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Review

Plant biotechnology: A key tool to improve crop production in Rwanda

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Rwanda's economy relies on agriculture; nevertheless, crop production remains insufficient for both local consumption and exportation. Overall, enough agriculture yields in Rwanda to ensure food security has not yet been achieved regardless of more than 87% population engaged in agriculture activities. In this context, this study aimed at gathering the information on Rwanda's agriculture based on different research reports and Rwandan's government established policies to identify constraints to agricultural production faced by farmers and applicability of plant biotechnology. It was revealed that intensive and appealing discussions about agriculture economic importance, production of improved crops and the use of all necessary resources to ameliorate agricultural production need more attention. This review attempts to discuss the current problems facing agriculture in Rwanda and feasible solutions stressing that planning strong-long term policies, promoting crop breeding and use of plant biotechnology tools together with modern agriculture resources can boost up and transform economic developmental progress of Rwanda.

Key words: Rwanda, plant biotechnology, improved crops, food security.

INTRODUCTION

The current population growth in Rwanda makes it impossible to keep the balance between food production and consumption rate. According to FAO reports, more than 2/3 of African countries are among the most vulnerable to adapt to climate uncertainties (Mikova, 2015). In Rwanda, as in all other Sub-Saharan African countries, food inadequacy is very obvious despite the availability of natural agricultural resources, and it is unlikely that the situation will change if new measures are not taken (FAO, IFAD, UNICEF, 2017). Food inadequacy causes numerous problems including malnutrition and low incomes that barely satisfy daily needs for those who entirely depend on agricultural income. The problem might be the people's culture itself that fail to adapt to the current needs of the people and speed of development, the political policies that are not practical, or merely the misallocation of funds (Ndiritu, 1999). In 2016, the number of chronically undernourished people in the world was estimated to have increased to 815 million, up from 777 million in 2015 although still down from about 900 million in 2000 (World Population Prospects 2017 Revision, 2017). Food scarcity has worsened particularly in sub-Saharan Africa, south-eastern Asia, and western Asia and this situation was mostly found in places with

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conflicts together with regions where droughts or floods have appeared (FAO, 2011).

It is indeed known that the solutions to the current and future need of food require producing much more food and that more productive agriculture is urgently needed especially in Sub Saharan African countries. Most African countries including Rwanda have significantly prioritized and allocated funding to the agriculture industry to increase food productivity. In Rwanda, current agricultural policies advocate crop intensification and production of high valued crops and the use of biotechnology in animal production (Gahakwa et al., 2012).

Rwanda's social economic development highly depends on agricultural production. Rwandan government policies on agriculture have always been prioritized because agriculture is the maindrive of economic growth and employing more than 87% of the population in 2017 (FAO, IFAD, UNICEF, 2017). Despite the efforts made in the past years, agriculture has faced challenging problems mainly shortage and inconsistent rainfall, soil infertility, soil salinity, lack of land management, urban development, abrupt climate change which causes erosion in some parts of the country because of Rwanda's mountainous landscape (Brink et al., 1998; Gahakwa et al., 2012; Mikova, 2015). All these challenges have negatively impacted agriculture performance causing low food production as well as low exportation. With all these challenges in place, Rwanda together with other Sub-Saharan African countries are urged to solve these problems with new methodologies to tackle this difficult task of eradicating hunger, malnutrition and all diseases related to being chronically undernourished (FAO, IFAD, UNICEF, 2017).

All documents (articles, reports and other information's) relevant to this study were accessed from different online database resources, and the main keywords were: Rwanda, crops improvement, agriculture in Rwanda, food security, plant biotechnology. In this narrative literature review article, different suggestions about current constraints facing agriculture and plant biotechnology implementation in Rwanda providing possible key solutions were critically discussed.

In Rwanda, current agriculture is dominated by conventional practices and mostly owned by smallholder farmers. However, due to food security demand in recent years and increasing population, there is urgent need for adequate quantity and quality of food. Unlike other countries where most revenue is from agricultures, in Rwanda it is the opposite (Figure 1). Despite 87% population practicing agriculture on a daily basis, only 17% is the agriculture's contribution to GDP (National Institute of Statistics of Rwanda, 2017).

CHALLENGES OF AGRICULTURE IN RWANDA

In Rwanda, the majority of the agricultural produce is food crops instead of export crops which results in generating low incomes for the farmers (Muvunyi et al., 2017). Primarily due to numerous challenges that result in low crop production including shortage of arable land and mismanagement of available land, inadequate rainfall (climate uncertainties), soil infertility, pest and disease control, limited technology base, to cite a few.

Shortage of cultivatable land and mismanagement of available land

Rwanda is known as one of the countries with the highest population densities in Africa; so, the Rwandan government has decided to put in place some policies that can alleviate the lack of land problems. These include land distribution, soil fertility management, industrialization of agriculture and use of agro-chemicals. Even though the implementation is in place, some problems arise as the increase of population of Rwanda in 2017 was 2.40% (World Population Prospects 2017 Revision, 2017). Urbanization and fast-growing industry of infrastructures are among the main reasons of arable land losses (National Institute of Statistics of Rwanda, 2018).

Lack of quality planting materials

Rwanda's farming suffers shortage of quality planting materials due to few production companies or organizations of good quality seeds (Musebe et al., 2017). Good quality planting material affects crop yield and income of farmers. It is desirable for farmers to use quality seed that are of high value that can benefit them. That is why more proper seed storage units, tissue culture production units and other possible alternative methods to increase the number of quality planting materials are needed.

Soil infertility and crop nutrient deficiency

Because Rwanda's soil is at high risk of erosion, soil fertility has been declining, and the rate of production in a local area where smallholder farmers use most cultivable land has also been affected. Malpractice of traditional agriculture, the low utilization of modern agricultural methods as well as the economic policies have practically done little to encourage agricultural transformation. Now research-based policies are in place to transform the production rate. Rwanda's soils need chemical fertilizer inputs since some parts of the country are characterized with low quantity of inorganic matter like nitrogen, sulfur, phosphorus and potassium along with the regular popular use of organic manures which are low in nutrient content (Ministry of Agriculture and Animal Resources, 2009,

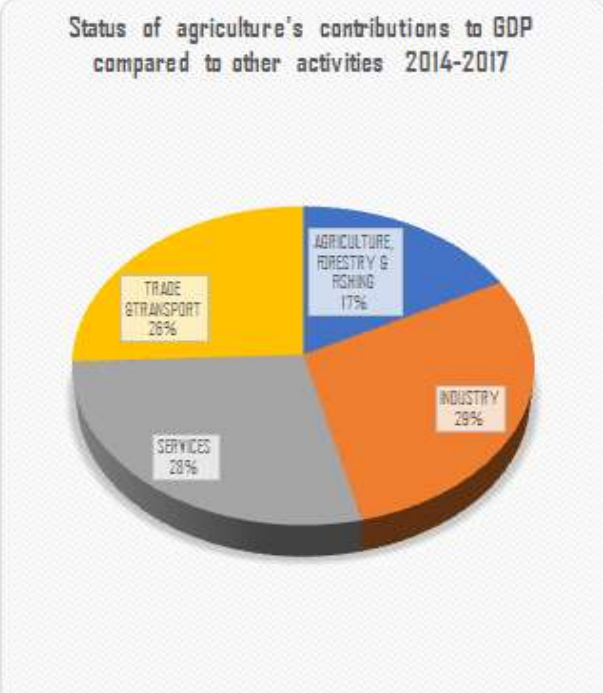

Figure 1. Status of agriculture's contribution to the gross domestic product (GDP) compared to other activities.
Data source: www.statistics.gov.rw (National Institute of Statistics Rwanda, 2017).

2018). In studied areas, in Rwanda, soil fertility was imbalanced and caused low yields in maize and groundnuts (Kabirigi et al., 2016). So frequent and intensive application of necessary fertilizers (N, P, and K) is to be encouraged, and it has started with distribution of agro-chemicals fertilizers rich in the organic nutrients to the local farmers to support one cow per low-income family program which involves distributing heifers to needy families and ensuring the use of both natural organic nutrients and agrochemicals for better yields of food crops by smallholder farmers (Ministry of Agriculture and Animal Resources, 2009, 2018). Modernization of agricultural practices and irrigation in different areas of the country is also an alternative that has started in different areas of the country, and this needs to be encouraged more. Research in the area of alternative means of fertilizing the soil, along with the use of herbicide tolerant varieties is also an area of interest and opportunity for young researchers.

