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Full Length Research Paper

Lipase and esterase activities of lactic acid bacteria isolated from different biotopes

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The lipolytic and esterase activities of fifteen strains of lactic acid bacteria isolated from different biotopes of Algeria and Mauritania were tested on MRS medium supplemented with lipidic substrates. Five of them showed maximum activity in the presence of tributyrin; the activity is therefore a tributyrin esterase. These strains were identified by MALDI-TOF in *Enterococcus faecium* and *Enterococcus durans*. The study of growth kinetics as a function of time shows a start of fatty acid production during the exponential phase to reach its maximum in the stationary phase. A better esterase activity is observed at between pH 6 and 9 and at an optimal temperature of 30 to 40°C for the five strains. The influence of metal and additive ions on the esterase activity varies between bacteria but generally, total inhibition was observed in all strains tested in the presence of SDS, NaN_3, $CuCl_2$, EDTA, $AgNO_3$ and $HgCl_2$.

Key words: Lipolytic activity, esterase activity, tributyrin, tributyrin esterase, MALDI-TOF, *Enterococcus*, growth kinetics, metal ions.

INTRODUCTION

Lipases (EC 3.1.1.3) and esterases (EC 3.1.1.1) are carboxyl esterases that hydrolyze the carboxylic ester linkages of triacylglycerols to release diglycerides, monoglycerides, free fatty acids and glycerol. The lipolytic and/or esterase activity of lactic acid bacteria contributes to the production of new foods or food supplements (García-Cano et al., 2019). These bacteria with probiotic potential can also produce conjugated fatty acids by hydrolyzing triacylglycerol thanks to their lipase activity (Kuhl et al., 2016). It is generally recognized that lactic acid bacteria play an important role in quality, flavor, and maturation in cured meat production (Dinçer and Kıvanç, 2018).

Carboxyl esterases act only in ester-water interface and are of considerable physiological and industrial importance (Martinelle et al., 1995). The water activity in the reaction medium controls the balance of the reactions (Borrelli and Trono, 2015). In case of low water activity, lipases catalyze other reactions (esterification, interesterification, acidolysis, alcoholysis, and aminolysis reactions) (Joseph et al., 2008; Bajaj et al., 2010).

Generally, esterases hydrolyze only short chain fatty acid triglycerides while lipases are active on water insoluble substrates and hydrolyze long chains to fatty acids (Kilcawley et al., 1998).

Microbial lipases are widespread in bacteria, especially

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Table 1. Bacterial strains used in this study.

Origin	Code of strain	Species
Camel's milk (Illizi, Algeria)	BH21	*Lactobacillus plantarum*
Camel's milk (Nouakchott, Mauritania)	CAM18	*Enterococcus* sp.
Camel's milk (Timimoun, Algeria)	CAT13; CAT18	*Enterococcus* sp.
Camel's milk (Béchar, Algeria)	CHBK320	*Leuconostoc mesenteroides ssp dextranicum*
Camel's milk (Tindouf, Algeria)	CHTD27	*Lactobacillus brevis*
Cow's Milk (Oran El-Kerma, Algeria)	LKV11	*Enterococcus* sp.
Olive brine (Sig, Algeria)	OV5	*Lactococcus lactis* ssp *diacetylactis*
Fresh beef meat (Mostaganem, Algeria)	V6-2 ; V17 ; V18	*Lactococcus lactis* ssp *lactis*
	V9	*Lactococcus lactis* ssp *cremoris*
Fresh sheep meat (Relizane, Algeria)	VO19	*Enterococcus* sp.

in Gram+ (Fickers et al., 2008). Among them are lactic acid bacteria, which are considered slightly lipolytic in comparison with other bacterial species (Brennan et al., 2002). This activity does not influence bacterial growth. In fact, these enzymes do not show any nutritional role (Fernández et al., 2000; Nardi et al., 2002). However, their presence in cheeses, and at higher concentrations and at precise periods, leads to the release of fatty acids responsible for the final taste (Das et al., 2005). *Lactobacillus plantarum* CCFM12 shows good esterase activity responsible for a considerable improvement in the production of ethyl esters and which leads to the fruity taste of camembert cheese (Hong et al., 2018).

Esterases of lactic acid bacteria preferentially degrade para-nitrophenyl or beta-naphthyl derivatives of C4 or C6 fatty acids, and a good activity was also observed in the presence of tributyrin. Esterasic activity decreases considerably with lengthening of the chain (Corrieu and Luquet, 2008).

Lipases and esterases enzymes of lactic acid bacteria are either extracellular or intracellular (Katz et al., 1997; Meyers et al., 1996), hence in the second case the need for cell lysis to promote their access to the substrate.

Several esterases of lactic acid bacteria such as *Lactococcus lactis, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus helveticus, Lactobacillus rhamnosus, Streptococcus thermophilus and Enterococcus faecium,* were previously characterized. These enzymes have an optimal activity in a temperature range of 30 to 45°C depending on the strains considered, as well as a neutral or slightly alkaline pH. Their activators are Zn^{2+}, Mg^{2+}, Ca^{2+} ions as well as NaCl. Phenylmethylsulfonyl fluoride (PMSF), para-chloro-mercuribenzoate (PCMB), and Hg^{2+}, Cu^{2+}, Ag^{2+} ions inhibit esterase activity (Tsakalidou et al., 1994; Holland and Coolbear, 1996; Fernendez et al., 2000; Nardi et al., 2002; Chich et al., 1997; Fenster et al., 2000; Gobbetti et al., 1997a; Castillo et al., 1999; Choi et al., 2004; Liu et al., 2001; Mobarak-Qamsari et al., 2011; Salwoom et al., 2019).

The objective of this research was to highlight the lipase and esterase activity of lactic acid bacteria cultivated in the presence of different lipid substrates. Studies of the kinetics of growth and production of esterases and/or lipases depending of various physico-chemical parameters (pH, temperature, surfactants, metal ions and other additives) will be useful in order to optimize the use of these enzymes.

MATERIALS AND METHODS

Bacterial strains

The 15 lactic acid bacterial strains in the genus *Lactococcus*, *Lactobacillus*, *Enterococcus* and *Leuconostoc* have been isolated by serial dilution plating on MRS medium (De Man et al., 1960) from raw camel or cow milk, olive brine and fresh meat collected in Algeria and Mauritania (Table 1). The bacterial cultures were maintained at -20°C in MRS broth containing 20% glycerol (v/v). Working cultures were prepared by two consecutive transfers in MRS broth at 30ºC for 18 h.

Bacterial identification

The bacteria were streaked on MRS agar plates and incubated at 30°C for 24 to 48 h. Single isolates were identified by Bruker Daltonic's MALDI-TOF Biotyper – CM according to the manufacturer instructions. The identification is carried out by mass spectrometry analysis coupled with a source of laser ionization assisted by a MALDI (Matrix Assisted Laser Desorption Ionization) and a TOF (Time of Flight) analyser. For the MALDI-TOF MS analysis, the strains were grown on MRS medium for 24 h. Each colony was smeared on the target and then covered with 1 µl of formic acid and 1 µl of the matrix. It is then identified by the MALDI-TOF.

Detection of intracellular lipolytic and esterase activity

Bacterial preculture was grown on MRS broth at 30°C until it reaches OD600 ~1.2. Tubes of 10 ml of fresh MRS broth were inoculated with 0.1 ml of the bacterial preculture and incubated at 30°C for 18 h. The intracellular enzymes from the isolate were extracted by using glass bead stirring. After centrifugation at 8000

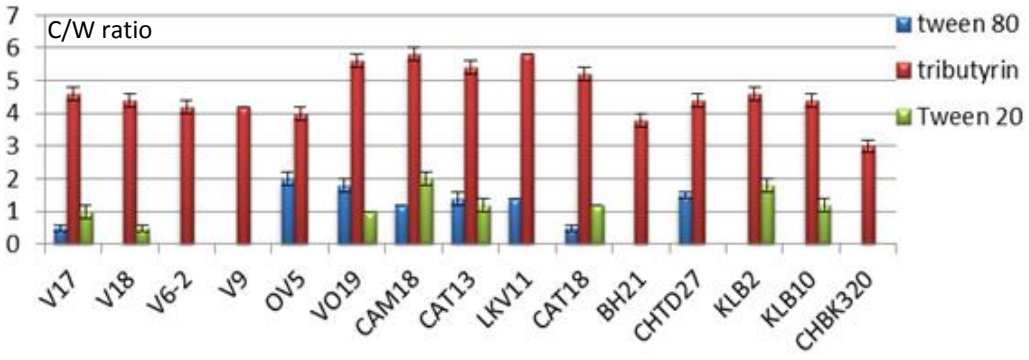

Figure 1. Lipolytic activity on artificial lipid substrates in lactic strains (C: clarification area, W: well diameter).

rpm, the supernatant is recovered. It constitutes the crude enzymatic extract.

The activity of enzymes was investigated according to the diffusion method in buffered agar at pH 7 (0.1 M phosphate buffer) containing various natural or artificial lipid substrates as follows:

1. 3% olive oil, almond oil, argan oil or oleic acid. The media are then opacified with $CaCO_3$ in order to visualize a possible lipase activity.
2. 0.25% of tributyrin is added and the emulsion is homogenized by sonication. In case degradation of tributyrin an observed clarification reflects the presence of esterases (Medina et al., 2004).
3. 3% Tween 80, 0.01% $CaCl_2$ and 0.5% NaCl. Under these conditions, the presence of lipases is manifested by opacity (Guiraud and Galzy, 1980).

Plates were incubated at 37°C for 72 h. The strains as well as the lipid substrate where the activity is maximal are selected for further work.

Kinetics of growth and release of fatty acids as a function of time

Tubes of 10 ml of fresh MRS broth were inoculated with 0.1 ml of the bacterial preculture as described above and incubated at 30°C. The kinetics of bacterial growth is determined by measuring OD600 nm every 4 h.

In parallel, 0.5 ml of enzymatic extract is mixed with 1 ml of pH 7 phosphate buffer and 1.5 ml of lipid substrate. After 60 min of incubation at 37°C with stirring, in order to promote lipid-lipase source contact, 1 ml of 95% ethanol is added to extract the free fatty acids. Titration with 0.05 M KOH solution in the presence of phenolphthalein was carried out to determine the concentration of free fatty acids. The control consists of the same reaction mixture but without enzymes (Ginalska et al., 2004). The results were expressed in µmol of fatty acid / ml of sample (Sokolovska et al., 1998).

pH and temperature determination for lipase activity

The lipase activity was measured in a pH range of 4 to 9. This pH range is obtained using 0.1 M buffer solutions of acetic acid-sodium acetate buffer (pH 3-5), or phosphate buffer sodium (pH 6-7), or Tris-HCl buffer (pH 8) or glycine-NaOH buffer (pH 9). The concentration of released fatty acids was carried out by titration in the same manner as previously described.

The optimal temperature for lipase activity was determined in the same way and in the presence of the optimal pH buffer obtained previously for each strain. To determine the optimal temperature for lipase activity, incubation was performed at 20, 30, 37, 40 and 45°C.

Effect of metal ions and additives on lipase activity

The enzymatic extract was incubated in the presence of 10 mM of various additives and metal ions for 1 h at a temperature of 37°C. The ions, additives and surfactants used are: Ascorbic acid, aspartic acid, folic acid, glutamic acid, nicotinic acid, serine, cysteine, riboflavin, EDTA, Triton X100, beta-mercaptoethanol, ammonium sulfate, barium sulfate, magnesium sulfate, lithium sulfate, sodium thiosulfate, SDS, copper chloride, silver nitrate, iron, calcium chloride, manganese chloride, mercury chloride, zinc chloride, urea, sodium azide, calcium carbonate, Tween 80, Tween 20, sodium molybdate and potassium permanganate. The reaction mixture was supplemented with lipid substrate and different buffers at optimal pH and then incubated for 60 min at optimal temperatures for each strain in order to obtain the released fatty acids.

RESULTS AND DISCUSSION

Intracellular lipolytic and esterase activities

The results of lipolysis obtained on medium supplemented with different lipid substrates are shown in Figures 1 and 2. An example of lipase effect on tributyrin is shown on Figure 3. The lactic acid bacterial strains degrade the lipid substrates differently with a maximum of activity when adding 0.25% tributyrin. This occurs especially in strains VO19, CAM18, CAT13, CAT18 and LKV11 of the genus Enterococcus (Table 1). Ginalska et al. (2004) and Gobbetti et al. (1997b) reported that the lipolytic activity is often observed in enterococci and it shows higher activity than strains of most other genera of lactic acid bacteria.

Our results agree with the work of Cardenas et al., (2001) who showed that bacterial lipases tend to reveal better hydrolytic activity on tributyrin, which is a

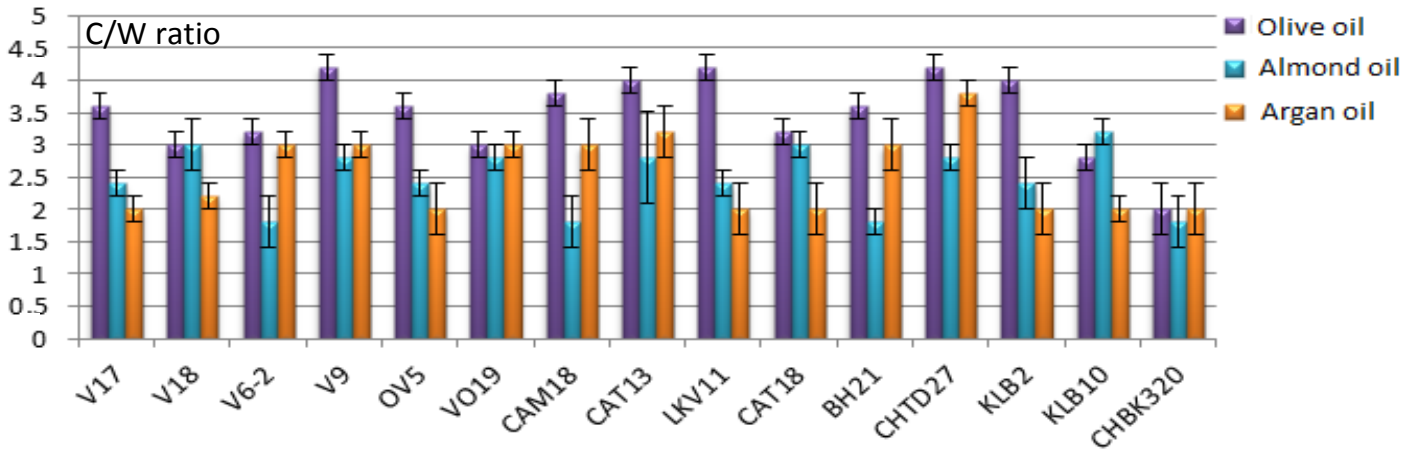

Figure 2. Lipolytic activity on natural lipid substrates in lactic strains (C: clarification area, W: well diameter).

Figure 3. Tributyrin esterase activity of lactic strains.

triglyceride composed of short chain fatty acid, namely butyric acid. Tributyrin can be easily broken down by esterases acting on short lipid chains (<10C). Likewise, the lipases of lactic acid bacteria have an optimal efficacy with tributyrin and lower with natural lipids (Talon and Montel, 1994; De Roissart and Luquet, 1994; Guiraud and Galzy, 1980), which has been observed in the presence of natural substrate, in particular olive oil. The lipase activity was less effective with olive oil where in comparison with tributyrin; on the other hand, the presence of olive oil leads to an increase in lipase activity compared to other oils supplemented. This is due to the presence of oleic acid in large quantities in olive oil (78%) because lipases are of the inductive type with a preference for monounsaturated long chain fatty acids. These results are consistent with several studies on microbial lipases showing a high production of lipase in the presence of olive oil among several oils tested (Feitosa et al., 2010; Sooch and Kauldhar, 2013; Iqbal and Rehman, 2015; Quian and Chun-Yun, 2009; Nwachukwu et al., 2017; Vishnupriya et al., 2010; Esakkiraj et al., 2010; Dandavate et al., 2009). An important lipolytic activity is also observed with 2% olive oil in other bacterial species, like *Pseudomonas aeruginosa* KM110 and *Bacillus* sp ZR-5 (Mobarak-Qamsari et al., 2011; Soleymani et al., 2017).

In the presence of Tween 80 and Tween 20, the lactic strains are weakly lipolytic (Figure 1), so the degradation of these two substrates differs depending on whether the bacteria hydrolyze the Tween 20 containing the lower chain lauric acid esters and the Tween 80 composed of oleic acid degraded respectively by esterases and lipases (Kumar et al., 2012). Therefore, the five strains of *Enterococcus* mentioned above therefore show better

Table 2. Identification of lactic strains by MALDI-TOF.

Analyte name	Organism (best match)	Score value
CAM18	*E. faecium*	2,306
CAT13	*E. faecium*	2,344
CAT18	*E. faecium*	2,333
LKV11	*E. durans*	2,151
VO19	*E. faecium*	2,177

degradation in the presence of tributyrin, the activity then turns out to be a tributyrin esterase.

These strains are retained for further work and are identified by MALDI-TOF; the results obtained are shown in the Table 2.

Kinetics of growth and release of fatty acids

The kinetics are carried out every 4 h by measuring the bacterial growth at 600 nm and assaying of freed fatty acids obtained by the action of intracellular tributyrin esterase on the tributyrin supplemented therefore 1 µmol of fatty acid released / ml / min corresponds to an enzyme unit (EU). The results obtained are shown on Figure 4a to e.

The results showed the presence of tributyrin-esterase in the intracellular content of the five strains tested from the exponential phase and with maximum production during the stationary phase. On the other hand, the enzymatic activity varies from one strain to another. In addition, according to the phases, a significant production is then observed in the CAT13 and VO19 strains during the exponential phase, which is maintained until the stationary phase. The production of lipases is therefore associated with cell growth, this agrees with studies showing that bacterial lipases are produced during the growth phase or late in the same phase (Papon and Talon, 1988; Makhzoum et al., 1995; Gupta et al., 2004). Other works on intracellular extracts of *Lactobacillus* species had shown that ester activity was present from the start of the exponential phase and then increased to reach a maximum value at the start of the stationary phase (El Soda et al., 1986; Khalid et al., 1990). Serio et al. (2010) also noted an ester activity on strains of *Enterococcus* in stationary phase; similar results are observed in our study where strains CAT18, LKV11 and CAM18 activity is moderate in the exponential phase with maximum production during the stationary phase.

Determination of optimum pH and temperature

Bacterial lipases are generally neutral (Dharmsthiti and Kuhasuntisuk, 1998; Dharmsthiti and Luchai, 1999; Lee et al., 1999), or slightly alkaline (Kanwar and Goswami, 2002; Schmidt-Dannert et al., 1994; Sidhu et al., 1998a, b; Sunna et al., 2002). These results are observed in five strains tested with an optimum pH between pH 6 and 9 (Figure 5b), which is consistent with the work of Esteban-Torres et al. (2014b) who reported a noticeable lipolytic activity at pH 7. Tributyrin esterase of *Lactobacillus plantarum* strain 2739 had an optimum pH of 7 (Gobbetti et al., 1996, 1997a), while *Lactobacillus plantarum* MF32 lipase shows maximum activity at a more alkaline pH (pH 9.3) (Andersen et al., 1995).

The bacteria studied in this work have an optimal temperature ranging from 30 to 40°C (Figure 5a), some work has shown that bacterial lipases have an optimal temperature of 30 to 60°C (Lesuisse et al., 1993; Wang et al., 1995; Dharmsthiti et al., 1998; Litthauer et al., 2002). This has been observed in lipases of *Lactobacillus plantarum* with an optimal temperature of 35°C (Andersen et al., 1995; Gobbetti et al., 1996; 1997a; Lopes et al., 2002), and in *Enterococcus faecium* where maximum activity occurs at 40°C (Ramakrishnan et al., 2016).

Effect of metal ions and additives on lipase activity

Several studies show the effect of metal and additive ions on lipase and esterase activity, despite their concentration and the mechanism of induction may vary from one species to another (Saxena et al., 1994). Supplemented metal ions and additives act differently on the esterase activity of the strains tested as shown in Figure 6. The activity is completely inhibited by SDS, sodium azide, EDTA, copper chloride, silver nitrate and mercury chloride in the five bacteria studied. Some studies showed a considerable decrease in activity in the presence of EDTA, which can influence the interfacial zone between substrate and lipase (Sztajer et al., 1992). The activity of lipase Lp_3562 is strongly inhibited by Hg^{2+}, Cu^{2+} and SDS (Esteban-Torres et al., 2014a). A significant inhibition is observed in Lp_1760 in the presence of Hg^{2+}, Zn^{2+}, Cu^{2+} and SDS (Esteban-Torres et al., 2014b). Significant tributyrin-esterase activity is detected when adding Tween 20 and barium sulfate in VO19 and cysteine in CAM18. It was reported that Tween 20, 40, 60 and Triton X-100 could activate lipases in

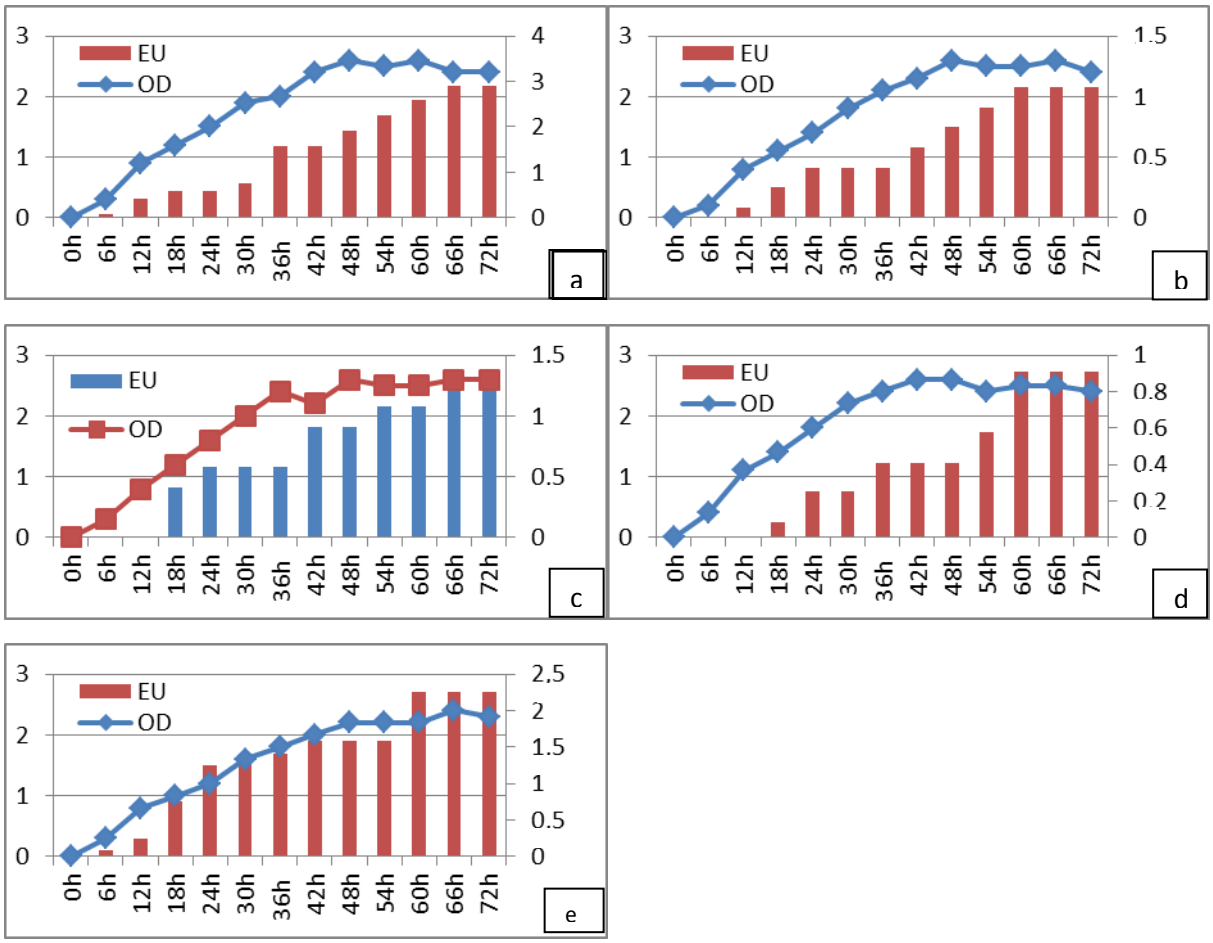

Figure 4. Growth kinetics and fatty acid production as a function of time. a: CAM18; b: LKV11; c: CAT13; d: CAT18; e: VO19. EU: Enzyme unit ; OD: Optical density.

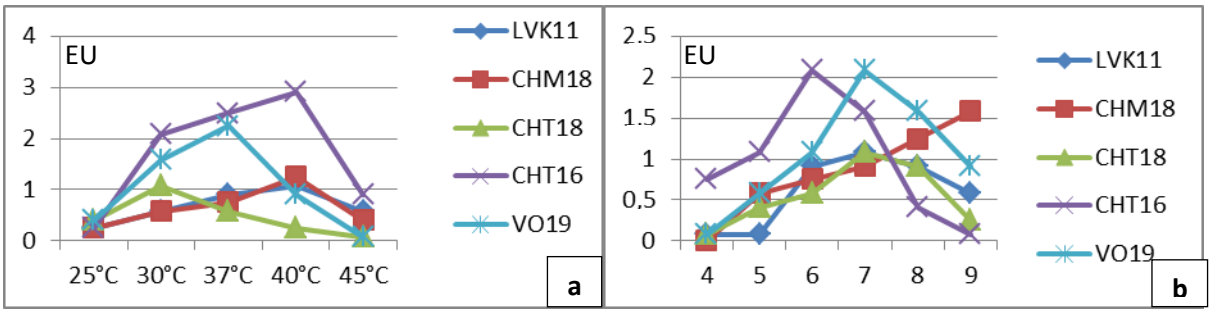

Figure 5. Study of optimal parameters (a: Optimal temperature, b: Optimal pH).

