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Endophytic *Luteibacter yeojuensis* strains stimulate banana plant growth

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Key words: Banana, fungal endophytes, *Luteibacter*, 16S rDNA, tissue culture.

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All relevant data are within the paper and its Supporting Information files.

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Abstract: This study isolated endophytic bacteria from tissue-cultured banana ultivars Grand Nain and Saba. The bacteria were further characterized and identified through morphological, cultural, and molecular analyses. The bacteria had morphological and colony characteristics resembling those of *Luteibacter* species. Colonies were white to yellow, round, and slightly raised, with the entire margin in nutrient agar medium. The bacterial isolates were Gram-negative based on the potassium hydroxide test (KOH) test. Phylogenetic analysis of the 16S ribosomal gene region grouped the three isolates in the *Luteibacter yeojuensis* clade. The three *Luteibacter yeojuensis* isolates were not pathogenic to banana 'Grand Nain,' 'Lakatan,' and 'Saba' in both wounded and unwounded assays conducted in controlled assays. No stunting, wilting, and corm tissue browning were observed 14 days post-inoculation when the bacteria were inoculated on tissue-cultured plants; two of the three isolates significantly increased plant height of cv. Lakatan ($p<0.05$) and one isolate, *L. yeojuensis* GN11-20, enhanced shoot proliferation in cv. Grand Nain. The study reports *L. yeojuensis* as an endophytic bacterium with growth-promoting activity in tissue-cultured banana plants. The endosymbiotic association of *L. yeojuensis* in bananas could enhance plant growth and resistance to banana diseases.

1. Introduction

Banana (*Musa* spp.) is a high-value fruit and cash crop widely grown as one of the staple foods in Asia (Rossman *et al*., 2012). The Philippines remains the top producer of bananas in Asia and the second largest exporter globally, following Ecuador (FAO, 2023). There are three major varieties of banana produced in the country: Cavendish (51%), Saba (29%), and Lakatan (10%) (Anzures *et al.,* 2022). Data from the Philippine Statistics Authority (2023) show that the Davao region is the top producer of bananas, with 868.19 thousand metric tons (mt) or 37.4% of the total banana production in the last quarter of 2023. This was followed by Northern Mindanao with 431.86 thousand mt (19%) and Soccsksargen Region with 279.20 thousand mt (12.3%) shares in production, respectively (Philippine Statistics Authority, 2023).

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Cultivated banana genotypes are triploids that are generally sterile and seedless (Uma, 2021). Thus, conventional production of banana planting materials is made through sword suckers. However, these vegetative materials may also harbour plant pathogens. They may subsequently result in the carry-over of diseases in succeeding planting materials; hence, plant tissue culture is a widely practiced method as an alternative for banana production. Aside from preventing the carry-over of diseases, the technology allows the plants to aseptic production with uniform genotypes in a relatively shorter period (Souza *et al*., 2000). The technology only requires a small portion (1-3 mm^2) of the plant parts (meristems) for *in-vitro* mass production and germplasm conservation (Agbadje *et al.*, 2021); this allowed bananas rapid propagation from a single corm with favourable pests and pathogen-free genotypes (Agbadje *et al.,* 2021). However, contamination with microorganisms that may act as endophytes, saprophytes, or asymptomatic pathogens is commonly observed (Cassells, 1991). Contamination may emanate from the plant's plant tissues (endophytes) and surfaces (Nair and Padmavathy, 2014). Proper growth media and explant sterilizations can easily control microbes on the explant's surface and those carried from the environment (Sivanesan *et al*., 2021). In contrast, endophytes are challenging to control because they are inside the plant tissue and are tolerant to surface sterilization (Hardoim *et al.,* 2015).

Endophytes like bacteria, fungi, and actinomycetes can colonize healthy living tissues and establish a symbiotic relationship with the host plant (Nair and Padmavathy, 2014). Host plants benefit from endophytes through plant growth promotion (Afzal *et al.,* 2019), pathogen and insect attack defence (Sturz and Matheson, 1996; Azevedo *et al.,* 2000; Pieterse *et al.,* 2014; Martínez-Hidalgo *et al.,* 2015; Oukala *et al.,* 2021), and increased tolerance to abiotic factors including salinity (Ali *et al.,* 2014), low temperature (Subramanian *et al.,* 2015), and heavy metals (Rajkumar *et al.,* 2009). Bacterial endophytes of bananas are known plant growth promoters and biocontrol agents. For instance, several bacterial endophytes from diverse communities form an antagonistic relationship against *Fusarium oxysporum f.sp. cubense* (*Foc*) (Jie *et al.,* 2009). Plant growth promotion in banana cv. Prata Ana has also been demonstrated in shoot tip cultures colonized with endophytic *Klebsiella pneumoniae* (Fernandes *et al.,* 2013). Thus, the utilization of endophytes could improve the banana production system. However, any new endophytes found from a plant must undergo pathogenicity testing to ensure that they do not cause infection to the host once removed from their natural system (within the host).

This study identified and characterized an endophytic bacterium isolated from healthy tissue-cultured banana 'Grand Nain' and 'Saba.' We hypothesize that endophytes from healthy banana plants do not harm host plants but function as plant growth promoters. Thus, these endophytes may be used directly or indirectly as potential bioinoculants under a green and sustainable agriculture production system.

2. Materials and Methods

Isolation and storage of the bacterial contaminants

White-to-yellow pigmented bacteria were observed in multiple shoot cultures of tissue-cultured banana cultivars Grand Nain and Saba. A loopful of bacterial cells growing from the stems was transferred onto a nutrient agar (NA) medium. Plates were stored at room temperature (28-30°C) for two days (with 14 hours of light in 24 hours cycle) (Cruz and Balendres, 2021). The bacterium was then purified and further characterized (see succeeding section). A loopful of the bacterium from a 48-hour-old culture was transferred to a fresh NA plate and incubated using the abovementioned conditions. Cultures were stored in microcentrifuge tubes containing 1 mL of sterile distilled water. The cultures were deposited at the Bacteria Repository of the Institute of Plant Breeding, Agriculture and Food Science College, University of the Philippines Los Baños, Laguna, Philippines.

Morphocultural characterization and PCR assay

The bacterial morphology of 48 to 72-hour-old cultures was assessed under a light microscope (Olympus CX23, Japan), and the colony characteristics were recorded. The bacterial genomic DNA was extracted using Chen and Kuo's procedure (Chen and Kuo, 1993) for molecular analyses. The isolated genomic DNA was standardized to 30 ng/µL and was subsequently used as a template for the succeeding polymerase chain reaction (PCR) assay, which amplifies the 16S ribosomal gene region. The PCR assay was performed in MyCycler™ Thermal

Cycler (Bio-Rad, USA) in a 15-µL reaction volume (Cruz and Balendres, 2021). The PCR cocktail mix consisted of 1x PCR Buffer (Invitrogen), 2.0 mM MgCl2 (Invitrogen), 0.2 mM dNTPs (Invitrogen), 0.2 µM each of the forward (27F, 5'-AGAGTTTGATCCTGGCTCAG-3') and reverse (1492R, 5'-GGTTACCTTGTTACGACTT-3') primers (Lane, 1991), one U Taq DNA Polymerase (Invitrogen), one µL of the bacterial genomic DNA, and DEPC-water to volume. The thermal cycling conditions were as follows: initial denaturation at 95°C for two min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for two min, and final extension at 72°C for seven min. The PCR products were resolved by gel electrophoresis in 1.0% Agarose (Vivantis) and 0.5X Tris-Acetate-EDTA (TAE) buffer and were sent to Apical Scientific Sdn. Bhd. (Malaysia) for DNA sequencing.

Molecular characterization and phylogenetic analysis

A consensus DNA sequence was derived from the resultant forward and reverse sequences using Geneious software. Sequence similarity analysis was performed in the NCBI BLASTN program (Zhang and Madden, 1997). Sequences were analysed based on the highest percent similarity, e-value, and query cover. The authentic 16S rDNA sequences of five species of Luteibacter available in Genbank (Table 1) were compared with the consensus sequences of the three *Luteibacter yeojuensis* SbM36C, GN11-20, SabaM36A isolates obtained from this study. The phylogenetic distance of the three bacterial isolates to eight *Luteibacter yeojuensis* isolates from other

Table 1 - *Luteibacter* species with the closest similarity to the 16S rDNA region of the bacterium were isolated in this study

Species	Strain	Source	Locality	16S Genbank Accession	Reference
Luteibacter yeojuensis	IHB B 6856	Aquilaria agallocha	India	KF668474.1	NCBI GenBank
Luteibacter jiangsuensis	JW-64-1	Soil	China	NR_132709.1	Wang *et al.* (2011)
Luteibacter anthropi	CCUG 25036	Human blood sample	Sweden	NR_116911.1	Kampfer *et al.* (2009)
Luteibacter rhizovicinus	LJ96	Hordeum vulgare	Denmark	NR_042197.1	Johansen *et al.* (2005)
Luteibacter pinisoli	MAH-14	Soil	South Korea	KY964279.1	Huq and Akter (2017)
Burkholderia vietnaminensis	LMG 10929	Oryza sativa	Vietnam	NR_041720.1	LiPuma *et al*. (1999)

IHB= Institute of Himalayan Bioresource Technology, Post Box No. 6, Palampur, Himachal Pradesh 176061, India;
JW= Nanjing Agricultural University, Tongwei Road 6, Nanjing, Jiangsu 210095, China;
CCUG= Culture Collection University of Gothenburg, Department of Clinical Bacteriology;
LJ= Department of Bacteriology, Danish Veterinary Institute, Buelowsvej 27, DK-1790, Denmark;
MAH= Food and Nutrition, Chung-Ang University, 4726, Seodong-daero, Daedeok-myeon, Anseong-si, Gyeonggi-do 17546, South Korea;
LMG= Ghent University, Belgium.

Table 2 - *Luteibacter yeojuensis* strains from other countries compared with the strains isolated in this study

Species	Strain	Source	Locality	16S Genbank Accession	Reference
Luteibacter yeojuensis	IHB B 6856	*Aquilaria agallocha*	India	KF668474.1	NCBI GenBank
Luteibacter yeojuensis	T-79	*Curcuma longa*	India	KM589043.1	Kandan *et al.* (2014)
Luteibacter yeojuensis	NBRC 106387	Not Available	Japan	AB682403.1	Nakagawa *et al.* (2011)
Luteibacter yeojuensis	HBU 72524	Not Available	China	MW365223.1	Lv (2020)
Luteibacter yeojuensis	R2A16-10	Soil	Korea	NR_043618	Kim *et al*. (2006)
Luteibacter yeojuensis	RT27	*Oryza sativa*	China	MK014251.1	NCBI GenBank
Luteibacter yeojuensis	OsEnb_ALM_B9	*Oryza sativa*	India	MN889326.1	Kumar *et al.* (2020)
Luteibacter yeojuensis	Z51	Rock	China	KM019785.1	Zhang (2014)
Burkholderia vietnaminensis	LMG 10929	*Oryza sativa*	Vietnam	NR_041720.1	LiPuma *et al.* (1999)

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countries (Table 2) was further determined. *Burkholderia vietnaminensis* (LMG 10929) was used as the outgroup in all analyses (LiPuma *et al.* 1999). The generated 16S rDNA sequences were aligned using CLUSTALW in MEGA X software (Kumar *et al.* 2018). The phylogenetic trees were constructed using the Tamura Nei model (Tamura and Nei 1993) with uniformly distributed rates and 1,000 bootstrap replicates.

Gram test using KOH

A potassium hydroxide (KOH) test was performed following Buck's procedure (Buck, 1982) to identify the gram reaction of the bacterial isolates. Therefore, a micropipette placed 10 µL of 3% KOH on the top of a clean microscope slide. A loopful of bacteria from a forty-eight to 72-hour-old was then transferred to the drop of KOH using a sterile wire loop. The mixture was constantly stirred for 15 seconds and observed for viscosity and formation of mucoidal string. Gram-negative bacteria turn vicious and form a mucoidal string. In contrast, Gram-positive bacteria do not turn viscous with no formation of mucoidal string. Three replicates were used for each isolate.

In-vitro pathogenicity testing and morphometric assessment

The pathogenicity of the bacterial isolates in tissue-cultured banana plantlets was assessed in vitro using a pseudostem injection assay; the four-week-old banana 'Lakatan' (AA), 'Grand Nain' (AAA), and 'Saba' (ABB) were surface-disinfected using 70% ethanol and air-dried. Pseudostems were cut and inoculated by injecting 200 µL of bacterial suspension (0.5, OD_{600}) in wounded and unwounded tissues. The pseudostems were injected with sterile distilled water (SDW) for the control treatment. Treated plantlets were maintained in the plant regeneration medium (basal Murashige and Skoog, 3 $mg \cdot l^{-1}$ 6-benzylaminopurine, 3% sucrose, 7 $g \cdot l^{-1}$ agar, pH 5.7) and exposed to 14-hour fluorescent light cycle at 20±5℃ temperature (Murashige and Skoog, 1962). Four replicate plantlets were used for each isolate and variety. Symptom development was assessed at 14 days post-inoculation. Morphometric characteristics - plant height, number of shoots, and roots formed - were evaluated from all treatments. Analysis of Variance (ANOVA) was performed using the IRRI Statistical Tool for Agricultural Research (STAR Nebula) software with a 95% confidence. The experiment was performed twice.

3. Results

Identity of the bacterial contaminants

Bacteria that are white to yellow were isolated from heathy-looking tissue-cultured bananas cv. Grand Nain and Saba (Fig. 1a to 1c). Colonies are white to yellow in color, round, and slightly raised, with the entire margin in NA medium. Cell shapes were bacilli, 1-2 µm x 7-10 µm size, containing monotrichous, amphitrichous, or lophotrichous flagella (Fig. 1d to 1f). The isolate colony and morphology resembled that of *Luteibacter spp.* The isolates were gram-negative based on the KOH test (Fig. 1 g to 1i). The bacterial isolates' identity was further validated through molecular analysis of the 16S ribosomal DNA region. The bacterial isolates had high similarity (>99%) to *Luteibacter yeojuensis* in BLASTN analysis and were grouped within the *Luteibacter yeojuensis* clade in the constructed phylogenetic tree (Table 3, Fig. 2). A distance tree also revealed that the three Philippine isolates have the closest similarity to *Luteibacter yeojuensis* strain IHB B 6856 from India (Fig. 3).

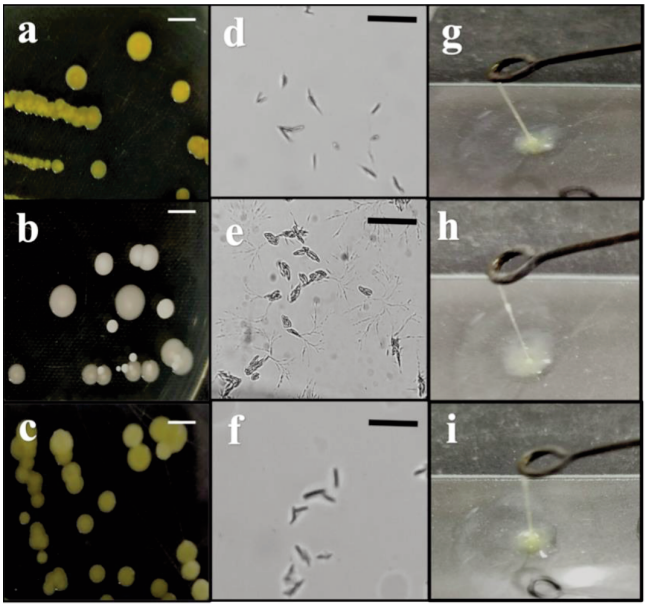

Fig. 1 - Morphological and cultural characteristics of the bacterial contaminants. Cultural characteristics of GN11-20 (a), SbM36A (b), and SbM36C (c) strains of 48-hour-old cultures grown in nutrient agar (NA) medium. The white bar represents 2 cm. Bacterial cell morphology for each isolate was also shown (d, e, and f). The black bar at the upper left represents 10 µm. Gram reaction of the bacterial contaminants using potassium hydroxide (KOH) test (g, h, and i) indicated the isolates were gram-negative through viscous string formation.

Table 3 - Percentage similarities of the three bacterial isolates associated with tissue-cultured banana based on BLASTN search

Isolate	Species	16S rRNA (Accession)
SbM36A	*Luteibacter yeojuensis* IHB B 6856	99.09% (KF668474.1)
GN11-20	*Luteibacter yeojuensis* IHB B 6856	99.64% (KF668474.1)
SbM36C	*Luteibacter yeojuensis* IHB B 6856	99.16% (KF668474.1)

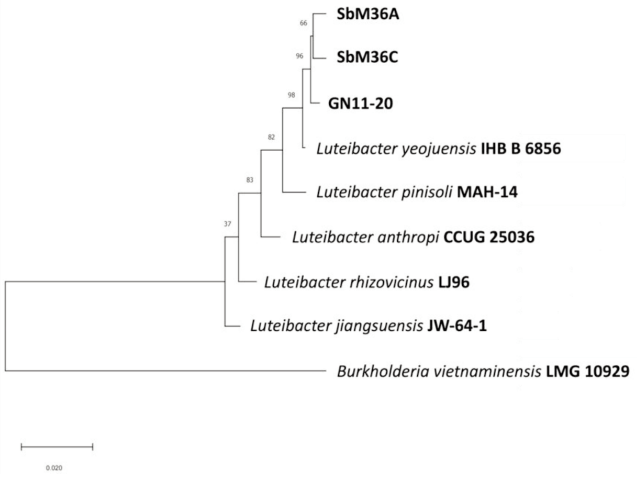

Fig. 2 - The phylogenetic position of the 16S rDNA of the three *Luteibacter yeojuensis* strains was isolated in this study with other bacterial species. The tree was constructed using the Tamura-Nei model (Tamura and Nei, 1993) with 1,000 bootstrap replicates. Burkholderia vietnamiensis (LMG 10929) served as an outgroup.

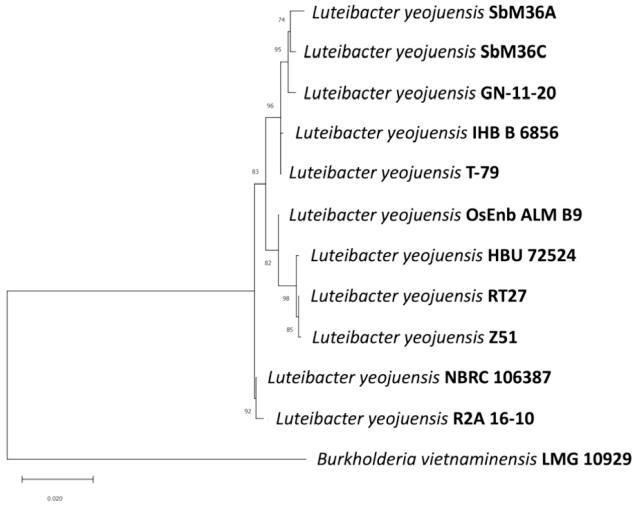

Fig. 3 - The distance tree of the 16S rDNA sequences of *Luteibacter yeojuensis* GN11-20, SbM36A, and SbM36C was isolated in this study with other strains from Asia. The tree was constructed using the Tamura-Nei model (Tamura and Nei, 1993) with 1,000 bootstrap replicates. *Burkholderia vietnamiensis* (LMG 10929) served as an outgroup.

Morphometric characters of the banana plants

Results demonstrated the potential of *L. yeojuensis* for increased plant height and shoot proliferation on three. Both *L. yeojuensis* GN11-20 and *L. yeojuensis S*bM36A increased plant height in 'Lakatan' plants (Table 4, Fig. 4). More shoots were consistently recorded in banana cv. Grand Nain inoculated with the three *L. yeojuensis* isolates compared to the control treatment. One isolate, *Luteibacter yeojuensis* GN11-20, significantly increased shoot proliferation of tissue-cultured 'Grand Nain', the cultivar where the bacterium was initially isolated (Table 4, Fig. 4). On the other hand, no significant differences were observed in the number of shoots formed in banana 'Lakatan' and 'Saba' were inoculated with the three *L. yeojuensis* isolates compared to the control treatment. The plant height, the number of shoots, and the roots of the three genotypes inoculated with *L. yeojuensis* SbM36C were not significantly different from the control treatment.

Non-pathogenicity of bacteria to tissue-cultured bananas

In-vitro pathogenicity tests showed that the three *Luteibacter yeojuensis* strains were non-pathogenic to the three banana cultivars (Fig. 4 and 5) in wounded and unwounded assays. There were no adverse effects observed in plants inoculated with *Luteibacter yeojuensis* SbM36C, GN11-20, and SbM36A as compared to the control treatments (Fig. 5). No stunting and wilting in any of the test banana plants were observed. When corm tissues were dissected, there was no browning in any inoculated plants, and the appearance of the corm was similar to that of the control treatment.

4. Discussion and Conclusions

Little is known about the endophytes of banana plants. This study isolated bacterial endophyte *L.*

Table 4 - Effect of inoculation of *Luteibacter yeojuensis* SbM36C, GN11-20, SbM36A strains on in-vitro shoot and root production of

Genotype	Treatment	Plant height (cm)		Number of shoots emerged		Number of roots formed	
		Mean±SD	P-value	Mean±SD	P-value	Mean±SD	P-value
Grand Nain	SbM36C	2.7 ± 0.5	0.7855 NS	4.3± 2.5 ab	0.0278 *	2.5 ± 1.9	0.4148 NS
	GN11-20	2.4 ± 0.6		7.3 ± 0.5 a		2.5 ± 1.3	
	SbM36A	2.4 ± 0.4		3.0 ± 2.7 b		4.3 ± 1.7	
	Control	2.7 ± 0.7		2.5 ± 1.7 b		2.5 ± 1.9	
Lakatan	SbM36C	2.3 ± 0.6 b	0.0224 *	5.0 ± 3.4	0.6799 NS	2.0 ± 0.8	0.0659 NS
	GN11-20	3.2 ± 0.2 a		3.8 ± 1.0		5.0 ± 0.8	
	SbM36A	3.5 ± 0.3 a		3.5 ± 0.6		2.5 ± 1.3	
	Control	2.9 ± 0.7 ab		5.0 ± 2.7		3.8 ± 2.5	
Saba	SbM36C	2.9 ± 0.5	0.5947 NS	4.3 ± 0.5	0.0851 NS	2.8 ± 1.7	0.2367 NS
	GN11-20	2.4 ± 0.3		2.5 ± 1.7		2.5 ± 1.3	
	SbM36A	2.6 ± 0.8		4.3 ± 0.5		2.0 ± 1.2	
	Control	2.5 ± 0.6		2.8 ± 1.3		1.3 ± 0.5	

Plantlets inoculated with water served as control. Asterisks (*) indicate significant differences between the treatments and the corresponding control by Least Significant Difference (LSD) test at α= 0.05;
NS= not significant. Different letters in the mean values for each genotype indicate significant differences between the treatments.

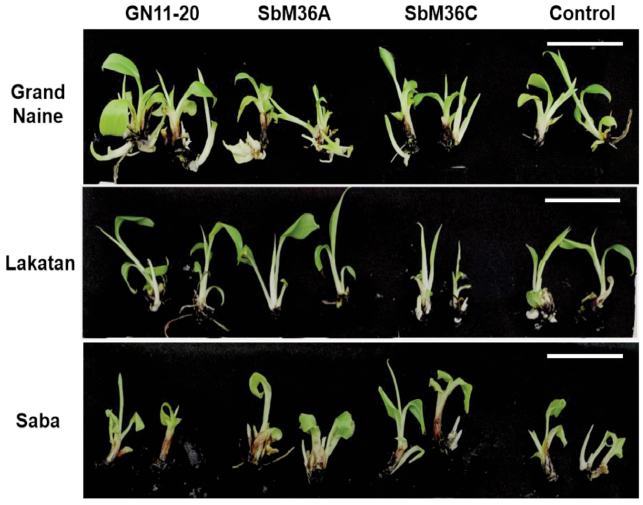

Fig. 4 - Pathogenicity of *Luteibacter yeojuensis* SbM36C, GN11-20, and SbM36A strains isolated in this study at 14 days post-inoculation (dpi). Data shows two plantlets for each treatment. Control plantlets (treated with sterile distilled water) were also shown (d). The bar at the upper left represents 2 cm.

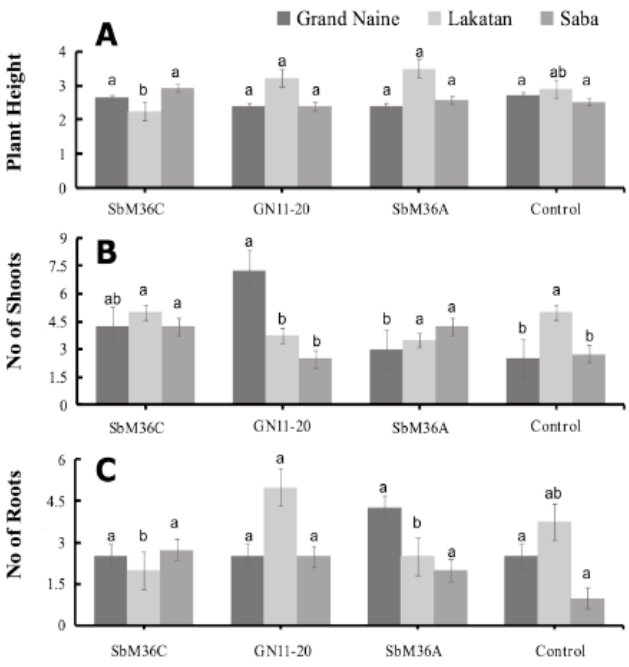

Fig. 5 - Morphometric characteristics of tissue-cultured banana plantlets inoculated with *Luteibacter yeojuensis* SbM36A, GN11-20, and SbM36C strains isolated in this study. Plantlets inoculated with sterile distilled water served as control. The plant height (a), number of shoots that emerged (b), and number of roots formed (c) of the three genotypes were collected 14 days post-inoculation. Different letters in each bar indicate significant differences ($p<0.05$).

yeojuensis isolates SbM36C, GN11-20, and SbM36A from healthy tissue-cultured banana cultivars Saba and Grand Nain. While the colony characteristics (size and pigmentation) of the three bacterial isolates varied among the isolates, all three isolates belonged to the same species, as confirmed by the 16S rDNA

sequence analyses. Bacterial species under the order Xanthomonadales, such as *Luteibacter sp.,* are gram-negative, aerobic, and carotenoid-producing species that provide the yellow-orange-red colour of the cultures (Saddler and Bradbury, 2005). The carotenoid pigments in Xanthomonadales are lipid-soluble and play a significant role in culture survival under low-temperature conditions and against UV radiation (Azman *et al.,* 2018). The bacterial isolates from this study were obtained from different sources of banana genotypes: GN11-20 was isolated from 'Grand Nain,' SbM36C and SbM36A were isolated from 'Saba,' respectively. These genotypes were of different ages from the time of isolation of the endophytes. Hence, they had a potentially varying exposure to temperature and UV radiation. This genotypic and environmental variation might have affected the levels of carotenoids found in the endophytes isolated in this study, thereby affecting pigmentation despite belonging to the same species (Dieser *et al.,* 2010). This study also highlights the importance of molecular assays, analyzing the 16S rDNA sequences, in identifying species when phenotypes of the bacteria are influenced by their response to the environment.

None of the *L. yeojuensis* isolates from this study resulted in infection in tissue-cultured banana 'Lakatan,' 'Grand Nain,' and 'Saba.' However, there were recorded differences in the morphometric characteristics of the three banana cultivars as influenced by the inoculation of three endophytes. Both *L. yeojuensis* GN11-20 and *L. yeojuensis* SbM36A have growth-promoting properties in tissue-cultured banana plants at 14 days post-inoculation; this positively affects plants and may increase if treatments are extended for a longer incubation time (e.g., a month). The study supports the hypothesis that bacterial endophytes from healthy plants benefit their host plant. Nevertheless, not all endophytes within the same species have plant growth-promoting properties, as demonstrated by *L. yeojuensis* SbM36C.

Two isolates - *L. yeojuensis* SbM36A and *L. yeojuensis* GN11-20 - significantly enhanced plant height in 'Lakatan'. The *L. yeojuensis* GN11-20 further improved shoot emergence in 'Grand Nain' plants. These results suggest a symbiotic relationship between the two *Luteibacter yeojuensis* isolates and bananas. On the other hand, *L. yeojuensis* SbM36C did not significantly affect the growth of banana plants regarding plant height, number of shoots, and roots. Several factors may have affected the performance of the three bacterial isolates or the endophytes' successful colonization in the plant host. These factors include plant genotype and tissue type (Hardoim *et al.*, 2015). The longevity of exposure to the endophyte could also have an effect. Extending the inoculation period from 2 weeks to 4 weeks might increase the plant growth-promoting activity of the endophyte. None of the *L. yeojuensis* isolates increased the number of roots in the three genotypes tested. Previous reports have recorded the potential of *Luteibacter rhizovicinus* for enhanced root development in barley, specifically leading to higher weight and length of the roots (Guglielmetti *et al.*, 2013). Hence, investigating the potential of the endophytes isolated for root development might also lead to a further understanding their plant growth-promoting activities.

The improved plant height and shoot production by *L. yeojuensis* strains isolated in this study might be stimulated by the increased IAA synthesis (Pieterse *et al.*, 2009). *Luteibacter sp.,* as an endohyphal bacterium (endophyte that forms a symbiotic relationship with a fungus) of *Platycladus orientalis,* increased indole-acetic acid (IAA) production, resulting in significantly higher seedling and root length (Hoffman *et al.,* 2013). The antagonistic property of *L. yeojuensis* to plant pathogens has also been associated with high indolic compound production by *L. yeojuensis.* However, further investigation is needed since endophytic bacteria can utilize several other mechanisms (e.g., phosphate solubilization, gelatinase, and chitinase production) for plant growth improvement (Liu *et al.,* 2017; Tang *et al.,* 2020).

Several *Luteibacter* species are endophytes of economically important crops such as rice (Raj *et al.,* 2019), and apple (Piagnini *et al.,* 2007) acting either as plant-growth promoters or biocontrol agents. In bananas, *Luteibacter sp.* has been previously detected as an endophyte of 'Gros Michel' (Köberl *et al.,* 2015) and was later found to have an antagonistic relationship with *Fusarium oxysporum* f. sp. *cubense* TR4 (Foc TR4) that causes severe wilt disease in bananas (Köberl *et al.,* 2017; Nakkeeran *et al.,* 2021). This study isolated and identified three strains of *Luteibacter* species, specifically *L. yeojuensis,* from healthy banana plants. This is the first confirmed report of *L. yeojuensis* as an endophyte of banana 'Lakatan', 'Grand Nain', and 'Saba'. The cultivar Grand Nain, a Cavendish

banana cultivar group member, is susceptible to Foc TR4. Hence, it would be worthwhile to test the bioactivity of the three *L. yeojuensis* strains in this study to Foc TR4 strains found in the country.

This study reports three *Luteibacter yeojuensis* strains as banana endophytes for the first time. It further demonstrates the growth-promoting potential of two *L. yeojuensis* strains in three tissue-cultured banana plants 'Grand Nain', 'Saba', and 'Lakatan'. The bacterial strains could be used to develop bioinoculants to improve plant growth in the future. However, its effect on other banana cultivars not used in this study should be further explored. Investigating the role of isolated endophytes as potential biological agents of diseases in bananas would be worthwhile research.

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Preliminary evaluation of nematode community responses to ground covers in jute leaf cultivation

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Abstract: Jute leaf (*Corchorus olitorius*) is an emerging ethnic crop in the Mid-Atlantic United States. No information is available on nematode associations and nematode community responses to jute leaf grown with ground cover. We conducted a preliminary field study in the summer of 2023 in Beltsville, Maryland to evaluate the responses of endemic nematode communities to three cultivars of jute leaf (Firebird, Molokhia, and USDA PI 404029) and four ground cover treatments (compost, compost and landscape fabric, compost and straw mulch, and compost and wood mulch). We extracted nematodes from soil samples collected before planting, at midseason, and at harvest. By the end of the season, plots with straw had higher counts of *Prismatolaimus, Mononchus* and total plant-parasites and plots with wood chips had higher counts of *Helicotylenchus*. Structure index and maturity index 2-5 were also higher in plots with straw at the end of the season. Cultivar had a relatively small impact on the nematode community with USDA PI 404029 plots having the highest *Plectus* counts and Firebird plots having the highest predator counts at midseason only. The channel and enrichment indexes showed a shift occurred with all four treatments: the soil environment became dominated by bacterial decomposition pathways with nutrient enriched conditions. However, plant biomass was not different between treatments. These results suggest ground covers can influence soil nematode communities in jute leaf production.

1. Introduction

Ethnic foods are increasingly in demand in the United States (US). Metropolitan areas such as Washington, DC are home to growing populations of ethnically diverse residents with a broad range of dietary preferences (Mangan *et al.*, 2008; Govindasamy *et al.*, 2022). Many

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tropical crops from Africa and Asia can be grown as annuals in the summer months in the mid-Atlantic US (Mangan *et al.*, 2010). Local production of ethnic crops on small-scale farms can increase accessibility of products that might otherwise be difficult to obtain at high quality due to long-distance transport (Trobe, 2001). Jute leaf (*Corchorus olitorius*) is an important crop in tropical countries in Africa and Asia and is in demand in the eastern US (Govindasamy *et al.,* 2007). It is primarily grown as a leafy vegetable and the leaves are often used to add flavor and thicken soups and stews (Islam, 2013). Roots can be used for medicinal purposes and stems can also be used for fiber production (Islam *et al*., 2013; Nyadanu *et al*., 2017).

A management practice useful for crop production is using ground covers and mulching. Physical barriers laid on the soil surface can create an impediment for weed development and aids in conserving moisture and regulating soil temperature (Flint, 2012; Richardson *et al*., 2023, 2024). Sustainable ground covers derived from organic materials used to suppress weeds can contribute to higher soil moisture content and higher yields of vegetables in the northeastern US (Gheshm and Brown, 2018; Larkin, 2020; Richardson *et al*., 2023). Urban centers produce large quantities of organic waste, some of which can be repurposed as compost or ground covers for agricultural use (Arcas-Pilz *et al.,* 2023). Wastes such as biosolids, yard waste, and food waste can provide nutrients to crops as compost, ground covers, or amendments (Wang *et al.,* 2008; Splawski *et al.,* 2016; Shrestha *et al*., 2020).