Inadequate rainfall

Inconsistent and poor rainfall leads to flooding and prolonged drought, and it has been a constraint to the growth of agriculture in Rwanda since 90% of agriculture production depends on rainfall (Mikova, 2015). The inconsistency of rainfall is attributed mainly to deforestation of the country since more than 80% of cooking energy comes from trees, and since wood and charcoal are the main source of fuel for Rwandan households (REMA, 2012). Luckily, overgrazing has been managed from the last decade when the government implemented a policy (Zero-grazing system) where cattles and other livestock have to be fed in order to allow improvement of pastures, increase of organic manure, promoting improved livestock breeds and reduction of environmental degrations problems (Twagiramungu, 2006). This policy has been useful, but more attention needs to be developed to be able to produce plant hybrids that can adapt to dramatic climate change (drought), and it can be more beneficial for Rwanda to be the hub and pioneers of these plant hybrids in Sub-Saharan African countries. Africa's harsh climatic conditions are affecting people's lives and need immediate attention (Kathiresan, 2011; National Institute of Statistics of Rwanda, 2017). Use of greenhouse technology to grow crops can also be an alternative method to resolve this inadequate rainfall problem.

Pest and diseases control

Recently, in Rwanda, crop-devouring caterpillars known as fall armyworms damaged 17% of maize crop in just a few months. The fall armyworm pest originated from

Americas but has recently spread throughout African countries: Nigeria, Saotome, Malawi, Zambia, Zimbabwe, South Africa, Namibia, Uganda and Democratic Republic of Congo. This has significantly directly affected the revenue of farmers and high loss of harvest (Goergen et al., 2016). In Rwanda, most cultivable land is apportioned for production of major consumable crops like cassava, beans, sweet potato, and maize and due to diseases attacking these crops from time to time their yield is still low (Nduwumuremyi et al., 2016). Many developing countries have been affected by the different outbreaks of diseases, and numerous crops have been affected (Munganyinka et al., 2017). Even though African countries spend enormous expenses purchasing herbicides, fungicides and insecticides, this does not inhibit the considerable crop losses due to pests and diseases. It proves that this option does not sustain the increase of productivity and opting for biotechnology based diseases control methods can be a better option.

Limited technological base and insufficient resources

The use of a hoe and other traditional agriculture methods has served the Rwandan Agriculture Industry for a long time, but these means prove to be time-consuming, frustrating and limiting the production capacity of the people (Gahakwa et al., 2012). This conventional practice of agriculture has not changed in quite a long time now due to the lack of trained workforce, difficulties in getting an agricultural credit, academic research results that do not reach the people. Therefore, urgent need of forward-thinking techniques to augment agricultural yield and diminish losses at the same time conserving the environment are highly required.

Post-harvest deterioration

As in many African countries, post-harvest management and handling is still quite a challenge due to a few industrial food processing units. Most produce deteriorates right after few days of harvest. In Rwanda, initiatives to manage the processing of pineapple post-harvest losses has started and should escalate to more food crops (Ministry of Agriculture and Animal Resources, 2018; Nduwumuremyi et al., 2016).

CROP PRODUCTION PERFORMANCE IN RWANDA

Crop production is practiced by the majority of Rwandese households. Each household produces at least one type of crop and the majority produce either vegetables or fruits. According to the data by National Institute of Statistics, the percentage of households engaged in agriculture does not make much contribution to the GDP (Figure 1) mainly due to the challenges discussed in this review.

Conventional agriculture is a term used to designate farming techniques that are done traditionally (United States Department of Agriculture, 2015). In East Africa and particularly in Rwanda, conventional agriculture methods are still the main drive of the agriculture industry, and this results in poor productivity causing Africa to depend on international food aid and agriculture assistance from developed countries to support small and large-scale farmers (Blein et al., 2013).

Most of the Rwandese population and labor force are engaged in traditional agriculture. Traditional agriculture practices are mainly characterised by crop rotation, use of compost and burning of fields to maintain soil fertility by increasing nitrification. In Rwanda, cultivation of most food crops has always been dominated by smallholder farmers who do it to survive and no surplus production for the market. As a result, the income of the farmer and the country, in general, is deficient compared to other Asian countries where Green agriculture revolution has been applied. Therefore, appropriate measures should be put in place to address these problems adequately (Kathiresan, 2011).

Although soil fertility in Rwanda has been maintained for a decade, due to rapid urbanization and increase in population, climate change and the traditional practices of agriculture lead to low production. In these circumstances, agriculture improvement is highly critical to the present and the future economic growth and the wellbeing of Rwandese and other developing countries population. Changes in agricultural production that meet the needs of people are urgently needed to raise the standard of living and to minimize poverty.

OPPORTUNITIES AND PROPOSED SOLUTIONS FOR RWANDAN AGRICULTURE

Rwanda has an arable soil and abundance of water that can surely promote agricultural production if used well, but to guarantee the nutritional and food security of Rwanda, it is a big challenge which requires the involvement of multisectoral firms (Kathiresan, 2011; Figure 2). With current population growth, young generation should consider the current challenges as an opportunity to enter into this sector and respond to the rising demand for agricultural products globally.

Improving agricultural production incomes can encourage the use of local products and services thereby promoting the growth in the rest of Rwanda's economy and moreover, potentially create jobs. Nearly all farmers still use traditional agricultural methods mainly because of lack of funds to buy the modern agriculture inputs such as agricultural machinery and chemical fertilizers, pest and disease control inputs; so, the introduction of new

