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Application of Biotechnology in Animal Genetics and Breeding

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Abstract

Biotechnology have contributed immensely in increasing livestock productivity and can help to alleviate poverty and hunger and ensure environmental sustainability in sub Sahara Africa. A wide range of biotechnological techniques are available and have already been used in animal genetics and breeding. The progress of genomic information increases the accuracy of selection and has a great impact on generation interval and more comprehensively rapidly replacing the traditional quantitative genetic approaches. Biotechnology has the potential to improve the productivity of animals through increase in growth rate, carcass quality, reproduction, improved nutrition and feed utilization, safety of food, improved health and welfare of animals and reduced waste through more efficient utilization of resources. Biotechnology applied in animal breeding and genetics are mainly geared towards increasing breeding efficiency of livestock especially within organized breeding schemes and animal genetic resources sustainability and maintaining genetic diversity, a major goal in animal breeding. Biotechnology development requires a high level of commitment to research and development, expensive equipment, heavy machines, expensive chemicals as well as high level of technological expertise. Not all available biotechnologies are appropriate or relevant in Sub Sahara Africa, there is need to adapt some of these technologies, develop capacity to maintain a strong base of applied in adaptive research centers and some level of training to keep abreast with new developments and to adapting such technology to local conditions so as to ensure sustainability and awareness to the rural farmers. The present paper is to review the molecular technologies in animal genetics and animal breeding.

Keywords: Molecular biotechnologies; Animal genetics; Animal breeding; Molecular Markers; Genotype.

Introduction

Livestock production constitutes about 43% of the total value of agricultural production and this is expected to increase (Jutzi, 2003). In developing countries, it accounts for more than a third of agricultural GDP (FAO, 2006a). Fast growing population and urbanization is changing the lifestyle and purchasing patterns with respect to food products and the world's population is expected to grow to 7.5 billion people in 2020 with developing countries taking the lead (IFPRI, 2001; FAO, 2002). It is projected that the demand for livestock will nearly double by 2030 (FAO, 2002).

Developing countries are faced with the major challenge to increase agricultural productivity in order to help feed their rapidly growing populations without depleting the natural resource base. Biotechnology is therefore a vital means to meet this objective through addressing the production constraints of small-scale or resource-poor farmers who contribute more than 70% of the food produced in sub-Sahara Africa (Rege, 2016).

Animal Biotechnology is the application of scientific and engineering principles to the processing or production of materials by animals to provide goods and services for the wellbeing of human population (Bin-Abdullah, *et a*l., 2011), while molecular genetics is the study of the genetic makeup of individuals at the DNA level; it involves the identification and mapping of genes and genetic polymorphisms. Biotechnology has the potential to improve the productivity of animals through increase in growth rate, carcass quality, reproduction, improved nutrition and feed utilization, safety of food, improved health and welfare of animals and reduced waste through more efficient utilization of resources. Therefore, the biotechnology of livestock production is growing faster than any other sectors. By the year 2020, livestock production is predicted to become the most important agricultural sector in terms of

value-added commodity (Madan, 2005). Biotechnology applied in animal breeding and genetics are mainly geared towards increasing breeding efficiency of livestock especially within organized breeding schemes and animal genetic resources sustainability and maintaining genetic diversity, a major goal in animal breeding.

The selection and evaluation of different breeds started with the domestication of animal species around 12000 years ago which led by the wish to obtain traits dictated by social, nutritional and environmental needs without understanding of the molecular processes involved (FAO, 2011). The objective of this paper is to review the application of biotechnology in animal genetics and breeding in sub-Sahara Africa.

I. APPLICATION OF BIOTECHNOLOGIES

Genomic information in Selection

Knowledge about the genome of livestock is increasingly becoming valuable. This improves the genotype of the animals and in conjunction with knowledge of estimated breeding value (EBV) of the animal has significant impacts in animal genetics and breeding. The application of genomic information such as sequence or DNA marker polymorphism for the selection of farm animals need the knowledge of the effect of physically mapped genes with effects on economically important traits or quantitative trait loci (QTL) (Montaldo, 2006). Genome based selection of farm animals have great prospective to reduce the inbreeding rates (Dekkers, 2007) and can help in quantitative genetic trait architecture (Daetwyler *et al.,* 2010).

