

Phenotypic Characterization of Citron Watermelon (*Citrullus lanatus* var. *citroides*) Genotypes for Bioenergy Production

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Abstract

Citron watermelon (*Citrullus lanatus* var. *citroides*) is an important climate smart crop, characterized by high oil content in the seeds and it is a popular fruit in southern Africa. To determine best performing genotypes eight phenotypic traits were assessed to identify cultivars with potential for biodiesel production. A field experiment was conducted at Sebele Agricultural Research Station, in Southern Botswana, during 2019 to 2020 cropping season. Four (4) melon genotypes were planted in a Randomized Complete Block Design replicated three times and eight (8) characters were assessed. Except seed oil content (26.6%) there was significant ($P < 0.05$) difference among all the traits, an indication of higher genetic diversity in the selected citron watermelon. SC1-Sesoswane, exhibited highest seed yield (1540 kg/ha) and oil yield (435.03 kg/ha) while MMB-280-Lerotse recorded lowest seed yield and oil yield of 548.1 kg/ha and 144 kg/ha respectively. There was a significant correlation of (0.9) between oil yield (kg/ha), number of fruits produced, and seed yield (kg/ha), an indication that increasing the seed yield will positively increase the amount of oil produced. This study revealed the potential bioenergy production of the four cultivars. However, SC1-Sesoswane appears a more promising alternative cultivar for feedstock for biodiesel production.

Keywords: bioenergy, biodiesel, citron watermelon, feedstock, genotypes

1. Introduction

Worldwide production and use of bioenergy as alternative source of energy is significantly increasing in response to environmental concerns posed by using fossil fuel (Hernandez & Capareda, 2019). The growth in the production of biodiesel has been phenomenal because of the general desire to cut down on the release of greenhouse gases into the atmosphere (Hanaki & Portugal-Pereira, 2018). According to Xavier (2020) the resources that are used to produce petrol and diesel are gradually getting depleted, it is evident that we need to look for alternate source of fuels. Botswana is a party to the United Nations Framework Convention on Climate Change (UNFCCC) and has adopted the Paris Agreement in 2015, hence the country has made voluntary commitment through its first Nationally Determined Contribution (NDC) to reduce the country's total greenhouse gas (GHG) emissions by 15% by the year 2030. Furthermore, the country is working towards providing affordable and clean energy to its general population to align with UN 2030 Agenda for Sustainable Development Goals, especially SDG7 (Government of Botswana, 2021).

Botswana relies on fossil fuel imports which are non-renewable and unstable due to rising costs, hence the government is seriously looking for alternative sources of energy. The Government of Botswana initiated the project of biofuel production about 10 years, in collaboration with the Japanese International Cooperation Agency (JICA)-Japan. The identified feedstocks as noted in Botswana National Development Plan 10 were sweet sorghum crop and Jathropha plant (Botswana National Development Plan 10). The potential of Jathropha plant as an alternative biodiesel production in Botswana was evaluated by Jonas et al. (2020), however since Jathropha is an exotic plant, efforts were intensified to conduct research on indigenous plants. Therefore, indigenous oil-bearing wild plant including *Sclerocarya birrea*, *Tylosema esculentum* and *Ximenia caffra* (Gandure & Ketlogetswe, 2011), and *Trichilia emetica* (Mashungwa & Mmolotsi, 2007) were explored.

In 2019, the Government of Botswana embarked on a new project on assessment of indigenous plants for potential biofuel production. The Biofuels Projects, whilst focusing on indigenous non-food plants, other feedstock such as animal fat and used cooking oil were included for evaluation. The project was undertaken by the Ministry of Mineral and Energy (MME) through the Department of Energy (DoE) in collaboration with National Agricultural Research & Development Institute (NARDI) in Ministry of Agriculture (MoA) and University of Botswana (UB). The latter two institutions provided research support and technical expertise. The NARDI was tasked specifically with Biofuel resource assessment, which includes collection and selection of indigenous oil producing plants with properties of high oil yield, such as *Croton megalobotrys* (Motsebe), *Hyphaene petersiana* (Mokolwane), *Ochna pulchra* (Monyelenyele), *Pappea capensis* (Mothata/Mopenweeng) and *Trichilia emetica* (Mosikiri). The characterisation, surveying areas where these five indigenous oils producing plant are found, and production of distribution map of these plants was undertaken through the Biofuel project.