Cryptococcus sp. (Thirunavukarasu et al., 2008). Tween 20 also increases the production of *Bacillus altitudinis* AP-MSU esterases (Palanichamy et al., 2012), but inhibition of lipase activity is detected in the presence of surfactants (Tween-20, Tween-80 and Triton X-100) tested on Celite-immobilized commercial lipase (Lipolase 100 L) (Sharma et al., 2016). It was also noted that lipases produced by *Pseudomonas aeruginosa* HFE733 are activated by beta-mercaptoethanol and cysteine (Jun et al., 2018). Other ions and additives tested had little or no effect on enzyme activity with a slight decrease or sometimes activation of tributyrin esterases. In some

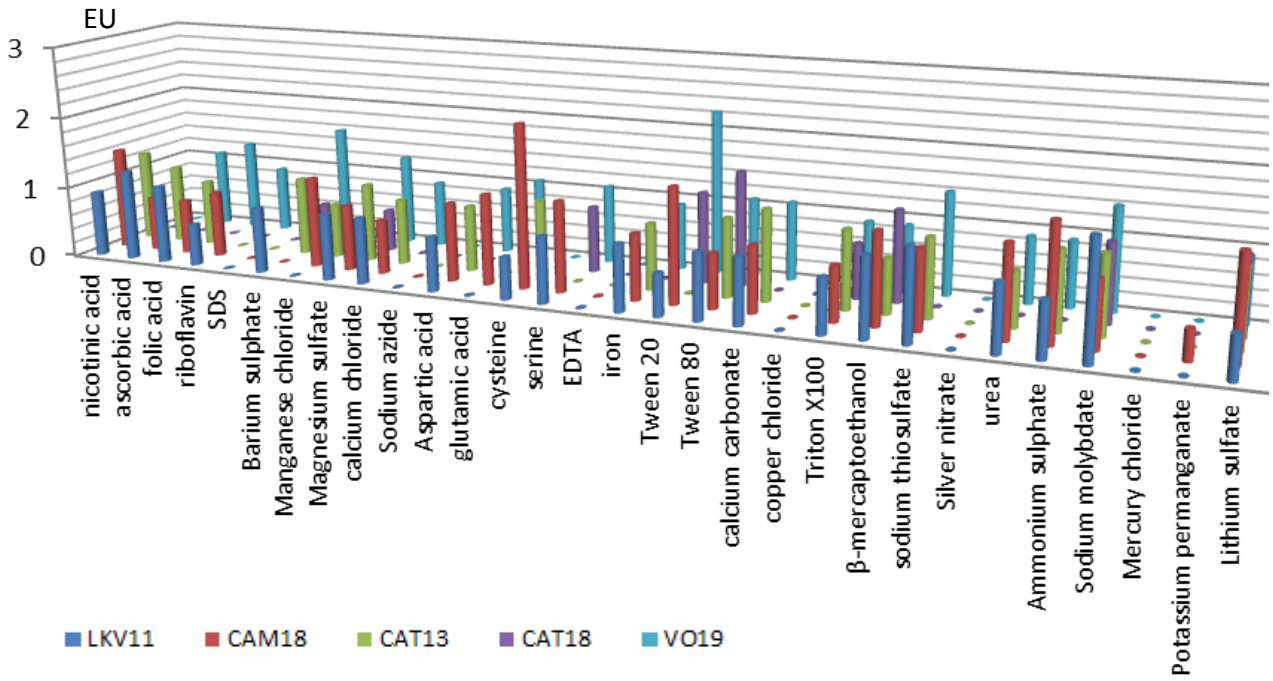

Figure 6. Effect of additives on the lipolytic activity of the five enterococcal strains.

cases the same ion or additive acts differently in the five strains tested by activating or inhibiting enzyme activity as shown in Figure 6.

Conclusion

Five enterococcal strains show a maximum esterase activity in medium supplemented of tributyrin. The production of intracellular tributyrin esterases can be related to bacterial growth or sometimes maximal during the stationary phase, requiring a neutral or slightly alkaline pH and an optimal temperature between 30 and 40°C. The tributyrin esterases act differently when metal ions and additives are added, while SDS, sodium azide, EDTA, copper chloride, silver nitrate and mercury chloride were found to inhibit enzyme activity.

CONFLICT OF INTERESTS

The authors declared no conflict of interests.

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Genetic variation via SSR polymorphic information content and ecological distribution of Nigerian sesame

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Sesame (*Sesamum indicum* L.) of the family Pedaliaceae is an important and old oil crop that is cultivated mainly in the tropical and subtropical regions of Asia and Africa for several economic values. Several molecular markers have been employed to study the genetic diversity of this important crop. The study focused on the genetic diversity through polymorphism information content (PIC) by the use of Simple Sequence Repeat markers among randomly collected 22 Sesame germplasm across 2 ecological zones stored in NACGRAB seed Genebank, Nigeria. The extraction procedure followed Cetyltrimethylammonium Bromide (CTAB) and the recovered DNA were good with average concentration of 337.00 ng/µL and average quality of 1.795. 30 primers were designed but only 12 with highest genome coverage were used to analyzed the genetic data with NTSYS pc ver.2.02 and Power Marker ver.3.5. The PIC ranged between 0.36 in Primer OTO2 and 0.76 in Primer OTO5. The evolutionary relationship was constructed based on the polymorphic primers and according to their ecological locations. The populations were divided to 2 major clads. Of the 2 ecological zones, the more diverse ecological zone is the derived Savana with 13 accessions, while the less diverse is the humid forest with 9 accessions.

Key words: Sesame, genetic diversity, polymorphism information content (PIC), ecological distribution, microsatellite.

INTRODUCTION

Sesame (*Sesamum indicum* L., 2n = 26), belonging to the Pedaliaceae family with 60 species organized into 16 genera (Singh et al., 2015) is one of the oldest cultivated plant, considered important for its edible oil (Anilakumar et al., 2010). Myanmar takes the lead position in sesame production closely followed by India and China (Koppelman et al., 2015). Sesame is very important to human being in so many ways, especially in dietary preparation and besides direct consumption, sesame seeds are also used as an active ingredient in cosmetic industry (Dossa et al., 2016), decorative elements, antiseptics, bactericides, viricides (Bedigian, 2010), disinfectants, moth repellants

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(Bedigian 2011) and antitubercular agents because they contain natural antioxidants such as sesamin, sesamoland sesamolin (Badril et al., 2014; Elleuch et al., 2007).

The presence of genetic diversity is a desirable requirement for any breeding program (Dossaet al., 2017a). Nigeria, being one of the most important Sesame producing countries aims to make systematic efforts to characterize and document the genetic variability of Sesame in the country. The insight of genetic variation between and within populations gives pivotal information which is indispensable for the formulation of management strategy channeled towards crop improvement program and conservation of biodiversity (Pandeyet al.,2015). Morphological and agronomic characteristics as well as isozyme and molecular marker analysis have been used to determine genetic diversity in crop species (Alemuet al.,2013). The use of morphological and agronomic characteristics is prone to strong influence of environmental interference. Molecular markers offer a reliable means of identification and to understand the genetic variability in crops as it overcomes the limitation of environmental influence (Wu et al., 2014) and sesame research has witnessed a rapid development of genetic tools particularly molecular markers and their application in genetic diversity studies and marker assisted breeding (Dossa et al., 2017b; Uncu et al., 2017).

Different molecular marker systems have been developed, with its merit and demerits in terms of ease of use and degree of information to study genetic diversity. The universal markers include the randomly amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), sequence-related amplified polymorphism (SRAP), inter-simple sequence repeats (ISSR), specie-specific marker such as simple sequence repeats (SSR), diversity arrays technology (DArT), restriction-site associated DNA sequencing (RADseq), single-nucleotide polymorphism (SNP), specific-locus amplified fragment sequencing (SLAFseq), random selective amplification of microsatellite polymorphic loci (RSAMPL) (Dossa et al., 2017a) and expressed sequence tags-SSR (EST-SSR). The choice of marker is determined by the kind of study to be undertaken. A suitable marker is characterized by high polymorphism, co-dominant inheritance, frequency of occurrence and even distribution throughout the genome (Welsing et al., 2005), selective neutral behavior, easy access, easy and fast assay, low cost and high throughput, high reproducibility, and transferability between laboratories, population and/or species (Woldesenbet et al., 2005).

Microsatellites or simple sequence repeat (SSR) is considered a good choice because of high abundance and reproducibility, easy scoring, low cost, extensive coverage, high polymorphic information content (PIC)and its co dominant nature (Wei et al.,2014; Pandey et al., 2015; Dossa et al., 2017a).

The efficiency of SSR is not faultless as its application was limited in sesame and the pioneer findings on sesame specific primers reported polymorphism in 10 SSRs in sesame (Dixit et al., 2005) compared to more than 1000 SSR loci in soybean (Xin et al., 2012; Song et al., 2004).This paradigm has however shifted recently since the completion of genome sequencing for this oil rich crop. The combination of agro-morphological and molecular markers is the best choice to characterize germplasm as it gives the opportunity to comparatively analyze the phenotypes from field experiments with molecular phenotypes and genotypes from laboratory studies (Pham et al., 2011).

Sesame research has witnessed three different periods: Germplasm collection era, classical breeding and genetics era and presently the Omics era. Advancement in Omic era has projected sesame research to a higher level of seed improvement (Dossa et al., 2017b). The completion and availability of the full nuclear genome sequence gives unlimited access to reference genome information for the study of genetic traits and comparative genomics (Wang et al., 2014).

The whole sesame genome was sequenced with the help of high-throughput next-generation sequencing and informative SSRs were identified to be distributed throughout the whole genome sequence (Dossa et al., 2017a). Botstein and others (Nagy et al., 2012)published one of the first reports where PIC became the popular scientific approach for calculation of genetic variabilities through a widely acceptable formula to measure the informationcontentofmolecularmarkers.PIC determines the value of a marker for detecting polymorphism within a population, depending on the number of detectable alleles and the distribution of their frequency (Wei et al., 2014).PIC gives an evaluation of the ability of molecular makers to discriminate a given population (Vilas, 2014).

This research was conducted to determine, using SSR markers, the extent of genetic variation or distance among twenty two accessions of sesame cultivars collected from the forest and savannah agro ecological zones in Nigeria. They are now conserved in the gene bank of the National Centre for Genetic Resources and Biotechnology.

MATERIALS AND METHODS

Sample collection

About 22 Sesame accessions from NACGRAB's seed genebank that their field collections were within eight States of the Federation (Nigeria) but restricted to only two ecological zones were collected with their Accession Numbersfor this studiesas recorded in Table 1. The samples were potted in glass house and allowed to grow for 3 weeks before collection for analysis.

DNA extraction procedure

About 2 g of leaves from 3 weeks old of potted sesame was ground

Table 1. List of sesame accessions, nucleic acid concentration and purity recovered.

S/N	Sesame accession number	State of collection/ ecological zone	Nucleic acid conc. (ng/µl)	260/280 purity
1	NGB00421	Imo/ HF	170.30	1.78
2	NGB00400	Kwara/ DS	68.60	1.84
3	NGB00402	Edo/ HF	777.00	1.76
4	NGB00368	Oyo/ DS	92.30	1.69
5	NGB00406	Nassarawa/ DS	86.70	1.74
6	NGB00420	Kogi/ DS	44.60	1.79
7	NGB00382	Ondo/ HF	175.50	1.75
8	NG512162	Anambra/ HF	143.60	1.88
9	NGB00379	Ondo/ HF	1056.30	1.89
10	NG512165	Edo/ HF	811.00	1.77
11	NG612166	Imo/ HF	690.10	1.82
12	NG512167	Kogi/ DS	165.80	1.76
13	NG512171	Anambra/ HF	1131.60	1.73
14	NG512173	Kogi/ DS	29.80	1.77
15	NG512174	Ondo/ HF	372.90	1.84
16	NGB00422	Nassarawa / DS	94.80	1.90
17	NGB00405	Kogi/ DS	49.60	1.76
18	NGB00425	Kwara/ DS	144.80	1.88
19	NGB00428	Nassarawa/ DS	155.10	1.86
20	NGB00433	Oyo/ DS	193.70	1.75
21	NGB01409	Kwara/ DS	490.20	1.80
22	NGB00419	Kogi/ DS	589.00	1.74

DS: Derived Savanna vegetation. HF: Humid forest vegetation.

after being surface sterilized with ethanol and 700µl of freshly prepared modified CTAB extraction buffer (200mMTris, pH 7.5; 50mM EDTA, pH 8.0; 2M NaCl; 2% CTAB; 1% beta-mercaptoethanol (just before use))was added in mortar and pestle. The resultant mixture was homogenized and incubated in 65°C water bath for 20min. It was allowed to cool for 7 min and 600µL chloroform: isoamylalcohol (24:1) was added in the tubes. The mixture was placed in an orbital shaker at 450 rpm for 1 h. The supernatant recovered after centrifugation at 10,000 rpm for 7 min was transferred into new tubes and up to 600µL Isopropanol was added and kept in -20°Cfreezer overnight for DNA precipitation. The pellet was collected by centrifugation at 10,000 rpm for 2 min and washed with 70% ethanol up to 3 times. Pellet was air-dried in hood until no further trace of ethanol. An average of 150 µL nuclease free water was added to elute the DNA.

DNA quantitation

Nanodrop spectrophotometer 2000 model was used to check the quality and quantity of the extracted DNA prior to amplification of the gene regions.

Gene amplification

Primers were designed from 3 gene regions of *S. indicum* with the following accession numbers: XM_011086313; XM_011086585 and XM_020696983. Up to 30 primers were designed and tested but only 12 most polymorphic, with widest genome coverage were used for the analysis (Table 2). The good amplification result was produced and retried for reproducibility in total reaction volume of 20 µL (2.5 µL of 10x PCR buffer, 1.0 µL 25 mM $MgCl_2$, 1.0 µL of 10µM forward primer, 1.0 µL 10µM reverse primer, 1.0 µL DMSO, 2.0 µL 2.5Mm DNTPs , 0.1 µL Taq 5u/ul , 3.0 µL 10ng/µl DNA, 8.4 µL H_2O) for the following PCR reactions: 94°C for 5min incubation, 94°C for 15 s denaturation; 58°C for 20 s annealing; 72°C for 25 s extension; 72°C for 7 min final extension and held finally at 4°C till samples taken out of PCR machine. The product was checked on gel electrophoresis and viewed with 100 bp ladder in trans-illuminator.

Data analysis

The data generated from the product of amplification and gel-electrophoresis were subjected to analysis using NTSYS pc ver.2.02 (Figure 1) and Power Marker ver.3.5 (Table 3).

RESULTS AND DISCUSSION

The mean value for DNA purity of 1.79 recovered (Table 1) is close to the acceptable figure of 1.80 which is a prerequisite for downstream analysis (Desjardins and Conklin, 2010). The 12SSR primers (Table 2) revealed polymorphic motifs by producing variable number of alleles which are different in sizes (Table 3).

Table 2. The 12 polymorphic SSR primers used for the analysis of 22 sesame accessions.

S/N	Forward	Reverse	Product size	Tm
OTO1	5'ATCCACGACAGCACACTCAG 3'	5'CCTCACTTTAGCCGGAACTG 3'	155	57.5
OTO2	5' ATCTTTAGCCCCGTTCTGGT 3'	5'TGCTCTGCCCTATCCTTCAT 3'	160	59
OTO3	5'TGTGATCTTTAGCCCCGTTC 3'	5'TGCTCTGCCCTATCCTTCAT 3'	118	58
OTO4	5'TGATCTTTAGCCCCGTTCTG 3'	5'TGCTCTGCCCTATCCTTCAT 3'	129	58
OTO5	5'TCAACAACAGCAGACCAGGG 3'	5'AAGTTTGAGACCCCGCAACA 3'	154	58
OTO6	5'TGGTCAACAACAGCAGACCA 3'	5'AAGAAGTTTGAGACCCCGCA 3'	160	57
OTO7	5'AGCATTATGGTCAACAACAGCA 3'	5'CTGCTGCGAGAACTGCTCT 3'	144	58
OTO8	5'CAGACCAGGGCGCCTCAC 3'	5'TTGAGACCCCGCAACAGGC 3'	156	60
OTO9	5'CTCGGCTTGCCAAGGAACA 3'	5'CATGTGCTCGTTCCTCCAGT 3'	146	59
OTO10	5'GACCTGAAACTGGCCCAGAT 3'	5'GATGCTTGCTTTGCCCGTG 3'	155	59
OTO11	5'CAGGTGGCAATCTGGAATCT 3'	5'TTGGAGGGGGTTCTTCTTTT 3'	150	59
OTO12	5'CTTTTCTCAACGATGCCACA 3'	5'CTCGTCTGGCTCGACATACA 3'	155	57

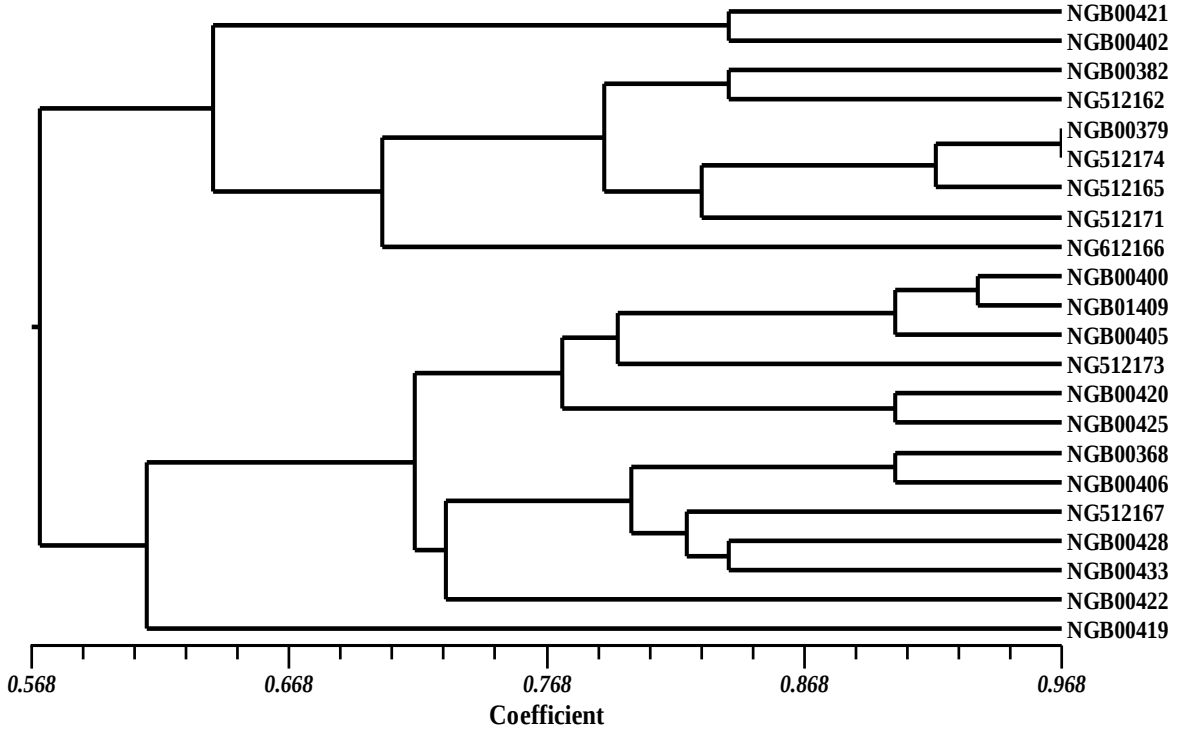

Figure 1. The phylogenetic analysis constructed based on SSR markers for 22 sesame accessions collected from 2 different ecological zones in Nigeria.

The total number of alleles identified at 12 primer loci across the 22 accessions of sesame is 47 (Table 3). These alleles ranged between 3 and 6 per locus. The average number of alleles per locus is 3.92. The loci with the highest number of alleles are found in OTO4 (6) and OTO10 (6). These two SSR loci with the highest variation of polymorphic motifs are best for discriminating the 22 accessions of sesame used in this study. In the same way, the frequency of the major alleles shared across the 22 accessions at each locus are between 27% (OTO4) and 77% (OTO2) covering about 50% (11 genotypes/accessions). A moderately high level of diversity was found among the 12 loci examined ranging between 0.38 and 0.79 with average of 0.62. The implication of the number of allele diversity and their frequency among the accessions is referred to as polymorphic information content (PIC) (Shahriar et al., 2014). PIC value of each marker is estimated on the

Table 3. Sesame microsatellite markers showing number of alleles, gene diversity and polymorphic information content (PIC) among 12 polymorphic markers for diversity studies.

Marker	Major allele frequency	Sample size	No. of obs.	Allele No.	Availability	Gene diversity	PIC
OTO1	0.5455	22.0000	22.0000	3.0000	1.0000	0.5950	0.5262
OTO2	0.7727	22.0000	22.0000	4.0000	1.0000	0.3843	0.3619
OTO3	0.5455	22.0000	22.0000	3.0000	1.0000	0.5826	0.5076
OTO4	0.2727	22.0000	22.0000	6.0000	1.0000	0.7934	0.7620
OTO5	0.5455	22.0000	22.0000	3.0000	1.0000	0.5620	0.4762
OTO6	0.4545	22.0000	22.0000	3.0000	1.0000	0.6405	0.5669
OTO7	0.5909	22.0000	22.0000	4.0000	1.0000	0.5826	0.5332
OTO8	0.5000	22.0000	22.0000	4.0000	1.0000	0.6405	0.5804
OTO9	0.5000	22.0000	22.0000	3.0000	1.0000	0.5744	0.4838
OTO10	0.3636	22.0000	22.0000	6.0000	1.0000	0.7479	0.7086
OTO11	0.4091	22.0000	22.0000	4.0000	1.0000	0.6983	0.6436
OTO12	0.5455	22.0000	22.0000	4.0000	1.0000	0.6116	0.5549
Mean	0.5038	22.0000	22.0000	3.9167	1.0000	0.6178	0.5588

basis of its alleles. There is significance in the variation of PIC for all the studied SSR loci. In this study, the level of polymorphism among the 22 sesame accessions was evaluated by calculating PIC values for each of the 12 SSR loci. The PIC values ranged from 0.36 (OTO4) to 0.76 (OTO2) with an average of 0.59 per locus (Table 3). The SSR primer loci with highest PIC is however more informative than what we had on the number of alleles. The PIC ofOTO4 (0.76) is higher than OTO10 (0.70) which clearly revealed that marker OTO4 becomes the best locus for discriminating the 22 accessions among the used markers followed by OTO10, OTO11, OTO8 and so on.

Furthermore, the Unweighted Pair-Group Method with Arithmetic mean (UPGMA) revealed 3 major clusters and one outlier (Figure 1). Two genotypes are however very close with up to 97% similarity (NGB00397 and NG512174). Following the site of these samples collection, they both came from Ondo state, humid forest vegetation. Although, they differ phenotypically and were collected from different locations in the same ecological zone, it was inferred that they possess similar genetic characters. In addition to this, the dendrogram separated into two major clusters on the node 57% based on the different ecological distribution of the genotypes. One cluster is made of 13 genotypes and are found to be the collections from the drier vegetation, the derived Savanna. While the second cluster comprise of 9 genotypes collected from the humid forest vegetation. These studies also proved certain patterns of association between genetic similarity and geographical proximity in sesame as also reported by Dossa et al. (2017b). Furthermore, it had been reported that the frequency of cross-pollination in sesame could be as high as 60% (Dossa et al., 2016). Hence the percentage similarity of geographically-close accessions may be insignificant and non-divergent. This is a plausible reason why breeders essentially must consider the geographical divergence of two parental genotypes if they really want to achieve any significant variation in their expected Sesame progenies which is similar to the report of Wei et al.(2014).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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ABBREVIATIONS

PIC, Polymorphism information content; **CTAB,** cetyltrimethylammonium bromide; **RAPD,** randomly amplified polymorphic DNA; **EDTA,** ethylene di-amine tetra-acetate.

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Genetic diversity for immature pod traits in Ethiopian cowpea [*Vigna unguiculata* (L.) Walp.] landrace collections

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This study was undertaken to evaluate the extent and pattern of genetic diversity for immature pod traits in Ethiopia cowpea landrace collections. Eighty one landraces and improved cultivars were tested in a 9 x 9 simple lattice design. Analysis of variance revealed highly significant (P<0.01) or significant (p<0.05) differences among the genotypes for all traits. The first four principal components were able to explain 81% of variation for quantitative traits and 76% for qualitative traits. The genotypes were grouped into three distinct clusters, the first, second and third clusters with 60, 15 and 25% of the genotypes in that order. The landraces were distributed all over the clusters while the improved cultivars were absent in the second cluster. Shannon-Weaver diversity indices also showed existence of adequate genetic variability among the genotypes for qualitative traits. Shannon-Weaver diversity indices ranged from the lowest of 0.50 for pod curvature to the highest of 0.99 for pod shape. The study clearly showed that, even if the genotypes were classified into a few cluster, there was adequate divergence among the clusters showing existence of considerable genetic variability for immature pod traits for exploitation in future breeding for better green pod yield and quality in cowpea.

Key words: Cowpea, diversity, genotypes, immature pod, qualitative traits, quantitative trait.

INTRODUCTION

Cowpea (*Vigna unguiculata (*L.*)* Walp) is one of the most important grain legumes native to Africa (Timko and Singh, 2008). Cowpea is globally grown in tropical Africa, Asia, Latin America and Southern USA (Trinidad et al., 2010) under a wide range of soil pH including under low soil fertility, acidic soils and under drought-prone conditions (Badiane et al., 2012). The global area coverage of cowpea is estimated to around 11 million hectare from which 6 million tons of grain is produced with the share of Africa being 96% of world cowpea grain production (FAOSTAT, 2018). In Ethiopia, cowpea is grown almost in all lowland areas but the major

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production complexes are located in the Southern and Western parts of the country (Bedru et al., 2019; Sisay et al., 2019).

Cowpea is grown by farmers for different purposes including the nutritious tender leaves, immature pods, fresh peas and dry grain (Chikwendu et al., 2014; Gerrano et al., 2017; Mamiro et al., 2011). In terms of nutritive value, cowpea is an excellent source of protein, particularly to the poor people who could not afford animal products, essential minerals, vitamins and folates (USDA, 2015). Cowpea hay is also an important feed for animals during the dry seasons (Timko and Singh, 2008). The crop has deep roots which help to stabilize the soil, protect the surface and preserve the moisture (NRC, 2006). Like other legumes, cowpea also restores soil fertility by fixing atmospheric nitrogen (Nielsen et al., 1997). Thus, this crop can contribute greatly towards meeting the food requirement of people in areas where food security and malnutrition are major challenges.