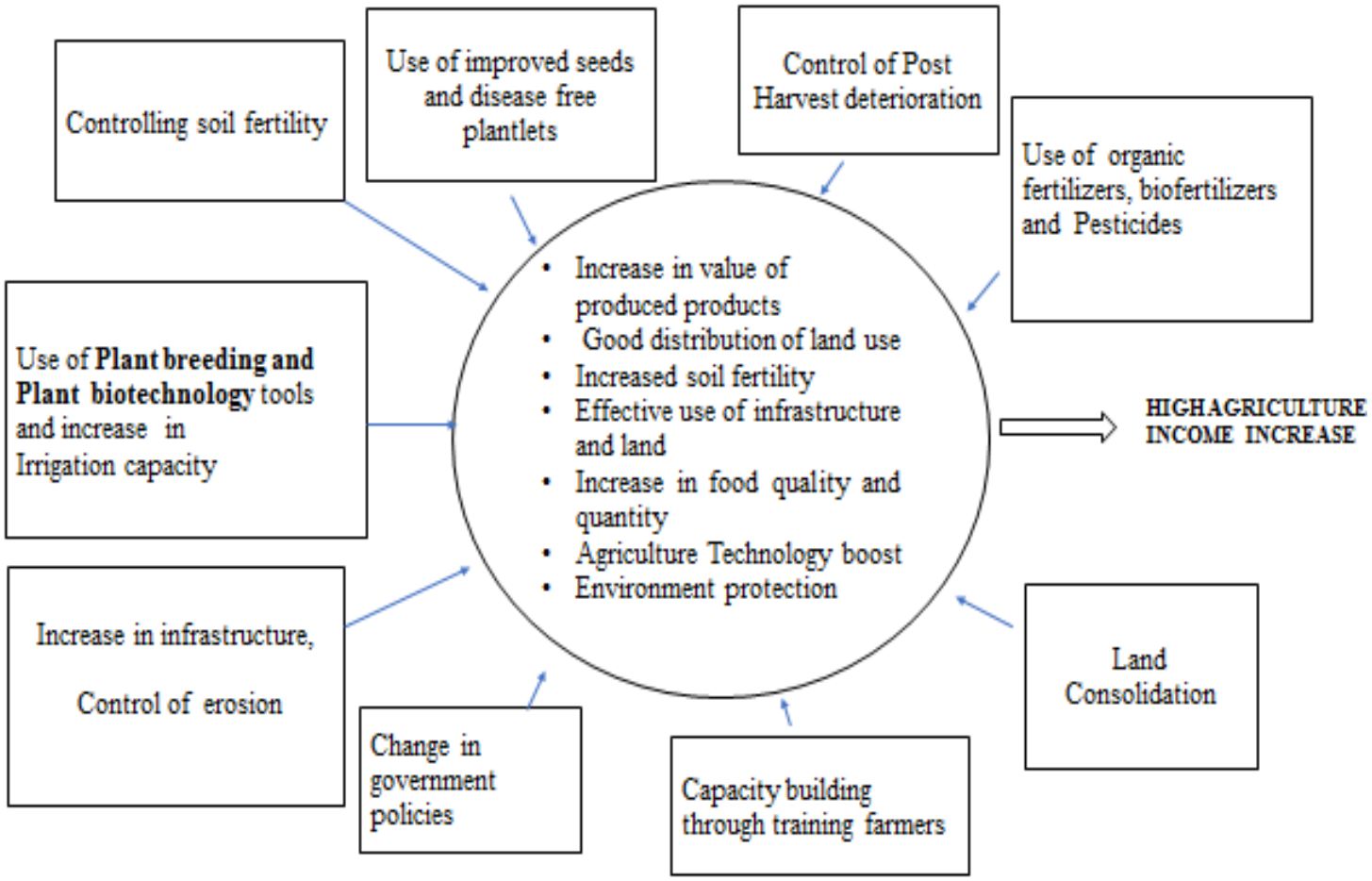

Figure 2. Potential solutions for Rwanda's agriculture.

alternatives for conventional agricultural practices is also an open opportunity (Ye et al., 2002).

Even though suitable policies are in place to tackle agriculture problem, their implementation needs a speed up, and new measures have to be put in place. Rwanda as any other Sub-Saharan African countries are in need of free-disease plantlets for highly cultivated crops and to achieve this, plant biotechnology holds the key to high agricultural productivity (Musebe et al., 2017). Use of plant biotechnology has to be highly considered as a means to solve some agri-related problems (Figure 2) since its benefits can speed up the economy and stimulate the research processes. China can be a good example with the Bt Cotton experience proving the direct and indirect benefits of its investments in plant biotechnology research and product development (Hautea and Escaler, 2004). In 2002, Bt Cotton was grown in 2.1 million hectares by around five million farmers in the world (Innes, 2006). The average Bt Cotton farmer has reduced pesticide sprays for the Asian bollworm from 20 to 6 times per year and produces a kilogram of cotton for 28% less cost than the farmer using non-Bt varieties in Asia (Huang et al., 2002). Instant detection of disease attacks by using ELISA and PCR techniques is also required for better management of farms. The use of biotechnology tools to protect seed distributed among farmers, biological control agents and testing varieties of seed identity and purity before their distribution are primary tools that can benefit African farmers. In this context, it is recommended for developing African countries to start thinking about pursuing gene transfer to breed-disease and introduction of pest-resistant varieties in order to meet the future food's needs.

Rwanda's current vision is to promote agricultural productivity through reforms of using modern agriculture methods and animal production, increase in agriculture budget, education of farmers on how to use new agricultural methodologies because the conventional agricultural research does not keep equal distribution between the high demand of food and the supply chain (Ministry of Agriculture and Animal Resources-Rwanda, 2009).

Plant biotechnology and genetic engineering are the

primary drive of agriculture progress in developed countries (Huang et al., 2002). Despite the difficulties in sharing information between scientists across the country, the information gathered about the current status of plant biotechnology in Rwanda from some researchers in Rwanda Agriculture Board (RAB) have reported the use of tissue culture: *in vitro* cultivation of cash crops like banana, coffee, potato, sweet potato, pineapple, passion fruit, Tamarillo also known as a tree tomato (Gahakwa et al., 2012). Several private companies (FAIM.CO) have also initiated *in vitro* production of crops including bananas. The effort made still does not provide enough for the high demand of plantlets from the farmers. Disseminating resistant varieties produced using plant breeding technology is highly recommended since most of the varieties that are brought from abroad sometimes fail to adapt (Gahakwa et al., 2012). More research is needed to identify and use suitable domestic breeding techniques for popular varieties in the country, and this should be widespread to other crops since the only crops receiving research attention are common beans, bananas, cassava and sweet potatoes (Karangwa, 2018).

After the genocide of Tutsi in 1994, plant breeding and tissue culture budget were merged. However, today efforts are made for the allocation of research funds to many biotechnology tools of which introduction of genetically modified hybrid research, use of DNA markers in plant breeding and optimization of current tissue culture protocol to minimize the cost of tissue culture products, therefore, improving the income of smallholder farmers and agriculture productivity in general (Ministry of Agriculture and Animal Resources, 2004).

MODERN AGRICULTURE AND PLANT BIOTECHNOLOGY STATUS IN RWANDA

Rwanda's plant biotechnology is mostly dominated by tissue culture of medicinal plants and micropropagation of disease-free food crops mainly bananas, potato, sweet potato and cassava (Nduwumuremyi et al., 2016). To ensure food security, appropriate measures to increase the capacity of plant biotechnology should be a priority.

Tissue culture practiced in Rwanda is one of the techniques that is believed can solve agriculture production problems because it has so many advantages, one of them being the high multiplication of plantlets in a short time and space (Smith, 2013). The plants produced with tissue culture techniques are also known to be free of viruses and other diseases; thus, are all with high survival rate in the field. They grow with uniformity, and as a result, they increase yield and quality (Hautea and Escaler, 2004). Currently, many developing countries are adopting this technique but it is not yet highly spread throughout because the plantlets resulting from tissue culture are still expensive and not every farmer has access to them. Unlike developing countries, developed countries have taken one step ahead from tissue culture techniques into high agricultural technologies like plant genetic engineering, plant breeding with DNA molecular markers and these techniques have replaced the conventional plant breeding. By mastering the above technologies, the capacity to start transgenic plants research will be achieved.

Conventional methods for food production in Rwanda do not suffice the market need, and it has been discovered that plant biotechnology tools can be used to alleviate current agricultural productivity problems (Roberts, 1984). In Rwanda as well as in other Sub-Saharan African countries, few institutions are conducting research and implementation of Agriculture Biotechnology. In Rwanda, University of Rwanda (UR), Rwanda Agriculture Board (RAB), INES-Ruhengeri, FAIM.CO are all among the few organizations that have undertaken the biotechnology program, and it has been a few years now, but the impact of that program on Rwandan people's livelihood is still debatable. Further, it is mainly because the research that is conducted does not initiate the production of affordable products that can reduce the need of costly agrochemicals and deleterious effect of diseases and weeds thus promoting agricultural productivity (Wandui et al., 2013).

For example, to embark on the problem of lack of free-disease planting material and rapid crop multiplication, tissue culture practice is now a common practice in most African countries including Rwanda. In Rwanda, there are a number of laboratories that are involved in multiplication of banana, pineapple, potato and coffee plantlets (Gahakwa et al., 2012). It has been done so because the demand of these products were high and it has become a source of high income for these plant growers. The practice now is targeting the small-scale farmers and it is hoped to increase productivity, therefore, contributing to the food security and poverty eradication. Choosing the right high productive and reliable breed of cultivated plants is very recommended and should be more exploited by all sectors involved in agriculture (Musoni, 2016; Muvunyi et al., 2017).

In Rwanda, some of them are to master the novel traditional biotechnology method: tissue culture techniques and aeroponics that can help in the multiplication of different essential plants. Adapting to tissue culture was because the above mentioned crops are among the most important in the country and are daily affected by numerous challenges.

