A review of biotechnological approaches towards crop improvement in African yam bean (*Sphenostylis stenocarpa* Hochst. Ex A. Rich.)

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- Plant biotechnology
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- Underutilised legume

**ABSTRACT**

Globally, climate change is a major factor that contributes significantly to food and nutrition insecurity, limiting crop yield and availability. Although efforts are being made to curb food insecurity, millions of people still suffer from malnutrition. For the United Nations (UN) Sustainable Development Goal of Food Security to be achieved, diverse cropping systems must be developed instead of relying mainly on a few staple crops. Many orphan legumes have untapped potential that can be of significance for developing improved cultivars with enhanced tolerance to changing climatic conditions. One typical example of such an orphan crop is *Sphenostylis stenocarpa* Hochst. Ex A. Rich. Harms, popularly known as African yam bean (AYB). The crop is an underutilised tropical legume that is climate-resilient and has excellent potential for smallholder agriculture in sub-Saharan Africa (SSA). Studies on AYB have featured morphological characterisation, assessment of genetic diversity using various molecular markers, and the development of tissue culture protocols for rapidly multiplying propagules. However, these have not translated into varietal development, and low yields remain a challenge. The application of suitable biotechnologies to improve AYB is imperative for increased yield, sustainable utilisation and conservation. This review discusses biotechnological strategies with prospective applications for AYB improvement. The potential risks of these strategies are also highlighted.

**1. Introduction**

Food security, climate change, and crop diversity are closely associated in complex ways. Without crop diversification, food security will be almost impossible to achieve (Marino, 2019). Globally, climate change is a major factor that contributes significantly to food insecurity, limiting crop yield and food availability (Jimenez-Lopez et al., 2020). Whereas efforts are being made to curb food insecurity, millions of people still suffer malnutrition due to insufficient protein intake and micronutrient deficiency (Khan et al., 2016). Diets deficient in proteins, vitamins, and minerals hinder the growth and development of infants. At the same time, steady consumption of calorie-dense foods can lead to obesity and overweight. There are about 250 million undernourished people in Africa and 500 million in Asia, making these two continents regions with the highest prevalence of malnutrition (Mustafa et al., 2021). Crop diversification can help improve human diets and livelihood while mitigating nutrition insecurity (Mango et al., 2018). About 30,000 edible crop species exist globally. However, only 103 crop species are consumed, with rice, wheat, and maize being the most cultivated (FAO, 2018; Mustafa et al., 2021). Reliance on only a few staple crops can pose an ecological, nutritional and economic risk (Dissanayaka et al., 2021).

Legumes are the second most important group of crops after cereals. They play a vital role in providing protein, oils, and minerals in human diets, thus boosting health and lowering the risk of several chronic diseases (Becerra-Tomás et al., 2019; Cui et al., 2021; Foyer et al., 2016). Legumes also offer advantages in utilisation as livestock feed and a source of income to small-scale farmers (Reddy et al., 2012). They enhance sustainable agriculture due to their nitrogen-fixing ability, limiting the use and impact of inorganic nitrogen fertilisers (Considine et al., 2017; Quilbé et al., 2021). With climate change affecting global agriculture,
orphan legumes are alternatives for crop cultivation and utilisation (Murthy and Paek, 2021). The term “orphan” crops refer to minor crops, which have received limited research attention globally. Consequently, orphan crops have remained economically significant only in specific local regions in most developing countries (Cullis and Kunert, 2017). Synonyms used to describe orphan crops include “underutilised,” “neglected”, “promising”, or “minor crops” (FAO, 2019). Underutilised legumes have the advantage of being climate-resilient and providing nutritional security (Paliwal et al., 2021). Some examples of underutilised legumes include African yam bean (Sphenostylis stenocarpa), Bambara groundnut (Vigna subterranean), Kersting’s groundnut (Macrotyloma uniflorum), etc. (Paliwal et al., 2020).

This review highlights the application of different biotechnological strategies and their prospects for improvement in African yam bean (AYB). The potential risks of these technologies are also discussed.

