CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

Department of Crop Sciences and Agroforestry

Effect of plant growth regulators on micropropagation of *Sphenostylis stenocarpa* and *Mucuna flagellipes*, crops of African origin

Bachelor's thesis

Prague 2024

Author: Markéta Smutná

Supervisor: Ing. Iva Viehmannová, Ph.D.

Declaration

I, Markéta Smutná, hereby declare that I have done this thesis entitled "Effect of plant growth regulators on micropropagation of *Sphenostylis stenocarpa* and *Mucuna flagellipes*, crops of African origin" independently, using the sources quoted and acknowledged by means of complete references according to Citation rules of the FTA.

In Prague, April 2024

....

Markéta Smutná

Acknowledgements

I would like to express my gratitude to my supervisor, Ing. Iva Viehmannová, Ph.D., for her support, guidance, and patience throughout the journey of writing my thesis. Her expertise, insightful hints, and constructive feedback were invaluable in the process of both laboratory work and writing.

I would also like to thank the Laboratory of Plant Tissue Cultures and the Botanical Garden of the Faculty of Tropical AgriScineces at the Czech University of Life Sciences Prague for providing the material and space for my experiments.

Abstract

Mucuna flagellipes and *Sphenostylis stenocarpa* are plants from the family Fabaceae originating in Africa. Both legumes produce seeds high in protein, *Sphenostylis stenocarpa* also produces edible underground tubers rich in starch. Despite their nutritional potential, they remain relatively unknown and underutilized. The aim of this thesis was to optimize the micropropagation of *Mucuna flagellipes* and *Sphenostylis stenocarpa*. For the establishment of *in vitro* cultures plant material surface sterilization was used; shoots of *Mucuna flagellipes* were treated with 70% EtOH for 60 s followed by 2% NaClO and seeds of *Sphenostylis stenocarpa* were sterilized using 96% EtOH for 10 s followed by 2% NaClO. The plant material of *Mucuna flagellipes* was subcultured on MS medium (Murashige & Skoog 1962) every six weeks. The experiment was carried out on MS medium with the addition of 6-benzylaminopurine (BAP) or zeatin (ZEA) at concentrations of 0.2, 0.5, and 1 $mg.l^{-1}$. The highest number of shoots (4.70 ± 0.19) was obtained on MS medium with 1 $mg.l^{-1}$ ZEA. For rooting, ½ MS medium with the addition of indole-3-butyric acid (IBA) at concentrations of 0.1 and 0.5 $mg.l^{-1}$ was tested. The highest number of roots (2.80 ± 0.21) was obtained on ½ MS medium without any plant growth regulators (PGRs). Well-rooted plants were then transferred *ex vitro*. The survival rate after three weeks in the greenhouse was 100%. For *Sphenostylis stenocarpa*, germinated seeds were subcultured on MS medium. Due to the production of callus, different basal media with various concentrations and combinations of BAP, ZEA, α – naphthaleneacetic acid (NAA), and adenine sulphate (AS) were tested. Growth of new shoots was observed on MS medium supplemented with 0.2 $mg.l^{-1}$ BAP and 30 $mg.l^{-1}$ AS. However, no combination of PGRs tested led to the callus inhibition and the growth of shoots in length that would allow further propagation.

Key words: Auxin, cytokinin, *in vitro* propagation, *Mucuna flagellipes*, *Sphenostylis stenocarpa*

Contents

1. Introduction **1**

2. Literature Review **2**

2.1. *Mucuna flagellipes* 2

2.1.1. Taxonomy and common names 2

2.1.2. Origin, geographic distribution and ecology 2

2.1.3. Morphology 3

2.1.4. Use and properties 5

2.1.4.1. Nutritional composition 5

2.1.4.2. Phytochemical composition 6

2.1.5. Cultivation and propagation 7

2.2. *Sphenostylis stenocarpa* 7

2.2.1. Taxonomy and common names 7

2.2.2. Origin, geographic distribution and ecology 8

2.2.3. Morphology 9

2.2.4. Use and properties 11

2.2.4.1. Nutritional composition 12

2.2.4.2. Phytochemical composition 13

2.2.5. Cultivation 14

2.2.6. Propagation 15

2.3. *In vitro* cultures in Fabaceae 15

3. Aims of the Thesis **18**

4. Material and methods **19**

4.1. Plant material 19

4.1.1. Establishment of *in vitro* culture 19

4.1.2. *In vitro* propagation 19

4.1.2.1. *In vitro* propagation of *M. flagellipes* 19

4.1.3. *In vitro* rooting of *M. flagellipes* 21

4.1.4. *Ex vitro* transfer of *M. flagellipes* 21

4.1.5. *In vitro* propagation of *S. stenocarpa* 21

4.1.6. Statistical evaluation of collected data 22

5. Results **23**

5.1. *In vitro* propagation and *ex vitro* transfer of *M. flagellipes* 23

5.1.1. Establishment of *in vitro* cultures 23

5.1.2. *In vitro* propagation 23

5.1.3. *In vitro* rooting 25

5.1.4. Transfer *ex vitro* 27

5.2. *In vitro* propagation of *S. stenocarpa* 28

5.2.1. Establishment of *in vitro* cultures 28

5.2.2. Propagation 28

6. Discussion **30**

6.1. *In vitro* propagation of *M. flagellipes* 30

6.2. *In vitro* rooting and *ex vitro* transfer of *M. flagellipes* 31

6.3. *In vitro* propagation of *S. stenocarpa* 31

7. Conclusions **33**

8. Recommendation **34**

List of figures

Figure 1: Distribution of *Mucuna flagellipes* **3**

Figure 2: Inflorescence of *M. flagellipes* **4**

Figure 3: Seeds of *M. flagellipes* **5**

Figure 4: Distribution of *Sphenostylis stenocarpa* **9**

Figure 5: Morphological traits of *S. stenocarpa*. a) adult plant, b) dry pods **10**

Figure 6: Seeds of *S. stenocarpa* **10**

Figure 7: Tubers of *S. stenocarpa* **11**

Figure 8: Nodal stem segment used for *in vitro* propagation **20**

Figure 9: Explants after 6 weeks of cultivation. a) MS medium, b) MS medium + 1 $mg.l^{-1}$ BAP, c) MS medium + 1 $mg.l^{-1}$ ZEA **25**

Figure 10: Rooting after 4 weeks of cultivation a) ½ MS medium, b) ½ MS medium + 0.1 $mg.l^{-1}$ IBA, c) ½ MS medium + 0.5 $mg.l^{-1}$ IBA **26**

Figure 11: Explants after 4 weeks of cultivation. a) ½ MS medium, b) ½ MS medium + 0.1 $mg.l^{-1}$ IBA, c) ½ MS medium + 0.5 $mg.l^{-1}$ IBA **27**

Figure 12: Transfer *ex vitro*. a) plant immediately after transfer *ex vitro*, b) plant after 3 weeks in the greenhouse **27**

Figure 13: Plantlet of *S. stenocarpa* after 3 weeks *in vitro* culture **28**

Figure 14: *S. stenocarpa* after 4 weeks of cultivation. a) MS medium b) DKW medium with 1 $mg.l^{-1}$ BAP + 0.1 NAA $mg.l^{-1}$ + 30 $mg.l^{-1}$ AS, c) MS medium with 1 $mg.l^{-1}$ BAP + 0.1 NAA $mg.l^{-1}$ + 30 $mg.l^{-1}$ AS **29**

List of tables

Table 1: Treatments on MS basal medium used for *in vitro* propagation of *M. flagellipes*.......... **20**

Table 2: Treatments used for *in vitro* propagation of *S. stenocarpa* **22**

Table 3: Effect of PGRs in MS medium on shoot and root formation in *M. flagellipes* **24**

Table 4: Effect of IBA in ½ MS medium on rooting after 4 weeks of cultivation........ **26**

List of the abbreviations used in the thesis

AS	Adenine sulphate
BAP	6-benzylaminopurine
DKW	Driver and Kuniyuki (1984) Walnut medium
IBA	Indole-3-butyric acid
KIN	Kinetin
MS	Murashige and Skoog (1962) medium
mT	Meta-Topolin
NAA	α – naphthaleneacetic acid
PGR	Plant growth regulator
TDZ	Thidiazuron

1. Introduction

Mucuna flagellipes and *Sphenostylis stenocarpa*, members of the Fabaceae family native to Africa, are perennial climbing vines predominantly found in West African countries such as Nigeria, Ghana, and Cameroon (Potter & Doyle 1992; POWO 2024). Seeds of both legumes have high protein content, varying between 19.53-29.53%, and are used for human consumption (Oshodi et al. 1995; Onuegbu et al. 2013; George et al. 2020). *Sphenostylis stenocarpa* also produces edible underground root tubers rich in starch, containing 47-67% of carbohydrates (Potter 1992; Konyeme et al. 2020). The tubers also contain amount of protein ranging between 11-19% which is six times more than the amount of protein in cassava and three times more than in sweet potato (Dakora 2013). *Mucuna flagellipes* and *Sphenosytlis stenocarpa* have the ability to grow in various agroecological conditions, thus they carry a great potential to broaden the food base and improve food security, especially in local communities (Jansen 2005; Adewale et al. 2008). However, they remain relatively unknown and underutilized compared to other leguminous crops such as soybean or cowpea and are considered minor or cover crops (Klu et al. 2001; Jansen 2005).

In spite of their nutritional and ecological significance, these crops have received little attention, particularly in terms of research for crop improvement and genetic resources conservation. The optimization of micropropagation is the basic premise not only for successful propagation at a large scale but also for *ex situ* conservation of plant biodiversity and plant breeding (Cruz-Cruz et al. 2013). Therefore, the objective of this thesis was to optimize the micropropagation protocol for *Mucuna flagellipes* and *Sphenostylis stenocarpa* which could be used to speed up the production of these plants, and thus the breeding process.

2. Literature Review

2.1. *Mucuna flagellipes*

2.1.1. Taxonomy and common names

Mucuna flagellipes is part of the kingdom Plantae, the order Fabales, the family Fabaceae, and the subfamily Faboideae (Wiriadinata et al. 2016). The name of the *Mucuna* genus comes from Tupa-Guarini where the word mucunã is a common name for plants of these species (Quattrocchi 1999). The genus consists of 114 accepted species distributed typically in tropical and subtropical forests of the world (POWO 2023).

Some of the English common names for plants of the *Mucuna* genus include cowitch and cowhage (Lampariello et al. 2012). In the Igbo language the *Mucuna flagellipes* species has the name Ukpo (Anyanwu 2021) but the name of the species differs depending on the location – épi in Central Africa, tsoko-mbele in Congo, gleemi, samante or tatwea in Ghana, blidué, blon dubu and eva in Ivory Cost as well as njo(-wi) in Sierra Leone (Quattrocchi 2012).

2.1.2. Origin, geographic distribution and ecology

Mucuna Adans. is a monophyletic genus which originated in the Paleotropics approximately 25 million years ago. Over time, the genus expanded into Africa as well as the Neotropics. Multiple events of oceanic dispersal of the seeds over long distances played a significant role in its pantropical distribution, as well as diversification (Moura et al. 2016).

Ancestor of *M. flagellipes* arrived in Africa through oceanic dispersal from South America (Moura et al. 2016). Today *M. flagellipes* is distributed in West and Central Africa (Fig. 1) from Sierra Leone, through to Cameroon and the Democratic Republic of Congo to Angola (POWO 2024). It grows at elevations ranging from sea-level up to the altitude of 1,400 m in wet and shaded areas with soil rich in hummus. It can be found in riverbanks, swamp forests, and the edges of mangrove vegetation (Jansen 2005).

Figure 1: Distribution of *Mucuna flagellipes* (source: POWO 2024)

2.1.3. Morphology

M. flagellipes is a large woody liana which can grow up to 30 meters in length. At the base, the stem can be up to 7 cm thick in diameter. Both the stem and branches are glabrous (Hennessy 1991). The leaves are alternate and ovate or elliptic in shape (Hennessy 1991; Nwankwo et al. 2022).