The genomic technology in livestock provides a major opportunity to address the challenges of agricultural production due to rapid increase in population. Animals form a distinctive genomics resource as a result of their significant- phenotypic diversity and of their population structure. The purpose of genomic technology is the characterization and mapping of the locus that affect the traits of interest provide a high quality and affordable source of protein for human. Since domestication, man has constantly modified the genomes of species through various selective breeding practices for characters, ranging from color, growth, composition and disposition (Koopaei and Koshkoiyeh, 2011). Animal genomics are very concerned with challenges in relation to the increase worldwide demand for high quality and healthy products, the prerequisite of sustainable economics and environments of breeding system and want to adapt to global changes (Charles *et al.,* 2010). Acquisition of standard knowledge on the structure and functioning of animal genome from molecular data using very efficient technologies has become a concern for researchers.

B. Molecular Marker Technologies

Molecular markers play a very vital role in livestock improvement through conventional breeding strategies. Different applications of molecular markers are short-range or immediate and long-range applications (Naqvi, 2007).

Molecular markers have now become a vital tool for the identification and characterization of animal species. According to FAO (2007), four countries in Africa (Nigeria, Cameroon, Chad and Togo) reportedly use molecular markers to characterize genetic resources. Molecular marker technology encompasses
protein based and DNA based techniques. The latter is generally preferred as compared to the earlier protein based markers because of showing low susceptibility to environmental or developmental influences. DNA markers correspond to a short sequence of DNA, the presence of which is linked to a desirable trait (Williams, 2005). Genetic markers can get a number of forms, polymorphic in nature which is associated with particular trait or genes (Williams, 2005). Molecular genetic markers are effectively used to estimate the genetic diversity within or between the breeds (Troy, et al., 2001; Hanotte, et al., 2002) and information concerning allelic variation at a given locus (Erhardt and Weimann, 2007). The use of genetic markers to illustrate the genetic makeup and forecast the performance of an animal is a powerful aid in animal breeding (Beuzen *et al.,* 2000).

In the last three decade number of marker techniques were consequently develop, in particular restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), randomly amplified polymorphic DNA (RAPD), microsatellite (simple sequence repeat), single nucleotide polymorphism (SNP) and mitochondrial DNA markers.

1). Restriction Fragment Length Polymorphisms (RFLP)

The Restriction Fragment Length Polymorphisms was the earliest form of deoxyribonucleic acid (DNA) marker used to build up the first true genomic map (Williams, 2005). This hybridization based marker technology uses synthetic oligonucleotides as probes, which are fluorescently labeled to hybridize DNA (Teneva, 2009). The RFLP technology was initially developed in 1980s (Botstein *et al*., 1980) and uses restriction enzymes that cut the DNA at specific site to visualize the differences at the level of DNA structure (Mburu

and Hanotte, 2005). Using RFLP differences are marked when the length of DNA fragments are different, it mean that the restriction enzymes (RE) cut the DNA at distinct locations. The change or polymorphism occurs due to mutation imply creation or eliminating of the RE site and make new RE site. The variations are identified by using a hybridization probe. Molecular biology lab apparatus gel electrophoresis is required for the identification of RFLP to separate the DNA fragments of different length to transfer the fragments to nylon membrane and radioactive labeled probe is used to visualize the DNA fragments exposed to an X-ray film (Lien, 2001).

2). Amplified Fragment Length Polymorphism (AFLP)

AFLP markers have found the widest application in analyses of genetic variation below the species level, particularly in investigations of population structure and differentiation. AFLP methods rapidly generate hundreds of highly replicable markers from DNA; thus, they allow high-resolution genotyping of fingerprinting quality. The time and cost efficiency, reproducibility and resolution of AFLPs are superior or equal to those of other markers (RAPD, RFLP and microsatellites) (Brumlop and Finckh, 2010). AFLP markers have emerged as a major new type of genetic marker with broad application in population genetics and quantitative trait loci (QTL) mapping (Mueller and Wolfenbarger, 1999). AFLPs are dominant bi-allelic markers (Vos *et al.,* 1995) and are unable to distinguish dominant homozygous from dominant heterozygous individuals (Vos *et al.,* 1995), the method is an ideal molecular approach for population genetics and genome typing, it is consequently widely applied to detect genetic polymorphisms, evaluate, and characterize animal genetic resources (Ajmone-Marsan *et al.,* 2007).

