# Genome resources for underutilised legume crops: genome sizes, genome skimming and marker development

Antonia Diakostefani1, Rania Velissaris1, Emilia Cvijanovic1, Robin Bulgin1, Andriana Pantelides1, Ilia J Leitch2 (0000-0002-3837-8186), Sahr Mian2 (0000-0001-7828-0629), Joseph A Morton2,3 (0000-0003-4400-4575), Marybel Soto Gomez2 (0000-0003-1812-7416), Mark A. Chapman1,\* (0000-0002-7151-723X).

1 Biological Sciences, University of Southampton, Life Sciences Building 85,Highfield Campus  
Southampton, SO17 1BJ, UK

2 Royal Botanic Gardens, Kew, Richmond, TW9 3AB, UK

3 School of Biological and Behavioural Sciences, Queen Mary University of London, Mile End Road, London, UK

\* Author for correspondence, email [m.chapman@soton.ac.uk](mailto:m.chapman@soton.ac.uk)

## Abstract

Underutilised crops suffer from under-investigation relative to more mainstream crops, but often possess improved stress tolerance and/or nutrition, making them potentially important for breeding programmes in the context of climate change and an expanding human population. Developing basic genome resources for underutilised crops may therefore catalyse analyses to facilitate their use, through improved understanding of population structure, phylogeny, candidate genes, and linkage mapping.

We carried out nuclear and plastid genome sequencing and assembly for five underutilised legumes: jack bean, sword bean, Kersting’s groundnut, moth bean, and zombi pea. Using only ‘off-the-shelf’, free-to-use bioinformatic tools, we also developed a simple but effective pipeline to identify thousands of markers, which could be applied in other species. We assembled 53–68% of the genome and 73- 95% of the gene space in the five legumes. The assemblies were fragmented but nevertheless useful for identifying between 34,000 - 60,000 microsatellites. Examination of 32 markers in zombi pea revealed 16 primer pairs which amplified in at least half of the eight accessions tested and were polymorphic. We also present nuclear genome size estimates for 17 legume taxa (12 for the first time), comprising the above five species as well as other domesticated legume species and crop wild relatives.

We aim for the newly developed markers and genome size estimates presented here to be useful for the research community by aiding genomic and population genetic studies for these taxa, and to provide information on approaches that can be applied for investigating other important yet underutilised crops.

## Keywords

Fabaceae, minor crop, microsatellites, simple sequence repeats (SSRs), C-value, underutilised crop

## Introduction

Climate change and a rapidly increasing population underlie major concerns over food security (Schmidhuber and Tubiello 2007; FAO 2016). Currently, there is an overreliance on a limited number of staple crops, with 90% of the global basic nutritional intake being derived from only fifteen crops (FAO 2020). This is leading to an increasingly homogenous food supply dominated by globally important cereal and oil crops, along with a decline of other cereal and tuber crops (Khoury et al. 2014). Though substantial efforts have been made to create climate- and disease-resistant varieties of staple crops that are also high yielding, these have a limited capacity to adapt to different growing conditions, and productivity gains from crop improvement are likely reaching a plateau (Grassini et al. 2013). Current agricultural practices, such as excessive fertiliser use, are unsustainable and major changes in food production will be needed to eradicate hunger and malnutrition whilst coping with a rising population and the changing climate conditions. Greater crop biodiversity is crucial for the development of stable crop production systems that harvest sufficient food (Campbell et al. 2016; Massawe et al. 2016; Renard and Tilman 2019).

Underutilised crops are domesticated, semi-domesticated, or wild species or varieties of plants that may be significant in local production systems, but are currently overlooked in research, breeding, and policy making (Chivenge et al. 2015; Massawe et al. 2015; Hunter et al. 2019; Li et al. 2020). Many of these species were previously widely grown but have fallen into disuse for a range of economic, agronomic, and cultural reasons. Globally, there are hundreds of underutilised plants that could offer alternative food sources; it is therefore important to investigate these species and identify solutions for negative attributes that may reduce their popularity, such as growing and storage difficulties and the presence of antinutrients (Li and Siddique 2020).