The inflorescences (Fig. 2) emerge from mature woody stems aged between 2 and 3 years in the axils of leaves, taking the form of pendant panicles. The peduncle measures between 8 and 20 cm in length (Hennessy 1991). The flowers are bisexual and yellow in colour (Nwankwo et al. 2022). The tube of the calyx is broadly cup-shaped, measuring approximately 10 - 15 mm, with five lobes. The vexillum is green and can be either smooth or covered in fine hair near its lower edges. The stamens are 45 mm in length and there are two types of them: adaxial and abaxial. Adaxial stamens are ovoid and covered in dense hair. The abaxial stamens are obelliptic and sparsely covered with hair. The ovary is densely covered in hair and carries three ovules. The style bends at midpoint, curving upwards and the stigma is cap-shaped and shaggy proximally (Hennessy 1991).

Figure 2: Inflorescence of *M. flagellipes* (source: Harris 2006)

M. flagellipes is a mammal pollinated species, in particular, it is pollinated by bats of the *Megaloglossus* genus (Von Helversen & Winter 2005). The pollen grains are triaperturate with a reticulate surface (Moura et al. 2018). The plant produces long ribbed pods with brownish hairs. Each pod contains one to three seeds (Fig. 3) which are brown in colour and have a slightly convex lens-like shape measuring 25 - 29 mm in length and 21 - 25 mm in width. There is a circular seem that encircles the seed, measuring about 50 mm in length (Hennessy 1991).

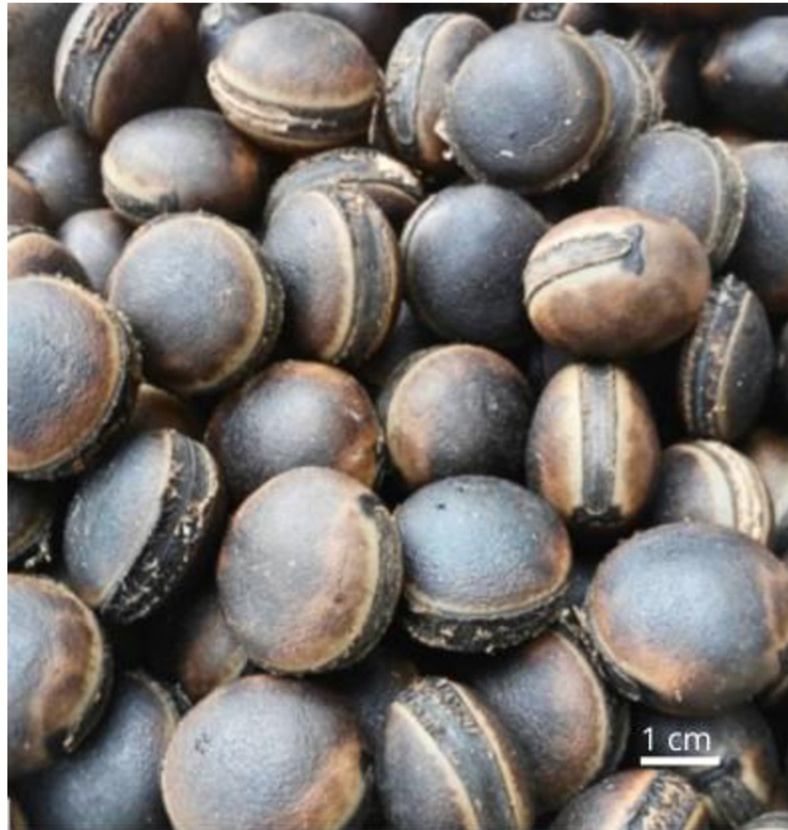

Figure 3: Seeds of *M. flagellipes* (source: Okwu 2020)

2.1.4. Use and properties

M. flagellipes produces edible seeds which must be cracked and dehulled to extract the edible kernel (Aviara et al. 2010). In Southeastern Nigeria, they are used as a soup flavouring and thickening agent (Okwu & Okoro 2006). The gum from the seeds can also be used in pharmacology as a suspending agent. Not only the seeds, but also stems and leaves are locally important due their ability to serve as a dye. By boiling the leaves and stems, they can dye wooden objects, ceramics, vegetable fibers, and cloth black or blue-black colour (Jansen 2005).

2.1.4.1. Nutritional composition

The seeds of *M. flagellipes* contain 39.24 – 61.49% of carbohydrates, 20.90 – 24.94% of protein, 1.84 – 3.77% of fat and 5.37 – 11.56% of fibre, which makes them a good source of protein and carbohydrates (Ene-Obong & Carnovale 1992; Onuegbu et al. 2013). In addition, due to their low moisture content, the seeds can be stored for long periods without spoilage (Ajayi et al. 2006).

Up to 50% of carbohydrates in the seeds of *M. flagellipes* are water solulable polysaccharides, also known as gum (Onweluzo et al. 1999). The gum from seeds of *M. flagellipes* contains D-galactose, D-mannose and D-glucose. It shows high emulsion activity as well as high water absorption capacity and therefore can be used as thickening or bulking agents (Onweluzo et al. 1995; Onweluzo et al. 1999).

The seeds of *M. flagellipes* are low in in sulphur containing amino acids, such as methionine. However, they are rich in aspartic acid, glutamic acid, lysine and trypthophan. The content of amino acid can be affected significantly by processing the seeds. Autoclaving showed to be the best method to preserve the protein content as denaturation occurs less compared to other methods (Ajayi et al. 2006).

The oil from the seeds of *M. flagellipes* contains significantly higher percentage of unsaturated fatty acids (77.97%) compared to saturated ones, which makes it nutritionally valuable. Concerning unsaturated fats, the most common is the oleic acid with 60.68%, followed by polyunsaturated linoleic acid with 15.03%. With 100 mg/100 g value of iodine, the oil from the seeds of *M. flagellipes* belongs to the semi-drying group of oils, which makes it suitable for cooking (Ajayi et al. 2006)

In contrast to the nutritional quality, the seeds also contain anti-nutritional compounds such as oxalate tannins, saponins, phenols and phytates (Onuegbu et al. 2013). These can be significantly reduced by boiling, roasting, soaking and boiling or autoclaving (Udensi et al. 2010; Onuegbu et al. 2013). Autoclaving gives the best results, however, as it may not be an available option for local people, soaking and boiling the seeds brings good results as well (Udensi et al. 2010).

2.1.4.2. Phytochemical composition

M. flagellipes is rich in phytochemicals such as alkaloids, flavonoids, glycosides, saponins, and tannins and has the potential to serve as a medicinal plant (Okwu & Okoro 2006; Jovita et al. 2017). The ethanolic extract from seeds can significantly reduce hypertension as they slow heart rate and regulate dyslipidemia (Jovita et al. 2017). The aqueous extract has the potential to be utilised as an antimicrobial agent (Ajayi et al. 2010). For prevention of destruction of proliferating cells in the bone marrow, the extract from leaves can be used. It also boosts blood production and can be used in treatment where anemia is a limiting factor (Ozioko & Ozor 2020).

2.1.5. Cultivation and propagation

M. flagellipes is a perennial plant which can flower and produce fruit throughout the whole year (Jansen 2005). For propagation, seeds are used. Soaking them improves germination (Agba 2021). For optimal yield, the plants should be cultivated in spacing of 1.0 x 0.6 m or 1.0 x 1.0 m (Agba et al. 2016). In order to increase the number of leaves and boost nodulation, combined application of lime and phosphorus is effective. It also increases overall yield, as the plants produce significantly more pods and seeds compared to plants where phosphorus nor lime have been applied (Agba et al. 2019). In addition, after harvest the soil accumulates more organic matter, nitrogen and potassium and therefore improves sustainable soil fertility. This can be effective in post-harvest management of agricultural land (Ekwealor et al. 2015; Agba et al. 2019). For small farmers, poultry manure can be effective as cultivation with it improves the nutritional as well as the medicinal value of plants (Agba 2021).

For large scale propagation of genetically identical plants, *in vitro* cultures are used (Reuther 1990). Protocols for rapid *in vitro* propagation of plants of the Mucuna genus, such as medicinally important *Mucuna pruriens,* had been done showing successful micropropagation of these plants. (Chattopadhyay et al. 1995; Faisal et al. 2006; Lahiri et al. 2012). Even though not much is known about cultivation, growth, and yields of *M. flagellipes* on a commercial scale (Agba 2021), studies on *M. pruriens* can indicate its potential for its successful micropropagation.

2.2. *Sphenostylis stenocarpa*

2.2.1. Taxonomy and common names

Sphenostylis stenocarpa (Hochst. ex A. Rich.) Harm belongs to the kingdom Plantae, the order Fabales, the family Fabaceae, and the subfamily Faboideae. Its name comes from the Greek words *spheno* = wedge and *stylis* = style after its style which is terete at the base but flattened and broadened apically, making the style a distinctive feature of the genus *Sphenostylis* and its closely related genus *Nesphostylis* Verdc. (Potter 1992). Although these two so-called sister genera share many similarities, they exhibit certain morphological distinctions, such as the variations in standard petals, distinctions in the bracteoles, stamens, and seeds (Potter 1992; Potter & Doyle 1994). Notably, the

apical dilation of the stamens as well as a tooth at the base of the vexillary stamen, bracteoles which are longer than the calyx as well as ariled seeds absent in *Sphenostylis* set these two genera apart (Potter & Doyle 1994).

As a result of these variations, the genus has undergone several changes (Potter & Doyle 1994). Today the *Sphenostylis* genus includes eight species (WFO 2023). Several of them are used in tropical and southern Africa by humans with *Sphenostylis stenocarpa* being the most significant for its wide distribution as well as economic importance (Potter 1992).

Sphenosytlis stenocarpa is commonly known as African yam bean or Yam pea (Terrell et al. 1986). Many dialectical synonyms of African origin are known, including for example azima, ahuma, girigiri and kashin kaji in Nigeria, nkhoma or cinkhoma in Malawi and sesonge, gundosollo or sumpelegu in Togo (Potter 1992).

2.2.2. Origin, geographic distribution and ecology

Sphenostylis stenocarpa is a crop endemic to tropical Africa (Allen & Allen 1981) grown for its edible seeds and tubers mostly in countries of West and Central Africa (Potter & Doyle 1992) (Fig. 4). The common ancestor of the plants in *Sphenostylis* genus evolved in southern central Africa in a region of today's Zambia (Potter & Doyle 1994).

S. stenocarpa grows in the latitudes of 15° North to 15° South and the longitudes of 15° West to 40° East of Africa (Adewale et al. 2008). Wide geographical range in the tropics is possible for its ability to grow in both dry and humid areas. With the exception of lowland tropical rainforest, *S. stenocarpa* can occur in various agroecological conditions, such as on rocks, in both open and wooded savannas or forests (Potter 1992; Adewale et al. 2008). It can also be found in cultivated fields as weed (Potter 1992). Due to its lower seed yields compared to for example soybean (Potter 1992), *S. stenocarpa* is highly underutilized (Klu et al. 2001). However, its ability to grow in various conditions gives it an advantage over most conventional legumes and makes it a crop with a potential to help better the food security in Africa and other tropical regions (Adewale et al. 2008).