3). Random Amplification of Polymorphic DNA (RAPD)

Randomly amplified Polymorphic DNA (RAPD) marker is a polymorphic assay based on the amplification of randomly sequenced DNA sequences using primers with arbitrary nucleotide sequence. The RAPD marker uses 10bp random primers to locate random segments of sequenced DNA to show polymorphism (Lopes, *et al.,* 2007). In the last decade, the RAPD technique based on the polymerase chain reaction (PCR) has been one of the most commonly used molecular techniques to develop DNA markers (Kumar and Gurusubramanian, 2011). The RAPD technology provides a quick and efficient screening for DNA sequence based polymorphism at a very large number of loci. RAPD does not require pre-sequencing of DNA (Nandani and Thakur, 2014).

The RAPD analysis has been extensively used for various purposes which include identification and classification of accessions (Fukuoka *et al.,* 1992), identification of breeds (Qian *et al.,* 1997) and genetic diversity analysis (Cao and Oard, 1997). The principle of RAPD is that, a single, short oligonucleotide primer which binds to many different loci is used to amplify random sequences from a complex DNA template. This means that the amplified fragment generated by PCR depends on the length and size of both the primer and the target genome (Nandani and Thakur, 2014). RAPDs technique depend on its simplicity and independence of any prior DNA sequence information (Edwards and McCouch, 2007), having several advantages compared to RFLP (Lynch and Milligan, 1994), the disadvantage of RAPD markers is that polymorphisms are detected only at the presence or absence of a band of a certain molecular weight, with no information on heterozygosity besides being dominantly inherited (Brumlop and Finckh, 2010).

4). Microsatellite Marker

Microsatellite are short sequences made up of a simple sequence motif, 2-6 bases long tandemly repeated and arranged head to tail without interruption by any other base or motif. Microsatellites loci are also known as short tandem repeats (STR's), simple sequence repeats (SSR's) and simple sequence tandem repeats (SSTR). They are interspersed throughout genome. The repeated units vary among individuals and they have adequately high mutation rate. Microsatellite markers of choice in livestock are of many kinds with molecular applications including, genetic characterization studies (Wajid *et al.,* 2013), analysis of population structure (Arora and Bathia, 2004), estimate genetic variability and inbreeding (Mateus *et al.,* 2004), disease diagnostics, forensic analysis, development of genetic map, and marker assisted breeding (Montoya *et al.,* 2007; Ritz *et al.,* 2000 and Naicy and Anilkumar, 2008).

5). Single Nucleotide Polymorphism (SNP)

SNP is an important and efficient molecular marker reported to have many progresses in whole genome sequencing, in the development of next generation sequencing technologies and advances in these technologies have led to the foundation of the high density single nucleotide polymorphism (HD-SNP) arrays as an up-to-the-minute implement for the genetic and genomic analysis of farm animals (Fan *et al.,* 2010). The SNP have represented one of the most interesting advances in genotyping. They are abundant in the genome, genetically stable, found in both coding and non-coding regions, biallelic co-dominant marker and to high-throughput automated analysis (Vignal *et al.,* 2002; Stoneking, 2001). SNP present in coding regions can be associated

with the protein function and as the inheritance pattern is more stable, they can be more suitable markers for selection over time (Beuzen, *et al.*, 2000). The importance of SNP in a restriction enzyme recognition site is also confirmed by PCR-RFLP, used as genotyping procedure and the PCR product as a result of restriction enzyme cutting will generate varying fragments that can be analyzed by gel electrophoresis. Due to the appropriate coverage and density over the genome, SNP could contribute to as a tool for proficient genotyping, species identification and evaluation and for further analysis of population structure and genetics, exploring the genetic mechanism of complex agricultural traits, more amenable tool improving selection method for genetic improvement of farm animal production. Illumina (2010), released two new genotyping SNP chips including a low-density chip (Bovine3K) having 2,900 SNP and a high density chip (Bovine HD) with 777,962 SNP (Illumina, 2010), these chips can provide genotypes that enhance the precision of genomic evaluation by better tracking of the loci responsible for genetic difference (VanRaden and Tooker, 2010).