Another crop with huge potential as a feedstock is citron watermelon (*Citrullus lanatus* var. *citroides*), a climate smart crop which originates from southern Africa (Chomicki & Renner, 2014), and this crop is well adapted to the semi-arid environment of Botswana. Citron watermelon plants belongs to family Cucurbitaceae, a prostrate monoecious annual, producing herbaceous stems which can grow up to 3 m long, the fruits are formed in different shapes, the flesh is firm and white, green-white or yellowish, while the seeds are varying in size, dicotyledonous, and typically red, white, or mottled seed coat colour (Meeuse, 1962). Different forms of watermelon occur: *C. lanatus* var. *lanatus* the sweet watermelons, *C. lanatus* var. *citroides*, the cow-melons cooking and seed or 'Sesoswane' types (Mujaju et al., 2010). Citron watermelon is a popular fruit in Botswana, traditionally called 'Marotse' it is consumed when the pulp is cooked fresh or dried as Thopi, or sundried as Mpale for the cooking type melon. The citron 'Sesoswane' has high seed oil content, and the seeds are used for extraction of oil. Crossbreeding between sweet watermelon and citron watermelon is common leading to the inter-varietal forms called 'Mokatse' which are abundant in the traditional agro-ecosystem (Mujaju et al., 2013), and these are essential as an animal feed during drought (Madibela et al., 2016). In Botswana watermelon production for both sweet watermelon and melon was 9,274 tonnes and 3,079 tonnes respectively, which is a total production of 12,353 tonnes, and this production is more than a combination of cereal grains (maize, sorghum, and millet) which had a total production of 2,126 tonnes for the 2019 growing season (Statistics Botswana, 2019).

Melon seed oil, like other vegetable oils can provide a useful source of biodiesel production, as it has a good energy source, and is a good candidate for transesterification (Ogunwa et al., 2015). In some instances, watermelon waste, especially the lignocellulosic material after drying can be blended with cow dung to produce renewable energy (Tambuwal & Okoh, 2018). The advent of second-generation (2G) technology used in the production of ethanol produced from lignocellulosic biomass of sugarcane waste (Pereira et al., 2015) can be explored on waste from citron watermelon to produce biofuel in Botswana. The local utilization of citron watermelon in biodiesel production will improve its production, markets, and generate employment for the small-scale farmers involved in growing the crop (Ogunwa & Achugasim, 2015), as an underutilized crop this will also add crop diversity, improve food security in the country (Mandizvo et al., 2021; Mayes et al., 2013). Despite the challenges for fossil fuel which has been implicated to have serious environmental issues, there is no documented information on citron watermelon as a potential feedstock to produce biodiesel in Botswana. Therefore, success of this study shall therefore aid at enhancing efficiency of marotse seed oil in the production of biodiesel as an alternative fuel which can replace the fossil fuels. Therefore, the objective of this study is to explore the potential of citron watermelon as feedstock for biodiesel production, by assessing seed yield, oil yield and yield related components and evaluate the relationships among the selected traits.

2. Materials and Methods

2.1 Plant Material and Experimental Design

Four genotypes of citron watermelon, that were sourced from National Plant Genetic Resource Centre (NPGRC), Gaborone, were used in the study. One is edible seed type SC1-Sesoswane (brown seed), and three are cooking types MMB-280-Lerotse (maroon seed), SC117-Lerotse (whitish-pink seed) and SC44-Lerotse (green seed), Figure 1. The field experiment was conducted at Sebele Research Station (24°33'S, 25°54'E, 994 m above sea level), Southeast part of Botswana. Soils are Eutric and Cambisols having medium to course texture (de Wit et al., 1990). The study was carried out during the rainy season of 2019/2020 from December 2019 to June 2020, in a randomized complete block design (RCBD) replicated three times. The plot size was 22.5 m², with planting of inter and intra spacing was 1.5 × 0.9 m, respectively. RCBD was used to improve precision of treatment comparisons and reduce variability among experimental units, the blocking allows for each block to be relatively

homogenous (Baird et al., 2022). The Generalized Linear Model regarding the Randomized Complete Block Design is shown below.

$$Y_{ij} = \mu.. + \rho_i + \tau_j + \varepsilon_{ij} \quad (1)$$

where, $i = 1, 2, \dots, n$; $j = 1, 2, \dots, r$; μ = overall mean response, averaging across all blocks and treatments; ρ_i : block effect, average difference in response for i -th block ($\sum \rho_i = 0$); τ_j treatment effect, average across blocks ($\sum \tau_j = 0$); $\varepsilon_{ij} \sim \text{iidN}(0, \sigma^2)$: random experimental error.