2. Methodology

Google Scholar and ScienceDirect were employed to search for the following keywords: African yam bean, Sphenostylis stenocarpa, biotechnological tools, biotechnological approaches, plant biotechnology, crop improvement and underutilised legumes. To enhance the likelihood of finding relevant articles, Boolean operators were used to combine keywords in the following manner: “biotechnological approaches and African yam bean”, “crop improvement and biotechnological approaches”, biotechnological approaches and underutilised legumes”, and “crop improvement and underutilised legumes”. Only studies that related to the topic of interest were included for the review. Also, only articles written in English were included. The publication date was a constraint limiting the articles used over the past 10 years to 11% based on relevance. Other publication dates were limited to the last ten years (2011–2021), accounting for 89%. Also, other articles used include original articles, review articles and information from institutional websites such as the Food and Agriculture Organization (FAO), The International Institute of Tropical Agriculture (IITA), etc. A total of 109 published articles were used for this review. The result of this review is categorised into three sections. The first section discusses the AYB crop, its benefits and limitation. The second section discusses the biotechnological tools that have been applied in AYB improvement, while the third section discusses future perspectives for AYB crop improvement.

3. African yam bean (AYB)

AYB is a tropical African orphan crop, which belongs to the family Fabaceae. It is a leguminous seed crop (Figure 1) with swollen shoots commonly found in West Africa (Nigeria), East Africa (Kenya), Southern Africa (Malawi), and Central Africa (Adewale and Odoh, 2013). It is cultivated in local communities of Africa for its edible tubers and seeds, which are rich in nutrients (Adewale and Odoh, 2013). The nutritional content of AYB compares favourably with those of other commonly consumed legumes. The protein content of AYB varies between 17 and 30% (George et al., 2020; Oluwole et al., 2020), higher than those of Bambara groundnut, common bean, chickpea, and pigeon pea (George et al., 2020). A study by Ajibola et al. (2016) revealed that the predominant proteins in AYB are globulin and albumin. Additionally, the crop is rich in starch, dietary fibre, and vital minerals (Aremu et al., 2019; Baiyeri et al., 2018; Oluwole et al., 2020).

AYB is used extensively for intercropping because of its nitrogen-fixing ability, which enriches the soil (Klu et al., 2001; Abdul-kareem et al., 2015). Arisa and Ogbueue (2007) reported that diabetic and hypertensive patients could consume AYB. In diabetic patients, the authors observed that AYB digests slowly, causing a gradual increase in blood sugar levels. Also, the enzymatic protein hydrolysate of the plant can serve as a potential ingredient in nutraceutical products (Esan and Fasasi, 2013). Nnamani et al. (2017) reported using AYB as a remedy for treating acute heart diseases in South-eastern Nigeria. Other uses of the plant include the preparation of animal feeds (Ahiyowu et al., 2007; Onuoha et al., 2017) and production of insecticides from its processed extracts (Omitogun et al., 1999; Okeola and Machuka, 2001). Biotechnology-assisted improvement of AYB is imperative, particularly with respect to reduced cooking time (to save cost), resistance to diseases, enhanced yield and other target traits. This could boost its social acceptance as a protein-dense legume and production given SDG goal 2, which aims to achieve zero hunger by 2030.

3.1. Factors that limit AYB productivity

Although AYB is a crop with high nutritional value, its production depends on the cultivation of landraces by local farmers. The plant’s breeding system is not yet fully understood; hence, improved varieties are yet to be released. Adewale and Adegbite (2018) posited that it is difficult to achieve hybridisation in AYB, given that the plant is more of an autogamous crop than an allogamous one in the ratio 9:1. Unlike the known staple crops, AYB has been neglected by the scientific community and industries because of little or no information about its economic value. Insufficient information on the nutritional benefits has hindered the consumption of the crop. Many of the farmers who are knowledgeable about the crop are aged and without formal education, leading to poor or no documentation on the crop (Nnamani et al., 2017). Therefore, younger farmers need to engage in its cultivation to prevent the loss of information.

Figure 1. Two accessions of African yam bean showing variation in seed size and colour. Source: Oluwole. A = TSs-66, B = TSs-47, TSs- Tropical Sphenostylis stenocarpa, Seeds were obtained from International Institute of Tropical Agriculture (IITA), Ibadan.
and value. Besides, several other factors have contributed to the limited productivity of AYB, including low yields, prolonged cooking duration, anti-nutritional factors present, and extended maturity period (Ojuederie et al., 2016; Nnamani et al., 2017). The cooking time of AYB can last for several hours, which is a discouraging factor. A reduced cooking time, as well as improved processing techniques, can bolster AYB utilisation. Having refined food products made from the crop can facilitate its utilisation if a functional value chain is available. Another limiting factor to AYB production is the requirement for staking. Since the plant is a climber, farmers require stakes to obtain a better yield. There is also insufficient information on the reproductive biology, pod-filling, and photoperiodic sensitivity that influence planting time and season of the crop (Adewale and Odoh 2013). In addition, AYB is susceptible to several diseases such as leaf spots and stem blights caused by *Sclerotinia sclerotiorum* (Akinlabi et al., 2015), flower bud and pod rot (Figure 2) caused by several fungi (Afolabi et al., 2019). Farmers are also faced with the problem of labour-intensive farming methods. The absence of farm machinery limits production and post-harvest activities such as dehulling. These limitations can be overcome to achieve enhanced productivity. The use of conventional breeding to improve AYB may take several years (Adewale et al., 2015); hence, biotechnological approaches are crucial for precise and efficient crop improvement (Kumar et al., 2015). It is vital to consider the suitability and local farming practices of AYB to identify lines with specific traits of interest (Muhammad et al., 2020). Table 1 shows a list of accessions with known phenotypes that can be deployed as parental materials.

