

The Sixth African Nutrition Epidemiology Conference held in Accra, Ghana July 21-25, 2014

## Africa Biofortified Sorghum for Nutrition

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### Abstract

The goal of African Biofortified Sorghum (ABS) Project is to develop and deploy sorghum with enhanced pro-vitamin A to farmers and end-users in Africa to alleviate vitamin A related micronutrient deficiencies diseases. Sorghum with enhanced pro-vitamin A will first be deployed in the three initial target countries of Nigeria in (English-speaking West Africa), Burkina Faso (French-speaking West Africa) and Kenya in (English-speaking East Africa). To achieve this goal the project technology development team led by DuPont Pioneer has developed several promising high pro-vitamin A sorghum events. ABS 203 event is so far the most advanced and well characterized lead event with about 10 µg pro-vitamin A/g tissue which would supply about 40-50% of the daily recommended vitamin A. Through gene expression optimization other events with higher amounts of pro-vitamin A including ABS 214, ABS 235, ABS 239 with 25µg, 30-40 µg, 40-50 µg of pro-vitamin A per g tissue, respectively, have been developed. Preliminary results of introgression of ABS pro-vitamin A traits into local sorghum varieties in Nigeria and Kenya shows stable introgression of ABS vitamin A into local farmer-preferred sorghum varieties. *Ex ante* economic analysis shows that ABS has a benefit-cost ratio that is at least 10 and cost per Disability Adjusted Life-Years (DALY) saved of less than US\$100, 20 times more cost effective than World Health Organization (WHO) and World Bank average. ABS gene Intellectual Property Rights (IPR) and freedom to Operate (FTO) have been

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Abbreviations: ABS- Africa Biofortified Sorghum, VAD- vitamin A deficiency, GMO-genetically modified foods

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donated for use royalty free for Africa. Prior to the focus on the current target countries, the project was implemented by 14 institutions in Africa and the USA. Currently, the ABS project is implemented by DuPont Pioneer, Africa Harvest Biotechnology Foundation International (Africa Harvest), the Institute of Agricultural Research (IAR) of Nigeria, the Kenya Agricultural and Livestock Research Organization (KALRO) and the National Biotechnology Development Agency (NABDA) of Nigeria and other partners.

**Keywords:** Africa Biofortified Sorghum, Vitamin A, Malnutrition

### **Introduction**

Vitamin A deficiency (VAD) is of public health significance in the developing world. VAD is the world's commonest cause of childhood blindness, with about 228 million children are affected sub-clinically and 500,000 children become partially or totally blind every year as a result of VAD<sup>(1)</sup>. More than half of the cases occur in developing countries and this is attributed to the consumption of vitamin A deficient diets<sup>(2)</sup>. Young children are the most vulnerable with globally 140 million children aged of 5 years, of whom nearly 100 million live in South Asia or sub-Saharan Africa, have low serum retinol concentrations ( $<0.7 \mu\text{mol/L}$ )<sup>(3)</sup>. Countries of eastern and southern Africa have the highest prevalence (37%) of preschool children with low serum retinol concentrations, followed by South Asia (35%) and Western and Central Africa (33%)<sup>(3)</sup>. In South Africa, 1 in 3 preschool children has a serum retinol concentration ( $<0.7 \mu\text{mol/L}$ )<sup>(4)</sup>, and 55–68% of children aged 1–9 years consume  $<50\%$  of the recommended dietary intake of vitamin A (700 $\mu\text{g}$  retinol equivalents)<sup>(5)</sup>; children living in rural areas are the most affected<sup>(4,5)</sup>. Vitamin A deficiency is caused by a habitual diet that provides too little bioavailable vitamin A to meet physiologic needs<sup>(6)</sup>. Rapid growth and frequent infections, which cause ineffective utilization of the vitamin, are also critical factors<sup>(7)</sup>.