Ground cover applications can have cascading effects on the soil ecosystem. Nematodes are microscopic worms that are ubiquitous in soil environments and are sensitive to additions of organic matter-based mulches and amendments (McSorley and Gallaher, 1996; Forge and Kempler, 2009; Waldo *et al.,* 2024). Plant-parasitic nematodes feed on plant roots and can negatively affect plant health and reduce yields in leafy vegetable crops, including jute leaf (Atungwu *et al.,* 2013; Mbogoh *et al.,* 2013; Kimaru *et al.,* 2014). Yield of jute leaf in Nigeria and India has been reduced by 52% and 68%, respectively, due to feeding injury from *Meloidogyne incognita* (Saikia and Phukan, 1986; Adepoju and Oluwatayo, 2016). Organic amendments can contribute to suppression of undesirable plant-parasitic nematodes by introducing and enhancing natural antagonistic organisms including predatory/parasitic fungi, collembola, tardigrades, mites and protozoa as well as releasing lethal compounds such as ammonia and organic acids that are byproducts of decomposition (Akhtar and Malik, 2000; Thoden *et al*., 2011; Timper, 2014; Rosskopf *et al.,* 2020). Some microorganisms such as *Bacillus* spp., *Pastueria* spp., *Pochonia* spp, and *Trichoderma* spp. have shown promise at reducing numbers of plant-parasitic nematodes and have been further studied as potential biocontrol agents of plant-parasitic nematodes in cropping systems (Meyer and Roberts, 2002; Pires *et al.,* 2022). Other nematode groups feed on a range of soil microflora and microfauna and can have positive effects on soil health (Yeates *et al.,* 1993; Neher, 2001). Bacterivore nematodes rapidly increase following additions of organic matter in response to bacterial blooms (Ferris and Bongers, 2006). Bacterivores contribute to nutrient cycling by culling bacteria, which releases carbon (C) and nitrogen (N) back into the soil that may otherwise be respired or immobilized during periods of high microbial activity (Akhtar and Malik, 2000; Wang and McSorley, 2005). Omnivorous and predatory nematodes also play important roles in soil ecosystems as biological indicators of food web structure. Increases in predatory nematode abundance are common in response to applications of organic materials, which is desirable in agricultural systems (Forge *et al.,* 2003; Oka, 2010; McSorley, 2011). Predatory nematodes consuming plant-parasitic nematodes and opportunistic nematodes can act as a check on unregulated population growth that could otherwise occur under nutrient enriched conditions (Bongers and Bongers, 1998; Ferris, 2010).

As farmers look for opportunities to diversify their crop production and reach underserved ethnic markets, they need an understanding of best practices for cultivating ethnic crops. However, it is unknown how ground covers influence yield of jute leaf and associated nematode communities in the Mid-Atlantic US. Information on impacts of ground covers on the nematode community in Maryland may help identify the structure of the soil food web and potential risks of plant-parasitic nematodes to jute leaf. To fill this gap in knowledge, we conducted a preliminary investigation to ascertain how ground covers influenced yield of jute leaf and the nematode community. We used four ground cover treatments (compost, compost and landscape fabric, compost and straw, and compost and wood mulch) to grow three cultivars of jute leaf (Firebird, Molokhia, and

USDA PI 404029) in Maryland.

2. Materials and Methods

Study site

We established experimental plots in May 2023 at the University of the District of Columbia's (UDC) Firebird Research Farm (39°3'11.1492 N, 76°52'52.716 W). The soil was classified as a Russett-Christiana complex, with a fine loamy, mixed, semiactive, mesic Aquic Hapludults for the Russett series, and a fine, kaolinitic, mesic Aquic Hapludults as part of the Christiana series. Soil texture was 39% sand, 36% silt, and 25% clay. Plots were solarized prior to planting to kill weeds. Plots were hand weeded as necessary during the study and no pesticides or fertilizers were applied.

Treatments

We used four ground cover treatments and three jute leaf cultivars in the study. Ground cover treatments were applied to 0.9 m by 9 m plots and were arranged as a randomized complete block design in a 4×3 factorial with four replicates (Supplementary materials - SM - Figs. 1S - 2S). Each ground cover plot was subdivided into three 0.9 m by 3 m subplots, with each subplot randomly being assigned one of the three cultivars. Fifteen centimeters of mushroom compost was applied on top of the ground of the entire study area with a C:N ratio of 9:1. The four ground cover treatments chosen were: mushroom compost only (compost), landscape fabric (fabric), straw, or wood chips. Each treatment was placed on top of the 15 cm of mushroom compost by the beginning of June (5 June 2023). Mushroom compost and straw were purchased from Purple Mountain Organics (Takoma Park, MD). Landscape fabric used in the study was Sunbelt Black Ground Cover 3.2oz (DeWitt, Sikeston, MO). Wood chip mulch was produced in 2019 from a mixture of local softwood and hardwood trees felled from Firebird Farm and chipped for use as mulch. A compost sample was sent to Waypoint Analytical Inc. (Leola, PA) to determine C:N content (Peters *et al.,* 2003). The three jute leaf cultivars included Firebird (developed at UDC), Molokhia (Egyptian spinach) (Kitazawa Seed Co, Oakland, CA), and USDA PI 404029 (USDA germplasm repository). Seeds were soaked overnight and planted in Fort Vee potting mix (Vermont Compost, Montpelier, VT) in 50 cell trays and grown under high tunnel conditions for 30 days prior to transplanting.

Soil sampling and data collection

Soil samples were collected after a soil solarization (pre-treatment) in early May (8 May 2023). We collected midseason and final samples on 10 August and 29 September, respectively. The experiment lasted a total of 144 days from soil pre-treatment to collection of final samples. We collected six 3 cm × 20 cm soil cores from the center of each plot. The six cores were combined and homogenized into a single composite sample per plot, placed in individual polyethylene sample bags, and then placed into a cooler for transportation to the lab where they were stored at 4.5°C. Soil cores were homogenized and nematodes were extracted from 100 cm^3 soil using centrifugal sugar floatation (Jenkins, 1964).

Nematodes were fixed in 2% formalin and the genera of a subset of 100 nematodes were identified from each sample using an inverted microscope (Zeiss, Oberkochen, Germany). Relative abundance was determined by multiplying the proportion of each genus in the sample by the total number of nematodes in the sample. Nematodes were categorized into functional groups, based on their diet, and colonizer-persister (cp) groups (Bongers, 1990; Yeates *et al.,* 1993). The cp numbers assigned to genera reflect life history traits. Numbers near one correspond with *r* strategists that are associated with nutrient enriched and disturbed ecosystems and numbers near five correspond to *K* strategists that are associated with stable ecosystems (Bongers, 1990; Yeates *et al.,* 1993; Ferris *et al.*, 2001). We calculated ecological indexes from nematode counts using the Nematode Indicator Joint Analysis (NINJA) online tool (Sieriebriennikov *et al*., 2014). We also measured fresh aboveground plant biomass at the end of the season by cutting plant stems 7.5 cm above the soil surface and measuring the mass of each plant.

Statistical analysis

We compared the effects of ground cover, cultivar, and the interaction of ground cover and cultivar on nematode abundance and index means using analysis of covariance (ANCOVA). Log_{10} transformations were performed on data prior to analysis to improve normality and homogeneity of variance. Mean relative abundance of nematodes from midseason and final sampling dates were each

analyzed individually using pre-treatment counts as a covariate. ANCOVA was selected to help account for seasonal variation by including pre-treatment counts as a covariate. Means of plant biomass across ground cover treatments and cultivars were compared using analysis of variance (ANOVA). Significant results ($P \leq 0.05$) were separated with Tukey's HSD. Significant differences presented at ≤0.05 occurred within an individual sampling date (midseason or final). Statistical analyses were conducted in R using 'Agricolae' package (R Core Team, 2019; de Mendiburu, 2021). Graphics were generated using ggplot2 (Wickham, 2016).

3. Results

We processed 144 soil samples during the study. Twenty-seven nematode genera were identified, with six classified as plant-parasitic nematodes, ten as bacterivores, five as fungivores, four as omnivores, and two as predators (Table 1). Bacterivores were

Table 1 - Nematode genera and proportion of all 144 soil samples that contain each genus from the study in Beltsville, MD

Functional group [z]	Genus	cp or pp value [y]	Proportion of samples genus was identified
Plant-parasite			
	Criconemella	3	<0.01
	Helicotylenchus	3	0.94
	Heterodera	3	<0.01
	Hoplolaimus	3	0.03
	Paratylenchus	2	0.28
	Pratylenchus	3	0.04
Bacterivore			
	Acrobeles	2	0.03
	Alaimus	4	0.15
	Butlerius	1	0.45
	Cephalobus	1	0.44
	Diploscapter	1	0.10
	Eucephalobus	1	0.80
	Panagrolaimus	1	0.03
	Plectus	2	0.26
	Prismatolaimus	3	0.60
	Rhabditis	1	1.00
Fungivore			
	Aphelenchoides	2	0.22
	Aphelenchus	2	0.79
	Diphtherophora	3	0.05
	Ditylenchus	2	0.44
	Tylenchus	2	1.00
Omnivore			
	Aporcelaimus	5	0.04
	Dorylaimoides	4	<0.01
	Eudorylaimus	4	<0.01
	Prodorylaimus	4	0.02
Predator			
	Ironus	4	0.10
	Mononchus	4	0.27

[z] Functional groups assigned to genera based on nematode genus primary dietary preference (Yeates *et al*., 1993).
[y] Colonizer-persister (cp) or plant-parasite (pp) number according to (Bongers, 1990). Cp and pp numbers near one correspond with *r* strategists that are associated with nutrient enriched and disturbed ecosystems and numbers near five correspond to *K* strategists that are associated with stable ecosystems (Ferris *et al.*, 2001).

the most abundant functional group followed by fungivores (SM Tables 1S, 2S). The most prominent genera were *Rhabditis* (58% of total nematodes) and *Tylenchus* (25% of total nematodes). *Helicotylenchus* was the most abundant plant-parasitic nematode genus, representing 93% of all plant-parasitic nematodes. *Paratylenchus* was the second most abundant genus, representing 6% of the total plant-parasitic nematodes.

Ground cover effects

Nematode counts and ecological indexes were not different among ground cover treatments at midseason sampling, but were during the final sampling period (Figs. 1, 2). Total plant-parasitic nematodes numbers were more than two times as abundant in straw plots than compost only plots (*P*= 0.02). Plots with wood chips had greater *Helicotylenchus* abundance than plots with compost only (*P*= 0.03). Straw plots had the highest counts of *Prismatolaimus* (*P*= 0.02) and cp 3 nematodes (*P*= 0.02) compared to fabric plots as well as the highest counts of *Mononchus* (*P*= 0.03), total predators (*P*= 0.03), and cp 4 nematodes (*P*= 0.04) compared to wood chip plots. Whereas, the range of count means for *Rhabditis* greatly increased from pre-treatment samples (55-150) to midseason samples (671-1044) (*P*= 0.7) and final samples (682-1004) (*P*= 0.6), no differences occurred among treatments (SM Table 1 S).

Maturity index 2-5 (*P*= 0.03) and structure index (*P*= 0.05) were also greater in plots with straw compared to those with fabric at the final sampling date (Fig. 2). Across all treatments at the end of the season, the range of enrichment index means were near the upper limit of possible values at the end of the study (87-91) (*P*= 0.4) and the range of channel index means were approaching the lowest limit of possible values at the end of the study (9-15) (*P*= 0.6), but no differences occurred among treatments for either index (Fig. 2). Mean biomass for the four ground covers ranged 1.39-1.69 kg, but was not statistically different among ground cover treatments (*P*= 0.4).

Effects of plant cultivars

Plectus and the predator functional group differed across cultivars (Fig. 3). *Plectus* counts at midseason were higher in plots with USDA PI 404029 than Molokhia or Firebird (*P*= 0.01). Predators were more numerous at midseason in Firebird plots than

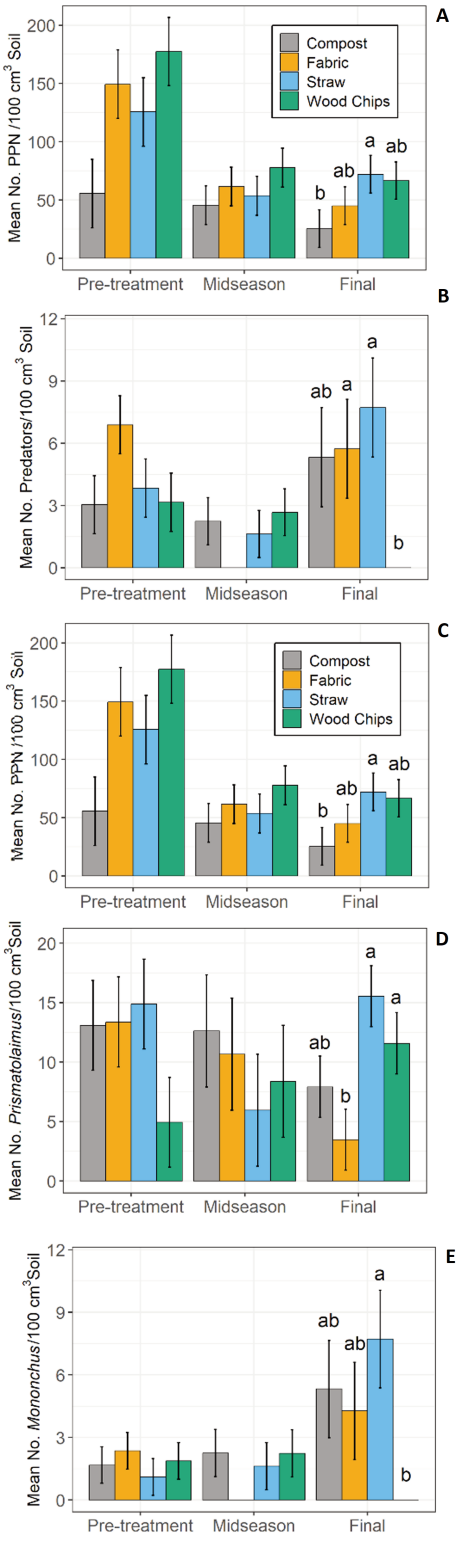

Fig. 1 - Mean number of nematodes categorized by functional group or genus, with standard errors of the mean, across three sampling periods (pre-treatment, midseason, and final) and four ground cover treatments: compost only; compost+fabric; compost+straw; compost+wood chips. Different letters indicate differences between treatments within a sampling date (Tukey HSD, P≤0.05). PPN = plant-parasitic nematodes.

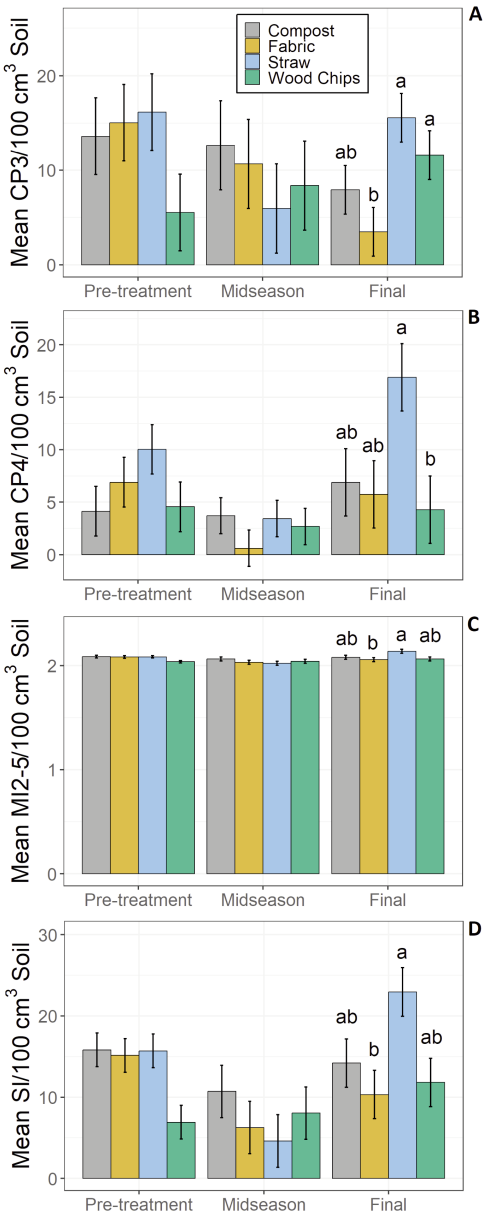

Fig. 2 - Mean number of nematodes that were categorized as colonizer persisters (cp) or ecological indexes. Means, with standard errors of the mean, were presented across three sampling dates (pre-treatment, midseason, and final) and four ground cover treatments: compost only; compost+fabric; compost+straw; compost+wood chips. Different letters indicate differences between treatments within a sampling date (Tukey HSD, P ≤ 0.05). cp 3 = colonizer-persister group 3; cp 4 = colonizer-persister group 4; MI2-5 = maturity index colonizer-persister groups 2-5; SI = structure index. Colonizer-persister groups are based on life history traits with values approaching one representing *r* selection strategists and values approaching five representing *K* selection strategists. MI2-5 is a measure of environmental disturbance with values approaching zero indicating high disturbance and values approaching five indicating low disturbance. SI is a measure of food web complexity with values approaching zero indicating low food web complexity and values approaching 100 indicating high food web complexity.

Molokhia and USDA PI 404029 (*P*= 0.02). No ecological indexes differed across cultivars (*P*>0.05) (SM Table 2S). Mean biomass ranged 1.42-1.72 kg, but the effect of cultivar was not significant (*P*= 0.2).

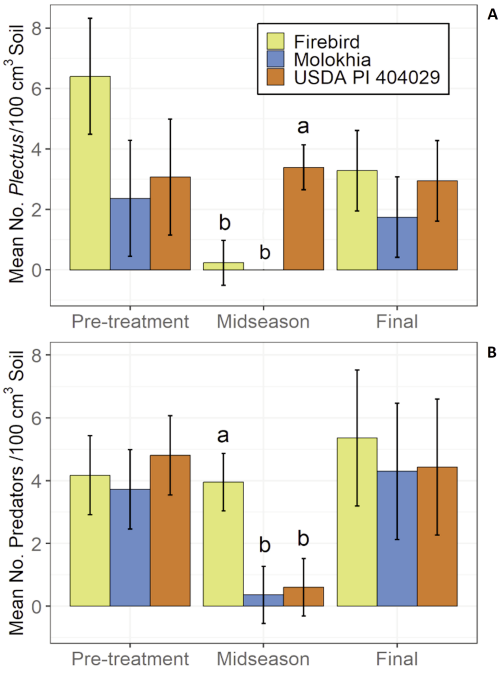

Fig. 3 - Mean relative abundance of nematode Plectus (A) and predatory nematodes (B), with standard errors of the mean, across three sampling periods (pre-treatment, midseason, and final) and three cultivars of jute leaf: Firebird; Molokhia; and USDA PI 404029. Different letters indicate differences between treatments within a sampling date (Tukey HSD, P≤0.05).

4. Discussion and Conclusions

In this study we demonstrate how ground cover treatments on jute leaf, has a direct impact on the nematode community. No single ground cover enhanced abundance of all free-living nematodes, but nematode abundance differed across the treatments. Plots with straw had higher counts of the free-living bacterivore *Prismatolaimus* and predator *Mononchus* by the end of the season than the others treatment. Higher counts of *Prismatolaimus* contributed to the increase of cp 3 nematodes and structure index in straw plots at the end of the study. Differences in abundance of *Mononchus* largely contributed to increases in cp 4 nematodes, predators, and maturity index 2-5 in straw plots at the end of the study. Organic mulch applications can stimulate increases of bacterivore and predatory nematode abundances (Ferris and Bongers, 2006;

Wang *et al.,* 2008; Pavao-Zuckerman and Sookhdeo, 2017). *Prismatolaimus* and *Mononchus* are indicators of preliminary structure development and become more common as enriched conditions transition to more stable conditions (Ferris *et al.,* 2001). Use of amendments with high carbon content such as straw can result in greater free-living nematode abundance compared to using nitrogen rich sources such as manures (Liu *et al.,* 2016). Soil amended with products that include straw can have positive effects on physical properties of soil that are favorable for nematodes such as increased moisture retention and enhanced soil porosity as well as providing high levels of carbon that stimulates growth of microbial food sources (Zhao *et al.,* 2009). Elevated nutrient levels occurring following applications of straw mulch can lead to an increase of *Prismatolaimus* abundance and omnivore-predator metabolic activity (Song *et al.,* 2020). Predator abundance has been shown to increase following application of compost mixtures with straw and leaf litter mulches in barley and tomato production systems (Renčo *et al.,* 2010; Petrikovszki *et al.,* 2021).

Helicotylenchus was the dominant plant-parasitic nematode in our study in line with what already presented in natural environment by other studies (Babatola, 1983; Atungwu *et al.,* 2013). Abundance of *Helicotylenchus* numerically declined across all ground cover treatments from pre-treatment to midseason, and remained low at final sampling. Compost only plots had lower abundance of *Helicotylenchus* than straw and lower total plant-parasitic nematode abundance than wood chips at the end of the season. Materials with high C:N content such as straw or wood chips can be less effective at suppressing plant-parasitic nematodes than low C:N content mulches like compost or manure (Liu *et al.,* 2016; Hornung *et al.,* 2020). Low C:N content materials may better facilitate growth of nematode antagonists or make soil conditions less favorable by altering pH through soil acidification, though these mechanisms need further evaluation (Liu *et al.,* 2016; Ye *et al.,* 2018; Martinez *et al.,* 2023). Despite the differences in plant-parasite abundance, no differences occurred in plant yield. Higher *Helicotylenchus* abundance in plots with wood chips was accompanied by zero presence of predatory nematodes. The lack of predatory nematodes may have resulted in less top-down regulatory pressure on *Helicotylenchus* reproduction in plots with wood mulch compared to other treatments.

Enriched conditions dominated by bacterial composition with low community structure were observed in this study, which are commonly associated with addition of organic amendments (Thoden *et al.*, 2011). Bacterial feeders in the genus *Rhabditis* greatly increased following applications of all ground cover treatments. The enrichment and channel indexes were strongly influenced by the drastic change in *Rhabditis* abundance. *Rhabditis* nematodes are categorized as cp 1 bacterial feeding nematodes which are opportunistic and respond quickly to nutrient enriched conditions (Bongers and Bongers, 1998; Ferris and Bongers, 2006). Flushes of bacterial growth in response to compost applications provides abundant resources for *r*-strategists like *Rhabditis* which often drives rapid population growth (Bulluck *et al.*, 2002; Ferris and Bongers, 2006; Fengjuan *et al.,* 2020).

In our study, densities of *Plectus* were greatest in USDA PI 404029 plots and predator densities were greatest in Firebird plots at midseason sampling. The counts were very low among the three cultivars at midseason and impacts were temporary and did not last to the end of the season. Plant species diversity can impact nematode abundance and diversity (Yeates 1999; Porazinska *et al.,* 2003; de Deyn *et al.,* 2004). For example, bacterivore abundance generally increases as plant species diversity increases, but responses of predatory nematodes can be more variable and take place more gradually (Viketoft *et al.,* 2011; Kostenko *et al.,* 2015; Cortois *et al.,* 2017; Dietrich *et al.,* 2021). Impacts of intraspecific plant diversity on functional groups of soil invertebrates are less well studied, but increases of plant diversity can influence soil communities, particularly at lower trophic levels (Koricheva and Hayes, 2018; Yan *et al.,* 2021). For example, omnivore-predator abundance varied between individual genotypes of *Phragmites australis*, whereas bacterivore nematode abundance was affected by the overall genetic diversity of *P. australis* genotypes (Yan *et al.,* 2021). The mechanism of how plant genetic diversity impacts free-living nematodes is not clearly understood. Resource quantity (plant biomass) and root quality (C:N ratio) may play a role in influencing nematode communities, especially where levels of organic matter in soil are low (Bezemer *et al.,* 2010; Cortois *et al.,* 2017; Dietrich *et al.,* 2021). Secondary compounds produced by roots may also influence microbial communities and could also shape

nematode communities from the bottom up (Bezemer *et al.,* 2010). Cultivar effect in our study could have been more pronounced earlier in the season, but likely had a lesser overall impact on the nematode community. Effects from organic matter decomposition from mulches may have been limited earlier in the season, but became more pronounced as nematode populations may have responded to changes in the availability of resources.

Our findings indicate that ground covers derived from organic materials and urban wastes can influence nematode communities in plots of jute leaf in Maryland. Our study was limited to a single year because of insufficient funding and capacity to continue for additional years. Future research incorporating multi-season trials in a broader geographic area could validate these findings and help growers select a ground cover that promotes nematode communities beneficial to soil health. Information is also needed on ecological impacts of producing other ethnic crops on the soil community in the Mid-Atlantic US.

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Supplementary materials

Preliminary evaluation of nematode community responses to ground covers in jute leaf cultivation

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Key words: Compost, ethnic crop, landscape fabric, mulch, straw, wood chips.

Abstract: Jute leaf (*Corchorus olitorius*) is an emerging ethnic crop in the Mid-Atlantic United States. No information is available on nematode associations and nematode community responses to jute leaf grown with ground cover. We conducted a preliminary field study in the summer of 2023 in Beltsville, Maryland to evaluate the responses of endemic nematode communities to three cultivars of jute leaf (Firebird, Molokhia, and USDA PI 404029) and four ground cover treatments (compost, compost and landscape fabric, compost and straw mulch, and compost and wood mulch). We extracted nematodes from soil samples collected before planting, at midseason, and at harvest. By the end of the season, plots with straw had higher counts of *Prismatolaimus, Mononchus* and total plant-parasites and plots with wood chips had higher counts of *Helicotylenchus*. Structure index and maturity index 2-5 were also higher in plots with straw at the end of the season. Cultivar had a relatively small impact on the nematode community with USDA PI 404029 plots having the highest *Plectus* counts and Firebird plots having the highest predator counts at midseason only. The channel and enrichment indexes showed a shift occurred with all four treatments: the soil environment became dominated by bacterial decomposition pathways with nutrient enriched conditions. However, plant biomass was not different between treatments. These results suggest ground covers can influence soil nematode communities in jute leaf production.

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Ground cover treatments
Wood chips + compost
Fabric + compost
Straw + compost
Compost only

Cultivars
A = Firebird
B = Molokhia
C = USDA PI 404029

C	A	A	C
B	B	C	B
A	C	B	A
C	C	B	A
A	B	C	B
B	A	A	C
A	B	B	A
B	A	C	C
C	C	A	B
C	B	B	B
A	A	C	C
B	C	A	A

Fig. 1S - Treatment layout in experimental plots. Each ground cover treatment plot was 8.1 m^2 and each cultivar plot was 2.7 m^2.

Fig. 2S - Experimental site and layout of plots planted with jute leaf.

Table 1S - Means (±standard error of the mean) of nematode relative abundance and ecological indexes across four ground cover treatments and three sampling periods in a jute leaf experiment in Beltsville, MD, USA. Genera found in less than 1% of samples were omitted from the table

Nematode group/ecological index	Sampling period											
	Pre-plant treatment				Midseason treatment				Final treatment			
	C†	F	S	W	C	F	S	W	C	F	S	W
Plant-parasites	56 (±9)	149 (±40)	126 (±26)	177 (±33)	46 (±10)	62 (±23)	54 (±13)	78 (±17)	25 (±10)	45 (±14)	72 (±18)	67 (±21)
Helicotylenchus	55 (±9)	142 (±41)	120 (±27)	172 (±33)	42 (±9)	59 (±23)	51 (±13)	77 (±17)	24 (±10)	43 (±15)	44 (±14)	55 (±15)
Hoplolaimus	0 (±0)	0 (±(±0)	1 (±(±0.4)	0 (±0)	1 (±0.7)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	3 (±3.3)
Paratylenchus	1 (±0.4)	7 (±1.9)	4 (±1.8)	5 (±1.8)	2 (±1.5)	2 (±1.7)	3 (±1.5)	0 (±0.4)	1 (±1.2)	2 (±1.3)	26 (±15)	5 (±3.3)
Pratylenchus	0 (±0)	0 (±0)	1 (±0.5)	0 (±0.5)	0 (±0)	0 (±0)	0 (±0)	0 (±0.5)	0 (±0)	0 (±0)	2 (±1.6)	3 (±3.3)
Bacterivores	117 (±18)	206 (±39)	156 (±40)	172 (±30)	732 (±144)	1085 (±191)	962 (±235)	915 (±157)	737 (±107)	1052 (±193)	1066 (±333)	1043 (±158)
Acrobeles	1 (±0.5)	0 (±0)	1 (±1.1)	2 (±1.6)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)
Alaimus	1 (±0.8)	0 (±0)	6 (±3.8)	1 (±0.7)	1 (±1.2)	1 (±0.6)	2 (±1.8)	0 (±0)	2 (±1.1)	0 (±0)	9 (±5.1)	4 (±2.2)
Butlerius	0 (±0)	0 (±0)	0 (±0)	0 (±0)	17 (±5.1)	15 (±7.2)	18 (±4.7)	29 (±9.2)	19 (±5)	14 (±4.8)	17 (±5.8)	18 (±6.1)
Cephalobus	12 (±3.4)	7 (±1.7)	12 (±4.1)	5 (±2.8)	8 (±3.1)	1 (±0.8)	9 (±4)	3 (±1.6)	8 (±3.6)	10 (±8.9)	3 (±1.1)	6 (±3.3)
Diploscapter	0 (±0.3)	0 (±0)	0 (±0.4)	0 (±0)	0 (±0)	1 (±1.4)	2 (±1.9)	0 (±0)	1 (±0.9)	4 (±2.9)	6 (±3.3)	1 (±1.2)
Eucephalobus	30 (±5.3)	31 (±10)	22 (±6.4)	19 (±3.2)	20 (±4.6)	10 (±2.5)	13 (±3.8)	29 (±9.5)	15 (±4.2)	14 (±4.3)	22 (±5.8)	12 (±4.7)
Panagrolaimus	0 (±0)	0 (±0)	0 (±0)	0 (±0)	1 (±1.2)	1 (±0.6)	2 (±1.8)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)
Plectus	4 (±3)	5 (±1.9)	2 (±0.8)	5 (±2.7)	2 (±1)	2 (±1.5)	1 (±0.6)	0 (±0.4)	2 (±1.4)	1 (±1)	3 (±1.6)	4 (±2)
Prismatolaimus	13 (±3.1)	13 (±4.5)	15 (±4.6)	5 (±2.5)	13 (±7)	11 (±5.2)	6 (±2.5)	8 (±2.6)	8 (±1.8)	3 (±1.4)	16 (±3.7)	12 (±2.8)
Rhabditis	55 (±10)	150 (±37)	98 (±30)	134 (±24)	671 (±145)	1044 (±191)	911 (±241)	845 (±150)	682 (±111)	1004 (±187)	991 (±334)	986 (±162)
Fungivores	264 (±36)	380 (±33)	333 (±50)	389 (±46)	252 (±34)	318 (±34)	345 (±49)	338 (±33)	231 (±32)	260 (±37)	316 (±31)	305 (±61)
Aphelenchoides	4 (±1.5)	5 (±2.7)	4 (±1.6)	2 (±0.9)	0 (±0)	1 (±0.7)	1 (±1)	7 (±3.8)	0 (±0)	6 (±4.5)	1 (±0.8)	5 (±4.2)
Aphelenchus	15 (±3.5)	22 (±5.4)	17 (±5.3)	34 (±10.4)	17 (±5)	16 (±3.1)	21 (±6.6)	31 (±9)	16 (±7.4)	22 (±8)	7 (±2.7)	33 (±12)
Diphtherophora	1 (±0.4)	2 (±1.2)	1 (±1)	1 (±0.6)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)
Ditylenchus	0 (±0)	0 (±0)	0 (±0)	0 (±0)	37 (±16)	49 (±12)	31 (±9)	20 (±10)	23 (±9.2)	10 (±5.4)	35 (±13)	15 (±5.6)
Tylenchus	244 (±34)	350 (±30)	311 (±48)	352 (±42)	198 (±25)	253 (±36)	293 (±42)	280 (±27)	192 (±25)	222 (±27)	272 (±34)	252 (±58)
Omnivores	1 (±0.9)	4 (±2.5)	1 (±0.5)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	1 (±0.7)	0 (±0)
Aporcelaimus	1 (±0.9)	4 (±2.5)	0 (±0.4)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	1 (±0.7)	0 (±0)
Dorylaimoides	0 (±0)	0 (±0)	0 (±0)	1 (±0.9)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)
Prodorylaimus	0 (±0.1)	0 (±0)	0 (±0.2)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	1 (±1.5)	0 (±0)	0 (±0)
Predators	3 (±1)	7 (±2)	4 (±1.3)	3 (±1)	2 (±1.3)	0 (±0)	2 (±1.2)	3 (±1.4)	5 (±3.5)	6 (±2)	8 (±2.6)	0 (±0)
Ironus	1 (±0.6)	5 (±1.7)	2 (±1.2)	0 (±0.4)	0 (±0)	0 (±0)	0 (±0)	0 (±0.4)	0 (±0)	0 (±0)	0 (±0)	0 (±0)
Mononchus	2 (±0.7)	2 (±1.1)	1 (±0.9)	2 (±0.8)	2 (±1.3)	0 (±0)	2 (±1.2)	2 (±1.4)	5 (±3.5)	4 (±1.7)	8 (±2.6)	0 (±0)
cp 1	55 (±10)	150 (±37)	99 (±30)	134 (±24)	689 (±146)	1061 (±192)	932 (±239)	874 (±155)	702 (±111)	1023 (±189)	1015 (±332)	1005 (±164)
cp 2	311 (±42)	421 (±38)	368 (±55)	420 (±46)	281 (±32)	331 (±34)	367 (±52)	370 (±38)	255 (±30)	285 (±42)	343 (±34)	327 (±60)
cp 3	14 (±3.3)	15 (±4.6)	16 (±5.2)	6 (±2.6)	13 (±7)	11 (±5.2)	6 (±2.5)	8 (±2.6)	8 (±1.8)	3 (±1.4)	16 (±3.7)	12 (±2.8)
cp 4	4 (±1.1)	7 (±2)	10 (±4)	5 (±1.1)	4 (±2.3)	1 (±0.6)	3 (±2)	3 (±1.4)	7 (±3.5)	6 (±2)	17 (±4.5)	4 (±2.2)
cp 5	1 (±0.9)	4 (±2.5)	1 (±0.5)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	1 (±0.7)	0 (±0)
MI	2 (±0)	2 (±0)	2 (±0)	2 (±0)	1 (±0)	1 (±0.1)	1 (±0.1)	1 (±0.1)	1 (±0)	1 (±0.1)	1 (±0.1)	1 (±0.1)
MI2-5	2 (±0)	2 (±0)	2 (±0)	2 (±0)	2 (±0)	2 (±0)	2 (±0)	2 (±0)	2 (±0)	2 (±0)	2 (±0)	2 (±0)
BI	36 (±1.5)	31 (±2.4)	34 (±1.6)	32 (±1.2)	10 (±1.4)	9 (±2.1)	12 (±2.3)	11 (±1.8)	9 (±1.2)	8 (±1.7)	13 (±2.4)	10 (±2.2)
CI	55 (±3.5)	46 (±4.9)	54 (±4)	45 (±3.3)	11 (±1.8)	11 (±2.8)	14 (±2.9)	13 (±2.8)	9 (±1.4)	9 (±2.1)	15 (±3.2)	11 (±2.6)
EI	61 (±1.6)	67 (±2.5)	63 (±1.7)	68 (±1.3)	89 (±1.4)	91 (±2.2)	88 (±2.4)	89 (±2)	91 (±1.3)	91 (±1.8)	87 (±2.5)	90 (±2.3)
SI	16 (±2)	15 (±2.5)	16 (±2)	7 (±1.6)	11 (±5.2)	6 (±2.6)	5 (±1.5)	8 (±2.4)	14 (±3.5)	10 (±2.5)	23 (±3)	12 (±3)

C = compost only; F = compost + fabric; S = compost + straw; W = compost + wood chips;
cp 1 = colonizer-persister group 1; cp 2 = colonizer-persister group 2; cp 3 = colonizer-persister group 3; cp 4 = colonizer-persister group 4; cp 5 = colonizer-persister group 5; MI = maturity index; MI2-5 = maturity index cp 2-5; BI = basal index; CI = channel index; EI = enrichment index; SI = structure index.