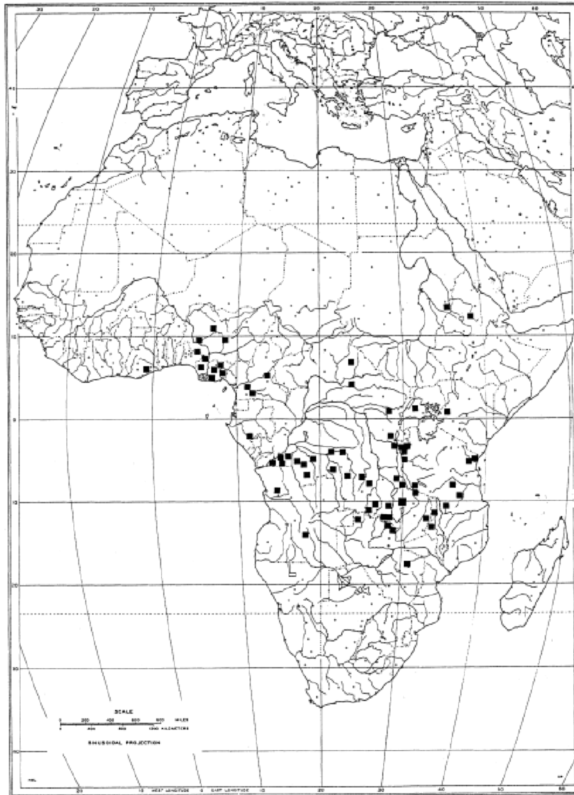

Figure 4: Distribution of *Sphenostylis stenocarpa* (source: Potter 1992)

2.2.3. Morphology

Being the most morphologically variable species of the *Sphenostylis* genus (Potter 1992), *S. stenocarpa* is a twining herbaceous vine (Fig. 5a), which can grow up to 3 meters in length (Adewale et al. 2011). The primary stem may or may not be pigmented (Adewale et al. 2011) and produces numerous branches that also twine tightly around available support structures (Adewale & Dumet 2011). The leaves are trifoliate with ovate, linear, lanceolate, or elliptic leaflets (Potter 1992).

The primary and secondary branches produce racemes on long peduncles (Adewale & Dumet 2011) with up to twelve attractive purplish or pinkish flowers with twisted standard petals (Potter 1992). The stamens and pistils are arranged to ensure self-pollination (Popoola et al. 2011) but overcrossing or pollination by insects is also effective and can bring seeds (Ojuederie et al. 2016). After pollination, up to three pods can arise on a peduncle (Adewale & Dumet 2011). The edible pods (Fig. 5 b) are linear, around 22 cm long, and can hold up to 20 edible seeds. When matured, the pods usually

shatter by splitting along both the dorsal and ventral sutures (Adewale et al. 2011; Adewale & Dumet 2011).

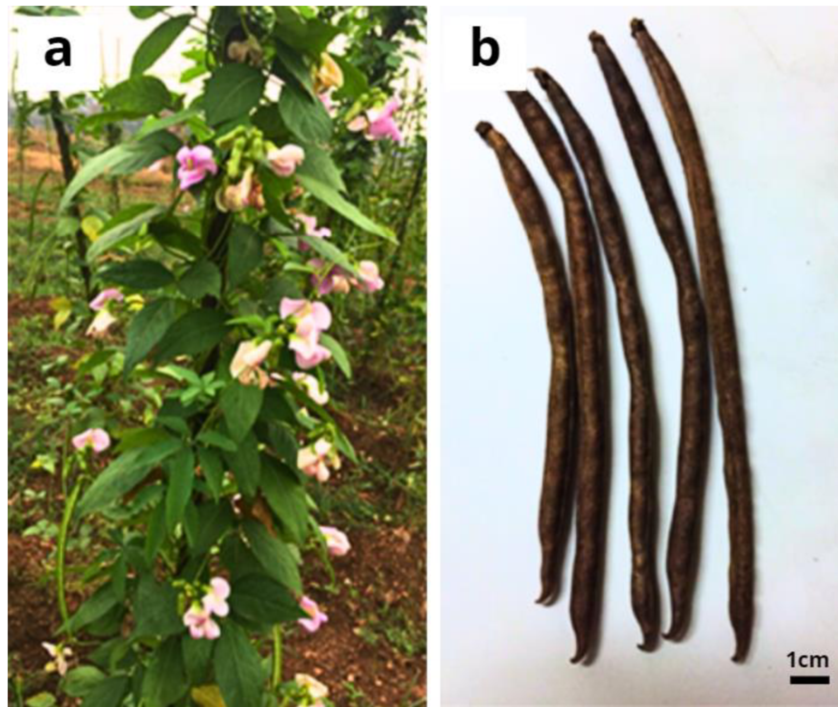

Figure 5: Morphological traits of *S. stenocarpa*. a) adult plant, b) dry pods (source: Aina 2021)

The seeds (Fig. 6) are approximately 1 cm long and can vary in both shape and colour. They can go from round to oval or oblong (Adewale et al. 2011). The colour variations include light cream colour as well as reddish brown or purple-black (Abdulkareem et al. 2015) and can serve as a morphological variable which can be used for the classification of *S. stenocarpa* (Adewale et al. 2011).

Figure 6: Seeds of *S. stenocarpa* (source: Aina 2021)

As well as edible pods and seeds, the plant also produces edible underground root tubers (Fig. 7), that are rich in starch (Potter 1992; Klu et al. 2001). During the dry season, when the above-ground parts of the plant die, they serve as an organ of perennation (Potter 1992). The tubers can grow to various sizes, shapes - such as round, ovate, spindle or irregular, and skin colours - from light cream and brownish orange to pink (Adewale & Dumet 2011).

Figure 7: Tubers of *S. stenocarpa* (source: Paliwal et al. 2021)

2.2.4. Use and properties

S. stenocarpa provides nutritional seeds and tubers for human consumption. However, the utilization varies depending on the cultivation area. Even though both tubers and seeds are edible, in Central and East Africa tubers are preferred by farmers. In West Africa the pods containing seeds are harvested, while tubers are in many cases just a neglected byproduct of the plant (Potter 1992; Klu et al. 2001).

The seeds of *S. stenocarpa* are known for their hard-to-cook nature. This can affect consumers' choice and gives it an economic disadvantage which can be a reason of their low consumption. With traditional ways of preparation, it can take up to 24 hours to prepare the seeds for consumption (Njoku et al. 1989). There are many ways of preparation, the seeds can be soaked for several hours and boiled, fried or ground into flour or paste. They can be eaten plain or in stews, the paste can be wrapped in banana leaves with seasoning and boiled (Potter 1992). The seeds of *S. stenocarpa* are also traditionally used for medical purposes as a cure for stomach aches or mixed with water as a remedy for acute drunkenness (Azuzu 1986). Germinated seeds can serve as a dietary

supplement for individuals with diabetes and hyperlipidaemia as they help to reduce complications associated with oxidative stress (Uchegbu 2015).

The tubers are also nutritionally rich and have a good flavour similar to potatoes. They can be boiled or roasted and eaten plain or in stews (Potter 1992).

2.2.4.1. Nutritional composition

The seeds of *S. stenocarpa* contain 49.88-63.51% of carbohydrates, 19.53-29.53% of protein, 1.39-7.53% of fat and 2.47-9.57% of fiber. Several studies with varying results have been done on the nutritional composition of seeds of *S. stenocarpa*. The reason for that is that the nutritional composition is influenced by many factors, with the most important being the genotype and environmental conditions in which the plant is cultivated (Oshodi et al. 1995; George et al. 2020).

The seeds are abundant in starch as well as non-starchy polysaccharides (Ade-Omowaye et al. 2015). Starch from legumes is digested more slowly than starch from cereals and tubers. This results in not spiking blood glucose and makes it a good dietary choice for individuals suffering from diabetes (Phillips 1993).

Although the protein content is significantly lower than that in soybean, it is similar to that of lima bean, bambara groundnut or pigeon pea. It has a great potential to serve as an important source of protein for humans as it is a high quality protein due to its amino acid composition and content (Apata & Ologhobo 1990; Oshodi et al. 1995). Depending on the cultivar, the total content of essential amino acids in *S. stenocarpa* ranges between 311 to 603 mg/g which is higher or comparable to soya bean with the total content of essential amino acids of 444 mg/g (Oshodi et al. 1995). In total, 8 essential and 9 non-essential amino acids can be found in the seeds of *S. Stenocarpa* (George et al. 2020). The seeds are a good source of the following amino acids - lysine phenylalanine, tyrosine, methionine, and cystine in amounts that meet the recommended amino acid requirements for infants and therefore for adults (Oshodi et al. 1995).

The oil from the seeds of *S. stenocarpa* contains a notable amount of polyunsaturated fatty acids, with a particular emphasis on linoleic acid (Adeyeye et al. 1999). This fatty acid is essential for promoting growth, supporting physiological processes, and maintaining the body's overall well-being (Koba & Yanagita 2014). The most concentrated fatty acids together with linoleic acid include stearic and palmitic acid

(Adeyeye et al. 1999). Even though saturated fatty acids as stearic are connected to human health problems like high blood cholesterol, a high concentration of stearic acid in *S. stenocarpa* is not a concern as it is converted into monosaturated oleic acid which appears to have no effect (Oshodi et al. 1995). Other fatty acids found in the oil in small quantities include caprylic, capric, lauric, myristic, palmitoleic, oleic, eicosenoic and erucic acids (Adeyeye et al. 1999).

Opposed to its nutritional qualities, raw seeds of *S. stenocarpa* contain anti-nutritional compounds, which are naturally occurring chemical constituents found in plant food. They modify nutrient absorption, slow down metabolic processes and may lead to flatulence in consumers (Oboh et al. 1998). Reported anti-nutrients include α-galactosides, inositol phosphate, lectin, phytate, tannin and trypsin inhibitor (Oboh et al. 1998; Fasoyiro et al. 2006). In order to make the seeds suitable for human consumption, they have to be processed. Methods like soaking, cooking, dehulling or fermentation not only make the seeds more suitable for consumption but also lower the amount of anti-nutrients significantly (Fasoyiro et al. 2006).

The tubers of *S. stenocarpa* contain 46.59-66.52% of carbohydrates which makes it a good source of food energy (Konyeme et al. 2020). The amount of protein in tubers ranges between 11-19% which is six times more than the amount of protein in cassava and three times more than in sweet potato (Dakora 2013). Compared to seeds, tubers contain fewer anti-nutrients and are completely safe for human consumption (Ojuederie et al. 2020).

2.2.4.2. Phytochemical composition

S. stenocarpa is rich in phytochemicals such as alkaloids, flavonoids, and saponins (Soetan et al. 2018). The flavonoids reported include apigenin, chrysoeriol, genistein, and tricin (Nyananyo & Nyingifa 2011). These secondary metabolites naturally occurring in plants act as natural antioxidants. Foods rich in those antioxidative compounds can help combat oxidative stress which harms the cellular structure and contributes to degenerative illnesses (Oboh et al. 2009). Thanks to these antioxidative qualities, phytochemicals play a role in different cell processes such as proliferation, differentiation, or programmed cell death. Thus, they can have preventative and therapeutic implications in combating conditions such as cancer and diabetes (Lim & Park 2022).

Through fermentation, the levels of free phenolics are increased. As free phenolics are digested earlier than bound phenolics, fermentation carries more benefits for human health (Oboh et al. 2009). Using aceton, greater amounts of phytochemicals can be extracted from *S. stenoacarpa* than using water (Soetan et al. 2018).

2.2.5. Cultivation

Even though the tubers serve as an organ of perennation and the plant can regrow from rootstock after the dry season, *S. stenocarpa* is mostly cultivated as an annual crop (Potter 1992). It is considered a minor crop, usually cultivated in fields with major crops like cassava and yam, and cared for mainly by women and children (Klu et al. 2001).

The major crops are planted on mounds first. A month later, in June or July, after the major crops have been established, *S. stenocarpa* is planted (Potter 1992; Klu et al. 2001). Two to three seeds are sown at the base or on top of the mound, where it can use support of the cassava stems while growing (Klu et al. 2001). Weed management done at least twice before the harvest of the major crops in September is beneficial for the plants. After seven to eight months, in December or January, the pods with seeds are ready to harvest. Tubers are ready for harvest after seven to eight months as well (Potter 1992; Klu et al. 2001).

In spite of the fact that the seed yields are not as high as those of some conventional legumes such as soybean, *S. stenocarpa* provides significant yields even in varied environmental conditions (Potter 1992). In addition, cropping system can impact the seed yield significantly. Intercropping systems show better results than solo cultivation (Adeniyan et al. 2007).