Most AYB accessions have been classified based on phenotypic traits, such as seed pattern and seed coat colour. This kind of classification contains limited genetic information, which may mislead and provide lean evidence on the available genetic diversity of the crop (Adewale and Odoh, 2013). Knowledge of the crop’s cytogenetics is paramount for overall improvement and genetic manipulation (Popoola et al., 2011). Popoola et al. (2011) and Adesoye and Nnadi (2011) reported the bivalent chromosomal status of AYB to include $2n = 18$, $20$, $22$, and $24$ with TSs-3 having $2n = 18$. Most of the AYB accessions had $2n = 22$.

4. Biotechnological approaches for AYB improvement

Biotechnological strategies useful in crop improvement include the following: (i) molecular markers for the assessment of genetic diversity and marker-assisted breeding, ii) plant tissue culture for mass propagation (iii) genetic modification for novel trait integration, and (iv) omics e.g. genomics, proteomics and metabolomics for unravelling gene function and regulation (Dawson et al., 2009; Obembe, 2019).

So far, there is a paucity of information on the genetic improvements of AYB (Popoola et al., 2011). The biotechnological approaches reported on AYB include the use of molecular markers for estimating genetic diversity and micropropagation (Table 2). Currently, no transgenic AYB has been reported, neither has genetic engineering nor marker-assisted breeding methods been exploited for improvement in the crop.

4.1. Applications of micropropagation techniques to AYB improvement

This section discusses the applications of micropropagation techniques for the *in vitro* regeneration of AYB. Aliyu and Adesoye (2007) demonstrated that 0.1 % mercuric chloride was most appropriate for AYB explant sterilisation. Otoseng (2005) developed a clonal propagation procedure for landraces of AYB using nodal explants. The author maintained nodal segments of stems on MS basal media fortified with several cytokinins (N-phenyl-N’-1,2,3 thidiazol-5-ylurea [TDZ]), 6-(γ,γ-dimethylallylamino) purine [2iP] and 6-benzylaminopurine [BAP]). Compared to the other cytokinins, BAP induced a more satisfactory effect in both culture establishment and shoot proliferation stages. The author also observed persistent callusing in both phases. In the same study, the application of growth regulators like gibberellic acid (GA3) and 2,3,5-triiodobenzoic acid (TIBA) known to inhibit callogenesis had no positive effect. Whereas erratic adventitious root formation was detected in *in vitro*, some shoots were rooted in the presence of the auxins α-naphthalene acetic acid (NAA) and indole-3-butyroric acid (IBA). IBA gave a more satisfactory effect than NAA, as it elicited more roots. Generally, the author noted that cuttings of AYB formed adventitious roots regardless of whether auxin was present or not.

Akande et al. (2009) investigated callus induction from the root, stem, and leaf explants of two AYB accessions, SSSWN56 (brown seed) and SSSWN75 (grey seed). According to the authors, stem explants from both accessions cultivated on medium augmented with 1.5 mg/L each of kinetin (KIN) and NAA elicited maximum callus percentage (100 %) with minimum callusing observed with root explants. Callus induction was not detected on plant growth regulator free (PGR)-free and indole acetic acid (IAA)-fortified media.