DNA molecular markers are also among the biotechnological techniques that can be applied in various forms to construct linkage maps of different crops thereby locating the particular gene of relevance to the improvement of the quality of crops; It can also influence rapid crop and livestock breeding production. Mapped markers are useful in speeding up selection of traits for use in conventional cross-breeding procedures (Ndiritu, 1999). In common bean improvement, some efforts are

also going on at RAB (Rwanda Agriculture Board) to improve efficiency in developing multiple constraint resistance and marketable bean varieties in Rwanda using marker assisted selection (MAS), and, to implement and strengthen capacity of scientists and technicians in applying MAS technologies (Annuarite and Alice, 2018; Tamara et al., 2018). With MAS, Pythium root rot resistance genes have been successfully introgressed in certain Rwandan popular bean varieties (Nzungize et al., 2011). Other MAS programs in common beans to produce the beans varieties resistant to Bean Common Mosaic Necrotic Virus are also being tried by Scientist at RAB (Worrall et al., 2015). The potential of MAS technologies to produce sweet potatoes and cassava varieties resistance to different viruses are also being tested in RAB (Munganyinka et al., 2017; Njeru et al., 2008). Even though the need to use biotechnology programs and its applications to benefit the people is urgent, a number of critical elements have to be reviewed because this new agriculture technology is very sophisticated, expensive and location-specific; therefore, policymakers have to set priorities that favor the growth of agricultural biotechnology industry (Hautea and Escaler, 2004). Funds need to be allocated to research to try and test both conventional and modern agricultural methods. Crops that are most affected by diseases and environmental challenges should gain more interest.

Plant biotechnology has increased the quality and quantity of agricultural production industry in developed countries as well as in developing countries for those who have chosen to use plant biotechnology products (Mackey, 2003). It has dramatically increased farm income, and has allowed the insertions of genes with desirable agriculture characteristics from one organism to another, which includes: increased level of proteins, fat and carbohydrates levels and stimulation of post-harvest maturations of plants. All biotechnology resources cannot be used to solve the current Agri-related problems in Rwanda. Policymakers should identify which technology can and cannot benefit the farmers and should also be aware that biotechnology advancement is not a short income process, is costly, and its benefits might not be noticeable in a short-term period.

REASON FOR CONTROVERSY ABOUT ADAPTING TO GM CROPS

Africa's agricultural development and growth have been slowing down for a long time now even though most biotechnologists prefer GMO crops over conventional crops and claim that GMO crops are potentially healthier and more productive (Huang et al., 2003); also, there are so many claims now that biotechnology products can revolutionize not only agriculture but also medicine and environmental problems. Conversely, critics of biotechnology argue that GMO crops might affect human health and damage the environment and that it might be little or nothing to facilitating the elimination of poverty in developing countries. Regardless of critics that are associated with GMO crops, their increase since 1990 has not diminished at all; from 1996 to 2010, it exceeded 1 billion hectares which are equivalent to the total area of USA or China, which demonstrate that biotech crops will be here for a long time (Clive, 2009).

The reason why farmers in most developed countries have adopted the use of GM crops is because they have seen a very positive income. Adopting GM crops will come with a lot of tangible benefits cutting down the number of herbicides, fungicides and other chemicals to control pests. With the concern and critics of GM crop's security, the technology has not stopped and continues to prosper in developing countries whereby now more precision technology to transform are in place. Whereas technologies like CRISPR/Cas9 allows scientist to target very specifically a desired loci in the plant genome, this technology allows making the tiny changes, therefore, eliminating the concerns of leaving exogenous DNA in the plant or another fingerprint (In Stewart, 2016). Using this particular technology will benefit both large and small scale farmers by both growing economy and employment rates as it has been the case in the US and Argentina (Burachik, 2012).

Applying biotechnology is looked up as costly, requires expertise, hard to accomplish, high technology-based, and argued that it probably comes with high risk to human health (FAO, IFAD, UNICEF, 2017); however, it was proved to improve and generate high production rate of inexpensive food for developed countries, and it has helped these countries in many ways to fight and eliminate the hunger and malnutrition problem (Wang and Zhang, 2001). Only a few African countries have managed the production of transgenic plants. In Tanzania, Mikocheni Agricultural Research in Dar es Salaam, a plant virologist has genetically transformed cassava to resist potential viruses like cassava mosaic virus though the products are still in field trials (Guardian Weekly, 2013); and for the moment, only three countries in Africa have GM crops that have reached the commercial level (Clive, 2009). While the effect of GM crops has positively affected the economic growth of developed countries by reducing the cost and introducing better farming practices that benefit both the farmers and the environment; in developing countries, it has not yet been achieved, and the quantity, quality, and safety of foods are currently the primary needs of the people. The great importance of plant biotechnology tools combined with other agricultural tools can solve hunger problem.

CONCLUSION AND RECOMMENDATIONS

Considering the potential benefit that plant biotechnology holds, it should be considered in the framework of the agricultural sector at large perceiving scientific, technical, regulatory, socio-economic and political evolution (Heffer,

2000).

To take a step further in the development of agriculture, hard choices from policymakers, government officials and the citizens have to be made because the economy of most developed and developing countries main drive is agriculture. Thus, it will be very wise to allocate necessary funds for experimentation and research of applicability of modern biotechnology programs: tissue culture, genetic engineering, use of GM crops, use of plant molecular markers especially in developing countries since the demand to apply that technology will always be high, and the future of agriculture will definitely depend on modern plant biotechnology. Biotechnology programs that deal with agriculture and health problems of the people should be supported and promoted.

To revolutionize plant biotechnology industry in Rwanda and Africa as a whole, initiatives to build strong long-term policies to promote this technology starting by training individuals and increasing the scientific capacities and infrastructures that specializes in plant biotechnology should be recommended. Rwandan government should reinforce its current agricultural policies: documenting the available plant breeds by increasing the number of community gene bank and installing proper research units in the whole country, renovating and improving the current plant breeding techniques and training the new generation of plant breeders, limiting the use of agrochemicals to protect the environment, soil management, plan for irrigation in cases of irregular rainfall, and of course implementation of plant biotechnology to ensure a substantial future agriculture are all among the few recommendations to enhance farmers' agriculture productivity. As for the production of modified food crops, it has allowed the production of improved crops resistant to disease and with improved resistance to environmental factors and their stability. The production of transgenic crops holds great promise for improved quality food crops and low production of pharmaceuticals and disease-free strains. Although this new technology can be useful to overcome different problems that agriculture faces in Rwanda, practicing this technology for the moment requires debatable and ethical considerations before full application.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Is the introgression of Lobi/Baoulé cattle by zebuine genes in Burkina Faso Lobi cattle threatened?

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Burkina Faso livestock is made up of two main cattle population, namely Zebuine and Taurine. Transhumance and settlement of Zebu cattle breeders in tsetse challenged areas lead to cross-breeding Zebu and Taurine. Introgression of the Zebu cattle may have changed the structure of the trypanotolerant Lobi/Baoulé breed. The objective of the present study was to appreciate the introgression of Zebu genes into Baoulé population by assessing the structure and the genetic diversity of cattle populations across the tsetse belt in Burkina Faso. Therefore, 450 blood samples were taken for genotyping in 29 villages of 3 main regions where Baoulé, Baoulé×Zebu and Zebu populations are found. Twenty five loci of 22 autosomes have been genotyped. The mean of observed alleles per locus was 12.44±4.31 while the mean of expected alleles was 4.67±1.48. The heterozygosity ranged from 0.34 to 0.76 and 0.36 to 0.87, respectively for observed and expected heterozygosity across loci. The average heterozygosity across population was 0.73±0.10. The mean estimates of *F*-statistics were F_{IS} = 0.117±0.019, F_{IT} = 0.158±0.019 and F_{ST} = 0.047±0.005. The phylogenetic tree showed the Baoulé South-West segregating apart from the other populations, Baoulé×Zebu being an intermediate genetic group between Baoulé South-West and Zebu North populations. The Baoulé West could not be differentiated from crosses. The Baoulé breed seems to be impacted by the introgression of Zebu genes to its biotope and pure Baoulé seems to be confined to the South-West with very few pure individuals in the West.

Key words: Burkina Faso, Zebu, Baoulé×Zebu, Baoulé, introgression, microsatellite.