Strategies to control vitamin A deficiency include dietary diversification, food fortification, and vitamin A supplementation<sup>(1, 6, 8)</sup>. Animal sourced foods though good sources of vitamin A are too expensive for poor communities to afford<sup>(9)</sup>. This leaves foods of plant origin as an important source of pro-vitamin A in developing countries. Dietary diversification includes the production of carotene-rich crops, such as orange-fleshed sweet potato (OFSP)<sup>(6)</sup>. However,

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dietary diversification has limited impact in arid and semi-arid regions of Africa because of frequent drought and lack of alternative foods. Of the 600 carotenoids found in nature, only 3 are important precursors of vitamin A in humans, namely,  $\alpha$ -carotene,  $\beta$ -carotene, and  $\beta$ -cryptoxanthin.  $\beta$ -Carotene is the major pro-vitamin A component of most carotenoid-containing foods<sup>10</sup>. The goal of Africa Biofortified Sorghum Project is to develop and deploy sorghum enhanced with pro-vitamin A as the first priority product targeting people living in arid and semi-arid areas where sorghum is the main staple food.

### Progress in Technology Development

To achieve the goal of alleviating malnutrition in Africa, the ABS Project has envisaged developing and deploying three ABS products in the following, decreasing order of priority: the first priority product is ABS with enhanced pro-vitamin A alone; the second will be ABS with enhanced pro-vitamin A and bioavailable zinc and iron; the third will be ABS with enhanced pro-vitamin A and bioavailable zinc and iron. It will also have improved protein quality and digestibility<sup>(11, 12, 13)</sup>.

However, because the development and deployment of GMO products is expensive and time consuming, the project will not embark on development of the three products simultaneously. Emulating and learning from the model used by Golden Rice Project, the ABS project is now focusing on development and deployment of ABS with enhanced pro-vitamin A alone, as the first product. In addition to product prioritization, the countries of ABS product deployment have been prioritized and the first three priority countries are Nigeria, Burkina Faso and Kenya<sup>(11, 12)</sup>.

To develop the first product, the ABS technology development team led by DuPont Pioneer had developed several promising events. Africa Harvest, working with teams in Nigeria (the IAR and NABDA) and Kenya (KALRO) are carrying out the confined field trials (CFT). ABS203 is so far the most advanced and well characterized lead event for commercialization. The event has about 10  $\mu\text{g/gm}$   $\beta$ -carotene which would supply about 40-50 % of the daily recommended vitamin A. In addition, the pro-vitamin A in ABS203 is stabilized by expression of vitamin E in the sorghum seeds. The event has been approved for CFT in Nigeria while a CFT application is

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under consideration in Kenya. To ensure ABS project sustainability, DuPont Pioneer and Africa Harvest are continuously developing and evaluating other improved ABS events for technology backstopping.

Through application of gene sorting and optimization of gene expression approaches, the DuPont Pioneer technology team has developed other improved ABS events that are under evaluation. These include ABS214, 220, 235 and ABS239. ABS214 and 220 are under CFT evaluation in Johnston, Iowa in USA. The purpose of this CFT is to evaluate the yield and other agronomic performances for these events of ABS214 and 220 that have with  $\beta$ -carotene accumulation up to 25 $\mu$ g/g. The CFT results demonstrated that higher  $\beta$ -carotene accumulation up to 25 $\mu$ g/g does not cause yield penalty. Through gene expression optimization events ABS235, ABS239 have been developed with stronger red-orange color than ABS214 (25 $\mu$ g/g  $\beta$ -carotene) which indicates much higher  $\beta$ -carotene accumulation. Preliminary data from DuPont Pioneer indicate that ABS239 events accumulate beta-carotene in a range between 40-50  $\mu$ g/g as shown by the chromatograms analysis, while ABS235 has 30-40  $\mu$ g/g  $\beta$ -carotenes. More detailed carotenoid analysis by HPLC for ABS239 and ABS235 events is in progress and will be validated by ISU.

The ABS pro-vitamin A, is similar to pro-vitamin A from all plant food sources such as carrots and spinach, as opposed to vitamin A from animals which is the retinol form of vitamin A.