In *in vitro* regeneration by direct organogenesis from an embryo, leaf, cotyledonary node, and shoot tips explant of AYB was studied by Adesoye et al. (2012). The authors observed 100 % multiple shoots induction when embryo explants were maintained on MS medium augmented with...
Table 1. Morphological characteristics of potential AYB accessions for use as parents in breeding program.

| ACC  | PD        | SS          | TT        | D50   | DSE   | Prot   | Oil   | SYP (g) | Origin |
|------|-----------|-------------|-----------|-------|-------|--------|-------|---------|--------|
| TSs-10 | Shattering | Round/globular | Smooth    | 77    | 5.83  | 19.13  | 15.34 | 31.62   | NG     |
| TSs-48 | Shattering | Round/globular | Rough     | 89    | 6     | 23.53  | 10.31 | 39.47   | NG     |
| TSs-49 | Shattering | Oblong      | Smooth    | 81    | 6.33  | 22.4   | 7.27  | 36.44   | NG     |
| TSs-57 | Shattering | Oval        | Smooth    | 84    | 5.5   | 17     | 18.9  | 45.03   | NG     |
| TSs-58 | Shattering | Rhomboid    | Smooth    | 81    | 6.25  | 17     | 10.08 | 39.43   | NG     |
| TSs-61 | Shattering | Oblong      | Wrinkle   | 84    | 6.25  | 18     | 12.67 | 43.35   | NG     |
| TSs-69 | Shattering | Oblong      | Wrinkle   | 86    | 6.33  | 20.33  | 15.22 | 43.36   | NG     |
| TSs-82 | Shattering | Oblong      | Smooth    | 89    | 6.5   | 17.63  | 11.79 | 39.43   | NG     |
| TSs-93 | Shattering | Oval        | Rough     | 77    | 7     | 22.57  | 12.62 | 48.78   | NG     |
| TSs-95 | Shattering | Oval        | Smooth    | 86    | 6.08  | 16.33  | 12.43 | 33.9    | NG     |
| TSs-96 | Shattering | Rhomboid    | Smooth    | 86    | 6     | 18.43  | 14.03 | 32.15   | NG     |

ACC: Accesions; PD: Pod dehiscence; SS: Seed shapes; TT: Testa texture; NG: Nigeria.
References: a: IITA, 2021; b: Adewale (2011); c: Aremu et al. (2019); d: IITA, 2021.

0.5 mg/L of BAP and 0.05 mg/L of NAA. Leaf explant produced callus without organ formation. Shoot tip and cotyledony node explants maintained on medium with 2.0 mg/L of BAP alone responded with a maximum shoot number of 4.75. However, minimum shoot responses were detected when explants were cultured on 0.1 mg/L of kinetin. Although multiple shoots derived from the embryos rooted directly on shoot induction medium, shoots derived from shoot tips rooted on medium amended with 0.5 and 0.25 mg/L of NAA. Cotyledonary node-derived shoots did not generate roots.

Ogunsola et al. (2016) reported in vitro morphogenic responses of two AYB accessions (TSs154 and TSs5) to PGRs. They maintained explants derived from mature embryos on PGR-free medium and NAA- and BAP-augmented medium. The authors also cultivated nodal cuttings derived from the shoots of regenerated embryos on a medium supplemented with different concentrations and combinations of BAP, KIN, and NAA. According to the authors, medium without PGRs supported embryo regeneration and growth than medium with growth regulators. Although both media enhanced shoot formation and rooting, none could elicit multiple shoots from embryo explants. Nodal explants of TSs154 produced multiple shoots with the maximum average number of shoots, roots, and leaves of 5.3 ± 2.3, 3.7 ± 2.9, and 7.7 ± 3.6, respectively. More so, root lengths of 3.1 ± 0.0 cm were formed on medium fortified with 0.03 mg/L and NAA 0.6 mg/L. BAP for TSs154, while for TSs5, the maximum average number of 3.2 ± 2.5 shoots and 5.9 ± 1.5 leaves were formed in medium with 0.05 mg/L of NAA and 2.0 mg/L of KIN.

4.2. Application of molecular markers technology for genetic diversity assessment in AYB

Recently, the assessment of genetic diversity in AYB using molecular markers has been on the rise. AFLP, SSR, RAPD, and SNP have all been deployed to estimate genetic diversity in the species (Table 3).

4.2.1. Analysis of genetic diversity

Whereas pre-breeding programs are ongoing, an improved variety of AYB is yet to be released. Many studies have focused on morphological characterisation, which is affected by environmental factors. Molecular studies are of importance in ensuring that variation is genetic and not environmentally induced. For AYB breeding, assessment of genetic diversity is pertinent. Accurate information on genetic variability is vital for the conservation and utilisation of germplasm resources.