INTRODUCTION

African cattle populations are said to be originated from 2 wild aurochs populations (Loftus et al., 1994, 1999; Bradley et al., 1994). *Bos taurus* (taurine), the humpless descendants of aurochs were domesticated in either the Near East or on the African continent (Epstein, 1971; Clutton-Brock, 1989; Bradley et al., 1996; Hanotte et al., 2002). Several investigations indicated that African Zebu cattle are an admixture of *Bos indicus* and *B. taurus* (MacHugh et al., 1997; Hanotte et al., 2002). Analysis of mitochondrial DNA sequences and microsatellites loci indicate that *B. indicus* may have diverged from *B. taurus* (Bradley et al., 1996; MacHugh et al., 1997; Hanotte et al., 2002). In West Africa, cattle populations are representative of both shorthorn (*B. taurus brachyceros*) and longhorn (*B. taurus longifrons*) humpless taurines, humped zebus (*B. indicus*) and Zebu/Taurine cattle (Gautier et al., 2009).

In Burkina Faso, indigenous cattle are very important for the subsistence and economic development of the country. These indigenous cattle provide essential food products, draft power, manure, and income for rural people. Indigenous breeds are well adapted to local environment thus they have developed disease tolerance and adaptation to harsh climatic conditions. This adaptation favoured the survival under stresses and exploitation of poor quality feeds stuff (Sodhi et al., 2005; Gautier et al., 2009).

With different drought episodes in 1973 and 1983 that occurred in Burkina Faso (Paturel et al., 1998) and the shift of the Northern limit lines of tsetse flies (Courtin et al., 2010) there has been an introgression of Zebu cattle genes through the movement of pastoralist people in the tsetse challenged areas (Grace et al., 2007) seeking for grass and water for livestock. In addition, some of the transhumant livestock keepers settled for long in the tsetse challenged areas rearing and crossing the Zebu breed to the local taurine to control the recurrent trypanosomosis disease. Local mixed livestock-crops farmers crossbreed also the Zebu to the local taurine since the 1920s to 1930s (Grace, 2005) to get hybrid animals that are used as draught animals. The intermediate sized animal is preferred because the local taurine is smaller and less powerful. These trends may have changed the structure of the populations in the tsetse challenged zones. It was therefore important to ascertain the introgression of Zebu genes into Baoulé breed in order to help guide decisions on improvement and conservation priorities. This is especially necessary owning to the husbandry systems practiced by local livestock farmers, which may affect diversity levels through high gene flow between breeds.

MATERIALS AND METHODS

Animals

1045 blood samples were taken from animals belonging to Baoulé also named Lobi cattle, Zebu and crossbred of Baoulé×Zebu cattle populations (471 males and 574 females) out of which 450 samples have been randomly selected as per location for genotyping. The animals have been sampled in 29 villages of three different regions and different altitudes. The North (5 villages) being a tsetse free region, the South-West and the West are tsetse challenged regions (Figure 1). Tsetse free is in upper part of the map and separated from the tsetse challenged regions by the northern limit lines of *Glossina tachinoides*, *Glossina morsitans submorsitans* and *Glossina palpalis gambiensis*

Six populations have been considered in the analysis (Table 1); Zebu of the challenged areas have been merged (Other Zebu) due to low number (4) of Zebu samples in the West. Baoulé×Zebu population in the West was the biggest sample out of the six populations. That results from crossbreeding the 2 main breed (Zebu and Baoulé) to control trypanosomosis disease in the tsetse challenged areas of Burkina Faso in general. Crossbreed population had the highest size in the genotyped sampled (158). On the other hand, the trypanotolerant Baoulé was more important in terms of size in the South-West than the other regions.

DNA extraction

Whole blood of each individual was dropped onto a Whatman FTA card according to Whatman protocol BD09. The samples were kept in multi-barrier pouch till punching day.

Three millilitres diameter Harris punch has been used to remove sample discs from the spotted cards. Genomic DNA was isolated according to a modified protocol of Whatman (Soudré, 2011).

DNA amplification

Microsatellites (31) primers were chosen for the amplification of the genomic DNA. 15 were donated by the International Livestock Research Institute, Nairobi, Kenya. PCR conditions were optimized and all the 31 microsatellites tested for polymorphism. A final panel of 25 polymorphic microsatellites has been used for genotyping of the cattle populations (BM1818, BM1824, BM2113, CSSM066, ETH3, ETH10, ETH185, ETH225, HAUT24, HAUT27, HEL1, HEL5, HEL9, HEL13, ILSTSS005, ILSTS006, INRA023, INRA032, TGLA53, TGLA122, TGLA126, TGLA227, AGLA293, ILSST033 and MGTG4B). Microsatellites were selected combining information from both the National Centre for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/) database and BOVMAP (http://locus.jouy.inra.fr/cgi-bin/bovmap/intro2.pl) covering 22 autosomal chromosomes regions. PCRs were then performed using Applied Biosystems GeneAmp® PCR System 9700 thermal cycler.

Genotyping process

The PCR products were diluted 1/10 in distilled water, and

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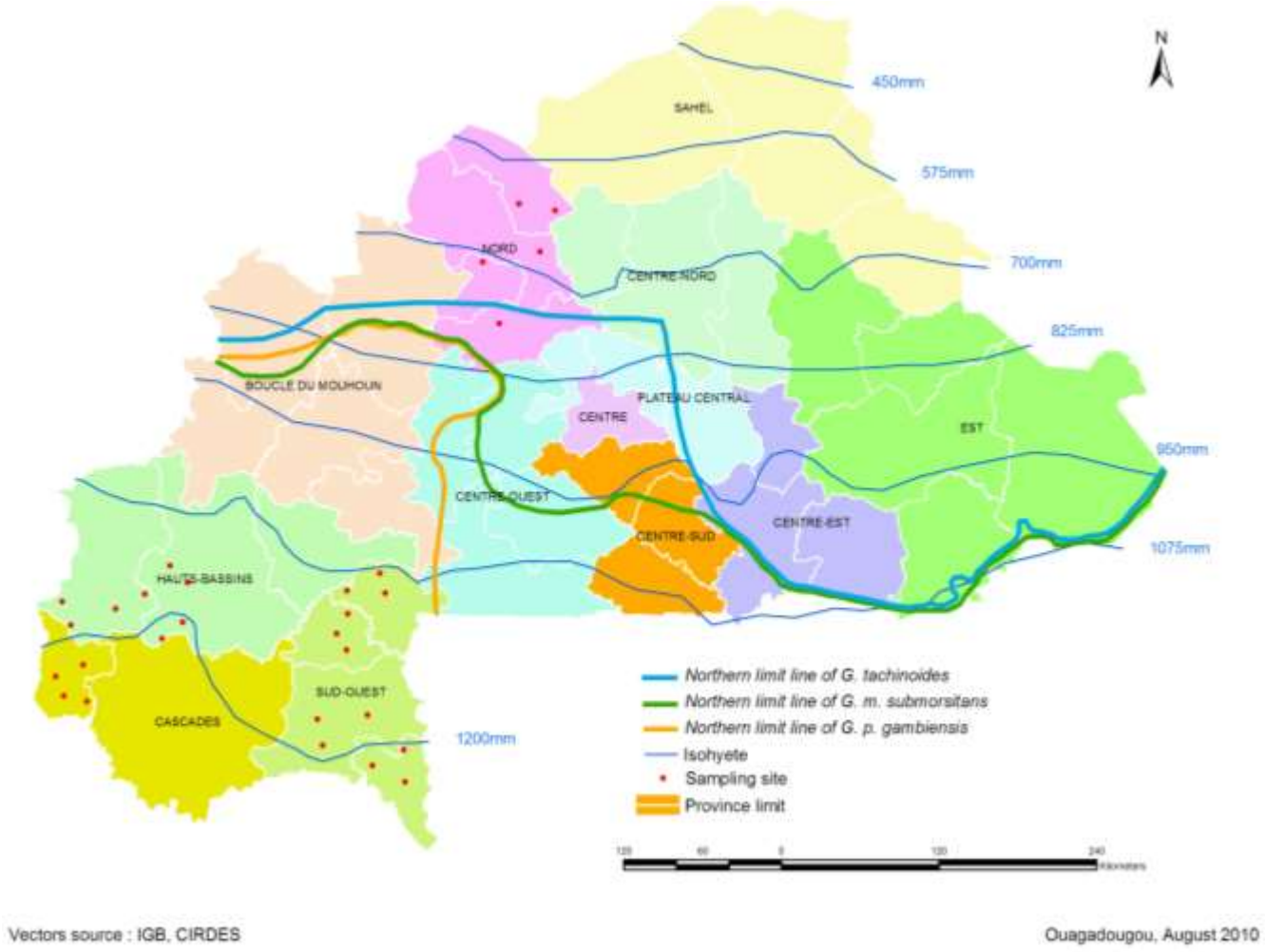

Figure 1. Northern limit lines of different tsetse flies species in Burkina Faso.

genotyping was performed on MegaBACE^{TM} 500, fluorescence-based DNA system utilizing capillary electrophoresis. Alleles were called and scored under MegaBACE^{TM} Genetic Profiler Software Suite v2.2 system.