Soil samples were collected for analysis from the site, the soil chemical properties were analyzed at the Soil and Plant Analytical Laboratory (SPAL) Botswana, using standard procedures. Soil chemical properties were available for P, K, and total N, organic carbon and soil pH from SPAL as follows, pH (7.13), Organic Content (%) 0.71, P (mg/kg) 886.4, Ca (cmol/kg) 5.25, Mg (cmol/kg) 2.3, K (cmol/kg) 0.84, Na (cmol/kg) 0.73. The soil is fertile which implies that the soil levels of organic matter are adequate, high phosphate and enough potassium levels. The observed 7.13 soil pH indicates that the soil is suitable for watermelon production. The experimental location had an average maximum and minimum temperature varying 33.1-34.7 °C and 19.2-19.5 °C, respectively and approximately a total rainfall of 200 mm was received during the cropping period.

2.2 Agro-morphological Traits Evaluated and Statistical Analysis

The morphological and agronomic traits selected were chosen from International Plant Genetic Resources Institute, for *Cucumis melon* (IPGRI, 2003). Data collected include days to 50% flowering, seed number per fruit, fruit number per plot, rind thickness (cm) and diameter (cm), seed yield (kg/ha), seed oil content and oil yield (kg/ha). Seed number per fruit was recorded from 10 fruits, and are categorized as low (> 10), medium (10-100) and high (< 100), the seed number was counted using an electronic seed counter (contador Pfeuffer GmbH). Fruits number per plot, was record of fruit in each plot. Fruit sizes were measured at seed harvest maturity for the rind thickness (cm) and diameter (cm) using a ruler. The seeds were extracted using a juice extraction machine developed at the Department of Agricultural Research, and seed was weighed using a digital scale (Mettler Toledo-balances), and this was converted to Kg/ha. For measurement of oil content (%), a protocol by Jonas et al. (2018) was used, basically oil was extracted from samples using Ankom XT15 extractor and petroleum ether (chromatography grade) was used as a solvent. The oil yield (kg/ha) was calculated as a function of oil content and grain yield. Collected data was subjected to analysis of variance (ANOVA) with the Statistical Analysis System (SAS). Probabilities equal to or less than 0.05 were considered significant. If analysis of variance indicated differences between treatments means an LSD test was performed to separate them, while Pearson correlation analysis was conducted to identify the relations among traits.



Figure 1. Samples of the four citron watermelon genotypes used for the study

3. Results and Discussion

There was a significant difference ($P < 0.05$) among all the assessed traits except for seed oil content, therefore evincing the presence of high genetic diversity among the four cultivars (Table 1). However, the lack of variation on the seed oil content is an indication of similarity among the cooking melon and seed type melon of the four

selected cultivars. SC1-Sesoswane performed best in most of the parameters measured, as it required the minimum days to 50% flowering (32 days), while SC117-Lerotse took maximum days to 50% flowering (40 days) (Table 1). Similarly, do Nascimento et al. (2018) observed a wide range of days to flowering of 33.7 to 48 days, they considered early flowering to have an advantage of faster return of investment to farmers and a key trait for escape to drought for watermelons.