S. stenocarpa can thrive even in regions with unfavourable conditions due to its ability of nodule formation and nitrogen fixation (Oagile et al. 2012). Even though the nodule formation is lower in soils deficient in phosphorus, it can be improved significantly by using P fertilizers which also increases the grain yields (Obiagwu 1995; Obiagwu 1997). The plant has potential as a cover crop as it can improve soil quality in cropping systems as well as protect the soil in the early rainy season while using minimum inputs (Obiagwu 1997).

2.2.6. Propagation

For propagation, seeds are usually used by farmers, but the plant can grow from tubers as well (Potter 1992). It is also possible to propagate *S. stenocarpa* by using all types of stem cuttings as the position of the cutting on the vine nor the size of the leaf affects rooting of the cutting. In fact, trimming the leaf to reduce its size increases mortality of the cuttings. Application of auxins can be beneficial as it speeds up rooting and increases the number of roots. However, it is not necessary to use auxins as both stem cuttings with and without auxin treatments grow into normal plants. Even though propagation by stem cuttings is more costly and therefore not as convenient for larger productions, it can still be beneficial for further research purposes (Oagile 2005). For speedy multiplication of genetically identical plants, *in vitro* culture techniques can be used (Reuther 1990). As *S. stenocarpa* has undergone little to no genetic improvement, these techniques can be used for plant breeding to improve its agronomic and nutritional qualities (Potter 1992; Ongusola et al. 2016).

2.3. *In vitro* cultures in Fabaceae

Micropropagation is a form of vegetative propagation that can be used to propagate plants with desirable traits at a large scale at lower costs, get disease-free material, and for conservation of plant biodiversity. The plants are grown under controlled conditions in sterile containers to avoid contamination. Compared to traditional forms of propagation, the cultures can be started from explants, which are small pieces of plants, or embryos. They can then be grown in small spaces regardless of seasonal changes. Factors such as temperature, light, nutrients, and growth regulators can be controlled and therefore ensure higher rate of propagation (George et al. 2008).

The methods of micropropagation are the following: propagation from axillary buds or shoots, direct organogenesis, indirect organogenesis, and direct and indirect somatic embryogenesis (George et al. 2008). The propagation from axillary buds or shoots is the most widely applicable and reliable method which allows large-scale clonal production of elite cultivars, thus speeding up their breeding process (George et al. 2008; Gatti et al. 2016). In direct organogenesis, the newly regenerated organs derive directly from the tissues of the explant without undergoing callus. This also applies to direct

somatic embryogenesis, where the embryos develop directly from somatic cells without the formation of callus. That is the main difference from indirect organogenesis and indirect somatic embryogenesis, where organs or embryos are formed on the callus, potentially resulting in genetic variation among plants and from the stock plant (George et al. 2008).

The optimization of micropropagation is the basic premise not only for successful propagation at a large scale but also for *ex situ* conservation of plant biodiversity and plant breeding (Engelmann 1997; Cruz-Cruz et al. 2013). However, when it comes to micropropagation, there has not been a great advancement of plants from the Fabaceae family. This is due to the diversity of responses when cultured *in vitro*. The use of tissue culture for genetic improvement is also limited by poor regeneration as well as high genotype diversity (Pratap et al. 2018).

As an example, plants of the *Phaseolus* species are generally considered unsuitable for *in vitro* culture due to their limited plant regeneration potential and excessive secondary callus production. Shoot regeneration from produced callus is very low in most cases, resulting in an inefficient process. Although leguminous species have difficulties regenerating, somatic embryogenesis or organogenesis has been successfully applied to species like *Cajanus cajan, Cicer arietinum, Lens culinaris, Lupinus sp., Phaseolus vulgaris, Pisum sativum, Vicia faba, Vigna mungo, Vigna radiata,* and *Vigna unguiculata* (Gatti et al. 2016).

As most of the conventionally grown legumes have a rather narrow genetic base, it is highly desirable to use species found in the wild to incorporate desirable traits and widen the genetic base (Gatti et al. 2016; Pratap et al. 2018). In recent years, there has been some transgenic development in species such as *Cajanus cajan*, *Cicer aretinum*, *Glycine max, Vigna radiata*, and *Vigna unguiculata* resulting for example in salinity or water stress tolerance. Transgenic varieties can bring solutions to problems we will face in the future, therefore it is necessary to develop stable regeneration protocols in order to exploit the potential *in vitro* technology brings (Pratap et al. 2018).

Few studies have been done on *in vitro* propagation of *Sphenostylis stenocarpa*. In the study by Adesoye et al. (2012), the effect of two cytokinins 6-benzylaminopurine (BAP) and kinetin (KIN) in combination with α-napthaleneacetic acid NAA was tested in MS (Murashige & Skoog 1962) medium. BAP in combination with NAA brought

better results compared to KIN in plants regenerated by direct organogenesis from embryo and leaf explants. Similar results were obtained by Ongusola et al. (2016) who tested the influence of plant growth regulators (PGRs) on nodal cutting derived from germinated zygotic embryos. The combination of BAP and NAA also proved to be effective, however, the study also highlighted the difference of morphological responses based on the genotype.

In the *Mucuna* genus, several studies have been done on micropropagation of *Mucuna pruriens*, an important medicinal plant. The effect of PGRs on plant regeneration through callus morphogenesis and from cotyledonary node explants was tested. BAP alone was found to be the most effective compared to KIN (Lahiri et al. 2012; Madkami et al. 2023). However, in a study by Faisal et al. (2006) combination of BAP and NAA was shown to be the most effective for shoot proliferation in plants regenerated from nodal explants. Similar results were obtained in a protocol for micropropagation of *Mucuna gigantea*, where four following cytokinins were tested – BAP, KIN, meta-Topolin (mT) and Thidiazuron (TDZ) in plants regenerated from nodal explants. As in previous studies, BAP has been shown as most efficient when it comes to shoot induction. The best results were obtained on MS media supplemented with BAP and NAA (Madkami et al. 2022).

3. Aims of the Thesis

The aim of the thesis was to optimize *in vitro* propagation and develop a micropropagation protocol for *Sphenosytlis stenocarpa* and *Mucuna flagellipes*, underutilized crops with edible seeds of African origin.

The partial objectives were following:

- Establishment of *in vitro* culture of *S. stenocarpa* and *M. flagellipes*
- Optimization of *in vitro* plant propagation
- Optimization of *in vitro* rooting

Objectives of this thesis were set under following hypotheses:

- Cytokinins BAP and ZEA are efficient for shoot proliferation.
- Auxin indole-3-butyric acid (IBA) induces rooting of *in vitro* shoots.

4. Material and methods

4.1. Plant material

For this experiment, seeds of *Sphenostylis stenocarpa* (Hochst. ex A.Rich.) Harms and young actively growing shoots of *Mucuna flagellipes* Vogel ex Hook.f. were obtained from the Botanical Garden of the Faculty of Tropical AgriSciences at Czech University of Life Sciences Prague.

4.1.1. Establishment of *in vitro* culture

For the establishment of *in vitro* culture surface sterilization of plant material was used. The seeds of *S. stenocarpa* were soaked in 96% EtOH for 10 s. For *M. flagellipes*, the shoots were cut into apical and nodal segments and sterilized with 70% EtOH for 60 s. Thereafter, the plant material (of both species) was transferred to a beaker with 2% NaClO with a drop of Tween 20 and was placed on a laboratory rotary shaker for 15 minutes. The plant material was then rinsed 3 times in sterile distilled water in a flow box and transferred into Erlenmeyer flasks (100 ml volume) containing 35 ml of MS media. The MS media was supplemented with 30 $g.l^{-1}$ sucrose, 8 $g.l^{-1}$ agar, and 100 $mg.l^{-1}$ *myo*-inositol. The pH was adjusted to 5.7 before autoclaving for 20 minutes at 120°C and a pressure of 100 kPa. The *in vitro* cultures were then incubated under a 16/8 light-dark regime at a temperature of 23/21°C (light/dark period).

4.1.2. *In vitro* propagation

4.1.2.1. *In vitro* propagation of *M. flagellipes*

In order to obtain a sufficient amount of plant material for the experiment, apical and nodal stem segments were propagated on MS medium every six weeks.

Thereafter the experiment was established using one nodal stem segments 0.5 – 0.7 cm in length (Fig. 8), always collected from the first position below the apical bud. The leaves were removed. The experiment consisted of 6 treatments with various concentrations of BAP and ZEA (used independently) (Table 1). As a control group, explants cultivated on MS medium without PGRs were used. Each treatment consisted of

20 explants and the evaluation was carried out after 6 weeks. The measured characteristics were the number of shoots and their length, the number of nodes, and the number and length of roots.

Table 1: Treatments on MS basal medium used for *in vitro* propagation of *M. flagellipes*

Treatment	BAP ($mg.l^{-1}$)	ZEA ($mg.l^{-1}$)
Control	0	0
1	0.2	-
2	0.5	-
3	1	-
4	-	0.2
5	-	0.5
6	-	1

Figure 8: Nodal stem segment used for *in vitro* propagation (source: author)

4.1.3. *In vitro* rooting of *M. flagellipes*

For rooting, cca 3 cm long top parts of shoots with 3 meristems from previous experiments were used. They were placed on ½ MS medium with the addition of IBA at concentrations of 0.1 $mg.l^{-1}$ or 0.5 $mg.l^{-1}$. As a control group, explants cultivated on ½ MS medium without PGRs was used. There were 20 explants in each treatment and the evaluation was carried out after 4 weeks. The measured characteristics were the number and length of roots and the mean size of callus formed.

4.1.4. *Ex vitro* transfer of *M. flagellipes*

Well-rooted plants with min. 2 roots 1 cm long were used for *ex vitro* transfer to the greenhouse of the Botanical Garden of the Faculty of Tropical AgriSciences at the Czech University of Life Sciences Prague. The explants were taken out of Erlenmeyer flasks and the roots were cleaned from the basal media under running tap water. Thereafter they were transferred in a substrate consisting of soil and perlite in a 1:1 volume ratio. The substrate was treated in a dry heat sterilizer at 180°C for 120 min before the transfer. After the transfer to the greenhouse, where the plants were kept at a temperature of 24°C, they were covered using perforated transparent cups. The cups were gradually removed during 2 weeks. After their removal, the plants were repotted and bamboo sticks were added to pots to support twining. The evaluation of the survival rate was carried out after 3 weeks.

4.1.5. *In vitro* propagation of *S. stenocarpa*

Nodal and apical segments of *S. stenocarpa* were transferred to MS medium 6 weeks after germination. As plants on MS medium without PGRs did not grow and started producing callus, different treatments of various concentrations of BAP, ZEA, NAA, and adenine sulphate (AS) were used in MS medium and DKW medium (Driver & Kuniyuki 1984) (Table 2) in order to reduce callus formation and induce growth of shoots.

Table 2: Treatments used for *in vitro* propagation of *S. stenocarpa*

Basal medium	BAP ($mg.l^{-1}$)	ZEA ($mg.l^{-1}$)	NAA ($mg.l^{-1}$)	AS ($mg.l^{-1}$)
MS	0	0	0	0
MS	0.2	-	-	-
MS	0.2	-	-	30
MS	0.5	-	0.05	-
MS	1	-	0.1	-
MS	1	-	0.1	30
MS	-	0.1	-	30
DKW	1	0.1	-	30

4.1.6. Statistical evaluation of collected data

Statistical evaluation of collected data was carried out using the analysis of variance (ANOVA) with a following Tukey's test to find significant differences between groups (p=0.05). The analysis was done in StatSoft STATISTICA 14.0.1.

5. Results

5.1. *In vitro* propagation and *ex vitro* transfer of *M. flagellipes*

5.1.1. Establishment of *in vitro* cultures

Sterilization of young actively growing shoots with 70% EtOH followed by 2% NaClO proved to be efficient as it led to no contamination. The survival rate of sterilized plant material was 80% (20% were necrotic and did not survive the sterilization process). After 6 weeks the plants grew into size suitable for further propagation. Through repeated subcultivations, the number of 140 plants needed for the experiment was reached.