Introducing a new crop into standard farming practices is challenging; it requires dedicated breeding programmes to establish these cultivars and make them economically viable. In developed countries, where crop breeding is mostly conducted by private companies rather than public communities, underutilised crops are rarely considered, and breeding programmes carried out by universities tend to have low impact on farming practices. This lack of investment slows the development of true-breeding, high-yielding cultivars of potential value for global food systems, while the continued high research interest and dissemination of major crops results in minor crop varieties and the associated indigenous knowledge being lost (Chivenge et al. 2015; Khoury et al. 2022). Improving an underutilised crop through breeding not only rescues the threatened crop varieties, but may also further increase interest and encourage investment by private and public sectors (Li et al. 2020), thus efforts to make these crops more marketable would be beneficial (Stamp et al. 2012).

Generating molecular markers for use in determining relationships between different populations and varieties, generating genetic maps, and identifying genomic regions controlling adaptive (or maladaptive) traits will help us begin to understand the genetics and attributes of underutilised crops and provide valuable resources to inform molecular breeding techniques. (Hodel et al. 2016a; Chapman 2019). The advent of high-throughput sequencing (HTS) and accompanying bioinformatic tools have reduced the cost and time required for assembling genome-scale sequences (e.g., whole genome assemblies and transcriptomes) and developing markers such as SSRs (i.e., simple sequence repeats or microsatellites) (Hodel et al. 2016b). These approaches have thus been adopted for hundreds of plant species in the last decade, including several underutilised crops (e.g., Chapman 2015; Vatanparast et al. 2016; Sathyanarayana et al. 2017; Singh et al. 2020). Furthermore, even draft quality genome assemblies can serve additional purposes, such as identifying genes of interest (e.g., loci associated with drought tolerance) and examining sequence evolution across taxa (Fisher et al. 2022).

Legumes (Fabaceae) comprise the second most important group of crops after cereals (Poaceae) and have a large impact on nutritional security due to their high protein content. Their ability to fix atmospheric nitrogen also means that they can often grow on poor soils without additional fertiliser input (Kebede 2021). Given the environmental and economic cost of synthetic fertilisers, the expansion of legume production could have wide benefits for farmers and the environment. In addition, several underutilised legumes are perennial, leading to an improved soil stability.

Here we present draft nuclear and plastid genomes and genome-derived SSR markers for five species of underutilised legumes with no available genomic data in public repositories as of time of publishing: jack bean (*Canavalia ensiformis* (L.) DC.), sword bean (*Canavalia gladiata* (Jacq.) DC.), Kersting’s groundnut (*Macrotyloma geocarpum* (Harms) Maréchal & Baudet), moth bean (*Vigna aconitifolia* (Jacq.) Marechal), and zombi pea (*Vigna vexillata* (L.) A. Rich). These are all nutrient rich and possess characteristics that give resistance to various environmental conditions under which staple and well-known legumes may struggle, as explained below.

Jack bean and sword bean are closely related climbing legumes in the genus *Canavalia*. The two species are similar in growth habit, but distinguished by seed colour and hilum length (Moteetee 2016). Jack bean is typically an annual plant native to South and Central America, with uses in both human diets and animal fodder. Young green pods are consumed as a vegetable in various parts of Asia; it is also farmed on a modest scale in non-Asian nations and can be grown in marginal soils with low water availability due to its deep roots. Sword bean is a perennial species that originated in East Asia and is now grown throughout the tropics (Haq 2011). It is generally considered a hardy species with potentially useful agricultural traits (Ekanayake et al. 2000; Haq 2011), including a deep root system that confers resilience to drought and waterlogging. Both species contain various antinutrients, including lectins, tannins, and protease inhibitors (Vadivel et al. 2010; Doss et al. 2011) that must be removed using specific preparation methods before safe consumption in high quantities. Nevertheless, jack bean and sword bean have potential to be used more widely in human nutrition as their protein quality is comparable to staple legume crops and they are rich in macro- and micronutrients. For example, jack bean contains a high quantity of protein (23-34%) derived from several (but not all) essential amino acids, and is additionally a good source of carbohydrates (ca. 55%) and micronutrients (Akpapunam and Sefa-Dedeh 1997). Due to its low-fat content (0.2% in fresh seeds), jack bean may represent a viable source of nutrition for those seeking a low-fat, high-protein fortified diet. Trials have demonstrated that jack bean can be incorporated in popular dishes typically made from other legumes (Karoli et al. 2017). Sword bean is used in various parts of Asia as a substitute for mashed potatoes or broad beans, the leaves have the potential to cure ailments such as skin rashes and constipation. The two species are phylogenetically distinct from many staple legumes, being found in the subtribe Diocleinae and therefore divergent from chickpea (Cajaninae), soybean (Glycininae) and the dozens of *Vigna* and *Phaseolus* crop species (Phaseolinae) (Kajita et al. 2001). This placement makes their investigation important from the standpoint of understanding the phylogenetics of the legumes more broadly.