Table 2S - Means (±standard error of the mean) of nematode relative abundance and ecological indexes across three cultivars of jute leaf and three sampling periods in a jute leaf experiment in Beltsville, MD, USA. Genera found in less than 1% of samples were omitted from the table

Nematode group/ecological index	Sampling period								
	Pre-plant			Midseason			Final		
	Cultivar			Cultivar			Cultivar		
	F†	M	U	F	M	U	F	M	U
Plant-parasites	142 (±21)	112 (±32)	128 (±28)	61 (±9.6)	59 (±17)	59 (±16)	47 (±17)	58 (±13)	52 (±13)
Helicotylenchus	139 (±21)	108 (±33)	120 (±28)	58 (±9.7)	56 (±17)	58 (±16)	41 (±12)	44 (±13)	40 (±11)
Hoplolaimus	0 (±0)	0 (±0.2)	0 (±0.3)	1 (±0)	0 (±0.6)	0 (±0)	0 (±2.4)	2 (±0)	0 (±0)
Paratylenchus	2 (±1.4)	3 (±0.9)	7 (±1.8)	2 (±1.2)	3 (±1.1)	2 (±1.2)	7 (±4.1)	8 (±6.1)	11 (±9.8)
Pratylenchus	0 (±0.4)	1 (±0)	0 (±0.3)	0 (±0)	0 (±0.4)	0 (±0)	0 (±2.6)	4 (±0)	0 (±0)
Bacterivores	169 (±29)	156 (±32)	164 (±28)	942 (±179)	877 (±120)	952 (±179)	1031 (±231)	1048 (±170)	845 (±146)
Acrobeles	1 (±0.8)	1 (±1.1)	1 (±0.5)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)
Alaimus	3 (±0.7)	1 (±2.6)	2 (±1.5)	1 (±0.9)	1 (±1.4)	0 (±0.4)	6 (±0.6)	1 (±3)	5 (±3.1)
Butlerius	0 (±0)	0 (±0)	0 (±0)	19 (±4.9)	21 (±5.5)	19 (±7.3)	16 (±3.4)	16 (±5)	20 (±5.3)
Cephalobus	11 (±2.7)	9 (±3.2)	9 (±2.4)	7 (±1.5)	4 (±3.1)	4 (±2.3)	5 (±6.6)	8 (±2.3)	6 (±2.9)
Diploscapter	0 (±0.3)	0 (±0)	0 (±0.2)	2 (±0)	0 (±1.7)	0 (±0)	2 (±0.7)	1 (±1.1)	6 (±3.2)
Eucephalobus	23 (±3.8)	21 (±4.3)	33 (±8.2)	20 (±3.1)	16 (±7.2)	18 (±4.7)	12 (±4.4)	19 (±3.8)	17 (±4.2)
Panagrolaimus	0 (±0)	0 (±0)	0 (±0)	1 (±0.9)	1 (±1.4)	0 (±0.4)	0 (±0)	0 (±0)	0 (±0)
Plectus	2 (±2.9)	6 (±1.1)	3 (±1.2)	0 (±0.2)	0 (±0)	3 (±1.3)	2 (±1.6)	3 (±1.2)	3 (±1.2)
Prismatolaimus	10 (±4)	12 (±2.7)	13 (±3.3)	5 (±5)	13 (±1.9)	10 (±4.4)	10 (±2.5)	8 (±2.5)	11 (±2.5)
Rhabditis	118 (±27)	106 (±28)	104 (±20)	886 (±180)	821 (±121)	896 (±180)	978 (±229)	991 (±173)	778 (±145)
Fungivores	340 (±38)	333 (±39)	352 (±37)	306 (±27)	308 (±34)	326 (±39)	315 (±20)	241 (±50)	277 (±34)
Aphelenchoides	4 (±1.1)	3 (±1.7)	4 (±1.9)	0 (±2.6)	4 (±0)	2 (±1.7)	2 (±0.4)	0 (±1.3)	7 (±4.4)
Aphelenchus	11 (±8.3)	32 (±2.7)	24 (±4.3)	18 (±3.3)	19 (±5.4)	27 (±7.2)	13 (±9.1)	20 (±5.3)	25 (±6.8)
Diphtherophora	1 (±0.3)	0 (±0.8)	2 (±0.9)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)
Ditylenchus	0 (±0)	0 (±0)	0 (±0)	27 (±10)	44 (±7.1)	31 (±13)	22 (±5.3)	16 (±9.9)	24 (±8.1)
Tylenchus	324 (±31)	297 (±38)	323 (±35)	261 (±24)	242 (±32)	266 (±33)	277 (±18)	205 (±47)	222 (±27)
Omnivores	0 (±1.5)	2 (±0.2)	2 (±1.4)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0.5)
Aporcelaimus	0 (±1.5)	2 (±0.2)	2 (±1.4)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0.5)
Dorylaimoides	0 (±0.7)	1 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)
Prodorylaimus	0 (±0.1)	0 (±0.2)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	1 (±0)	0 (±1.1)	0 (±0)
Predators	4 (±1)	4 (±1.2)	5 (±1.5)	0 (±1.5)	4 (±0.4)	1 (±0.4)	4 (±2.7)	5 (±2.2)	4 (±1.5)
Ironus	2 (±1)	2 (±1.1)	2 (±1)	0 (±0.3)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)
Mononchus	2 (±0.7)	2 (±0.8)	2 (±0.8)	0 (±1.5)	4 (±0.4)	1 (±0.4)	3 (±2.7)	5 (±2)	4 (±1.5)
cp 1	118 (±27)	106 (±28)	104 (±19)	909 (±180)	843 (±121)	915 (±181)	996 (±228)	1009 (±174)	804 (±145)
cp 2	376 (±40)	369 (±40)	395 (±42)	333 (±27)	328 (±37)	352 (±40)	333 (±24)	272 (±50)	303 (±33)
cp 3	11 (±4)	13 (±2.6)	14 (±4)	5 (±5)	13 (±1.9)	10 (±4.4)	10 (±2.5)	8 (±2.5)	11 (±2.5)
cp 4	7 (±1.2)	5 (±2.5)	7 (±2.4)	2 (±2)	5 (±1.4)	1 (±0.6)	10 (±2.7)	6 (±3.2)	9 (±3.1)
cp 5	0 (±1.5)	2 (±0.2)	2 (±1.4)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0.5)
MI	2 (±0)	2 (±0)	2 (±0)	1 (±0)	1 (±0)	1 (±0.1)	1 (±0)	1 (±0.1)	1 (±0.1)
MI2-5	2 (±0)	2 (±0)	2 (±0)	2 (±0)	2 (±0)	2 (±0)	2 (±0)	2 (±0)	2 (±0)
BI	33 (±1.6)	33 (±1.8)	33 (±1.2)	9 (±1.9)	12 (±1.3)	11 (±1.8)	10 (±1.4)	9 (±1.9)	11 (±1.8)
CI	50 (±3.5)	50 (±4.2)	50 (±2.9)	9 (±2.6)	14 (±1.5)	12 (±2.3)	11 (±1.6)	9 (±2.6)	12 (±2.2)
EI	65 (±1.8)	65 (±1.9)	64 (±1.3)	91 (±1.9)	88 (±1.3)	89 (±1.8)	90 (±1.4)	91 (±2)	89 (±1.9)
SI	12 (±2.3)	14 (±1.6)	14 (±2)	4 (±2.9)	11 (±1.4)	7 (±3.4)	15 (±2.5)	13 (±2.7)	16 (±3.2)

F = Firebird Farm; M = Molokhia; U = USDA PI 404029;

cp 1 = colonizer-persister group 1; cp 2 = colonizer-persister group 2; cp 3 = colonizer-persister group 3; cp 4 = colonizer-persister group 4; cp 5 = colonizer-persister group 5; MI = maturity index; MI2-5 = maturity index cp 2-5; BI = basal index; CI = channel index; EI = enrichment index; SI = structure index.

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Continuous lighting improves the leaf quality of sweet basil (*Ocimum basilicum* L.) grown in a controlled environment

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Abstract: In vertical farms, continuous lighting (CL) with lower light intensity (photosynthetic photon flux density, PPFD) is a method to reduce the investment costs for the lighting system. Continuous lighting has both negative and positive effects on crop performance, depending on the plant species. In this study, we investigated the effect of CL on plant growth and leaf quality in sweet basil (*Ocimum basilicum* L. cv. Tigullio) cultivated in a growth chamber with light emitting diode (LED) (R:B:G=3:1:1). Basil plants were grown hydroponically for 14 days with a photoperiod of 16 h d^{-1} (control) or 24 h d^{-1} with a PPFD of 220 or 147 µmol m^{-2} s^{-1}, respectively. The daily light integral was 12.7 mol m^{-2} d^{-1} in both treatments. Plant growth was not significantly affected by the light regime. Compared with the control, CL increased the leaf antioxidant capacity and concentration of total chlorophylls, flavonoids and phenols, and reduced the nitrate level. Continuous lighting would slightly increase or decrease electricity costs compared to 16-hour illumination, depending on the daily schedule of the standard lighting regime.

1. Introduction

In many countries, high-value fresh vegetables and herbs are increasingly produced in plant factories with artificial light, also known as vertical farms. In vertical farms, crop yield is enhanced due to the use of multi-tiered cultivation systems and the maintenance of optimal environmental conditions for plants (Zhu and Marcelis, 2023). In a controlled environment, plant growth is primarily determined by the daily light integral (DLI) (Warner *et al.*, 2023), which is the light level plants receive over a day and thus depends on the photosynthetic photon flux density (PPFD) and the photoperiod. Generally, crop yield and quality are

boosted by increasing DLI to a certain level. In vertical farms, typical DLI values are 12 to 17 mol m^{-2} d^{-1} for leafy greens and herbs, and 15 to 40 mol m^{-2} d^{-1} for fruiting crops (MechaTronix Horticulture Lighting, 2025). The photoperiod is generally 16 or 18 h d^{-1} with PFFD ranging between 150 and 300 µmol m^{-2} s^{-1}) (Nájera *et al.*, 2022).

The main drawback of vertical farms is the high electricity requirement for artificial illumination and air conditioning (Zhu and Marcelis, 2023). Despite the adoption of energy-efficient light-emitting diode (LED) fixtures, the lighting system remains one of the main components of the capital costs of vertical farms and accounts for approximately 80% of the total electricity demand (Cai *et al.*, 2024). Therefore, the key to reducing energy costs and/or enhancing energy use efficiency in vertical farms is innovating lighting systems and strategies (Liu *et al.*, 2022).

Continuous lighting (CL; i.e., 24-hour photoperiod without dark interruption) with lower PFFD is a cost-effective strategy to achieve the same DLI in vertical farms, as it reduces the investment costs for the lighting system (fewer fixtures are necessary) and the operational costs for illumination in case of a time-based pricing scheme for electricity (Lanoue *et al.*, 2022).

Long photoperiod (>18 h d^{-1}) can enhance plant production by increasing leaf photosynthesis. Under the same DLI, longer photoperiods with lower PPFD increase yield in most species (Warner *et al.*, 2023), since crop light use efficiency rises with decreasing irradiance (e.g., Palmer and van Iersel, 2020; Carotti *et al.*, 2021). This explains why many studies have been conducted on the effect of CL in greenhouse crops, which started nearly one century ago (Arthur *et al.*, 1930), and more recently in vertical farms (Velez-Ramirez *et al.*, 2011; Shibaeva *et al.*, 2023 a). However, leaf injury and growth inhibition were observed in some species grown under greenhouse conditions with CL for a relatively long period, such as tomato, eggplant, pepper, and cucumber (Velez-Ramirez *et al.*, 2011; Shibaeva *et al.*, 2023 a). Although the mechanism of leaf damage under CL has not yet been completely elucidated, it has been attributed to carbohydrate accumulation in leaf tissues and disruption of endogenous circadian rhythms, which results in early senescence and reduced photosynthesis (Velez-Ramirez *et al.*, 2011; Shibaeva *et al.*, 2023 a). In contrast to fruiting crops, leafy vegetables and herbs have a short growing cycle and generally, they are not negatively affected by long photoperiod or CL (Shibaeva *et al.*, 2023 a); positive effects have also been reported (Table 1S). For instance, in rocket CL enhanced plant growth and improved leaf quality by reducing nitrate concentration and increasing the concentration of antioxidants (Proietti *et al.*, 2021).

Sweet basil (*Ocimum basilicum* L.) is an economically important herb cultivated worldwide (Camlica and Yaldiz, 2023) due to its adaptability to different growing conditions and systems. Its short cycle, rapid growth, and easy cultivation make basil one of the most used species in vertical farms; basil is the most studied species for controlled environment agriculture after lettuce (Dsouza *et al.*, 2023). There exists a reasonable consensus on the optimal DLI for basil grown indoors, which ranges between approximately 13 and 15 mol m^{-2} d^{-1} (Liaros *et al.*, 2016; Dou *et al.*, 2018; Pennisi *et al.*, 2020), but contrasting results have been found on the response of this species to CL, since positive (Islam *et al.*, 2010; Lanoue *et al.*, 2022; Fayezizadeh *et al.*, 2024), negative (Beaman *et al.*, 2009), or no (Pennisi *et al.*, 2020) effects have been reported.

In this work, the impact of CL on plant growth and leaf quality was studied in sweet basil cultivated hydroponically in a growth chamber with LED light under a photoperiod of 16 or 24 h d^{-1} with the same DLI (12.7 mol m^{-2} d^{-1}). Based on the results in the literature and a preliminary experiment, in which only growth parameters were measured, we hypothesized that CL does not affect plant growth but improves leaf quality by reducing nitrate accumulation and increasing the concentration of antioxidant compounds.

2. Materials and Methods

Plant material and growing conditions

Two experiments were conducted with sweet basil (cv. Tigullio; Franchi Sementi, Grassobbio, Italy) grown in a floating raft system in a growth chamber at the University of Pisa.

Basil seeds were sown in 240-cell trays with stone wool plugs and 41 days after sowing, the seedlings with two pairs of true leaves were transplanted in plastic tanks with 14 litres of aerated nutrient solution. The solution had a pH of 6.0 and an electrical conductivity of 2.39 dS m^{-1}, and contained the following concentration of nutritive elements: $N\text{-}NO_3$ 10.0 mM, P 1.5 mM, K 9.0 mM, Ca 4.5 mM, Mg

2.0 mM, Fe 40.0 µM, B 40.0 µM, Cu 3.0 µM, Zn 10.0 µM, Mn 10.0 µM, and Mo 1.0 µM. Twelve plants were grown in each tank and crop density was approximately 96 plants m^{-2}. Air temperature and relative humidity were kept at 24.0°C and 65% - 70%, respectively.

Basil plants were illuminated by red, blue, and green (R:B:G=3:1:1) (Fig. 1) LED tubes (Circular Natural Indoor, C-Led, Imola, Italy) with a 16-hour photoperiod at 220 µmol m^{-2} s^{-1} PPFD, till transplantation in hydroponic tanks. Afterwards, one group of plants was kept under the same light regime (control) while another group was grown with a 24-hour photoperiod at 147 µmol m^{-2} s^{-1} PPFD (CL). The lowest PPFD was achieved by using fewer lamps and slightly adjusting the distance between lamps and the plant canopies. An opaque plastic screen was used to avoid contamination between the two light treatments, which lasted 14 days. Each treatment had four replicates, each consisting of one hydroponic tank.

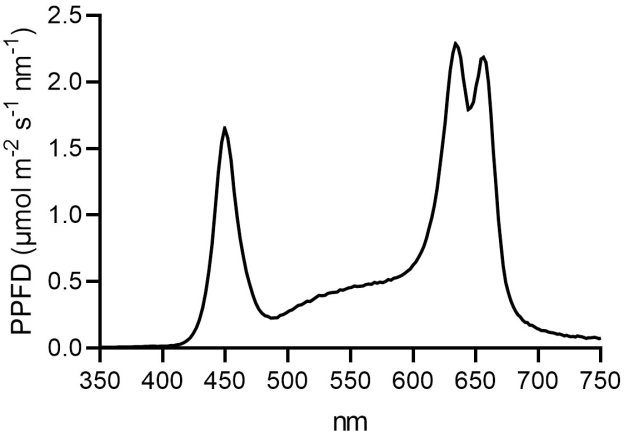

Fig. 1 - The spectral distribution of the LED tubes (Circular Natural Indoor, C-Led, Imola, Italy) used in the experiments with sweet basil in a growth chamber. The spectrum was measured using a portable spectroradiometer (SpectraPen LM 510, Photon Systems Instruments, Drásov, Czech Republic).
PPFD= photosynthetic photon flux density.

Determinations

Leaf fresh (FW) and dry weight (DW), stem and root DW, plant height, and leaf area were measured in plant samples collected in each replicate; each sample consisted of six individual plants. Dry weight was determined in plant samples that were dried in a ventilated oven at 70°C until they were of constant weight. Leaf area was measured using a digital planimeter (DT Area Meter MK2, Delta T-Devices). Leaf area index (LAI) was computed as the average leaf area per plant divided by the area occupied by one plant.

Twelve days after the beginning of the experiment, stomatal behaviour was assessed by measuring the abaxial leaf diffusion conductance (g_s) with a diffusion porometer (AP4, Delta-T Devices, Cambridge, UK). The g_s was measured on two individual plants in each replicate of both treatments at two times corresponding to midday and midnight of the control treatment.

Plant water uptake was determined by measuring the weight of each hydroponic tank at the beginning and the end of each experiment. The weight difference was assumed to be equal to the plant water absorption because the tank was covered by the polystyrene floating raft and thus direct evaporation was insignificant.

Leaf concentration of mineral elements was determined in dried samples whereas the antioxidant capacity and the concentration of total chlorophylls, carotenoids, flavonoids, and phenols were examined in fresh samples. Each sample comprised all leaves from six individual plants collected from each tank.

To determine leaf mineral concentration, finely ground samples underwent mineralization in a 5:2 v/v mixture of 65% HNO_3 and 35% $HClO_4$ at 240°C for one hour or were extracted with distilled water at room temperature for two hours. The mineralized samples were utilized for assessing the concentrations of K, Ca, Mg, Cu, Fe, Mn, and Zn through atomic absorption spectroscopy. At the same time, P levels were determined spectrophotometrically with Olsen's method. Water extracts were analysed spectrophotometrically for nitrate concentration using the salicylic-sulfuric acid method (Puccinelli *et al.*, 2023).

Methanol (99% v/v) was used to extract fresh samples, followed by a 60-minute sonication (frequency 28-34 kHz, power peak 350 W). The samples were stored at -18°C for 24 hours; afterwards, the concentrations of total chlorophylls, carotenoids, phenols, and flavonoids were determined spectrophotometrically as previously reported (Puccinelli *et al.*, 2023). The antioxidant capacity of leaf samples was measured in methanol extracts using the ferric-reducing ability of plasma (FRAP) assay (Benzie and Strain, 1996) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Blois, 1958).

Electricity costs of light regimes

The electricity cost of the light regimes tested in this work was calculated every week using the

scheme for time-of-use pricing of electricity in place in Italy, which differentiates the prices by single-hour rate and time slot (F), as follows (ENEL, 2025 a):
F1: from 8.00 am to 7.00 pm from Monday to Friday, excluding national holidays;
F2: from 7.00 am to 8.00 am and from 7.00 pm to 11.00 pm from Monday to Friday, Saturday from 07.00 am to 11.00 pm, excluding national holidays;
F3: from 11.00 pm to 7.00 am from Monday to Saturday, Sunday, and national holidays.

The prices used for calculation were 0.1783, 0.1716 and 0.1485 € kWh^{-1} for F1, F2, and F3, respectively. They are mixed costs because they have variable and fixed components. The variable component is the PUN Index GME (PUN is the Italian acronym for Prezzo Unico Nazionale, "National Single Price"), which is the weighted average of electricity prices in different areas of Italy and at different times of the day. The fixed part is the contribution paid to the energy provider. We used a fixed component of 0.020 € kWh^{-1} (ENEL, 2025 a) while the PUNs were the mean values recorded in January 2025 for the three time slots (ENEL, 2025 b).

The weekly lighting cost was computed for three light regimes with the same DLI (12.7 mol m^{-2} d^{-1}): 220 µmol m^{-2} s^{-1} PPFD for 16 h d^{-1} from 11:00 pm until 03:00 pm of the next day (LR1) or from 08:00 am until 12:00 pm (LR2); 147 µmol m^{-2} s^{-1} PPFD for 24 h d^{-1} (LR3). The consumption of electricity was estimated using a photon efficacy of LED lamps of 3.1 µmol J^{-1}.

Statistical analysis

Since the two experiments were conducted in the same growing conditions and the results were quite similar, the data were pooled, subjected to 1- or 2-way ANOVA, and reported as the mean values (±SE) of eight replicates. Leaf diffusion conductance data were subjected to 2-way ANOVA, with the time of measurement and light regime as variability factors. Data were tested for the normality of the distribution using the Shapiro-Wilk test and for the homogeneity of variances using Levene's test. Statistical analysis was performed using JMP statistical software.

3. Results

Plant growth

In our study, the light regime did not affect basil growth as no significant differences (P>0.050) were found between the controls and CL plants regarding plant height, leaf and stem FW (yield), and leaf, stem, root, and total DW (Table 1). The production for the market of bunched fresh herbs was 0.96 kg m^{-2}, a noticeable yield for a crop that lasted only two weeks. The leaf area index has an average value of 2.16 and was not significantly affected by the light regime (data not shown)

Plant water and mineral relations

The cumulated water uptake during the experiments was not significantly influenced by the light regime and averaged 34.8±2.1 L m^{-2}. This result is consistent with the absence of significant differences in LAI between the controls and CL plants, and the measurements of g_s, which did not vary significantly between the two plant groups at both midday and midnight of the control light regime

Table 1 - Leaf and stem fresh (FW) and dry weight (DW), root and total DW and plant height, in sweet basil plants grown hydroponically for 14 days in a growth chamber under two LED light regimes with the same daily light integral (12.7 mol m^{-2} d^{-1}): a 16-hour photoperiod and 220 µmol m^{-2} s^{-1} PPDF (Control); 24-hour photoperiod and 147 µmol m^{-2} s^{-1} PPDF (CL)

	Light regime		ANOVA significance
	Control	CL	
Leaf FW (kg m^{-2})	0.783±0.044	0.748±0.005	NS
Stem FW (kg m^{-2})	0.198±0.018	0.195±0.012	NS
Leaf DW (kg m^{-2})	0.059±0.003	0.058±0.003	NS
Stem DW (kg m^{-2})	0.015±0.004	0.015±0.004	NS
Root DW (kg m^{-2})	0.013±0.001	0.012±0.001	NS
Total DW (kg m^{-2})	0.087±0.004	0.084±0.004	NS
Plant height (cm)	11.9±0.2	11.5±0.3	NS

Mean values (±SE) of eight replicates.
PPFD= photosynthetic photon flux density. Significance level: NS = not significant.

(Fig. 2). However, on average g_s was significantly higher at midday (290.0±8.6 mmol H_2O m^{-2} s^{-1}) that at midnight (187.7±12.1 mmol H_2O m^{-2} s^{-1}) .

In both experiments, no plant revealed evident mineral deficiency symptoms and the leaf concentration of nutritive elements (Table 2) was within or above the adequate levels reported for sweet basil (Bryson *et al.*, 2014). Nonetheless, CL plants showed a significantly lower leaf concentration of Mg and Mn, and a higher concentration of Zn than the control plants. In Japanese mugwort, CL induced a lower leaf concentration of K, Mn, and Zn, in partial agreement with our findings (Hata and Kawamura, 2023).

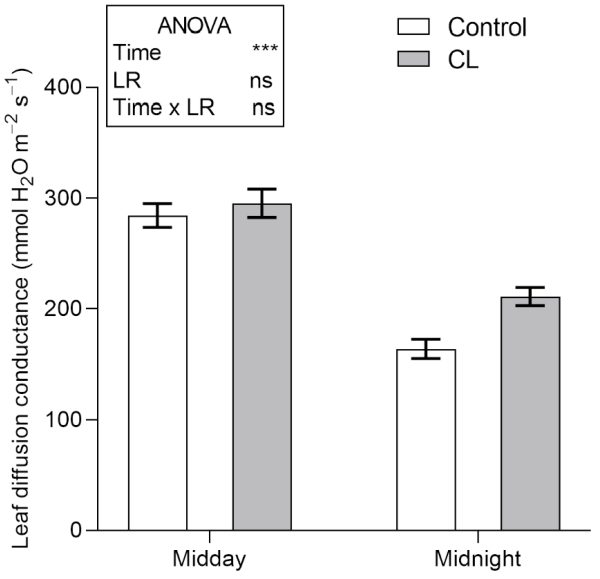

Fig. 2 - Abaxial leaf diffusion conductance in sweet basil plants grown hydroponically for 14 days in a growth chamber under two LED light regimes (LR) with the same daily light integral (12.67 mol m^{-2} d^{-1}): a 16-hour photoperiod and 220 µmol m^{-2} s^{-1} PPDF (Control); 24-hour photoperiod and 147 µmol m^{-2} s^{-1} PPDF (CL). The measurements were taken at midday and midnight of the control light regime 12 days after the beginning of the experiment. Mean values (±SE) of eight replicates. Significance level: *** P ≤ 0.001; ** P ≤ 0.01; * P ≤ 0.05; NS = not significant.

Leaf quality

We evaluated basil quality by measuring leaf moisture content, which influences the produce's response to post-harvest processing and storage (Clarkson *et al.*, 2003), and concentration of several substances associated with the visual (chlorophylls), nutritional (antioxidant compounds), or safety (nitrate) quality.

Table 2 - Leaf concentration (on a fresh weight basis) of macro and microelements in sweet basil plants grown hydroponically for 14 days in a growth chamber under two LED light regimes with the same daily light integral (12.7 mol m^{-2} d^{-1}): 16-hour photoperiod and 220 µmol m^{-2} s^{-1} PPDF (Control); 24-hour photoperiod and 147 µmol m^{-2} s^{-1} PPDF (CL)

	Light regime		ANOVA significance
	Control	CL	
Ca (g kg^{-1})	1.138±0.079	0.922±0.122	NS
K (g Kg^{-1})	5.938±0.411	5.019±0.575	NS
Mg (g kg^{-1})	0.342±0.020 A	0.240±0.025 B	**
P (g kg^{-1})	0.840±0.025	0.771±0.022	NS
Cu (mg kg^{-1})	1.812±0.106	1.640±0.084	NS
Mn (mg kg^{-1})	6.946±1.177 A	3.687±0.328 B	*
Fe (mg kg^{-1})	16.233±1.552	14.061±3.366	NS
Zn (mg kg^{-1})	4.185±0.268 B	6.254±0.786 A	*

Mean values (±SE; n = 8) flanked by different letters are significantly different at the 5% level. Significance level: *** P ≤ 0.001; ** P ≤ 0.01; * P ≤ 0.05; NS = not significant. PPFD= photosynthetic photon flux density.

In our experiments, leaf moisture content was not affected by the photoperiod (Fig. 3A). Compared to the 16-hour photoperiod, CL significantly increased the leaf concentration of total chlorophylls (+35%), flavonoids (+40%), and phenols (+44%), and the antioxidant capacity, which was measured using both FRAP (+47%) and DPPH (+63%;) assay (Fig. 3). Conversely, no significant differences in carotenoids concentration were found between the controls and CL plants (Fig. 3D) and the nitrate level was slightly but significantly reduced by CL (Fig. 3B) A significant positive correlation was found between the total antioxidant capacity measured with the two assays (R^2 = 0.856; n=8) and between the antioxidant capacity and the level of phenols (R^2=0.756 and R^2=0.845 for FRAP and DPPH assay, respectively) or flavonoids (R^2=0.956 and R^2=0.845).

Electricity consumption and cost

In our simulation, two reference light regimes were compared to CL: from 11:00 pm until 03:00 pm the following day (LR1), or from 08:00 am until 12:00 pm (LR2, Table 3).

The calculated electricity consumption for artificial lighting was 7.95 kWh m^{-2} $week^{-1}$ across all three scenarios, with weekly electricity costs estimated to range from 1.30 to 1.39 € m^{-2} $week^{-1,}$ based on the time-of-use pricing model in Italy (Table

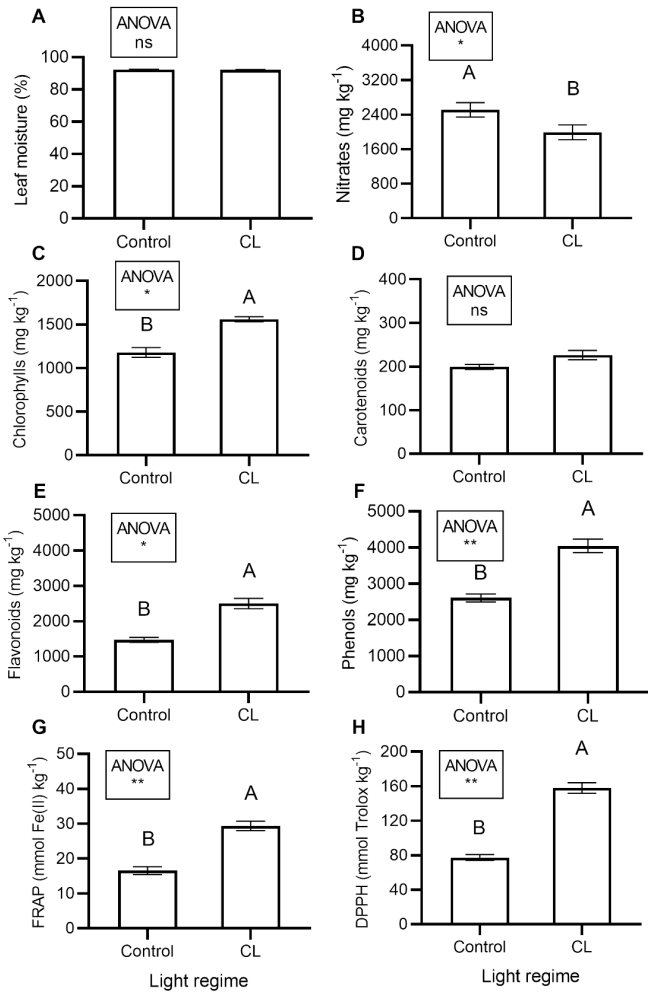

Fig. 3 - Leaf moisture content (A) and concentration (on a fresh weight basis) of nitrates (B), total chlorophylls (C), carotenoids (D), flavonoids (E), and phenols (F), and antioxidant capacity measured by FRAP (G) and DPPH (H) assay, in sweet basil plants grown hydroponically for 14 days in a growth chamber under two LED light regimes with the same daily light integral (12.67 mol m^{-2} d^{-1}): 16-hour photoperiod and 220 µmol m^{-2} s^{-1} PPDF (Control); ii) 24-hour photoperiod and 147 µmol m^{-2} s^{-1} PPDF (CL). Mean values (±SE) of eight replicates. Significance level: *** P≤0.001; ** P≤0.01; * P≤0.05; NS = not significant. Abbreviations: PFFD, photosynthetic photon flux density; FRAP the ferric reducing ability of plasma (FRAP) assay w; DPPH, the 2,2-diphenyl-1-picrylhydrazyl assay.

3). When evaluating CL against a 16-hour light regime, we found a minor increase (+2.6%) or decrease (-4.4%) in electricity costs, which depended on the daily schedule of the latter system.

4. Discussion and Conclusions

According to Beaman *et al.* (2009), basil needs a dark period for optimal growth since CL may cause leaf chlorosis and necrosis, which were not observed in our experiments. In an experiment conducted in a growth chamber with the same PPFD level (250 µmol m^{-2} s^{-1}) from LED lamps, fresh and dry biomass did not significantly differ in basil plants grown with a photoperiod of 16, 20, or 24 h d^{-1}, notwithstanding the large differences in DLI (14.4, 18.0, and 21.6 mol m^{-2} d^{-1}, respectively) (Pennisi *et al.*, 2020). In contrast, in greenhouse-grown basil the supplementation of natural radiation with artificial light (from high-pressure sodium lamps) for 24 h d^{-1} increased shoot DW compared to illumination for 16 or 20 h d^{-1} (Islam *et al.*, 2010). Islam *et al.* (2010) used the same PPFD (150 µmol m^{-2} s^{-1} from HPS lamps) in the three light treatments and therefore the artificial DLIs were different (8.64, 10.80, and 12.96 mol m^{-2} d^{-1}), and this probably accounts for the differences between their and our findings. Continuous lighting also increased the biomass of green basil microgreens compared to a 16-hour photoperiod with the same DLI (14 or 24 mol m^{-2} d^{-1}) (Lanoue *et al.*, 2022). Positive effects of CL on plant growth were also observed in Chinese cabbage (Kang *et al.*, 2024), rocket (Proietti *et al.*, 2021), Japanese mugwort (Hata and Kawamura, 2023), and microgreens of different species (Lanoue *et al.*, 2022; Shibaeva *et al.*, 2023 b).

The significant reduction of g_s detected at midnight in both treatments (Fig. 2) suggested that a circadian rhythm of stomatal movements was present in plants grown under CL (Dodd *et al.*, 2004). Similar results were found in Chinese cabbage (Kang *et al.*, 2024). In

Table 3 - Leaf concentration (on a fresh weight basis) of macro and microelements in sweet basil plants grown hydroponically for 14 days in a growth chamber under two LED light regimes with the same daily light integral (12.7 mol m^{-2} d^{-1}): 16-hour photoperiod and 220 µmol m^{-2} s^{-1} PPDF (Control); 24-hour photoperiod and 147 µmol m^{-2} s^{-1} PPDF (CL)

Light regime	PPFD (µmol m^{-2} s^{-1})	Photoperiod (h d^{-1})	Daily light integral (mol m^{-2} d^{-1})	Lighting schedule	Costs (€ m^{-2} $week^{-1}$)
LR1	220	16	12.7	11:00 pm - 03:00 pm	1.30
LR2	220	16	12.7	08:00 am - 12:00 pm	1.39
CL	147	24	12.7	Continuous light	1.33

PPFD= photosynthetic photon fluence rate.

contrast, in soybean (Kassai, 2008) and potato (Wheeler *et al.*, 2019) grown in a growth chamber, midday g_s was lower in plants grown with CL than in those grown with a photoperiod of 10-12 h d^{-1}.

Compared with the control, CL increased the leaf antioxidant capacity and concentration of total flavonoids and phenols and reduced the nitrate level (Fig. 3). In general, our results agree with previous findings (Table 1S). For instance, an increase in pigment concentration was observed in rocket plants grown with CL (Proietti *et al.*, 2021). In lettuce, CL improved leaf quality by reducing nitrate concentration and increasing the concentration of phenolic compounds, even when it was applied only for 1-3 days before harvest (Bian *et al.*, 2018; Yang *et al.*, 2022; Zhang *et al.*, 2021). In contrast, in several species in the Amaranthaceae, the leaf concentration of pigments and total polyphenols, and the antioxidant capacity were invariably lower in CL plants than in those grown with shorter photoperiods (Ali *et al.*, 2009).