5.1.2. *In vitro* propagation

In the experiment, the highest number of shoots (4.70 per explant) after 6 weeks of cultivation was obtained on a medium supplemented with the highest concentration of ZEA tested (1 $mg.l^{-1}$). Although the mean length of shoots of this treatment was only 5.52 cm, which is not significantly different from the control group, it produced the highest number of nodes (19.30 per explant). These results are not significantly different from the results obtained with the highest concentration of BAP tested (1 $mg.l^{-1}$). However, explants on medium with ZEA showed more vital growth compared to media supplemented with BAP where browning of the leaves was observed (Fig. 9 b). In contrast, explants on medium without PGRs did not grow any new shoots, were relatively short (2.77 cm), and produced low number of nodes (4.55 per explant).

The shoot length decreased with higher concentrations of PGRs. Contrary to this fact, the number of nodes increased with the cytokinin concentration in the medium. In general, during the first four weeks, the initial shoot elongated, and new shoots started to form. However, additional two weeks were needed for the elongation of new shoots to obtain a size suitable for further propagation. Overall, treatments with ZEA produced more shoots than those with BAP. Even though the mean shoot length of explants was higher in BAP, with a maximum of 10.05 cm on medium with 0.2 $mg.l^{-1}$ BAP, explants on medium with ZEA had a higher number of nodes per explant. Their shorter length of shoots did not limit the ability for further propagation through stem cuttings. Plants on medium with ZEA produced healthier-looking shoots with bigger leaves compared to

explants with BAP treatment where the size was extremely shortened (Fig. 9 b, c). When compared to the control group, the leaf size of explants on media with PGRs was much smaller and they were also yellowish in colour (Fig. 9). In addition, with a high concentration of PGRs, especially in ZEA, the plants gained red pigmentation (Fig. 9 c). However, this did not impact their further growth or rooting. Upon transfer to ½ MS medium, the leaves gained back the large size and dark green colour.

In the control group, 100% of explants produced roots. On average 2.40 roots per explant of 8.89 cm mean length. The roots were thin and branching. The growth of roots was inhibited by the use of both BAP and ZEA even in low concentrations. Explants on media supplemented with PGRs did not produce any roots and grew callus in 100% of explants instead. Formation of callus was also observed in the control group but only in 40% of explants, and it did not affect further production of roots.

Table 3: Effect of PGRs in MS medium on shoot and root formation in *M. flagellipes*

PGRs ($mg.l^{-1}$)	**Number of shoots per explant (mean ± S.E.)**	**Length of shoots (cm) (mean ± S.E.)**	**Number of nodes (cm) (mean ± S.E.)**	**Number of roots per explant (mean ± S.E.)**	**Length of roots (cm) (mean ± S.E.)**
0	1.00 ± 0.00 a	2.77 ± 0.23 a	4.55 ± 0.28 a	2.40 ± 0.40	8.89 ± 2.62
0.2 BAP	1.25 ± 0.10 ab	10.05 ± 0.62 b	8.00 ± 0.29 a	n/a	n/a
0.5 BAP	1.70 ± 0.13 ac	9.57 ± 0.58 bc	9.15 ± 0.66 a	n/a	n/a
1 BAP	3.60 ± 0.21 de	7.10 ± 0.54 bd	18.25 ± 0.79 b	n/a	n/a
0.2 ZEA	2.20 ± 0.14 bce	7.60 ± 0.46 bd	15.35 ± 0.54 b	n/a	n/a
0.5 ZEA	2.95 ± 0.22 ce	6.58 ± 0.46 cd	15.25 ± 0.88 b	n/a	n/a
1 ZEA	4.70 ± 0.19 d	5.52 ± 0.34 ad	19.30 ± 0.57 b	n/a	n/a

Numbers in the same column followed by the same letter are not significantly different. (Tukey's test, $p \leq 0.05$)

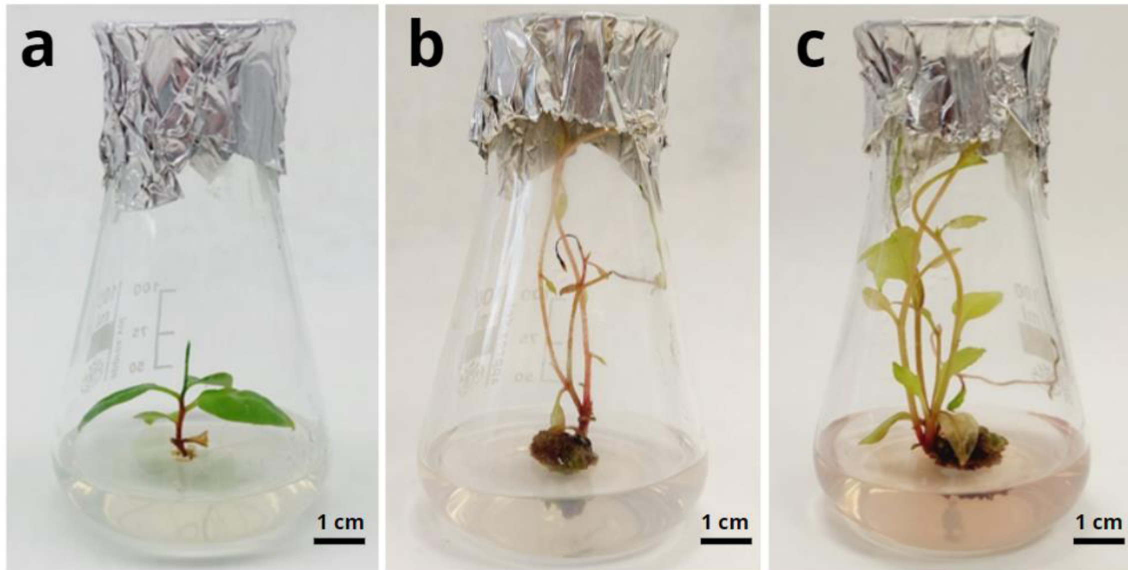

Figure 9: Explants after 6 weeks of cultivation. a) MS medium, b) MS medium + 1 mg.l^{-1} BAP, c) MS medium + 1 mg.l^{-1} ZEA (source: author)

5.1.3. *In vitro* rooting

For *in vitro* rooting, ½ MS medium without any PGRs showed the best results with 2.80 roots per explant with a mean length of 9.88 cm after 4 weeks of cultivation. With the addition of IBA to the medium, even at the lowest concentration of 0.1 mg.l^{-1}, the explants formed a significantly lower number of roots which were also significantly shorter. Furthermore, the addition of IBA induced the growth of callus at the end of the stem cutting where the plant was touching the medium. In ½ MS medium, roots were formed in 100% of explants, and none of them formed callus. The roots were thin, long, and branching (Fig 10 a). The rate of rooting was much lower in media with IBA, 40% in medium with 0.1 mg.l^{-1} IBA, and only 15% in medium with 0.5 mg.l^{-1} IBA. Roots formed on medium with IBA were shorter but thicker and showed less branching (Fig 10 b).

There was also a significant difference in the overall appearance of explants on each media (Fig. 11). Explants cultivated without PGRs produced large dark green leaves contrary to media with IBA, where the plants produced small leaves of purplish colour that in some explants started to fall down.

Table 4: Effect of IBA in ½ MS medium on rooting in after 4 weeks of cultivation

IBA ($mg.l^{-1}$)	Number of roots per explant (mean ± S.E.)	Length of roots (cm) (mean ± S.E.)	Size of callus (cm) (mean ± S.E.)	Rooting (%)
0	2.80 ± 0.21 a	9.88 ± 0.64 a	n/a	100
0.1	0.80 ± 0.17 b	1.26 ± 0.32 b	0.85 ± 0.05 a	40
0.5	0.40 ± 0.22 b	0.76 ± 0.13 b	0.87 ± 0.03 a	15

Numbers in the same column followed by the same letter are not significantly different. (Tukey's test, $p \leq 0.05$)

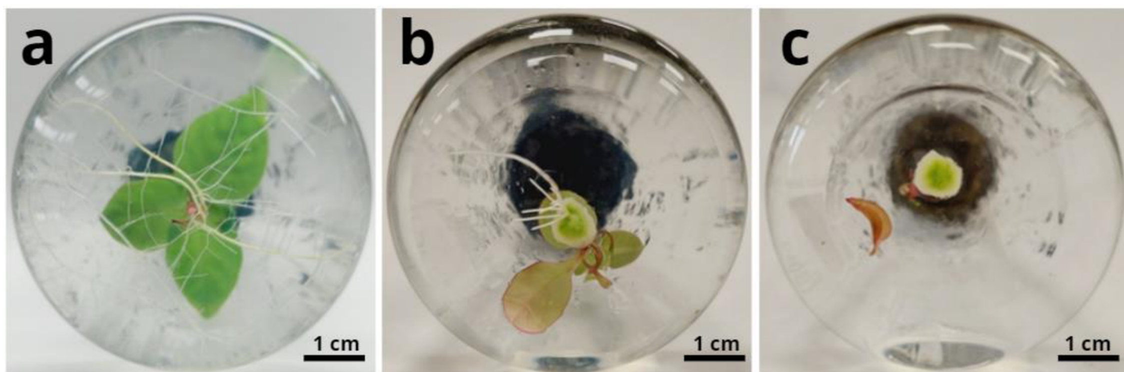

Figure 10: Rooting after 4 weeks of cultivation a) ½ MS medium, b) ½ MS medium + 0.1 $mg.l^{-1}$ IBA, c) ½ MS medium + 0.5 $mg.l^{-1}$ IBA (source: author)

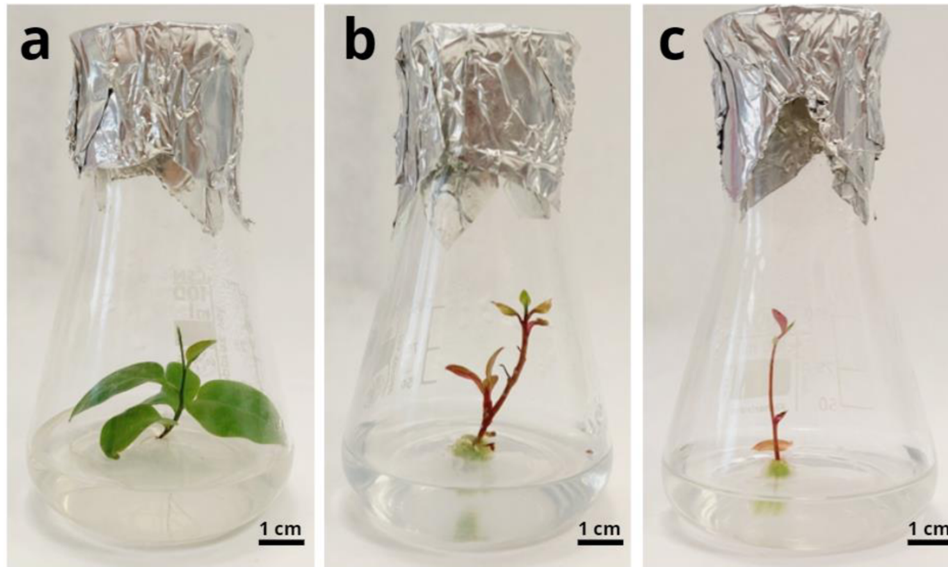

Figure 11: Explants after 4 weeks of cultivation. a) ½ MS medium, b) ½ MS medium + 0.1 $mg.l^{-1}$ IBA, c) ½ MS medium + 0.5 $mg.l^{-1}$ IBA (source: author)

5.1.4. Transfer *ex vitro*

Well-rooted plants from the *in vitro* rooting experiment were transferred into the greenhouse (Fig. 12 a). The survival rate of plants after 3 weeks in the greenhouse was 100%. The plants grew in length, produced new leaves, and looked healthy 3 weeks after the transfer (Fig 12 b).