Kersting’s groundnut, widely grown in West Africa, is a geocarpic legume, i.e. pods develop underground, the same growth habit observed in peanuts and Bambara groundnut. It is drought resistant and higher in essential minerals and the amino acids lysine, arginine and methionine than other legumes (Assogba et al. 2016; Ayenan and Ezin 2016). Kersting’s groundnut has a palatable taste and has potential for use in infant food formulations, as a source of iron for anaemic patients, as well as helping prevent malnutrition (Garcia-Casal et al. 2018). Despite these benefits, Kersting’s groundnut, especially as a food, is becoming increasingly neglected (Chivenge et al. 2015) – yield is low relative to other similar crops, harvesting the underground pods is physically demanding, and the seeds are highly susceptible to Bruchid infestations post-harvest, leading to large losses. Therefore, the beans must be sold quickly and cannot be relied upon during food shortages (Chivenge et al. 2015). Nevertheless, it is used in rituals by some groups, which is helping to conserve the species. Although relatively understudied, recent genetic analyses have (1) revealed the relationships between Kersting’s groundnut and two other underutilised crops in the genus (i.e. *Macrotyloma axillare* and *M. uniflorum* (Fisher et al. 2022)), and (2) demonstrated significant population structure but low genetic diversity overall in Kersting’s groundnut (Kafoutchoni et al. 2021).

Moth bean and zombi pea are species in the genus *Vigna*, which encompasses multiple well-known food crops such as cowpea, adzuki bean, mung bean and dozens of minor legume crops (Delgado-Salinas et al. 2011). Moth bean has been identified as a potentially significant crop for the future due to its high content of digestible protein and micronutrients (especially iron and zinc), nutraceutical properties (as an anti-diabetic, antioxidant and anti-hypertensive), and medicinal properties (as a pain reliever) (Adsule 1996; Bhadkaria et al. 2022). Moth bean is grown primarily in the arid regions of India and Pakistan, and its high drought tolerance highlights its potential for cultivation in parts of Africa and other water-limited areas, especially under the predicted future climate (Baath et al. 2018). Nevertheless, it remains as an underutilised crop due to the low yield of existing cultivars and difficulty of harvesting (mowers cannot be used due to the shape of the plants therefore sickles are typically used). Moth bean also contains antinutrients, but these can be reduced via traditional processing methods.

Zombi pea is a perennial climbing legume of African origin that is mainly used for food, forage, and erosion control (Karuniawan et al. 2006). There are two domesticated forms: a seed form that is grown in Africa, and a tuberous root form found in a small region around Indonesia (Dachapak et al. 2018). Wild populations of the species are found throughout the tropics and subtropics, where they may be harvested for local use (Amkul et al. 2020). Zombi pea is used primarily as a backup crop due to its reliability during periods of both high and low rainfall, when more popular crops such as sweet potato and cassava cannot be grown (Karuniawan et al. 2006). Moreover, the tubers tend to be high in protein compared to traditional root crops (Chandel 1972, cited in (Dachapak et al. 2017). The wide distribution of zombi pea and its resilience to varying water availability could make it an excellent candidate crop for future improvement (Karuniawan et al. 2006).

Despite their current status as underutilised, the five focus crops of this study represent agronomic and nutritional traits of interest for future-proofing the global food supply. To aid in future genomics-based investigations, we generated short-read HTS data for one accession of each of the five crops, which we used to identify nuclear genome SSR markers and to produce draft plastid genomes. In addition, we estimated the genome size of these and 12 other underutilised legume crops and wild relatives. This information is critical for undertaking successful whole-genome assemblies by enabling the amount of sequencing required to capture the entire genome at a given coverage depth to be estimated, and later serving as a benchmark to evaluate assembly completeness. Additionally, genome size may provide insights into crop resilience, as this trait has long-recognized impacts at the nuclear, cellular, and whole-plant level, which ultimately can play a role in influencing where and how plants grow (reviewed in Pellicer et al. 2018). We anticipate that the genomic resources presented here will be used to address diverse research questions. In addition, by using off-the-shelf, free-to-use bioinformatic tools, our pipeline should encourage other researchers to adopt this strategy for other underutilised species.