Notwithstanding that nitrate may have positive effects on human health, nitrate intake with diet is linked with some health risks (Karwowska and Kononiuk, 2020). Therefore, the nitrate level in drinking water and food is strictly regulated and some agronomic practices have been proposed to reduce nitrate content in vegetables, which represent the primary source of nitrate in the human diet (Colla *et al.*, 2018). Sweet basil is prone to accumulate high levels of nitrate (Corrado *et al.*, 2020). The CL-induced reduction in leaf nitrate concentration observed in our study agrees with previous findings in other crops (Bian *et al.*, 2016; Proietti *et al.*, 2021; Shibaeva *et al.*, 2023 b). It is known that light regulates nitrate reductase (Lillo and Appenroth, 2001); CL can increase the activity of nitrate reductase (NR) and consequently the reduction of nitrate in plant leaves. In lettuce, the reduction of leaf nitrate concentration induced by short-term (two days) CL was associated with increased activity and gene expression of NR and nitrite reductase (Bian *et al.*, 2016, 2018). In contrast, CL augmented leaf nitrate levels in purslane (He *et al.*, 2023) and this was associated with a reduction in NR, compared with a 12-hour photoperiod.

Continuous illumination can increase the generation of reactive oxygen species (ROS) and induce oxidative stress (Haque *et al.*, 2015; Huang *et al.*, 2019; Liu and Liu, 2024). The balance between ROS production and the plant's ability to scavenge these molecules largely determines the extent of CL-induced plant damage (Kumar *et al.*, 2022). If this balance is achieved, injury-free production is possible under CL, with the additional improvement of produce quality resulting from a higher content of antioxidants (e.g. phenolics) and other health-promoting compounds, as was found in this and other works (Bian *et al.*, 2016; Hata and Kawamura, 2023). As such, applying CL for indoor cultivation of short-cycle crops can be considered an eustressor capable of promoting crop yield and improving produce quality (Vázquez-Hernández *et al.*, 2019).

For the same DLI, the use of CL can markedly decrease the number (-33% compared to the 16-hour photoperiod, in this work) and the installation costs of light fixtures. Moreover, in countries that implement a time-of-use pricing model of the electricity market, the operational costs of lighting are lower during off-peak periods. Electricity prices are generally lower early in the day, overnight, and during the weekends and holidays.

For basil production in vertical farms, illumination is generally operated for 16 continuous hours per day, switching on LEDs during the nighttime, when energy prices are lower than in the daytime (Avgoustaki and Xydis, 2021). According to our simulation, CL would slightly increase or decrease electricity costs compared to 16-hour illumination, depending on the daily schedule of the standard lighting regime (Table 3).

For microgreens production in vertical farms, compared to a 16-hour photoperiod, CL reduced electricity costs (expressed per unit of fresh biomass) by 8% to 38%, when DLIs were the same (Lanoue *et al.*, 2022). However, Lanoue and colleagues (Lanoue *et al.*, 2022) did not clarify when the light was switched on in the control treatment.

Vertical farms use powerful air conditioning systems to regulate air temperature and humidity for optimal crop production (Zhu and Marcelis, 2023). Continuous illumination at relatively low PPFD reduces the heat generated by LED lamps and the moisture released through plant transpiration, thus reducing the demand for air cooling and dehumidification (Cai *et al.*, 2024). In addition, CL could provide beneficial effects for pest and disease management. For instance, CL was found to suppress the sporulation of *Peronospora belbahrii,* which is the causal organism of basil downy mildew, one of the most destructive diseases of sweet basil (Radetsky *et al.*, 2020). Continuous light also reduced whiteflies'

infestation (*Trialeurodes vaporariorum)* in greenhouse roses (Johansen, 2009).

This work confirms the excellent adaptability of sweet basil to vertical farms since abundant yield can be achieved in a couple of weeks.

Continuous illumination at relatively low light intensity can be used to reduce the capital costs for the lighting system with no critical effects on crop yield and electricity costs in vertical farms. Leaf quality was improved by continuous lighting due to a lower nitrate level and higher antioxidant capacity and concentration of total flavonoids and phenols.

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Supplementary materials

Continuous lighting improves the leaf quality of sweet basil (*Ocimum basilicum* L.) grown in a controlled environment

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Key words: Artificial light, hydroponics, LED, 24-hour photoperiod, vertical farms.

Abstract: In vertical farms, continuous lighting (CL) with lower light intensity (photosynthetic photon flux density, PPFD) is a method to reduce the investment costs for the lighting system. Continuous lighting has both negative and positive effects on crop performance, depending on the plant species. In this study, we investigated the effect of CL on plant growth and leaf quality in sweet basil (*Ocimum basilicum* L. cv. Tigullio) cultivated in a growth chamber with light emitting diode (LED) (R:B:G=3:1:1). Basil plants were grown hydroponically for 14 days with and a photoperiod of 16 h d^{-1} (control) or 24 h d^{-1} with a PPFD of 220 or 147 µmol m^{-2} s^{-1}, respectively. The same daily light integral was 12.7 mol m^{-2} d^{-1} in both treatments. Plant growth was not significantly affected by the light regime. Compared with the control, CL increased the leaf antioxidant capacity and concentration of total chlorophylls, flavonoids and phenols, and reduced the nitrate level. Continuous lighting would slightly increase or decrease electricity costs compared to 16-hour illumination, depending on the daily schedule of the standard lighting regime.

Table 1S - A summary of the studies conducted on the effect of continuous lighting on some leafy vegetables and herbs grown in a controlled environment (growth chamber or vertical farm). The photoperiod, photosynthetic photon fluence density (PPFD), daily light integral (DLI, mol m^{-2} d^{-1}), and type of light used in the experiments are shown for the regular light regime (control) and continuous lighting (CL) along with experiment duration

Crop (species)	Regular light regime (control)	Continuous lighting (CL)	Treatment duration (d)	Effect of CL on plant growth and/or leaf quality compared with the control	Reference
Amaranth (*Amaranthus tricolor* L.) microgreens	Photoperiod: 16 h d^{-1} PFFD: 250.8 and 376.8 µmol m^{-2} s^{-1} DLI: 14.5 and 21.7 mol m^{-2} d^{-1} Light: LED	Photoperiod: 24 h d^{-1} PPFD: 166.6 and 247.6 µmol m^{-2} s^{-1} DLI: 14.4 and 21.5 mol m^{-2} d^{-1} Light: LED	11	**Positive**: higher FW and better and leaf quality (higher pigment concentration)	Lanoue *et al.*, 2022
Amaranth (*Amaranthus tricolor* L.), leaves	Photoperiod: 6, 12, and 18 h d^{-1} PFFD: 540 µmol m^{-2} s^{-1} DLI: 11.66, 23.33, and 34.99 mol m^{-2} d^{-1} Light: fluorescent and incandescent	Photoperiod: 24 h d^{-1} PPFD: 540 µmol m^{-2} s^{-1} DLI: 46.65 mol m^{-2} d^{-1} Light: fluorescent and incandescent	21	**Negative**: reduction of plant FW and DW, leaf antioxidant capacity, and concentration of pigments and polyphenols.	Ali *et al.*, 2009
Broccoli (*Brassica oleracea* var. *italica* Plenck), microgreens	Photoperiod: 16 h d^{-1} PFFD: 270 µmol m^{-2} s^{-1} DLI: 15.6 mol m^{-2} d^{-1} Light: LED	Photoperiod: 24 h d^{-1} PFFD: 180 and 270 µmol m^{-2} s^{-1} DLI: 15.6 and 23.3 mol m^{-2} d^{-1} Light: LED	12	**Positive**: higher yield and nutritional value (higher concentration of anthocyanins, flavonoids, carotenoids, and proline) and lower nitrate concentration. Short-term (3 d) CL before harvest	Shibaeva *et al.*, 2022
Calendula (*Calendula officinalis*), edible	Photoperiod: 16 h d^{-1} PFFD: 267 µmol m^{-2} s^{-1} DLI: 17.26 mol m^{-2} d^{-1} Light: LED	Photoperiod: 24 h d^{-1} PFFD: 200 and 400 µmol m^{-2} s^{-1} DLI: 17.25 and 34.56 mol m^{-2} d^{-1} Light: LED	Till 55 days after sowing	**Positive:** higher phenolic content, antioxidant capacity, and energy use efficiency.	Munyanont *et al.*, 2024
Chicory (*Cichorium intybus*), plants	Photoperiod: 16 and 20 h d^{-1} PFFD: 250 µmol m^{-2} s^{-1} DLI: 14.4 and 18 mol m^{-2} d^{-1} Light: LED	Photoperiod: 24 h d^{-1} PPFD: 250 µmol m^{-2} s^{-1} DLI: 21.6 mol m^{-2} d^{-1} Light: LED	21	**Negative**: lower plant FW (no effect on DW).	Pennisi *et al.* 2020
Chinese cabbage (*Brassica campestris L.* ssp. *chinensis* var. *utilis*), plants	Photoperiod: 12 h d^{-1} PFFD: 200 and 300 µmol m^{-2} s^{-1} DLI: 8.64 and 12.96 mol m^{-2} d^{-1} Light: LED	Photoperiod: 24 h d^{-1} PPFD: 200 and 300 µmol m^{-2} s^{-1} DLI: 17.28 and 25.92 mol m^{-2} d^{-1} Light: LED	21	**Positive**: higher plant DW.	Kang *et al.*, 2024
Collard microgreens *(Brassica oleracea var. viridis*), microgreens	Photoperiod: 16 h d^{-1} PFFD: 250.8 and 376.8 µmol m^{-2} s^{-1} DLI: 14; 21 mol m^{-2} d^{-1} Light: LED	Photoperiod: 24 h d^{-1} PPFD: 166.6 and 247.6 µmol m^{-2} s^{-1} DLI: 14; 21 mol m^{-2} d^{-1} Light: LED	11	**No effect**	Lanoue *et al.*, 2022

FW= fresh weight; DW= dry weight; LED= Light-emitting diode; EFF= External electrode fluorescent light.

Crop (species)	Regular light regime (control)	Continuous lighting (CL)	Treatment duration (d)	Effect of CL on plant growth and/or leaf quality compared with the control	References
Ice Plant (*Mesembryanthemum crystallinum* L.), plants	Photoperiod: 8, 12, 16 and 20 h d^{-1} PFFD: 220 µmol m^{-2} s^{-1} DLI: 6.34, 9.50, 12.67 and 15.84 mol $m^{-2}d^{-1}$ Light: LED	Photoperiod: 24 h d^{-1} PPFD: 220 µmol m^{-2} s^{-1} DLI: 19.01 mol m^{-2} d^{-1} Light: LED	28	**No or negative effect** on plant FW and DW; smaller, thicker, darker and curlier leaves; lower leaf K content.	Xia and Mattson, 2022
Japanese mugwort (*Artemisia princeps* Pamp.) leaves	Photoperiod: 12 h d^{-1} PFFD: 130 µmol m^{-2} s^{-1} DLI: 5.62 mol m^{-2} d^{-1} Light: fluorescent	Photoperiod: 24 h d^{-1} PPFD: 130 µmol m^{-2} s^{-1} DLI: 11.24 mol m^{-2} d^{-1} Light: fluorescent	28	**Positive**: higher leaf FW and DW: increase in leaf concentration of ascorbic acid and polyphenols. Appearance of leaf browning in CL plants.	Hata and Kawamura, 2023
Kale (*Brassica oleracea* var. *acephala*)	Photoperiod: 6, 12 and 16 h d^{-1} PFFD: 500 µmol m^{-2} s^{-1} DLI: 10.80, 21.60 and 28.80 mol m^{-2} d^{-1} Light: metal halide	Photoperiod: 24 h d^{-1} PFFD: 500 µmol m^{-2} s^{-1} DLI: 43.20 mol m^{-2} d^{-1} Light: metal halide	21	**Positive**: higher plant FW and DW; increased leaf concentration of pigments.	Lefsrud *et al*., 2006
Kale (*Brassica oleracea* var. *acephala*)	Photoperiod: 14, 16 and 20 h d^{-1} PFFD: 235, 210, and 165 µmol m^{-2} s^{-1} DLI: 11.9 mol m^{-2} d^{-1} Light: (R:B, 3:1) LED	Photoperiod: 24 h d^{-1} PFFD: 140 µmol m^{-2} s^{-1} DLI: 11.9 mol m^{-2} d^{-1} Light: metal halide	21	**Positive**: greater plant FW and DW; higher light use efficiency.	Zauli *et al.*, 2024
Lettuce (*Lactuca sativa* L.), leaves	Photoperiod: 12 h d^{-1} PFFD: 200 µmol m^{-2} s^{-1} DLI: 8.64 mol m^{-2} d^{-1} Light: LED	Photoperiod: 24 h d^{-1} PPFD: 200 µmol m^{-2} s^{-1} DLI: 17.28 mol m-^{2} d^{-1} Light: LED	2-3 (pre-harvest)	**Positive**: higher leaf antioxidant capacity and concentration of pigments and phenolic compounds; lower nitrate level.	Bian *et al.*, 2016, 2018
Lettuce (*Lactuca sativa*L.), leaves	Photoperiod: 12, 16, and 20 h d^{-1} PFFD: 320 µmol m^{-2} s^{-1} DLI: 13.82, 18.43 and 23.04 mol m^{-2} d^{-1} Light: Metalhalide	Photoperiod: 24 h d^{-1} PPFD: 320 µmol m^{-2} s^{-1} DLI: 27.65 mol m^{-2} d^{-1} Light: Metalhalide	14	**Positive**: higher plant FW and DW; higher leaf chlorophylls concentration.	Inada and Yakumoto, 1989
Lettuce (*Lactuca sativa* L.), plants	Photoperiod: 12, 16, and 20 h d^{-1} PFFD: 150 and 200 µmol m^{-2} s^{-1} DLI: 6.48, 8.64, 10.8, 8.64, 11.52, and 14.4 mol m^{-2} d^{-1} Light: EEFLs	Photoperiod: 24 h d^{-1} PPFD: 150 and 200 µmol m^{-2} s^{-1} DLI: 12.96 and 17.28 mol m^{-2} d^{-1} Light: EEFLs	21	**Positive**: higher plant FW and DW; higher leaf antioxidant capacity and concentration of phenolic compounds with low PPFD.	Cho *et al*., 2020
Lettuce (*Lactuca sativa* L.), plants	Photoperiod: 16 h d^{-1} PFFD: 120, 150, 180, and 270 µmol m^{-2} s^{-1} DLI: 6.9, 8.6, 10.4, and 15.6 mol m^{-2} d^{-1} Light: LED	Photoperiod: 24 h d^{-1} PPFD: 120, 150, and 180 µmol m^{-2} s-1 DLI: 10.4, 13.0, and 16.6 mol m^{-2} d^{-1} Light: LED	18	**Positive**: higher plant FW and DW; greater leaf pigmentation in red lettuce.	Kelly *et al*., 2020

Crop (species)	Regular light regime (control)	Continuous lighting (CL)	Treatment duration (d)	Effect of CL on plant growth and/or leaf quality compared with the control	Reference
Lettuce (*Lactuca sativa* L.), plants	Photoperiod: 16 and 24 h d^{-1} PFFD: 350 and 415 µmol m^{-2} s^{-1} DLI: 20.16 and 23.90 Light: Fluorescent	Photoperiod: 24 h d^{-1} PPFD: 350 and 216 µmol m^{-2} s^{-1} DLI: 30.24 and 22.46 Light: Fluorescent	21	**Positive**: increase in FW and DW.	Koontz and Prince, 1987
Lettuce (*Lactuca sativa* L.), plants	Photoperiod: 16 h d^{-1} PFFD: 200 µmol m^{-2} s^{-1} DLI: 11.52 mol m^{-2} d^{-1} Light: LED	Photoperiod: 24 h d^{-1} PPFD: 200 µmol m^{-2} s^{-1} DLI: 17.28 mol m^{-2} d^{-1} Light: LED	30	**Positive**: higher plant FW and DW.	Liu *et al.*, 2020
Lettuce (*Lactuca sativa* L.), plants	Photoperiod: 18 h d^{-1} PFFD: 360 µmol m^{-2} s^{-1} DLI: 23.328 mol m^{-2} d^{-1} Light: LED	Photoperiod: 24 h d^{-1} PPFD: 270 µmol m^{-2} s^{-1} DLI: 23.328 mol m^{-2} d^{-1} Light: LED	12	**Positive**: higher plant FW and DW leaf quality (higher sugar concentration). **Negative**: leaf chlorosis and necrosis.	Liu Jand Liu, 2022
Lettuce (*Lactuca sativa* L.), plants	Photoperiod: 18 h d^{-1} PFFD: 200, 300, and 400 µmol m^{-2} s^{-1} DLI: 12.96, 19.44, and 25.92 mol m^{-2} d^{-1} Light: LED	Photoperiod: 24 h d^{-1} PFFD: 200, 300, and 400 µmol m^{-2} s^{-1} DLI: 23.328 mol m^{-2} d^{-1} Light: LED	16 - 12	**Positive**: higher plant FW and DW leaf quality (higher antioxidants concentration).	Liu and Liu, 2024
Lettuce (*Lactuca sativa* L.), plants	Photoperiod: 16 h d^{-1} PFFD: 120 µmol m^{-2} s^{-1} DLI: 6.91 mol m^{-2} d^{-1} Light: fluorescent and LED	Photoperiod: 24 h d^{-1} PPFD: 120 µmol m^{-2} s^{-1} DLI: 10.37 mol m^{-2} d^{-1} Light: LED	31	**Positive**: higher plant DW.	Ohtake *et al.*, 2018
Lettuce (*Lactuca sativa* L.), plants	Photoperiod: 16 and 20 h d^{-1} PFFD: 250 µmol m^{-2} s^{-1} DLI: 14.4 and 18 mol m^{-2} d^{-1} Light: LED	Photoperiod: 24 h d^{-1} PPFD: 250 µmol m^{-2} s^{-1} DLI: 21.6 mol m^{-2} d^{-1} Light: LED	21	**No effect** on plant FW and DW.	Pennisi *et al.*, 2020
Lettuce (*Lactuca sativa* L.), plants	Photoperiod: 16 h d^{-1} PFFD: 225 µmol m^{-2} s^{-1} DLI: 12.96 mol m^{-2} d^{-1} Light: LED	Photoperiod: 24 h d^{-1} PPFD: 150 µmol m^{-2} s^{-1} DLI: 12.96 mol m^{-2} d^{-1} Light: LED	14	**Positive**: higher plant FW and DW.	Shao *et al.*, 2022
Lettuce (*Lactuca sativa* L.), red leaves, plants	Photoperiod: 16 h d^{-1} PFFD: 225 µmol m^{-2} s^{-1} DLI: 12.96 mol m^{-2} d^{-1} Light: LED	Photoperiod: 24 h d^{-1} PPFD: 150 µmol m^{-2} s^{-1} DLI: 12.96 mol m^{-2} d^{-1} Light: LED	2 – 4 (pre-harvest)	**Positive**: better nutritional and flavor quality due to greater sweetness and lesser bitterness.	Shen *et al.*, 2024

Crop (species)	Regular light regime (control)	Continuous lighting (CL)	Treatment duration (d)	Effect of CL on plant growth and/or leaf quality compared with the control	References
Lettuce (*Lactuca sativa* L.), plants	Photoperiod: 14 h d^{-1} PFFD: 200 µmol m^{-2} s^{-1} DLI: 10.08 mol m^{-2} d^{-1} Light: LED	Photoperiod: 24 h d^{-1} PPFD: 200 µmol m^{-2} s-1 DLI: 17.28 mol m^{-2} d^{-1} Light: LED	1-3 (pre-harvest)	**Positive** on lettuce quality: lower nitrate concentration and higher concentrations of sugars, pigments, and phenolic compounds; greater sweetness and lesser bitterness.	Yang *et al.*, 2023
Lettuce (*Lactuca sativa* L.), plants	Photoperiod: 12 h d^{-1} PFFD: 240 µmol m^{-2} s^{-1} DLI: 10.37 mol m^{-2} d^{-1} Light: LED	Photoperiod: 24 h d^{-1} PPFD: 120 µmol m^{-2} s^{-1} DLI: 10.37 mol m^{-2} d^{-1} Light: LED	15	**Positive**: higher FW and DW; higher concentration of ascorbic acid.	Zha *et al*., 2019
Mizuna (*Brassica rapa* ssp *nipposinica* (LH Bailey) Hanelt), , microgreens	Photoperiod: 16 h d^{-1} PFFD: 270 µmol m^{-2} s^{-1} DLI: 15.6 mol m^{-2} d^{-1} Light: LED	Photoperiod: 24 h d^{-1} PFFD: 180 and 270 µmol m^{-2} s^{-1} DLI: 15.6 and 23.3 mol m^{-2} d^{-1} Light: LED	12	**Positive**: higher yield and nutritional value (higher concentration of anthocyanins, flavonoids, carotenoids, and proline) and lower nitrate level. Short-term (3 d) CL before harvest increased nutrition-	Shibaeva *et al*., 2022
Mung bean (*Vigna radiata* L.), seedlings	Photoperiod: 12 h d^{-1} PFFD: 400 µmol m-2 s^{-1} DLI: 17.28 Light: fluorescent	Photoperiod: 24 h d^{-1} PPFD: 400 µmol m^{-2} s^{-1} DLI: 34.56 Light: fluorescent	12	**Positive**: higher plant FW and leaf chlorophyll concentration.	Kumar *et al.,* 2022
Nasturtium (*Tropaeolum majus* L.), plants	Photoperiod: 16 h d^{-1} PFFD: 300 µmol m^{-2} s^{-1} DLI: 17.3 mol m^{-2} d^{-1} Light: LED	Photoperiod: 24 h d^{-1} PPFD: 200 µmol m^{-2} s^{-1} DLI: 17.3 mol m^{-2} d^{-1} Light: LED	21	**Positive**: higher plant FW and DW; higher leaf antioxidant capacity.	Xu *et al.*, 2021
Pak-choi (*Brasica rapa* var. *chinensis*), plants	Photoperiod: 12 h d^{-1} PFFD: 100 µmol m^{-2} s^{-1} DLI: 4.32 mol m^{-2} d^{-1} Light: LED	Photoperiod: 24 h d^{-1} PPFD: 100 µmol m^{-2} s^{-1} DLI: 8.64 mol m^{-2} d^{-1} Light: LED	30	**Positive**: higher plant FW and DW.	Harun *et al.*, 2019
Purslane (*Portulaca oleracea* L.), plants	Photoperiod: 12 and 18 h d^{-1} PFFD: 240 and 480 µmol m^{-2} s^{-1} DLI: 20.37 and 20.74 mol m^{-2} d^{-1} Light: LED	Photoperiod: 24 h d^{-1} PPFD: 240 µmol m^{-2} s^{-1} DLI: 20.74 mol m^{-2} d^{-1} Light: LED	16	**Negative**: lower plant FW and DW at the same DLI; higher leaf nitrate concentration.	He *et al.*, 2023
Radish (*Raphanus sativus* L.), plants	Photoperiod: 12, 16, and 20 h d^{-1} PFFD: 320 µmol m^{-2} s^{-1} DLI: 13.82, 18.43 and 23.04 mol m^{-2} d^{-1} Light: metal halide	Photoperiod: 24 h d^{-1} PPFD: 320 µmol m^{-2} s^{-1} DLI: 27.65 mol m^{-2} d^{-1} Light: metal halide	14	**Positive**: higher plant FW and DW; higher leaf chlorophylls concentration.	Inada and Yakumoto, 1989
Radish (*Raphanus sativus* var. *radical* Pers.), microgreens	Photoperiod: 16 h d^{-1} PFFD: 270 µmol m^{-2} s^{-1} DLI: 15.6 mol m^{-2} d^{-1} Light: LED	Photoperiod: 24 h d^{-1} PFFD: 180 and 270 µmol m^{-2} s^{-1} DLI: 15.6 and 23.3 mol m^{-2} d^{-1} Light: LED	12	**Positive**: higher yield and nutritional value (higher concentration of pigments, flavonoids, and proline) and lower nitrate level. Short-term (3 d) CL before harvest increased nutritional value	Shibaeva *et al*., 2022

Crop (species)	Regular light regime (control)	Continuous lighting (CL)	Treatment duration (d)	Effect of CL on plant growth and/or leaf quality compared with the control	References
Red beet (*Beta vulgaris L.* ssp. *vulgaris*), leaves	Photoperiod: 6, 12, and 18 h d^{-1} PFFD: 540 µmol m^{-2} s^{-1} DLI: 11.66, 23.33, and 34.99 mol m^{-2} d^{-1} Light: fluorescent and incandescent	Photoperiod: 24 h d^{-1} PPFD: 540 µmol m^{-2} s^{-1} DLI: 46.65 mol m^{-2} d^{-1} Light: fluorescent and incandescent	21	**Negative**: reduction of plant FW and DW, and leaf antioxidant capacity and concentration of pigments and polyphenols.	Ali *et al.*, 2009
Rocket (*Eruca sativa* L.), plants	Photoperiod: 16 and 20 h d^{-1} PFFD: 250 µmol m^{-2} s^{-1} DLI: 14.4 and 18 mol m^{-2} d^{-1} Light: LED	Photoperiod: 24 h d^{-1} PPFD: 250 µmol m^{-2} s^{-1} DLI: 21.6 mol m^{-2} d^{-1} Light: LED	21	**No effect** on plant FW and DW.	Pennisi *et al.*, 2020
Rocket (*Eruca vesicaria* L.), leaves	Photoperiod: 12 h d^{-1} PFFD: 600 µmol m^{-2} s^{-1} DLI: 25.92 mol m^{-2} d^{-1} Light: LED	Photoperiod: 24 h d^{-1} PPFD: 300 µmol m^{-2} s^{-1} DLI: 25.92 mol m^{-2} d^{-1} Light: LED	30	**Positive**: higher plant FW and DW; higher leaf concentrations of pigments and antioxidants; lower nitrate level.	Proietti *et al.*, 2021
Rocket (*Eruca vesicaria* sp. *sativa* Mill.), microgreens	Photoperiod: 16 h d^{-1} PFFD: 270 µmol m^{-2} s^{-1} DLI: 15.6 mol m^{-2} d^{-1} Light: LED	Photoperiod: 24 h d^{-1} PFFD: 180 and 270 µmol m^{-2} s^{-1} DLI: 15.6 and 23.3 mol m^{-2} d^{-1} Light: LED	12	**Positive**: higher yield and nutritional value (higher concentration of anthocyanins, flavonoids, carotenoids, and proline) and lower nitrate content. Short-term (3 d) CL before harvest increased nutritional value and decreased nitrate level.	Shibaeva *et al.*, 2022
Spinach *(Spinacea oleracea* L.), leaves	Photoperiod: 6, 12, and 18 h d^{-1} PFFD: 540 µmol m^{-2} s^{-1} DLI: 11.66, 23.33, and 34.99 mol m^{-2} d^{-1} Light: fluorescent and incandescent	Photoperiod: 24 h d^{-1} PPFD: 540 µmol m^{-2} s^{-1} DLI: 46.65 mol m^{-2} d^{-1} Light: fluorescent and incandescent	21	**Negative**: reduction of plant FW and DW, leaf antioxidant capacity, and concentration of pigments and polyphenols.	Ali *et al.*, 2009
Sweet basil (*Ocimum basilicum* L.) plants	Photoperiod: 16 and 20 h d^{-1} PFFD: 250 µmol m^{-2} s^{-1} DLI: 14.4 and 18 mol m^{-2} d^{-1} Light: LED	Photoperiod: 24 h d^{-1} PPFD: 250 µmol m^{-2} s^{-1} DLI: 21.6 mol m^{-2} d^{-1} Light: LED	21	**No effect** on plant FW and DW.	Pennisi *et al.*, 2020
Sweet basil (*Ocimum basilicum* L.), microgreens	Photoperiod: 16 h d-1 PFFD: 250.8; 376.8 µmol m^{-2} s^{-1} DLI: 14; 21 mol m^{-2} d^{-1} Light: LED	Photoperiod: 24 h d^{-1} PPFD: 166.6; 247.6 µmol m^{-2} s^{-1} DLI: 14; 21 mol m^{-2} d^{-1} Light: LED	19	**Positive**: higher FW at the same DLI in green basil. No effects on red basil.	Lanoue *et al.*, 2022
Swiss chard (*Beta vulgaris L.* ssp. *cicla*), leaves	Photoperiod: 6, 12, and 18 h d^{-1} PFFD: 540 µmol m^{-2} s^{-1} DLI: 11.66, 23.33, and 34.99 mol m^{-2} d^{-1} Light: fluorescent and incandescent	Photoperiod: 24 h d^{-1} PPFD: 540 µmol m^{-2} s^{-1} DLI: 46.65 mol m^{-2} d-1 Light: fluorescent and incandescent	21	**Negative**: reduction of plant FW and DW, leaf antioxidant capacity and concentration of pigments and polyphenols.	Ali *et al.*, 2009

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Glucose exogenous increases biochemical and physiological responses in *Beta vulgaris* L.

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Key words: Antioxidant activity, elicitation, glucose, leaf pigments, phenolic compounds, secondary metabolism, yield.

Abstract: Glucose can act as a bioregulator in plants, influencing physiological and metabolic processes. This investigation aimed to analyses how the application of glucose impacts the photosynthetic rate, growth, and concentration of bioactive compounds with antioxidant potential in beet. The current investigation evaluated the effect of glucose (0, 5, 10, 20, and 30 mmol L^{-1}) on beet plants 10 days after transplantation. Glucose elicitation in beet plants increases levels of bioactive compounds, resulting in higher antioxidant potential. In addition to antioxidant benefits, glucose also plays a hormonal role, leading to increases in biomass. These effects are correlated with enhanced photosynthetic efficiency, elevated carbohydrate concentrations, and positive modulation of the plant antioxidant system.

1. Introduction

Beta vulgaris L (beet) belonging to the Chenopodiaceae family, is a nutritious vegetable often used in salads and juices. It is an excellent source of nutrients, including vitamins (folic acid, and vitamin A, B1, B2, B6, and C), minerals (Sadowska-Bartosz and Bartosz, 2021), fiber, proteins (Fu *et al.,* 2020 a), and sugar (sucrose) (Tayyab *et al.,* 2023). Additionally, beet is considered a great health food, due to the high levels of bioactive compounds present, such as betalains, polyphenols, and flavonoids (Gorni *et al.,* 2023), which are powerful antioxidants associated with cardiovascular health and the reduction of cancer cells, among other diseases (Chhikara *et al.,* 2019). In addition to its nutritional properties, beets are valued for their diverse culinary applications. It can be consumed raw, grated in salads, cooked, roasted, grilled, and even turned into juices and smoothies.

Beet production faces major challenges due to climate change. Rising

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temperatures and changes in rainfall patterns affect both its growth, development, and the formation and quality of roots (Tayyab *et al.,* 2023). In addition, water shortages and the emergence of pests and diseases are becoming more frequent, putting productivity at risk (Baryga *et al.,* 2020). To deal with these problems, new techniques are being adopted to improve agricultural practices, making crops more sustainable and stimulating the plant to biosynthesize more bioactive compounds, which results in increased plant resistance to climate changes.

Elicitation has been used as a technique that involves the induction of responses in plants, generally in response to biotic (such as pathogens or herbivores) or abiotic (such as climate variations) stresses (Kandoudi and Németh-Zámboriné, 2022). Elicitors can act at several levels, such as activating plant defence mechanisms. For example, elicitation technique can be used to increase the amount of phytocompounds in plant, improving their functionality, this practice has been applied to increase the yield of secondary metabolites, biological activities (antioxidant properties) and mainly to induce plant growth (Baenas *et al.*, 2019; Siddiqui *et al.*, 2020; Gorni *et al.,* 2023) and can also stimulate cellular signaling pathways, leading to the expression of genes related to defence and strengthening of cellular structure (Sami *et al.,* 2021).

Among the various elicitors, glucose (Glc) has been studied due to its important roles in plants, to increase the biosynthesis and accumulation of secondary metabolites, resulting in increased plant yield (Hennion *et al.,* 2019). Regarding the elicitors that act in secondary metabolism, it is known that Glc is an essential sugar that plays a vital role in plant metabolism, acting as one of the main sources of energy (Saddhe *et al*., 2021). Produced during photosynthesis, it not only feeds plants, but is also essential for their health and growth (Fu *et al.,* 2020 b). In addition to providing energy, Glc is an important component in the formation of several biomolecules necessary for plant development (Wang *et al.,* 2019).

Given this information, some researchers have observed that the application of Glc acted as a growth inducer for plants, which was correlated with increased photosynthesis (Siddiqui *et al.,* 2020), seed germination (Gorni and Polimento, 2023), regulates antioxidant metabolism (Sami and Hayat, 2019), activates key enzymes involved in secondary metabolism (Zahid *et al.,* 2018), increases the biosynthesis of phenols, flavonoids, anthocyanins, and antioxidant activity (Xu *et al.,* 2016; Baenas *et al.,* 2019), gene expression (Rezaee *et al.,* 2018), and alleviates stresses (Sami *et al.,* 2021).

From this perspective, Glc is a substance that promotes plant growth and enhances quality by increasing the biosynthesis of bioactive compounds and reducing ROS concentration (Baenas *et al.*, 2014; Sami and Hayat, 2019). Given this information, the goal is to improve the quality and yield of beet roots through Glc application at different concentrations, aiming for a biostimulant effect. This approach could potentially enhance the growth and development of the crops, increase yield, and improve root quality. Therefore, this investigation aimed to analyses how the application of Glc impacts the photosynthetic rate, growth, and concentration of bioactive compounds with antioxidant potential in beet.

2. Materials and Methods

Experimental location and plant material

The experiment was conducted using beet plants in pots inside a greenhouse on a wooden bench, kept open sky, covered by shading which provided 50% of solar radiation located at the Escola Superior de Agronomia de Paraguaçu Paulista (ESAPP), Brazil (22°41'76" S, 50°58'33" W). The Köppen classification identifies the region's climate as Aw, the climate data is presented in figure 1. The experiment was conducted between April 19 and June 18, 2024 (60 days).

Fifteen-day-old beet seedlings were transplanted into pots with sandy soil. The soil was analysed and

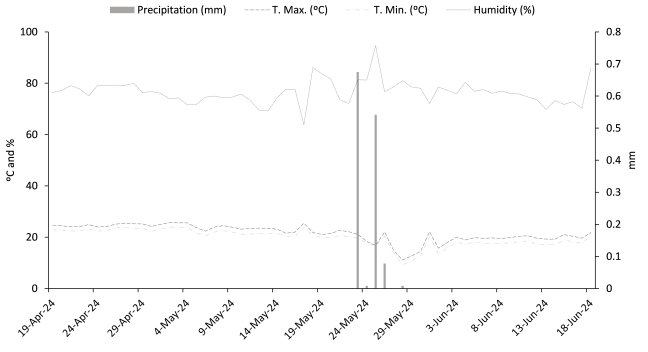

Fig. 1 - Climatic data of Paraguaçu Paulista, São Paulo, during the experimental period (April 19 to June 18, 2024). Source: INMET - Instituto Nacional de Meteorologia.

corrected according to Bulletin 100 (Cantarella *et al.,* 2022). The pots were irrigated by sprinklers twice a day during the experimental period. Every 15 days, cattle manure was applied as coverage fertilization. At 10 days after transplantation, the treatments were applied at concentrations of 0 (control), 5, 10, 20, and 30 m mol L^{-1} of Glc. Each treatment was replicated four times.

All analyses below were performed at the end of the experiment at 60 DAT.

Pigments analysis and quantification

Photosynthetic pigments were extracted in 95% ethanol and calculations were performed according to Lichtenthaler (1987). Fresh tissues (0.2 g) were added to 8 mL of 95% ethanol and shaken, leaving them to rest for 24 h at 4°C. After this process, readings were performed at wavelengths of 470, 647 and 663 nm.