Despite the manifold merits of cowpea in Ethiopia, the national production and productivity is far below the potential partially due to the biophysical challenges constraining productivity in smallholder farms and the inadequate technological interventions. Information on the cowpea production and productivity is scantly but a few sources show that the average national yield of cowpea in Ethiopia is estimated to be 400 kg ha^{-1} (Bedru et al., 2019), while yields of 2200 to 3200 kg ha^{-1} have been commonly recorded from improved varieties with proper crop management and protection practices (MoA, 2012). It showed the comparative advantages of genetic and agronomic interventions. From the breeding efforts by the research system hitherto, however, only six improved varieties were released (MoA, 2018), indicating the potential for further research particularly in cowpea breeding efforts.

It is obvious that genetic diversity in plant genetic resources provides an opportunity for plant breeders to develop new and improved cultivars with desirable characteristics including for farmers' preferred traits (Govindaraj et al., 2015). The success of crop breeding programs by and large depends on the magnitude of genetic diversity present in the breeding source materials, heritability of the trait under consideration and the level of selection intensity applied (Falconer, 1989). Genetic diversity is a prerequisite for the identification of superior genotypes, which may be recommended for direct release as commercial cultivars for wider production, but also for the identification of desirable parents to use as source materials in breeding programs (Rajaravindran and Natarajan, 2011). Ethiopia is believed to have considerable genetic diversity for cowpea both at phenotypic and genotypic levels (Belayneh et al., 2016; Mulugeta et al., 2016; Sisay et al., 2019; Tesfaye et al., 2019). Apart from genetic diversity for the regular morpho-agronomic traits at phenotypic level and for molecular diversity at genotypic level, information on the magnitude and pattern of phenotypic diversity for immature pod traits in Ethiopian landrace collections is scanty. Thus, this study was proposed to determine the extent and pattern of genetic diversity among cowpea collections using immature pod related phenotypic traits.

MATERIALS AND METHODS

Plant material and experimental design

Eighty one cowpea genotypes including 77 Ethiopian landraces collections kindly provided by the Ethiopian Institute of Biodiversity and Melkassa Agricultural Research Center and four improved varieties were selected for the study (Table 1). The experiment was conducted in the experimental field of Melkassa Agricultural Research Center in Ethiopia under irrigated conditions. Complete block design like randomized complete block design become less efficient as the number of treatment increases, primarily block size increases proportionally with the number of treatments, and the homogeneity of experimental plot within a large black is difficult to maintain (Gomez and Gomez, 1984). To this effect, 9 x 9 simple lattice design was employed. The seeds were directly sown on a 2.25 m x 3.0 m plot of three rows with 75 cm inter row and 20 cm intra row spacing. All crop management and protection practices were kept constant as per the recommendation for the site.

Data collection

Quantitative characters including days to 50% flowering, days to first pod picking, terminal leaflet length, terminal leaflet width, number of primary branches, green pod length and width, average green pod weight and pod yield per plant were recorded. All data were taken on plant basis except days to 50% emergence and days to first pod picking which were recorded on plot basis. Qualitative variables including growth pattern, pod attachment to peduncle, pod color, immature pod pigmentation pattern, absence or presence of secondary color in the pod, hue of secondary color, pod shape, pod curvature, pod suture string and pod texture of the surface were also scored using the standard descriptors for cowpea germplasm characterization (IBPGR, 1983).

Statistical analysis

Analysis of variance was performed based on the model for simple lattice design following Gomez and Gomez (1984). SAS version 9.2 statistical package was used for the analysis (SAS Institute Inc., 2010). Records on the quantitative variables were pre-standardized to means of zero and variances of unity before clustering to avoid bias due to differences in measurement scales (Manly, 1986). The optimum number of clusters in data set were determined by using gap statistics (Tibshirani et al., 2001). Analysis were done using different packages of R in R environment (R Development Core Team, 2019). Hierarchical cluster analysis was used to group the genotypes based on their similarities. The distance was measured using Euclidean distance and the distance matrix was used to construct the dendrogram using ward D^2 linkage method. Intercluster distances were calculated based on the standardized Mahalanobis's D^2 statistics as:

$$D^2_{ij} = (xi - xj)' \, cov^{-1} \, (xi - xj)$$

Table 1. Genotypes, collection site and source of the genotypes.

No.	Genotype	Collection region	Source	No	Genotype	Collection region	Source
1	208776	Oromia	EBI	42	NLLP-CPC-07-27	Oromia	MARC
2	211441A	Gambela	EBI	43	NLLP-CPC-07-28	SNNPRS	MARC
3	211441B	Gambela	EBI	44	NLLP-CPC-07-29	Gambela	MARC
4	211441C	Gambela	EBI	45	NLLP-CPC-07-31	SNNPRS	MARC
5	211490	SNNPRS	EBI	46	NLLP-CPC-07-32	Gambela	MARC
6	211491B	SNNPRS	EBI	47	NLLP-CPC-07-33	Oromia	MARC
7	211557	Amhara	EBI	48	NLLP-CPC-07-39A	Gambela	MARC
8	216749A	Gambela	EBI	49	NLLP-CPC-07-39B	Gambela	MARC
9	216749B	Gambela	EBI	50	NLLP-CPC-07-42	Oromia	MARC
10	220575	Amhara	EBI	51	NLLP-CPC-07-45	SNNPRS	MARC
11	222867	Gambela	EBI	52	NLLP-CPC-07-46A	SNNPRS	MARC
12	222890	Gambela	EBI	53	Dass 007	Gambela	MARC
13	223402	Oromia	EBI	54	NLLP-CPC-07-46B	SNNPRS	MARC
14	228624	Amhara	EBI	55	NLLP-CPC-07-47	SNNPRS	MARC
15	233403	Amhara	EBI	56	NLLP-CPC-07-48B	SNNPRS	MARC
16	235122B	Tigray	EBI	57	NLLP-CPC-07-82	Tigray	MARC
17	235122A	Tigray	EBI	58	NLLP-CPC-07-83	Tigray	MARC
18	244804	SNNPRS	EBI	59	NLLP-CPC-07-49	SNNPRS	MARC
19	Dass 001	Gambela	MARC	60	NLLP-CPC-07-51	SNNPRS	MARC
20	NLLP-CPC-07-01	Amhara	MARC	61	NLLP-CPC-07-52	SNNPRS	MARC
21	NLLP-CPC-07-02	Tigray	MARC	62	NLLP-CPC-07-53	Oromia	MARC
22	NLLP-CPC-07-03	Tigray	MARC	63	NLLP-CPC-07-54	Oromia	MARC
23	NLLP-CPC-07-04	Amhara	MARC	64	NLLP-CPC-07-48A	SNNPRS	MARC
24	NLLP-CPC-07-05	Amhara	MARC	65	NLLP-CPC-07-55	Oromia	MARC
25	Dass 002	Gambela	MARC	66	NLLP-CPC-07-56	Oromia	MARC
26	NLLP-CPC-07-07	Amhara	MARC	67	NLLP-CPC-07-58	Oromia	MARC
27	NLLP-CPC-07-09	Amhara	MARC	68	NLLP-CPC-07-60	Oromia	MARC
28	NLLP-CPC-07-10	Amhara	MARC	69	NLLP-CPC-07-64	Oromia	MARC
29	NLLP-CPC-07-101	Tigray	MARC	70	NLLP-CPC-07-69	Amhara	MARC
30	NLLP-CPC-07-11	Amhara	MARC	71	NLLP-CPC-07-72	Amhara	MARC
31	NLLP-CPC-07-12	Amhara	MARC	72	NLLP-CPC-07-75	Amhara	MARC
32	NLLP-CPC-07-14A	Tigray	MARC	73	NLLP-CPC-07-77	Tigray	MARC
33	NLLP-CPC-07-14B	Tigray	MARC	74	NLLP-CPC-07-78	Tigray	MARC
34	NLLP-CPC-07-16A	Oromia	MARC	75	NLLP-CPC-07-85	Tigray	MARC
35	NLLP-CPC-07-16B	Oromia	MARC	76	NLLP-CPC-07-89	Tigray	MARC
36	Dass 005	Gambela	MARC	77	NLLP-CPC-07-97	Gambela	MARC
37	NLLP-CPC-07-16C	Oromia	MARC	78	Kenketi	Improved	MARC
38	NLLP-CPC-07-18	SNNPRS	MARC	79	Bole	Improved	MARC
39	NLLP-CPC-07-23	Tigray	MARC	80	Black eye bean	Improved	MARC
40	NLLP-CPC-07-24	Tigray	MARC	81	TVU	Improved	MARC
41	NLLP-CPC-07-26	Oromia	MARC				

EIB=Ethiopian Institute of Biodiversity, MARC= Melkassa Agricultural Research Center.

Where, D^2ij = the distance between cases i and j; xi and xj = vectors of the values of the variables for cases i and j; and cov^{-1} = the pooled within groups variance-covariance matrix. The significance of D^2 values was tested by comparing the D^2 values between any two clusters against tabulated chi-square (χ^2) values at p = -1 degrees of freedom where p refers to the number of quantitative characters considered.

The diversity index (H') of Shannon and Weaver (1949) was used to measure the level of genetic variation for qualitative traits. The index (H') was estimated as follows:

$$H' = 1 - \sum_{i=1}^{n} pi \log_e pi$$

Table 2. Range, genotypes showing the extreme values, mean value from analysis of variance and coefficient of variance of 10 quantitative traits in 81 cowpea genotypes.

Trait	Minimum		Maximum		MS	Grand mean	CV (%)
	Value	Genotype	Value	Genotypes			
1	42	222867, 216749B, NLLP-CPC-07-29	99	NLLP-CPC-07-56	286***	58	5.1
2	76	NLLP-CPC-07-82	236	NLLP-CPC-07-77	1373***	149	13.5
3	54	222867	110	NLLP-CPC-07-04, NLLP-CPC-07-56	280.2***	70	5.6
4	3.4	235122A	8.4	NLLP-CPC-07-48B	0.9***	5.9	10.2
5	7.2	NLLP-CPC-07-39A	13.1	NLLP-CPC-07-16B	1.6***	10.3	5.1
6	4.3	NLLP-CPC-07-39A	8.72	Black eye bean, NLLP-CPC-07-32	1.1***	7.0	7.2
7	9	NLLP-CPC-07-69	16.8	NLLP-CPC-07-97	2.7***	12	7.9
8	2.6	NLLP-CPC-07-69	6.8	TVU	0.4*	4.0	13.1
9	0.6	NLLP-CPC-07-69	4.5	NLLP-CPC-07-97	0.6***	2.0	25.0
10	30	NLLP-CPC-07-24	747	NLLP-CPC-07-54	32348***	305	32.0

1=Days to 50% flowering, 2= Plant height (cm), 3= Days to 1st pod picking, 4= Terminal leaflet length (cm), 5= Terminal leaflet width (cm), 6= Number of primary branches, 7= green pod length (cm), 8= green pod width (mm), 9=Av. weight of green pod (g), 10= Pod yield (g/plant).

Where: H' = Shannon diversity Index; pi = the proportion of accessions in the ith class of an n–class character; n = the number of phenotypic classes of traits. Each diversity index value was divided by its maximum value ($\log_e n$) and normalized, to keep the values between 0 and 1. Principal components based on correlation matrix were calculated using the same software as in clustering.

RESULTS AND DISCUSSION

Analysis of variance

The range, mean square and CV of 10 quantitative characters of 81 cowpea genotypes are presented in Table 2. The range in the period from sowing to 50% flowering and days to first pod set was almost equal to 57 and 56 days, respectively. Similarly, the difference between the maximum and minimum mean value of plant height, terminal leaflet length, terminal leaflet width, pod length, pod width, average pod weight and pod yield were 160 cm, 5 cm, 5.9 cm, 4.4, 7.8 cm, 4.2 mm, 3.9 g and 717 g, respectively. The mean number of days to 50% flowering ranged from 42 days for genotypes 222867, 216749 B and NLLP-CPC-07-29 to 99 days for genotype NLLP-CPC-07-56. For days to first pod picking, the mean number of days ranged from 54 days for genotype 222867 to 110 days for genotypes NLLP-CPC-07-04 and NLLP-CPC-07-56. These phenological traits, 37% of the genotypes had taken more number of days to flowering compared to the grand mean (58 days) while 28% of the genotypes had taken more number of days to first pod picking compared to the grand mean (70 days). A wide range of variation in immature pod yield was recorded, which ranged from 30 g per plant for genotype NLLP-CPC-07-24 to 747 g per plant for genotype NLLP-CPC-07-54. The wide range for most of the traits indicated the diversity among the cowpea genotypes and also suggests the possibility of improving these traits through selection. The range and mean of days to 50% flowering and first green pod picking, plant height, number of primary branch, average pod weight and immature pod yield were generally larger than the previous reports (Khanpara et al., 2015; Shanko et al., 2014; Srinivas et al., 2017).

Most of the characters studied showed very highly significant ($P<0.001$) differences among genotypes except immature pod width which was significant at $p<0.05$. The analysis of variance revealed significant differences among the genotypes for the ten quantitative characters indicating the presence of adequate variability, which can be exploited through selection. Different reports indicated there is substantial phenotypic variability among cowpea varieties for different characters (Animasaun et al., 2015; Cobbinah et al., 2011; Gerrano et al., 2015; Lazaridi et al., 2017; Menssen et al., 2017; Molosiwa et al., 2016).

Diversity based on quantitative traits

Principal component analysis (PCA) reflects the importance of the largest contributor to the total variation at each axis of differentiation (Sharma, 1998). To decide how many components to maintain; different methods were recommended, an eigenvalue greater than one indicates that PCs account for more variance than accounted by one of the original variables in standardize data. This is commonly used as a cutoff point for which PCs are retained (Kaiser, 1960). An alternative method to determine the number of PCs is to look at a scree plot, which is the plot of eigenvalues ordered from the largest to the smallest. The number of component is determined by the point beyond which the remaining eigen values are

Table 3. Eigenvectors and eigenvalues of the first four principal components (PCs) for 10 quantitative pod characters of 81 cowpea genotypes.

Trait	Principal components			
	PC1	PC2	PC3	PC4
Eigenvalue	2.79	2.38	1.59	1.33
Proportion	27.9	23.8	15.9	13.3
Cumulative	27.9	51.7	67.6	80.9
Eigenvectors				
Days to 50% flowering	0.44	0.69	-0.45	0.32
Plant height (cm)	-0.19	0.62	-0.10	-0.32
Days to 1st pod picking	0.36	0.73	-0.46	0.30
Number of primary branches	0.32	-0.45	-0.14	0.54
Terminal LL length(cm)	0.04	0.44	0.77	0.29
Terminal LL width(cm)	-0.16	0.62	0.65	0.10
Pod length (cm)	0.86	0.02	0.28	-0.06
Pod width (mm)	0.74	-0.10	0.06	-0.55
Av. weight of green pod (g)	0.91	0.02	0.10	-0.26
Pod yield (g/plant)	0.42	-0.46	0.22	0.52

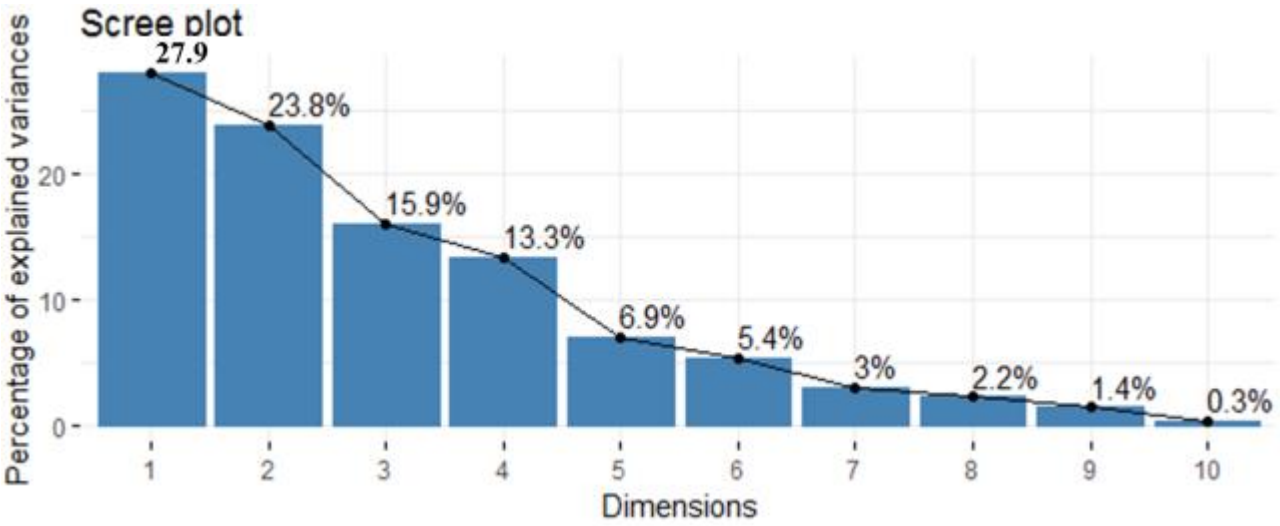

Figure 1. Scree plot of principal components (PCs) for 10 quantitative traits in 81 cowpea genotypes.

all relatively small and of comparable size (Jolliffe, 2002; Peres-neto et al., 2005). Accordingly, based on the scree plot (Figure 1) and eigenvalues (Table 3) four principal components (PCs) having eigenvalues between 1.33 and 2.79, extracted a cumulative of about 81% of the total phenotypic diversity maintained (Table 3). Manggoel and Uguru (2011) reported the first three components contributed 78.11% of the variability among the 10 cowpea accessions using 10 quantitative traits evaluated.

According to Chahal and Gosal (2002) characters with the largest absolute values closer to unity within the principal components influence the clustering more than those with lower absolute values closer to zero. The first principal component explains up to 27.9% of the total variance, and this was due mainly to variations in pod characters (average pod weight, pod length and width (Table 3). High contribution of pod character reported by Tesfaye et al. (2019) and in their report, about 77% of the total variance in 18 quantitative traits of 324 Ethiopian cowpea accessions could be explained on the basis of seven principal components and the first 22.6% of the total variance was due to mainly to pod length, seed

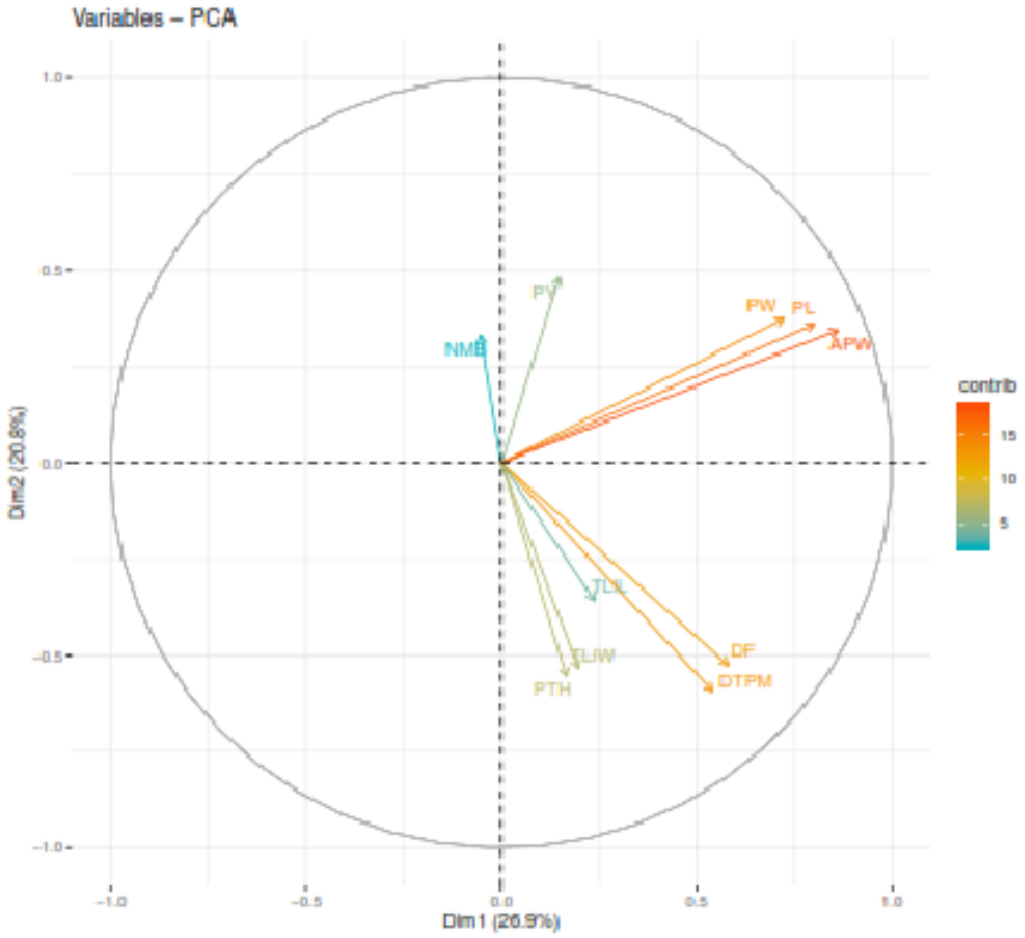

Figure 2. Variable correlation plot showing the relationships between 10 quantitative traits in 81 cowpea genotypes collected from different region of Ethiopia. DF=Days to 50% flowering, PH= Plant height, DTPM= Days to 1st pod picking, TLLL= Terminal leaflet length, TLLW= Terminal leaflet width, NMB= Number of primary branches, PL= immature pod length, PW= immature pod width, APW=Average weight of immature pod, PY= Pod yield.

length, seed width and seed yield per plot. Days to 50% flowering, days to first pod picking, plant height, and terminal leaf width were the main contributor for the variation in the second PC, which contribute 23.8% from the total variations. Similarly, the proportion of the total phenotypic variance of the genotypes accounted for by the third and fourth PCs were about 15.9 and 13.3%, respectively. The major contributor character for the third PC were variations mainly in the terminal leaflet length and width; and by less extent the phenological traits while variations in pod width, number of primary branches and pod yield contribute to the fourth PC. The PCA confirmed that the collected Ethiopian cowpea landraces have high diversity and all of the traits considered appeared to have high contributions towards the total phenotypic variability.

In the variable correlation plot (Figure 2), variables that are closed to the center of the plot are less important for the first components. Accordingly, a number of primary branches (NMB) and terminal leaflet length (TLLL) contribute less for principal components 1 and 2. Pod characters such as pod length (PL), pod width (PW), and average pod weight (APW), and crop maturity parameters (days to 50% flowering (DF) and first pod picking (DTPM)) were the major variables contributed for the variance of the first two components.

To observe the overall distribution pattern and correlations among the data attribute cluster analysis was done based on ten morphological traits related to the immature pod character of cowpea. The optimum number of clusters as determined by gap statistics was 3 (Figure 3a). The average linkage clustering method classified the 81 cowpea genotypes into three distinct clusters (Figure 3b). The number and name of genotypes in each cluster with their collection regions are presented in Table 4.

Cluster I was the largest group having 49 (60%) genotypes comprised from all collection regions, improved

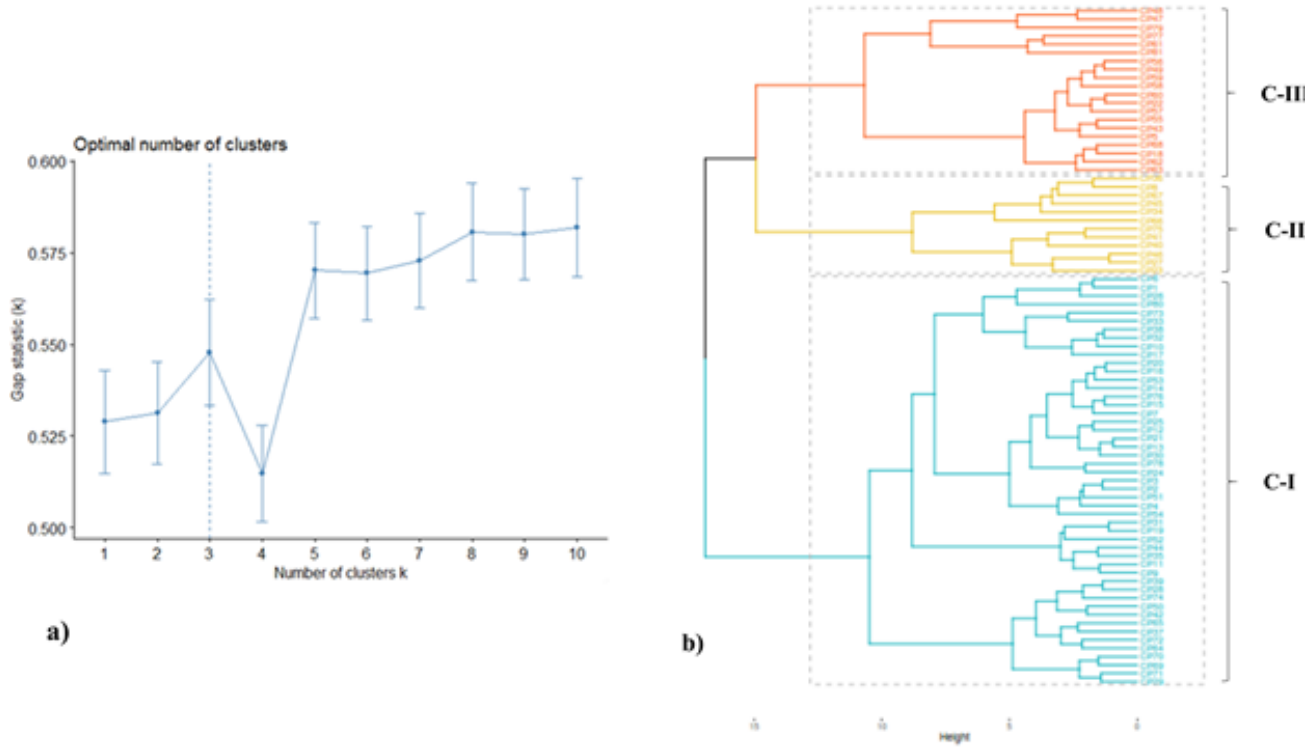

Figure 3. Cluster analysis: (a) Gap statistics to determine the number of clusters (K). (b) Dendrogram showing the relationships among the 81 cowpea genotypes evaluated for 10 quantitative traits. (Blue, yellow and red color represent cluster I, II and III, respectively).

cultivars and are characterized by early maturing genotypes, with the mean value of 51 days and 64 days for 50% flowering and first pod picking, respectively. The pod length, width and weight of this group were smaller. The second cluster consisted of about 15% of the genotype but all are landraces from all collection regions. This group was characterized by high yield and yield component traits (pod length, pod width, average pod weight and a number of the primary branch). The third group had 20 (25%) genotypes with large leaf areas (leaflet length and width), highest plant height and late maturing genotypes. The third cluster with high yield and genotypes in this cluster is a good source of pod yield per plant. The mean value of the 10 quantitative characters in each cluster is indicated in Table 5.