Statistical analysis

Estimates of total number of alleles, mean number of alleles, effective number of alleles, observed heterozygosity (*Ho*), and unbiased gene diversity (expected unbiased heterozygosity, *He*) for each population were obtained with POPGENE program version 1.31 (Yeh et al., 1999). *He* the most common measure of variability (Petit et al., 1998; Caballero and Toro, 2002) was estimated using the algorithm of Levene (1949), which is the same as Nei's (1987) unbiased heterozygosity. Convert package version 1.31 (Glaubitz, 2004) has been used to determine the allele frequencies and detect breed-specific alleles.

Deviations from Hardy-Weinberg Equilibrium (HWE) probability exact test with unbiased exact *P*-value of Guo and Thompson (1992) was performed using GENEPOP package version 4.0.10 of Rousset (2008) according to the Markov Chain parameters, dememorization (1000), batches (100), and iteration per batch (1000).

Using the variance-based of Weir and Cockerhan (1984), *F*-statistics (F_{IS}, F_{IT}, F_{ST}) for each locus and overall values were calculated using FSTAT version 2.9.3.2 (Goudet, 2002). Significance tests on the estimates *F*-statistics for each microsatellite locus were obtained by constructing 95 and 99% confidence intervals based on the standard deviations estimated by jackknifing across populations using FSTAT.

POPULATION 1.2.30 software (Langella, 1999) was used to construct a phylogenetic tree of populations with bootstrap on locus using Reynolds et al. (1983) least squares. That was run using UPGMA and 1000 trials. The tree was visualized with TreeView 1.6.6 software (Page, 1996).

The Bayesian clustering method, as implemented by the STRUCTURE 2.3.3 program (Pritchard et al., 2000; Falush et al., 2003, 2007; Hubisz et al., 2009) was run five times with burnin period of 5.10^4 iterations followed by 10^5 number of MCMC repeats after burnin assuming k=2. The admixture model was used with the sampling locations as a prior. Correlated allele frequencies model was used as well. The clustering has also been performed with Bayesian Analysis of Population Structure (BAPS) package version

Table 1. Allele numbers, heterozygosity, deviation from HWE, intra-population diversity the 6 populations.

Breed/Population	N	TNA	MNa	MNe	Ho	He	HWE	F_{IS}
Zebuine	**117**	**229**	**9.56 (3.03)**	**4.22 (1.19)**	**0.66 (0.11)**	**0.774 (0.11)**	**-**	**0.102**
Zebu North	66	218	8.72 (2.96)	4.20 (1.15)	0.65 (0.13)	0.74 (0.13)	4	0.116
Other Zebu	51	205	8.20 (2.40)	4.02 (1.29)	0.67 (0.11)	0.73 (0.11)	2	0.084
Taurine	**145**	**284**	**10.04 (3.45)**	**3.80 (1.46)**	**0.60 (0.13)**	**0.70 (0.13)**	**-**	**0.121**
Baoulé South-West	124	239	9.56 (3.44)	3.56 (1.31)	0.60 (0.14)	0.68 (0.14)	6	0.122
Baoulé West	21	176	7.04 (2.09)	4.03 (1.40)	0.65 (0.15)	0.74 (0.11)	1	0.120
Crosses	**158**	**364**	**11.20 (3.96)**	**4.69 (1.39)**	**0.67 (0.11)**	**0.77 (0.09)**	**-**	**0.122**
Baoulé×Zebu South-West	35	205	8.20 (2.87)	4.28 (1.26)	0.63 (0.17)	0.75 (0.12)	5	0.157
Baoulé×Zebu West	153	270	10.80 (3.82)	4.70 (1.45)	0.68 (0.10)	0.77 (0.08)	8	0.114

N, Sample size; TNA, total number of alleles; MNA, mean number of alleles observed; MNE, mean number of effective alleles; *Ho*, observed heterozygosity; *He*, expected heterozygosity; (2.96), standard deviation; HWE, locus-population deviation (P<0.0001); F_{IS}, intra-population heterozygosity deficiency.

5.3 (Corander et al., 2008) that showed the same pattern as STRUCTURE.

RESULTS

Alleles (311) were detected from the 25 loci surveyed, giving a mean number of alleles 12.44 ± 4.31 observed and mean of effective alleles number was 4.67 ± 1.48 (Table 1). The number of alleles ranged from 3 at CSSM066 to 22 at TGLA122 (Table 2). The lowest number of effective allele number was observed at CSSM066 (1.57) and the highest number at TGLA53 (7.65). The total number of alleles per population ranged from 176 to 270 alleles, respectively for Baoulé in the West and Zebu Baoulé in the West. The lowest number of alleles in Baoulé West population may be due to the small size of the Baoulé population (21) from this region in the sample. The total number of allele in taurine was somehow higher than in Zebuine may be because most of the primers are taurine based designed. Many have been designed from Hereford for example.

Some 7% of the total alleles have been detected as breed-specific alleles using Convert package, 12 private alleles of Zebu at 11 loci and 10 private alleles of Baoulé at 9 loci.

For loci in the study, 14 of them have deviated from HWE (P<0.0001 using the probability exact test of Fisher) as shown in Table 2; markers displaying a highly significant deviation from HWE are in italics. Furthermore, deviations from HWE were statistically significant (P<0.0001) for 26 locus-population combinations. Out of the 25 loci analyzed in each population, 1 to 8 deviated significantly from HWE. All populations also deviated from HWE (P<0.0001) probably because of heterozygote's deficiency.

Expected heterozygosity has been generally higher than observed heterozygosity not only at marker level but also at population level. At population level, the most diversified population was the Zebu Baoulé in the West with the highest observed and expected heterozygosity (*Ho* = 0.68±0.10, *He* = 0.77±0.08) and the less diversified the Baoulé from the South-West (*Ho* = 0.60±0.14, *He* = 0.68±0.14). In the overall population, the crosses as expected are more diversified (*He* = 0.77±0.09) than the pure breeds (Zebu and Baoulé) followed by Zebu (*He* = 0.74±0.11). Observed heterozygosity ranged from 0.34 (CSSM066) to 0.76 (INRA032 and TGLA227). The most variable marker in this study was TGLA53 (*He* = 0.87) compared to CSSM066 (*He* = 0.36). The marker which contributed much to the variability was INRA032 and HEL 9 (Ave. Het. = 0.82). The mean number of migrants per generation for all loci estimated based on the formula Nm = $0.25(1-F_{ST})/F_{ST}$ as implemented in POPGENE was 6.06.

Baoulé×Zebu population from the South-West showed the highest value of F_{IS} (0.157). Comparisons of F_{IS} of the 3 groups were not statistically significant as well as the comparisons of the F_{ST} values.

The locus ETH10 (0.121) contributed the most to the population differentiation. But the overall F_{ST} being < 0.15, the population differentiation seems to be moderate. The mean global F_{ST} ranged from 0.012 (MGTG4B) to 0.121 (ETH10) among different microsatellite loci with an estimated mean value of 0.047 (P<0.01), indicating 4.7% of the total variation being attributed to between breed differences.

A neighbor-joining dendogram constructed based on unbiased genetic distances showed 2 main clusters, one cluster composed of Baoulé South-West and the second

Table 2. Alleles number per locus, observed, expected, *P*-values, *F*-Statistics (Weir and Cockerhan, 1984), standard errors for each locus across populations.