6). Mitochondrial DNA (mtDNA) Markers

The mtDNA is an extra-chromosomal genome in the cell mitochondria that resides outside of the nucleus, and is inherited from mother with no paternal contribution (Avise, 1994). Due to higher evolutionary rates of mtDNA relative to the nuclear genome (Wan *et al*., 2004), it is preferred in constructing phylogenies and inferring evolutionary history, ideal for within and between-species comparisons (DeYoung and Honeycutt, 2005). The disadvantages of mtDNA analyses include hybridization, introgression and incomplete lineage sorting. mtDNA is of little use in investigating the recent loss of genetic variation as well as individual-level events such as identity, individual dispersal, and mating systems (Wan *et al.,* 2004).

II. Reproductive Technology in Animal Breeding

Biotechnology has been used primarily towards increasing reproduction rates, as foremost concern over the years for scientist and researchers. Advances in assisted reproductive technologies (ART) like artificial insemination (AI), in vitro production, super ovulation (SO), embryo transfer ET), multiple ovulation with embryo transfer (MOET), transgenesis and cloning have become a vital tool in livestock breeding and have been introduced to overcome reproductive problems (Vikrama and Balaji, 2002). All these technologies speed up genetic changes due to shorter generation interval and improving accuracy in selection program (Anonymous, 1992). The aim of reproductive technology in animal breeding is to increase the accuracy of predicting the true genetic merit of breeding animals. (AI) and (ET) are probably the most well-known methods that have been adopted in developed and developing countries for livestock improvement (Kahi and Rewe, 2008). The recent advances in biotechnology in reproduction included production of transgenic animals and cloning (Smidt and Niemann, 1999). ART has effects on animal breeding as it increases the rate of reproduction and decrease the generation interval (Aminoor Rahman, *et al.*, 2008). The most successful reproductive technology like AI and ET necessitated their application to some large extent. Some emerging biotechnologies such as MOET, *in vitro* fertilization (IVF) and cloning also provide prevailing tool for rapidly changing the animal populations genetically.

1). Artificial Insemination (AI)

Artificial insemination is among the best biotechnological techniques that have been used to increase reproductive capacity and it has received widespread application in large farm animals, no other technology in agriculture, except hybrid seed and fertilizer use, has been so widely adopted globally as AI (FAO, 2006b).

Progress in semen collection and dilution, and cryopreservation techniques now enables a single bull to be used simultaneously in several countries for up to 100,000 inseminations a year (Gibson and Smith, 1989) and widely used both in developing and in developed countries, large number are performed globally each year, more than 100 million cattle, 40 million pigs, 3.3 million sheep and 0.5 million goats (FAO, 2006b).

However, although AI is widely available in developing countries it is used far less, particularly in Africa, than in developed countries. Its use has been limited largely to "exploratory" purposes mainly by research institutions. A few countries including Nigeria, Botswana, Ethiopia, Ghana, Malawi, Mali, Senegal and Sudan have taken the technology to the field, mostly for programmes of "upgrading" indigenous stock and as a service to a limited number of commercial farmers keeping exotic dairy cattle breeds and few others have used the technology more widely. Kenya and Zimbabwe for example, have elaborate AI systems which include national insemination services incorporating progeny testing schemes (Rege, 2016). The Republic of South Africa is probably the biggest user of AI technology in Africa in terms of number of inseminations, this country also has what is perhaps the best organized progeny testing scheme on the continent (Rege, 2016). However, success of AI technology depends on accurate heat detection and timely insemination. The former

requires a certain level of experience among farmers while the latter is dependent on good infrastructure, including transport network, and availability of reliable means of transport (Rege, 2016).

However, owing to a number of technical, financial, infrastructural and managerial problems, its applicability in Africa has not yet matched that of its success in the developed countries (Van, 2011).The conception rate in field AI programmes in sub-Sahara Africa is very low and therefore the desired effect in terms of animal improvement has not been achieved.

2).Multiple Ovulations and Embryo Transfer (MOET)

Multiple ovulation embryo transfer (MOET) is an advance technology which includes super ovulation, fertilization, embryo recovery, short - term in vitro culture of embryos, embryo freezing and embryo transfer (Rege, 2016). The reproductive cycle of ruminant female is such that the ovarian follicle of a non-pregnant female matures and releases single egg at a time. Normally ovulation occurs as a result of circulation of gonadotropin hormone. But by increasing the concentration of hormone the number of egg production gets increased. However, this depends on health, nutrition, breed of animals and environment in which they live.