The pairwise generalized squared distance (D^2) among the three clusters based on Mahalanobis's D^2 statistics revealed the maximum and highly significant genetic distance was recorded between cluster I and III (D^2= 34.73) followed by cluster II and cluster III (D^2= 22.15). Non-significant (P>0.05) inter-cluster distance was observed between clusters I and II (D^2=9.96), indicating that genotypes in these two clusters had relatively little genetic divergence (Table 6). Thus, the crossing of genotypes from these two least distanced clusters produces little heterotic expression in the F1 population and low range of variability in subsequent segregating populations.

Diversity based on qualitative traits

In the principal component analysis (PCA) of 10 qualitative traits in 81 cowpea genotypes, four principal components were retained based on eigenvalues and scree plot (Table 7 and Figure 4). The qualitative traits showed that the first four PCs explained 35, 19, 12 and 10%, respectively of the total variance. The correlation between the variables and components for PC1 was mainly due to pod secondary color, the hue of secondary color, immature pod pigmentation, and pod attachment to peduncle and pod suture string. The texture of the pod surface and pod shape contributes to the variance in the PC2. The third component was related to the Intensity of green color in the immature pod and pod degree of curvature while PC4 was mainly due to the growth pattern of the genotypes.

Frequency distribution patterns, percent of proportion and Shannon-Weaver Diversity Index (H') were estimated for 81 cowpea genotypes from 10 qualitative traits (Table 8). The dominant (81%) growth pattern among the genotypes was indeterminate growth habit and 68% of

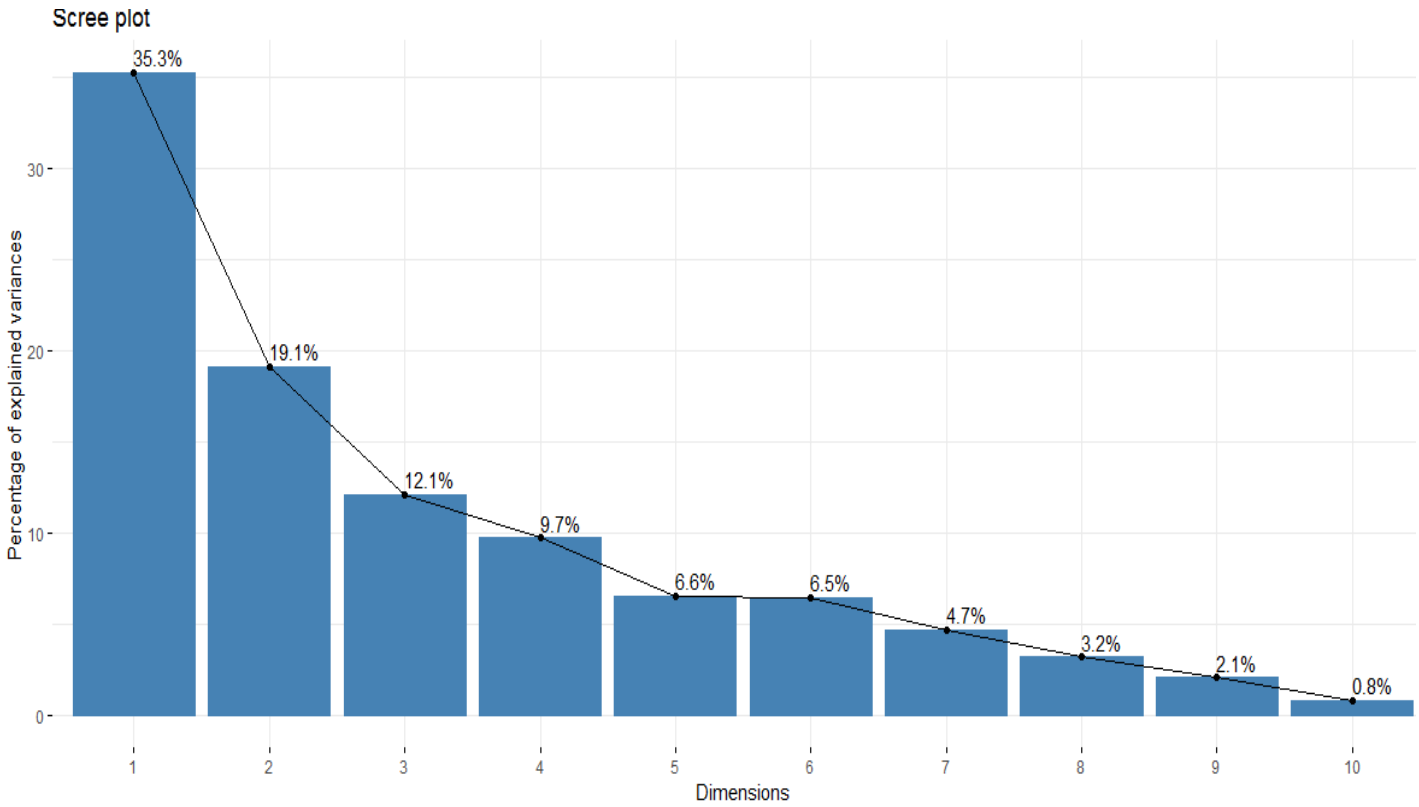

Figure 5. Scree plot of principal Components (PCs) for 10 qualitative traits in 81 cowpea genotypes.

Table 4. Clustering of 81 cowpea genotypes into four clusters using mean of 10 agro-morphological characters.

Cluster	Number of accessions	Accessions included	Collection region
I	49	211441A, 211491B, 220575, 222867, 223402, 228624, 233403, 211441B, 211441C, 222890, 235122A, 208776, 211557, 235122 B, 216749, Dass 001, Dass 002, Dass 007, Kenketi, Black eye Bean, NLLP-CPC-07-01, NLLP-CPC-07-02, NLLP-CPC-07-05, NLLP-CPC-07-07, NLLP-CPC-07-10, NLLP-CPC-07-101, NLLP-CPC-07-11, NLLP-CPC-07-12, NLLP-CPC-07-14A, NLLP-CPC-07-14B, NLLP-CPC-07-16B, NLLP-CPC-07-16C, NLLP-CPC-07-18, NLLP-CPC-07-23, NLLP-CPC-07-27, NLLP-CPC-07-29, NLLP-CPC-07-42, NLLP-CPC-07-45, NLLP-CPC-07-46A, NLLP-CPC-07-46B, NLLP-CPC-07-48A, NLLP-CPC-07-55, NLLP-CPC-07-64, NLLP-CPC-07-69, NLLP-CPC-07-72, NLLP-CPC-07-75, NLLP-CPC-07-77, NLLP-CPC-07-78, NLLP-CPC-07-89	Amhara, Gambella, Oromia, SNNPRS, Tigray, Improved
II	12	216749A, Dass 005, NLLP-CPC-07-04, NLLP-CPC-07-09, NLLP-CPC-07-16A, NLLP-CPC-07-24, NLLP-CPC-07-26, NLLP-CPC-07-31, NLLP-CPC-07-32, NLLP-CPC-07-56, NLLP-CPC-07-58, NLLP-CPC-07-85	Amhara, Gambella, Oromia, SNNPRS, Tigray,
III	20	244804, 211490, NLLP-CPC-07-03, NLLP-CPC-07-28, NLLP-CPC-07-33, NLLP-CPC-07-39A, NLLP-CPC-07-39B, NLLP-CPC-07-47, NLLP-CPC-07-48B, NLLP-CPC-07-82, NLLP-CPC-07-83, NLLP-CPC-07-49, NLLP-CPC-07-51, NLLP-CPC-07-52, Bole, TVU, NLLP-CPC-07-53, NLLP-CPC-07-54, NLLP-CPC-07-60, NLLP-CPC-07-97	Gambella, Oromia, SNNPRS, Tigray, Improved

Table 5. Mean value of immature pod yield and yield components of 81 cowpea genotypes in each cluster.

Character	Clusters		
	I	II	III
Days to 50% flowering	50.98	61.65	79.79
Plant height (cm)	150.44	135.12	164.78
Days to 1st mature pod	63.63	72.53	91.42
Number of primary branch	5.70	6.40	5.64
Terminal LL length(cm)	10.28	9.89	10.98
Terminal LL width(cm)	7.05	6.45	7.80
Pod length (cm)	11.61	13.02	12.58
Pod width (mm)	3.90	4.31	4.00
Av. weight of green pod (g)	1.74	2.45	2.17
Pod yield (g/plant)	286.29	394.80	232.48

Table 6. Average inter-cluster distance among clusters.

Clusters	I	II
I		
II	9.96^{ns}	
III	34.73**	22.15**

χ^2=16.92.0 and 21.67 at 5 and 1% probability level, respectively; **highly significant, at p<0.01. ns=not significant.

Table 7. Eigenvectors and eigenvalues of the first four principal components (PCs) for 10 qualitative pod characters of 81 cowpea genotypes.

Variable	Principal components			
	PC1	PC2	PC3	PC4
Eigenvalue	3.5	1.9	1.2	1.0
Proportion	35.3	19.1	12.1	9.7
Cumulative	35.3	54.4	66.5	76.2
		Eigenvectors		
Growth Pattern	-0.39	-0.18	-0.26	0.75
Pod attachment to peduncle	-0.70	0.42	-0.02	0.21
Immature pod pigmentation	0.75	0.47	-0.17	-0.16
Intensity of green color in immature pod	-0.19	0.53	0.55	0.32
Pod secondary color	0.81	0.47	-0.16	0.16
Hue of secondary color	0.79	0.49	0.07	0.16
Pod shape	-0.52	0.55	0.11	-0.01
Pod degree of curvature	0.13	-0.29	0.84	-0.09
Pod seture string	0.67	-0.25	0.26	0.38
Texture of pod surface	0.55	-0.55	-0.06	0.22

the genotypes did not have pigment in its immature pod. The proportion of genotypes with preferred immature pod characters like medium to dark green pod color, with the absence of secondary color in its pod, round shape cross-section, straight pod and fibreless were 27, 72, 51, 89 and 52%, respectively. Immature pod with very

Table 8. Estimate of frequency, proportion and Shannon-Weaver diversity index (H') of qualitative traits of 81 Ethiopian cowpea genotypes.

Trait	Description	Code	Frequency	Proportion (%)	H'
Growth pattern	Determinate	1	15	19	0.80
	Indeterminate	2	66	81	
Pod attachment to peduncle	Pendent	3	38	47	0.96
	30°-90° down from erect	5	23	28	
	Erect	7	20	25	
Immature pod pigmentation	None	0	55	68	0.57
	Pigmented tip	1	8	10	
	Pigmented sutures	2	7	9	
	Pigmented valves, green sutures	3	3	4	
	Splashes of pigment	4	8	10	
Intensity of green color in immature pod	Very light green	1	19	23	0.79
	Light green	3	40	49	
	Medium green	5	19	23	
	Dark green	7	3	4	
Pod secondary color	Absent	0	58	72	0.91
	Present	1	23	28	
Hue of secondary color	Pink	1	19	83	0.79
	Red	2	4	17	
Pod shape	Round	1	41	51	0.99
	Flat	3	40	49	
Pod degree of curvature	Straight	0	72	89	0.50
	Slightly curved	3	6	7	
	Curved	5	3	4	
Pod seture string	Absent	0	42	52	0.73
	Moderately stringy	1	37	46	
	Very stringy	3	2	2	
Texture of pod surface	Very smooth	1	8	10	0.78
	Smooth	3	27	33	
	Moderately rough	5	29	36	
	Rough	7	15	19	
	Very rough	9	2	2	

smooth and smooth surfaces were 10 and 33%, respectively. Proulx et al. (2010) indicated that for fresh pod quality the acceptable pod quality is pods with bright green color with no secondary color, tender, and firm, snap very easily, no sign of shriveling and abnormality. Thus, this result indicated the existence of considerable diversity to develop varieties for green pod purposes. For most genotypes, the pod attached to the peduncle was pendent (47%). Those genotypes with secondary color in their pods, the hue of secondary color were dominantly pink (83%) and only 17% of the genotypes were red colored.

Shannon-Weaver diversity index (H') was used to compare diversity among qualitative traits. The value of the Shannon diversity index ranged from 0.50 to 0.99. Higher value of H' observed for pod shape (0.99) followed by pod attachement to peduncle (0.96) and pod secondary color (0.91). The frequency class of pod curvature was unbalanced, 72, 6 and 3 for straight, slightly curved and curved pods, respectivelly and due to

this the H' was low (0.50). A low H' indicates extremely unbalanced frequency classes for an individual trait and a lack of genetic diversity (Perry and Mclntosh, 1991).

Conclusion

Ethiopian cowpea landraces were found to be divers for immature pod characters based on the quantitative and qualitative traits tested. Thus, there is an opportunity to develop cowpea varieties for immature pod purposes from available local germplasm collections either through selection or hybridization. Genotypes with good quality for immature pod purposes can be used as a source of new cultivars with improved features or as a gene pool of useful traits in breeding programs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Review

Algae: Potential biotechnological source in the Arabian Gulf

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Arabian Gulf is characterized by being nutrient-rich, which contributes to the biodiversity of marine macro- and microalgae. Marine algae are a potentially prolific source of many valuable products that may represent useful leads in developing the quality of human life. The studies on the bioactivity of marine algae in the Arabian Gulf region are very recent; thus, there are not many studies on this field. This review paper is focus on the importance of algae in various fields, and the benefit of such organisms to solve many environmental and health problems to develop the economy, and healthcare of surrounding Gulf countries by taking advantage of many common algal species present in the Arabian Gulf waters. Studies focuses on potentially using local algal species to control common environmental problems in the region such as harmful algal blooms, oil spills, and in wastewater treatment. Furthermore, they were a potential source of food and feed, and can be used in medical field for the treatment of many common diseases. This review describe researches involving the most common algal species found in Arabian Gulf contributing for the bioactive compound's isolation, for its productivity in large scale, and for its potential biotechnological applications.

Key words: Biotechnology, Marine algae, seaweeds, algae processing, pharmacologically active compounds, algae as food, aquaculture feed, algae oil content.

INTRODUCTION

Arabian Gulf characterized by the semienclosed and shallow marginal sea that has a mean depth of 35 m and a 100 m maximum depth at Strait of Hormuz (Figure 1). It covers an area of 226,000 km^2 that extends from the northwest of the Shatt Al-Arab river to southwest of strait Hormuz (Held and Cummings, 2018; Reynolds, 1993). Arabian Gulf has a sub-tropical climate because it is located between 24 and 30° north of the equator, with a low annual rainfall of an average of less than 5 cm in the coastal areas (Glennie, 1998). It has a mean surface salinity of about 41 practical salinity unit (PSU) and ranges from 35 PSU in the area of Shatt Al-Arab input to 70 PSU near Qatar (Al-Yamani et al., 2004; Brewer and Dyrssen, 1985).

The Arabian Gulf has a highly diverse marine environment that comprises genetically and biologically different organisms. It contains a wide range of coastal and marine ecosystems such as coral reefs, mangrove swamp, seagrass beds, sand, and mudflats that supports the growth of diverse marine organisms by forming feeding and nursery grounds for them (Naser, 2011). These marine ecosystems are considered of high value

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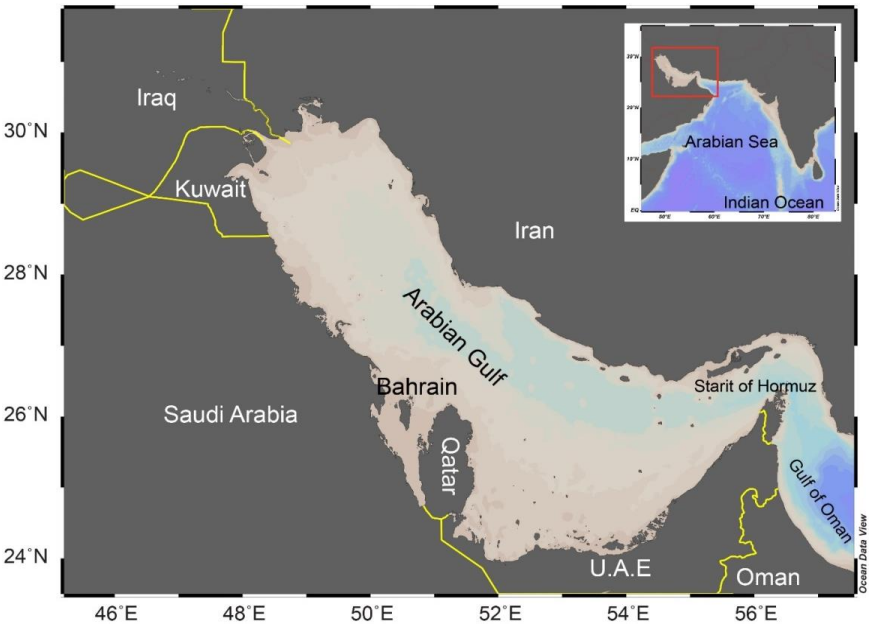

Figure 1. The map shows the location of the Arabian Gulf and the surrounding countries [Based on the Arabian Gulf map, available free of charge in https://www.google.com/maps/@27.6521528,56.1652784,6z (Accessed on March 2, 2020)]

as they provide ecologically important goods and services to the Arabian Gulf countries (Treweek, 1999). Thus, the Arabian Gulf marine ecosystems represent a major source of food and play an important role in nutrients cycling, primary production and erosion, and sedimentation control (Naser, 2014).

In the Arabian Gulf, the biological value of marine plants especially “algae” is still rarely considered, despite the fact that it contains important compounds that is used commercially in the pharmaceutical, medical, cosmetic, food and agricultural industries (Sohrabipour and Rabiei, 2006). Different marine algae and floral species can be ecologically important for the country when concerning their bioactivities.

The goal of this review is to give an overview of the most dominant algae species in the Arabian Gulf showing their importance in solving environmental questions and as a source of nutritional supplements and bioactive compounds potentially adequate for health issues.

METHODOLOGY

The information was selected from the following databases: PubMed, Web of Science, PubChem, SciFinder and LILACS. All original articles were included indexed in the period 1985-2019. The following uniterms were used: Arabian gulf, Biotechnology; Marine algae; seaweeds; algae processing; pharmacologically active compounds; algae as food; aquaculture feed; algae oil content.

BIOLOGY OF ALGAE

Algae are photosynthetic organisms that uses sunlight to produce energy through photosynthesis process. They are made up of eukaryotic cells that contains nuclei and organelles. All algae have plasmids that contains chlorophyll for photosynthesis process. However, they differ with chlorophyll combinations and carried pigments (Howe et al., 2008). Also, they vary in size ranging from unicellular microscopic algae (microalgae) such as cyanobacteria to more visible and complex multicellular algae (macroalgae or seaweeds). Marine algae comprises a variety of species, types, and groups that are unique in their characteristics. With over 150,000 species already identified and with many more yet to be identified, algae are classified into multiple major groupings as follows: Cyanobacteria (Cyanophyceae), green algae (Chlorophyceae), red algae (Rhodophyceae), brown algae (Phaeophyceae), yellow-green algae (Xanthophyceae), golden algae (Chrysophyceae), dinoflagellates (Dinophyceae), diatoms (Bacillariophyceae), and ‘pico-plankton’ (Prasinophyceae and Eustigmatophyceae) (Algaebase, 2020). These plants proliferate in aquatic environment, mainly in the sea, with some species having the ability to grow in freshwater environment (Fitzsimons et al., 1996).

Marine macroalgae

Marine macroalgae also known as seaweeds are macro-autotrophic, multicellular marine plants that are found

from intertidal to shallow subtidal zones. The macroscopic algae are classified into three major groups: *Chlorophyta* (green algae), *Rhodophyta* (red alga), and *Phaeophyceae* (brown algae) (Koch et al., 2013). They play a major role in marine coastal ecosystems food web by their ability of recycling nutrients. In addition, they support the growth of different associated species by providing them with a physical structure (Koch et al., 2013; Leopardas et al., 2014). Marine macroalgae have been used as food sources and crude pharmaceutical products in most East Asian countries especially Japan and China. It has been used in the treatment of many diseases such as iodine deficiency (Basedow's disease, goiter, and hyperthyroidism). Some seaweeds were provided as a source of vitamins to support other treatments for patients with various intestinal disorders (such as vermifuges, and as hypocholesterolemic and hypoglycemic agents) (El Gamal, 2012). In addition, macroalgae extracts were included in many industrial and biotechnological applications like, cosmetics, organic fertilizers, medicine against various microbial infections, biofuel and more (Salehi et al., 2019).

Marine macroalgae use

Marine algae are considered as the most important marine resources in the world. They represent a source of food, different types of feed, and medicine in the east as well as in the west countries (Kovac et al., 2013). Marine macroalgae in the Arabian Gulf were distributed along the seashore of the surrounded countries. The biomass and diversity of marina algae in the Arabian Gulf region have been studied and documented. In the Arabian Gulf, 299 species of algae were cited (Algaebase, 2020) and about 44% of them have some potential biotechnological application (Table 1). Seaweeds in this region were tested to be used in different applications:

Pharmaceutical applications: Marine algae play a major role in the pharmaceutical industry due to their bioactivity. They may produce low and high molecular weight bioactive metabolites that can be used as a prototype of new medicines. The synthesized bioactive compounds by algae includes pigments (example, carotenoids, xanthophylls, chlorophyll and terpenoids), vitamins, saturated and polyunsaturated fatty acids, amino acids and antioxidants (example, polyphenols, alkaloids and halogenated compounds) and polysaccharides (example, agar, carrageenan, proteoglycans, alginate, laminaran, rhamnan sulfate, galactosyl glycerol and fucoidan) (Dhargalkar and Pereira, 2005; Smit, 2004). Many of these compounds were discovered to have anti-bacterial, anti-fungal, algicidal, anti-viral, anti-protozoan and various pharmaceutically-relevant activities (Lakshmi et al., 2006; Bowman, 2007) that can treat cancer, diabetes, arthritis, acquired immune deficiency syndrome (AIDS), and other diseases (de Almeida et al., 2011). The composition and content of these bioactive compounds mainly depend on the algae species and the environmental factors such as season and temperature variation (Sardari and Nordberg Karlsson, 2018)

1. Antimicrobial activity: Seaweeds are known for their antimicrobial activity against different pathogens such as bacteria, fungi, protozoa and viruses. Many studies were done on the most dominant algal species in the Arabian Gulf region to test their microbicidal activity. *Sargassum latifolium* is an example of marine flora that can produce an antibacterial agent that acts against shrimp pathogenic bacteria (Dashtiannasab et al., 2012). While different brown, green and red algae such as *Caulerpa sertularioides*, *Gracilaria corticata*, *Gracillaria salicornia* and *Sargassum oligocystum* species have an anti-Leishmanial activity (anti-protozoan) against Leishmania protozoa (Fouladvand et al., 2011). *Sargassum oligocystum* (collected from the Arabian Gulf) was also tested in another study showing its property to produce antibacterial activity against *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 14990), *Pseudomonas aeruginosa* (ATCC 27853), and *E. coli* (ATCC 25922) when extracted in hot water rather than alcoholic extract (Tajbakhsh et al., 2011a). Also, it was discovered that *Sargassum oligocystum* have remarkable antitumor activity against K562 and Daudi cell lines at concentrations 500 and 400 µg/ml of the algal extract (Zandi et al., 2010). Different studies were done on brown algae genus *Cystoseira* showed that different species showed different bioactivities against microbial community. A research done by Tajbakhsh and colleagues showed that *Cystoseira trinodis* have activity against *S. aureus, S. epidermidis*, *E. coli*, and *P. aeruginosa* bacterial species (Tajbakhsh et al., 2011b). While *Cystoseira myrica* has virucide activity against herpes simplex virus type 1 (Zandi et al., 2007).

2. Antioxidants: Marine plants and algae play a major role in human diet due to its richness in vitamins and minerals and the existence of phenolic compounds and flavonoids in all parts of the plant that acts as anti-oxidants in the human body (Khan and Satam, 2003; Mathew and Abraham, 2006). Plant-derived secondary metabolites, such as antioxidant compounds, help in cleaning the accumulated free radicals in the human body that may result in DNA and cell damage (Bergamini et al., 2004). Few studies were available on the antioxidant property of macroalgal species extracts from Arabian Gulf. It was discovered that different species of green, brown and red algae studied such as *Laurencia snyderia, Acanthophora nayadiformis, Gracilaria corticata, Sargassum tenerrimum,* and green algae *Chaetomorpha*

Table 1. Main algae species found in Arabian Gulf, its principal uses and isolated substances.