Photosynthetic parameters

Chlorophyll fluorescence parameters were measured using a portable chlorophyll fluorometer (Opti-Sciences, F_V/F_M Meter). Beet leaves were placed under dark adaptation clips for 30 min prior to measurement to allow complete accumulation of chlorophyll energy. After the adaptation period, measurements of initial fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence (F_v), maximum photochemical efficiency of PSII (F_v/F_m), and absorbed energy conversion (F_v/F_0).

Biochemical assessment of enzymes activities and protein

Frozen samples were extracted in 0.1 M potassium phosphate buffer (pH 7.5) containing EDTA and PVP. The supernatant was utilized for enzyme activity assays, included those SOD (EC 1.15.1.1) was measured by the inhibition of nitroblue tetrazolium (NBT) reduction by superoxide, which is generated by the reaction of riboflavin under light (Giannopolitis and Ries, 1977), CAT (EC 1.11.1.6) is determined by the decomposition of hydrogen peroxide, which can be monitored spectrophotometrically (240 nm) (Azevedo *et al.,* 1998), and APX (EC1.11.1.11) is measured by the decrease in absorbance of ascorbic acid in the presence of H_2O_2 (Moldes *et al.*, 2008).

For phenylpropanoid pathway enzymes, leaves were extracted using 0.5 mM Tris-EDTA buffer (pH 8.5) containing EDTA. The supernatant was used to measure the activity of phenylalanine ammonia lyase (PAL, EC 4.3.1.5) following the method of Hyodo *et al.* (1978), and chalcone synthase (CHS, EC 2.3.1.74) according to Moustafa and Wong (1967). The PAL assay was conducted at 40°C for 1 hour in a reaction mixture (1 mL) consisting of 0.5 M Tris-HCl (pH 8.5), enzyme extract, and 30 µM L-phenylalanine. The reaction was stopped in an ice bath for 5 minutes. PAL activity was quantified spectrophotometrically by measuring the concentration of trans-cinnamic acid produced at 290 nm. The CHS assay was conducted at 30°C for 1 hour in a reaction mixture (1 mL) consisting of 5 mM Tris-HCl (pH 7.8), 10 mM KCN, enzyme extract, and chalcone was added to ethylene glycol monomethyl ether. CHS activity was measured by the production of chalcone at 370 nm.

Protein concentration was determined using the Bradford method (1976), where the supernatant was added to the Bradford solution, after 2 minutes the reading was taken at 590 nm, using bovine serum albumin as standard.

Biochemical assessment of ROS

To determine ROS, samples were extracted in 0.1% trichloroacetic acid. The supernatant used for the hydrogen peroxide (H_2O_2) was evaluated according to Alexieva *et al.* (2001), samples were added to a solution of 0.1 M potassium phosphate buffer (pH 7.5) and 1 M potassium iodide solution. The mixture was incubated on ice for 1 h. The reading was performed at a wavelength of 390 nm. Using H_2O_2 standards. Lipid peroxidation was by the malondialdehyde (MDA) method by Heath and Packer (1968), supernatant was mixed with a solution containing 0.5% TBA (thiobarbituric acid) in 20% TCA (trichloroacetic acid). This mixture was incubated at 95°C for 30 min, and the reaction was stopped with an ice bath. The absorbances were 535 nm and 600 nm. Superoxide radical ($O_2^{\cdot-}$) content was evaluated according to Elstner and Heupel (1976), sample was added to a 65 mM potassium phosphate buffer solution (pH 7.8) and a 10 mM hydroxylamine hydrochloride solution and incubated at 25 ºC for 20 min. After incubation, 1% (m/v) sulfanilamide in hydrochloric acid (2.4 N) and 0.02% N-(1-naphthylethylenediamine) dihydrochloride were added. The reaction mixture was homogenized and incubated again at 25°C for 20 min. Finally, ethyl ether was added, the solution was centrifuged at 1500 rpm for 5 min, and the wavelength used was 530 nm.

Determination of nitrogen compounds

For extraction, leaf samples were macerated in 80% ethanol solution. The material was macerated and centrifuged, and the supernatant was used for analysis.

Total soluble sugar content was determined according to Dubois *et al.* (1956), sample was mixed with a phenolic reagent (5%) and then sulfuric acid was added. After cooling, the absorbance was read at 390 nm, using glucose as a standard.

Reducing sugar content was determined using Somogyi-Nelson (Bezerra Neto and Barreto, 2004), sample was mixed with the stirred Somogyi reagent and incubated in boiling water for 15 minutes. After cooling on ice, the Nelson reagent was added, mixed and left to stand for 20 minutes. After the addition of water, the absorbance was read at 760 nm, glucose was used as a standard.

Sucrose content was determined according to Van Handel (1968), sample was mixed with a KOH reagent (30%) and then sulfuric acid was added. The mixture was incubated at 100°C for 10 minutes, after cooling the absorbance was read at 490 nm, using sucrose as a standard.

Starch content was determined using anthrone reagent (Sadasivam and Manickam, 1996). Samples were mixed in anthrone reagent and boiled for 10 minutes, the mixture was cooled, and the absorbance read at 650 nm, glucose was used as a standard.

Total amino acids content was determined according to Yemm *et al.* (1955). Samples were mixed in sodium citrate (0.2 M), ninhydrin solution (5%) and KCN (0.2 mM). The mixture was homogenized and heated at 100°C for 20 minutes. After cooling in running water, 60% ethanol was added and the absorbance was read at 570 nm, using methionine as a standard.

Determination of proline

Proline was determined by Bates *et al.* (1973). For extraction, leaf samples were macerated in a 5-sulfosalicylic acid solution (3%), the material was macerated and centrifuged, and the supernatant was used for analysis. The sample was mixed with a solution of acid ninhydrin and acetic acid, which was incubated at 100°C for 1 h. Afterwards, the proline was partitioned with the addition of toluene and the mixture was shaken. Absorbance was read at 520 nm, using proline as a standard.

Biochemical analysis of tuberous root

Fresh beet root (5 g) was subjected to a washing and peeling process and extracted with a processor for two minutes. After this procedure, the juice was filtered through sieves (2 mm) and stored in a refrigerator before analysis.

Total soluble solids

The analysis of total soluble solids was performed using an analogue refractometer, where the samples were centrifuged, and a drop deposited on the refractometer prism and the values were collected. The results were expressed in degrees Brix (°Brix).

Total polyphenols, flavonoids, anthocyanins, carotenoid, and betalains concentrations

Polyphenols concentrations was determined according to the Folin-Ciocalteu method and 25% sodium carbonate solution (Stagos *et al.,* 2012), using gallic acid as standard. Flavonoids concentrations were measured according to Yao *et al*. (2013), using 5% $NaNO_2$, 10% $AlCl_3$, and NaOH (1 M), using rutin as standard. Anthocyanins were determined according to the Francis method (1982), in 95% ethanol extraction solution acidified with HCl, the mixture was left to rest for 24 hours at 4°C, protected from light, after which it was filtered and the absorbance read at 535 nm. The carotenoid content was extracted from fresh leaf samples with chloroform/acetone/ethanol (2:1:1, v/v/v) (Sadler *et al*., 1990). For determination of β-carotene, absorbance was measured at wavelengths of 450 nm. Quantification of betalains followed the methodology of Nilsson (1970), root samples were homogenized, and absorbance readings were performed at wavelengths of 480 nm and 540 nm.

DPPH activity

DPPH radical scavenging was determined by Blois (1958). Samples were mixed with ethanol, acetate buffer (0.1 M, pH 5.5) and a DPPH solution (0.5 mM) was added. After resting in the dark for 30 minutes, absorbance was read at 517 nm. The results were expressed as a percentage (%).

Growth and yield parameters

The measured traits included the number of leaves. Using a caliper, measurements were taken of the root diameter, and length. For shoot, root, and total fresh weight a precision scale was used.

Experimental design and statistical analysis

Analysis of the data was performed using Sisvar (Ferreira, 2019). ANOVA was performed on the data to determine the Tukey's test ($p \leq 0.01$). A multivariate analysis of variables was performed for all parameters using SigmaPlot software.

3. Results

Beet plants treated with Glc showed significant results in chlorophyll fluorescence (Fig. 2). The F_0 values exhibited reductions of 15.6%, 14.9%, 16.48%, and 12.2%, respectively, compared to the control (Fig. 2 a). Increases were observed for F_m by 5.5%, 9.8%, 1.5%, and 4.6% (Fig. 2 b), F_v by 10.8%, 16.1%, 6.0%, and 8.9% (Fig. 2 c), F_v/F_m by 5.1%, 5.36%, 4.5%, and 4.1% (Fig. 2 d), and F_v/F_0 by 31.3%, 36.5%, 27.3%, and 24.4% (Fig. 2 e), respectively, compared to untreated plants.

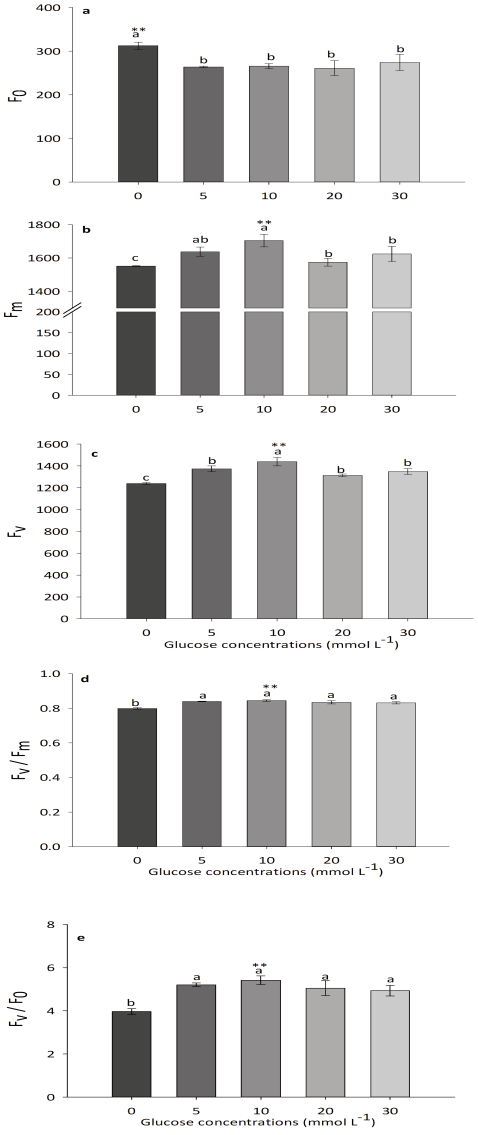

Fig. 2 - *F0* (a), *Fm* (b), *Fv* (c), *Fv/Fm* (d), and *Fv/F0* (e) in beet plants treated with different glucose concentrations. **= significant at a probability level of p≤0.01 using the Tukey's test at 5% probability. Bars represent standard error of the mean; n = 4.

Chlorophyll concentrations treated with 5 and 20 mmol L^{-1} of Glc increased by 47.2% and 20.2% for Chlorophyll *a*, 42.6% and 17.4% for Chlorophyll *b*, 46.2% and 19.6% for Total Chlorophyll, and 39.9% and 13.6% for Carotenoids, respectively, compared to the control. However, when the Glc concentration was 10 and 30 mmol L^{-1}, the Chlorophyll a, Chlorophyll b, Total Chlorophyll, and Carotenoids were lower than those obtained without Glc treatment (Fig. 3).

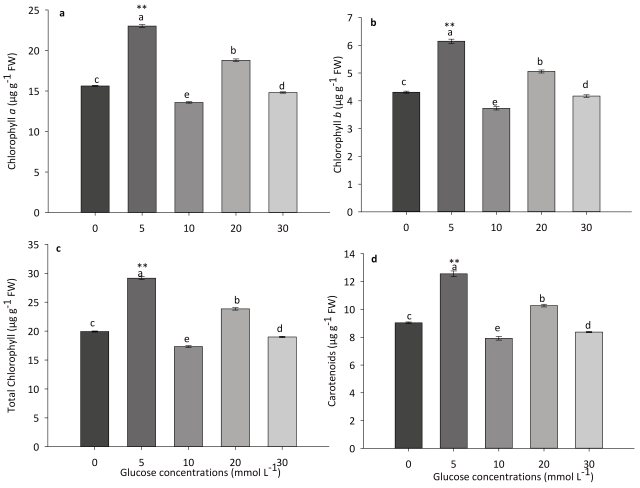

Fig. 3 - Chlorophyll a (a), chlorophyll b (b), total chlorophyll (c) and carotenoids (d) concentrations in beet plants treated with different glucose concentrations. **= significant at a probability level of p≤0.01 using the Tukey's test at 5% probability. Bars represent standard error of the mean; n = 4. FW = fresh weight.

Beet plants treated with Glc showed reductions in MDA concentrations of 82.5%, 31.9%, 40.9%, and 38.9%, respectively, compared to the control (Fig. 4 a). However, H_2O_2 concentrations decreased by 27.2% and 13.1%, respectively, in plants treated with 5 and 10 mmol L^{-1} of Glc compared to the control (Fig. 4b), and concentrations of 20 and 30 mmol L^{-1} of Glc, did not differ from the control. As for O_2^- concentrations, there was an increase of 15.3% in plants treated with 20 mmol L^{-1} of Glc, while concentrations of 5 and 10 mmol L^{-1} decreased by 18.1% and 62.2%, respectively, compared to the

control (Fig. 4 c).

Regarding the activity of the SOD enzyme, treatments with 5, 10, and 30 mmol L^{-1} of Glc showed increases of 37.9%, 15.0%, and 12.4%, respectively, compared to the control (Fig. 5 a). However, when the Glc concentration was 20 mmol L^{-1} was lower than those obtained without Glc treatment. For CAT and APX, there was an increase in activity in plants treated with 5, and 10 mmol L^{-1}, respectively, compared to the control (Fig. 5 b, 5 c). However, the application of concentrations of 20 and 30 mmol L^{-1} showed lower activity than those obtained without treatment with Glc.

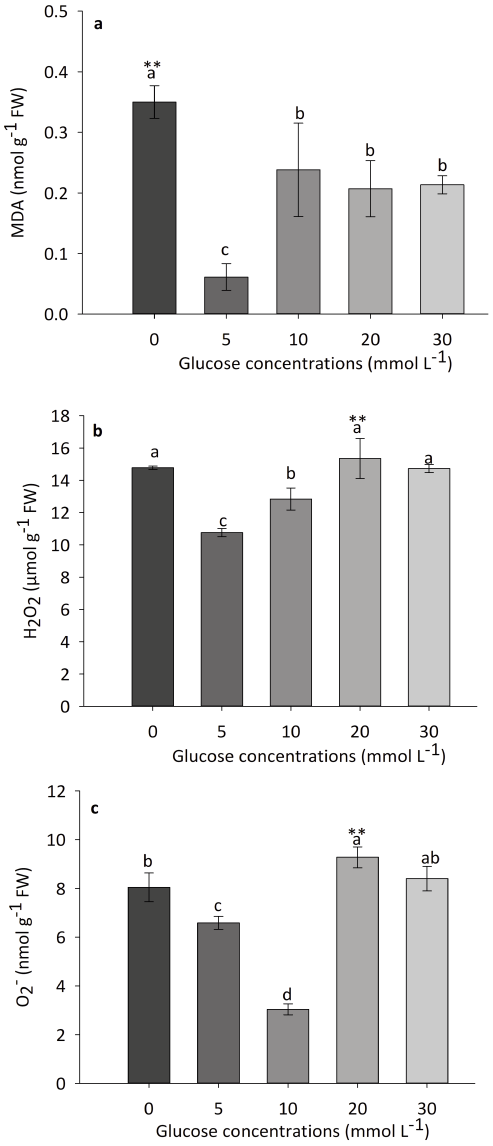

Fig. 4 - MDA (a), H_2O_2 (b), and O_2^- (c) concentrations in beet plants treated with different glucose concentrations. **= significant at a probability level of p≤0.01 using the Tukey's test at 5% probability. Bars represent standard error of the mean; n= 4. FW = fresh weight.

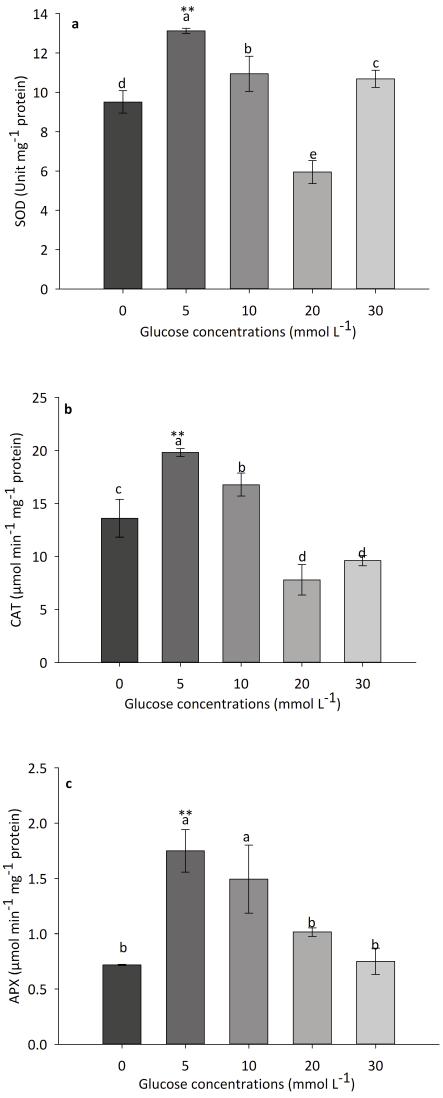

Fig. 5 - Specific activity of SOD (a), CAT (b), and APX (c) in beet plants treated with different glucose concentrations. **: significant at a probability level of p ≤ 0.01 using the Tukey's test at 5% probability. Bars represent standard error of the mean; n = 4.

Total sugar concentrations in plants treated with 10 and 30 mmol L^{-1} of Glc showed increases of 5.4% and 11.3%, respectively, compared to the control (Fig. 6 a). For reducing sugar concentrations, applications of 10, 20, and 30 mmol L^{-1} of Glc increased by 61.6%, 41.1%, and 53.8%, respectively, compared to the control (Fig. 6 b). Sucrose concentrations increased by 14.4% and 32.9%, respectively, in plants treated with 10 and 20 mmol L^{-1} of Glc compared to the control (Fig. 6 c). Starch concentrations were higher by 9.3%, 30.7%, and 18.9%, respectively, in plants treated with 10, 20, and 30 mmol L^{-1} of Glc compared to the control (Fig. 6 d), however, when the Glc concentration was 5 mmol L^{-1}

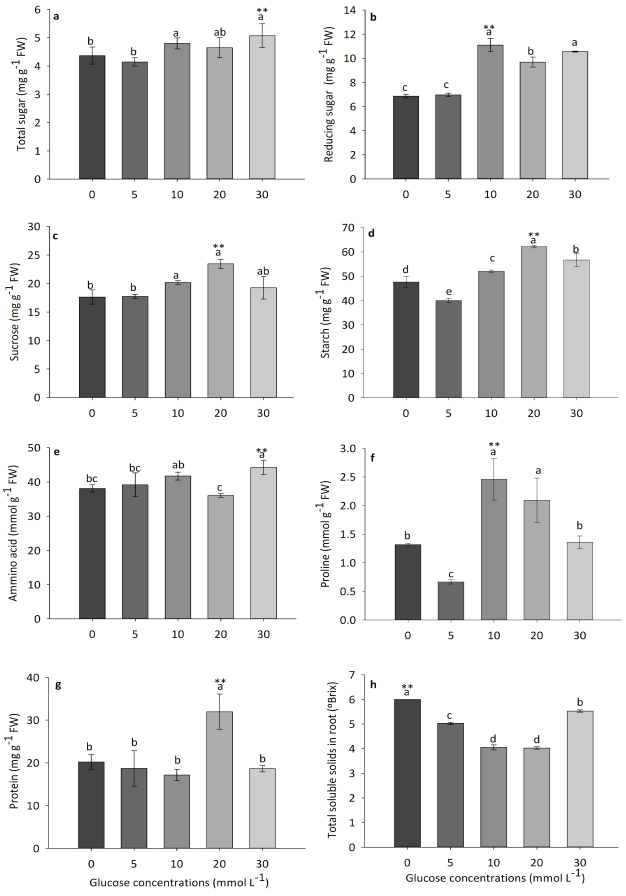

Fig. 6 - Total sugar (a), reducing sugar (b), sucrose (c), starch (d), amino acid (e), proline (f), protein (g), and total soluble solids in root (h) concentrations in beet plants, and total soluble solids in juice (f) treated with different glucose concentrations. **= significant at a probability level of p≤0.01 using the Tukey's test at 5% probability. Bars represent standard error of the mean; n= 4. FW= fresh weight.

was lower than those obtained without Glc treatment. For amino acid concentrations, only plants treated with 30 mmol L^{-1} of Glc showed an increase of 16.1% compared to the control (Fig. 6 e). However, proline concentrations increased by 86.5% and 58.7%, respectively, in plants treated with 10 and 20 mmol L^{-1} of Glc compared to the control (Fig. 6 f), however, when the Glc concentration was 5 mmol L^{-1} was lower than those obtained without Glc treatment. Protein content increased by 26.6% in plants treated with 20 mmol L^{-1} of Glc compared to the control (Fig. 6 g). However, total soluble solids in tuberous roots decreased in all treatments compared to the control (Fig. 6 h).

The PAL enzyme activity showed increases of 30.3%, 48.8%, and 42.0%, respectively, in plants treated with 5, 10, and 30 mmol L^{-1} of Glc compared to the control (Fig. 7 a). However, the CHS enzyme activity increased by 7.9%, and 17.2%, respectively, in plants treated with 10, and 30 mmol L^{-1} of Glc compared to the control (Fig. 7 b). However, the application of concentration of 20 mmol L^{-1} showed lower activity than those obtained without treatment with Glc. The application of glucose at 30 mmol L^{-1} resulted in increases of 21.2% for polyphenols, 34.6% for flavonoids, 22.7% for β-carotene, 26.9% for anthocyanins, and 28.7% for betalains, respectively, compared to the control (Fig. 8 a-e). However, the application of concentrations of 5, 10, and 20 mmol L^{-1} showed lower activity than those obtained without treatment with Glc respectively. However, increases of 27.6%, 99.4%, and 20.4% were observed at concentrations of 5, 20, and 30 mmol L^{-1}, respectively, in the antioxidant activity by the DPPH

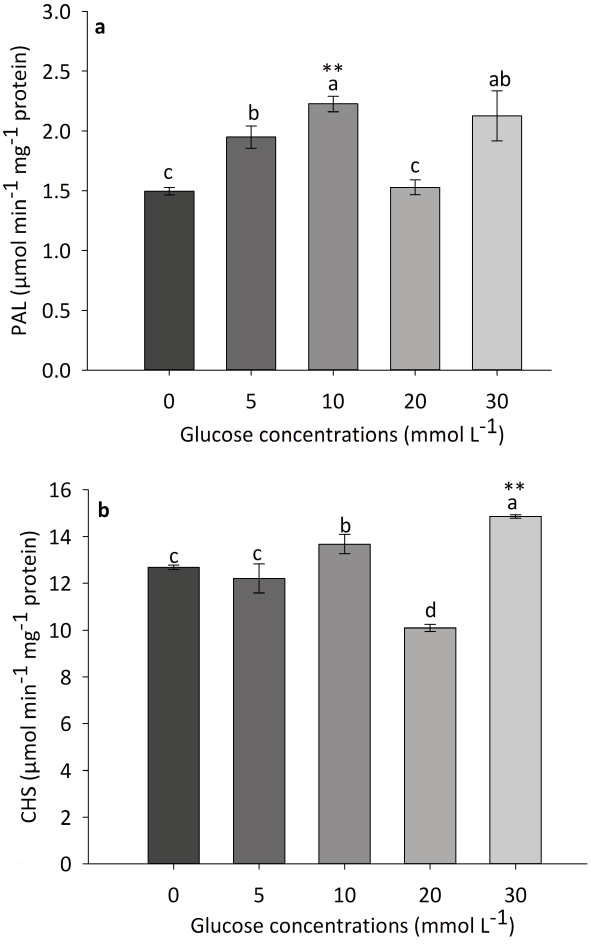

Fig. 7 - Specific activity of phenylalanine ammonia lyase (PAL) (a), chalcone synthase (CHS) (b) in beet plants treated with different glucose concentrations. **= significant at a probability level of p≤0.01 using the Tukey's test at 5% probability. Bars represent standard error of the mean; *n* = 4.

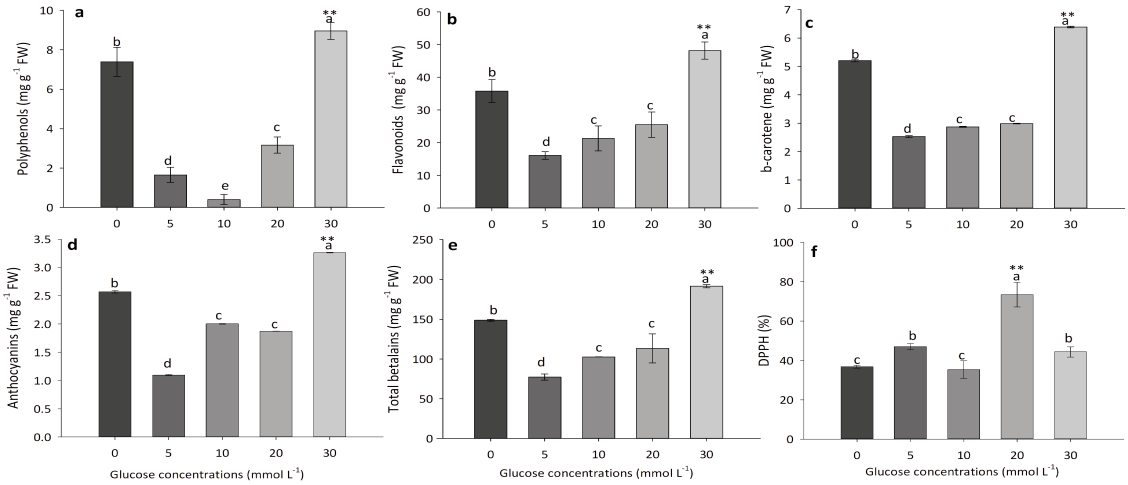

Fig. 8 - Polyphenols (a), flavonoids (b), β-carotene (c), anthocyanins (d), total betalains (e), and DPPH (f) concentrations in beet plants treated with different glucose concentrations. **: significant at a probability level of $p \leq 0.01$ using the Tukey's test at 5% probability. Bars represent standard error of the mean; n = 4. FW = fresh weight.

method compared to the control plants (Fig. 8 f).

For growth analysis, plants treated with 20 mmol L^{-1} of Glc showed increases in leaf fresh weight by 113.2% compared to the control (Fig. 9 a). Root fresh weight increased by 45.9% in plants treated with 10 mmol L^{-1} compared to the control (Fig. 9 b). Total fresh weight increased by 24.6% and 26.6%, respectively, in plants treated with 5 and 20 mmol L^{-1}

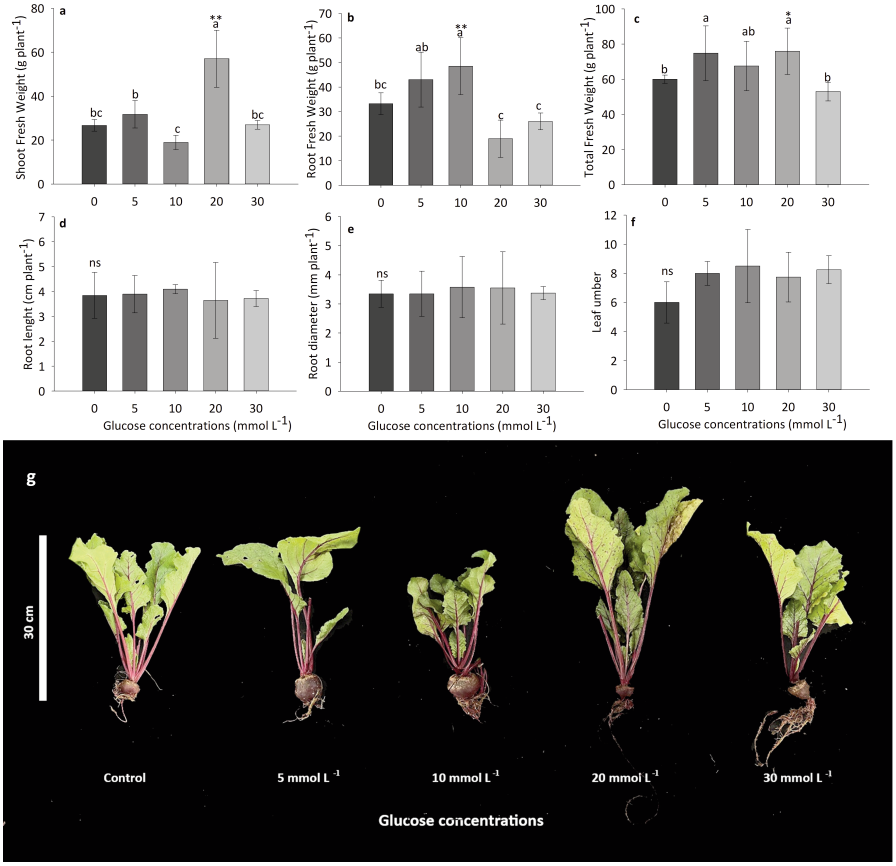

Fig. 9 - Shoot fresh weight (a), root fresh weight (b), total fresh weight (c), root length (d), and root diameter (e), and leaf number (f), and visual growth development (g) in beet plants treated with different glucose concentrations. ** and *= significant at a probability level of $p \leq 0.01$ and $p \leq 0.05$; NS= not significant using the Tukey's test at 5% probability. Bars represent standard error of the mean; n = 4.

of Glc compared to the control (Fig. 9 c). However, root length, root diameter, and number of leaves did not show significant responses to glucose application (Fig. 9 d-f).

PCA was conducted to evaluate the relationship between morpho-physiological traits and Glc treatments (Fig. 10). The variables were depicted along two principal axes, which together explained 57.81% of the total variance (PC1: 32.18%; PC2: 25.63%). This analysis indicated a positive correlation between biometric characteristics and the 20 mmol L^{-1} concentration. In contrast, physiological and biochemical parameters were associated with the 5, 10, and 30 mmol L^{-1} concentrations.

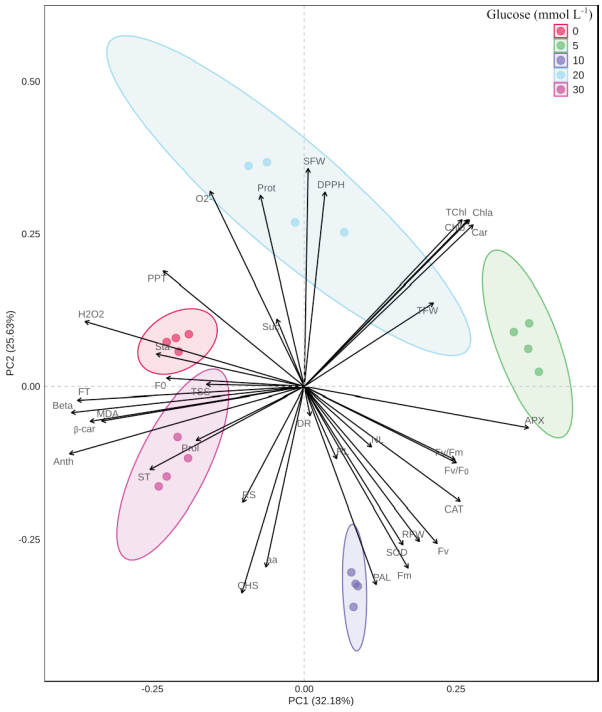

Fig. 1 - PCA of the analyzed parameters from beet plants treated with different glucose concentrations. Minimum fluorescence (F0), maximum fluorescence (Fm), variable fluorescence (Fv), maximum photochemical efficiency (Fv/Fm), variable fluorescence/initial fluorescence (Fv/F0), chlorophyll a (Chla), chlorophyll b (Chlb), total chlorophyll (TChl), carotenoid (CAR), malondialdehyde (MDA), hydrogen peroxide (H2O2), superoxide radical (O2-), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), proline (Pro), amino acids (aa), total sugar (ST), reducing sugar (RS), sucrose (SUC), starch (STA), phenylalanine ammonia lyase (PAL), chalcone synthase (CHS), total flavonoids (FT), total polyphenols (PPT), anthocyanin (Anth), β-carotene (β-car), 2,2-diphenyl-1-picrylhydrazyl (DPPH), total soluble solids (TSS), protein (Prot), root size (SR), root diameter (RD), number of leaves (NL), shoot fresh weight (SFW), root fresh weight (RFW), and total fresh weight (TFW).

4. Discussion and Conclusions

In the present study, the data show that chlorophyll fluorescence differed among treatments (Fig. 2). However, chlorophyll fluorescence analysis has been widely used to assess photosynthetic efficiency in plants, as this technique is sensitive to changes in the environment and plant metabolism (Jiang *et al.,* 2012). Based on the results found, Glc can act as an energy substrate for plant metabolic processes, particularly for photosynthesis (Sinha and Roitsch, 2001). With increased Glc availability in the plant, there was consequently an increase in photosynthetic rate, thereby enhancing energy production and improving light absorption efficiency, resulting in higher chlorophyll fluorescence (Wang *et al.,* 2019).

The use of Glc showed positive responses in several crops, such as promoting germination and vegetative growth, inducing greater plant yields (González-Hernández *et al.,* 2020; Gorni and Polimeno, 2023). Although Glc is a byproduct of photosynthesis, its exogenous application can stimulate the synthesis of photosynthetic pigments (Siddiqui *et al.,* 2020), which supports our findings (Fig. 3). Furthermore, studies indicate that elicitation with Glc enhances electron transfer from PSII to PSI, as well as increases ATP and NADPH production, crucial for various biochemical reactions in plants, thereby influencing carbohydrate, lipid, and protein synthesis and regulating biochemical processes (Rineau *et al.,* 2013; Siddiqui *et al.,* 2020). Similar results confirm that Glc application stimulates pigment accumulation in plants, as observed in mustard (*Brassica juncea*) (Sami *et al.,* 2021), triticale (× *Triticosecale Wittmack*) (Wang *et al.,* 2019), and cucumber (*Cucumis sativus*) (Yusuf *et al.,* 2021).

During photosynthesis and other aerobic metabolic processes, plants generate reactive oxygen species (ROS) as part of their normal metabolism and in response to environmental stresses. However, factors such as biotic and abiotic stresses can induce higher ROS production in plants as part of their defense mechanisms, leading to cellular damage and oxidative stress (Das and Roychoudhury, 2014). Our results demonstrated that Glc was effective in reducing ROS accumulation compared to the control (Fig. 4). Considering these findings, Glc elicitation induced an adaptive response in beet plants, increasing the activity of SOD, CAT, and APX (Fig. 5), which correlated with reduced ROS levels,

contributing to a more stable redox balance and fewer ROS-induced cellular damages (Siddiqui *et al.,* 2020). Similar results have shown increased activity of antioxidant enzymes and reduced ROS levels following Glc elicitation in mustard and cucumber plants (Sami *et al.,* 2021; Yusuf *et al.,* 2021).