Moyib et al. (2008) and Popoola et al. (2017) used RAPD markers in the genetic analysis of AYB, respectively. The similarity indices recorded in these studies ranged from 0.42 – 0.96 and 0.72-0.93. According to Moyib et al. (2008), cluster analysis for the 24 accessions were grouped into eight clusters at a similarity index of 0.80. The principal component

Table 3. Molecular markers used in the assessment of genetic diversity in AYB.

| Molecular marker used | No of AYB accessions | No. of Loci/Polymorphic fragments | Citation |
|-----------------------|----------------------|----------------------------------|----------|
| RAPD                  | 24                   | 53                               | Moyib et al. (2008), Popoola et al. (2017). |
| AFLP                  | 77                   | 227                              | Adewale et al. (2015), Ojuederie et al. (2014). |
| SSR                   | 67                   | 55                               | Shitta et al. (2016). |
| ISSR                  | 17                   | 107                              | Nnamani et al. (2019). |
| SNP                   | 137                  | 3.6K SNP                         | Oluwole et al. (2020). |

Table 2. Biotechnological approaches deployed to date for AYB improvement.

| S/N | Biotechnology technique | Application | Reference |
|-----|-------------------------|-------------|-----------|
| 1   | Plant tissue culture    | - Clonal propagation via nodal explant<br>- Callus induction (from leaf, root, and stem explants)<br>- Explant sterilisation<br>- Direct organogenesis (from the embryo, leaf, cotyledony node, and shoot tip explants)<br>- In vitro morphogenetic response in mature embryo explant | Otsoseng (2005), Akande et al. (2009), Aliyu and Adesoye (2007), Adesoye et al. (2012), Ogunsola et al. (2016). |
| 2   | Molecular markers       | - Genetic diversity assessment based on random amplified polymorphic DNA (RAPD)<br>- Evaluation of genetic diversity using amplified fragment length polymorphism (AFLP)<br>- Transferability of cowpea simple sequence repeat (SSR) for the evaluation of genetic diversity in AYB<br>- Genetic diversity assessment using inter simple Sequence Repeat (ISSR) markers | Moyib et al. (2008), Popoola et al. (2017), Ojuederie et al. (2014), Adewale et al. (2015), Shitta et al. (2016), Nnamani et al. (2019). |
analysis (PCA) showed the first three principal components contributing 30.20, 22.17, 8.60, respectively and it is about 60.98 % of the total variation observed among the twenty-four accessions of AYB. The PCAs were consistent with phylogenetic tree and structure results. The separation of the accessions into these groups was based on phenotypic differences, mainly tuber formation, tuber flesh colour, and seed shape (showing a predictive relationship between genotype and phenotype. In the latter study with ten accessions, RAPD markers detected three clusters.

Adewale et al. (2015) evaluated the inter-specific diversity in 77 accessions of AYB using a total of five EcoRI/MseI primer combinations. About 26 % (59) of the 227 bands produced were polymorphic. E-.ACT/M-CAG had a polymorphic efficiency of 85 % and E-AGC/M-CAG with the least polymorphic efficiency of 80.6 % out of all primer combinations. Among the AYB accessions studied, the Jaccard genetic distance ranged between 0.048 and 0.842. The accessions were grouped into four major clusters consisting of 8, 20, 21, and 28 accessions, respectively. The mean fixation and mean expected heterozygosity indices of 0.203 and 0.284 revealed a large genetic base in the AYB accessions. Ojuederie et al. (2014) used four AFLP primer combinations in assessing genetic diversity in forty AYB accessions. The primers amplified 1730 fragments, out of which 1647 were polymorphic fragments (95.20 %). E-AGC/M-CAG produced the most number of bands (520). Polymorphic information content ranged from 0.9447 to 0.9626. Two primer combinations E-AAC/M-CAG and E-ACT/M-CAG recorded 100 % polymorphism. The forty accessions of AYB were grouped into two major clusters with similarity indices of 0.66–0.91.

Presently, there is no AYB-derived simple sequence repeat (SSR) markers. Shitta et al. (2016) used 36 cowpea-derived SSR primers (16 genomic, 10 unigene, and 10 EST-SSR) to screen AYB for genetic diversity. The amplification ability of the 36 primers was tested across genomic DNA extracted from 67 accessions of AYB used in the study. Thirteen of the SSRs (36%) were able to amplify AYB, while only eight out of these SSRs gave an amplification rate of above 60% in AYB genomic DNA and were thus used for the study. The polymorphic fragments generated from the primers were 55, with an average of 6.9 per primer. The simple matching coefficient ranged from 0.458 to 1.00. A dendrogram drawn based on UPGMA produced three main clusters, with cluster 1 being the most diverse with a dissimilarity range of 0.517–1.000, suggesting a large amount of genetic diversity in the AYB accessions to be exploited for AYB improvement.