Loci	NA	NE	Ho	He	P-value§	F_{IS}	F_{IT}	F_{ST}
BM1824	9	3.45	0.65	0.71	0.0476	0.059 (0.045)	0.098 (0.036)	0.042 (0.033)
BM2113	17	6.19	0.67	0.84	0.0000	0.173 (0.046)	0.227 (0.083)	0.063 (0.051)
INRA023	11	4.52	0.70	0.78	0.0000	0.056 (0.035)	0.114 (0.072)	0.060 (0.046)
MGTG4B	15	5.12	0.73	0.81	0.0000	0.084 (0.017)	0.095 (0.017)	0.012 (0.007)
AGLA293	15	5.84	0.74	0.83	0.0090	0.074 (0.014)	0.124 (0.022)	0.055 (0.037)
ETH10	10	4.75	0.65	0.79	0.0000	0.099 (0.037)	0.211 (0.112)	0.121 (0.096)
ILSTS006	9	3.47	0.52	0.71	0.0000	0.266 (0.050)	0.292 (0.038)	0.036 (0.028)
HEL9	11	6.18	0.75	0.84	0.0000	0.085 (0.036)	0.114 (0.050)	0.032 (0.028)
ETH225	13	3.96	0.65	0.75	0.0020	0.064 (0.029)	0.148 (0.048)	0.092 (0.072)
ILSTS005	9	3.01	0.60	0.67	0.0004	0.076 (0.025)	0.109 (0.047)	0.035 (0.032)
INRA032	17	5.96	0.76	0.83	0.0086	0.068 (0.010)	0.094 (0.023)	0.029 (0.019)
HEL13	7	3.01	0.58	0.67	0.0167	0.061 (0.025)	0.141 (0.072)	0.085 (0.061)
ILSTS033	12	3.19	0.54	0.69	0.0000	0.153 (0.076)	0.241 (0.125)	0.100 (0.076)
CSSM066	3	1.57	0.34	0.36	0.0181	0.051 (0.134)	0.159 (0.169)	0.106 (0.045)
HEL1	8	5.04	0.44	0.80	0.0000	0.417 (0.039)	0.466 (0.063)	0.082 (0.058)
TGLA53	19	7.65	0.60	0.87	0.0000	0.278 (0.025)	0.321 (0.041)	0.059 (0.043)
ETH185	13	3.85	0.63	0.74	0.0000	0.148 (0.034)	0.163 (0.028)	0.018 (0.011)
TGLA227	16	6.07	0.76	0.84	0.0000	0.055 (0.007)	0.107 (0.042)	0.055 (0.041)
ETH3	11	3.36	0.69	0.70	0.7610	-0.005 (0.011)	0.028 (0.027)	0.033 (0.025)
TGLA126	9	4.40	0.75	0.77	0.0722	0.023 (0.019)	0.044 (0.024)	0.021 (0.016)
HEL5	12	5.41	0.67	0.82	0.0000	0.153 (0.034)	0.168 (0.038)	0.018 (0.011)
TGLA122	22	3.30	0.65	0.70	0.0000	0.061 (0.042)	0.083 (0.057)	0.022 (0.017)
HAUT24	19	6.90	0.71	0.86	0.0000	0.133 (0.047)	0.177 (0.033)	0.052 (0.017)
BM1818	12	6.33	0.74	0.84	0.0068	0.087 (0.028)	0.133 (0.023)	0.050 (0.033)
HAUT27	12	4.32	0.65	0.77	0.0003	0.127 (0.037)	0.163 (0.031)	0.042 (0.017)
Total	311	-	-	-	-	-	-	-
Mean	12.44	4.67	0.65	0.76	-	-	-	-
St. Dev	4.30	1.48	0.10	0.10	-	-		-
Overall	-	-	-	-	-	0.117 (0.019)**	0.158 (0.019)	0.047 (0.005)*

NA, Observed number of alleles; NE, effective number of alleles; *Ho*, observed heterozygosity; *He*, expected heterozygosity; §Fisher's probability exact test across all populations with deviations from HWE (P<0.0001); *, 95% confidence interval. **, 99% confidence interval.

being composed of the remaining populations (Figure 2). In the second cluster, the populations clustered further into 3 genetic groups; the first group had Baoulé×Zebu South-West and Other Zebu. That group had the smallest genetic distance (D_A = 0.0434 in Table 3). The last group had only Zebu North. The unbiased genetic distance between Baoulé South-West and Zebu North was the longest one (D_A = 0.3390). A phylogenetic tree (Figure 2) supports it with a bootstrap value of 75%. The bootstraps showed the Baoulé South-West segregating from the other populations with 100% of replicates.

Using STRUCTURE, the most likely K is that where ln Pr(G/K) is maximized. The maximum value of ln Pr(G/K) was obtained at K = 2 (Figure 3), that provided an explanation of the genetic structure and levels of admixture for the populations. This assumption has been supported by farmers' assumption as well about clusters on the field. The clusters shown in Figure 2 have been confirmed using BAPS program.

DISCUSSION

The genetic diversity of Burkina Faso cattle populations sampled from different regions across the country was assessed. The mean number of observed alleles was almost similar to the 11.4 alleles per locus reported by Loftus et al. (1999) but considerably higher than the 8.4 reported by MacHugh et al., (1997), 9.7 reported by Thévenon et al. (2007) in the Southern-West of Burkina Faso, 4.59 and 4.37 reported in Pakistan breeds by Rehman and Khan (2009), 7.11, 7.41 and 6.74 reported in Arabic Zebu, Bororo Zebu and Kuri cattle, respectively (Grema et al., 2017). This difference may reflect an

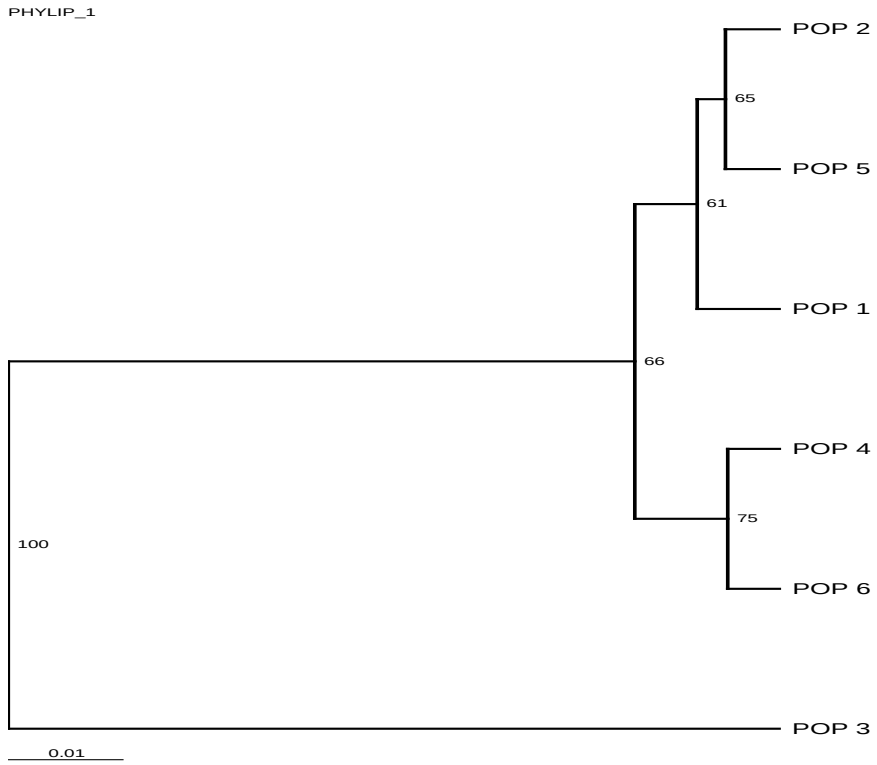

Figure 2. Neighbor joining tree summarizing genetic distances among 6 cattle populations (POP 1: Zebu North; POP 2: Other Zebu; POP 3: Baoulé South-West; POP 4: Baoulé West; POP 5: Baoulé×Zebu South-West; POP 6: Baoulé×Zebu West). Bootstrap values indicating the degree of support for each branch point are shown beside the node as the percentage of replicates in which the cluster to the right of the node was recovered.