## Materials and Methods

**Genome size measurements**

Genome size (expressed as 1C-values, the DNA content in an un-replicated gametic nucleus) was measured for 31 individuals representing 17 legume taxa, using a one-step flow cytometry procedure. Approximately 1 cm2 fresh, mature leaf tissue was co-chopped in a petri dish with the internal standard *Solanum lycopersicum*L. “Stupiké polní rané”, 958.44 Mb/1C (Doležel et al. 1992) using a new razor blade in 1 ml of buffer. A further 1ml of buffer was added to the sample and the contents gently mixed. Samples were prepared using General Purpose Buffer (GPB) (Loureiro et al. 2007), Galbraith’s buffer (Galbraith et al. 1983) or Cystain PI OxProtect (Sysmex UK Ltd), depending on the individual species to obtain the lowest coefficient of variation (CV) (Pellicer et al. 2021a) (see Supplementary Table 1). Polyvinyl pyrrolidone (PVP) and β-mercaptoethanol were also added to the GPB and Galbraith buffer as described (Pellicer et al. 2021b) to improve the quality of the flow histograms.

The sample was then passed through a 30 μm nylon filter, stained with 100 μl propidium iodide (1 mg/ml) and incubated on ice for 15 minutes. One sample was prepared from each individual and three replicates were run, recording up to 1,000 nuclei per fluorescence peak using a Sysmex CyFlow Space (Sysmex Europe GmbH, Norderstedt, Germany) flow cytometer fitted with a 100 mW green solid state laser. The resulting histograms were analysed with the WindowsTM-based FlowMax software (v. 2.9 2014, Sysmex GmbH) and the average of each sample was used to estimate the genome size.

**Nuclear genome assembly**

Two accessions of the five species selected for genome sequencing were grown in the greenhouse at the University of Southampton, and one was selected for DNA extraction and sequencing (Table 1). DNA extraction was carried out using a modified CTAB method (Doyle and Doyle 1990); DNA quality and quantity were estimated using agarose gel electrophoresis and NanoDrop 2000 (Thermo Scientific, UK). Samples were treated with RNase and sent to Novogene (UK) for sequencing on a HiSeq X10. Using as references the genome sizes available in the Kew Plant DNA C-values database (Pellicer and Leitch 2020) and other literature, we aimed to achieve ≥ 10X coverage and therefore generated different amounts of data for each species depending on their genome size. The amounts varied from 6 Gb for jack bean (based on a genome size of another *Canavalia* species, *C. rosea*, estimated at 267 Mb/1C (Lin et al. 2021) to 11 Gb for the zombi pea (based on a previously recorded genome size of 587 Mb/1C for the species (Pellicer and Leitch 2020).

Raw sequencing data in native format (fastq) was quality checked using Trimmomatic (Bolger et al. 2014) and cleaned using the settings ILLUMINACLIP 2:30:10, LEADING:5, TRAILING:5, SLIDINGWINDOW:4:15. In Trimmomatic, we also excluded from consideration both unpaired reads and those shorter than 72 bp .

We performed nuclear genome assembly using ABySS ver. 2.0.2 (Jackman et al. 2017) and for all species a range of kmer options from 64 to 120 in steps of 8 were examined. For each species, we used the largest N50 value in combination with total assembly length to select a single assembly for SSR marker development, although we acknowledge that other metrics can be used to identify the ‘best’ assembly. However, given that we are aiming to generate a draft assembly for marker development, we feel this is appropriate and intuitive to implement across the five species. We assessed gene space coverage using BUSCO ver5.3.2 (Benchmarking Universal Single-Copy Orthologs; (Simao et al. 2015) to determine the proportion of eudicot conserved genes (eudicots\_odb10 gene set) present in each of the five assemblies and whether these recovered loci were complete (in single or multiple copy), fragmented or missing.

**Plastid genome assembly**

Plastid genomes (plastomes) were assembled using NOVOplasty ver. 4.1 (Dierckxsens et al. 2016) based on 15M paired-end reads. The plastid gene *rbc*L for each of the five species was downloaded from GenBank (Accession numbers U74238, MW960581, LC375226, MH391992 and KX087537) and used as the ‘seed’ sequence. Plastomes were annotated using GeSeq (Tillich et al. 2017) and visualised using OGDRAW (Greiner et al. 2019).

**Marker identification**

Simple sequence repeat (SSR) markers were exclusively identified from contigs >500 bp in length, a cut-off used to avoid potential problems associated with primer design for SSRs in short contigs. Markers were identified using MISA (Thiel et al. 2003; <http://pgrc.ipk-gatersleben.de/misa/>) with a minimum of eight repeat units required to identify dinucleotide repeats, six for trinucleotide repeats, and four for tetra-, penta- and hexanucleotide repeats.