4.2.2. Application of advanced genomic tools in AYB

Several advanced genomic tools can be applied in AYB improvement. Diversity array technology sequencing (DArTseq) has been deployed for high-throughput genetic analysis of different traits in AYB. The DArTseq procedure is based on genome complexity reduction using a suite of restriction enzymes. Microarray hybridisation is used in the detection of the presence or absence of individual fragments in genomic representations. This technology is increasingly being used in the characterisation and diversity studies of several crops for enhanced conservation in genebanks. Some tropical crops and a few orphan crops have been analysed using this cost-effective technology (Huttner et al., 2005).

Genome-wide association studies (GWAS) is a tool used to assess the relationship between single-nucleotide polymorphisms (SNP) and variation in a particular phenotypic trait. This technique overcomes some limitations associated with conventional breeding (Luo et al., 2020) and has been applied in several crops to identify genes controlling specific characteristics (Table 5). In using GWAS, consideration is given to the magnitude of linkage disequilibrium (degree of non-random association of alleles at different loci); and likely spurious association derived from the population structure and genetic relatedness (Zeng et al., 2017). The rate of false-positive errors can be corrected using a mixed linear model (MLM) and compressed MLM (Zeng et al., 2017). GWAS identifies the loci responsible for a specific trait via single-nucleotide polymorphism (SNP) and phenotypic variation based on linkage disequilibrium. GWAS on AYB is currently ongoing at the Genetic Resources Center (GRC) of the international institute of tropical agriculture (IITA), Ibadan, Nigeria (Palival et al., 2020). In a preliminary study by Oluwole et al. (2020), GWAS of nutritional traits in AYB generated 3.6K SNPs using the DArTseq. The authors identified about 50 putative QTLs linked with seed starch, protein, and oil contents.

5. Future perspectives

Several modern biotechnological tools have been employed to improve different legume crops (Table 4). With the advancement of high-throughput techniques, it is crucial to consider integrating various omics technologies to initiate a global view rather than a single-omics approach (Narayana and von Wettberg, 2020). Omics technologies such as transcriptomics, genomics, proteomics, and metabolomics should be deployed in AYB to further understand and improve the crop (Popoola et al., 2019). The various omics tools are helpful in improving crop nutrition quality, gene analysis, protein modelling, and developing varieties resistant to biotic and abiotic stress (Shaﬁ et al., 2019). Le Signor et al. (2017) reported success in combining GWAS and proteomics in identifying genes responsible for the synthesis of globulin in Legumes.

| Biotechnological tools                          | Function/application                                      | Example of crops                  | References                               |
|------------------------------------------------|-----------------------------------------------------------|-----------------------------------|------------------------------------------|
| Bioinformatics                                  | Identification of DNA sequences and specific motifs       | Soybean                           | Yuan et al. (2017)                       |
| Proteomic technology                           | Identification of stress responses                        | Common bean                       | Larraínzar and Wienkoop (2017)          |
| Genetic modification                           | Used to modify complex traits, e.g. resistance to pests and diseases | Cowpea, Chickpea                  | Beit et al. (2017), Alok et al. (2020)  |
| Genome-wide associated studies (GWAS)          | Used to identify genetic loci for various traits in various crop species | Soybean, Common bean              | Do et al. (2019); Raggi et al. (2019)   |
| Genome editing- CRISPR/Cas9                    | Used to improve specific traits in plants via inducing mutation. | Cowpea (Vigna unguiculata), Medicago truncatula | Meng et al. (2019); Ji et al. (2019)    |
5.1. Genomics

Genomics is the study of genomes, and it is applicable in the analysis of gene regulation, genomic variations, genome evolution, genomic variations, gene regulation, and genome sequence information (Zheng et al., 2019). So far, not all aspects of genomics have been applied for AYB improvement. For a robust breeding program, the whole genome sequencing of the crop is essential. As such, the Alliance for Accelerated Crop Improvement in Africa (ACACIA) is currently undertaking the whole genome sequencing project of AYB (ACACIA, 2020). The availability of a complete whole genome sequence would significantly improve marker discovery and precise detection of various QTL positions in the AYB genome (Paliwal et al., 2020). Molecular markers associated with specific traits in AYB need to be developed to aid improvement. Through translational research (Jacob et al., 2018), valuable traits of AYB can be transferred to major crops or vice-versa through biotechnological strategies. Also, identifying transposable elements, which are usually found in the genomes of flowering plants, can help identify specific genes for crop improvement (Popoola et al., 2019).