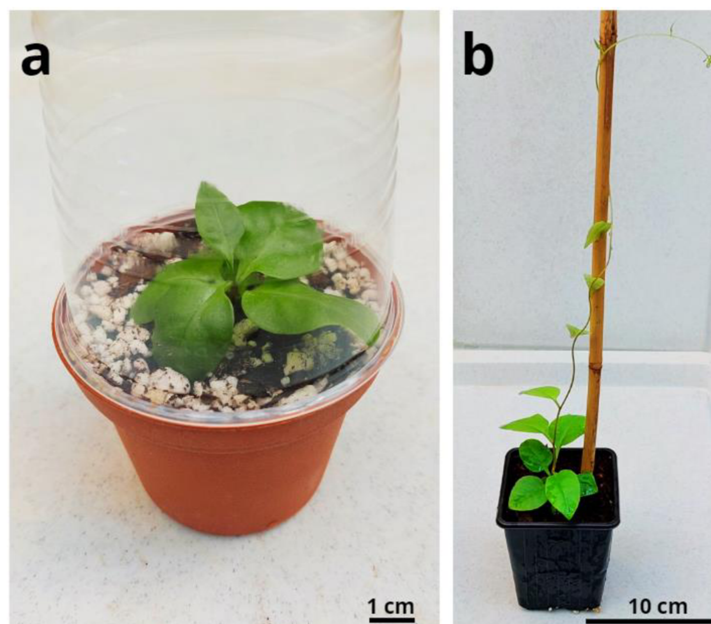

Figure 12: Transfer *ex vitro*. a) plant immediately after transfer *ex vitro*, b) plant after 3 weeks in the greenhouse (source: author)

5.2. *In vitro* propagation of *S. stenocarpa*

5.2.1. Establishment of *in vitro* cultures

Treating seeds of *S. stenocarpa* with 96% EtOH followed by 2% NaClO was proven to be effective for sterilization of the material as the contamination rate was only 2.5%. However, only 10% of seeds germinated. The germination started 2 weeks after sowing seeds on the medium (Fig. 13). After 6 weeks, the plants grew into size suitable for further propagation.

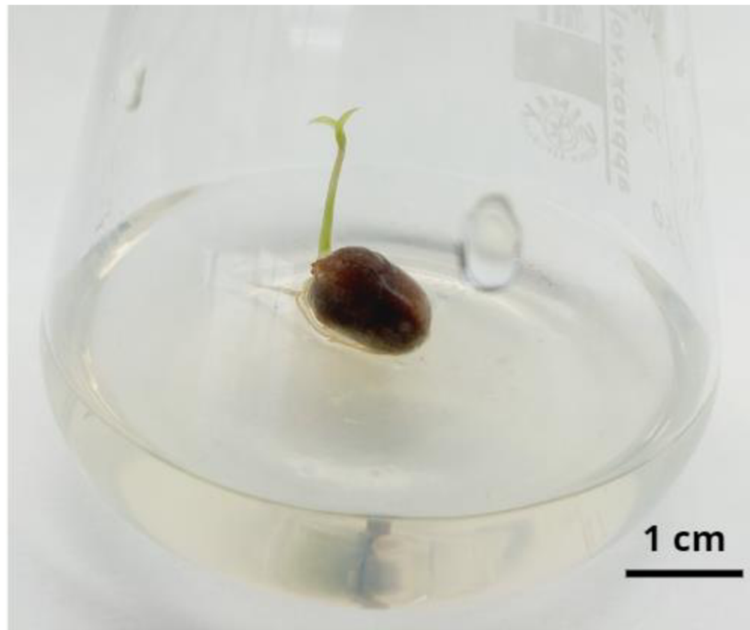

Figure 13: Plantlet of *S. stenocarpa* after 3 weeks *in vitro* culture (source: author)

5.2.2. Propagation

After the first subculture of plants grown from the seeds, the apical segment transferred on new MS media grew in length and formed roots. However, after the second subculture, the explants lost their ability to grow and started producing callus instead. The nodal stem segments of *in vitro* seedlings did not grow at all and started producing callus right after the first propagation. Therefore, various concentrations and combinations of PGRs on different types of basal media were tested in order to stimulate the growth of shoots.

On MS medium supplemented with 0.2 mg.l^{-1} BAP and 30 mg.l^{-1} AS, growth of new shoots was observed. However, BAP also stimulated fast growth of callus, and thus the newly developed shoots were transferred to MS medium without PGRs. After the transfer, they did not grow in length and formed callus again (Fig. 14 a). In this case, AS showed to be crucial as explants on MS media with just 0.2 mg.l^{-1} BAP did not grow at all and turned brown after 4 weeks.

Different concentrations of BAP, NAA, and AS in MS medium were shown to be ineffective. In all treatments tested, explants developed callus on the plant base, and it was growing intensively (Fig. 14 c) compared to explants on PGRs-free MS medium. A combination of 1 mg.l^{-1} BAP with 0.1 NAA mg.l^{-1} and 30 mg.l^{-1} AS on DKW medium also led to the growth of callus but of smaller size than that on MS medium with the same concentration of PGRs (Fig. 14 b).

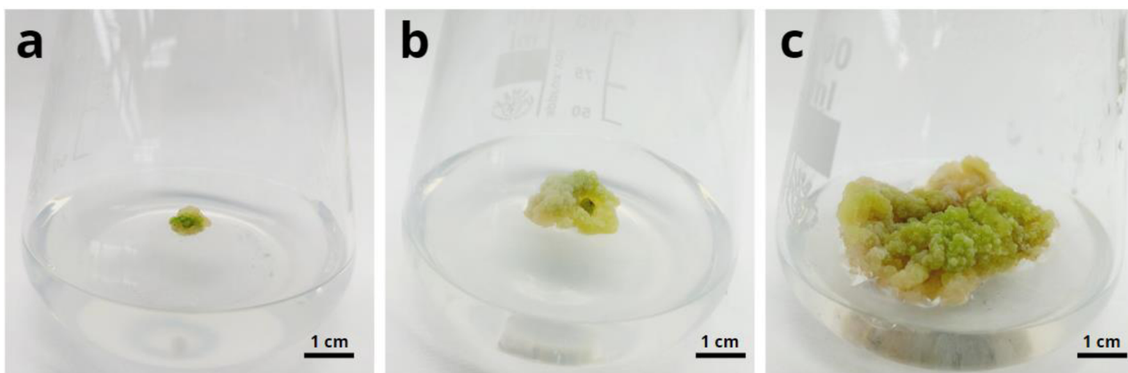

Figure 14: *S. stenocarpa* after 4 weeks of cultivation. a) MS medium b) DKW medium with 1 mg.l^{-1} BAP + 0.1 NAA mg.l^{-1} + 30 mg.l^{-1} AS, c) MS medium with 1 mg.l^{-1} BAP + 0.1 NAA mg.l^{-1} + 30 mg.l^{-1} AS (source: author)

6. Discussion

6.1. *In vitro* propagation of *M. flagellipes*

This study presents the first *in vitro* propagation protocol for *Mucuna flagellipes*. In this research, explants of *M. flagellipes* on MS medium without PGRs developed into compact unbranched plants. Therefore, for the enhancement of shoot production, the effect of two cytokinins, BAP and ZEA, was tested. There are a few previous studies on *in vitro* propagation of other plants from the *Mucuna* genus where the effect of cytokinins or cytokinin in combination with an auxin was tested. The use of PGRs proved to be necessary for the successful micropropagation of plants from the *Mucuna* genus (Faisal et al. 2006; Madkami et al. 2023).

In studies on *Mucuna pruriens* and *Mucuna gigantea*, BAP alone or in combination with NAA has proven effective in inducing shoot proliferation (Faisal et al. 2006; Lahiri et al., 2012; Madkami et al. 2022; Madkami et al., 2023). Similarly, in our study, MS medium supplemented with 1mg.l^{-1} BAP provided promising results (3.60 shoots per explant), aligning with previous findings regarding the efficiency of BAP in shoot induction across *Mucuna* species. However, in previously mentioned studies, the effect of ZEA on shoot proliferation has not been tested. Although addition of 1 mg.l^{-1} ZEA in MS medium did not lead to significantly higher production of newly developed shoots in comparison to 1 mg.l^{-1} BAP (4.70, and 3.60 shoots per explant respectively), its role in promoting the production of vital shoots was crucial. In recent years, the use of ZEA is becoming more popular (Jameson 2023) and our study suggests its potential for enhancing micropropagation outcomes in *Mucuna* species.

In addition, the leave size of newly regenerated leaves in explants on media with PGRs was significantly smaller compared to explants on PGRs-free MS medium. This could be attributed to rapid cell division caused by both used cytokinins, which leads to smaller, more compact leaves rather than large ones with extensive cell expansion (Skalák et al. 2019). However, upon transfer to the rooting medium, the leaves gained back their large size and dark green colour.

6.2. *In vitro* rooting and *ex vitro* transfer of *M. flagellipes*

In this study, explants of *M. flagellipes* on multiplication media supplemented with cytokinins did not develop any roots. Therefore, it was necessary to find optimal medium for *in vitro* rooting. Based on previous study on *M. pruriens* by Faisal et al. (2006), where the highest number of roots was obtained on ½ MS medium with 0.2 $mg.l^{-1}$ IBA, ½ MS media with different concentrations of IBA were tested. Contrary to Faisal et al. (2006), in this study, the highest number of roots (2.80 per explant) and 100% rooting rate was obtained on ½ MS medium without IBA. These outcomes align with those reported by Madkami et al. (2023), where the greatest root regeneration (7.0 per explant) in *M. pruriens* occurred on ⅛ MS medium without any PGRs, indicating that PGR supplementation may not be imperative for successful rooting.

The survival rate of plants of *M. flagellipes* transferred *ex vitro* into soil and perlite in 1:1 volume ratio was 100% after 3 weeks of cultivation. This is in agreement with other studies on plants from the *Mucuna* genus, where the transfer of well-rooted plants *ex vitro* leads to high survival rate, ranging between 80-100% (Faisal et al. 2006; Lahiri et al. 2012; Madkami et al. 2023).

6.3. *In vitro* propagation of *S. stenocarpa*

In vitro propagation of *S. stenocarpa* on MS medium without PGRs led to the production of callus. Therefore, the concentration of PGRs from previous micropropagation protocols for *S. stenocarpa* was tested to induce shoot production and elongation. MS medium with 0.5 $mg.l^{-1}$ BAP and 0.05 $mg.l^{-1}$ of NAA was tested. However, in our study, this treatment led to excessive callus production. This does not corroborate with studies by Adesoye et al. (2012) and Ongusola et al. (2016), who both reported very similar treatments to be effective in shoot induction in *S. stenocarpa*. According to Ongusola et al. (2016), the highest number of shoots per explant (5.3) was obtained on MS medium supplemented with 0.6 $mg.l^{-1}$ BAP and 0.03 $mg.l^{-1}$ NAA. Adesoye et al. (2012) obtained 4.5 shoots per explant and 100% multiple shoot induction on MS medium supplemented with 0.5 $mg.l^{-1}$ BAP and 0.05 $mg.l^{-1}$ of NAA.

In studies on other species from the Fabaceae family, the combination of BAP and NAA also proved to be efficient in multiple shoot induction (Kartha et al. 1981; Yadav et

al. 2010; Singh et al. 2019). However, in this study, the combination of BAP and NAA in various concentrations led to callus production. Therefore, it was necessary to induce shoot production from the callus. According to Veitía et al. (2015), 40 $mg.l^{-1}$ AS stimulated the formation and elongation of shoots from callus in the indirect organogenesis of *Phaseolus vulgaris.* Thus, to stimulate the formation and elongation of shoots from the callus in *S. stenocarpa*, different treatments with 30$mg.l^{-1}$ AS were tested. In this study, MS medium supplemented with 0.2 $mg.l^{-1}$ BAP and 30 $mg.l^{-1}$ AS showed growth of new shoots. However, upon their transfer to PGRs-free MS medium, they did not grow in length and produced callus instead. In other treatments with AS no production of new shoots was observed.