However, benefits from MOET Include increasing the number of offspring produced by valuable females, increasing the population base on rare or endangered breeds or species, ex situ preservation of endangered populations, progeny testing of females and increasing rates of genetic improvement in breeding programme. Genetic improvement of ruminants in developed countries has made much progress in the last 35 or so years through the use of large-scale progeny testing of males.

Open nucleus breeding system (ONBS) may be especially valuable for developing countries where the use of AI has been a failure. The concept is based on a scheme with a nucleus herd/flock established under controlled conditions to facilitate selection (Smith, 1988). The nucleus is established from the "best" animals obtained by screening the base (farmers) population for outstanding females. These are then recorded individually and the “best” individuals chosen to form the elite herd/flock of the nucleus. If ET is possible, the elite female herd is used through MOET with superior sires to produce embryos which are carried by recipient females from the base population. The resulting offspring are reared and recorded and the males among them are evaluated using, as appropriate, the performance of their sibs and paternal half sibs and their own performance. From these, an elite group of males with high breeding values for the specific trait is selected and used in the base population for genetic improvement through natural service or AI. It should be noted that, while MOET improves the rate of progress substantially, it is possible to operate an ONBS without ET technology, especially in species, such as small ruminants, with high reproductive rates. Such schemes are being tried for sheep in West Asia by FAO (Jasiorowski, 1990) and in Africa (Yapi *et al.*, 1994). However, availability of AI and ET, in addition to increasing rates of genetic gain, enhances the flexibility of the system. For example, germplasm from other populations can be introduced easily through semen or embryos. One of the advantages of a nucleus herd is that it provides opportunity to record information on more traits than is possible in a decentralized progeny testing scheme.

Application of MOET in nucleus breeding schemes could increase animal genetic gains by 30-80% (Nicholas and Smith, 1983), average number of live progeny per donor flushed ranges 2-3 in sheep and cattle and 6-8 in goats (Macmillan and Tervit, 1990). Considerations of these figures suggest that MOET could increase annual genetic gains by 1020% in large nucleus breeding schemes (Rege, 2016).

However, costs of operating such schemes in sub-Sahara Africa need to be evaluated before they can be recommended. In any case, the generally small herds or flocks and uncontrolled breeding in communal grazing situations preclude implementation.

3). Semen and Embryo Sexing

Although the semen and embryo sexing techniques do not dramatically increase the rate of genetic gain, they can increase production efficiency. They are being developed and refined in a number of research institutions in developing countries. The involvement of private companies providing these services is likely to increase their accessibility in developing countries where AI is already established. With few exceptions, they are not widely used by breeders or farmers in developing countries (FAO, 2007). Sexed sperm is commercially available in several developing countries, including Argentina and Brazil (Rath, 2008).

4).In Vitro Fertilization (IVF)

The term *in vitro* simply means in glass or in artificial conditions and IVF refers to the fertilization of egg by sperm that occurred not in uterus but outside the uterus at artificially maintained condition. The IVF technology involves

taking out the eggs from ovaries of female donor, in vitro maturation of egg cultures kept in an incubator, fertilization of the eggs in test tubes by semen obtained from superior male and implantation of seven days old embryos in reproductive tract of other recipient female which acts as foster mother or surrogate mothers. These are used only to serve as animal incubator and to deliver offspring after normal gestation period. The surrogate mothers do not contribute anything in terms of genetic makeup since the same comes from the egg of donor mother and semen from artificial insemination (Kefeyalew and Addis, 2015).

In recent years, IVF technology has revolutionized the field of animal biotechnology because of production of more animals as compared to animal production through normal course. However, an animal produces about 4-5 offspring in her life through normal reproduction, whereas through IVF the same can produce 50-80 offspring in her life. Therefore, the IVF holds a great promise because a large number of animals may be produced and gene pool of animal population can also be improved.