#### **Biochemical forms of vitamin A- retinol and pro-vitamin A**

The ABS 'vitamin A' is referred as pro-vitamin A. This is because vitamin A found in foods occurs into two major categories-retinol the form of vitamin A found in food of animal origin and is readily used by the body. In plant vitamin A occurs in form of carotenes that include: alpha-carotene, beta-carotene, gamma-carotene; and the xanthophyll beta-cryptoxanthin<sup>(14)</sup>. The most important of these is  $\beta$ -carotene because it is more readily converted into retinol that is used by the human body<sup>(15)</sup>. The rate of conversion varies from plant to plant and for ABS this is being determined but conservative factor of 6:1 (i.e. 6 molecules of  $\beta$ -carotene give 1 molecule of retinol) is currently being used. The advantage of humans eating vitamin A in form of pro-vitamin A rather than retinol is because when the body has had adequate amounts of vitamin A, it automatically stops conversion pro-vitamin A into retinol, avoiding the problem of vitamin A

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toxicity called hypervitaminosis A <sup>(14,16,17)</sup>. High doses of beta-carotene (up to 180 mg/day) have been used to treat erythropoietic protoporphyria, a photosensitivity disorder, without toxic side effects <sup>(15)</sup>. This why we don't get vitamin A toxicity when we eat high pro-vitamin A foods such as carrots or spinach.

It is also noteworthy that the human body will only obtain nutritional benefits from ABS when it is eaten and converted into retinal. That is why information on what quantities should be eaten and what the conversion factor of  $\beta$ -carotene into retinol is are so critical.

#### **How much Africa Biofortified Sorghum should the consumer eat?**

Although vitamin A is mostly known for improved human vision it has two other beneficial nutritional functions in human body: first, it is important for growth and development and second, it is critical for the maintenance of the immune system. The vitamin A used for improved vision is called retinal.

However, one pertinent question is how much the consumer should eat to derive nutritional benefits from ABS. The amounts that one eats to get benefits from pro-vitamin A from ABS sorghum depends on five factors:

- Stability of vitamin A when cooked,
- Stability of vitamin A when stored,
- Bio-availability of pro-vitamin in the body,
- Conversion factor of  $\beta$ -carotene from ABS into retinal and
- Age of the person consuming <sup>(18, 19, 20, 21, 22)</sup>.

There are ongoing studies at various ABS collaborating institutions involving competent nutritionists to determine the importance these factors in determining the nutritional benefits of ABS. Preliminary results using conservative figures show that ABS203 with 12  $\mu\text{g/g}$   $\beta$ -carotene will meet 40-50% of RDA for children while other events such as ABS235 and 239 will give higher benefits.

**Biosafety of ABS genes and gene products.** The ABS project will develop a full regulatory biosafety package that will be submitted to the National Biosafety Authorities (NBA) in Kenya,

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Nigeria and Burkina Faso for deregulation and commercialization. The regulatory package will cover the standard core and event specific regulatory data packages. However, preliminary biosafety data shows that the genes and their gene product used in developing ABS have no likelihood of eliciting toxicological and allergenic reaction when consumed.

ABS events are based on use of similar genes used in development of Golden Rice including *crt1*, *pmi* and *psyl*, except for vitamin E gene *hv-hggt*. The *psyl* gene that encodes Phytoene Synthase (PSY1) and *crt1* that encode Carotene Desaturase I (CRT I) have been introduced in the ABS to catalyze the biosynthesis of pro -vitamin A. The ABS events did not use herbicide resistance genes but instead used *pmi* gene which encodes phosphomannose isomerase (PMI) as the selectable marker gene and the later has been deregulated. Bioinformatics analysis and digestibility studies for these proteins, PMI, CRT1 and PSY1 protein that have been done for Golden rice development showed that the expressed proteins were neither toxic nor allelgenic.

#### **Approaches for wide adoption and impact of ABS**

Factors determining adoptions of new agricultural technologies especially in the developing countries are widely studied and documented. These include: economic benefits, availability of technology transfer supporting packages such as seeds and fertilizer, availability of market pull, credit to facilitate farmers to purchase inputs, supporting government policies and regulatory framework<sup>(23, 24, 25, 26)</sup>. Although the importance and magnitude of the influence of these factors will be country and target-area specific, we expect the same factors to influence the adoption of ABS.

The ABS project has proactively embarked on positive modulation measures of some selected factors to spur wide adoption of high nutritious pro-vitamin A sorghum in target countries. Key to this includes deploying sorghum in already commercialized and widely adopted open pollinated sorghum varieties, developing sorghum seeds system for deployment of quality seeds to farmers in partnership with other stakeholders and examining the future potential of deploying high pro-vitamin A sorghum through sorghum hybrids that will ensure high yields, better maintenance of seed and trait quality.