Protocols for micropropagation of *S. stenocarpa* by Adesoye et al. (2012) and Ongusola et al. (2016) had been conducted using MS medium. However, compared to MS medium, DKW medium has greater sulphate concentration which can be beneficial to woody plants (Phillips & Garda 2019). Therefore, the effect of PGRs in DKW medium in explants of S. *stenocarpa* was tested as well. While explants cultivated on DKW medium supplemented with 1 $mg.l^{-1}$ BAP, 0.1 NAA $mg.l^{-1}$ and 30 $mg.l^{-1}$ AS produced smaller-sized callus compared to explant on MS medium with the same concentration of PGRs, it still did not lead to shoot growth.

S. stenocarpa, as oberved by Ongusula et al. (2016), exhibits a significant degree of genotype variability, which directly influences its response to various micropropagation treatments. This genetic diversity underscores the challenges in standardizing effective protocols for its cultivation *in vitro*. Despite the utilization of previously successful methods documented in both *S. stenocarpa* and other Fabaceae species, the outcomes in this study were notably inefficient. The challenging nature of micropropagating Fabaceae plants is well-known for their genotype variability and poor regeneration ability (Pratap et al. 2018). Consequently, the inability to reproduce the results of previous micropropagation protocols highlights the complex relationship between genotype-specific reactions and the necessity for customized micropropagation techniques within this specific group of plants.

7. Conclusions

For establishment of *in vitro* cultures of *M. flagellipes* and *S. stenocarpa*, surface sterilization of plant material with 70% EtOH for 60 s or 96% EtOH for 10 s followed by 2% NaClO was successful.

Considering the fact that explants of *M. flagellipes* on MS medium without PGRs did not produce any new shoots, the application of cytokinins is crucial for multiple-shoot induction. Although both tested cytokinins, BAP and ZEA, were efficient in the formation of new shoots and meristems, explants on medium with 1 $mg.l^{-1}$ ZEA produced more vital-looking shoots compared to explants on medium with 1 $mg.l^{-1}$ BAP. Therefore, the best medium for shoot proliferation was MS medium supplemented with 1 $mg.l^{-1}$ ZEA resulting in 4.70 shoots per explant.

For *in vitro* rooting of *M. flagellipes*, the highest number of roots (2.80 per explant) and 100% rooting rate was obtained on ½ MS medium without PGRs. The addition of auxin IBA led to the production of callus and a decreased rooting rate with a lower number of short roots. Therefore, the positive effect of IBA on rhizogenesis was not confirmed in this species.

Ex vitro transfer of well-rooted plants of *M. flagellipes* into sterilized soil perlite mixture in 1:1 volume ratio was successful. The plants grew in length, produced new leaves, and looked healthy 3 weeks after the transfer. The survival rate was 100%.

Therefore, the micropropagation protocol for M. *flagellipes* was successfully developed and can be used for large-scale propagation of this species.

For *in vitro* propagation of *S. stenocarpa*, no treatment tested resulted in shoot proliferation nor elongation of existing shoots. In all treatments, except for 0.2 $mg.l^{-1}$ BAP on MS medium which led to browning, callus was produced. DKW medium led to production of smaller-sized callus compared to MS medium with the same combination of PGRs. However, no plant regeneration was achieved. Treatments previously used for micropropagation of *S. stenocarpa* in different studies were not successful. The reason can be high genotype variability and poor regeneration ability, typical for plants from the Fabaceae family.

8. Recommendation

Within further research on *M. falgellipes*, more types of cytokinins at different concentrations and combination of cytokinins with auxins for the improvement of shoot proliferation should be tested. Genetic stability of newly developed plants should be assessed using molecular markers.

In *S. stenocarpa*, the effect of various types of PGRs and other organic compounds might be tested to improve shoot proliferation. Given the extensive callus production, optimizing indirect morphogenesis could be a feasible method for obtaining a large number of new plants.

9. References

Abdulkareem KA, Animasaun DA, Oyedeji S, Olabanji OM. 2015. Morphological characterization and variability study of African yam beans [*Sphenostylis stenocarpa* (Hochst Ex A. Rich)]. Global Journal of Pure and Applied Sciences **21**:21-27.

Adeniyan ON, Akande SR, Balogun MO, Saka JO. 2007. Evaluation of crop yield of African yam bean, maize and kenaf under intercropping systems. American-Eurasian Journal of Agricultural and Environment Science **2**:99-102.

Ade-Omowaye BIO, Tucker GA, Smetanska I. 2015. Nutritional potential of nine underexploited legumes in Southwest Nigeria. International Food Research Journal **22**: 798-806.

Adesoye AI, Emese A, Olayode OM. 2012. *In vitro* regeneration of African Yam Bean (*Sphenostylis stenocarpa* (Hochst ex. A. Rich.) Harms by direct organogenesis. Kasetsart Journal (Natural Science) **46**:592-602.

Adewale BD, Dumet DJ, Vroh I, Kehinde O, Ojo D, Adegbite A, Franco J. 2011. Morphological diversity analysis of African yam bean (*Sphenostylis stenocarpa* Hochst. ex A. Rich.) Harms and prospects for utilization in germplasm conservation and breeding. Genetic Resources and Crop Evolution **59**:927-936.

Adewale BD, Dumet DJ. 2011. Descriptors for African yam bean. International Institute of Tropical Agriculture. Available from www.iita.org (accessed August 2023).

Adewale BD, Kehinde OB, Odu BO, Dumet DJ. 2008. The potentials of African yam bean (*Sphenostylis stenocarpa* Hochst. ex. A. Rich) Harms in Nigeria: character distribution and genetic diversity. Pages 265-276 in Smart J, Haq N, editors. 5th International Symposium on New Crops and Uses: their role in a rapidly changing world. University of Southampton, Southampton.

Adeyeye AA, Oshodi KO, Ipinmoroti EI. 1999. Fatty acid composition of six varieties of dehulled African yam bean (*Sphenostylis stenocarpa*) flour. International Journal of Food Sciences and Nutrition **50**:357-365.

Agba OA, Adiaha MS, Asiegbu JE, Mbah BN. 2016. Effect of plant population on the growth and yield of *Mucuna flagellipes* (Vogel Ex Hook) in an ultisol, South Nigeria. World Scientific News **60**:1-12.

Agba OA, Eze SC, Atugwu AI, Awere SU, Chukwu C. 2019. Influence of combined application of P fertilizer and lime on *Mucuna flagellipes* nodulation, growth and yield. African Journal of Plant Science **13**:47-58.

Agba OA. 2021. Investigation of poultry manure on growth and proximate composition of some indigenous vegetables Ukpor (*Mucuna flagellipes*) and Bitter leaf (*Vernonia amygdalina*). Journal of Research in Agriculture and Animal Science **8**:9-15.

Aina A, et al. 2021. Predictive genotype-phenotype relations using genetic diversity in African yam bean (*Sphenostylis stenocarpa* (Hochst. ex. A. Rich) Harms). BioMed Centra Plant Biology **21**:1-12.

Ajayi IA, Nwozo SO, Adewuyi A. 2010. Antimicrobial activity and phytochemical screening of five selected seeds from Nigeria. International Journal of Biomedical and Pharmaceutical Science **4**:104-106.

Ajayi IA, Oderinde RA, Kajogbola DO, Uponi JI. 2006. Oil content and fatty acid composition of some underutilized legumes from Nigeria. Food chemistry **99**:115-120.

Allen ON, Allen EK. 1981. The Leguminous. A source book of Characterization, Uses and Nodulation. Page 461. Macmillan Publisher Ltd., London.

Anyanwu GO, Anzaku D, Donwell CC, Usunobun U, Adegbegi AJ, Ofoha PC, Rauf K. 2021. Chemical composition and *in vitro* antiobesity and *in vivo* anti-hyperlipidemic effects of *Ceratotheca sesamoides, Jatropha tanjorensis, Mucuna flagellipes, Pterocarpus mildbraedii* and *Piper guineense*. Phytomedicine Plus **1**:1-7.

Apata DF, Ologhobo AD. 1990. Some aspects of biochemistry and nutritive value of African yam bean seed (*Sphenostylis stenocarpa*). Food Chemistry **36**:271-280.

Aviara NA, Onuh OA, Ehiabhi SA. 2010. Physical properties of *Mucuna flagellipes* nuts. Global Science Books seed Science and Biotechnology **4**:59-68.

Azuzu IU. 1986. Pharmacological evaluation of the folklore use of *Sphenostylis stenocarpa*. Journal of Ethnopharm **16**:263-267.

Chattopadhyay S, Datta SK, Mahato SB. 1995. Rapid micropropagation for *Mucuna pruriens f. pruriens* L. Plant cell reports **15**:271-273.

Cruz-Cruz C, González-Arnao M, Engelmann F. 2013. Biotechnology and Conservation of Plant Biodiversity. Resources **2**:73-95.

Dakora FD. 2013. Biogeographic distribution, nodulation and nutritional attributes of underutilized indigenous African legumes. Acta Horticulturae **979**:53-64.

Driver JA, Kuniyuki AH. 1984. *In vitro* propagation of Paradox walnut rootstock. HortScience **19**:507-509.

Ekwealor KU, Okeke CU, Iroka CF, Joe ON, Ike ME, Ann MO, Chukwuma MO. 2015. Comparative Influence of *Ipomoea batatas, Luffa cylinderica* and *Mucuna flagellipes* in Nutrient Recovery and Post-Harvest Soil Management of Anambra Basin, Southeastern Nigeria. International Journal of Basic and Life Sciences **3**:204-213.

Ene-Obong HN, Carnovale E. 1992. Nigerian soup condiments: traditional processing and potential as dietary fibre sources. Food chemistry **43**:29-34.

Engelmann F. 1997. Present development and use of *in vitro* culture techniques for the conservation of plant genetic resources. Acta Horticulturae **447**:471-476.

Faisal M, Siddique I, Anis M. 2006. *In vitro* rapid regeneration of plantlets from nodal explants of *Mucuna pruriens*–a valuable medicinal plant. Annals of applied biology **148**:1-6.

Fasoyiro SB, Ajibade SR, Omole AJ, Adeniyan ON, Farinde EO. 2006. Proximate, minerals and antinutritional factors of some underutilized grain legumes in south-western Nigeria. Nutrition & Food Science **36**:18-23.

Gatti I, Guindón F, Bermejo C, Espósito A, Cointry E. 2016. *In vitro* tissue culture in breeding programs of leguminous pulses: use and current status. Plant Cell, Tissue and Organ Culture **127**:543-559.

George EF, Hall MA, Klerk GJD. 2008. Micropropagation: uses and methods. Pages 29-64 in George EF, Hall MA, Klerk GJD, editors. Plant Propagation by Tissue Culture. Springer, Dordrecht.

George TT, Obilana AO, Oyeyinka SA. 2020. The prospects of African yam bean: past and future importance. Heliyon **6**:1-10.

Harris DJ. 2006. *Mucuna flagellipes* Hook. f. West African Plants. Available from http://www.westafricanplants.senckenberg.de (accessed August 2023).

Hennessy EFF. 1991. Erythrineae (Fabaceae) in southern Africa. Bothalia **21**:1-25.

Jameson PE. 2023. Zeatin: The 60th anniversary of its identification. Plant Physiology **192**:34-55.

Jansen PCM. 2005. *Mucuna flagellipes* Hook.f. PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale), Wageningen. Available from https://prota.prota4u.org (accessed August 2023)

Jovita EE, Ani CO, Uzoma IC, Pamela AS, Kelechi OC, Ayowumi AM, Daniel NC. 2017. Antihypertensive effects of ethanolic extract of *Mucuna flagellipes* seed on salt-induced hypertension on Sprague-dawley rats. African Journal of Pharmacy and Pharmacology **11**:582-592.