The quality of the identified markers was estimated by synthesising and testing 32 primer pairs on eight different accessions of the zombi pea, representing two individuals from four varietal groups (*V. vexillata* var. *vexillata*, var. *angustifolia* (Schumach. & Thonn.) Baker, var. *ovata* (E.Mey.) B.J.Pienaar and var. *macrosperma* Maréchal, Mascherpa & Stainier; Table 2). There were 12, 12 and 8 primers spanning dinucleotide, trinucleotide and tetranucleotide repeats, respectively. Loci for primer design were selected randomly from those with a moderate length repeat unit (20mer for di- and trinucleotide repeats and 8-10mer for tetranucleotide repeats). We followed previously published methods (Yang et al. 2018; Chapman 2019) for DNA extraction, PCR and genotyping.

## Results

**Genome size variation**

The genome size values for the 17 legume taxa ranged between 372 Mb/1C in *Macrotyloma uniflorum* and 802 Mb/1C in *Vigna vexillata* var. *macrosperma*, representing a ~2-fold variation (Online Resource 1). These genomes were relatively small and narrowly distributed compared to 920 estimates for Fabaceae (mean = 2,034 Mb/1C, ~98-fold variation) and 10,770 angiosperms (mean = 5,020 Mb/1C, ~2,440-fold variation). Apparent intraspecific variation was relatively low in the nine species for which we measured more than one individual, ranging from 0 (in *Pachyrhizus erosus*) to 151 Mb (in *Vigna subterranea*), although the number of replicates was also low (i.e., 2 – 8 individuals). The range in CV values across all estimates was 3.03-5.25%.

**Nuclear and plastid genome assembly**

We obtained between 26.2 and 46.5 M raw reads for each of the five individuals sequenced (Table 1). Raw reads have been deposited in the NCBI SRA under bioproject PRJNA882244. After quality control and trimming, ca. 97% of the reads were retained. We achieved the desired ≥ 10X coverage for Kersting’s groundnut (11.5X) and moth bean (14.9X), but lower coverage for jack bean and sword bean (ca. 6X), and zombi pea (8.4X).

After assembling the data using multiple kmer settings we aimed to select the assembly with the highest contig N50 and total assembly length. The assembly with the highest N50 was not necessarily the longest length assembly (Online Resource 2; Online Resource 3), hence we made a compromise. For jack bean and sword bean we used kmer = 64 and for the other species we used kmer = 80. For the five selected assemblies, the lowest N50 (less than 5 kb) was determined for the three species with the lowest coverage (jack bean, sword bean and zombi pea). For Kersting’s groundnut and moth bean the N50 was 15.5 kb and 11.5 kb respectively (Online Resource 2). Across the five species, there was a negative correlation between genome size and N50 (Pearson’s correlation ρ = -0.890, *P* = 0.043). Based on the assembly length from ABySS and the genome size measurement from flow cytometry, we estimate that we assembled 53.0% to 68.4% of the (haploid) genome for the five species. Nevertheless, the BUSCO analysis based on 2,326 conserved eudicot genes suggests that we sequenced 73-95% of the gene space in these species (Figure 2). Therefore, while the assemblies are incomplete and fragmented, a large portion of the gene space is covered. Nearly half of the BUSCOs were complete and single copy in the two *Canavalia* species, and this rose to 70% in the zombi pea and >85% in both Kersting’s groundnut and moth bean. Assemblies with the larger N50 had a greater proportion of complete BUSCO~~s~~ loci (Pearson’s correlation ρ = 0.888, *P* = 0.044).

The plastid genomes assembled from these five species ranged in size from 149,321 bp (zombi pea) to 158,144 bp (jack bean). All exhibited the expected quadripartite structure (Online Resource 4).

**Marker identification**

We identified SSRs in each of the genome assemblies (after removing short contigs < 500 bp). The number of SSRs varied from 34,092 (moth bean) to 59,756 (sword bean) (Figure 3). This equated to one SSR per 6.6, 6.8, 7.5, 9.1, 11.7 kb in Kersting’s groundnut, sword bean, jack bean, moth bean and zombi pea, respectively. Dinucleotide repeat SSRs predominated in four species (46-60% of SSRs) with the exception being zombi pea where trinucleotide repeat SSRs were most common (39% of SSRs). Penta- and hexanucleotide repeat SSRs were the least common summing to less than 10% of all SSRs across all five species (Figure 3). To estimate the level of success in our primer design, we tested 32 markers in a panel of eight zombi pea accessions (Table 3). Of these, 17 amplified in more than half the individuals and all but one of these (i.e., 16) were polymorphic. The number of alleles at these 16 loci ranged from 2-6 (average 4.1).