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### **Introgression of High Pro-vitamin A in widely adopted Open Pollinated Sorghum varieties**

High pro-vitamin A sorghum will be with be deployed in local adopted NARS/ICRISAT sorghum varieties with wide adoption through introgression <sup>(12)</sup>. Two primary regions for deployment selected include: i) East-Africa/Anglophone and ii) West Africa/Franco-phone and West-Africa/Anglo-phone <sup>(13, 27)</sup>. There are also plans to extend deployment to Southern Africa and Northern Africa. Nigeria (through the ICRISAT/NARS system) has selected sorghum varieties that have wide regional adaptation and acceptance. These include SAMSORG 40, SAMSORG 14 and SAMSORG 70 <sup>(28)</sup>. These varieties were developed through joint efforts of ICRISAT and NARS. SAMSORG 14, 17 and 40 were selected for the West Africa Anglo-phone <sup>(13)</sup>. SAMSORG 40 (ICSV 400) is a short season variety (matures in 95-100 days) adapted to Sudan Savannah Ecology (Figure 1). It yields 2.5-3.5 tons per hectare. SAMSORG 17 (KSV3 (SK5912)-is a long season variety (matures in 165-175 days) adapted to Southern Guinea Ecology. It yields 2.5-3.5 tons per hectare. SAMSORG 17 (KSV3 (SK5912) is a long season variety (matures in 165-175 days) adapted to Southern Guinea Ecology. It yields 2.5-3.5 tons per hectare. SAMSORG 14 (KSV8) is a medium season variety (matures in 130-140 days) adapted to Northern Guinea Savannah Ecology <sup>(28)</sup>. The Guinea Savannah and Sudan Savannahs are vast stretch of African savannah land that spreads across 10 countries in West Africa has the potential to turn several African nations into global players in bulk commodity production, according to a study just published by FAO and the World Bank (Figure 1).

For Eastern and Southern Africa ABS traits will be introgressed into Tegemeo (2KX 17/B/1), Macia (SDS 3220), KARI Mtama I and Gadam sorghum varieties. The improved sorghum variety Macia (SDS 3220) was released on 14 Dec 1999 by the Tanzania National Variety Release Committee. Macia is a high-yielding, early maturing, white-grained variety developed jointly by ICRISAT and national scientists in southern Africa. It has so far been released in five SADC countries of Mozambique, Botswana (under the name Phofu), Zimbabwe, Namibia, and Tanzania. It is suitable for areas with a growing season of 3-4 months and yield up to 4 tons per hectare <sup>(29, 30, 31)</sup>.

KARI Mtama I sorghum variety is mainly grown in Kenya and matures in 3 to 3.5 months, white grain color and has wide adaptation. It grows in moist mid- latitudes of Kenya (Busia, Siaya, and

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Homa Bay), semi-arid low lands (Kitui, Makuweni, Mwingi, and Ntharaka) and humid Coast (Kwale, Kilifi, and Taita Taveta) <sup>(32)</sup>. Tegemeo sorghum variety was released in Tanzania in 1986 and on-station yield of 4.2 tons per hectares have been reported in Tanzania with a yield advantage of 114% over the local unimproved cultivars and is also grown in Kenya.

#### **Development of Sorghum Seed Systems**

Prerequisite to availability of quality seeds for small scale farmers is the development of a functional formal seed system especially when dealing with biotech crops. In general a functional seed system comprises organizations, individuals and institutions involved in different seed system functions that include the development, multiplication, processing, storage, distribution and marketing of seeds. Rules and regulations such as variety release procedures, intellectual property rights, certification programs, seed standards, and contract laws influence the structure, coordination and performance of the seed system <sup>(33)</sup>.

A quick appraisal of Sorghum Seed systems in ABS target countries shows that Kenya has an established formal Seed System dealing with hybrid maize consisting of several companies that are now diversifying into sorghum seed <sup>(34, 35)</sup>. The Kenya Plant Health Inspectorate Services (KEPHIS) implements the regulatory role consisting of seed certification and registration. Among ABS target countries, Kenya has established Seed companies marketing sorghum including- Kenya Seed company, Western Seed Company <sup>(35)</sup>.