Glc is a simple sugar that, when applied to plants, can stimulate carbohydrate metabolism, including increased synthesis of other carbohydrates such as sucrose and reducing sugars (Siddiqui *et al.,* 2020). Our results demonstrate that Glc application increased concentrations of total sugars, reducing sugars, sucrose, starch, and proteins in beet plants (Fig. 6). These findings indicate that Glc application was effective, as when absorbed by plants, this substance can be converted into starch as a form of energy storage, resulting in increased availability of carbohydrates and proteins (Siddiqui *et al.,* 2020). Additionally, there were increases in amino acid and proline concentrations (Fig. 6 e-f). The exogenous application of Glc results in a significant increase in amino acid concentration, as Glc serves as a substrate for the synthesis of various compounds, including amino acids. Moreover, exogenous Glc application can induce glutamine synthetase and glutamate dehydrogenase enzymes involved in amino acid synthesis (Forde and Lea, 2007; González-Hernández *et al.,* 2020). Furthermore, Glc can also modulate the enzyme P5CS (pyrroline-5-carboxylate reductase), leading plants to increase proline production as part of their adaptive strategies (Sami and Hayat, 2019).

Glucose application increases PAL and CHS activities in plants, which are responsible for the production of phenolic and flavonoids compounds (Wei *et al.,* 2011). These compounds play crucial roles in defense against biotic and abiotic stresses, flower and fruit pigmentation, and other essential physiological functions for plant development and adaptation to the environment (Baenas *et al.,* 2019). Studies on Glc application in plants have been shown to stimulate the expression of genes encoding enzymes, particularly plant defense enzymes (PAL, CHS, CHI), thereby inducing increases in the concentration of secondary metabolites. This approach is considered more efficient for enhancing the production of desired secondary metabolites and for manipulating biochemical and metabolic pathways (Guo *et al.,* 2011; Zahid *et al.,* 2018). Our results demonstrate that Glc application induced increases in PAL and CHS activity (Fig. 7), as well as an increase in secondary compounds at a concentration of 30 mmol L^{-1} (Fig. 8a-g), resulting in enhanced plant antioxidant activity (Fig. 8h). These compounds have garnered significant interest due to their positive impacts on various fundamental cellular processes, in addition to possessing antioxidant, anti-inflammatory, anticancer, among others (Chen, 2016). However, Glc application stimulated the increase of these compounds, making these plants more valuable for human consumption due to their rich source of bioactive nutrients, promoting a healthy and balanced diet.

The use of Glc can induce improvements in vegetative growth and yield in plants (Sami and Hayat, 2019; Sami *et al.,* 2021). In the present study, the beneficial role of Glc was confirmed due to the increase in fresh shoots, roots, and total weight (Fig. 9 a-c). Exogenous application of Glc promotes plant growth by acting as an energy source for cells, thereby significantly enhancing metabolic activity and positively regulating the expression of genes related to photosynthesis and hormonal signaling pathways (Mao *et al.,* 2018). These effects positively influence carbohydrate metabolism, stimulating the synthesis of reserves such as starch and sucrose, which translates into enhanced plant growth (Wang *et al.,* 2019; Gorni and Polimeno, 2023).

The results of this study show that the application of Glc provides significant improvements in the growth, development and productivity of beetroot, in addition to favoring the accumulation of bioactive compounds. These findings indicate that Glc not only serves as an energy source, but also acts as a stimulator of the plant's physiological responses, increasing its resistance to environmental stresses. The incorporation of Glc into agricultural management practices may therefore represent an effective strategy to optimize beet production, contributing to the nutritional quality and sustainability of crops. Future research may deepen the understanding of the mechanisms underlying these responses, expanding the potential of Glc in agricultural systems. This study underscores the importance of exogenous Glc application not only as an essential nutrient but also as a key modulator of biochemical processes that control plant development. It can thereby optimize agricultural management and crop yield.

Glucose application had a positive impact on the development of beets, stimulating different processes according to the concentration used. At 5

and 10 mmol L^{-1}, there was greater plant growth, increased chlorophyll fluorescence and activation of antioxidant enzymes. Higher concentrations (30 mmol L^{-1}) favored the production of pigments and bioactive compounds. These results indicate that Glc can act as a metabolic signal, helping the plant grow better and strengthen its defense mechanisms

Although the results of this study are promising, some limitations should be considered. The experiment was carried out in a greenhouse. To advance this line of research, future studies should test the application of glucose under field conditions and evaluate how different environmental factors affect its benefits for beet. Another important factor is the specific stage of the plant at which glucose application is more efficient, in addition to better exploration of the molecular processes involved in this response. These investigations can contribute to making this strategy even more efficient and applicable to real farming systems.

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Exploring grafting to propagate and conserve *Garcinia kola* a vulnerable species in Côte d'Ivoire

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Key words: Domestication, cultivation, *Garcinia kola*, grafting, season.

Abstract: *Garcinia kola*, a high-socioeconomic and multipurpose fruit tree, has been shown to have a low germination and long juvenile phase, which limit its cultivation initiative. Our study aimed to determine successful *G. kola* grafting conditions to promote its cultivation. So trials were carried out in June and August, testing four grafting methods: simple whip, top cleft, side cleft, and chip budding made on 12- and 36-month-old rootstock. The grafts were unwrapped at 15, 22, and 29 days after grafting. The results showed that the month of June is the favorable period for *G. kola* grafting. The best grafting success was observed with the chip budding and top cleft grafting methods applied on the 36- and 12-month-old rootstocks, respectively. The graft unwrapping time at 29 days after grafting promotes the best grafting success. The combined effect showed that the best graft success rate was recorded in June on 36-month-old rootstock (43.33%) with the chip budding and side cleft grafting method and 83.33% for the graft made on 12-month-old rootstock with the top cleft grafting method, respectively. This study is an important step for good plant material for domestication and cultivation of this resource.

1. Introduction

Garcinia kola is a native tree found in the tropical rainforests of West and Central Africa and belongs to the Clusiaceae family. *G. kola* is recognized as one of the non-timber forest products of great socio-economic importance (Assogbadjo *et al*., 2017; Codjia *et al*., 2018). The

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fruits, seeds, stems, roots, and bark are used for food and medicine for the treatment of several diseases such as diarrhea, laryngitis, gonorrhea, headache, and gastritis (Maňourová *et al*., 2019). The seed trade is an activity of great economic importance (Kouame *et al*., 2016; Maňourová *et al*., 2019).

Despite the importance of the species, no enhancement program is undertaken. However, *G. kola* is one of the species identified as a priority agroforestry species to benefit from a domestication program in West Africa (Franzel *et al*., 1996). In addition, *G. kola* is an agroforestry species, which contributes through the exploitation of its fruits and seeds to the diversification of farmers' income and the reduction of famine (Maňourová *et al*., 2019). However, the cultivation of the species by farmers remains very difficult due to the low germination capacity of the seeds and the time required for the plant to enter into flowering and fruiting, which is between 20 and 30 years (Yakubu *et al*., 2014; Agwu *et al*., 2018). It is therefore urgent to find effective strategies for the regeneration of elite trees that can induce early fruiting of the species. According to Sanou *et al*. (2004) and Leakey *et al*. (2017), domestication and genetic improvement strategies are necessary to conserve genetic material and improve fruit production. Leakey and Simons (2000) reported the growing interest in the genetic improvement of tropical forest trees aimed at developing tree cultivars with fruits with desirable characteristics.

Vegetative propagation of plants plays an important role in the production of high-quality plants for the domestication of forest trees (Leakey and Akinefessi, 2008). Cuttings, grafting, budding, and layering are vegetative macropropagation methods mainly used for the regeneration of several species (Love *et al*., 2017). Among these methods, grafting is the most used to improve the quality of plants. Grafting technique mainly involves joining parts of plants (scion and rootstock) in a manner to unite both parts so they can form a plant. A large number of research works have been carried out on agricultural fruit trees using grafting. Akinnifesi *et al*. (2009) reported that grafted trees of *Uapaca kirkiana* began to produce fruits only after 2-3 years, while those derived from seedlings took 12-15 years. Grafting techniques were also successfully used on *Allanblakia floribunda* Oliv. (Clusiaceae) to reduce the long juvenile phase of about 10-12 years to less than 5 years (Asaah *et al.,* 2012).

Concerning *Garcinia kola*, grafting is a recent area to explore. In Nigeria, Yakubu and Akinyele (2021) indicated the suitability of *G. kola* for grafting with 52% of grafting success. But these authors investigate only one source of the grafts using a single grafting technique. However, several research studies have shown that the success of grafting depends on several factors such as the grafting technique, the grafting period, and the age of the rootstock (Munjunga *et al*., 2013; Akter *et al*., 2016; Nguyen and Yen, 2018). In Côte d'Ivoire, works on vegetative propagation of *G. kola* have until now focused on cutting (Kouakou *et al*., 2016; Dao *et al*., 2020). More in-depth investigations to ensure efficient production of quality plants are necessary on *G. kola* grafting ability. The objective of this study is to determine the conditions favorable to the successful grafting of *G. kola*.

2. Materials and Methods

Site of study

The study was carried out from June 2018 to February 2020 in Abidjan at the University Nangui Abrogoua (UNA) research station (05°23´N, 04°00´W). This site is located in the forest zone where the rainfall pattern is bimodal with two dry seasons (from December to March and from July to August) and two rainy seasons (from April to June and from September to November). Mean annual rainfall varies between 1800 and 2000 mm. The mean monthly temperature varies between 27 and 30°C, whereas the mean relative humidity ranges from 70 to 84% (Kouakou *et al*., 2016).

Plant material

Plant materials were constituted of 12- and 36-month-old seedlings of *Garcinia kola* obtained in the nursery of the experimental research station of UNA. The average height and collar diameter of the rootstocks were 84.1 ± 10.04 cm and 1.31 ± 0.18 cm for 36-month-old seedlings and 43 ± 5.74 cm and 0.84 ± 0.10 cm for 12-month-old seedlings, respectively. These plants were used as rootstocks in this study. The mature fruiting tree located in Ahouabo (06°22´N, 03°82´W) was used for scion' collection.

Rootstock preparation and scion collection

Mature fruits were naturally collected under a

single tree in a cocoa field in Ananguié village (06°33´N, 03°67´W). The seeds from this fruit were used for the production of seedlings in the nursery. Seedlings of 24 and 36 months old were selected based on their vigor for use as rootstocks. The preparation of the rootstock concerned rootstocks aged 36 months old and consisted of reducing the lateral branches one week before the grafting operation in order to allow an accumulation of sap. In fact, the sap allows the scion, once placed, to maintain its vigor and accelerates its fusion with the rootstock. But no reducing branches were made on 12-month-old rootstock because there are no ramifications. The rootstock was watered every day one week before the grafting operation.

For scion collection, non-flowering lateral shoots of current season's growth were cut from a superior mature tree selected according to farmers' criteria, which included good physical and sanitary characteristics (such as vigorous growth and absence of parasites) as well as the quality of the fruits and nuts (tasty pulp and size of seeds). These shoots (15-20 cm long) were collected early in the morning (between 6 and 7 am) using disinfected scissors with 70% alcohol. To avoid dehydration of the shoots, they were stored in a damp, moistened gunny bag and packed in a cooler before transportation to the nursery. They were free from disease and unignified. They were used to provide the scions or buds for grafting onto the rootstocks depending on the grafting technique being tested. Grafting on rootstocks was carried out early in the morning, the day after the collection of the grafts, due to the long distance between the collection location and the nursery.

Grafting methods and experimental design

In order to determine the most favorable period for successful grafting of *Garcinia kola*, the grafting operation was carried out over two periods. The first trial was carried out in June (main rainy season) and the second in August (short dry season). At each trial period, four grafting techniques were tested in order to identify the optimized grafting success. These techniques were simple whip, top cleft, side cleft, and chip budding and were made according to Takoutsing *et al*. (2014). The grafting operations were made on 36-month-old and 12-month-old rootstock. All the grafting techniques were made on 36-month-old rootstocks. But only simple whip and top cleft techniques were applied on 12-month-old rootstock due to their small diameter (<1 mm), inapt for chip budding and side cleft technique application. The scions constituted of shoots were used for simple whip, top cleft, and side cleft. While buds were used for chip budding. To determine the time required for the graft to weld for each technique, the graft was unwrapped at 15, 22, and 29 days after grafting.

The experimental design adopted was a completely randomized block with two factors: the grafting technique and the unwrapped time for each trial period and the type of rootstock used. For the grafting carried out on 36-month-old rootstocks and at each trial period, 12 treatments were carried out (4 grafting techniques x 3 unwrapping times) and 6 treatments for 12-month-old rootstocks (2 grafting techniques x 3 unwrapping times). However, due to the limited availability of rootstock plants, the treatments were repeated twice. Each treatment consisted of 15 grafted plants. Over the two trial periods, a total of 720 plants were grafted from 36-month-old rootstocks (2 periods x 4 grafting techniques x 3 unwrapping times x 15 grafted plants x 2 replicates) and 360 plants grafted with 12-month-old rootstocks (2 periods x 2 grafting techniques x 3 unwrapping times x 15 grafted plants x 2 replicates).

Monitoring and data collection

The monitoring consisted of keeping the plot clean and regularly pulling out the weeds in the bags by hand in order to avoid any competition with the rootstocks. The unwrapping process, which consists of removing the ligature strips from the contact zone between the scion and the rootstock, was carried out at 15, 22, and 29 days after grafting in order to determine the time necessary for good welding between the scion and the rootstock. One week after each stripping operation, the rootstocks of the chip budding and the side graft were pruned at 2 cm above the grafting point without leaving any knots (Soloviev and Gaye, 2004). The data collection included the number of sprouted grafts and the days taken for each graft to sprout. Parameters evaluated during the experiment were the graft success, recovery time, and graft survival rate. The graft success rate (GsR) reflects the ratio of the number of successful grafts to the number of grafts performed and is expressed by the following formula:

$$GsR\ (\%) = \frac{(\text{number of successful grafts})}{(\text{number of grafts performed})} \times 100$$

The graft recovery time (GrT) corresponding to the sum of the recovery times of each graft, compared to the total number of successful grafts. It is expressed by the following formula:

$$GrT\ (day) = (\sum tr)/N$$

where *tr is* recovery time per graft and N the total number of successful grafts.

The survival rate of grafted plants was evaluated for 12 months after grafting taking into account the grafting technique and the age of the rootstock.

Statistical analysis

Data was subjected to analysis of variance (ANOVA) with respect to grafting season, technique, graft unwrapping time, and their interaction. When a significant difference was observed between the means (P <0.05), the ANOVA is supplemented by the least significant difference (LSD at P≤0.05) for separation of means. All the analyses were processed using the software Statistica 7.1.

3. Results

Effect of seasonality on the success of Garcinia kola grafting

The results indicated in Table 1 show that the graft success rate and the average recovery time of grafts carried out on rootstocks aged 36 and 12 months varied depending on the grafting period. The highest success rates for grafts carried out on rootstocks aged 36 months (34.02±2.47%) and rootstocks aged 12 months (48.33±5.00%) were recorded in the month of June. The lowest values of graft success rate were observed in the month of August. As for the average recovery time, the shortest (28.02±0.39 days and 20.72±0.69 days) were observed in grafts carried out in the month of June, respectively, with aged rootstocks of 36 and 12 months old. The month of June favors the success of *Garcinia kola* grafting with a higher success rate and an earlier recovery time. Furthermore, rootstocks aged 12 months showed good aptitude for grafting with an early graft recovery, unlike rootstocks aged 36 months old.

Effect of grafting technique on the success of Garcinia kola grafting

Grafting technique significantly influenced the graft success rate in 36-month-old rootstocks (P= 0.006) and 12-month-old rootstocks (P<0.011). On the other hand, no significant difference was observed in the average recovery time, whatever the technique, in the two types of rootstocks (Table 2).

Table 1 - Comparison of average values of grafting parameters according to period and age of rootstocks

Grafting period	Graft success (%)		Graft recovery time (day)	
	36 month old rootstock	12 month old rootstock	36 month old rootstock	12 month old rootstock
June	34.02 ± 2.47 a	48.33 ± 5.00 a	28.02 ± 0.39 b	20.72 ± 0.69 b
August	21.66 ± 1.46 b	27.77 ± 3.38 b	30.00 ± 0.52 a	23.27 ± 0.59 a
F	50.45	47.20	8.55	7.53
P	<0.001	<0.001	0.005	0.011

In a column, the values followed by different letters are significantly different at P<0.05.

Table 2 - Comparison of average values of grafting parameters according to grafting technique and rootstock age

Grafting techniques	Graft success rate (%)		Graft recovery time (day)	
	36 month old rootstock	12 month old rootstock	36 month old rootstock	12 month old rootstock
Simple whip	26.94 ± 2.83 b	32.77 ± 3.11 b	29.33 ± 0.56 a	21.94 ± 0.69 a
Top cleft	28.88 ± 3.34 b	43.33 ± 5.99 a	29.33 ± 0.48 a	22.05 ± 0.69 a
Chip budding	32.22 ± 3.66 a	-	27.83 ± 0.77 a	-
Side cleft	23.33 ± 2.80 c	-	29.55 ± 0.86 a	-
F	4.55	12.44	1.38	0.01
P	0.006	<0.001	0.258	0.906

In a column, the values followed by different letters are significantly different at P<0.05. - Not evaluated.

The highest graft success rate (32.22±3.66%) was obtained with the chip budding technique on rootstocks aged 36 months. While on 12-month-old rootstocks, the highest graft success rate (43.33±5.99%) was recorded with the simple whip technique. Concerning the average graft recovery time, it was between 27 and 29 days with grafts carried out on rootstocks aged 36 months, respectively, for the chip budding technique and the other techniques. While for plants aged 12 months, the average recovery time is 21.94±0.69 days for the simple whip technique and 22.05±0.69 days for the top cleft grafting technique. However, no significant difference is observed between these two average recovery times. The chip budding and top cleft grafting techniques are more favorable in rootstocks aged 36 and 12 months, respectively. Figure 1 shows an overview of the recovery of grafted plants with the different grafting techniques applied.

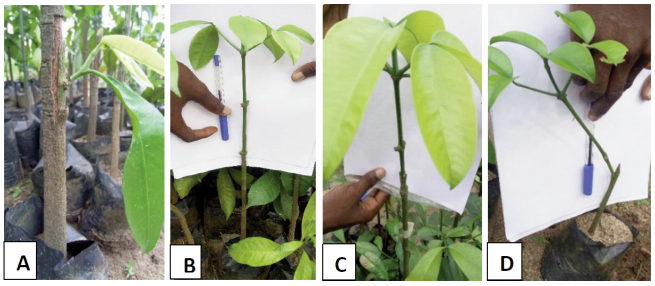

Fig. 1 - Successful grafts from different grafting techniques: (A) Chip budding ; (B) Simple Whip ; (C) Top cleft ; (D) Side cleft.

Effect of graft unwrapping time on the success of Garcinia kola grafting

The results presented in Table 3 show that only the graft success rate was significantly influenced ($P < 0.001$) by the graft unwrapping time. The highest percentages of graft success (38.75±2.71% and 55.83±5.42%) were obtained when the grafts were unwrapped at 29 DAG on 36- and 12-month-old rootstocks, respectively. While the lowest graft success rate (16.66±1.33% and 24.16±3.57%) was recorded when the grafts were unwrapped 15 days after grafting on 36- and 12-month-old rootstocks, respectively. The average recovery time for rootstocks aged 36 months is between 28 and 29 days, while that of rootstocks aged 12 months is between 21 and 22 days. The graft unwrapping time of 29 days is favorable to the success of grafting *Garcinia kola* in both types of rootstocks.

Combined effect of seasonality, grafting technique and graft unwrapping time on Garcinia kola grafting success

The interaction of season, grafting technique, and graft unwrapping time significantly influenced the graft success rate regardless of the age of the rootstock (Table 4). On the other hand, no significant difference was observed in terms of graft recovery time. For grafts carried out in the month of June on rootstocks aged 36 months, the highest graft success rates (43.33±8.81% and 43.33±3.33%) were obtained with the chip budding and side cleft techniques, respectively, and were unwrapped at 29 days after grafting. Concerning the grafts carried out in the month of June on rootstocks aged 12 months, the best graft success rate (83.33±3.33%) was recorded with the top cleft technique when unwrapped at 29 days after grafting. While the lowest graft success rate (23.33±3.33%) was obtained with the simple whip technique when the grafts were unwrapped 15 days after grafting. The graft recovery time varies from 24 to 30 days and 19 to 22 days after grafting with grafts performed in June on 36-month-old rootstocks and 12-month-old rootstocks,

Table 3 - Comparison of average values of grafting parameters according to graft unwrapping time and rootstock age

Graft unwrapping time (day)	Graft success rate (%)		Graft recovery time (day)	
	36 month old rootstock	12 month old rootstock	36 month old rootstock	12 month old rootstock
15	16.66 ± 1.33 c	24.16 ± 3.57 c	29.66 ± 0.58 a	22.41 ± 1.05 a
22	28.12 ± 2.01 b	34.16 ± 4.83 b	28.58 ± 0.47 a	21.58 ± 0.91 a
29	38.75 ± 2.71 a	55.83 ± 5.42 a	28.79 ± 0.71 a	22.00 ± 0.62 a
F	53.70	39.03	0.96	0.26
P	<0.001	<0.001	0.386	0.767

In a column, the values followed by different letters are significantly different at P<0.05.

Table 4 - Comparison of mean values of grafting parameters depending on the interaction of season, grafting technique, graft unwrapping time, and rootstock age

Grafting period	Grafting Technique	Graft unwrapping time (Day)	Graft success rate (%)		Graft recovery time (day)	
			36 month old rootstock	12 month old rootstock	36 month old rootstock	12 month old rootstock
June	Simple whip	15	15.00 ± 2.88 d	23.33 ± 3.33 e	28.00 ± 1.15 a	20.66 ± 2.40 a
		22	16.66 ± 4.40 d	33.33 ± 3.33 d	28.00 ± 1.15 a	20.66 ± 2.40 a
		29	28.33 ± 4.40 c	50.00 ± 5.77 b	28.66 ± 0.66 a	21.00 ± 1.52 a
	Top cleft	15	25.00 ± 2.88 c	40.00 ± 5.77 c	30.00 ± 1.15 a	19.33 ± 1.33 a
		22	31.66 ± 6.00 c	56.66 ± 8.81 b	29.33 ± 1.76 a	20.00 ± 2.30 a
		29	4.00 ± 5.77 b	83.33 ± 3.33 a	28.00 ± 1.15 a	22.66 ± 0.66 a
	Chip budding	15	23.33±3.33 c	-	26.00 ± 1.15 a	-
		22	40.00 ± 5.77 b	-	28.00 ± 1.15 a	-
		29	43.33 ± 8.81 a	-	24.33 ± 2.02 a	-
	Side cleft	15	13.33 ± 3.33 d	-	28.66 ± 0.66 a	-
		22	30.00 ± 5.77 c	-	28.00 ± 1.15 a	-
		29	43.33 ± 3.33 a	-	29.33 ± 1.76 a	-
	F		*12.87*	*1.18*	*0.852*	*0.34*
	P		*0.003*	*0.013*	*0.543*	*0.713*
August	Simple whip	15	10.33 ± 3.33 e	20.00 ± 5.77 d	32.66 ± 1.76 a	23.66 ± 1.45 a
		22	23.33 ± 3.33 c	26.66 ± 3.33 c	28.66 ± 0.66 a	22.00 ± 1.15 a
		29	30.00 ± 5.77 b	36.00 ± 5.77 b	30.00 ± 1.15 a	23.66 ± 1.45 a
	Top cleft	15	13.33 ± 3.33 d	13.33 ± 3.33 e	30.00 ± 1.15 a	24.00 ± 1.15 a
		22	20.00 ± 5.77 c	20.00 ± 5.77 c	28.00 ± 1.15 a	23.66 ± 1.45 a
		29	23.33 ± 3.33 c	46.66 ± 6.66 a	30.66 ± 0.66 a	20.66 ± 0.88 a
	Chip budding	15	20.00 ± 5.77 c	-	32.00 ± 2.30 a	-
		22	26.66 ± 3.33 c	-	28.66 ± 0.66 a	-
		29	36.66 ± 3.33 a	-	28.00 ± 1.15 a	-
	Side cleft	15	13.33 ± 3.33 f	-	30.00 ± 1.15 a	-
		22	16.66 ± 3.33 e	-	30.00 ± 3.05 a	-
		29	26.66 ± 3.33	-	31.33 ± 4.37 a	-
	F		*1.10*	0.13	*0.51*	2.95
	P		*0.038*	<0.001	*0.788*	0.116

In a column, the values followed by different letters are significantly different at P<0.05. - Not evaluated.

respectively. While for grafts performed in August, the graft recovery time varies from 28 to 32 days and 20 to 24 days after grafting for grafts performed on 36-month and 12-month rootstocks, respectively, 36-month-old, regardless of the grafting technique and graft unwrapping time.

Grafted plants survival rate

The survival rate was not significantly influenced by the grafting technique (Fig. 2). The grafting techniques tested on both types of rootstocks showed a survival rate of 100% at 12 months after grafting. The figure 3 presented a grafted plant of *Garcinia kola*.

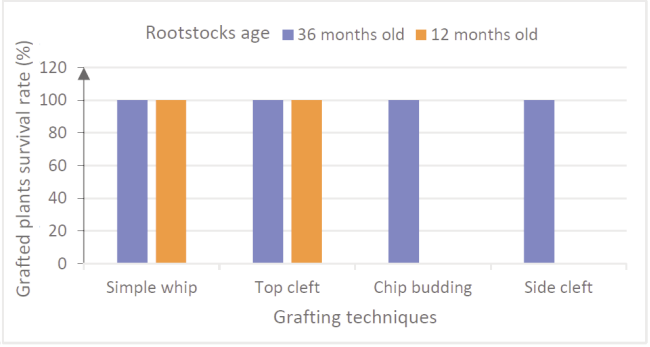

Fig. 2 - Survival rate of *Garcinia kola* grafted plants with differents grafting techniques on 36 and 12 month old rootstoks.

Fig. 3 - *Garcinia kola* grafted plant with the chip budding technique 12 months after grafting.

4. Discussion and Conclusions

In this study, The higher graft success observed in June with 12- and 36-month-old rootsocks and their earliness in graft recovery time, unlike grafts performed in August, would be due to the optimum humidity in the month of June (Karadeniz, 2005). Indeed, June was characterized by an abundance of rain (361 mm) with an average temperature of 26°C. These conditions promote the well growth of plants and grafts success. This finding is in agreement with the results of Chipojola *et al*. (2013), who obtained similar results for *Anarcadium occidentale* in Malawi. While August is characterized by the short, dry season. The low rainfall and high temperatures during the August season slow down the physiological activity of the rootstock and favor the scions drying. These results are in agreement with those of Djaha *et al*. (2012), who observed a low success of cashew grafting in November in Korhogo, in the north of Côte d'Ivoire. According to these authors, the dry season would not favor the vegetative phase of the plant because of the harmattan and the scarcity of rains. Similar observations were made by Soloviev *et al*. (2004), who obtained low success rates on the grafting of African plum (*Sclerocarya birrea*) in the cold dry season and in the middle of the hot dry season. Ondo *et al*. (2018) reported a low success rate in the grafting of two clones (GT1 and PB 217) of *Hevea brasiliensis* (H.B.K.) (Muell. Arg) during the dry season in Gabon. Negative effects of dry season on graft success are also reported in mango by Sivudu *et al*. (2014) and in *Vitex payos* (Lour.) Merr. by Bala *et al*. (2017).

The best graft success rate recorded with the chip budding method on 36-month-old rootstocks, unlike the other grafting methods, could be explained, on the one hand, by the presence of latex at the level of the scion and of the rootstock. Indeed, notches made on the rootstock during the operation do not affect the wood. It could promote the welding between the bud scion and the rootstock in the chip budding method. Concerning the other grafting methods, the contact zone between the rootstock and the scion is largely between the lignified parts. This makes it difficult to weld between the rootstock and the scion. According to Takoutsing *et al*. (2014), the success of the grafting process depends on the level of lignification of the rootstock and the grafting method. These authors reported a 100% graft success rate of *Garcinia lucida* Vesque using the top cleft method on six-month-old rootstocks.

The graft unwrapping time at 29 days after grafting enhances graft success more than those unwrapped at 15 and 22 days, whatever the rootstock's age. This means that this duration is sufficient for the establishment of the weld between the rootstock and the scion. Indeed, the realization of the anatomical welding is an essential condition for the success of the grafting (Scheidecker, 1961). In rubberwood (Hevea brasiliensis), grafts were generally unwrapped 21 days after grafting (Udayakumara and Seneviratne, 2005; Ondo *et al*., 2018).

The formation of new tissues by the rootstock and the scion at the level of the contact zone is done gradually over time. In this study, the average sprouting time differs according to the age of the rootstock, with 22 and 28 days for 12- and 36-month-old rootstocks, respectively, regardless of the graft unwrapping time. Similar graft recovery time was reported by Yao *et al*. (2019) in shea butter (*Vittelaria*

paradoxa), with a graft recovery time between 21 and 30 days. The reestablishing of the connection between the rootstock and the scion is manifested by the budding of dormant buds on the scion. The lower graft success rate observed with grafted plants unwrapped at 15 days after grafting can be explained by an incomplete welding of the two parts. Indeed, graft unwrapping leads to the separation of the rootstock and the scion. This opening of the contact zone causes it to dry out on the one hand and the proliferation of several pathogens, such as fungi, on the other hand, which accelerates the death of the graft. The combined effect of season, method, and unwrapping time significantly influenced the graft success rate. The best graft success rate was recorded on grafts made in June on 36-month-old rootstock (43.33%) with chip budding and side cleft grafting methods with grafts unwrapped at 29 days after grafting and 83.33% for grafts made on 12-month-old rootstock with the top cleft grafting method with grafts unwrapped at 29 days after grafting. This result would mean that this combination of season, grafting method, and unwrapping time depending on the age of the rootstock optimizes the success of the grafting. These results could be explained by favorable climatic conditions in June. Indeed, the month of June is in the great rainy season. In addition, chip budding and side cleft grafting methods were adapted to older rootstock because this method does not affect the wood (Yakubu *et al*., 2014). While the top cleft method promotes well-grafting to unlignified stems, such as the 12-month-old rootstock apical part. In this study, young *G. kola* plants, whatever their age, have a good ability for grafting when the grafts are unwrapped at 29 days after grafting. The short graft recovery time observed with 12-month-old rootstocks is in agreement with several authors who reported that younger rootstocks presented good ability for grafting in papaya (Nguyen and Yen, 2018) and in mango (Mahunu *et al*., 2009; Upadhya *et al*., 2014). Other trees, such as *Garcinia xanthochymus* (Hook. f.) [Syn. *G. tinctoria* (Wight)] (Krishnamoorthy *et al*., 2006), *Garcinia lucida* Vesque (Takoutsing *et al*., 2014), and Vittelaria paradoxa (Sanou *et al*., 2004), were successfully grafted. The best result obtained by using the top cleft method in this study suggests that this grafting method promotes well-grafting success. Several species were successfully grafted by the top cleft method, such as *Allanblakia parviflora* in Ghana (Ofori *et al*., 2008) and *Allanblakia stuhlmannii*, with grafting success over 70% (Munjunga *et al*., 2013).

The survival rate (100%) of all the grafted plants after 12 months indicates that grafting is an alternative for the production of quality plants for Garcinia kola. However, monitoring must be extended until flowering to confirm the long-term success of this technique.

This study has shown that it is possible to propagate elite trees of *Garcinia kola* by using the four grafting methods tested. Chip budding and side cleft were better on 36-month-old rootstocks, while the top cleft method is appropriate on 12-month-old rootstocks. The determination of adequate season (in rainy season) and graft unwrapping time (29 days after grafting) for the grafting success is very important to promote the utilization of this strategy by farmers for the species domestication and cultivation. The present study is the first in Côte d'Ivoire to systematically explore grafting techniques for the propagation and conservation of this vulnerable species with significant socio-economic value. Results of this study can be exploited by forest management structures to produce high-quality plants for agroforestry programs with several objectives, such as the mitigation of the effects of climate change and the improvement of farmers' incomes.

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Association analysis of intragenic molecular markers related to fiber quality and tensile strength of abaca (*Musa textilis* Nee)

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Key words: Genome association and prediction integrated tool (GAPIT), Hierfstat, intragenic molecular markers, *Musa textilis* Nee, population structure.

Abstract: Abaca are leaf-fiber plants found predominantly in the Philippines. Our country holds most of the Manila hemp market, but the unknown genetic architecture of the fiber hinders the crop's improvement. We developed intragenic molecular markers from genes related to fiber development and linked them to abaca fiber quality, with the goal of increasing precision of breeding. Pearson's correlation package of the R programming software revealed a high positive relationship between the pseudostem's top and middle girth (r=0.91), while a low negative correlation between the percent fiber percent fiber strain and the number of suckers (r= -0.42). The analysis also showed that the ultimate tensile strength was highly correlated with percent fiber percent fiber strain (r=0.33) and dry weight (r=0.34). Three subpopulations were determined using the STRUCTURE software, while Hierfstat computed an average 0.0648 F_{st} value, indicating moderate genetic diversity. Eight significant marker-trait associations (p-value <0.005) were identified with positive effects and >0.6% phenotypic variance explained (PVE). Eight markers from the COBRA-like protein, expansin, cellulose synthase, and auxin gene families were identified as linked to fiber quality and tensile strength. Our study identified nine abaca accessions with the trait of interest and the candidate genes. The significant molecular markers will be used to identify the hybrids with good fiber quality.

1. Introduction

There are eight widely known plant fibers, sisal, coir, cotton, flax,

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hemp, jute, ramie, and lastly, abaca (*M. textilis* Nee) (FAO, 2009). The latter is an herbaceous perennial *Musa* highly praised for its mechanical strength and industrial applications. In contrast to its close relative banana, this *Musa* species has thinner, glossier pseudostems, and instead of fruits, the succulent stems are the main commodity from abaca. These stems are harvested and processed into fibers internationally known as Manila hemp. There are multiple applications for plant fibers, but the boom of synthetic fiber industries slowly changed the demand for natural fibers (Bemiller, 2007). Nevertheless, natural fibers are sought after because of their sustainability and inexpensive production cost compared to their synthetic counterparts (Radhakrishnan, 2014). Abaca belongs to the leaf or cordage fibers. Their pseudostems are composed of multicelled fibers. These cells provide strength and mechanical resistance to the abaca fibers. The strands have weak bonds and can be easily separated through scraping. Cellulose and hemicellulose content are critical to the tensile strength and quality of fiber crops. The increased cellulose content indicates higher tensile strength. Meanwhile, increased hemicellulose, tends to decrease the mechanical potential of the fibers by lowering their tensile strength due to unstable monomers. Therefore, those plant fibers with high mechanical properties are chosen for structural builds (Djafari Petroudy, 2017). Still, at the molecular level, other factors can affect the cell wall biosynthesis and the strength of the fiber cells, especially when exposed to different environmental conditions and stress.