Seed number per fruit was higher (783) for SC1-Sesoswane, an indication that it has a potential for production of higher plant population compared to other cultivars but was only significantly different from MMB-280-Lerotse with lowest seeds of 353 (Table 1). Although, SC1-Sesoswane had a significantly ($P < 0.01$) higher fruit number, it exhibited lower fruit diameter and rind thickness, which is typical of a seed melon type. Fruit diameter varied from 14.6 cm to 30.3 cm with an average of 25.9 cm, and this is relatively bigger than those of the 37 melons accession evaluated in Brazil which had a diameter range of 8.0 cm to 17.7 cm and average of 12.95 (Gomes et al., 2021). However, rind thickness of 2.0 cm observed in their study was in accordance with those recorded in this research with an average of 2.4 cm. This could be attributed to the dominating traits of smaller rind thickness that was largely contributed by the smaller fruit sized and seed producing type melon. Our findings agree with the local melon grown in Tunisia with fruit length of 31.8 cm, and fruit diameter of 38.3 cm (Chikh-Rouhou et al., 2021). Selection for suitable fruit sizes could be some adaptive traits for citron watermelon under dry environments and may possibly be preferred characters by consumers. The high proportion of genetic variation recorded under fruit number, fruit diameter and rind thickness are an indication of high response to selection and evidence of higher agro-biodiversity among the citron melon crop species (Mandizvo et al., 2021). These results are in accordance with those observed in citron watermelon in Zimbabwe, which has extensive variation in color, sizes, shape, colour pattern and rind sizes which suggest valuable breeding can be gained through selection (Mujaju et al., 2013). Citron watermelon genotypes significantly ($P < 0.001$) influenced the seed yield and oil yield. SC1-Sesoswane exhibited highest seed yield 1540.3 kg/ha, while MMB-280-Lerotse recorded lower seed yield of 548.1 kg/ha (Table 2). Makinde et al. (2007) reported relatively lower seed yield among the citron watermelon in Nigeria, with a range of 347.2 kg/ha to 812.5 kg/ha. As expected, the SC1-Sesoswane produced the highest seed yield compared to other cultivars because farmers have selected the citron watermelon largely for seed production. Generally, all the genotypes produced a higher number of seeds more than 100 seeds per plant. An indication that the selected material has the potential to be used in biofuel production. Moreover, the aim of biodiesel production is to reduce the over dependence on fossil fuel and support agricultural industries to ensure sustainable economic development through the exploitation of abundant citron melon crop seeds. Major drivers for biofuel development are concerns about energy security, climate change and environment and rural development. In Botswana there is potential for development of biofuels without adverse effect on food security mainly due to availability of idle land, which farmers can be incentivized to grow energy and food crops (Kgati et al., 2012).

A summary of the seed oil content is provided in Table 2, there was no significant difference observed among the four cultivars in seed oil content with an average of 26.6%. A wide range of oil values from 22.1-53.5% from melons were reported by Milovanović and Pićuric-Jovanović (2005), an indication of a higher genetic diversity in this trait. Yunus and Kyari (2017) reported more than 44% oil yield of melon seeds, while Ogunwa et al. (2015) observed a higher oil yield of 52.2% of melon seeds in Nigeria, hence there is a need for further exploration of local citron watermelon accessions, mainly by increasing number of lines for evaluation. A wide oil range production was observed for SC1-Sesoswane which produced the highest oil yield of 435.03 kg/ha, while MMB-280-Lerotse exhibited lower oil yield of 144 kg/ha (Table 2). The difference in the oil yield between the genotypes is attributed to the seed yield. There was a strong positive significant correlation obtained between oil yield (kg/ha) and number of fruits produced (0.923), and with the seed yield (kg/ha) (0.99) (Table 3). This shows that selecting higher seed yielding cultivars will inherently lead to an increase in the amount of oil that will be generated. However, the number of fruits and days to 50% flowering had a negative correlation with melon diameter (-0.692) and rind thickness (-0.707) respectively. An indication that these two traits are important in the selection of the size of the melon, hence the quality of the crop is affected. Meru and McGregor (2013) reported seed oil percentage in watermelon is correlated with kernel percentage and seed size. It is well established that seed yield is a complex trait and depends on other characters directly or indirectly. This observation agrees with results obtained by Narayanankutty et al. (2006) who identified high positive direct effects for fruit weight, fruit number, days to first harvest and seed weight in snake gourd.

