, growth medium and fruit sampling period on the mineral contents, with low mineral contents found in the fruits, except for K and Mg.

The Na content was higher for the 50 mg dm^{-3} dose with the ammonium sulfate application and higher for the 200 mg dm^{-3} dose with the use of urea and calcium nitrate (Table 1). The presence of NH_4^+ tends to reduce the absorption of cations because of competition for absorption sites. This probably did not significantly occur

Table 1. Mean values of ºBrix, pH, titratable acidity (%), K (g kg^{-1}) Na (mg kg^{-1}), lycopene and total carotenoids content (µg g^{-1}) in tomatoes as a function of the sources and doses of nitrogen. Rio Paranaíba – Minas Gerais State, Brazil.

Dose (kg ha^{-1})	Source[1]				Mean	$F_{sources}$	F_{doses}	$F_{interaction}$	CV (%)
	Urea	AS	AN	CN					
ºBrix									
0	4.8 **								
100	5.3	5.1	5.3	5.3	5.2^{b}				2.2
400	6.2	6.1	6.1	6.1	6.1^{a}	1.71	333.26**	1.14^{ns}	
Mean	5.8^{A}	5.6^{A}	5.7^{A}	5.7^{A}					
pH									
0	4.0^{ns}								
100	4.1^{Aa}	4.0^{Bb}	4.1^{Ab}	4.0^{Bb}	4.0		42.08**	26.03**	
400	4.0^{Cb}	4.1^{Ba}	4.3^{Aa}	4.0^{BCa}	4.1	57.14**			0.5
Mean	4.0	4.0	4.2	4.0					
Titratable acidity (%)									
0	0.06 **								
100	0.07^{ABb}	0.06^{Bb}	0.07^{Bb}	0.08^{Aa}	0.07				
400	0.10^{Ba}	0.09^{Ca}	0.12^{Aa}	0.08^{Ca}	0.10	22.15**	378.72**	34.67**	4.3
Mean	0.09	0.08	0.09	0.08					
K (g kg^{-1})									
0	19.3 ^{ns}								
100	22.4	21.3	19.5	13.4	19.1^{a}				
400	21.8	22.6	18.5	17.8	20.1^{a}	3.46*	0.38	0.57^{ns}	20.4
Mean	22.1^{A}	21.9^{A}	19.0^{A}	15.6^{A}					
Na (mg kg^{-1})									
0	250*								
100	250^{Bb}	340^{Aa}	260^{Ba}	260^{Ba}	280				
400	280^{Aa}	240^{Bb}	240^{Ba}	250^{ABa}	250	10.70**	27.03**	28.26**	4.7
Mean	270	290	250	260					
Lycopene (µg g^{-1})									
0	81.8^{ns}								
100	66.2	74.5	73.3	63.2	69.3				
400	62.1	59.3	79.8	62.8	66.0	1.99	0.56	1.08^{ns}	0.5
Mean	64.1	66.9	76.6	63.0					
Carotenoids (µg g^{-1})									
0	108.33 ^{ns}								
100	88.1	99.1	97.5	84.1	92.2				
400	82.6	78.9	106.3	83.5	87.8	2.00	0.57	1.08^{ns}	12.6
Mean	85.3	89.0	101.9	83.8					

[1] AS, ammonium sulfate; AN, ammonium nitrate; CN, calcium nitrate. Means of the sources and doses followed by the same capital letter on the row and lowercase letter in the column did not differ by the Tukey and F tests at a 5% significance level. Mean of control treatment followed by ** or * indicate the significance of the contrast between this mean and the mean of the other treatments following the t test at 1% and 5%. The value of $F_{interaction}$ followed by **, * or ^{ns} indicate significance at 1%, 5% and non-significance.

in this work.

There was no difference in the lycopene and total carotenoids content in relation to the sources and doses of N (Table 1). Kuscu et al. (2014) observed that the N application caused an increase in the lycopene and total carotenoids content until the 120 kg ha^{-1} of N dose and values were reduced with the 180 kg ha^{-1} N application.

Conclusions

The 400 kg ha^{-1} N dose provided higher ºBrix and titratable acidity values in tomato fruits. The sources containing NH_4^+ resulted in higher pH values. The sources and doses of N did not influence the K, licopene and total carotenoids content in the fruits.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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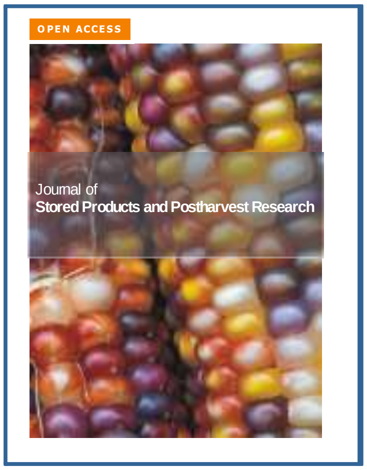

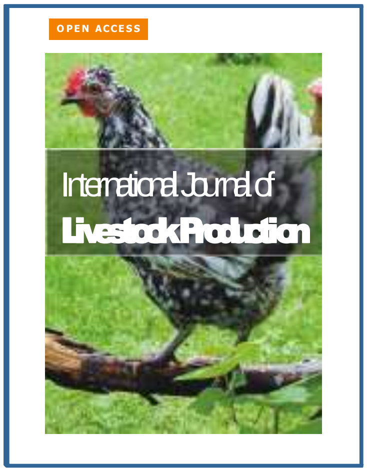

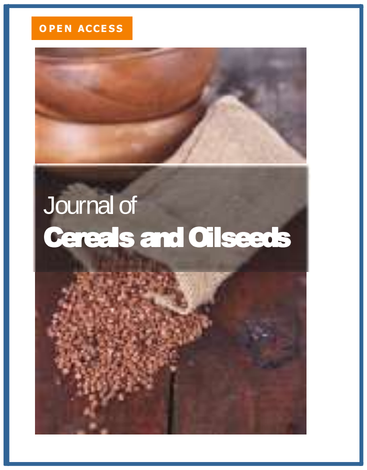

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