Nigeria has a rudimentary formal seed system with fewer seed companies involved in marketing quality seeds. Seed certification in Nigeria is done by the Plant Quarantine Service of the Federal Department of Agriculture. In Kenya, ABS in partnership with other stakeholders needs to help streamline the seed system to manage the deployment of biotech seeds. In Nigeria considerable efforts will be required to get quality ABS seeds to the farmers.



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### **Deployment of High Pro-vitamin A sorghum hybrids**

Sorghum hybrid will be ideal for deployment for ABS pro-vitamin A traits. Hybrids ensure high economic benefits of the technology and better quality maintenance of ABS traits compared to OPV. However, sorghum hybrids development and deployment in Africa is at its infancy. The grain productivity increased by 47% in China and 50% in India, which corresponds well with adaptation of hybrids in these countries <sup>(36)</sup>. In USA, Australia and China, over 95% of sorghum area is planted with hybrid sorghum. In India, over 85% of the rainy season sorghum is planted with hybrids. The hybrid vigor was discovered in 1927 and the germplasm used to develop hybrid sorghum in other countries originated from Africa, which is the center of origin of sorghum. It is a paradox that in Africa the development and commercialization of hybrid sorghum is at infancy.

Experience of the West African Sorghum Hybrids Adaptation Trials (WASHAT) by ICRISAT, in the mid-1980's in 17 West Africa countries <sup>(37)</sup> is that the low yields inherent in OPV varieties can be raised by shift to hybrid sorghum. Virtually everywhere that sorghum hybrids and inbred varieties were compared, hybrids showed a yield advantage, commonly of 20 to 60% over the inbred varieties <sup>(38)</sup>.

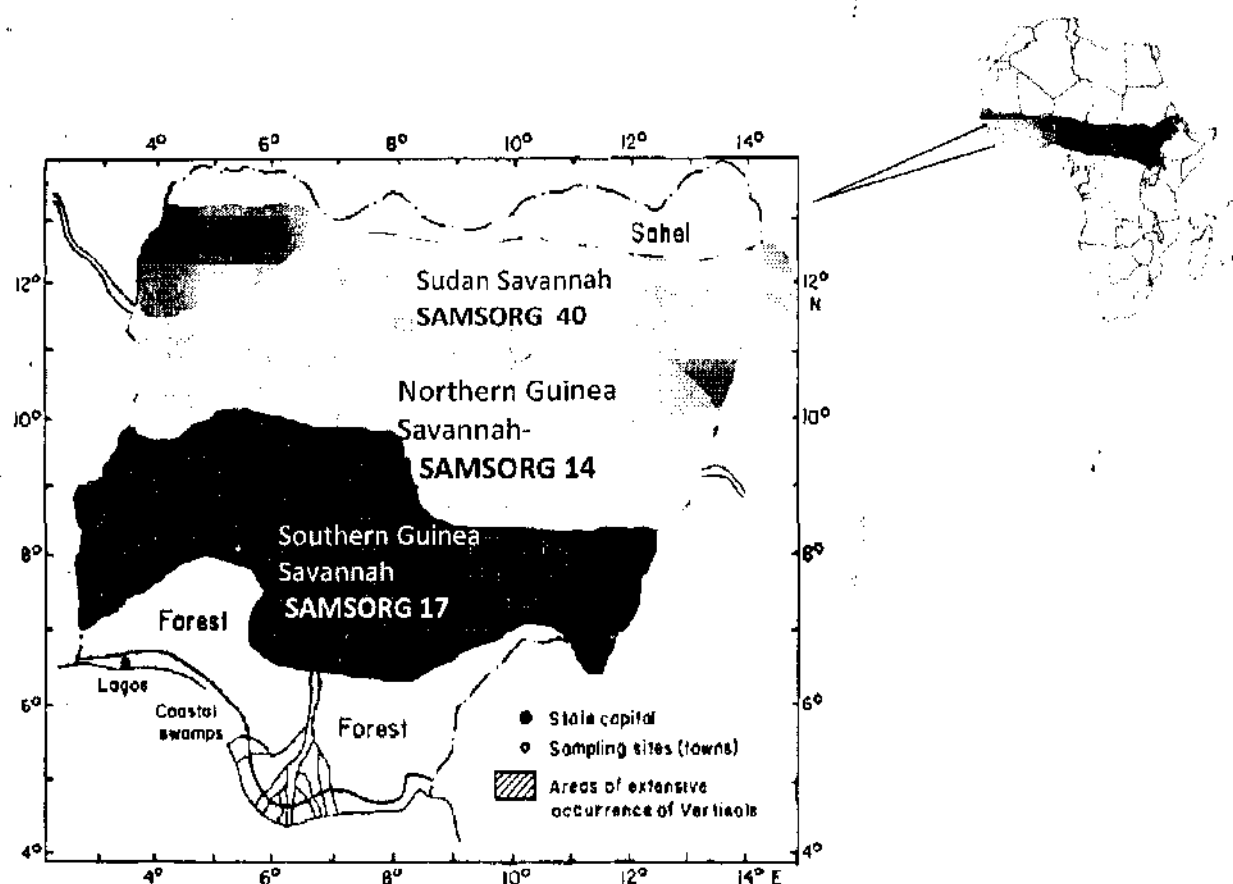
As growing conditions become more stressed, the yields of hybrid, and OPVs landraces decline, but the yield differences between hybrids and OPV varieties become proportionately larger in favor of the hybrids <sup>(38)</sup>. Out of the 17 sorghum-growing countries in West and Central Africa, only Mali, Nigeria and Niger have formally released sorghum hybrids. In Mali for example, four sorghum hybrids including 'Fadda' Sigui-kumbe, Sewa and Bensema were released in 2008. Large scale testing of this and other hybrid is ongoing in Niger and Nigeria <sup>(39, 40)</sup>.