Since its discovery in 1989 by Litt and Luty, SSR markers have been extensively used on many species. The availability of new software and whole genome sequences eased SSR mining, making new SSR studies robust and relevant. This co-dominant marker type is versatile and reliable for evolutionary analysis, genetic diversity, and marker-assisted breeding. It has helped hasten the classic plant breeding works (Victoria *et al*., 2011) and fingerprinted closely related species (Vinarao *et al.*, 2019). These markers can be developed from genic portions, miRNA regions, chloroplast sequences, expressed sequence tags, and whole genome assembly (Victoria *et al.*, 2011; Sagwal *et al.,* 2022). In addition, SSR markers were also used to uphold the breeder's right over the marketed seeds and prevent variety mislabeling in the field (Palumbo and Barcaccia, 2018). There are several published works on the use of SSRs on abaca (Boguero *et al.,* 2016; Yllano *et al.,* 2020; Mendoza *et al.,* 2024). However, these studies are focused on the genetic diversity of the crop rather than the markers' relationship with specific traits. The genomic markers used by Yllano *et al*. (2020) and Boguero *et al*. (2016) are even designed from other *Musa* species that lessens their specificity for association analysis. This study identified the intragenic SSR markers related to tensile strength and fiber quality. We also analyzed phenotype-genotype trait association using candidate gene-based SSR markers and selected abaca accessions that show promising traits. The information can be used in the genomic selection of elite accessions abaca hybrid production.

2. Materials and Methods

Phenotyping

The abaca gene bank composed of 73 accessions collected from 11 administrative regions of the Philippines, was used in the study (Suppl. Materials Table 1S). A total of 56% of this collection was planted in 2020, while the remaining 44% was established in 2016. The pseudostem were harvested when the flag leaf emerged. The number of suckers (S) were counted from each hill while and the leaf sheaths (LS) were counted from the harvested pseudostems. The circumference of the base of the crown leaf crown (GT), the middle girth (GM), and the base (GB) of the pseudostem was measured in centimeters. The stem length (SL) was measured from end to end of the pseudostem. The fresh weight (FW) was measured in kilograms, while the fiber dry weight (DW) was obtained in grams using an analytical balance after air drying. The tensile strength and percent fiber strain were obtained using the Shimadzu AGX series tensile strength machine. The ultimate tensile strength (*UTS*) was computed using the formula:

$$UTS = F/A$$

Where: *F* is the force applied and *A* the fiber cross sectional area.

DNA extraction and polymerase chain reaction

A total of 34 genes related to fiber development and quality were obtained from the list of transcripts identified by Reamillo (2018) and other related studies (Table 1). The identified sequences were

Table 1 - Profile of the intragenic molecular markers that were used for association mapping of fiber traits in Abaca (*M. textilis* Nee)

Marker	Motif	Annealing temperature	Product size	Functional identity
51	AGAGAGAG	59.9	309	Cellulose Synthase-like protein D2
52	TATATATA	59.95	307	Cellulose Synthase-like protein D2
53	TCTTCTTCT	60.15	306	Cellulose Synthase-like protein D2
54	TCTCTC	60.35	301	Cellulose Synthase-like protein D2
55	GAGAGA	60.25	302	Cellulose Synthase-like protein D5
56	AGGAGGAGGAGGAGGAGG	59.45	201	Cellulose Synthase-like protein E6
57	TCTCTC	59.55	301	Cellulose Synthase-like protein D2
58	TCTCTC	59.45	405	cobra-like protein 4
59	CGTCGTCGT	59.8	203	Expansin A2
60	CAACAACAA	60.9	205	Expansin A10
61	TTCTTCTTCTTCTTCTTCTTCTTC	58.9	306	Expansin A10
62	TCTCTCTC	59.95	336	Expansin A10
63	GCTGCTGCTGCT	59.6	403	Expansin A4
64	AGAGAG	59.2	268	Auxin response factor 9
65	TCTCTCTC	59.7	407	Auxin-responsive protein IAA6-like
66	CTCTCT	59.5	309	Auxin responsive IAA30 like protein
67	GTCGTCGTC	59.85	316	Auxin-induced protein 22D
68	GAGAGA	59.7	302	putative protein auxin response 4
69	CCTCCTCCTCCTCCT	59.8	201	AP2_like_ethylene_responsive_transcription_factor_AIL5
70	CTCCTCCTC	60.4	207	AP2-like ethylene-responsive transcription factor AIL5
71	AGAGAGAG	58.9	323	Ethylene insensitive 3-like protein
72	AGAGAG	59.7	313	probable_ethylene_response_sensor_1
73	AAGAAGAAGAAGAAGAAGAAG	59.9	224	glycolipid_transfer_protein_3
74	AGAGAGAG	60.15	331	Cellulose Synthase-like protein D2_5'UTR
75	TATATATA	60	322	Cellulose Synthase-like protein D2_5'UTR
76	TCTTCTTCT	60.1	246	Auxin_response_factor_9_5'UTR
77	TCTCTC	60.6	323	auxin-responsive protein IAA6-like_5'UTR
78	GAGAGA	59.3	244	Ethylene insensitive 3-like protein_5'UTR
79	AGGAGGAGGAGGAGGAGG	59.1	301	Ethylene insensitive 3-like protein_5'UTR
80	TCTCTC	58.9	306	Expansin A10
81	TCTCTC	58.7	201	auxin-responsive protein IAA30-like
82	CGTCGTCGT	58.5	215	putative protein auxin response 4
83	CAACAACAA	59.9	224	AP2-like ethylene-responsive transcription factor AIL5
84	TTCTTCTTCTTCTTCTTCTTC	58.7	112	glycolipid_transfer_protein_3

scanned from the NCBI website (https://www.ncbi.nlm.nih.gov/). The BLASTN command line version was used to determine the percent identity of the sequences and high bit score with the whole genome sequence of *Musa textilis* Nee cv. Abuab (Galvez *et al.,* 2021). Only those that passed the modified BLASTN threshold (e-value = 0.005, high identity and bit score) were scanned for tandem repeats using the microsatellite finder (http://insilico.ehu.es/mini_tools/microsatellites/). The molecular markers were generated from the 250 bp upstream and downstream of the tandem repeats using the primer3 website (https://primer3.ut.ee/). The parameters used in the development of the primers were: 18-27 primer size, 57°C to 63°C primer Tm, -10.0 to 110 primers bound %, and 20%-80% percent GC content.

The Doyle and Doyle (1990) CTAB DNA extraction protocol, modified by Sandoval (2011), was used to isolate the DNA. The 10 µL PCR reaction comprised of 1x PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs and primers (forward and reverse), and a 0.05 U/µL *Taq* polymerase cocktail. The running conditions for each reaction were 94°C for 4 minutes, then 35 cycles at

94°C for 30 s, 58-60°C for 30 s, 72°C for 45 s, and a final extension at 72°C for 10 min. The amplified bands were visualized using 6% polyacrylamide gel stained using GelRed and photodocumented in the GenoSens photo-documentation system. The amplified polymorphic bands were scored using the GelAnalyzer v.23.1 software (Lazar and Lazar, 2023).

Data analysis

Pearson's correlation between the observed traits was computed in the R software. STRUCTURE software v.2.3.4 (Pritchard *et al.,* 2000) generated the population structure and the q matrix used for association mapping. Each K was run over in ten replications in a burn-in period of 20,000 with 50,000 Monte Carlo Markov Chain (MCMC) replicates. The best grouping was determined using the R package Pophelper v.2.3.1 (Francis, 2017) (https://github.com/royfrancis/pophelper). The Hierfstat package was used to determine the F_{st} value between the populations. The q matrix generated by the STRUCTURE software was used to analyze the association between markers and traits using the FarmCPU (Fixed and Random Model Circulating Probability Unification) of the GAPIT (genomic association and prediction tool) package (Lipka *et al*., 2012).

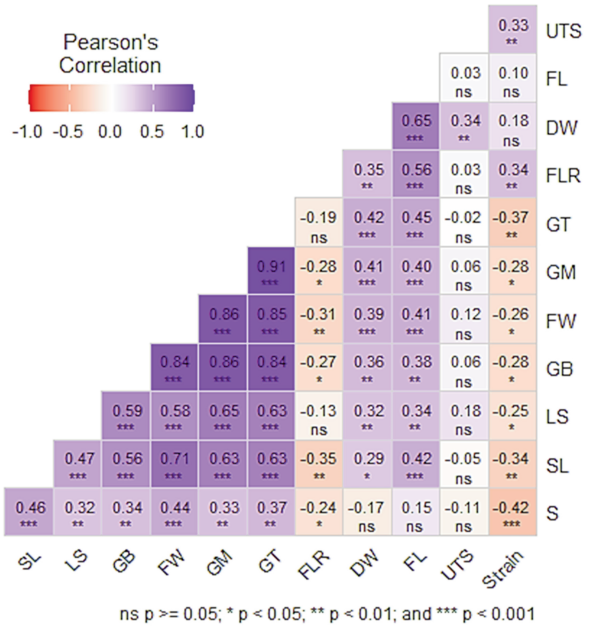

Fig. 1 - Pearson's correlation shows the correlation between the morphological traits of the 73 abaca (*Musa textilis* Nee) accessions. The highest positive correlations were found among the girth measures while the percent fiber strain and fiber length recovery (FLR) exhibited the lowest negative correlations among the traits.

3. Results

Figure 1 shows the results from Pearson's correlation analysis. The highest positive correlation was obtained from the girth measurements of the pseudostem's middle and top sections (r= 0.91). In contrast, the lowest negative correlation (r= -0.42) was obtained between the percent fiber strain and the number of suckers (S). The number of suckers also has a positive correlation with seven out of ten morphological traits. Pseudostem length (SL) correlation with fresh weight (r= 0.71) was its strongest correlation among the other characters. The leaf sheath (LS) shows an average positive correlation of r= 0.61 with girth and fresh weight characters. A strong mean correlation (r= 0.85) was observed among the base girths and fresh weight. Percent fiber strain and fiber length recovery (FLR) are the traits with the most negative correlations with other traits. The percent fiber strain and dry weight (dry weight) are the only traits that have a positive correlation with the ultimate tensile strength at r= 0.33 and r= 0.34, respectively. The correlation shows the trait values that increase together and characters with negative relationships.

Population structure

The F_{st} value indicates the allele frequency distribution between the subpopulations or clusters of abaca. The F_{st} value of >0.25 indicates high genetic differentiation and suggests that the accessions came from different genera or completely different species. The mean F_{st} value obtained from the abaca subpopulation in this study was F_{st} = 0.0648, suggesting moderate genetic variation between the three populations (Li *et al.,* 2014; Luo *et al.,* 2019). The lowest F_{st} value at 0.0229 was obtained between cluster 1 and cluster 2 followed by F_{st}=0.0728 between cluster 1 and 3. The highest F_{st} value of 0.0988 was obtained between cluster 2 and 3. All the computed F_{st} values indicate that the subpopulation are moderately diverse. Most members of cluster 2 are abaca accessions from Mindanao, while members of subpopulation 1 and 3 are from Luzon, particularly

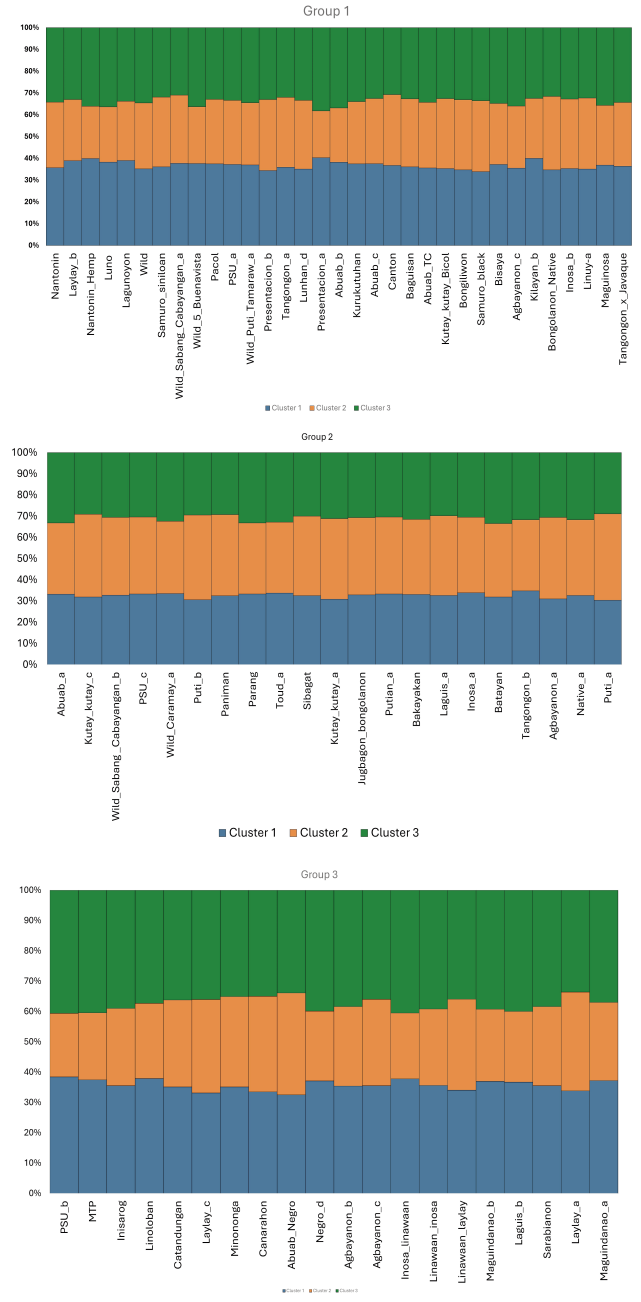

Fig. 2 - The inferred subpopulation assignment of the 73 abaca accessions showing three groupings. Each bar and percent color represent the allelic distribution possessed by each individual in each cluster.

from Region 5 (Fig. 2). The best K clustering was three clusters, the markers used are gene-specific markers hence, a few clusters are expected (Fig. 3).

Association mapping

All the marker-trait associations (MTAs) that are less than the threshold (p-value ≤0.005) were

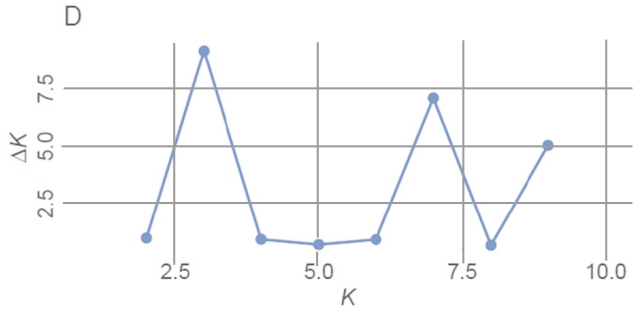

Fig. 3 - Three groupings identified by pophelper package of Rstudio as best k clustering for the 73 abaca accessions. The results for K=3 was then used as Q matrix or kinship in the GAPIT analysis.

considered significant in this study (Fig. 4). Table 2 shows the significant MTAs identified using the FarmCPU model implemented in the GAPIT package of the R software. This model was able to control the false positives effectively by iterative running of the fixed and random effects. The Q-Q plot showed the relationship of the expected and observed p-values for each trait (Fig. 4). The data points that lie beyond the gray area or the 95% confidence interval of the qqplot indicate rejection of the null hypothesis of no association. Eight significant MTAs for five traits were considered for this study. The number of suckers exhibited the highest number of associated markers with no negative effects (MK59, MK58, and MK69). The percent phenotypic variance explained (%PVE) by the markers on the number of suckers ranged from 0.6-57% while its minor allele frequency or MAF value ranges from 0.01-0.06. MK55 and MK63 were significantly associated with the ultimate tensile strength exhibited the highest positive effect among the MTAs. Their explained phenotypic variance was 35.91 and 7.16, respectively. Only one marker (MK55) was strongly associated with the girth size (middle) which explained 42.99% of the observed phenotypic variation. The markers associated with dry weight and percent fiber strain showed the lowest positive effect among the MTA at 0.01 and 0.4, respectively. Overall, by combining the results from phenotyping and the significant marker trait association, accessions that possess genes of interest and exhibits high agronomic trait values are PSU a and b for the number of suckers, Tangongon and Laylay for girth, Native and Parang for dry weight, Kutay-kutay and Native for ultimate tensile strength, and Laylay for percent fiber strain. The team had

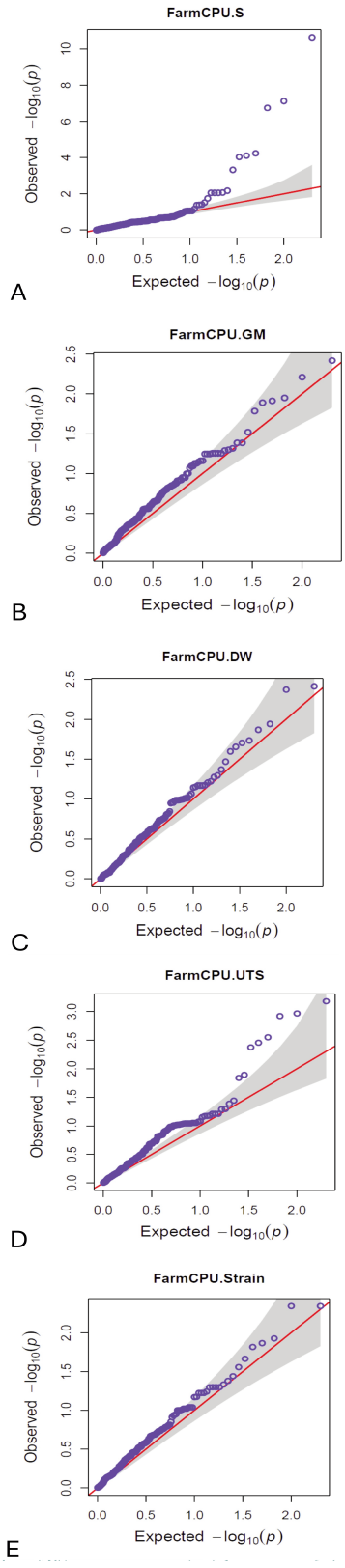

Fig. 4 - The QQ plot generated for each trait using the FarmCPU model. A. suckers, B. Girth (middle), C. Dry weight, D. Ultimate tensile strength, E. Percent fiber strain. The markers that are above the regression line (red line) and beyond the 95% confidence interval (gray area) are significantly related to the traits- of-interest.

Table 2- The significant marker-trait-associations (MTAs) related to the important agromorphological traits of abaca (Musa textilis Nee) determined using the association mapping tool GAPIT

Traits	Marker	Product size (bp)	P. value	Minor allele frequency (MAF)	Effect	%Phenotypic variance explained	Functional identification
Number of suckers	MK59	400	7,81E-05	0.013699	8.735656	0.603816	Expansin A2
Number of suckers	MK58	550	7,46E-08	0.027397	10.50474	7.864861	cobra-like protein 4
Number of suckers	MK69	250	2,25E-11	0.068493	7.862466	57.61	AP2_like_ethylene_responsive_transcription_factor_AIL5
Girth (Middle)	MK55	315	0.003813	0.054795	4.982266	42.99	Cellulose Synthase-like protein D5
Dry weight	MK63	400	0.003858s	0.082192	0.013531	51.2039	Expansin A4
Tensile strength	MK55	390	0.000658	0.082192	263.4273	35.91	Cellulose Synthase-like protein D5
Tensile strength	MK63	400	0.00281	0.08219	222.399	7.16	Auxin response factor 9
Strain	MK66	160	0.004529	0.013699	0.46045	9.89	Auxin responsive IAA30 like protein

produced 49 crosses out of the selected parentals, however only 15 hybrids survived. Applying the markers with high correlation with the traits of interest can hasten the breeding work on abaca. The hybrids that show amplicons for these markers will be considered for advanced trial. The marker aided selection increases the accuracy of crop improvement for abaca and remains a crucial step in breeding.

4. Discussion and Conclusions

Abaca fiber composition

The cell wall thickness was not only the determining factor for tensile strength. Sisal (*Agave sisalana*), a fiber plant species, with 73% cellulose showed a higher tensile strength of 484MPa than jute (*Corchorus capsularis*) with 249MPa and has 65% cellulose content (Alves Fidelis *et al*., 2013). The study of Saragih *et al.* (2020) focused on the thermal analysis of the fibers and their chemical composition. Natural fibers are composed of 66.43% cellulose, 24.7% hemicellulose, and 13.6% lignin. It can be deduced that the amount of cellulose in the fibers is higher than the other two fiber components and contributes much to the strength of the fibers. With their results, they also found the potential of abaca as bioplastic fillers. Different industrial applications require various chemical components for abaca fiber. For instance, the pulping industry would require abaca fibers with low ignin and ash content while having high α-cellulose concentration (Moreno and Protacio, 2012). An amount of >99% cellulose is considered pure. This is achievable through processing but difficult for certain fiber crops like abaca. Only cotton which is composed of 93% cellulose and 0% lignin would require just hot alkali treatment to produce high grade pulps (Bemiller, 2007). The input cost for locally producing cotton is higher than importing them causing a decline in the cotton industry of the Philippines (Asis, 2017). Looking for other pulp sources such as abaca and optimizing the protocol by adding or removing a few steps during production of good pulp can be a solution.

SSR Markers for association mapping

The candidate gene mapping using gene-derived SSR markers have been used in maize (Dubey *et al*., 2009), barley (Matthies *et al.,* 2012), sorghum (Wang *et al*., 2011), bread wheat (Mehta *et al*., 2021), cotton (Buyyarapu *et al*., 2011; Baytar *et al.,* 2022), durum wheat (Alsaleh, 2022), rice (Pritesh *et al*., 2023; Pradhan *et al*., 2023), potato (Schumacher *et al*., 2021), wheat (Singh *et al.*, 2018), flax (Nag *et al.*, 2020) and sugarcane (Divakar *et al*., 2023). These studies used varying sample sizes and different numbers of molecular markers; Divakar *et al*. (2023) even used a set of 70 highly related sugarcane that exhibited moderate genetic diversity. Their number is similar to this study where we used 73 abaca germplasm collection for determining the association of fiber traits with newly developed molecular markers. Nag *et al*. (2020) used only 28 molecular markers on flax to determine significant MTAs with different fiber quality traits. Matthies *et al*. (2012) also used 22 SSR markers to determine important association with barley's kernel quality. The polymorphic nature of SSR markers makes them effective in distinguishing genotypes from the same genome (Lin *et al*., 2012). These show that even with limited sample sizes and molecular markers significant associations can be determined.

The intragenic molecular markers were also found related to different plant architecture traits and grain yield components of rice. Seventeen cgSSR markers with varying percent explained PVE (4% to 17%) were observed to be linked with panicle length and weight, number of primary branches, and grain yield. The associated markers increase the accuracy of rice breeding (Sah *et al*., 2023). Bareto *et al*. (2019) also utilized SSR markers to improve the breeding work and elite genotype selection in sugarcane. A total of 23 significant MTAs were highly associated with not only sugar content but also the crop's plant architecture, including stalk height, weight, and number, and can yield. Further investigation on the MTAs role in traits should be done in future studies. Divakar *et al*. (2023) also looked into the association of 30 EST-SSR markers with yield trait components in 70 sugarcane accessions. The marker SEM407 was found to be highly associated with %Brix, sucrose content, and percent sugar recovery. In fiber crops, a total of 28 SSR markers were used to characterize the genetic structure of the fiber genes of flax. Mixed linear model analysis revealed that Lu10-600 and LU15-300 were observed associated with fiber length while LU7-150 was the lone allele correlated with fiber strength. Four markers (LU5-50, LU7-150, LU10-600, and LU15-300), on the other hand, were observed highly related to fiber yield. These markers

can be utilized to screen flax genotypes with good fiber quality potential for breeding (Nag *et al*., 2020). Qin *et al*. (2015) evaluated the relationship of genic SSR markers with 3 fiber quality traits in cotton. They found that 26 molecular markers were positively correlated with quality and fiber strength, exhibiting most of the association (12 markers). The genome-wide SSR markers were also used in association mapping in cotton. A total of 106 gene markers were determined to be significantly associated with six fiber quality traits, including fiber strength, fiber upper half length, short fiber, micronaire value, and fiber uniformity. The fiber strength had the greatest number of correlated markers (61 markers) in the study of Nie *et al*. (2016). Marker-assisted breeding (MAB) programs are especially challenging when few available markers cover the upland cotton's complex genetic background (Qin *et al*., 2015). With the increase in the identified correlated molecular markers, hybrids were developed from upland cotton, and 52 significant loci were found related to six fiber quality traits from the parents and hybrids (Huang *et al*., 2018) showing the success of gene specific markers in marker assisted breeding.

Auxin

Significant MTAs from the CDS of auxin response factor 9 and auxin-responsive IAA30-like protein were positively correlated with tensile strength and percent fiber strain. Four abaca accessions, namely, Tangongon, Laylay, Kutay-kutay, and Native have showed amplification in these markers and exhibited high girth and tensile strength. Auxin promotes fiber elongation in cotton (Zhu *et al*., 2022). Its accumulation in fiber cells during growth is also responsible for the development of cotton fiber cells (Zhang *et al*., 2017). In a gene expression analysis, auxin was observed to control the activity of reactive oxygen species and secondary wall deposition in fiber cells of cotton (Zhang *et al*., 2020). Buyyarapu *et al.* (2011) used cgSSR markers from auxin to determine its relationship with fiber development in cotton and these markers were found polymorphic for the 26 species of cotton, suggesting their varying number and effect in the cotton genome. The SAUR gene family, also known as Small Auxin-up RNA genes are responsible for the growth of plant tissues at different stages in flax (*L. usitatissimum* L.) The genome-wide analysis of the SAUR genes revealed 86 *Lus*SAUR genes. It was also found that these genes are highly influenced by the application of exogenous IAA. Some of the identified *Lus*SAUR genes were also found to be regulated in the cell wall thickening of the phloem bundle and the salt response of flax (Bao *et al*., 2024). The response of the 15 randomly selected SAUR genes of ramie was also observed under drought and high-temperature conditions. Eight of the *Bn*SAUR genes were regulated by both abiotic stress but mostly downregulated during high temperatures. Most notably, BnSAUR43 showed enhanced expression when subjected to drought conditions. These identified SAUR genes can be used for early detection of auxin-influenced traits in ramie (Huang *et al*., 2016). Various *SAUR* genes are differentially expressed in the leaves of Agave indicating their role in the leaf development of the crop (Deng *et al*., 2019). The studies mentioned revealed not only the possible role of auxin during stress conditions for fiber crops but also auxin's accumulation in the fiber cells meant a better fiber strength. The markers identified in this study can be used later to determine the relationship of abiotic stress with the fiber quality of abaca. The preliminary nature of this study also calls for further studies on expression analysis of auxin.

Cellulose synthase genes

In this study, the molecular marker generated from cellulose synthase-like protein D5 explains 35% of the phenotypic variation in the tensile strength of the abaca accessions. Cellulose is an essential carbohydrate that makes up the plant cell wall. It is a chain typically composed of 36 β-1,4-glucose units synthesized from the *CesA* proteins produced by the cellulose synthase genes. The functions of *CesA* genes were first studied in *Arabidopsis* using mutation. From the study of Beeckman *et al*. (2002), it appears that out of the 10 *CesA* genes, only four - *CESA1, CESA2, CESA3*, and *CESA9* - function in synthesizing primary cell walls in the embryo of *A. thaliana* while *AtCesA 4,7, and* 8 are responsible for secondary cell wall synthesis. The latter set of genes was confirmed to be upregulated in the secondary cell walls of cotton fibers (Betancur *et al*., 2010). Based on differential gene expression analysis, four cellulose synthase genes have been identified in flax, namely LusCESA1, LusCESA3-B, LusCESA4, and LusCESA8-A while ramie has CESA3 and CESA8 (Xie *et al*., 2020). These genes were observed to be upregulated during cell wall biosynthesis in these fiber crops.

Elucidating the history of gene families can result

to better functional analysis for *Musa* species, given the transcriptomic analysis for the crop is limited (Cenci *et al*., 2014). In abaca, only *CesA 7-5* and *CesA 9-7* were upregulated in Abuab while expansin and sucrose synthase genes were upregulated in Pacol. Based on the generated gene expression data, BC_1 resembles Abuab while BC_2 and BC_3 are more like the Pacol parent (Reamillo, 2018). The mutation in *CesA7* was found to show reduced fiber cell wall thickness and cellulose content as well as the absence of secondary cell walls (Maleki *et al*., 2016). This shows the importance of cellulose synthase genes in the strength and thickness of the fibers.

Tinawagang Puti is an abaca variety from the Bicol region and is one of the recommended varieties of PhilFIDA. Increasing its alpha cellulose and hemicellulose content was seen possible through addition of phosphorus sources (Bondad *et al*., 1979); however, the reason behind this physiological response was not fully identified Elucidating the underlying molecular mechanism would provide more valuable information and a more systematic approach to improving such abaca accessions. Because there are few studies on *M. textilis* Nee transcriptomics, focusing on the gene sequences of prominent fiber gene families and their probable phylogenetic relationship with model species will provide a better understanding of the expression control of the plant. In our study, the markers designed from the cellulose synthase genes studied by Reamillo (2018) exhibited an association with the abaca accessions' tensile strength and pseudostem girth size. With these molecular markers, simpler molecular approaches (i.e. PCR amplification) can be applied to identify accessions with the cellulose synthase gene and good fiber quality.

Expansin genes

The expansin markers explained the phenotypic variation of the number of suckers and dry weight by 0.6% and 57%, respectively. Cell walls must be sturdy enough to avoid damage brought by cell enlargement or external force and should also be flexible enough to permit some impact and induce wall relaxation. This ability is known as cell wall loosening. Expansin proteins are released to mediate the cell wall loosening (Sampedro and Cosgrove, 2005). These proteins are also used by grass pollens to soften the maternal cell walls of stigmas (Cosgrove, 2016). They also affect the germination and the fruiting stage of plants. Expansin, like *CesA* and sucrose synthase, is a large gene family composed of four subfamilies, namely, α-expansin, β-expansin, expansin-like A, and expansin-like B (Ding *et al*., 2016). The expansin genes that belonged to the α and β evolved from a common ancestor and shared similar introns. Based on the analysis of rice expansin genes, the promoter possessed responsive elements to hormones (Lee *et al.*, 2001). The relationship of expansin proteins with hormones was also observed in poplar trees (*Populus nigra* L.) and aspen (*Populus tremula* L.). The increase in salt stress caused the decrease of *PtrEXPA3* and *PnEXPA3* activities resulting in decreased size of petioles, leaves, and internodes but increased size of epidermal cells (Kuluev *et al.*, 2017). This shows that expansin proteins are responsible for stress-related events. Phylogenetics provided vital information and association analysis of expansin genes to distantly related species. This also directs researchers to a more systematic approach when manipulating *EXP* genes. Screening genomic libraries and PCR amplification revealed that out of the six expansin genes of cotton (*GhExp1-GhExp6*), *GhExp1* and *GhExp2* are involved in the elongation of the cotton cells during fiber development (Harmer *et al.*, 2002). In bast fibers like flax, expansin was observed to be upregulated during cell wall thickening, indicating its role in fiber cell rearrangement during maturation (Mokshina *et al*., 2020). Expansin genes are upregulated during oxidative and osmotic stresses such as drought and high salt conditions (Gao *et al*., 2018). The genes identified in the abaca accessions were Expansin A2 and A4 and were highly correlated with the number of suckers and dry weight. This indicates that using simple laboratories and computation techniques can link genes to their functions and accessions with the trait of interest.

COBRA-like genes

This protein family is glycosylphosphatidylinositol-chained proteins that modulate the orientation of cell expansion and cellulose crystallinity. The tetraploid *G. hirsutum* has 14-15 COBL genes more than the *G. raimondii* and *G. arboretum*. *GhCOBL9* and *GhCOBL13* were upregulated with cellulose synthase genes during secondary cell wall biosynthesis. Based on QTL analysis, *GhCOBL9* is correlated with fiber quality (Niu *et al.*, 2015). Expression analysis also revealed that *GhCOBL9* was constantly upregulated during secondary cell wall thickening in cotton (He *et al*., 2025). Mechanical resistance to lodging in sorghum (*Sorghum bicolor*)

was observed reduced by only one mutation in *SbBC1* genes that encode COBRA-like proteins. Not only did it affect mechanical strength but also the cellulose content and lignin content. These components decreased and increased, respectively. This suggests that *SbBC1* genes are involved in cell wall biosynthesis in sorghum (Li *et al*., 2019). This protein was found along the sides of *Arabidopsis* roots where there is an increased elongation activity (Schindelman *et al*., 2001). COBRA acts as a "polysaccharide chaperone" that binds to individual β-1,4-linked glucan units resulting in cellulose crystallization (Sorek *et al*., 2014). *In-silico* analysis revealed thirteen glycophosphatidylinositol anchors encoding genes from nine *Hevea* genomes that share similar functions with their *Arabidopsis* counterparts. Out of these thirteen genes, nine clustered in the COBRA gene subfamily-1 while the rest clustered with COBRA gene subfamily-II (Putranto *et al*., 2017). The mutated *SlCOBRA*-like proteins of tomato caused cell wall degradation by upregulating wall-degrading genes while overexpressed *SlCOBRA*-like proteins resulted in the thicker cell wall of fruits. This indicates that COBRA-like proteins participate in cell wall biosynthesis (Cao *et al*., 2012). In flax, their role is vague, but are upregulated during the primary, secondary, and tertiary cell wall formation (Mokshina *et al*., 2020). Based on expression analysis, a similar role was also observed in hemp, where the CBL 4 gene is active during cell wall formation (Koziel, 2010). In poplar trees, the cobra-like genes are involved in cellulose microfibril orientation (Gritsch *et al*., 2015). In the study of Reamillo (2018), COBRA-like proteins were upregulated in Abuab and downregulated in the backcrosses. In our work, only the cobra-like protein 4 was highly correlated with the number of suckers, explaining 7.8% of the phenotypic variation in the abaca collection. This trait showed a negative relationship with tensile strength and can be used to determine the fiber quality even at the juvenile stage of abaca.

This study explores the use of intragenic molecular markers to identify significant MTAs (p-value <0.005) related to fiber quality and tensile strength of abaca. Eight MTAs related to five traits were found to have a positive significant effect of up to 263 and percent phenotypic variance explained ranging from 0.6% to 57%. The molecular markers from the auxin, cellulose synthase, expansin, and COBRA-like gene families related to fiber development were identified in this study. However, it is suggested that these genes should undergo subsequent experiments to solidify their role in the abaca fiber quality and tensile strength. The markers identified in this study can be used to fast-track the crop improvement of abaca, for only the hybrids that possess the gene of interest shall be tested in advanced yield trials. The marker-assisted selection increases the precision of breeding in abaca and lowers agricultural input waste during field testing. With this research, we determined nine abaca accessions that possess both traits and markers of interest and were used to generate 49 seeds. These hybrids will be evaluated using morphological and molecular markers in a future study.