5.1.1. Genome editing

Genome editing, one of the game-changing technologies in biological sciences, can also be applied towards AYB improvement. It enhances the precise and targeted modification of specific traits of interest, thus accelerating crop improvement. Genome editing technologies such as CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 can alleviate the limitations of conventional breeding, thus increasing food production in a cheaper and faster manner (Gao, 2018). Recent advancements in CRISPR technologies have brought about precise and targeted genetic manipulation of crops, accelerating the shift towards crop enhancement via precision breeding (Zhang et al., 2020). The CRISPR/Cas9 method of editing has been applied in the improvement of about 20 different species of crops for several traits, which include disease resistance, tolerance to biotic and abiotic stresses, and yield improvement (Ricroch et al., 2017). This can also be applied for gene expression regulation, modification of epigenetic loci, and precise optimisation of traits in AYB to improve its resistance to diseases and maximise productivity especially under adverse and erratic weather conditions, even to reduce the prolonged cooking time in AYB. Genome editing of AYB can also reduce the crop’s extended maturity period and anti-nutritional compounds (Table 5). The nutraceutical properties of AYB can be improved for proper utilisation through the application of CRISPR/Cas9.

Recent studies have shown that genetic engineering to improve photosynthetic processes can improve yield potential vital for meeting future demand for food (Foyer et al., 2017; Simkin et al., 2019). Some biotechnological blueprints which can be adopted for improving yield potential include enhancing the kinetics of RuBisCO (Ribulose-1, 5-bisphosphate carboxylase-oxygenase). RuBisCO is a vital enzyme in plants, and it is the point of entry for carbon dioxide (CO₂) into the Calvin–Benson–Bassham cycle. Other strategies include increasing the mesophyll conductance, modulating the photosynthetic pathway, improving photon utilisation and reducing photodamage (Singer et al., 2020). The application of these biotechnological strategies can contribute to increased plant productivity in AYB.

5.2. Proteomics

Proteomics is another very useful omic tool for studying the cellular units of proteins under a specific condition (Afzal et al., 2019). It can be used to monitor candidate genes/proteins responsible for biological processes at a particular stage or condition in a specific tissue. Proteomics can be used to determine the function of several genes in a single experiment as well as discover new genes useful in providing solutions to biotic and abiotic stress (Zargar et al., 2017). Combining the omics approach with breeding programs can potentially facilitate legume sustainability, including AYB.

5.3. Transcriptomics

Transcriptomics is the study of all the RNA transcripts in an organism at a particular time. The genetic make-up of an organism is encoded in the DNA of the genome, which is then expressed via transcription (Lowe et al., 2017). Plant transcriptomics makes use of Next Generation Sequencing (NGS), which provides detailed information on the genome sequence of any crop, including non-model crops. Transcriptomics has been applied in non-model plants to optimise in vivo and in vitro biosynthesis of essential oils, biodiesel feedstock, and medicinal compounds (Stander et al., 2020). There is a need to discover new transcript genes as well as develop molecular markers for specific traits, which can be exploited to improve legume breeding programs. NGS is economical and provides excellent insight into transcriptomics (Afzal et al., 2019). Wang et al. (2015) and Ferreira-Neto et al. (2019) reported the application of transcriptomics in soybean. Ferreira-Neto et al. (2019) confirmed that the expression of Raffinose Family Oligosaccharides (RFO) and INS (inositol and inositol phosphates) pathways were involved in the under root dehydration of various soybean accessions while Wang et al. (2015) provided an understanding into the modulation of abiotic stress responses and development in soybean. Transcriptomics can be used for disease diagnosis and profiling in AYB. RNA-sequencing (RNA-Seq) can be used to detect disease-associated SNPs to help scientists understand the disease causal variant. It can also be used to study plant-pathogen interaction to develop efficient control measures. The use of dual RNA-Seq can be used to simultaneously profile the expression of both the plant (host) and pathogen. The advent of transcriptomics has made it possible to identify genes and pathways that can detect abiotic and biotic stress; and identify genes responsible for specific phenotypes (Lowe et al., 2017).

5.4. Metabolonomics

The field of metabolomics has developed powerful tools useful in plant and food science (Llorach et al., 2019). Metabolonomics was introduced to analyse compounds with low molecular weight in various biological systems (Pereira Braga and Adamec, 2019). The metabolome possesses components identified to be end-products of gene expression, biological systems, and post-translational modifications. The advent of metabolomics technologies has allowed for the analysis of compounds involved in various biological processes, including metabolic pathways, enzyme activities, and gene expression. Metabolonomics has been used to study the metabolic response of plants to various environmental stresses, such as drought, salinity, and heavy metals. This information is useful for the development of crops that are more resistant to these stresses.