5). Cryopreservation Technology

Larger population of livestock breeds (>20 percent) are at risk of extinction (FAO, 2007). Semen and embryo cryopreservation have been effectively used for conserving rare livestock breeds (Long, 2008). An evaluation of country reports indicates that over one third of countries use in vitro conservation (FAO, 2007). Cryopreservation of gametes, embryos, DNA or cells (for example skin fibroblasts) is a cost-effective approach for the conservation of endangered species. Although using DNA or non-germ cells to regenerate an extinct breed is still a major problem with available technologies. It has been suggested that cryopreserved cells of each breed should be stored long-term in secure locations and accessed if and when the need arises in the future, either to sequence their DNA to understand genetic differences among breeds or to use the cells in cloning to regenerate extinct breeds (Hodges, 2005). Conservation of indigenous genetic resources is one of the top priorities of developing countries and several country reports noted the potential use of AI and ET for cryo-conservation purposes (FAO, 2007). Due to changes induced by global warming, there is need in the sub-Sahara Africa to emphasize in improving the indigenous animal to meet the growing challenges.

6). Cloning Technology

Cloning is an asexual reproduction of genetically identical organism and can be achieved by nuclear transfer (NT) or by embryo splitting (Aminoor Rahman, *et al.,* 2008). It is an important tool in breeding, considering a perfect way to improve the performance of farm animals. One of the purposes of cloning is to increase the number of species in a population with superior characteristics. Cloning technology has concerned the interest of breeders for many years.

Animal cloning is the most topical development of selective assisted breeding in livestock (Wells, 2003). It has been used to replicate elite breeding animals (Plume, 2009), as Dolly the sheep was the first animal to be cloned in 1996 (Wilmut *et al.,* 1997) using Somatic cell nuclear transfer (SCNT). Since then, any other species have been cloned by the same process. There are around 6000 farm animals' clones worldwide (Plume, 2009). The technology has been applied in the breeding of elite cattle (Kato *et al.,* 1998), goat (Baguisi *et al.,* 1999), pig (Polejaeva *et al.,* 2000), horse (Galli *et al.,* 2003), buffalo (Shi *et al.,* 2007), camel (Wani *et al*., 2010), Rabbit (Chesne *et al.,* 2002) and other pet species like dog, cat, rat, mouse (Wakayama *et al.,* 1999; Roslin, 2003; Lee *et al.,* 2005; Li *et al.,* 2006 and Shin *et al.,* 2002).

High cost of cloning limits its use in practical animals breeding (Hugo, 2006). As compared to other assisted reproductive technology its effectiveness remains significantly little.

7*). Molecular Genetics for Disease Control*

Disease resistance is particularly an important characteristic of livestock in low-input livestock production systems in sub-Sahara Africa. Resistance to infectious diseases is often the critical determinant of the sustainability of such systems. Improving resistance is perceived as a primary target for genetic improvement programmes (Gibson and Bishop, 2005). The control of infectious disease of livestock is currently achieved by a number of mechanisms, including; chemical intervention such as anthelmintic (for nematode parasite control), acaricides (for tick control), antibiotics (for the control of many bacterial diseases), vaccination, sanitation, disinfection, culling, isolation and control of the movements of animal or animal products. Disease control or management using host genetic resistance (i.e. exploiting genetic variation in disease resistance amongst hosts) is becoming a popular component of effective disease control, complementing or sometimes replacing existing strategies. Breeders and agricultural industries in the developed world have a variety of incentives to genetically improve host resistance to disease. Host resistance to disease is a low-cost and usually sustainable approach to disease control. Increasingly, other measures are failing as parasites evolve to resist chemical or vaccine control measures. Important examples include the evolution of resistance to anthelmintic by nematodes in all major sheep-producing

countries, the evolution of resistance to antibiotics by bacteria and the evolution of resistance to vaccines by the virus causing Marek's disease. Legislative changes in many countries are also increasingly restricting the use of antibiotics and other therapeutics in animal production systems.

III. CONCLUSION

Genetic improvement programmmes for livestock can be enhanced by the use of molecular genetic information in introgression, genotype building and recurrent selection programmes. From these review, it is well understood that, biotechnology addressed the improvements of animal performance through molecular genetics approach. However, Biotechnology development requires a high level of commitment to research and development, expensive equipment, heavy machines, expensive chemicals as well as high level of technological expertise. Not all available biotechnological techniques are appropriate or relevant in Sub Sahara Africa, there is need to adapt some of these technologies, develop capacity to maintain a strong base of applied in adaptive research centers and some level of training to keep abreast with new developments and to adapting such technology to local conditions so as to ensure sustainability and awareness to the rural farmers.

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