Species	Substance	Activity	Reference
Sargassum latifolium (Turner) C.Agardh	Ethanolic and chloroformic crude extracts	Antibacterial agent against shrimp pathogens (*Vibrio alginolyticus*, *V. parahaemolyticus* and *V. Harveyi*)	Dashtiannasab et al., 2012
Sargassum oligocystum Montagne	Polyhy-droxylated fucophlorethol.	Antibacterial agent against human pathogens such as *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 14990), *Pseudomonas aeruginosa* (ATCC 27853), and *Escherichia coli* (ATCC 25922)	Tajbakhsh et al., 2011a
	Polysaccharides	Antitumor activity against K562 and Daudi cell lines	Zandi et al., 2010
	Halogenated compounds	Anti-protozoan (Leishmanial)	Fouladvand et al., 2011
Sargassum tenerrimum J.Agardh	Phenol and flavonoid	Anti-oxidant activities	Movahedinia and Heydari, 2012
Sargassum swartzii C.Agardh	-	Cytotoxic activity (antitumor) against Caco-2 & T47D cell lines	Khanavi et al., 2010
Sargassum ilicifolium (Turner) C.Agardh	C20:5n-3 (EPA) fatty acid	Nutritional supplement (regulating blood clotting and blood pressure, and develop brain and nervous system functions)	Rohani-Ghadikolaei et al., 2012
Caulerpa sertularioides (S.G.Gmelin) M.Howe (Appendix Figure 1)	-	Anti-protozoan (Leishmanial)	Fouladvand et al., 2011
Cystoseira trinodis (Forsskâl) Agardh (=*Sirophysalis trinodis* (Forsskâl) Kützing)	α-pinene	Antibacterial activity against human pathogens such as: *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 14990), *Pseudomonas aeruginosa* (ATCC 27853), and *Escherichia coli* (ATCC 25922)	Tajbakhsh et al., 2011b
Cystoseira myrica (=*Polycladia myrica* (S.G.Gmelin) Draima, Ballesteros, F.Rousseau & T.Thibaut)	-	Anti-viral activity	Zandi et al., 2007
	-	Cytotoxic activity (antitumor) against T47D cell line.	Khanavi et al., 2010
Ulva lactuca Linnaeus	High protein, lipids, and polysaccharides	Nutritional supplement	Rohani-Ghadikolaei et al., 2012
	Algae tissue	Removing toxic materials like ammonia & phosphate from fish farms	Al-Hafedh et al., 2012
Chaetomorpha linum (O.F.Müller)Kützing (Appendix Figure 2)	Phenolic and flavonoid contents	Anti-oxidant activities	Farasat et al., 2013
Colpomenia sinuosa (Mertens ex Roth) Derbès & Solier (Appendix Figure 4)	C20:5n-3 (EPA) fatty acid	Nutritional supplement (regulating blood clotting and blood pressure, and develop brain and nervous system functions)	Rohani-Ghadikolaei et al., 2012
	High protein, lipids, and polysaccharides	Nutritional supplement	Mohammadi et al., 2012

Table 1. Contd.

Enteromorpha intestinalis (=Ulva intestinalis Linnaeus) (Appendix Figure 6)	High protein content	Nutritional supplement	Rohani-Ghadikolaei et al., 2012
Jania rubens (Linnaeus) J.V.Lamouroux	High protein, lipids and polysaccharides	Nutritional supplement	Mohammadi et al., 2012
Laurencia papillosa (C. Agardh) Greville	High protein, lipids and polysaccharides	Nutritional supplement	Mohammadi et al., 2012
Laurencia snyderia E.Y.Dawson	Phenolic and flavonoid contents	Anti-oxidant activities	Hosseinzadeh et al., 2015
Acanthophora spicifera (M.Vahl) Børgesen	High protein, lipids and polysaccharides	Nutritional supplement	Mohammadi et al., 2012
Acanthophora nayadiformis (Delile) Papenfuss	Phenolic and flavonoid contents	Anti-oxidant activities	Hosseinzadeh et al., 2015
Champia parvula (C.Agardh) Harvey (Appendix Figure 3)	High protein, lipids and polysaccharides	Nutritional supplement	Mohammadi et al., 2012
Hypnea cervicornis (J.Agardh) Kuntze 1891	High protein, lipids and polysaccharides	Nutritional supplement	Mohammadi et al., 2012
Hypnea valentiae (Turner) Montagne (Appendix Figure 5)	High protein contents	Nutritional supplement	Rohani-Ghadikolaei et al., 2012
Gracilaria corticata (J.Agardh) J.Agardh	Agar	Gelling and stabilizing agent in the medical, cosmetics and food industries.	Yousef et al., 2013
	Halogenated compounds	Anti-protozoan (Leishmanial)	Fouladvand et al., 2011
	Phenol and flavonoid	Anti-oxidant activities	Movahedinia and Heydari, 2012
	High protein, lipids, and polysaccharides	Nutritional supplement	Mohammadi et al., 2012
Gracillaria salicornia (C.Agardh) E.Y.Dawson	Halogenated compounds	Anti-Leishmanial activity	Fouladvand et al., 2011
Gracilaria arcuata Zanardini	Thalli and tissue	Removing toxic materials like ammonia & phosphate from fish farms	Al-Hafedh et al., 2012

C20:5n-3= Eicosapentaenoic acid (omega-3 fatty acid); K56 = a human erythroleukemic cells; Caco-2 = human intestinal epithelial cells; T47D = a human breast cancer cell line.

Genus showed antioxidant activity (Farasat et al., 2013; Hosseinzadeh et al., 2015; Movahedinia and Heydari, 2012). Several studies on Arabian Gulf seaweeds revealed the fact that *Sargassum* sp. has the highest antioxidant activity (2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and Fe^{3+} reducing power) when compared with other tested seaweeds (Moubayed et al., 2017; Sadati et al., 2011). From Horincar's study, the antioxidant activity of marine algae depend mainly on algal species and the solvent used in extraction (Horincar et al., 2011). That, there is a positive correlation between the increase in algae phenolic content and antioxidant properties, because phenolic compounds act as electron donors and help in balancing the reactions created by free radicals in the body (Moubayed et al., 2017; Sadati et al., 2011). A recent study on seaweeds collected from the Kuwait coast revealed that the phenolic extracts of 26 tested seaweeds showed high antiradical and reducing power. In which the ethanol was efficient in extracting polyphenols, steroids, glycosides, tannins, and saponins, while water was efficient in extracting polysaccharides and proteins. The results showed that the seaweeds from this region with extreme climatic conditions produce more flavonoids than phlorotannins when compared with the same species at temperate climate. All *Sargassum* sp. and some other species like *Canistrocarpus cervicornis*, *Feldmannia irregularis, Polysiphonia platycarpa, Chondria* sp., and *Cladophora* sp., showed high antioxidant activity

and they were rich in polyphenolic compounds. Moreover, almost all the tested seaweeds contained hydroquinone, which is known as a skin whitening agent. Thus, the properties of seaweeds from this region qualify them to be used in various industries (Farvin et al., 2019a). Then, to increase the yield of bioactive compounds extraction from the same tested seaweeds, Farvin et al. (2019b) used five carbohydrases and three proteases enzymes to screen seven selected brown seaweeds for antioxidant and antimicrobial activity. The results showed that bioactivity defers among seaweeds and enzymes used for extraction. As an example, *Sargassum boveanum*-Viscozymes and *Sargassum boveanum*-Alcalase showed high antioxidant activity, while most of the seaweeds extracted by Flavourzyme showed antimicrobial activity. Thus, the study concluded that each seaweed species needs a specific enzyme to increase the yield of bioactive compounds.

3. Cytotoxic compounds: Tropical and subtropical algae were an important source of many natural products that can help in developing many drugs against cancer, microbial infections and inflammation (Cardozo et al., 2007; Stein et al., 2011). They also act as a source of protein, agar, carrageenan, alginates, vitamins and minerals that can be used in medical fields for the treatment of some diseases and related symptoms such as children fever, digestive disorders, joint pains, and cancer and used as sedatives and antibiotics (Iwashima et al., 2005; Mosaddegh et al., 2014). It was discovered that certain compounds in marine algae such as fucoidans, laminarians, and terpenoids have cytotoxic property against cancer cells by inhibiting their proliferation (Smit, 2004). The cytotoxic effects of algal extracts on different cell lines are currently being studied (Isnansetyo et al., 2017; Tannoury et al., 2017). Within these studies, cytotoxic activity against cancer and tumor cell lines, as well as immunomodulatory activity, has become one of the most important specificities of algae (Anastyuk et al., 2017; Khalifa et al., 2016). The bioactive compounds in algal extracts and isolates were used in the medical field in treating toxic cells in human body such as cancer cells by following three different scenarios. They can cause necrosis (cell lysis), apoptosis (a genetically controlled form of cell death), and decrease in cell viability by stopping the division of actively growing cells (Fadeel, 2004). The recent studies in this field are mainly focusing on elucidating the specific compounds that demonstrate such bioactivities. Also studying the synergistic effect of different bioactive compounds within algal extracts have attracted the interest of the scientific community, since the interactions between these substances increase the bioactive capacity (Choi et al., 2017).

The cytotoxic activity studies of seaweeds from the Arabian gulf region against human cell lines have started since 2010. A study on three different brown algal species *Sargassum swartzii*, *Cystoseira myrica* and *Colpomenia sinuosa* collected from the Arabian Gulf water showed that different fractions of these algae exhibited cytotoxicity against different cancer cell lines (HT-29 (human colon carcinoma), Caco-2 (human epithelial colorectal adenocarcinoma cells), T47D (breast cancer cell) and NIH 3T3 (mouse embryonic fibroblast cell)). The cytotoxicity of these algal extracts activates an estrogen receptor-independent mechanism in breast cancer cells, which promote cell proliferation and tumor progression (Khanavi et al., 2010). Another study on the cytotoxicity of Arabian gulf seaweeds species against cancer cells showed that the alcoholic extracts of *Gracillaria corticata*, *Ulva fasciata*, and *Sargassum ilicifolium* were antiproliferative against all the tested cancer cell lines (MCF-7 (human breast carcinoma cell line), MDA-MB-231 (human epithelial breast cancer cell), HeLa (cervical carcinoma), HepG-2 (human hepatocellular carcinoma cell line), and HT-29 (human colon carcinoma). While the methanolic extract of *Gracillaria corticata* showed the highest inhibition activity against MCF-7 cell line, which could induce apoptosis in human breast cancer in time and dose depended manner (Namvar et al., 2014). Ghannadi et al., (2016) did a study to compare the cytotoxicity of two *Gracillaria* species (red seaweed) collected from the Arabian Gulf water against different human cell lines. The obtained results showed that both *Gracillaria* species (*G. corticata* and *G. salicornia)* have cytotoxic activity against all the tested cell lines (HT‑29, HeLa, and MCF‑7), but *G. salicornia* activity was a little bit higher (HT‑29= 68.2 µg/ml, HeLa= 125.5 µg/ml and MCF‑7= 185.8 µg/ml). The production of algal extracts for these two species is easy and cost-effective; thus, the study suggested to use them as a therapeutic tool in the form of food or drug against human cancer (Ghannadi et al., 2016). The cytotoxicity of *G. salicornia* was also tested by Efrani showing a higher toxicity effect (IC_{50}>400 µg/ml) against MCF-7 cel line (Erfani et al., 2015) when compared with Ghannadi study (MCF‑7= 185.8 µg/ml) (Ghannadi et al., 2016). The differences in the activity results between both studies may be a consequence of different solvents used for extraction, season and time of collection, and also different methods used.

Food applications: Marine algae are known for their nutritional value because they are rich in protein, lipids, carbohydrates, and other essential elements. They are used as food and feed sources for fish farming due to their added-value dietary ingredients. Besides their nutritional value, seaweeds contain a number of pigments, defensive and storage compounds, and secondary metabolites that could have beneficial effects on farmed fish (Wan et al., 2018). Many studies were done on different macroalgae species collected from the Arabian Gulf to study their nutritional composition. Six seaweeds collected from the Arabian Gulf evaluated for their nutritional value and if these macroalgae can be used as

a potential source in aquaculture nutrition. The study showed that all the tested species have high nutritional value, rich in protein, lipids, carbohydrates, and essential elements. The collected seaweeds were high in carbohydrate (31.8 to 59.1% dry weight), ash (12.4 to 29.9%) and minerals (K, Mg, Fe, Mn, Cu, Zn and Co) but low in lipid content (1.5 to 3.6%). The tested red (*Gracilaria corticata* and *Hypnea valentiae*) and green seaweed (*Ulva lactuca* and *Enteromorpha intestinalis*) have high protein content ($p < 0.05$) compared to brown seaweeds. Moreover, the green algae *U. lactuca* was rich in carbohydrates (59.1%) and lipids (3.6%). While the brown algae (*Colpomenia sinuosa* and *Sargassum ilicifolium*) were rich in C20:5n-3 (EPA) fatty acid (5.4 ± 0.86 and 1.9 ± 0.22, respectively) compared with the other tested seaweeds, which is health-beneficial eicosapentaenoic acid that used as nutritional supplement for human diet, helping in regulating blood clotting and blood pressure, and develop brain and nervous system functions (Rohani-Ghadikolaei et al., 2012). The tested seaweeds were rich in essential elements that were absorbed from the seawater and accumulated in seaweed thalli (Azmat et al., 2006). The differences in their mineral composition and concentration are due to species and location specificity. Therefore, they concluded that these seaweed species could be a potential source of food or feed additive. Agar is also an important polysaccharide that broadly used as a gelling and stabilizing agent in different applications like food, cosmetics and medical industries (Villanueva et al., 2010). Some macroalgal genera that are common in these regions, such as *Gelidium* and *Gracilaria* are known as agar-containing seaweeds. These two genera give high-quality agar that commercially produced by France, Indonesia, the Republic of Korea, Mexico, Morocco, Portugal and Spain (McHugh, 2003). *Gracilaria* sp. is common in the northern part of the Arabian Gulf and considered as the major source of commercially valuable agar around the world (Marinho-soriano and Bourret, 2003). A study done by Yousef et al., (2013) to test the best conditions for agar extraction from *Gracilaria corticata* revealed that agar properties are highly affected by experimental variables except for socking time that had less effect. Thus, a soaking time of 1 h at 40°C with a ratio of 1:200 of algae-to-water and then extraction for 2.5 h at 80°C is recommended to achieve a better quality agar. However, to increase agar yield, a soaking time of 0 h at 40°C with a 1:100 algae-to-water ratio followed by extraction for 2 h at 60°C must be done.

Another study by Mohammadi et al., (2012) on eight seaweed species (*Caulerpa sertulariodes*, *Colpomenia sinuosa*, *Acanthophora spicifera*, *Champia parvula*, *Hypnea cervicornis*, *Gracilaria corticata*, *Jania rubens* and *Laurencia papillosa*) belonging to different orders (Rhodophyta, Chlorophyta and Phaeophyta) showed that the eight analyzed seaweeds have valuable nutritional compositions. This was due to their high levels of proteins, carbohydrate and fat owning in their cells (Mohammadi et al., 2012). Thus, they can provide dietary alternatives to the human diet to improve the nutritive value.

Seaweeds act as biofilters that increase the sustainability of intensive fish farming by filtering and recycling fish waste. Some studies on common seaweeds in the Arabian Gulf water concluded that *Ulva lactuca* and *Gracilaria arcuata* species were both suitable for integrated aquaculture and bioremediation by removing toxic materials like ammonia and phosphate from fish farms (Al-Hafedh et al., 2012).

Controlling harmful algal blooms (HABs): Harmful algal blooms (HABs) occur more frequently in the Arabian Gulf waters. The bloom of these microalgal species causes harm to marine organisms due to their ability to produce toxins. HAB species toxins kill marine organisms either directly or by accumulating in the food web causing mortality for fish, seabirds, marine mammals and humans when consuming affected seafood products (Al Shehhi et al., 2014). The Arabian Gulf area has experienced serious economic losses (Kuwait, Saudi Arabia, Iran, UAE, Oman, Bahrain and Qatar) during 2008-2009 when dinoflagellate algae known as *Cochlodinium polykrikoides* bloomed and killed thousands of tons of fish. This affected the economy of the Arabian Gulf countries by impacting tourism, blocking desalination plants, obstruct traditional fisheries and damaged coral reefs (Mindy et al., 2010). To control such problem, a study was done on polyunsaturated fatty acids (PUFAs) in green algae (*Ulva fasciata;* collected from Japan) showed an algicidal activity against harmful algal blooms caused by different micro-algal species such as *Chattonella antiqua, C. marina, Fibrocapsa japonica*, *Heterosigma akashiwo*, *Karenia mikimotoi*, *Heterocapsa circularisquama*, *Prorocentrum minimum*, *P. sigmoides*, *Scrippsiella trochoidea*, *Alexandrium catenella* and *Cochlodinium polykrikoides*. The activity of the isolated PUFAs (C16:4 n-3, C18:3 n-3, and C18:4 n-3) is limited to the specified microalgae without causing any harm to the surrounding marine living organisms. The produced allelochemical (PUFA's) were highly recommended for HAB'S controlling mechanism because of their high inhibition effect at very low concentration, ecologically accepted and, their high biodegradability (Alamsjah et al., 2008). Thus, they can be used in the Arabian Gulf region to end frequently occurring harmful algal blooms (HAB) without affecting the surrounding ecosystem.

Marine microalgae

The Arabian Gulf is known for its unique hypersaline, semi-arid and nutrient-rich environment (Quigg et al., 2013). The abundance and the diversity of marine microalgae (phytoplankton) in the regional waters were

well studied. The identified phytoplankton taxa in the Arabian Gulf waters and Oman Sea reached up to 376 in 2016 as described by Polikarpov et al., (2016), dominated by *Dinophyta* phylum that represents the most diverse group. Every year new microalgal species were introduced to the Arabian Gulf water via transporting ships ballast water discharge, that when proliferate can cause a serious economic and environmental problem (Al-Yamani et al., 2015). Generally, phytoplankton normally exists in the Arabian Gulf waters, but at certain favorable environmental conditions, they bloom affecting the surrounding marine organisms. Some species may produce toxins that can cause immortality for marine organisms, while others may discolor the surface water, thus blocking the sunlight for deeper organisms (Richlen et al., 2010). In 1999, a massive fish kill occurred in Kuwaiti waters due to the bloom of *Gymnodinium* species that produce a toxin called gymnodimine (Heil et al., 2001). Again in 2008-2009, the bloom of *Cochlodinium polykrikoides* resulted in killing thousands of tons of fish due to the secretion of biologically active metabolites such as cytotoxic agents and mucus substances (Richlen et al., 2010). On the other hand, the proliferation of microalgae can be beneficial by producing high levels of oxygen, participating in the global carbon cycle by fixing carbon and, act as a feed source for other higher organisms like fish larvae (Shams et al., 2012). Most of the phytoplankton species are known for their richness in bioactive compounds like pigments and lipids (Mimouni et al., 2015), that have anti-oxidant, anti-inflammatory, anti-mutagenic, anti-cancer, anti-obesity, anti-allergic activities, and cardio-neuro-, hepato- and photoprotective effects when combined together or either alone (Heydarizadeh et al., 2013).

Lipid contents of microalgae

Microalgae is a promising energy source for biofuel production in the Persian/Arabian Gulf. Since 2010, the Arabian Gulf countries focused on finding an alternative biofuel source rather than petroleum due to the depleting supplies. Many microalgal species in the Gulf area can accumulate high levels of lipids that can be extracted and converted to biodiesel, green diesel, or green jet fuel (Najafi et al., 2011; Wilson et al., 2012). Some microalgal species like *Nannochloropsis, Chlorella*, and *Dunaliella sp.* (common in the Arabian Gulf waters) were studied in a small indoor scale since 2004, started from Kuwait for commercial use and biofuel production. The advantage of producing biofuel from microalgae is summarized by their higher growth rates and productivity per unit land area, with the ability to use the non-fertile land for their reproduction (Williams and Laurens, 2010). Algal biofuel production mainly depends on algal biomass that varies among species (Ben-Amotz et al., 1987) and growth conditions (Fernandezreiriz et al., 1989). This was supported by research done on three *Dunaliella* species isolated from Qatar seawater (Arabian Gulf) showed that *Dunaliella bardawil* was highly productive and showed higher carbon sequestration and photosynthetic efficiencies than *D. salina* and *D. parva*. *D. bardawil* and *D. salina* were able to thrive in Gulf seawater under high salinity and hot, sunny climate, showing high oil contents in their biomass. Thus, they can be used as a biodiesel feedstock for Qatar (Wilson et al., 2012). In addition, other microalgal species isolated from Arabian Gulf and tested by Moazami et al. (2011) for possible biofuel production, and they were *Nanochloropsis* (PTCC 6016), *Nanochloropsis* (PTCC 6003), *Nitzschia* (PTCC 6001) and *Chlorella* (PTCC 6022). It was shown that the tested species have high oil contents, high biomass, and high productivity compared with the other tested species (Table 2). They concluding that, the two-isolated *Nanochloropsis* sp. proved to be suitable for commercial biofuel production (Moazami et al., 2011). *Nannochloris* sp. (strain QUCCCM31) is another promising biodiesel producing strain in the Arabian Gulf region. This strain was isolated from Qatar seawater and can thrive under high temperature (up to 45°C) and high salinity (35-100 ppt). It has high lipid productivity under harsh conditions and can produce nervonic acid, which is a straight fatty acid having high pharmaceutical potentials (Saadaoui et al., 2016).

To enhance biodiesel production in some microalgal species, certain products can be added to the supported media to increase their lipid productivity. The addition of myoinositol (200 mg/l); which is a stereoisomer of inositol; enhances the lipid productivity of *Dunaliella salina* to up to 50% and biodiesel quality by increasing auxin and phosphatidylinositol accumulation in the cells (Talebi et al., 2015). While another study on *Nanochloropsis* sp. showed an increase in biomass productivity when nano-chitosan flocculant agent was added to the culture media; rather than chitosan; to up to 9%. This will result in high biomass recovery that will lead to an efficient cost-effective technique for harvesting microalgae for biofuel production (Farid et al., 2013).

Carotenoid pigments of microalgae

The carotenoid pigment in marine microalgae plays an important role in food, feed, pharmaceutical, and nutraceutical industries due to their strong coloring ability and antioxidative activity (Fraser and Bramley, 2004). Microalgae are known to be the best natural source of carotenoids, especially strains belonging to Chlorophyta such as *Dunaliella salina*, *Nannochloropsis* sp. and various *Chlorella* species (James and Al-Khars, 1990; Zhang et al., 2014). These strains are common in the Arabian Gulf waters especially *Dunaliella* spp. (El-Gammal et al., 2017). *Dunaliella* spp. are common in the Arabian Gulf due to its ability to tolerate high salinities

Table 2. Comparison of oil content (%), average biomass concentration, maximum biomass concentration and lipid productivity of different microalgal species isolated from the Arabian Gulf.

Strains	Oil content (%)	Average biomass conc. (g/l)	Maximum biomass conc. (g/l)	Productivities (mg/l/d)	Reference
Nanochloropsis (PTCC 6016)	52	50.0	64.2	46.5	Moazami et al., 2011
Nanochloropsis (PTCC 6003)	46	21.7	48.6	32.6	
Chlorella (PTCC 6022)	38	12.6	28.1	19.3	
Nitzschia (PTCC 6001)	32	5.1	13.9	13.0	
D. salina (Persian Gulf)	22	0.15	-	33	Talebi et al., 2015
D. salina (Shariati)	18.9	0.05	-	10.26	

(halotolerant), and their blooms will be noticed by the brownish-red color (Al-Hasan and Sallal, 1985). The most dominant species are *D. salina* (Abu-Rezq et al., 2010a; Saburova et al., 2009) and *D. viridis* (Alshuaibi et al., 2011). *Dunaliella* spp. are known for mass production of *β*-carotene that is used as a food preservative (El-Baky and El-Baroty, 2011). Also, *β*-carotene in *Dunalliela* sp. showed a decrease in several types of cancer and degenerative diseases incidents due to the presence of 9-*cis* and all-*trans* stereoisomers (comprising 40 and 50% of *β*-carotene, respectively) (Ben-Amotz, 1999). For that reason, intensive researches were done to test the optimum conditions to increase *β*-carotene accumulation and harvesting from certain producing species. *β*-carotene accumulation in *Dunaliella salina* was discovered to increase when the light intensity (Abu-Rezq et al., 2010b) and salinity increases (Talebi et al., 2013). These two important factors were tested by Kitto and Reginald (2011) to check the best climatic conditions for harvesting *β*-carotene from *D. salina* for industrial purposes in UAE (United Arab Emirates). The productivity of *D. salina* increased from 120,000 cells/ml to 1.2 million cells/ml in day 12 at 43 PSU in outdoor culture ponds during summer. And the *β*-carotene production was the highest during summer (at 100,000 lux) reaching up to 98.34 pg/cell (Beta-carotene concentration) at 243 PSU salinity due to high light intensity (Kitto and Reginald, 2011).

It was discovered that under certain environmental stresses, some marine microalgal species surprisingly increase their pigments production. Since *D. salina* and *C. vulgaris*, microalgal species were commonly found in Arabian Gulf waters due to their high ability to tolerate salinity; Talebi et al. (2013) found that there is a correlation between species tolerance to salinity fluctuation and intracellular structure changes. Thus, *D. salina* resists the extra sodium ions (Na+) in the surrounding environment to up to 2 M by ejecting them externally to maintain the potassium ions (K+) internally balanced and the accumulation of β-carotene was surprisingly increased in order to protect their photosynthetic apparatus. While freshwater *Chlorella* strain (*Chlorella Vulgaris)* showed successful adaptation and increased fitness to high salinity levels rather than the saline strain (*C. salina*) that decreased (Talebi et al., 2013).

Chlorella species are known for their green colour due to the high amount of chlorophyll in their cells which make up 1 to 4% of their weight (Shim et al., 2008). Other pigments like alpha and beta carotenoids, neoxanthin, violaxanthin, zeaxanthin and lutein pigments are commonly found in their cells, except some keto-carotenoids like canthaxanthin and astaxanthin are only found in certain *Chlorella* sp. (Ip and Chen, 2005). *Nannochloropsis* sp. is recognized as a great source of lipids and pigments. It contains a variety of pigments with commercial interests like chlorophyll-a, violaxanthin, vaucheraxanthin, zeaxanthin, canthaxanthin, astaxanthin, antheraxanthin and beta-carotene (Jorge et al., 2003; Neto et al., 2018).

Pigments extraction (chlorophylls and carotenoids) from microalgal species could be achieved by four methods, such as ultrasound-assisted extraction (UAE), ethanol as a co-solvent, supercritical fluid extraction (SFE), microwave-assisted (MAE), and conventional Soxhlet extraction (Poojary et al., 2016).These techniques are strongly dependent on the type of extraction, the solvent used, temperature and duration of the process. Thus, the novel extraction techniques (MAE and UAE) were shown to provide a higher yield and, in consequence, lower costs compared to traditional solvent extraction techniques like Soxhlet extraction and SFE (Fabrowska et al., 2017). These pigment extraction techniques were tested worldwide on *Chlorella* and *Nannochloropsis*, in which these two species are common in the Arabian Gulf waters. However, in the Arabian Gulf region pigments extraction for these two strains yet to be done.