### Table 5. Traits that can be improved in AYB through the CRISPR approach.

| AYB traits for improvement | Crops with similar traits that have been successfully modified | Specific Approach | References |
|---------------------------|---------------------------------------------------------------|-------------------|-----------|
| Day-length sensitivity and plant architecture | Wild tomato | Modification of coding sequences using CRISPR, cis-regulatory regions (function of these regions, and uORFs (upstream open reading frame) of genes | Li et al. (2018); Zhang et al. (2020) |
| Growth period | Soybean | Gene knock out via CRISPR/Cas9 system and Agrobacterium-mediated transformation | Han et al. (2019); Lu et al. (2020) |
| Anti-nutritional factors | Soybean | Gene Editing to Eliminate Anti-Nutritional Factors in Soybean Seeds (On-going research) | USDA (2021) |
| | | | Camerlengo et al. (2020) |

O.O. Oluwole et al. Helixy 7 (2021) e08481
which help define the biochemical phenotype of a tissue or cell. The measurement of the metabolites gives a broad perspective of the biochemical make-up of plants, which can be used to assess gene function (Chhatak et al., 2018). Improved mass spectrometry (MS), ultraviolet-visible spectroscopy (UV-Vis), and nuclear magnetic resonance (NMR) are majorly used in metabolomics analysis because they provide detailed characterisation and structural exposition of the agents. Ramalingam et al. (2015) applied quantitative mass spectrometry to evaluate the metabolomics diversity in Medicago symbionts. Llorach et al. (2019) also reported a metabolomics study that compared the bioactive compounds of chickpeas, beans, and lentils. So far, the use of metabolomics in legumes is limited (Afzal et al., 2019). While advances have been achieved in various platforms of analytical chemistry that provide highly efficient techniques for metabolome analysis, they are not yet fully understood (Saleem et al., 2020).

6. Putative impacts and risks of using biotechnological techniques

Biotechnological techniques have varying benefits and risks depending on economic, environmental, cultural, or social factors. The application of plant biotechnological tools such as genome editing (GE) offers great potentials for developing new crops (Harfouche et al., 2021). The application of advanced biotechnology to develop engineered crop varieties can make crops with better yields available throughout the year (Dwivedi et al., 2018; Ionescu et al., 2017). Engineered AYB varieties can help improve AYB adaptation for shortened growing seasons. Another positive impact of biotechnology is the development of crops that are resistant to pests and diseases. Gene editing technology supports targeted and high-precision restructuring of plant genomes, reducing the cost of product development (Lassoued et al., 2019).

Although plant biotechnology has many benefits, it also has its disadvantages. One of the major challenges is that consumers are not willing to accept genetically modified foods because their long-term effects on their health are unknown, such as toxicity, intolerance, allergies, and antibiotic resistance (Wieczorek, 2003; Lassoued et al., 2019). Another challenge of biotechnology is that developing countries would always have to depend on developed countries for biotechnological tools because the tools are not readily available in developing countries. There is also the issue of monopoly by producers. For example, Monsanto is the leading seed company globally, which could result in negative effects on consumers (Panzarini et al., 2015). Other challenges of biotechnology (GMOs) includes genetic pollution and gene transfer in the environment (Hansson, 2019; Massabni and De Souza, 2020). Gene editing tools can also be used as biological weapons if such technology gets to the wrong hands; there could be effects on non-target species and loss of diversity in crops (Khan, 2019).

7. Conclusion

The improvement of underutilised legumes can achieve a robust agricultural system that addresses food and nutrition security. Underutilised legumes still have a lot of untapped potentials, and there is a need for an extensive focus on improving such legumes, like AYB, for food and income purposes. Biotechnological approaches that have been deployed to improve staple crops can be transferred to these underutilised crops. The International Institute of Tropical Agriculture as well as independent researchers are working on how AYB can be improved using genomic and biotechnological tools. This review identified some bottlenecks in AYB improvement, and with the aid of biotechnological tools, discovering the genetic basis of any trait of interest would be easier and faster. Advancement has been made in genetics and genomics; however, other omics technologies are yet to be explored in AYB. Modern techniques need to be applied for yield improvement, reduced cooking time and maturity period, as well as resistance to pests and diseases to make the crop suitable for food and income security in sub-Saharan Africa. The integration of all the omics technologies can facilitate the assessment of the complex cellular life of AYB. The application of biotechnology can help unravel the genetic potential of AYB for crop improvement, which can lead to further research opportunities.

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Oluwole et al. Heliyon 7 (2021) e08481

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