The seeds extraction or manual processing of melon in this study was cumbersome so appropriate mechanization is critical. Technology and smart mechanization process are developed by the National Agricultural Research and Development Institute through the Department of Agricultural Biosystem Engineering Department from

Botswana. The physical and mechanical properties of the fruits, seeds, and kernels are key components to designing and construction of the machines. Similarly, in Nigeria, Giwaa and Akanbi (2020) observed that the utilization and development of novel techniques for the extraction of oil from melon seeds, fruits, and kernels are still at lower stages and need to be advanced. Botswana transport sector relies on petroleum fuel of approximately 825.6 million litres imported from South Africa, this over reliance of imported fuels call for the country to tap into indigenous sources for energy supplies (Mabuza, 2018; Ketlogetswe, 2011). An estimated 2000 hectares of citron watermelon can supply approximately 1 million litres of oil which can be key component in production of blended fuel, hence a significant reduction of importation of petroleum in the country.

Table 1. Effect of genotype on citron watermelon yield components

Genotypes	Days to 50% flowering	Seed number per fruit	Fruit number per plot	Rind thickness (cm)	Diameter (cm)
SC1-Sesoswane	32.3c	782.7a	463.0a	1.8c	14.6c
MMB-280-Lerotse	37.0b	353.3b	136.0b	2.2b	30.3ba
SC117-Lerotse	40.0a	741.7a	155.0b	2.3b	25.4b
SC44-Lerotse	27.6d	570.7ba	175.0b	3.3a	33.3a
Average	34.2	612.1	232.3	2.4	22.3
Significance	*	**	**	***	**
LSD (P<0.05)	2.43	223.08	140.3	0.34	7.38

Note. *, **, *** Significant at P = 0.05, 0.01, 0.001, respectively. Means separated using the Least Significant Difference (LSD) at P = 0.05; means within column followed by the same letter(s) are not significantly different.

Table 2. Effect of genotypes on seed yield, seed oil content and oil yield of citron watermelon

Genotypes	Seed yield (kg/ha)	Seed oil content (%)	Oil yield (kg/ha)
SC1-Sesoswane	1540.3a	27.9a	435.03a
MMB-280-Lerotse	548.1b	26.3a	144.0b
SC117-Lerotse	917.0b	26.4a	239.27b
SC44-Lerotse	1007.4ab	25.7a	257.4b
Average	1003.2	26.6	268.9
Significance	**	ns	*
LSD (P<0.05)	601.4	3.27	163.8

Note. *, ** Significant at P = 0.05, 0.01, ns: non-significant, respectively. Means separated using the Least Significant Difference (LSD) at P = 0.05; means within column followed by the same letter are not significantly different.

Table 3. Correlation analysis of yield components and Oil content parameters

Parameters	No of fruits:	Seed per fruit	Seed yield (kg/ha)	Oil content (oil)	Oil yield (kg/ha)	DFF	Rind Thickness (cm)	Diameter (cm)
No. of fruit:	1							
Seed per fruit	0.45ns	1						
Seed yield (kg/ha)	0.92***	0.173*	1					
Oil content (%)	0.383ns	0.524*	0.209*	1				
Oil yield (kg/ha)	0.923***	0.501*	0.990***	0.328ns	1			
DFF	-0.198	-0.198	-0.289	0.102ns	-0.261	1		
Rind thickness (cm)	-0.007	0.173ns	0.183ns	-0.223	0.126ns	-0.707	1	
Diameter (cm)	-0.692	-0.627	-0.545	-0.238	-0.583	-0.115	0.375	1

Note. Values for correlation coefficient are significant at P ≤ 0.05 according to Pearson correlation. * P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001; ns= not significant; DFF: days to 50% flowering.

4. Conclusions

The results of this study revealed that a wide variability existed among cultivated citron watermelons genotypes, hence there is a huge potential in bioenergy production. It is recommended that more citron watermelon genotypes from different agro-ecological zones of the country be evaluated for morphological and physio-chemical properties. This will allow further identification of more lines with potential to use in biofuel production. The success of this project will increase the contribution of bioenergy to diversify energy mix in the country. In addition, it will promote investment in infrastructure to produce energy from agro-processing using, feedstock which are abundant in the country.

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Authors Contributions

Ms. Bareeleng was responsible for methodology, data analysis and drafted the manuscript, while Ms. Masukujane, Ms. Radikgomo and Mr. Orebotswe contributed to study design and validation, Ms. Kgokong S. B., Mr. Keabotse, Ms. Kgokong K. and Ms. Molubi were responsible for field experimentation and data collection, and Dr. Molosiwa was responsible for conceptualization, supervising and writing. All authors read and approved the final manuscript and authors contributed equally to the study.

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