Marine microalgae use

Microalgae have the ability to utilize several compounds, including pesticides, hydrocarbons, endocrine disruptors, and cyanides as carbon and nitrogen sources. Microalgae are known for their cell wall that is made up of

carbohydrate structures, which helped in the bio-absorption of toxic chemical agents in wastewater. For that reason, microalgae were used to reduce several compounds including nitrogen, phosphorus, heavy metals, and other pollutants in wastewaters (Hammad et al., 2016).

Wastewater treatment: *Chlorella* is known for the production of various organic macromolecules of interest (proteins, lipids, starch, carotenoids, minerals, vitamins, and pigments) that need a specific technique to be targeted and producing biomass (Safi et al., 2014). Among *Chlorella* species, *C. vulgaris* is the fastest-growing microalgae that can uptake certain nutrients like nitrogen and phosphorus from the surrounding waters, thus it was used in wastewater treatment plants (Kim et al., 2010). Many studies demonstrated that *C. vulgaris* has two cultivation modes: autotrophic and heterotrophic, and in the two modes can be used in wastewater treatment (Wang et al., 2009). A research done by Ansari et al., (2016) showed that *Chlorella vulgaris* showed a higher removal rate of total nitrogen (TN), alkalinity and chemical oxygen demand (COD) compared with other species. The removal rate for TN, alkalinity, and COD in the primary treated wastewater was 54, 24-28 and 78-82%, respectively. And the removal efficiency increased in the secondary wastewater, allowing the treated wastewater to be used for other industrial processes (Ansari et al., 2016). In addition, the productivity of *C.vulgaris* in municipal wastewater was noticed to increase under the elevated concentration of sodium hydrogen carbonate and higher light intensity until a certain value and then decreased. However, both cell biomass and lipid productivity increased under long exposure to daily illumination (Ebrahimian et al., 2014). Qatar municipal wastewater was successfully biotreated by *Chlorella* sp. and *Scenedesmus* sp. that showed high biomass yield and higher nitrogen and phosphorus uptake. Then, the microalgae biomass was used as a biofertilizer showing a noticeable increase in plant growth when compared with conventional inorganic fertilizer (Abdul Hakim et al., 2016). The manipulation of some microalgal species may help in enhancing wastewater treatment. This was supported by Ebrahimi et al. (2016) work that modified green algae *Cladophora sericioides* (harvested from the Arabian Gulf) with 3 mg/L L-cysteine resulting in increasing the copper adsorption from 65 to 95% with an initial concentration of 20 mg/L copper from the wastewater. Thus, bio-treatment by microalgae becomes an attractive process because of their photosynthetic capabilities, converting solar energy into useful biomasses and mitigating eutrophication by integrating nutrients like carbon, nitrogen and phosphorus.

Oil spills bioremediation: Seawater contamination with oil spills is a serious problem that attracted the attention of many environmental organizations due to their negative effect on the marine ecosystem (Roy et al., 2014). Such a problem if not treated very fast, it will cause the death of millions of organisms in seawater (Gu et al., 2003; Sverdrup et al., 2002). This problem will be mainly a concern to oil-producing countries such as those in the Arabian Gulf region (Dashti et al., 2018), in which oil wastes input in the seawater were continuously increasing because of chronic and careless habits in the use of oil and oil products (Pashaei et al., 2015). The biogeochemical process in the seawater increases the availability of hydrocarbons to benthic organisms as well as organisms in the water column through the sediment-water interface (Perelo, 2010). Hydrocarbons consist of carcinogenic compounds like polyaromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), which become a serious problem when they enter the food-chain (IARC, 1983). Therefore, bioremediation was the best technique to restore the natural environment and treat the contaminated area including water, soil and subsurface materials (Rosenberg, 1993). Bioremediation by microalgae was under concern especially in the Arabian Gulf region, and several tests were done on certain common microalgal strains to enhance their hydrocarbon degradation activity. A study was done on two microalgal species (*Scenedesmus obliquus* and *Chlorella vulgaris*) showed their biodegradation rate under heterotrophic conditions of 0.5, 1, 1.5 and 2% crude oil. The results show that *S. obliquus* has the highest growth rate at 0.5% crude oil, while *C. vulgaris* was at 2% under the same heterotrophic conditions. And both algal species were able to degrade oil effectively at low concentrations of oil (El-Sheekh et al., 2013). Also, a significant increase in *C. vulgaris* biomass was noticed as the crude oil concentrations increased indicating their high ability of crude oil hydrocarbons remediation. And it was noticed that approximately 94% of the light and 88% of heavy compounds were degraded by *C. vulgaris* in 14 days (Kalhor et al., 2017).

Microalgae can work in a symbiotic relationship with bacteria by supplying oxygen and in return up-take carbon dioxide (Oswald, 1988). Furthermore, they produce biosurfactants and extracellular enzymes to enhance bacterial biodegradation of pollutants (Muñoz et al., 2003). They also can accumulate hydrocarbons and make it available for hydrocarbon-degrading bacteria (Radwan, 2005). Microalgal species (cyanobacteria) of *Synechococcus, Synechocystis, Pleurocapsa,* and *Dermocarpella* were found in Arabian Gulf to be associated with other heterotrophic bacteria capable of degrading hydrocarbon mainly belonging to *Caulobacter, Acinetobacter* and *Pseudomonas* genera. These microalga species accumulate different hydrocarbons in their cells from the surrounding environment, thus resulting in increasing the inter-thylakoid spaces, and as a result, they will contribute to hydrocarbon pollutants removal from this water body (Al-Hasan et al., 2001). A study on hydrocarbon-degrading *Marinobacter* isolates

supports the cyanobacteria-bacteria association, in which it was noticed that this hydrocarbon-degrading bacteria grow better and consume more oil (crude oil, *n*-octadecane and phenanthrene) in the presence of their cyanobacteria partner than in their absence. Thus, they play an important role in the bioremediation of oil spills in the Arabian Gulf waters (Al-Wahaib et al., 2016).

Aquaculture feed: Unicellular microalgae form the base in the food web in the marine environment and are traditionally utilized for aquacultural purposes. Recently, these unicellular organisms were introduced to the aquaculture industry in two forms, liquid paste or as a dry powder. Unicellular microalgae are carefully chosen according to their nutritional value, including high levels of protein, carotenoids, minerals, vitamins and fatty acids (Ben-Amotz et al., 2009).

The studies on microalgae in the Arabian Gulf as a feed source in aquaculture began in the late 70s (Higuchi, 1978), and commercial fish production began in Kuwait in 1992 (Bishop, 2002). James et al., (1989) tested the best local seawater microalgal species for their nutritional value with the best conditions to increase their growth and productivity to use as fish feed. First, they evaluated the nutritional quality of *Chlorella* and *Nannochloropsis* species isolated from Kuwait's seawater under different subjected temperature. It showed that *Chlorella* strain MFD-1 have a higher growth rate in all tested temperatures from 15 to 35°C, and a clear increase in growth rate as the temperature increases than *Nannochloropsis* strain MFD-2. The total amino acid composition for both strains was the highest at 30°C with similar patterns in the amino acid profile. And the total essential w3 highly unsaturated fatty acids (HUFA) content in *Chlorella* strain MFD-1 increased when the temperature decreases. On the other hand, the total w3 HUFA in *Nannochloropsis* strain MFD-2 increased when the temperature increases up to 25°C, showing that, the thermophilic strains (*Chlorella*) are more productive than mesophilic strains (*Nannochloropsis*) of algae even under controlled conditions (James et al., 1989). Then another study was conducted by James and Al-Khars (1990) to test the growth and the productivity of these 2 strains under intensive continuous culture system using tubular photobioreactors. The results showed that *Nannochloropsis* Strain MFD-2 is more suitable for aquacultural purposes than *Chlorella* Strain MFD-1 because the total w3 HUFA and the essential fatty acid eicosapentaenoic acid (EPA) content were significantly higher ($P < 0.001$) in the former since EPA is mandatory for the feeding of marine fish larvae. Recently, *D. salina* is used in shrimp and fish aquaculture in Kuwait. This species was chosen because it has the ability to thrive under Kuwait's water harsh environment (high temperature, light intensity, and salinity) and due to its nutritional value (Abu-Rezq et al., 2010a, b). However, local halotolerant *Nannochloropsis* sp. was selected as a fish feed in Qatar due to its richness in various compounds that provide vital nutrition to the fish and ability to thrive under harsh environmental conditions such as high temperature, light intensity, evaporation rate and salinity (Das et al., 2015).

To enhance the production of certain shrimp species of economic value in the Arabian Gulf (Al-Harbi and Dimaano, 2010), a study on three microalgal species (*D. tertiolecta*, *T. suecica* and *N. oculata*) showed that *D. tertiolecta* was the best feed for the brine shrimp *Artemia urmiana.* It enhanced the length growth, survival rates and reproduction outcomes of this shrimp species rather than the other 2 tested microalgal species. The mean growth rate of the shrimp was 5.171 mm and the mean survival rate was 90% at day 8, and *A. urmiana* produced more cysts and nauplius when fed on *D. tertiolecta* with a mean of 12.87 cysts and 8.36 nauplius during the experiment period (20 days). Moreover, this was due to the high nutritional value of *D. tertiolecta* that comprises high lipid, carbohydrates, proteins, and *β*-carotene pigment (Mohebbi et al., 2015).

CONCLUSION

This review focus on the most common micro- and macroalgal species found in the Arabian Gulf region and how the Arabian Gulf counties can benefit from such resources in different fields. This is demonstrated by reviewing the most recent studies on the micro- and macroalgal species isolated from Arabian Gulf waters. Arabian Gulf waters are rich in the algal community that can be used as biofuel, food and feed resources due to their richness in protein, lipids, carbohydrates, and other essential elements. Also, the recent studies showed the cytotoxicity and the anti-oxidant activity of the most common algae in the Arabian Gulf that can be used in different pharmaceutical industries. Although there are several ongoing studies investigating the use of micro- and macroalgal communities in the regional waters to enhance healthcare, ecology, and economy of the surrounding countries, still further research needed to benefit from these organisms. Still, until now, there was no large commercial-scale operation in any of the Arabian Gulf countries. Recently, Arabian Gulf counties had set a five years plan, started from 2018 for finding an alternation eco-friendly fuel (biofuel) using microalgae, as mentioned in the "Regional workshop on production of biofuel and value-added products from microalgae" that was done in Kuwait at November-2018. Arabian Gulf countries must focus now on the commercial use of these algal species in a large scale, to help increase the economy of the country since the continuous use of fossil fuel causing many environmental problems.

Future areas of research will need to focus also on bioremediation activity by the current microalgal community, algal carotenoid pigments extraction such as

lutein and astaxanthin pigments that were poorly studied in this region, although they have an important value in different industries. In addition, more studies are needed to focus on increasing the productivity of lipids and pigments of common microalgal species under certain stresses and essential nutrient deprivation. Also, the bioactivity of lipid contents requires more attention to evaluate their acitivity specially those with algicidal activity that can be used against HABs, which is a serious problem in the Arabian Gulf that frequently occurs around the year. That can affect fisheries and the economy of the surrounded countries.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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APPENDIX

Pictures showing the common microalgal (seaweeds) species in the Arabian Gulf region.

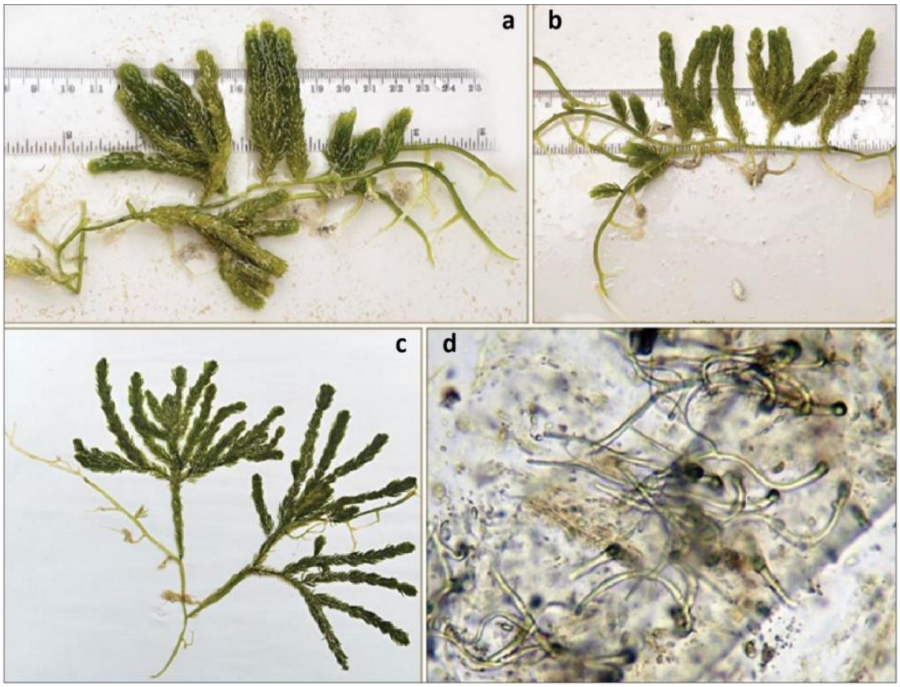

Figure 1. *Caulerpa sertularioides* (S.G.Gmelin) M.Howe: a-c – views of the whole plants; d- firm wall, braced internally by a system of trabeculae.
Source: Al-Yamani et al. (2014).

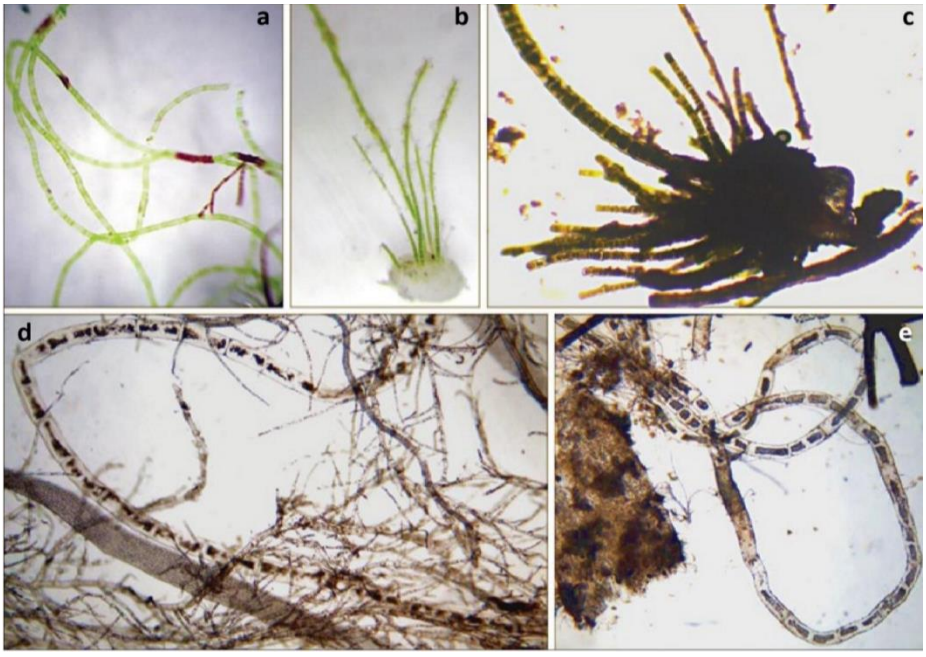

Figure 2. *Chaetomorpha crassa*: **a-** fragment of the thallus under microscope; *Chaetomorpha indica:* **b**- views of the whole thallus; c-fragment of the thallus under microscope; *Chaetomorpha linum:* **d,e** – fragments of the thallus under microscope.
Source: Al-Yamani et al. (2014).

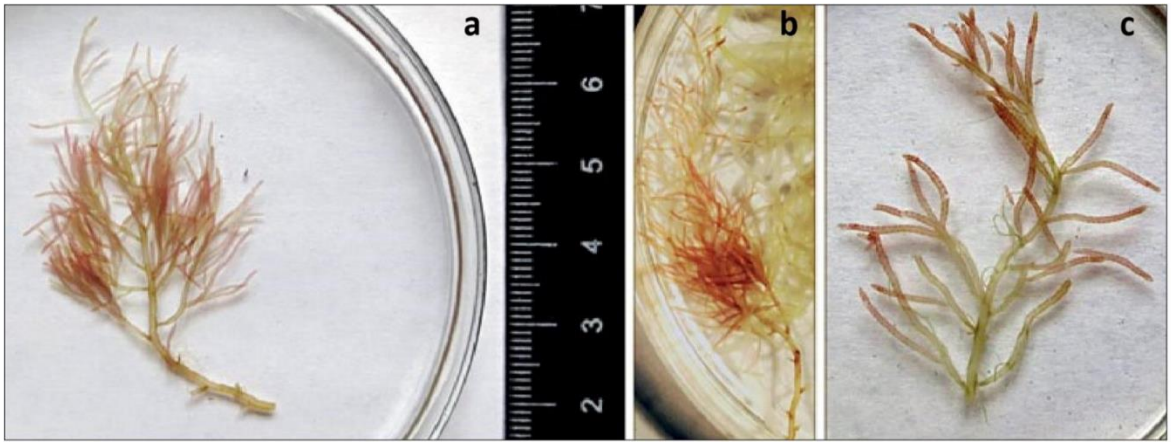

Figure 3. *Champia parvula*; **a,b** – views of the thallus; **c** – fragment of the thallus.
Source: Al-Yamani et al. (2014).

Figure 4. *Colpomenia sinuosa*: a-d – Thallus in the intertidal zone; e- view of the thallus; f- cross section with plurilocular sporangia.
Source: Al-Yamani et al. (2014).

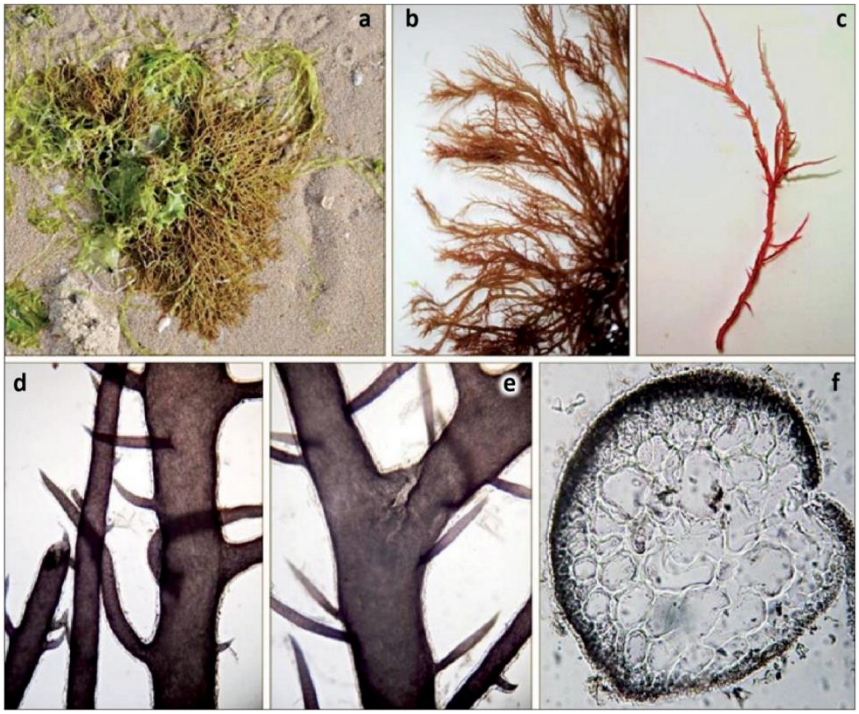

Figure 5. *Hypnea valentiae*: **a** – the thallus washed up on the beach; **b** – view of the thallus; **c** – fragment of the thallus; **d,e** – fragments of the thallus with short spiniform ramuli; **f** – cross section of the thallus. Source: Al-Yamani et al. (2014).

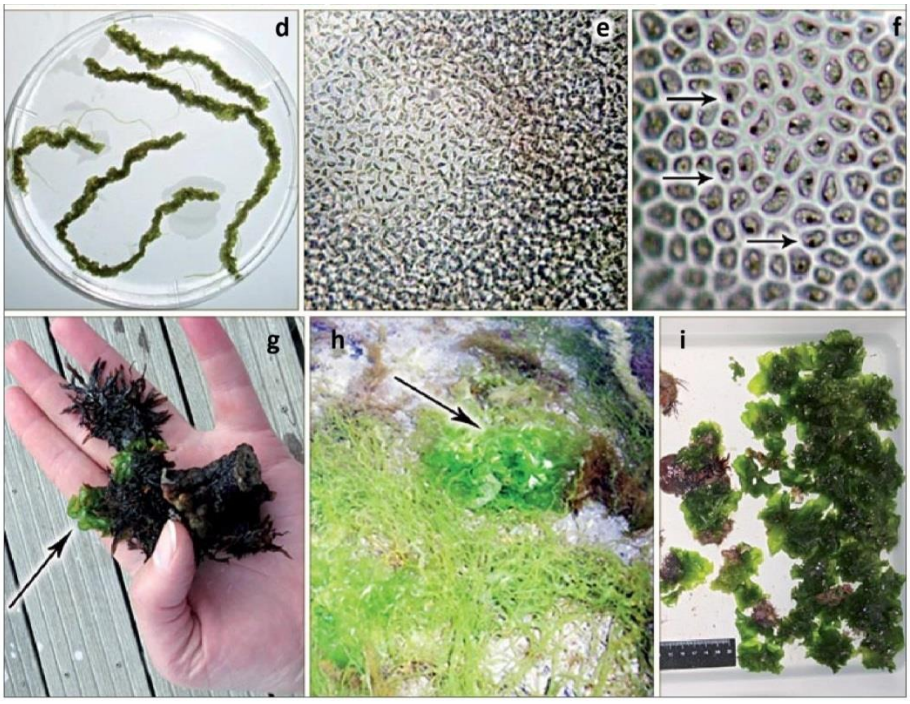

Figure 6. *Ulva intestinalis*: a-d – views of the wrinkled thallus; e – cup-shaped chloroplasts in margin of the cells; f – irregular arrangement of the cells on the surface of the thallus, cells include single pyrenoid (arrows); *Ulva lactuca*: : g,i – algae collected from the algal-fouling community of plastic pier; h – a mix *Ulva flexuosa* and *Ulva lactuca* (arrow) at the intertidal zone.
Source: Al-Yamani et al. (2014).

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Estimates of genetic variability, heritability and genetic advance for agronomic and yield traits in soybean (*Glycine max* L.)

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A study with 20 soybean genotypes was conducted in 2015 and 2016 to determine the genetic variability, heritability and genetic advance for some agronomic and yield traits in some soybean genotypes for selection criteria in a breeding programme. The field experiments were laid out in a randomized complete block design (RCBD) and replicated three times. Analysis of variance (ANOVA) revealed significant variation among the genotypes indicating that the planting materials were genetically divergent from each other. The estimates of genetic variability showed that phenotypic coefficient of variation (PCV) were higher than the genotypic coefficient of variation (GCV) for all the traits. Portraying the importance of environmental factors in the variations shown. High value of PCV and GCV were observed for traits such as plant height, number of leaves, number of pods and seed yield indicating the presence of sufficient genetic variation for selection in these traits. High heritability coupled with high genetic advance observed for plant height and number of pods suggests that selection could be effective for these traits.

Key words: Genetic advance, heritability, soybean, traits, variability.

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is one of the most valuable and most widely cultivated crops among the grain legumes. It has the highest protein content (38-42%) and edible oil (18-25%), therefore generally viewed to be one of the most foremost pulses and oil seed crops. Soybean occupies a pivotal place in Nigeria and Sub-Saharan Africa agriculture as a result of the insatiable demands for cheap source ofprotein from food and fodder. Soybean as a leguminous crop also has the capacity for soil fertility replenishment particularly in the Guinea Savanna (Yusuf et al., 2006), since it is able to fix approximately 300kg Nha^{-1} of nitrogen from the atmosphere into the soil (Keyser and Li, 1992). Soybean leftovers such as haulms so obtainedalso help in improving the soil condition of the farm, as on decay it supplies nutrients to subsequent crops more especially in a crop rotation system.

The improvement of genetic architecture of any crop is

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determined by the magnitude of genetic differences in a population ready to be taken advantage of and the extent to which the desirable traits are passed from one generation to the other (Tiwari et al., 2011). Variability can be defined as the availability of differences among the individuals of plant population. Variation usually arises as a result of differences either in genetic makeup of the individuals of a plant population or in the environment in which the plants are grown (Kavera, 2008). The existence of genetic variability is essential for performance of selection in any breeding programme. Selection as a breeding method will be meaningful if there is an appreciable quantity of genetic differences within the various genotypes used in the breeding programme. The selection of potential candidates among a particular germplasm, making use of them in the hybridization programme and subsequently picking the outstanding segregants in the segregating population is the usual breeding method in a highly self-pollinating crop like soybean.

Heritability estimates reveal the extent of transmission of quantitative traitssuch as plant height and seed yield from one generation to the other, as continuity in performance of selection is based on the transmittable aspect of the differences. Estimate of heritability help the breeder to effectively assign the necessary strategies to be adopted for a successful selection of the desired traits and to achieve the highest genetic gain within the shortest possible time and resources (Patil et al., 2015). Broad sense heritability is estimated using the total genetic variance. Researchers have observed that traits with higher heritability can be more easily manipulated by selection and breeding compared to traits with lower heritability.In the same manner, genetic advance is also a useful tool in forecasting the gain to beina specified selection intensity. However, when genetic advance is considered along with heritability, it becomes a more important measure in predicting responses to selection than the heritability estimates alone (Johnson et al., 1955). Grain yield is the most important trait in breeding soybean, depending on both the phenotypic potential and environmental conditions. Grain yield being a complex trait comprised of aspects of quantitative characters, whose expression is measured by the interaction of the genetic and environmental factors. This means that variability of quantitative trait is caused by genetic variability, environmental variability and variability of their interactions (Bosand Caligari, 1995; Soldati, 1995). Therefore, this study was undertaken to estimate the extent of genetic variability and heritability in soybean for effective selection in a breeding programme.