## Discussion

An important first step in understanding underutilised crops, which are often stress tolerant and/or nutritionally beneficial, is to quantify their genetic variation (Somta et al. 2011; Robotham and Chapman 2015; Dachapak et al. 2017; Minnaar-Ontong et al. 2021; Sserumaga et al. 2021), identify close relatives (Yang et al. 2018; Fisher et al. 2022), and ascertain variation within ‘inbred’ lines or varieties (Ho et al. 2016). Microsatellites (or SSRs) are molecular markers commonly used for this purpose, and their development is now relatively routine once genetic, genomic and/or transcriptomic resources are available (Hodel et al. 2016a; Chapman 2019). In this study we selected five underutilised legume crops with scant genomic resources and used low level genome sequencing (6-14X) to identify SSR markers that will be useful for both investigating the population genetic variation within these crops and for genetic mapping of quantitative traits.

We identified between ca. 34,000 and 60,000 SSRs per species. Our approach to assembling the genome was not designed to optimise the assembly (except that a range of kmers were used) and the number of reads used was relatively small. We therefore assume that some potential SSR markers could be from misassembled genome contigs and hence will not translate into usable markers. However, we do not believe this to be a significant problem; when we tested 32 SSR markers in eight zombi pea accessions, 16 amplified across at least half of the samples and were polymorphic. We also note that we did not optimise the PCR conditions, so further markers may amplify if we did this. Our results are similar to a previous study examining SSR design from *de novo* assembled genomes using small numbers of reads (similar to the approach employed here) which demonstrated that 14 of 18 SSR markers amplified in at least 50% (and on average 95%) of 12 individuals in a panel representing cultivated tomato and its wild relative, and all were polymorphic (Chapman 2019). Nevertheless, it should also be noted that even markers designed from whole genome assemblies and enriched for markers that should amplify across divergent accessions do not always amplify (e.g., Bhattarai et al. 2021).

The newly designed markers here should be of immediate use in their respective crops. Previous genetic mapping analyses of zombi pea and moth bean have used SSR markers from related legumes (Dachapak et al. 2018; Yundaeng et al. 2019). In both, hundreds of markers were screened because markers from the target species were not available. Of these, between 13.6 to 79.2 % of markers amplified in moth bean (Yundaeng et al. 2019) and 19.2 to 51.4 % in zombi pea (Dachapak et al. 2018). Whilst SSR markers from related species are clearly valuable, amplification success decreases as phylogenetic distance increases, and so having SSR markers developed from these focal species will hopefully be of immediate use. Population genetic variation in Kersting’s groundnut has recently been investigated using HTS-derived markers (DArT-Seq™; Kafoutchoni et al. 2021), but as far as we are aware, SSR markers have not been developed, and these are expected to be more cost-effective where a smaller number of markers would suffice. For jack bean and sword bean, population genetic variation has not been examined as far as we are aware; hence our work will potentially stimulate the investigation of, and ultimately investment in, these crops.

Whole genome sequence data, even when only assembled into partial drafts, can also provide valuable genetic information of use to researchers in addition to molecular markers such as SSRs. For example, we recently used a draft assembly of the perennial horse gram genome to identify gene orthologues across the legume family and test for positive selection (Fisher et al. 2022). This study revealed a number of gene ontology categories that appeared to be under positive selection in the family, as well as contributing towards identifying lineage-specific genes (Fisher et al. 2022).

Finally, the estimated genome sizes of several underutilised legume crops and related wild relatives provide useful information for researchers planning full genome sequencing of these taxa as the genome size is useful for estimating the amount of sequencing data needed to achieve the required coverage and hence the budget and time required for sequencing and assembling a genome (e.g., Li and Harkess 2018). Moreover, genome size has long-recognised impacts at the nuclear-, cellular- and organism-level that in turn plays a role in setting the thresholds on the plasticity of an organism’s functional traits independently of the information encoded in the DNA (Suda et al. 2015). These so-called “nucleotypic” effects are of considerable ecological significance as they influence where, when and how plants grow (reviewed in Greilhuber and Leitch 2013; Faizullah et al. 2021), and may therefore provide useful insights for crop plants concerning resilience and adaptive potential.