Kartha KK, Pahl K, Leung NL, Mroginski LA. 1981. Plant regeneration from meristems of grain legumes: soybean, cowpea, peanut, chickpea, and bean. Canadian Journal of Botany **59**:1671-1679.

Klu GYP, Amoatey HM, Bansa D, Kumaga FK. 2001. Cultivation and Uses of African yam bean (*Sphenostylis stenocarpa*) in the Volta Region of Ghana. The Journal of Food Technology in Africa **6**:74-77.

Koba K, Yanagita T. 2014. Health benefits of conjugated linoleic acid (CLA). Obesity research & clinical practice **8**:525-532.

Konyeme TE, Nyananyo BL, Tanee FBG. 2020. Diversity in proximate analysis of tubers of some African yam bean (*Sphenostylis stenocarpa*) (Hochst Ex. A. Rich.) harms (Fabaceae) accessions. Journal of Applied Sciences and Environmental Management **24**:1787-1793.

Lahiri K, Mukhopadhyay MJ, Desjardins Y, Mukhopadhyay S. 2012. Rapid and stable *in vitro* regeneration of plants through callus morphogenesis in two varieties of *Mucuna pruriens* L.–an anti Parkinson's drug yielding plant. The Nucleus **55**:37-43.

Lampariello LR, Cortelazzo A, Guerranti R, Sticozzi C, Valacchi G. 2012. The magic velvet bean of *Mucuna pruriens*. Journal of Traditional and Complementary Medicine **2**: 331-339.

Lim HM, Park SH. 2022. Regulation of reactive oxygen species by phytochemicals for the management of cancer and diabetes. Critical Reviews in Food Science and Nutrition **63**:5911-5936.

Madkami SK, Moharana A, Barik DP. 2023. Rapid micropropagation protocol of *Mucuna pruriens* var. utilis using cotyledonary node explant: A cultivated medicinal edible legume. Legume Research-An International Journal **46**:243-250.

Madkami SK, Moharana A, Mishra RC, Barik DP. 2022. Rapidi *in vitro* plant regeneration from nodal explant of *Mucuna gigantea*—an endangered medicinal legume. International Journal of Pharma and Bio Sciences **13**:1-8.

Moura TM, Bogler D, Miranda JMD, Gaglioti AL, Lewis GP. 2018. Morphological variation in pollen grains of *Mucuna* (Leguminosae): new biogeographic and evolutionary patterns. Plant Systematics and Evolution **304**:861-869.

Moura TM, Wilmot-Dear M, Vatanparast M, Fortuna-Perez AP, Tozzi AM, Lewis GP. 2016. A new infrageneric classification of Mucuna (Leguminosae-Papilionoideae): supported by morphology, molecular phylogeny and biogeography. Systematic Botany **41**:606-616.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum **15**:473-497.

Njoku HO, Eli I, Ofuya CO. 1989. Effect of pretreatment on the cooking time of the African yam bean (*Sphenostylis sternocarpa*). Journal of Food Science **54**:758–759.

Nwankwo OE, Odewo SA, Ajani BA, Soyewo LT, Nwefuru MS. 2022. Could the three be edible and natural sources of levodopa? Morphological characterization of three taxa of *Mucuna* (Fabaceae) in Ebonyi state, southeastern Nigeria. Agricultural Science Digest-A Research Journal **42**:468-471.

Nyananyo BL, Nyingifa AL. 2011. Phytochemical investigation on the seed of *Sphenostylis stenocarpa* (Hochst ex A. Rich.) Harms (Family Fabaceae). Journal of Applied Sciences and Environmental Management **15**:419-423.

Oagile O, Mmolotsi R, Segwagwe A, Babili TP. 2012. African yam bean (*Sphenostylis stenocarpa*) nodulates promiscuously with rhizobium indigenous to soils of Botswana. Journal of Plant Studies **1**:109-113.

Oagile O. 2005. African yam bean: morphology, clonal propogation and nitrogen fixation [PhD thesis]. University of Nottingham, Nottingham.

Obiagwu CJ. 1995. Effect of fertilizer treatment and planting time on the yield of some grain legumes of Benue River Basins of Nigeria. Journal of Plant Nutrition **18**:2075-2080.

Obiagwu CJ. 1997. Screening process for ideal food legume cover crops in the tropical cropping systems. (II) Application of the selection method for grain legume crops of the Benue River Basins of Nigeria (BRBN). Journal of Sustainable Agriculture **10**:15-31.

Oboh G, Ademiluyi AO, Akindahunsi AA. 2009. Changes in polyphenols distribution and antioxidant activity during fermentation of some underutilized legumes. Food Science and Technology International **15**:41-46.

Oboh HA, Mazquiz M, Burbano C, Cuadrado C, Pedrosa MM, Ayet G, Osagie AU. 1998. Anti-nutritional constituents of six under-utilized legumes grown in Nigeria. Journal of Chromatography A **823**:307-312.

Ojuederie OB, Ajiboye JA, Babalola OO. 2020. Biochemical and histopathological studies of key tissues in healthy male Wistar rats fed on African yam bean seed and tuber meals. Journal of Food Quality **2020**:1-10.

Ojuederie OB, Balogun MO, Abberton MT. 2016. Mechanism for pollination in African yam bean. African Crop Science Journal **24**:405-416.

Okwu DE, Okoro E. 2006. Phytochemical composition of *Brachystegia eurycoma* and *Mucuna flagellipes* seeds. Medicinal and Aromatic Plant Science and Biotechnology **26**:1-4.

Okwu F. 2020. Ukpo. Unusual4tune. Available from https://unusual4tune.wordpress.com/2020/11/18/ukpo/ (accessed August 2023)

Ongusola KE, Ojuederie OB, Emmanuel B. 2016. *In vitro* morphogenic responses of African yam bean (*Sphenostylis stenocarpa* (Hochst ex. A. Rich.) Harms) accessions to plant growth regulators. Plant Cell, Tissue and Organ Culture **127**:613-622.

Onuegbu NC, Nworah KO, Essien PE, Nwosu JN, Ojukwu M. 2013. Proximate, functional and anti-nutritional properties of boiled ukpo seed (*Mucuna flagellipes*) flour. Nigerian Food Journal **31**:1-5.

Onweluzo JC, Leelavathi K, Haridas Rao P. 1999. Effect of *Detarium microcarpum* (Dm) and *Mucuna flagellipes* (Mf) gums on the quality of white bread. Plant Foods for Human Nutrition **54**:173-182.

Onweluzo JC, Onuoha KC, Obanu ZA. 1995. Certain functional properties of gums derived from some lesser known tropical legumes (*Afzelia africana, Detarium microcarpum* and *Mucuna flagellipes*). Plant Foods for Human Nutrition **48**:55-63.

Oshodi AA, Ipinmoroti KO, Adeyeye EI, Hall GM. 1995. Amino and fatty acids composition of African yam bean (*Sphenostylis stenocarpa*) flour. Food Chemistry **53:**1-6.

Ozioko OM, Ozor II. 2020. Prophylaxis potential of *Mucuna flagellipes* against cyclophosphamide-induced bone marrow suppression. Journal of Experimental Research **8**:8-13.

Paliwal R, Adegboyega TT, Abberton M, Faloye B, Oyatomi O. 2021. Potential of genomics for the improvement of underutilized legumes in sub-Saharan Africa. Legume Science **3**:1-16.

Phillips GC, Garda M. 2019. Plant tissue culture media and practices: an overview. In Vitro Cellular & Developmental Biology **55**:242-257.

Phillips RD. 1993. Starchy legumes in human nutrition, health and culture. Plant Foods for Human Nutrition **44**:195-211.

Popoola JO, Adegbite AE, Obembe OO, Agbolade JO. 2011. Reproductive mechanisms and pollen characterization in some accessions of an underutilized legume:(*Sphenostylis stenocarpa* Hochst Ex. A. Rich) harms. International Journal of Biodiversity and Conservation **3**:185-192.

Potter D, Doyle JJ. 1992. Origins of the African Yam bean (*Sphenostylis stenocarpa*, Leguminosae): evidence from morphology, isozymes, chloroplast DNA, and linguistics. Economic Botany **46**:276-292.

Potter D, Doyle JJ. 1994. Phylogeny and systematics of *Sphenostylis* and *Nesphostylis* (Leguminosae: Phaseoleae) based on morphological and chloroplast DNA data. Systematic Botany **19**:389-406.

Potter D. 1992. Economic botany of *Sphenostylis* (Leguminosae). Economic Botany **46**:262-275.

POWO. 2023. *Mucuna* adans. Plant of the World Online. Available from http://www.plantsoftheworldonline.org/ (accessed November 2023).

POWO. 2024. *Mucuna flagellipes* Vogel ex Hook.f. Plants of the World Online. Available from http://www.plantsoftheworldonline.org/ (accessed January 2024).

Pratap A et al. 2018. Potential, constraints and applications of *in vitro* methods in improving grain legumes. Plant Breeding **137**:235-249.

Quattrocchi U. 1999. CRC world dictionary of plant names: common names, scientific names, eponyms, synonyms, and etymology. CRC Press, New York.

Quattrocchi U. 2012. CRC world dictionary of medicinal and poisonous plants: common names, scientific names, eponyms, synonyms, and etymology. CRC Press, New York.

Reuther G. 1990. Current status and future prospects of large scale micropropagation in commercial plant production. Food Biotechnology **4**:445-459.

Singh RK, Anandhan S, García-Pérez LM, Ruiz-May E, Nava Pérez E, Quiroz-Figueroa FR. 2019. An efficient protocol for *in vitro* propagation of the wild legume *Cicer microphyllum Benth*., a crop wild relative of chickpea (*Cicer arietinum* L.). In Vitro Cellular & Developmental Biology-Plant **55**:9-14.

Skalák J, et al. 2019. Multifaceted activity of cytokinin in leaf development shapes its size and structure in Arabidopsis. The Plant Journal **97**:805-824.

Soetan KO, Olaiya CO, Karigidi KO. 2018. Comparative *in vitro* antioxidant activities of six accessions of African yam beans (*Sphenostylis stenocarpa* L.). Annals: Food Science & Technology **19**:455-461.

Terrell EE, Hill SR, Wiersema JH, Rice WR. 1986. A checklist of names for 3000 vascular plants of economic importance. Agriculture Handbook 505. US Department of Agriculture, USA.

Uchegbu NN. 2015. Antioxidant activity of germinated African yam bean (*Sphenostylis stenocarpa*) in Alloxan diabetic rats. International Journal of Nutrition and Food Engineering **9**:239-243.

Udensi EA, Arisa NU, Ikpa E. 2010. Effects of soaking and boiling and autoclaving on the nutritional quality of *Mucuna flagellipes* ("ukpo"). African Journal of Biochemistry Research **4**:47-50.

Veitía N, García LR, Martirena-Ramírez A, Bermúdez-Caraballoso I, Collado R, Torres D, Romero C. 2015. Adenine sulfate effect on shoot formation of *Phaseolus vulgaris* L. cultivar'CIAP7247F'via indirect organogenesis. Biotecnología Vegetal **15**:123-126.

Von Helversen O, Winter Y. 2005. Glossophagine Bats and Their Flowers: Costs and Benefits for Plants and Pollinators. Page 357 in Kunz TH, Fenton MB, editors. Bat ecology. University of Chicago Press, Chicago.

WFO. 2023. *Sphenostylis* E. Mey. Available from www.worldfloraonline.org/taxon/wfo-4000036056 (accessed July 2023).

Wiriadinata H, Ohashi H, Adema F. 2016. Notes on Malesian Fabaceae (Leguminosae-Papilionoideae) 16. The genus *Mucuna*. Blumea - Biodiversity, Evolution and Biogeography of Plants **61**:90-124.

Yadav SK, Sreenu P, Vanaja M, Venkateswarlu B. 2010. Efficient shoo Regeneration from double cotyledonary node explants of green gram *Vigna radiata* (L.) Wilczek. Indian Journal of Biotechnology **9**:403-407.