MATERIALS AND METHODS

The experimental material for the study comprised of twenty (20) soybean genotypes: Five (5) from the International Institute for Tropical Agriculture (IITA), Ibadan and fifteen (15) from various locations in Nigeria. The research was conducted during a two-year period (2015 and 2016) from July to November of each year at the experimental field of theDepartment of Crop Science, University of Nigeria, Nsukka (Lat. 06° 52' N, long. 07°24' E and 447.26 masl) which is located in a derived Guinea Savanna Zone. The soil type of the study area was sandy clay loam and of acidic pH (4.9).The experiment was laid out in a randomized complex block design (RCBD) in three replications on a plot size of $4m^2$ and the soybean seeds were planted at the spacing of 15cm between stands and 30cm between rows. Acceptable management and cultural practices of soybean were carried out as required in each year of the trial. Data were collected in five plants that were randomly selected on days to 50% flowering, plant height, number of leaves, number of pods, number of branches, pod weight (g) and seed yield (g).The data collected were subjected to analysis of variance (ANOVA) using the generalized linear model (GLM) procedure of Statistical Analysis System (SAS) and significant means were compared using Least Significant Difference (LSD)at 5% level of probability. Phenotypic and genotypic coefficients of variation were estimated as per the formulaprescribed by Burton and Devane (1952).

$$GCV = [\delta^2 g / \bar{X}] \times 100$$

$$PCV = [\delta^2 p / \bar{X}] \times 100$$

Where; GCV = genotype coefficient of variation; PCV = phenotype coefficient of variation; $\delta^2 g$ = genotypic standard deviation; $\delta^2 g$ = phenotypic standard deviation; $\bar{X}$= population mean

Heritability in broad sense was calculated using the formula given by Singh and Chaudhary(1985) and the expected genetic gain was calculated using the procedure outlined by Johnsonet al. (1955).

$$h^2 b\ (\%) = [\delta^2 g / \delta^2 p] \times 100$$

where; $h^2 b$ = heritability in broad sense; $\delta^2 g$ = genotypic variance; $\delta^2 p$ = phenotypic variance;

$$\text{Genotypic variance, } \delta^2 g = [MSG - MSE] / r$$

where; MSG = mean sum of square for genotype; MSE = mean sum of square for error; r = number of replicate; $\delta^2 g$ = genotypic variance

RESULTS

Genotypic differences were highly significant ($p<0.01$) for all the traits, indicating the considerable amount of variability (Table 1). The mean performance of the genotypes indicates that the genotype *Vom* had the maximum mean values for days to 50% flowering, plant height, number of leaves and number of branches. However, the genotype *Ashuku* recorded the maximum pod weight and seed yield (19.4 and 13.0 g, respectively). TGX1485-ID was the earliest to attain 50% flowering while *Vom* was the last to attain 50% flowering and was comparable with the genotype, Lau. In general, the genotype *Agbonkagoro* recorded the minimum mean values for most of the traits measured as revealed in Table 1. In the present study, the genotypes *Ashuku*, *Mangu* and *Akwanga* gave significantly higher seed yield

Table 1. Mean values of agronomic and yield traits in soybean.

Genotype	DF	PH (cm)	NL	NB	NP	PW (g)	SY (g)
AgbonKagoro	45.2	28.5	**57.3**	**2.8**	**27.8**	**9.9**	**6.1**
Akwanga	43.8	32.3	65.2	3.2	50.4	18.4	12.1
Andaha	47.4	**28.5**	67.9	3.2	38.2	13.5	8.5
Ashuku	44.8	36.3	76.5	3.5	57.5	**19.4**	**13.0**
Dadinkowa	46.0	31.5	71.9	3.1	32.2	13.0	8.6
Garkawa	44.3	34.0	73.2	3.3	40.5	13.8	9.2
Gwantu	44.8	33.8	70.9	3.8	41.3	14.2	9.3
Kafanchan	44.0	30.9	68.7	3.1	38.5	14.9	10.0
Kagoro	45.2	35.5	74.7	3.7	46.1	16.0	11.2
Langtang	46.7	32.0	89.5	3.6	43.6	17.7	11.8
Lau	51.0	34.8	81.8	3.7	54.7	17.6	11.6
Mangu	44.3	35.7	85.9	3.5	52.2	19.0	12.5
Mararaba	45.0	34.1	73.1	3.5	45.0	15.4	10.2
TGX1485-ID	**42.3**	30.8	78.3	3.7	40.3	14.8	9.6
TGX1448-2E	42.8	34.9	71.1	3.5	45.4	14.4	9.5
TGX1987-10F	44.0	33.0	69.2	3.0	37.0	15.7	10.1
TGX1835-10e	42.7	31.9	65.2	3.2	34.2	12.9	8.6
TGX1987-62F	47.3	37.1	72.8	2.9	43.7	14.6	9.7
Tiv Local	44.9	34.6	68.9	3.5	49.1	16.6	11.1
Vom	**51.1**	**50.4**	**109.6**	**4.5**	**63.9**	17.0	9.9
Grand mean	45.4	34.0	74.6	3.4	44.1	15.4	10.1
Range	42.3-51.1	28.5-50.4	57.3-109.6	2.8-45	27.8-63.9	9.9-19.0	6.1-12.5
LSD@5%	0.84	1.46	4.96	0.17	5.44	2.03	1.35
CV%	7.31	16.82	23.66	17.85	38.81	41.19	42.18

DF= days to 50% flowering, PH = plant height, NL = number of leaves, NB = number of branches, PW = pod weight, SY = seed yield.

Table 2. Analysis of variance for agronomic and yield traits.

Parameter	Mean	CV (%)	MS	Error
DF	45.37	9.19	46.52**	17.38
PH	34.02	13.09	101.99**	19.82
NL	74.57	23.65	955.27**	310.94
NB	3.38	17.08	1.15**	0.33
NP	44.10	34.53	1159.911**	245.41
PW	15.43	36.77	154.26**	32.17
SY	10.11	38.46	70.12**	15.13

DF= days to 50% flowering, PH = plant height, NL = number of leaves, NB = number of branches, NP = number of pods, PW = pod weight, SY = seed yield, CV = Coefficient of variance, MS = mean square.

per plant compared to the grand mean (10.1g), therefore, may be utilized for the improvement of soybean yield.

The analysis of variance (ANOVA) showed that mean square due to genotypes were highly significant ($p<0.01$) for all the traits under investigation (Table 2), indicating the enormous genotypic variability among the genotypes. This suggests that there is enough reasons for selection of traits from among the different sources of planting materials for yield and yield component traits.

The level of varietal differences present in the soybean genotypes were estimated on the basis of genetic parameters viz., genotypic and phenotypic coefficients of variation, heritability in broad sense and genetic advance coupled with different traits are presented in Table 3. A long range of differences was observed for all the traits. Phenotypic variance was greater than the genotypic

Table 3. Estimates of variability, heritability and genetic advance for the traits.

Trait	δ^2_p	δ^2_g	PCV	GCV	Hb^2 (%)	GA	GA (%)
DF	51.21	48.81	15.92	15.55	95.31	14.06	31.28
PH	85.58	79.46	27.10	26.11	92.85	17.69	51.82
NL	527.72	392.50	27.93	24.09	74.38	13.90	16.90
NB	0.46	0.35	22.69	16.30	76.09	1.07	24.36
NP	323.38	249.27	32.67	28.68	77.08	28.55	51.86
PW	26.20	16.41	26.47	20.95	62.63	6.61	34.18
SY	11.60	6.59	27.07	20.41	56.81	3.99	31.72

DF= days to 50% flowering, PH = plant height, NL = number of leaves, NB = number of branches, NP = number of pods, PW = pod weight, SY = seed yield, δ^2_p = phenotypic variance, δ^2_p = genotypic variance, PCV = phenotypic coefficient of variation, GCV = genotypic coefficient of variance, Hb^2 = broad sense heritability, GA = genetic advance.

variance for the yield and yield component traits indicating the important effects of environment on these traits. The phenotypic variance and genotypic variance ranged from 0.46 and 0.35 for number of branches to 527.72 and 392.50 for number of leaves, respectively. Similarly, the estimates of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) showed that the values of PCV were higher than GCV for all the traits and this may be partly due to the interactive effect of the genotypes with the environment or other environmental factors influencing the expression of these traits (Table 3). PCV and GCV ranged from 15.92 and 15.55 for days to 50% flowering to 32.67 and 28.68 for number of pods, respectively. Moderate PCV (10-20) was recorded for days to 50% flowering whereas other traits showed high PCVs (> 20). The narrow difference observed between the PCV and GCV for days to 50% flowering indicated that this trait was less influenced by the environment. High magnitude of GCV observed in traits that includeplant height, number of leaves and pod weight indicates the presence of wide variation for these characters to be allowed for further improvement by selection of the individual traits.

With GCV only, it is impossible to ascertain the extent of differences which is heritable. Thus, the knowledge of heritability of a character helps the plant breeder in predicting the genetic advance for any quantitative characters and aid in exercising necessary selection procedure. In this current study, the highest heritability values were recorded for days to 50% flowering, plant height, number of pods, number of branches and number of leaves. The availability of high heritable variation of these characters would be useful to plant breeder in the improvement of these traits. Heritability in broad sense estimates coupled with genetic advance will be more effective and reliable in predicting the response to selection (Johnson et al., 1955). Heritability in broad sense involves both the additive and non-additive gene effects. High heritability in broad sensetogether with high genetic advance for the following traits; number of pods per plant and plant height suggests that selection can be effective for these traits based on phenotypic expression. High magnitude of broad sense heritability and low magnitude of genetic advance was observed for number of leaves which may be due to lack of genetic variability for that trait. In such a situation, advancement in this trait through usual selection may not be effective. However, recombination breeding and recurrent selection is advocated for improvement of such trait.

DISCUSSION

The highly significant variations for all the traits indicate that both the genotypes and the environmental factors had enormous effects on the agronomic and yield traits. This observation is in agreement with the findings of Adugna and Labuschgne (2003) who also observed highly significant variation in both agronomic and yield traits in cowpea. Similarly, Jandong et al. (2019) observed highly significant variation in most parameters among the soybean genotypes signifying the existence variability.The range of phenotypic variability was high for all the traits, indicating the different sources of the genotypes. A wide range of variability for different traits has also been reported by Narayanankutty et al. (2005). The highly significant mean squares due to genotypes signified the presence of variability within the genetic materials used for the research. The estimates of PCV and GCV showed that the values of PCV were higher than that of the GVC. This portrayed the importance of environment in the variation exhibited and confirms the results reported by Nath and Alam (2002). The closer difference between the PCV and GCV for days to 50% flowering expressed the little influence of environmental factors for that trait. This finding is in agreement with the report of Karnwal and Singh (2009). The low magnitude of PCV and GCV for days to 50% flowering is in agreement with the finding of Bangaret al. (2003) and Baraskaret al. (2014). High values of PCV and GCV for number of pods per plant observed in this present studyis in conformity with the result of Gohil et al. (2007) which

is supported by Baraskar et al. (2014).Similarly, Olayiwola and Soremi (2014) reported high estimates of PCV and GCV for number of pod per plant in cowpea.

The high values of phenotypic and genotypic coefficients of variation obtained for plant height in the present study is in conformity with the findings of previous researchers like Karnwal and Singh (2009) and Neelima et al. (2018). The highest heritability values were observed for days to 50% flowering (95.3%), plant height (92.9%), mean number of pods (77.1%), mean number of branches (76.1%) and mean number of leaves (74.4%). This finding validate the results of Baraskar et al. (2014) who reported high heritability estimates for plant height, days to 50% flowering, number of branches per plant and number of pods per plant in soybean. Similarly, Patel et al. (2016) also reported high heritability for plant height, number of branches per plant and number of pods per plant in cowpea. Some researchers also reported high magnitude of heritability for yield and its components in soybean (Bangar et al., 2003; Malik et al., 2006; Neelima et al., 2018). This indicates that selection based on phenotypic levels would be useful for the improvement of the traits by repeated mass selection or hybridization between selected genotypes, showing varying degree of variation for such traits. It should be noted that broad sense heritability only cannot be a true reflection of genetic gain that is expected from selection because it involves both the additive and non-additive aspects of the variability (Singh et al., 2013).

The importance of estimate of genetic advance in percentage is to ascertain the real development made by isolating outstanding genotypes for a given trait (Allard, 1960). In the study being reported, it was discovered that genetic advance as a percentage of the mean (GAM) for number of pods per plant was of relatively low heritability (77.1%) when compared with plant height (92.9%). Similarly, seed yield per plant had relatively low heritability (56.8%) comparable to GAM. This suggest that plant height and days to 50% flowering had greater non-additive variance and environmental influence compared with number of pods per plant and seed yield per plant per plant respectively (Pradeepkumar et al., 2001).According to Johnson et al. (1955), high heritability estimates in conjunction with high genetic advance is indicative of additive gene action and selection based on these parameters would be more effective and reliable.

Conclusion

In the present investigation, the analysis of variance revealed the enormity of phenotypic variability that exists among the genotypes. On the basis of mean seed yield performance, the genotypes *Ashuku*, *Mangu* and *Akwanga* exhibited high seed yield per plant. Estimates of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) indicated that the values of PCV were greater than those of GCV, revealing the significant effect of environmental factors. Estimates of heritability were shown to be high for days to 50% flowering plant height, mean number of pods, mean number of branches and mean number of leaves. However, high magnitude of genetic advance was observed for number of pods per plant and plant height whereas pods weight per plant exhibited moderate genetic advance. High heritability estimates coupled with high genetic advance expressed as percentage of mean were observed for plant height and number of pods per plant, could be ascribed to the predominance of additivegene effects and high selective index and thus, possibilities of effective selection for improvement of these traits.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effects of *Ambrosia maritima* (Damsissa) ethanolic extract on phenylhydrazine hydrochloride-induced anaemia in rabbits (*Lepus cuniculus*)

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This work was performed to evaluate the anti-anaemic activity of the ethanolic extract of *Ambrosia maritima* (Damsissa) in phenylhydrazine hydrochloride (PHZ)-induced anaemia in rabbits. Twenty-five adult rabbits of different sexes were equally divided into 5 groups. The induction of anaemia was performed by subcutaneous administration of PHZ at a dose of 30 mg/kg body weight and maintained a dose of 15 mg/kg body weight. Ethanolic extract of *A. maritima* was orally administered to the groups at different dose rates (250, 500 and 1000 mg/kg body weight) and blood samples were collected at different intervals of time for heamatological examinations. The investigated haematological parameters included Hb concentration, total erythrocytes count (RBCs), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC). The results revealed a significant ($p<0.05$) decrease in Hb concentration, RBCs count and PCV as well as a significant increase in MCV and MCH in response to PHZ-treatment.No significant changes were observed in MCHC throughout the duration of the experiment.The data demonstrated that *A. maritima* ameliorated hemolytic anemia (anti-anaemic effect). Oral treatment with *A. maritime* extract did not demonstrate any toxic effect at the given doses as marked by hematological data.

Key words: Hemolytic anemia, phenylhydrazin, *Ambrosia maritima*, anti-anaemic effect.

INTRODUCTION

Medicinal plants, since time immemorial, have been used virtually in all cultures as a source of medicine. The widespread utilize of herbal remedies and health care preparations, as those described in ancient texts such as the Vedas and medicinal plants have been traced to the occurrence of natural products with medicinal properties (Akerele, 1988).

Anaemia is a condition in which the number of circulating red blood cells is decreased, either the amount of haemoglobin, or the volume of packed red cells

causing a reduction in the blood's ability to provide enough oxygen to body tissues and organs. Certain diseases, such as malaria, malnutrition, protozoal infections and physiological conditions such as pregnancy are among the various conditions that may lead to anaemia in both the adults and children. WHO epidemiological studies revealed that almost more than 1/4 of the world population suffered from anemia (Adusi-Pokuyet al., 2008).

Ambrosia maritima is a member of the family Asteraceae. It is known in Sudan as “Damssisa”, and is a widely distributed weed in Northern and Central Sudan especially near water catchment and Nile Bank (El Ghazali et al., 1994). Traditionally, the decoction of the whole plant is used to cure gastrointestinal disturbances, abdominal pain, kidney inflammation and renal colic, whereas the leaves are used for diabetes and hypertension cure. In addition, its curative properties are extended to include molluscicidal, antimalarial and antitumor activities (Diraret al., 2014).

As most plants of Asteraceae family, the plant is rich in sesquiterpene lactones, such as neoambrosin, ambrosin, and damsin which have molluscicidal and cytotoxic activities. In addition, this plant has been shown to contain several phytoconstituents such as coumarins, flavonoids, sterols and tannins, and exhibit considerable antioxidant activity (Said et al., 2018).

The current work aimed to investigate the effects of *A.maritima* (Damsissa) ethanolic extract on the Phenylhydrazine hydrochloride-induced anaemia and to determine the main active principles responsible for its anti-anaemic activity.

MATERIALS AND METHODS

Collection, identification and preparation of plant material

A. maritima shoots were collected from their natural habitat from the river bank of Elmatmma locality, River Nile State, Sudan. The plant was authenticated by Medicinal and Aromatic Plants Research Institute (MAPRI), Sudan. The plant material was cleaned from dirt, shade dried, and then ground into coarse powder.

Preparation of the ethanolic extract of *Ambrosia maritima*

Extraction was performed according to the method depicted previously by Sukhdev et al. (2008) as follows; 2000 g *A. maritima* shoots was ground using mortar and pestle and successively extracted by soaking in 80% ethanol for about 72 h with daily filtration and evaporation. Solvent was evaporated under reduced pressure to dryness using rotary evaporator apparatus and the yield weight was 138.5 g.

Proximate and chemical analysis of *A. maritima*

A. maritima shoots were analyzed for their proximate composition and mineral contents. The proximate analysis was done for crude fibre, crude protein, dry matter, ash, ether extract, and nitrogen free extract. The mineral contents include: sodium, potassium, calcium, magnesium and iron.

Phytochemical screening of *A. maritima*

Phytochemical analysis tests were carried out usingspecific standard methods. Ethanolic extract of *A. maritima* was divided into several portions for identification of tannins, terpenoids, cardiac glycosides, alkaloids, saponins, and anthraquinones (Boham et al., 1994; Debiyi and Sofowora, 1978; Harborne, 1973; Obadoni and Ochuko, 2002; Sofowora, 1993; Wagner and Bladt, 1996).

Test for tannins: 5 ml of bromine water was mixed with 0.2 g ethanolic extract. Decoloration of bromine water demonstrated the entity of tannins (Boham et al., 1994).

Test for terpenoids: 2.0 ml of chloroform was added to 5 ml of the ethanolic extract, evaporated on a water bath and then heated with 3 ml of concentratedH_2SO_4. The formation of a grey color is an indication of the presence of terpenoids (Debiyi and Sofowora, 1978; Sofowora, 1993).

Test for alkaloids: Crude extract (0.3 g) was added to 2 ml of concentrated HCl. A small amount of amyl alcohol was added to the mixture and filtered at room temperature. Few drops of Dragendorff’s reagent (solution of potassium bismuth iodide) were addedto the acid layer and a reddish-brown precipitate was observed (Harborne, 1973; Obadoni and Ochuko, 2002).

Test for cardiac glycoside (Kedde test): Part of the ethanolic extract (3 ml) was added to a small amount of Kedde reagent (Mix equal volumes of a 2% solution of 3, 5 dinitrobenzoic acid in menthol and a 7.5% aqueous solution of KOH).Appearance of a blue or violet color indicated the presence of cardinolids (Debiyi and Sofowora, 1978; Sofowora, 1993).

Test for saponins (Froth Test): Crude extract (0.5 g) was dissolved in 5 mL distilled water. The mixture was shaken vigorously and stable persistent froth was obtained (Wagner and Bladt, 1996).

Test for anthraquinones (Borntrager’s Test): Crude extract (0.5 g) was taken into the first test tube, and 5 mlchloroform was added while shaking for 5 min. The extract was filtered to the second test tube and shaken with an equal volume of 100% ammonia solution. The development of pink or red color in the ammonia layer (lower layer) indicated the presence of anthraquinones (Debiyi and Sofowora, 1978; Sofowora, 1993).

Experimental animals

Twenty-five apparently healthy adult rabbits (*Lepus cuniculus*) of different sexes weighing 0.9 to 1.7 kg were obtained from Omdurman Local Market, Sudan. The rabbits were identified by ear

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Table 1. Phytochemical screening of ethanolic extract of *Ambrosia maritima*.

Secondary metabolite	Results
Terpenoids	+
Alkaloids	-
Saponins	+
Anthraquinones	-
Cardiac glycosides	-

+, presence; −, absence.

tags, housed in cages in the Department of Nutrition, Faculty of Animal Production, University of Khartoum and maintained under standard environmental condition; controlled temperature, relative humidity with free access to water and *Medicago sativa* (Alfalfa) hay. The experiment in rats was done in accordance with the ethical principles in animal research, approved by the Committee for Ethics at Sudan Veterinary Council, Ministry of the Cabinet.

Experimental design

After adaption period for 30 days, the rabbits were weighed, and assigned randomly into five groups; A, B, C, D, and E (5 rabbits/each). Groups B, C, D and Ewere injected subcutaneously with a single dose of phenylhydrazine hydrochloride (30 mg/kg body weight)and with a maintained dose of 15 mg/kg body weight for 2 days after administration of the first dose. *A. maritime* ethanolic extract was administered orally to rabbits of groups B, C, Dat doses 250, 500 and 1000 mg/kg body weight/day, respectively starting from day 8. Animals in Group E served as phenylhydrazine control group while Group A served as control one (without phenylhydrazine and without *A. maritima*).

Blood samples collection

The area of blood sampling was shaved scrubbed by disinfectant (70% ethanol) before the jugular vein was punctured. The collection of blood samples was achieved at days 0, 7, 14, 22 and 30 of the experimental periods, for haematological analysis, which include: Hemoglobin concentration (Hb), Hematocrit (PCV), Erythrocyte count (RBCs), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC).

Statistical analysis

One-way analysis of variance (ANOVA) was utilized for the analysis of data. Duncan's multiple range test was used for determining the significance. A probability value of $p<0.05$ was considered as significant (Snedecor and Cochran, 1989).

RESULTS

Phytochemical screening

The results of phytochemical constituents of *A. maritime* ethanolic extract are demonstrated in Table 1. The phytochemical screening showed the entity of triterpenes, saponins and tannins as well asthe absence of alkaloids, anthraquniones and cardiac glycosides.

Proximate analysis

The proximate analysis of the *A. maritime* ethanolic extract demonstrated the presence of all the macronutrients (Table 2).

Mineral content evaluation

Table 3 exhibited that major trace elements and minerals are present in *A. maritima*, in relatively high concentrations. The highest mineral concentration (141.50 ppm) was that of potassium (K), mean while, the lowest concentration (9.55 ppm) was that of magnesium (Mg).

Haematology analysis

The haematological values of Hb, RBCs, PCV, MCV, MCH, and MCHC, are presented in Figures 1 to 6, respectively. The data did not show any significant differences among the experimental groupsat day zero of the experiment.At day 7, all groups except group A (negative control), ofthe haematological parameters revealed significant ($P<0.5$) reduction in the values of Hb concentration (Figure 1), RBCs count (Figure 2) and PCV (Figure 3); meanwhile, there were significant ($p<0.05$) increase in MCV (Figure 4) and MCH (Figure 5) in PHZ-treated groups compared to the negative control (Group A). However, these groups (B, C, D and E) did not exhibit any significant differences in MCHC (Figure 6) compared to the negative control (Group A).

At day 14, Groups B, C and D which were given *A. maritima* showed an increase in Hb concentration compared to Group E. Nevertheless, this increase was significant in Group D, in which the Hb concentration was not significantly different from that of Group A (Figure 1). The RBCs count also showed significant ($p<0.05$)

Table 2. Percentage proximate composition of *Ambrosia maritima.*

Macronutrient	Composition (%)
Crude protein	6
Crude fiber	27.51
Ether extract	1.42
Ash	13.47
Nitrogen free extract	45.28
Dry matter	**93.58**

Table 3. Mineral contents of *Ambrosia maritima.*

Minerals	Concentration (ppm)
Sodium (Na)	57.50
Potassium (K)	141.50
Calcium (Ca)	25.86
Magnesium (Mg)	9.55
Iron (Fe)	19.7

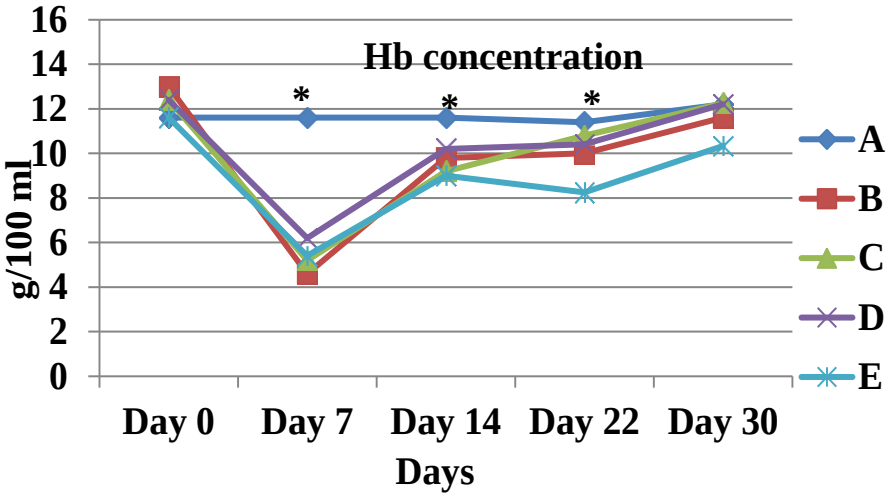

Figure 1. Effect of ethanolic extract of *Ambrosia maritime* on haemoglobin concentration (g/dl) in phenylhydrazine HCL (PHZ)-induced anaemic rabbits (Mean±SEM). A: Control without phenylhydrazine and without *Ambrosia maritima* extract. B: PHZ +250 mg *Ambrosia maritime* ethanolic extract/kg body weight. C: PHZ +500 mg *Ambrosia maritime* ethanolic extract/kg body weight. D: PHZ +1000 mg *Ambrosia maritime* ethanolic extract/kg body weight. E: PHZ.

increase in all the *A. maritima* treated groups (B, C and D) compared to that of group E. Noteworthy, the *A. maritima* treated groups did not demonstrate any significant differences in RBCs count when compared to Group A (negative control group). The PCV values increased in GroupsB, C andD to a levelthat was significantly higher than that of group E, but was not significantly different from that of group A. The MCV values decreased significantly in Groups B, C andD in comparison to that of Group E. MCV values in these groups were comparable to that in control group and did not show any significant differences compared to Group A. The MCH values in *A. maritima* treated groups decreased to a level, which was not significantly different compared to that of Group A. On the other hand, group E still showed significant rise in MCH value compared to

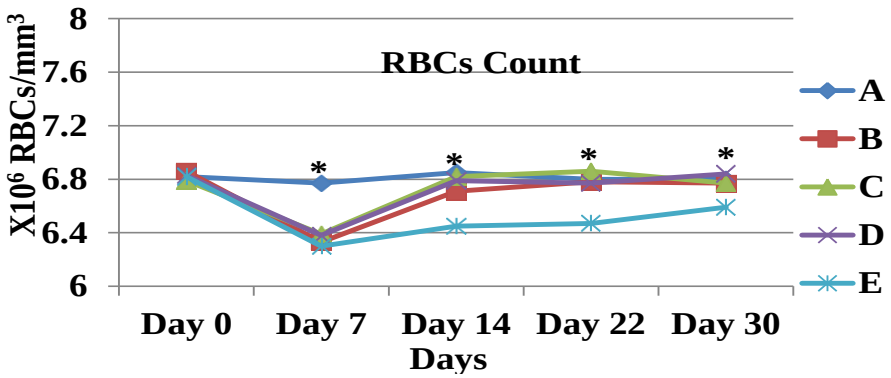

Figure 2. Effect of ethanolic extract of *Ambrosia maritime* on total erythrocyte count in phenylhydrazine HCL (PHZ)-induced anaemic rabbits (Mean±SEM). A: Control without phenylhydrazine and without *Ambrosia maritima* extract. B: PHZ +250 mg *Ambrosia maritime* ethanolic extract/kg body weight. C: PHZ +500 mg *Ambrosia maritime* ethanolic extract/kg body weight. D: PHZ +1000 mg *Ambrosia maritime* ethanolic extract/kg body weight. E: PHZ.