Going forward, we hope that by demonstrating the ease at which a partial and draft genome can be sequenced and assembled using publicly available scripts and limited bioinformatic experience, we encourage others to adopt similar practices for other species. Application of these methods to the species studied here, as well as other underutilised crops, could be an important step in rescuing and improving threated crop varieties, and to increasing the resilience of our food production systems In the face of climate change.

## Statements and Declarations

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## Competing Interests

Nothing to declare

## Data Availability

Raw reads have been deposited in the NCBI SRA under bioproject PRJNA882244. Assemblies and SSR lists are available from the corresponding author upon request. Genome will be added to the next release of the Kew Plant DNA C-values database (https://cvalues.science.kew.org/).

## Tables

**Table 1 – Species, accessions, number of reads before and after QC trimming and estimated genome size.**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species (common name)** | **Species (Latin)** | **Species (code)** | **Accession** | **Source\*** | **Country of origin** | **Number of raw reads** | **Number of trimmed reads** | **Percentage retained** | **Genome size (Mbp/1C)\*\*** |
| Jack Bean | *Canavalia ensiformis* | *Cen* | AGG90720 | AGG | Unknown | 28,441,183 | 27,545,274 | 96.8 | 694 |
| Sword Bean | *Canavalia gladiata* | *Cgl* | AGG309492 | AGG | Nigeria | 26,232,033 | 25,376,591 | 96.7 | 641 |
| Kersting's groundnut | *Macrotyloma geocarpum* | *Mge* | TKg\_12 | IITA | Unknown | 30,085,640 | 29,221,512 | 97.1 | 391 |
| Moth Bean | *Vigna aconitifolia* | *Vac* | PI426980 | USDA-ARS | Pakistan | 46,515,535 | 45,147,373 | 97.1 | 469 |
| Zombi Pea | *Vigna vexillata* var. *macrosperma* | *Vve* | TVNu-240 | IITA | Central African Republic | 45,064,166 | 43,747,476 | 97.1 | 802 |

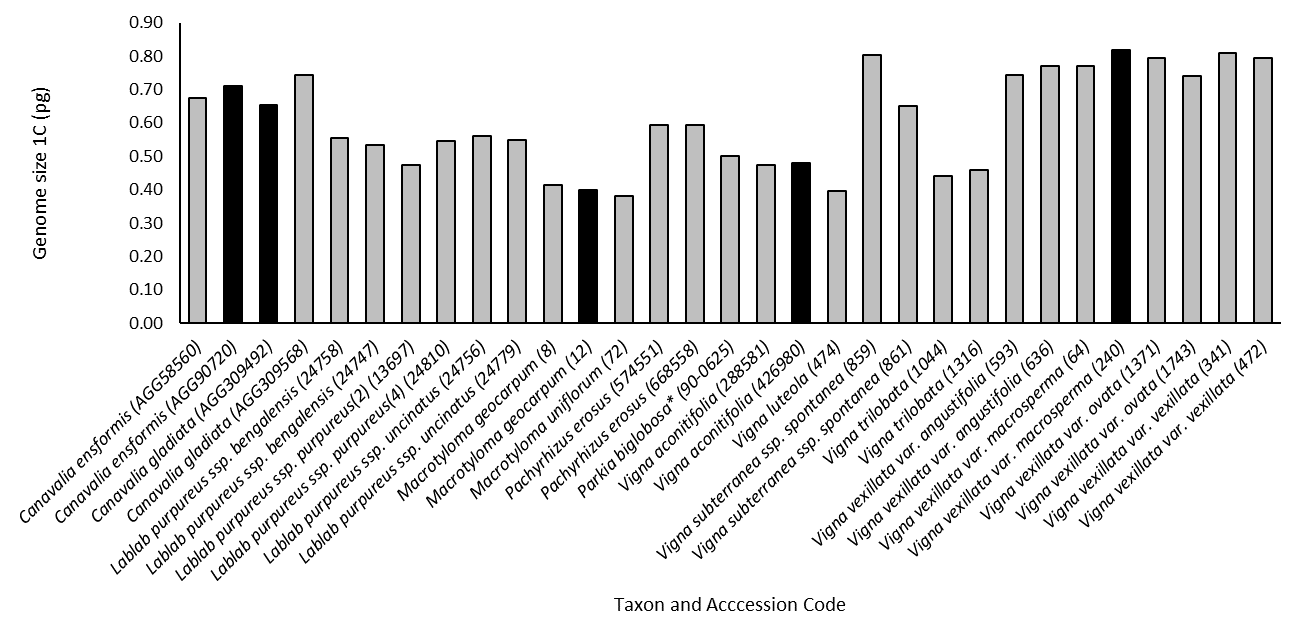
\* Source of the seed: USDA-ARS, USDA Agricultural Research Service; IITA, International Institute of Tropical Agriculture; AGG, Australian Grains Genebank