Table 3. Nei's (1978) unbiased genetic identity (above diagonal) and genetic distance (below diagonal).

Parameter	Zebu North	Other Zebu	Baoulé South-West	Baoulé West	Baoulé×Zebu South-West	Baoulé×Zebu West
Zebu North	-	0.9500	0.7125	0.8868	0.9425	0.9362
Other Zebu	0.0513		0.7469	0.8974	0.9575	0.9384
Baoulé South-West	0.3390	0.2918	-	0.8562	0.8456	0.8272
Baoulé West	0.1202	0.1083	0.1552	-	0.9209	0.9569
Baoulé×Zebu South-West	0.0592	0.0434	0.1677	0.0824	-	0.9548
Baoulé×Zebu West	0.0659	0.0636	0.1897	0.0441	0.0462	-

unexpected bias in the selection of the loci but also the absence of selection pressure in cattle in Burkina Faso. In such a situation, direct comparisons may not be possible because of the markers sets and techniques used. Compared to Thévenon et al. (2007), the results were almost similar when considered only the mean number of alleles per locus from tsetse challenged area from where samples were taken as well.

In Burkina Faso, Zebu population is known to consist of Fulani Zebu, M'Bororo Zebu, Azawak Zebu originated from Niger, and a few years ago Gudali Zebu which was formally from Nigeria. Individuals from these Zebu types are thought to be included. Also, the analyses showed that a certain number of animal migrated per generation in the present populations. That is very common in diversity studies and it may be due to cattle movement along with human. In Burkina Faso, it may be due to the transhumance. The deviation in Baoulé population may

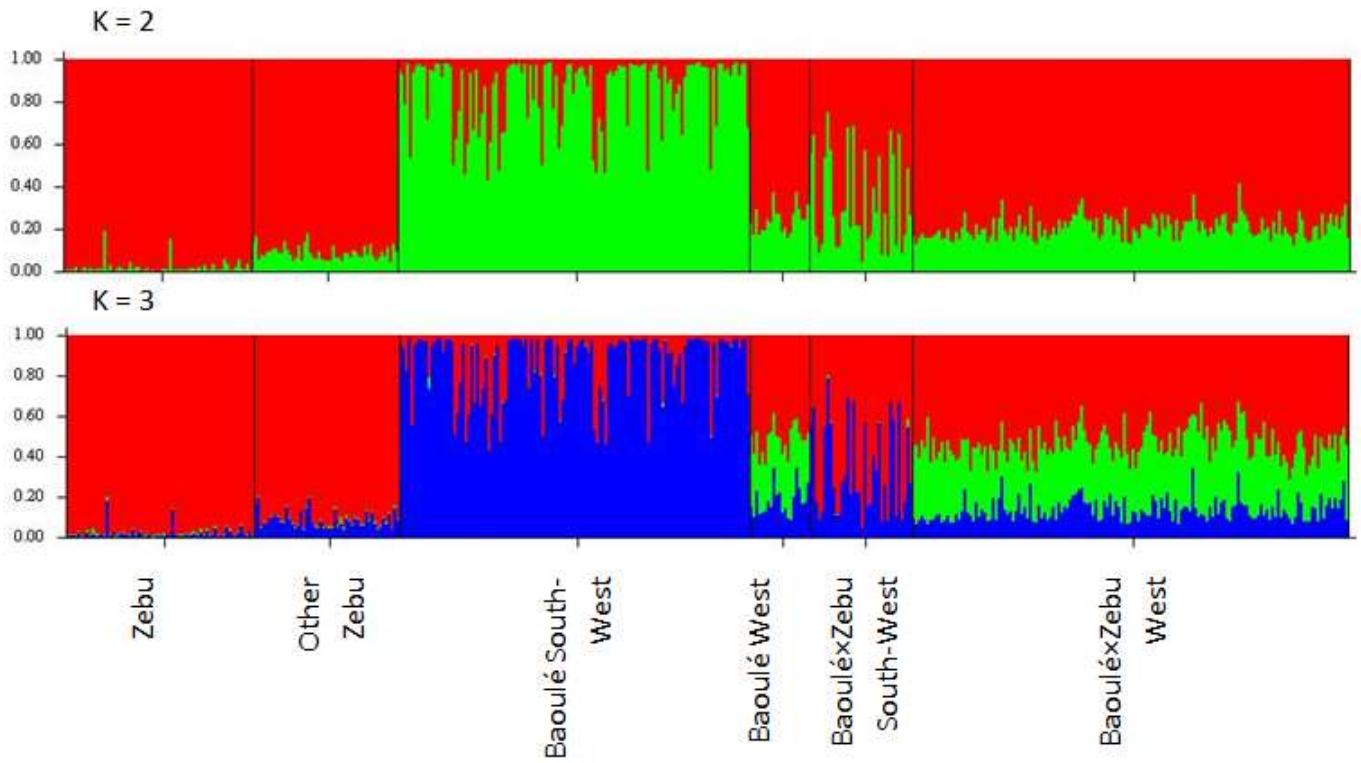

Figure 3. Genetic structure of the six cattle populations. Each individual animal is represented by a single vertical line divided into K colours, where K is the number of clusters assumed and the length of the coloured segment represents the individual's ancestry proportion of membership to a particular cluster estimate. Black lines separate individual populations whose names are indicated below the diagram.

result from misclassifying N'Dama type or their cross bred in Baoulé type. N'Dama cattle were present in the South-West in the International Centre for Research and Development in Animal Husbandry in Subhumid Zones (CIRDES) research farm for scientific experiences and some individuals have been introduced in farmers' herds. One more reason of departure from HWE could be the admixture linkage disequilibrium, the correlations that arise between linked markers in admixed populations, as described by Falush et al. (2003).

Observed and expected heterozygosity across populations were similar or comparable to those reported by Moazami-Goudarzi et al., (1997), Ibeagha-Awemu et al. (2004) in West and Central African cattle populations, Sodhi et al. (2005) in Indian cattle populations, Zerabruk et al., (2007) and Dadi et al. (2008) in Ethiopian indigenous cattle populations. But lightly different from Martin-Burriel et al. (2007) may be because the populations in the study were endangered. Average heterozygosity was within the range of 0.3 to 0.8 as suggested by Takezaki and Nei (1996) to be useful for measuring genetic variation. The overall F_{ST} revealed a moderate level of genetic differentiation among the populations in the study. The overall value of F_{ST} observed is similar to that observed in Ankole cattle in Uganda (Kugonza et al., 2010), lower than that reported in 2 Indian cattle populations (Sodhi et al., 2005) greater than that observed in Ethiopian populations (Dadi et al. 2008), in Ankole cattle in the African Great lakes region (Ndumu et al., 2008). The moderate genetic differentiation could be a result of gene flow from other populations. In the Northern part the animals are reared without any trypanosomosis pressure therefore there is less or no crossing with the taurine breed. But in the tsetse challenged regions where trypanosomosis is the most important disease in cattle (Soudre et al., 2009) crossbreeding is frequent. In addition, the pastoral production systems, long distance migrations within and across countries, utilization of communal pastures, exchange of breeding animals, uncontrolled mating facilitate constant gene flow.

Conclusion

Moderate genetic differentiation among indigenous cattle populations in Burkina Faso across the loci makes possible to use these breeds to improve the genetic for

production and conservation and diversity in general. Regarding trypanosomosis, it may help to improve the tolerance of Zebu breed to trypanosomosis in the tsetse infested regions. Added to these advantages, little is known about the genetic diversity, structure and degree of admixture among Burkina Faso cattle populations. This supports the statement of Hanotte and Jianli (2005), knowledge of both the global diversity of the breeds and admixture events will be needed in order to be able to make sound priority decisions. Actions should be drawn to conserve the Baoulé breed which is threatened by the introgression of Zebu breed to its biotope. The Baoulé West as reported by the study cannot be differentiated from the crosses. The introgression of Zebu in the Southern areas of Burkina Faso will be perhaps more important with the climate change.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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