These successful model of sorghum hybrid technology transfer to smallholder farmers show that the technology is appropriate for increasing food security and nutrition among smallholder farmers in Africa. However, the coverage of these hybrids insignificant in West and East Africa remains insignificant. For Nigeria, hybrids were developed for the malting industry, in Kenya preliminary research shows that sorghum hybrids can yield up to 50% more than open pollinated varieties (OPV's). Despite the demonstrated yield superiority of hybrid sorghum, sorghum-

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farming systems in Africa are still dominated by the low yielding varieties. It cannot therefore be overemphasized that future deployment of ABS nutrients in sorghum hybrids will greatly enhance the economic benefits of the technology.

**Figure 1.**



### Conclusions and way forward

The project has made tremendous progress towards development of ABS product. To date several ABS events with optimized amounts of B-carotene have been developed. The key task left include introgression of ABS traits into local sorghum varieties, development of full biosafety and regulatory data package, nutritional studies, development of seed systems for commercialization of final ABS product. To fully realize these key objectives the project urgently needs further multi-donor financial support.

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### Acknowledgments

We wish to express our gratitude to the International Crops Research Institute for the Semi-Arid-Tropics (ICRISAT)/ National Agricultural Research Stations (NARS) of Kenya, Burkina Faso and Nigeria for providing the genotypes used for ABS introgression. The Bill and Melinda Gates Foundation for funding phase I of ABS Project and the Howard Buffet Foundation for funding phase II of ABS project. We are grateful to other ABS partners not mentioned in this paper: the Institut de l'Environnement et de Recherches Agricoles (INERA), the Agricultural Research Council (ARC) of South Africa, the Council for Scientific and Industrial Research (CSIR) in South Africa, the African Agricultural Technology Foundation (AATF), the University of Pretoria Department of Food Science, the Centre Africain pour la Recherche et le Developpement Agricoles (CORAF) for their various contributions to the project success. The institutions that donated gene for ABS traits including DuPont, which donated the initial technology and training of African scientists; Syngenta, which donated pro-vitamin A genes and selectable markers and Japan Tobacco, which donated technology for transformation. We give further gratitude to ABS host countries- the Governments of Nigeria, Kenya and Burkina Faso for their support to the project.

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# **Legends**

**Figure1.** The Guinea Savannah and Sudan savannah regions of Nigeria and their extension into other Africa countries <sup>(41)</sup>.