\*\* Measured using flow cytometry (see Online Resource 1)

**Table 2 – Accessions of zombi pea (*Vigna vexillata*) analysed with 32 SSRs**

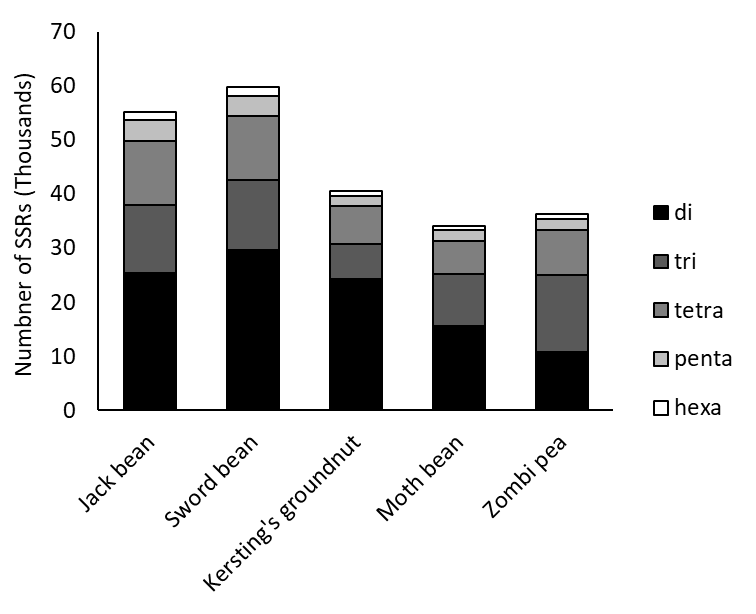
|  |  |  |
| --- | --- | --- |
| **Accession** | **Var.** | **Origin** |
| TVNu-593 | *angustifolia* | Niger |
| TVNu-636 | *angustifolia* | Congo |
| TVNu-64 | *macrosperma* | Australia |
| TVNu-240 | *macrosperma* | Central African Republic |
| TVNu-1371 | *ovata* | South Africa |
| TVNu-1743 | *ovata* | South Africa |
| TVNu-327 | *vexillata* | Zambia |
| TVNu-378 | *vexillata* | Cameroon |

## Figures

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**Figure 1 – Genome size estimates of 31 legume accessions comprising 17 taxa, obtained using flow cytometry.** For exact values see Supplementary Table 1. The five individuals selected for genome sequencing are shown in black. \* This estimate should be treated as preliminary as it was impossible to obtain a clean extraction using GPB, Galbraith, or Cystain PI OxProtect.

**Figure 2 – Results of BUSCO (Benchmarking Universal Single-Copy Orthologs) analysis to assess gene space coverage**. Genes identified in the five genomes were compared to 2,326 conserved genes from the eudicots and are reported as complete - single copy, C(S); complete - duplicated, C(D); fragmented (F); or missing (M).



**Figure 3 – Numbers of SSRs in each genome, subdivided by repeat type.**

# Online Resources

**Online Resource 1 – Accessions and genome size estimates.**

**Online Resource 2 – Assembly metrics.** N:500, number of contigs greater than 500 bp; N50, length (bp) of the shortest contig for which longer and equal length contigs cover at least 50 % of the assembly; sum, total assembly length (Mb). Highlighted rows indicate the assemblies used for SSR identification.

**Online Resource 3 – Assembly metrics for the five species across multiple kmers.** The x-axis labels give the three-letter code for the species followed by the kmer length (Cen = *Canavalia ensiformis*; Cgl = *C. gladiata*; Mge = *Macrotyloma geocarpum*; Vac = *Vigna aconitifolia*; Vve = *V. vexillata*).

**Online Resource 4 – Plastid genome assemblies of five underutilised legume species**. *Cen, Canavalia ensiformis* (jack bean); *Cgl, Canavalia gladiata* (sword bean); *Mge, Macrotyloma geocarpum* (Kersting’s groundnut); *Vac, Vigna aconitifolia* (moth bean); and *Vve, Vigna vexillata* (zombi pea).

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