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Table 1S - The site of collection of the 73 abaca (*Musa textilis* Nee) used in the study

Accession	Region	Accession	Region
Sarabianon	Region XIII	Lunhan_d	Region IX
Laguis_b	Region XIII	Putian_a	Region IX
Laylay_a	Region XIII	Jugbagon_bongolanon	Region IX
Batayan	Region XIII	Kutay-Kutay_a	Region IX
Tangongon_b	Region XIII	Bakayakan	Region IX
Laguis_a	Region XIII	Tangongon_a	Region IX
Inosa_a	Region XIII	Kutay-kutay_c	Region IV-B
Sibagat	Region XIII	Presentacion 2	Region IV-B
Maguindanao_b	Region XII	PSU_b	Region IV-B
Maguindanao_a	Region XII	PSU_a	Region IV-B
Maguinosa	Region XII	PSU_c	Region IV-B
Tangongon_x_Javaque	Region XII	Wild_Puti_Tamaraw_a	Region IV-B
Linawaan_inosa	Region VIII	Wild_2_Sabang-cabayangan	Region IV-B
Inosa_b	Region VIII	Wild1_sabang_cabayangan	Region IV-B
Inosa_linawaan	Region VIII	Wild_1_Caramay	Region IV-B
Canarahon	Region VIII	Pacol	Region IV-B
Linuy-a	Region VIII	Wild5_Buenavista	Region IV-B
Linawaan_laylay	Region VIII	Samuro Siniloan	Region IV-A
Bongolanon_native	Region VII	Lagunoyon	Region III
Puti_a	Region VII	Abuab_a	Region III
Negro_d	Region VI	Wild	Region III
Agbayanon_b	Region VI	Laylay_b	CAR
Agbayanon_a	Region VI	Nantonin_hemp	CAR
Bisaya	Region VI	Luno	CAR
Agbayanon_c	Region VI	Nantonin	CAR
Native_a	Region VI	07-002	Region VII
Presentacion_a	Region V	Catandungan	Region V
Tuod_a	Region V	Paniman	Region V
Bagwisan	Region V	Abuab_b	Region V
Abuab_c	Region V	Samuro Black	Region V
Kutay-kutay_Bicol	Region V	MTP	Region V
Minononga	Region V	Abuab_Negro	Region V
Inisarog	Region V	Bongiliwon	Region V
Parang	Region V	Puti_b	Region V
Linoloban	Region V	Kurukutuhan	Region V
Abuab TC	Region V	Laylay_c	Region V
Canton	Region V		

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Review paper

Role of vertical farming for sustainable urban horticulture: A review

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Key words: Hydroponics, production system, soilless farming, sustainability, urban agriculture.

Abstract: New constraints such as urbanization, food security, farmland scarcity, and escalating greenhouse gas emissions underscore the importance of vertical farming. This eco-friendly method offers a promising solution to traditional farming, aiding a growing global populace in securing sustenance. Resource use efficiency of vertical farming and the ability to produce premium agricultural goods are driving its global appeal. Particularly beneficial in areas with limited soil and water resources, vertical farming could play a vital role in sustaining fruit and vegetable production. Vertical agriculture emphasizes the critical need for urban centers to combat pollution and escalating food expenses by prioritizing self-reliance through local food production. Advanced cultivation techniques like hydroponics and aeroponics make vertical farming viable for urban environments, requiring minimal oversight and yielding higher outputs. Despite its potential, vertical farming encounters obstacles such as steep upfront and operational costs, complexity, and maintenance demands for optimal growth conditions. To promote urban agriculture, there is a need for enhanced extension services to educate and train growers and farmers on vertical farming techniques for producing diverse horticultural yields.

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no conflict of interests.

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1. Introduction

What is urban horticulture?

With each one-degree centigrade increase in temperature, an estimated 10 percent of the current cropping area is predicted to become unusable (Bouteska *et al*., 2024). This scenario raises concerns about the potential scarcity of land for farming to adequately feed the global population shortly (Clemson University, 2011). Moreover, research indicates that roughly 80 percent of the currently cultivated land worldwide is actively being utilized (Clemson University, 2011). Urban horticulture, involving the precise cultivation of crops for the production, processing, and distribution of food and other goods within urban and peri-urban settings, offers a solution to this challenge. Definitions of

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urban and horticultural practices vary across nations, regions, and local cultural contexts (CDRF, 2010). Horticulture involves creating gardens and growing plants, while agronomy focuses on managing forests, trees, and their products, along with the cultivation of cereals, animal feed, and other agricultural activities (Abegunde, 2014). Horticulture, which encompasses the production of crops for food, medicine, or aesthetics, falls under the umbrella of agriculture. Conversely, agronomy, primarily concerned with field cropping, centers around producing durable goods like fiber, oilseeds, and legumes.

Urban horticulture encompasses the cultivation of edible and medicinal plants such as fruits, vegetables, herbs, and mushrooms in urban areas and their environs, especially in land-constrained settings (Orsini *et al*., 2013). Its applications range from landscaping public spaces to therapeutic uses for individuals with disabilities (Moustier and Danso, 2006). This practice varies in scale and scope, spanning from high-tech intensive methods to small-scale soilless cultivation with localized irrigation systems (Drescher *et al.*, 2006). In developed nations, urban horticulture often occurs on small state-owned, private, or public plots like community gardens, offering not just fresh produce but also social engagement and recreational benefits (Van Leeuwen *et al*., 2017; Nugent, 2000). Conversely, in developing countries, urban horticulture serves as a means to establish self-sustaining enterprises, generating income and fostering social stability (Van Leeuwen *et al*., 2017). Furthermore, urban horticulture enhances access to fresh, nutritious foods through local gardens and plays a significant role in combating obesity and related health issues. By cultivating fruits and vegetables locally, urban communities can increase their intake of nutritious foods. This approach not only offers scarce food resources to migrant communities but also fosters a sense of community and belonging among residents through urban horticulture projects. Community gardens in particular, provide opportunities for social interaction, shared responsibility, and the establishment of support networks, which can contribute to overall well-being and healthier lifestyles. These advancements address malnutrition prevalent in many urban settings (Lovell, 2016). Urban horticulture plays a vital role in stimulating employment opportunities within agriculture, marketing, and processing domains, thereby enhancing livelihoods and boosting supplemental income for all essential participants within the horticultural value network (Orsini *et al*., 2013). Local food production minimizes intermediaries, transportation, storage costs, and handling, thereby reducing the economic and environmental footprint of food production (FAO, 2014). Furthermore, urban horticulture can help alleviate food deserts in underserved communities and provide affordable food options (De Leever, 2010). By offering recreational spaces, preserved environments, and biodiversity, urban horticulture enhances the quality of life for urban residents (Van Leeuwen *et al*., 2017). Pace *et al*. (2018) observed that concerning postharvest storage, lettuces grown in soilless conditions demonstrated superior qualitative and microbiological performance compared to those cultivated in soil. Specifically, the soilless growing system enhanced the storability of lettuces and facilitated the production of uncontaminated raw materials.

What is vertical farming?

Vertical farming, initially implemented by American geologist Gilbert Ellis Bailey in 1915 (Kretschmer and Kollenberg, 2011) and it was revolutionized by Patrick Blanc, who invented and patented a geometric approach that maximizes production efficiency within a compact footprint. This method entails vertically aligning animals and plants for food or other purposes in a soil-free, space-saving design (Anirudh, 2014) that capitalizes on acreage volume, expanding development possibilities in three dimensions compared to traditional farming practices. Vertical farming systems can range from stacked containers to interconnected networks sprawled over multiple acres, adaptable for indoor and outdoor settings. Utilizing biodegradable and reusable mediums, these systems can be powered by alternative energy sources for pumps and sustainably nourished with organic materials. The definition of vertical farming varies based on factors like size, density, control level, layout, structure, location, and objectives, leading to diverse stakeholder perspectives, from viewing it as a minor crop activity to a pivotal component for future food security. The interchangeable use of “vertical farming” as both an activity and a term further complicates its perception (Waldron, 2018).

Essentially, “vertical farming” refers to cultivating plants in multiple layers to maximize yield within a

limited surface area. Chin *et al*. (2017) note that vertical gardening serves various purposes, including ecological and financial benefits. For plant enthusiasts, growing plants vertically in confined spaces provides an engaging activity for both plant enthusiasts and experts. For educators, vertical garden cultivation offers a unique and stimulating opportunity for teachers to practically impart science, basic arithmetic, and finance concepts. For Health-Conscious Consumers, vertical planting, based on organic principles, yields cleaner, fresher fruits, and vegetables with minimal chemical input, appealing to those focused on healthy eating. For Business-oriented Individuals, vertical farming provides businesses with a sustainable and efficient approach to food production, creating valuable opportunities for growth and innovation. This controlled setting offers numerous benefits, such as decreased risks of disease and pest outbreaks, reduced environmental impact, and the ability to uphold produce quality while conserving water (Van der Schans *et al*., 2014). Vertical farming systems minimize water usage and streamline food sales and distribution within urban areas, lowering associated costs. Vertical gardening acts as a sustainable substitute for conventional crop cultivation, aiding in the conservation of land for traditional farming methods. Recognized as a cost-effective food production method (Jansen, 2017), vertical farming proves invaluable in land-scarce scenarios, making it an ideal solution for terrace kitchen gardens. Furthermore, vertical farming plays a significant role in promoting organic farming practices (Anirudh, 2014), allowing growers to sidestep land-related challenges like pollution, environmental rehabilitation, and zoning issues through the use of indoor vertical farming techniques (Anderson *et al*., 2012). Moreover, this technique would reduce the reliance on synthetic pesticides (Garg and Balodi, 2014).

Other advantages of vertical farming include year-round produce distribution, immunity to yield losses due to adverse weather, job creation in urban settings, water conservation, and reuse, and decreased food spoilage risks from mishandling. The utilization of controlled environments in vertical farming enhances its resilience against the effects of climate change (Germer *et al*., 2011). Crops in vertical gardens are typically more manageable for cultivation and harvesting, with lower susceptibility to diseases and pests (Utami *et al*., 2012). The absence of soil in vertical farming systems generally prevents weed growth, reducing labor costs in this aspect. Plants receive optimal aeration, and the visual appeal of the garden is enhanced through vertical farming practices. Comparatively, higher yields are often achieved with this method than with traditional farming practices. For example, Chinese cabbage yield using vertical farming reached 45 tons per hectare, surpassing the 10 to 25 tons per hectare range seen in field cultivation of the same crop (Utami *et al*., 2012). According to the opinion of Kannaujia *et al*. (2021), the benefits of vertical farming encompass increased net returns per unit area, accelerated growth leading to higher yields, reduced water and fertilizer usage, job opportunities, enhanced farmer incomes, continuous year-round production, environmentally friendlier processes compared to traditional methods, decreased risks of crop failures, minimized reliance on pesticides for pest control, progress towards nutritional security, and immunity to adverse environmental conditions. Vertical structures primarily cultivate plants that grow upright, are not sprawling, and bear fruits along their stems. These include vegetables like lettuce, tomatoes, cucumbers, bell peppers, mint, cilantro, spinach, strawberries, various herbs, and certain medicinal plants that demand minimal vertical space (Kannaujia *et al*., 2021).

2. Vertical farming types, techniques, and production systems

Outdoor vertical farming type

Vertical farming outdoors entails cultivating vegetables using vertical support systems constructed from readily available materials, such as bamboo structures supplemented with wires to assist the vegetables in climbing upwards (Kannaujia *et al*., 2021).

Indoor vertical farming type

Indoor vertical farming involves a range of permanent structures that support plant growth, typically within polyhouses, net houses, shade nets, and bamboo structures covered with plastic sheets. This method encompasses hydroponics, aeroponics, and aquaponics (Kannaujia *et al*., 2021). An indoor multilayer plant cultivation system meticulously

manages all growth factors like temperature, light, carbon dioxide levels (CO_2), humidity, water, and nutrients to yield abundant, high-quality fresh produce consistently throughout the year, independent of natural light or outdoor conditions (Sharathkumar *et al*., 2020). Vertical farming involves growing crops or vegetables in vertically stacked layers or on vertical structures that promote upward growth, typically within a controlled environment tailored for optimal plant growth. It frequently employs soilless farming methods like hydroponics, aquaponics, and aeroponics (Kannaujia *et al*., 2021).

Hydroponics

Hydroponics entails cultivating plants without soil, with their roots submerged in nutrient-rich liquid solutions comprising vital macronutrients like nitrogen, phosphorus, and potassium, along with trace elements such as iron, manganese, and zinc. Inert materials like gravel, sand, sawdust etc. are used to support the roots in hydroponic systems (Fig. 1). By maintaining controlled environments and adhering to strict certification standards, hydroponic farms present a pragmatic method for sustainable food production by eradicating the necessity for harmful chemicals like pesticides. This innovative farming method is not just a concept but is actively being integrated into sustainable agriculture practices to meet the increasing global food demand (Debangshi, 2021). Hydroponics involves plants growing without soil, instead utilizing inert mediums like coco peat and nutrient-rich water solutions to support and nourish the roots, and can conserve about 70 percent more water than traditional farming methods (Bhanu Murthy *et al.*, 2022).

It presents a viable production alternative that vegetable growers can explore, with setups possible even in small spaces like a 400 ft² area or a small shed (USDA, 2016). In hydroponic systems, plant roots are immersed in a nutrient solution tailored to meet their nutrient requirement (Despommier, 2014).

Aquaponics

Aquaponics represents a type of hydroponics where plants are cultivated alongside aquatic organisms in a closed-loop system that emulates natural processes. A device for solids removal filters the nutrient-rich wastewater from fish tanks before channeling it to a biofilter, where harmful ammonia is converted into nutrient-rich nitrate. The plants absorb these nutrients, cleansing the wastewater before it returns to the fish tanks. Additionally, plants can absorb carbon dioxide (CO_2) from the air to perform photosynthesis, which is essential for their growth. In an aquatic environment like a fish tank or aquaponic system, fish release carbon dioxide into the water through respiration (Fig. 2). Therefore, in

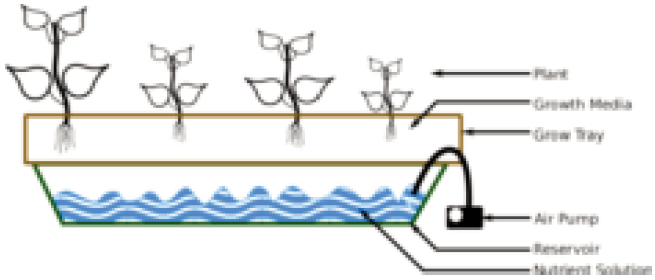

Fig. 1 - A model of hydroponics. Adapted from: https://images.app.goo.gl/jshegzQxE9uQnUk3A

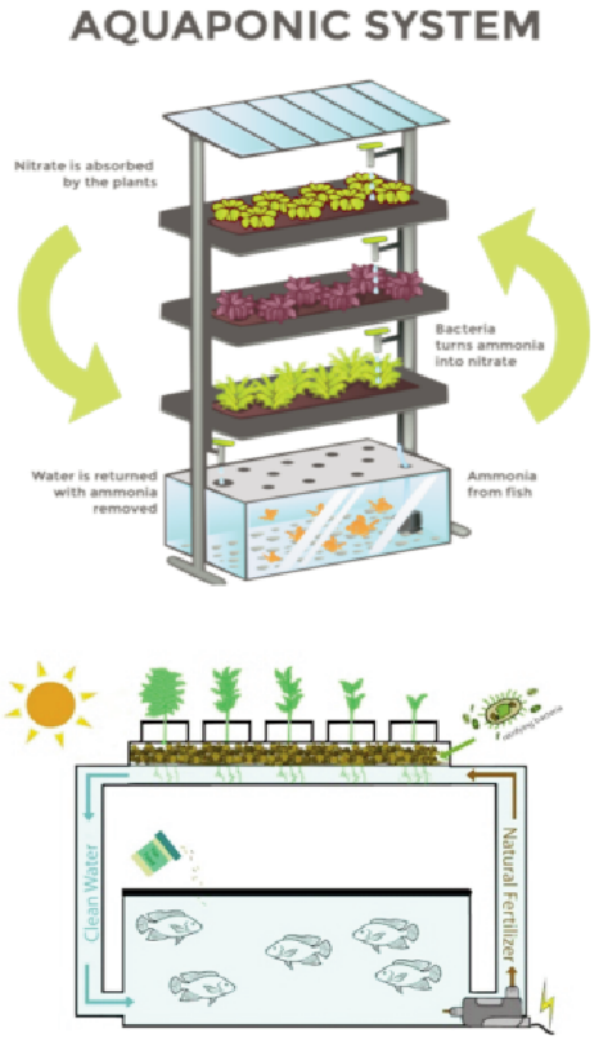

Fig. 2 - A model of aquaponics (Adapted from: https://images.app.goo.gl/6sKPJmTEfi1Z4MgZ7).

an aquaponic system or a similar setup where fish and plants coexist, it is important for the plants to uptake CO_2 from the fish to help maintain the balance of gases in the water and promote the well-being of both the aquatic life and the plants. Despite its aquacultural aspect, aquaponics is not as commonly employed as traditional hydroponics in commercial vertical farming setups, which typically focus on cultivating select fast-growing vegetable crops (Debangshi, 2021). Aquaponics functions as an ecosystem built on mutual relationships between plants and fish. The concept revolves around establishing a semi-self-sustaining system where fish waste nourishes plants in a vertical farming environment (Diver, 2021).

Notably, aquaponics utilizes only 2 percent of the water required in traditional farming, and operates as a closed-loop system with recyclable outputs, thereby generating minimal waste (McCollow, 2014). For instance, AeroFarms in New Jersey reportedly consumes 95 percent less water compared to field cultivation to produce equivalent volumes of leafy greens (Peters, 2015). Filtration poses a significant challenge for aquaponic businesses, as the accumulation of debris can endanger the fish and cause a chemical imbalance in water which is crucial for supporting plant growth (McCollow, 2014).

Aeroponics

Aeroponics originated from NASA's project in the 1990s, aiming to develop an efficient method for plant cultivation in space. Unlike hydroponics and aquaponics, aeroponics operates without a solid or liquid medium for plant growth. However, a liquid nutrient solution is being sprayed on plant roots in sprinkler form. It stands out as the most environmentally sustainable soilless cultivation technique, requiring no medium replenishment and consuming up to 90 percent less water than highly efficient traditional hydroponic systems. No growing medium is necessary for aeroponics, as the system is designed vertically, leading to energy savings as excess liquid naturally drains away due to gravity, unlike traditional horizontal hydroponic systems that often rely on water pumps to manage surplus solutions (Fig. 3). While aeroponic systems are not prevalent in current vertical farming practices, they are gradually gaining popularity (Debangshi, 2021). Aeroponics supports the growth of vegetables, flowers, and fruits, offering potentially higher nutrient quality and absorption of vitamins and nutrients compared to other methods (Birkby, 2016). Despite the use of modern pumps and control systems, aeroponics requires less automation.

These systems are favored over hydroponics for their water conservation benefits and reduced disease susceptibility (Boston, 2014). The vertical growth pattern in aeroponics makes it ideal for producing a large volume of plant materials in limited spaces, with plants either growing vertically in a tube-like structure or suspended in containers.

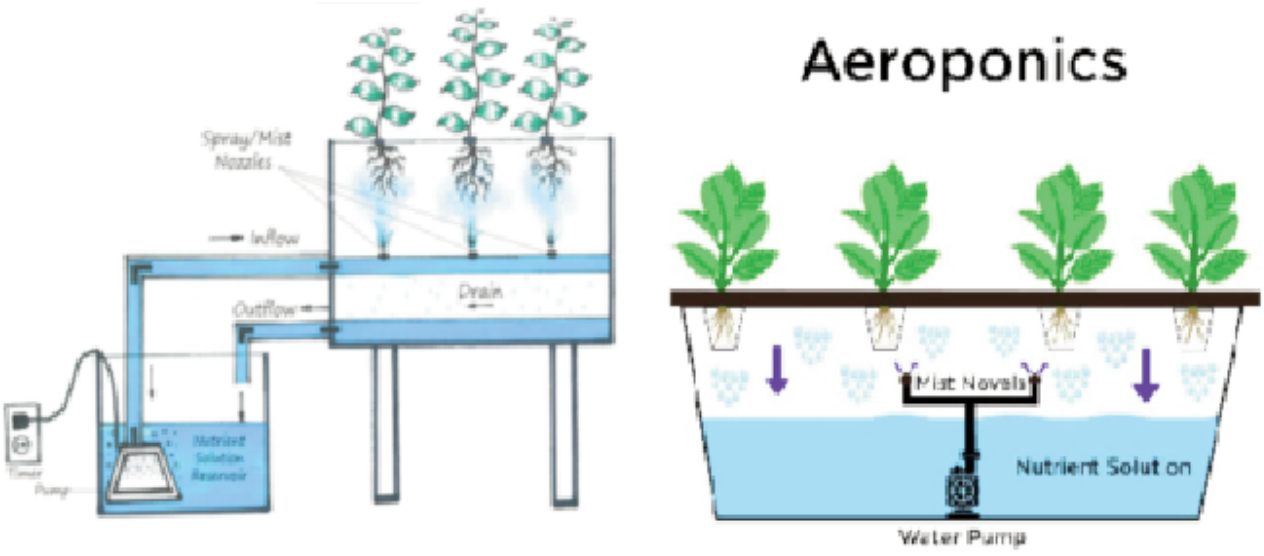

Fig. 3 - A model of aeroponics (Adapted from: https://images.app.goo.gl/9JCB4eRgYPya8vsa8)

3. What are the opportunities and importance of vertical farming for sustainable urban horticulture?

Globally, urban regions exhibit higher population densities compared to rural areas, accommodating 54 percent of the total population in 2014 (Fig. 4). The rapid economic growth over the last thirty years has coincided with a significant increase in the urban population. In 1950, urban dwellers constituted 30 percent of the global populace; projections indicate that by 2050, this figure will swell to 66 percent. With the world's population expected to approach nine billion by 2050, the role of vertical farming in fostering sustainable urban horticulture and ensuring food security has emerged as a pivotal topic for discussion. Urbanization entails a demographic shift where a larger share of a nation's populace resides in cities due to migration from rural areas to regions that subsequently witness declining populations, leading to escalated land utilization (Satterthwaite *et al*., 2010). The repercussions of urbanization on agriculture are poised to revolutionize how food is distributed and marketed (Kennedy *et al*., 2004). Notably, urban locales are witnessing a rising inclination towards the consumption of fresh produce such as fruits, vegetables, meat, and dairy (Agnes, 2014). Forecasts suggest that urbanization will amplify job opportunities in food processing, transportation, and retail sectors while diminishing agricultural employment (Cohen and Garret, 2010). Consequently, urban residents will be compelled to offer high-quality products for consumption or commercial purposes through urban agriculture to sustain their livelihoods (Redwood, 2009).

In the contemporary era, we are witnessing a swift surge in global population, particularly in developing regions like Africa, Asia, and South America.

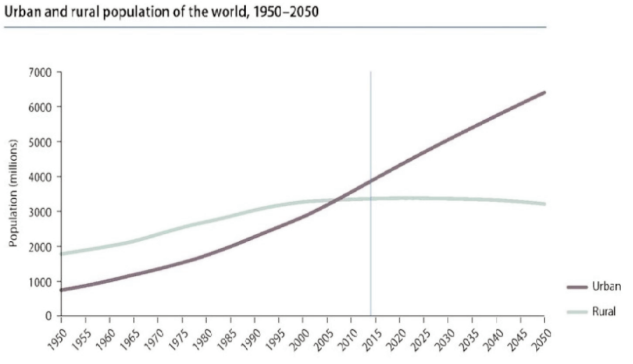

Fig. 4 - World urban and rural population, 1950-2050. Source, Nwosisi and Nandwani (2018).

Projections by United Nations indicate that by 2100, the global population is expected to surpass 21 billion individuals. UN estimations suggest that by 2050, 66 percent of the world's populace will reside in urban areas, with Europe reaching 82 percent urbanization (United Nations, 2017). The rapid expansion of the population invariably translates into heightened food requirements. Presently, 80 percent of the world's arable land is under cultivation, while the remaining 20 percent lies barren, its agricultural potential nearly squandered due to inadequate land management in recent decades (Eigenbrod and Gruda, 2015). The global population is swiftly escalating, alongside the pace of urbanization. UN projections from 2015 forecast a population increase exceeding double by 2050, urbanization trends continue to evolve, with more people moving to cities globally. By 2050, the United Nations projects that nearly 68% of the world's population will live in urban areas. As urban regions contribute to more than 70 percent of worldwide CO_2 emissions, resulting in pollution and negative environmental consequences, ensuring food security emerges as a critical issue in urban environments. As urban populations swell, the conventional cultivation of fresh fruits and vegetables faces challenges due to nutrient-deficient soil and limited usable land, posing a significant threat to the supply of fresh produce (Zaręba *et al*., 2021). Vertical farming, a soilless agricultural technique for cultivating fresh vegetables, is swiftly gaining traction and holds a promising future. Researchers and scientists are exploring this method of farming as a viable option for urban dwellers.

Despite its longstanding presence, vertical farming remains relatively unknown to many, with only a minority having harnessed its potential for growing fresh produce. This innovative approach has the potential to significantly enhance food production while reducing the environmental impact of agriculture by utilizing less land, water, pesticides, and fertilizers, thereby enhancing overall efficiency (Barui *et al.*, 2022). The rising popularity of vertical farming globally is attributed to its efficient resource management and ability to yield high-quality foods. Particularly in regions with limited access to soil and water resources, vertical farming could revolutionize fruit and vegetable production. Across all crops, the

use of vertical farming technology results in a substantial increase in yields. However, the degree of improvement varies significantly, with crops like potatoes and tomatoes showcasing the most potential for yield enhancement. Peas and spinach reflect minimal benefits from the vertical farming technology (Table 1). To combat issues like congestion, pollution, and escalating food prices, urban areas facing scarcity of land and high real estate costs must generate sufficient food to sustain their populations (Mir *et al*., 2022). Factors such as rapid urbanization, natural calamities, climate change, and indiscriminate use of chemicals and pesticides have collectively contributed to deteriorating soil fertility. Consequently, soil productivity has plummeted, soil health has deteriorated, and the available land per person has dwindled (Lal, 2015). Projections suggest that by 2050, with the global population expected to reach 8.9 billion, there will be a 50 percent surge in food demand, necessitating additional arable land that is simply not accessible (FAO, 2011).

The anticipated arable land per capita by 2050 is forecasted to be less than 0.20 hectares, marking a decline to less than one-third of the 1970 levels (FAO, 2011) (Fig. 5). The challenges posed by these issues pose significant threats to conventional soil-based agricultural systems, rendering food production a formidable task in the present day. To address these challenges, traditional soil-based farming methods need to be augmented with more efficient and environmentally sustainable modern farming practices (Lehmann, 2010). In transitioning to

Table 1 - Estimated yield of a vertical farm compared to traditional farming. Source: Barui *et al*., 2022

Crops	Yield in vertical farming (tons/ha)	Field yield (tons/ha)
Carrots	58	30
Radish	23	15
Potatoes	150	28
Tomatoes	155	45
Pepper	133	30
Strawberry	69	30
Peas	9	6
Cabbage	67	50
Lettuce	37	25
Spinach	22	12
Total (average)	71	28

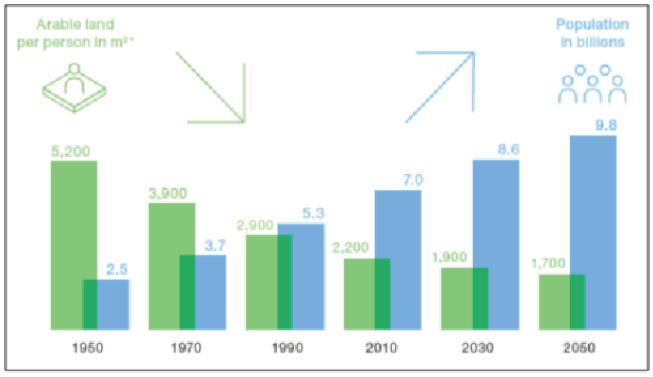

Fig. 5 - Scenario of global population and cultivable land with their projection up to 2050. Source: FAO (2011).

innovative vertical farming techniques, factors such as diminishing soil productivity, limited soil nutrient reserves, insufficient irrigation water availability, and the impacts of climate change must all be taken into account. Vertical farming methods, serving as an alternative to conventional soil-based farming systems, have the potential to serve as a supplementary solution in mitigating the scarcity of fertile arable lands and water resources (Texier, 2013).

The human population, grappling with the impacts of climate change, must forge a new realm of spaces that ensure environmental sustainability and foster sustainable urban horticulture. In the future, the rise of vertical urban farms will increase crop production, lower costs, and improve the quality of food in urban areas. This will aid in reducing the harmful environmental effects of urbanization (Thomaier *et al*., 2015).

Vertical urban farms offer numerous advantages for the natural environment, including the autonomy of vertical crops from the polluted urban surroundings, aligning with the principles of sustainable development for environmental well-being, minimal water usage that enhances sustainable water management, and the potential utilization of organic waste generated (Despommier, 2013). Crucially, advanced vertical farming surpasses traditional farming in food production. Recent plant factory technologies can cultivate fresh, safe, and nutrient-rich produce year-round within cities, despite climate fluctuations, exhibiting high water and land efficiency while requiring less labor than traditional methods (Kozai and Niu, 2015). The proliferation of horticultural offerings in urban

settings grants individuals more opportunities to engage with greenery and fosters community bonds. Many city dwellers, for instance, partake in activities like tending flower beds to enhance their city's green spaces and landscapes, showcasing how plants can cultivate communal ties. Moreover, these undertakings not only enhance the aesthetic appeal of cities but also significantly impact the well-being and quality of life of participants (Wakefield *et al*., 2007).

Vertical farming emerges as a promising strategy to address a range of Sustainable Development Goals (SDGs) established by the United Nations General Assembly in 2015, covering "good health and well-being, sustainable cities and communities, responsible consumption and production" (United Nations, 2020). Nature-related hobbies such as gardening and horticultural pursuits are renowned for their stress-reducing effects. Recent research underscores that engaging in gardening or horticultural activities can alleviate stress (Van den Berg and Cluster, 2011), boost self-esteem, nurture social connections (Cammack *et al*., 2002), and enhance cognitive well-being (Cimprich, 1993). Vertical farming is swiftly gaining prominence as a preferred cultivation method among various urban agricultural practices (Agrilyst, 2017). Vertical farming represents an advanced form of soilless cultivation that pushes the boundaries of traditional agriculture, evolving into a fusion of urban and rural practices. These vertical farms serve as hubs for the self-production of food, recreating ideal climatic conditions inside structures to support the growth of diverse plant varieties. Here, vegetables thrive without soil, drawing nutrients from water solutions under LED lighting systems. Supporters of vertical farming and urban horticulture emphasize that by functioning within controlled environments, these farms can protect crops from insect infestations and diseases, eliminating the necessity for pest control measures (Despommier, 2013). A key objective of vertical farming is to maximize crop yields per unit of land area utilized. However, when determining the optimal number of plants to cultivate within a given building space, careful attention must be paid to plant spacing. The density of plants is a critical factor in managing pests and diseases, as it affects the microclimate around the plants (Burdon and Chivers, 1982).

Achieving complete prevention of disease transmission would require impractically large plant spacing (Burdon and Chivers, 1982). Therefore, the quest for maximizing crop production per unit area must strike a balance between plant density and avoiding overcrowding for optimal crop growth and health. Even traditional open-field growers face mounting pressure to enhance productivity. The planet is showing signs of strain, with escalating disease pressures underscoring the critical need for sustainable solutions like vertical farming in regions striving for self-reliance. As we face the daunting challenge of feeding a burgeoning global population over the next two decades, the strategy involves harnessing space efficiently, expanding skyward, and repurposing existing urban areas (Kumar *et al*., 2018). Urban food security hinges on factors such as food availability, accessibility, and quality, all of which stand to benefit from the implementation of urban vertical farming techniques. Various gardening methods can significantly bolster the food security of communities and households. Whether at a personal or communal scale, cultivating food can enhance food and nutrition security across all economic strata (Kortright and Wakefield, 2011). The integration of urban horticulture in future city planning holds the promise of elevating food security levels, fostering a more balanced food distribution between rural and urban regions. Despite the advancement of global urban horticulture, rural agriculture will continue to play a pivotal role in ensuring global food security (Dubbeling *et al.*, 2010). Vertical farming epitomizes sustainability, with the ability to stack farms vertically to amplify productivity per unit of land, thereby conserving arable land for alternative uses. Moreover, these farms eliminate transportation costs by bringing crops closer to urban markets, while also facilitating the recycling of energy and water within their structures. Additionally, the utilization of methane digesters can convert organic waste into energy, powering the operations within the building itself (Kumar *et al*., 2018).

4. Challenges in vertical farming systems

The study conducted by Jasim *et al*. (2016) revealed that the majority of vertical farms interviewed lacked systematic tracking of key metrics such as monthly energy and water usage, precise yield, waste management, air quality, and the extent of pesticide or fertilizer application. These deficiency poses significant obstacle in establishing a

performance baseline for these operations. Light poses a critical challenge as light-emitting diodes must substitute natural sunlight, potentially leading to heightened energy consumption, operating expenses, capital outlay, and overall investment costs, particularly in large-scale commercial setups, thereby impacting profitability (Kretschmer and Kollenberg, 2011). Vertical farming primarily focuses on cultivating rapidly maturing vegetables and crops like herbs and salad greens. Slow-growing leafy vegetables and grains are less lucrative due to lower profitability.

Without natural insect populations, manual pollination is necessary in vertical farms, requiring additional labor and attention (Birkby, 2016). A key concern lies in the initial expenses associated with implementing vertical farming systems, encompassing costs for remote monitoring systems, automation technologies for stacking, climate control mechanisms, and other infrastructure (Barui *et al.*, 2022). According to Vashi and Dubei (2020), the potentials of vertical farming include catering to the rising demand for safe and organic produce, fostering community and hobby farming benefits, and repurposing historical structures that require management and restoration. Additionally, due to the diverse climatic and nutritional needs of various crops, mixed farming is impractical in vertical setups, and not all crops suitable for horizontal cultivation can thrive vertically. Meeting market or customer demands within specific timeframes poses challenges, as produce may mature ahead of or behind schedule, resulting in surplus yield from vertical structures that either go to waste or are undersold (Kannaujia *et al*., 2021).

Choosing a crop for vertical farming involves assessing two key factors: technical feasibility and market acceptance. Establishing and running a vertical farming system customized for a particular crop poses notable technical challenges, with the marketability of the produced goods being essential. While in theory, any crop can be grown vertically, the majority encounter notable technical and growth-related obstacles. Due to their small size, quick growth cycles, and minimal energy needs, several vertical farming systems specialize in leafy greens such as leaf lettuce, head lettuce, and herbs (Beacham *et al*., 2019). In contrast, energy-intensive crops such as large vining plants (e.g., melons) or tree fruits often require specialized designs different from standard vertical farming systems, making them less common in vertical farms. Companies specializing in vertical farming technologies play a key role in developing unique designs tailored to accommodate the specific needs of diverse crops in vertical farming systems.

5. Summary and future line of works

Vertical farming involves cultivating crops in confined areas where plants receive regular nutrients and water. Quick-growing green leafy vegetables such as mint, amaranthus, and lettuce thrive in vertical systems due to the accurate nutrients and water they get. This efficient growth results in high-quality produce and good yields, making vertical farming a promising solution for urban areas facing land scarcity and high food demand, aiming to combat pollution and escalating food prices. The outlook for vertical farming seems promising, particularly with the utilization of advanced technologies like hydroponics, aeroponics, and aquaponics.

These contemporary farming techniques are ideal for urban settings as they boost productivity significantly while vertical farms often utilize automation technologies such as sensors, artificial intelligence, and robotic systems to monitor and control factors like lighting, temperature, humidity, and nutrient delivery. This automation can reduce the need for constant manual intervention and monitoring. The decreasing availability of cultivable land in urban areas has worsened the difficulty of supplying fresh, high-quality vegetables. Hence, vertical farming emerges as an ideal alternative for cultivating fresh leafy vegetables and fruits in the times ahead. Further research into vertical farming is crucial to enhance production and lower operational expenses compared to traditional soil-based agriculture. To sustain urban horticulture, there is a pressing need to focus on extension programs to train farmers on vertical farming techniques and cultivate various crops in vertical structures. This education will be crucial in encouraging the extensive uptake of vertical farming techniques for a more sustainable urban food production system.

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