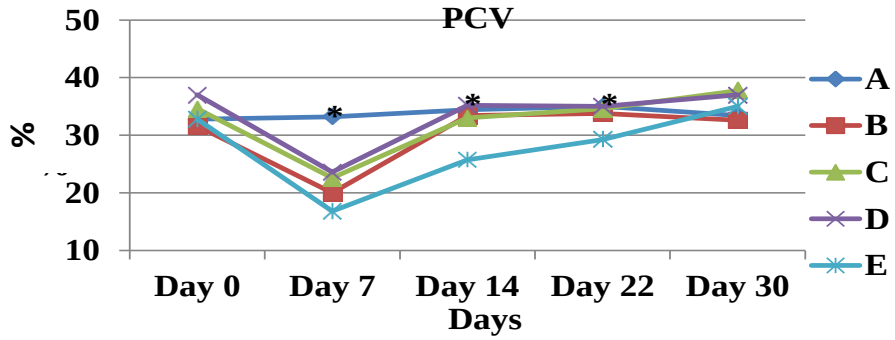

Figure 3. Effect of administration of ethanolic extract of *Ambrosia maritime* on packed cell volume (PCV %) in phenylhydrazine HCL (PHZ)-induced anaemic rabbits (Mean±SEM). A: Control without phenylhydrazine and without *Ambrosia maritima* extract. B: PHZ +250 mg *Ambrosia maritime* ethanolic extract/kg body weight. C: PHZ +500 mg *Ambrosia maritime* ethanolic extract/kg body weight. D: PHZ +1000 mg *Ambrosia maritime* ethanolic extract/kg body weight. E: PHZ.

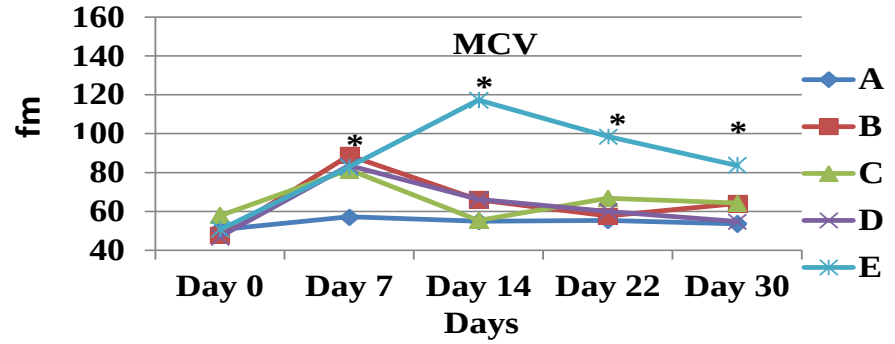

Figure 4. Effect of ethanolic extract of *Ambrosia maritime* on mean corpuscular volume (MCV) fm, in phenylhydrazine HCL (PHZ)-induced anaemic rabbits (Mean±SEM). A: Control without phenylhydrazine and without *Ambrosia maritima* extract. B: PHZ +250 mg *Ambrosia maritime* ethanolic extract/kg body weight. C: PHZ +500 mg *Ambrosia maritime* ethanolic extract/kg body weight. D: PHZ +1000 mg *Ambrosia maritime* ethanolic extract/kg body weight. E: PHZ.

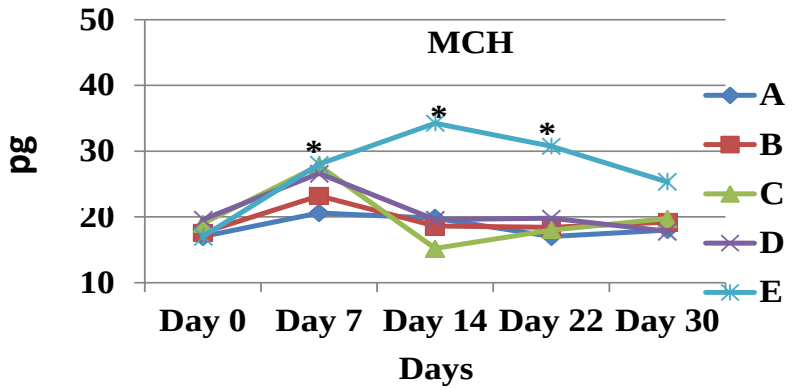

Figure 5. Effect of ethanolic extract of *Ambrosia maritima*on mean corpuscular haemoglobin (MCH) pg, in phenylhydrazine HCL (PHZ)-induced anaemic rabbits (Mean±SEM). A: Control without phenylhydrazine and without *Ambrosia maritima* extract. B: PHZ +250 mg *Ambrosia maritima*ethanolic extract/kg body weight. C: PHZ +500 mg *Ambrosia maritima*ethanolic extract/kg body weight. D: PHZ +1000 mg *Ambrosia maritima*ethanolic extract/kg body weight. E: PHZ.

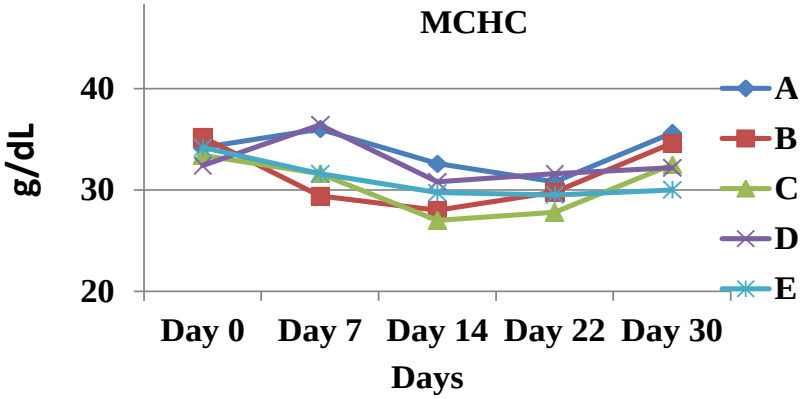

Figure 6. Effect of ethanolic extract of *Ambrosia maritima*on mean corpuscular haemoglobin concentration (MCHC) g/dl in phenyl hydrazine HCL (PHZ)-induced anaemic rabbits (Mean±SEM). A: Control without phenylhydrazine and without *Ambrosia maritima* extract. B: PHZ +250 mg *Ambrosia maritime* ethanolic extract/kg body weight. C: PHZ +500 mg *Ambrosia maritime* ethanolic extract/kg body weight. D: PHZ +1000 mg *Ambrosia maritime* ethanolic extract/kg body weight. E: PHZ.

that of the other experimental groups (A, B, C, and D). MCHC values at day 14 did not show any significant difference among all the experimental groups.

At day 22, no significant differences in Hb concentrations were noticed between the *A.maritima* treated groups (B, C, and D) and control group (Group A). However, the PHZ-treated group (group E) still showed significantly lower value compared with the control group (group A) and to the *A.maritima* treated groups, with the exception of group B (which was treated with 250 mg *A. maritima*). The same trend was noticed for RBCs count, the *A. maritima* treated groups did not show any significant difference compared to control group (Group A). However, the PHZ-treated group (Group E) showed significantly lower value of RBCs count compared to control group (A) and to that of all *A.maritima* treated groups (B, C, and D). PCV value was not significantly different from that of all experimental groups. On the other hand, MCV in *A. maritima* treated groups did not show any significant difference compared to control (A).Same trend was noticed for MCH; no significant differences were noticed between *A. maritima* treated groups and the control, meanwhile PHZ-treated group (E) showed significantly higher MCH compared to control group and *A. maritima* treated groups (B, C, and D). Moreover, all experimental groups (including control

one) did not exhibit any significant differences in MCHC when compared to each other.

At day 30, all experimental groups did not show any significant differences in Hb concentration, PCV, MCH, and MCHC. However, RBCs count in Group E (phenylhydrazin group) was still significantly lower compared to negative control group (Group A) and *A. maritima* treated groups (Groups B, C, and D). MCV value in group Ewas significantly higher compared to Groups A and D; however, it did not demonstrate any significant difference compared to Groups B and C. Noteworthy among the treated groups, Group D had MCV which numerically coincided with that in control group and significantly different from that in Group E.

DISCUSSION

The data of the proximate analysis of *A. maritime* (Table 2) showed that crude protein content of the extract was 6%which is comparable to those reported for *Grewia tenax* fruits (Ali, 2009).

On the other hand, the data of the proximate analysis demonstrated that the crude fibre content (Table 2) was higher than the determined fibre content of*G.tenax*fruits (Ali, 2009) and *Telfairia occidentalis* (Ogbe et al*.,* 2010).

This has been beneficial because food fibre has been documented to potentiatethe absorption of trace elements in the intestine and decreasecholesterol absorption (Le-veille and Sanberlich, 1966). This makes the plant suitable for combating anaemia.

The ash content is anindicator for plant's mineral content. The higher ash content (13.47%) indicated that the plant contains appreciable amount of mineral elements. The value was higher than that reported for *Jatropha tanjorensis* leaves (Iduet al., 2014) and *G. tenax* fruits (Ali, 2009).

Evaluation of the mineral content (Table 3) showed that *A. maritima* contains high values of iron. This is consistent with the results of Yagi et al. (2013) who reported that *A. maritima* leaves have higher value of iron (590 ± 1 ppm) compared to *G. tena x*(200 ± 4 ppm), which is used as anti-anaemic plant in Sudan.Iron is an essential element for human beings and animals for the synthesis of haemoglobin. It facilitates the oxidation of carbohydrates, proteins and fats to control body weight, which is very important factor in diabetes (Yagi et al., 2013).

On the other hand, *A. maritima* contains high level of calcium (Table 3). Calcium is the main constituent of the skeleton andis essential for regulating many vital cellular activities such as nerve and muscle functions, hormonal actions, blood clotting and cellular mortality (Yagi et al., 2013). Furthermore, the proximate analysis revealed high potassium content, which is essential for different physiological processes including enzyme activation and protein synthesis. Potassium also participates actively in the maintenance of the cardiac rhythm (Martin et al., 1985).

Haemolytic anaemia could be induced in rabbits after subcutaneous administration of phenylhydrazine hydrochloride at a dose of 30 mg/kg body weight with maintained dose of 15 mg/kg body weight of the same drug 2 days after the administration of the first dose (Prasong and Maitree, 1994; Ogbe et al*.,* 2010).

Phenylhydrazine hydrochloride has been earlier used to induce anaemia in rats (Bowman and Rand, 1980). These authors reported that anaemia was observed after 6 days of injection. The recovery from anaemia occurs at day 9. After six days of exposure, Phenylhydrazine was reported to cause the formation of Heinz bodies on RBC membranes (Bowman and Rand, 1980; Gordon-Smith, 1980). Akah et al*.* (2009) reported that oral administration of 10 mg/kg phenylhydrazine for 8 days reduces haematological indices by 50%. In further studies, phenylhydrazine was found to decrease haemoglobin concentration, red blood cell count and haematocrit (Agbor et al*.,* 2005; Berger, 2007).

In the present study, the anemia developed by phenylhydrazine was macrocytic hypochromic anaemia, which is common in haemolyticanaemia (Lee et al., 2014).

The effect of administration of *A. maritime* ethanolic extract at 250, 500 and 1000mg/kg body weight on haematological parameters in anaemic rabbits, clearly indicated that there were significant increase in the values of Hb, RBC and significant decrease in MCH, MCV values, which might indicate significant improvement of these parameters by *A. maritima* extract. Treatment of anaemia by plant extracts has been well documented by many research works. Oladijiet al*.*, (2007) reported that administration of aqueous extract of *Sorghum bicolor* stem bark results in significant increase in haemoglobin concentration and PCV. Similarly, Pawar et al. (2010) reported significant increase in erythrocytes count, haemoglobin concentration, leukocytes count and haematocrit in anaemic rats treated with *Asteracantha longifolia*. Moreover, Akahet al. (2009) demonstrated previously that the orally administration of *Brillantai sianitens* extract to PHZ-treated rats elevated Hb, RBCs count, and PCV within one week.

Nevertheless, the extract did not show any alteration in MCHC values throughout this study. Thus, the MCHC values remain in the normal level which may be further evidence for macrocytic anaemia or due to releasing of reticulocytes. Similar result has been reported by Ali (2009) in the treatment of hemorrhagic anaemic rats with *Azanza garckeana* aqueous extract.

Furthermore, the PCV values increased in GroupsB, C and Dto a level which was significantly higher than that in Group E, but was not significantly different from that in Group A.

Nevertheless, a significant reduction in Hb and RBCs count in response to intramuscular injection with *A.*

maritima extract and feeding *A. maritima* to chicks in diet has been reported (Bakhiet and Adam, 1996; Azza, 2003). The discrepancy between the present results and the previous ones might be due to source of the plant, method of extraction, route of administration and animal species used; however, the oral route is the most common in folk medicine. Noteworthy, oral administration of water extract of *A. maritima* has been found to increase RBCs count and Hb concentration significantly in anaemic rats (Azza, 2003).

Conclusion

The study concluded that *A. maritime* ethanolic extract showed anti-anaemic effect on phenylhydrazine induced anaemia in rabbits and this was clearly proved by increasing the hematological values.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Descriptive sensory evaluation of "gari" produced from fermentation of cassava using some selected *Rhizopus species*

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Two local varieties of cassava (*Manihot esculenta* crantz) pulps named "okoiyawo" and "odongbo" were fermented with pure *Rhizopus oligosporus, R. nigricans and R. oryzae.* The products were subsequently processed in to "gari" (a roasted fermented cassava meal), the form in which it is popularly consumed in Nigeria. The selected process variables are cassava varieties, starter culture and fermentation time (2, 4 and 6 days). Fifteen randomly selected and trained panelists in the University carried out descriptive sensory analysis which characterized color, taste, flavor, smoothness, moldability and consistency of "gari" and "eba". The sensory evaluation of the samples showed that the creamy-white is the most dominant color. A peculiar flavor, fine, and sour "gari", consistent and moldable "eba" was also obtained. The optimum fermentation time for sensory quality is 48 h (2 days). The samples produced from "odongbo" are of good quality than the ones produced from "okoiyawo". The selected *Rhizopus species* had a significant effect on the taste, flavor, and moldability of "eba". There were significant differences ($p<0.05$) in the consistencies of the "eba" samples. The highly consistent ones are "odongbo" products while the ones made from "okoiyawo" are mildly consistent. There were no significant differences ($p<0.05$) in the moldability of "eba" samples.

Key words: Gari, Sensory evaluation, *Rhizopus* species.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) belongs to the family Euphorbiaceae. It is a major carbohydrate staple crop in Nigeria, engaging over four million farmers in production and providing food for over 100 million persons (FAO, 2016). The consumption of cassava (*Manihot esculenta* Crantz) have been on the increase and planting of cassava has expanded to areas where cassava was not cultivated many years ago (Ogunnaike et al., 2015). It is one of the most important staple food crops grown in the tropical Africa, and plays a major role in effort to alleviate the African food crisis because of its efficient production of food energy, year-round availability, tolerance to extreme stress conditions, and suitability to present farming and food system in Africa (FAO, 2016)

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and can be processed into various forms such as gari, fufu and tapioca. Cassava tuber is known to have low protein but rich in vitamin C (35 mg per 100 g fresh weight), and contain traces of niacin, thiamine, riboflavin and vitamin A (Ehirim, 2018; Sineenart et al., 2018).

Solid state fermentation has been used to detoxify cyanide content of cassava and improving its protein values. The most commonly used microorganisms in Solid State fermentation (SSF) are filamentous fungi (Adejuyitan et al., 2019). Filamentous fungi are the most important group of microorganisms used in SSF processes due to their physiological, enzymatic and biochemical properties. Among the microorganisms most used are those belonging to the genus *Rhizopus*, which have been used for the production of products such as Tempeh, due to the organoleptic and nutritional characteristics that it provides to the product (Olaoye et al., 2015).

Several non-pathogen micro-fungi such as Rhizopus species were used in enriching cassava product with protein. Londono-Hernandez et al., (2018) reported the effect of *Rhizopus oryzae* on the chemical composition, physical-structural characteristics and nutritional value of sorghum grains. He found out that physical structure of the sorghum starch granules was modified during the fermentation process due to the action of the enzymes, mainly amylases, produced by the microorganism. The fermentation process improved the nutritional characteristics of the sorghum. Fermentation also improved the sensory properties of cassava products such as “gari”, fufu etc.

Gari (a roasted fermented cassava meal) is the most popular cassava product consumed in West Africa and the most important food product in the diet of millions of Ghanaians and Nigerians (Ehirim, 2018). The process of ’’gari’’ production from cassava includes fermentation which may last between one to five days, depending on the region where it is being produced. The longer the time of fermentation, the more desirable its sensory characteristics and the more appealing to the customer. “Gari” has a slightly sour taste and it could be white or cream depending on the variety of cassava used and the processing method adopted. The objective of this study was to determine the sensory characteristics of “gari” and “eba” produced from fermentation of cassava using *R. oligosporus, R. oryzae and R. nigricans* at different time (2, 4 and 6 days). And also, to determine the effects of processing variables on sensory attributes of the samples.

MATERIALS AND METHODS

Raw material sourcing

Manihot utilissima and Manihot palmata (that is, odongbo and okoiyawo) were obtained from Ladoke Akintola University of Technology (LAUTECH) farm, Ogbomoso, Oyo State. The roots were between 10-12 months old. Pure culture of *R. oligosporus, R. oryzea, and R. nigricans* were obtained from the microbiology laboratory of Department of food Science and Engineering, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

Production of “gari”

Freshly harvested cassava roots were peeled, washed and grated. The mash was then sterilized for 30 min according to the method described by Oguntimein et al., (1994) using ultraviolet (u.v) sterilizer. 0.5 mL of aliquot of each culture suspension (*Rhizopus oligosporus*, *R. oryzae*, and *R. nigricans*) in nutrient solution as described by Oboh and Akindahunsi (2003) were used as inocula for every 100 g of sterilized cassava mash. The mash was allowed to ferment for 2, 4 and 8 days. The water content in the mash was removed using a screw press. The resulting cake was finally sieved and fried to “gari”. Control sample were also made for each cassava variety without the use of the microorganisms fermented for 3 days. The samples were stored prior to the analyses.

Sensory analysis

Descriptive method

The sensory evaluations were conducted by 15 trained panelists consisting of students who were experienced with the products and terminology. During orientation sessions, the panelists were provided with a glossary of terms and questionnaire which was developed and used throughout the study. Sensory characteristics of the sample were divided into four main categories that included 13 sensory attributes: Color: white, creamy-white, yellow, brown; smoothness: fine, coarse, small lumps, big lumps; flavor: intense “gari” flavor, mild “gari” flavor; taste: strongly sour, slightly sour and bland. The samples were arranged randomly and presented to the panelists in white paper cups to evaluate the aforementioned sensory attributes and indicate the level that describe their perception. Each sample was duplicated and coded in such a way that the panelists could not be biased by the coding system as a set of three digits of random numbers were assigned to each sample. Water was provided to each panelist for use in rinsing mouth between samples. The “gari” samples were then poured in boiling water (gari/water ratio 1:3), covered for a while and turned to “eba” using local turning stick and assessed for the same attributes stipulated above with these exception: Small lumps, big lumps for smoothness. Consistency: Highly consistent, moderately consistent, not consistent and moldability: moldable, not moldable were also examined in “eba” made from the “gari” sample.

Statistical analysis

Data obtained from the panelists were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS) at 5% significant level. The mean values of the samples were separated using alphabetical letters to indicate significant difference between the samples.

RESULTS AND DISCUSSION

The descriptions of sensory quality attributes of “gari” and “eba”.

The descriptions of sensory attributes of “gari” and “eba” produced from fermentation of cassava using some

Table 1. The description of sensory attributes of "gari" produced from fermentation of cassava using some selected *Rhizopus species.*

Sample	Color	Smoothness	Flavour	Taste
ODL2	Creamy-white	Coarse	Mild gari flavor	Strongly sour
ODL4	Creamy-white	Fine	intense gari flavor	Strongly sour
ODL6	White	Fine	Mild gari flavor	Slightly sour
ODN2	Creamy-white	Coarse	Mild gari flavor	Strongly sour
ODN4	Creamy-white	Coarse	Mild gari flavor	Strongly sour
ODN6	White	Fine	Mild gari flavor	Bland
ODZ2	Creamy-white	Coarse	Mild gari flavor	Slightly sour
ODZ4	Creamy-white	Coarse	Mild gari flavor	Strongly sour
ODZ6	White	Fine	Mild gari flavor	Bland
ODCT	Creamy-white	Fine	Mild gari flavor	Bland
OKL2	Creamy-white	Coarse	Mild gari flavor	Slightly sour
OKL4	Creamy-white	Coarse	Mild gari flavor	Strongly sour
OKL6	Creamy-white	Coarse	Mild gari flavor	Slightly sour
OKN2	Creamy-white	Fine	Intense gari flavor	Strongly sour
OKN4	Brown	Coarse	Intense gari flavor	Bland
OKN6	Creamy-white	Fine	Mild gari flavor	Bland
OKZ2	Creamy-white	Coarse	Mild gari flavor	Strongly sour
OKZ4	Brown	Coarse	Intense gari flavor	Slightly sour
OKZ6	Brown	Coarse	Intense gari flavor	Slightly sour
OKCT	Creamy-white	Coarse	Intense gari flavor	Slightly sour

ODL2= Gari produced from odongbo fermented with *R. oligosporus* for 2 days. ODL4= gari produced from odongbo fermented with *R. oligosporus* for 4 days. ODL6= gari produced from odongbo fermented with *R. oligosporus* for 6 days. ODN2= gari produced from odongbo fermented with *R. nigricans* for 2 days. ODN4 = gari produced from odongbo fermented with *R. nigricans* for 4 days. ODN6= gari produced from odongbo fermented with *R. nigricans* for 6 days. ODZ2 = gari produced from odongbo fermented with *R. oryzae* for 2 days. ODZ4= gari produced from odongbo fermented with *R. oryzae* for 4 days. ODZ6= gari produced from odongbo fermented with *R. oryzae* for 6 days. ODCT= control sample produced from odongbo; OKL2= gari produced from okoiyawo fermented with *R. oligosporus* for 2 days. OKL4= gari produced from okoiyawo fermented with *R. oligosporus* for 4 days. OKL6= gari produced from okoiyawo fermented with *R. oligosporus* for 6 days. OKN2 = gari produced from okoiyawo fermented with *R. nigricans* for 2 days. OKN4= gari produced from okoiyawo fermented with *R. nigricans* for 4 days. OKN6= gari produced from okoiyawo fermented with *R. nigricans* for 6 days. OKZ2= gari produced from okoiyawo fermented with *R. oryzae* for 2 days. OKZ4 = gari produced from okoiyawo fermented with *R. oryzae* for 4 days. OKZ6= gari produced from okoiyawo fermented with *R. oryzae* for 6 days. OKCT = "Okoiyawo" control.

selected *Rhizopus species* are shown in Tables 1 and 2. Table 3 showed the percentage of each sensory attributes of "gari". In term of color, creamy-white had the highest percentage (70%), while brown and white colour had the same percentage (15%). The dominant texture is coarse (70%), followed by fine texture (30%). In term of flavor, 85% had mild "gari" flavor and 15% had intense "gari" flavor. Moreover, 45% of the samples were strongly sour when tasted, 40% were slightly sour, 15% tasted bland. Similarly, Table 4 showed the percentage of peculiar sensory attributes of "eba" samples. In term of colour, creamy-white had the highest percentage (70%), followed by white (15%), brown (15%), and yellow had nil (0%). Fine texture had the highest percentage (90%), followed by coarse (10%). In term of flavor, 55% had intense "eba" flavor while mild "eba" flavor had 45%. Moreover, 30% of the "eba" samples are strongly sour, 60% are slightly sour while 10% are bland. 25% of are highly consistent, while 75% are mildly consistent. The moldability of the "eba" samples showed that all are moldable (100%).

Effects of process variables on color

The descriptions of color "gari" as well as "eba" samples are shown in Tables 1 and 2. Creamy-white is the most dominant color, followed by white while the least is brown. The statistical analysis showed that the color of "gari" ranged from 1.00 to 3.50. The control sample produced from "odongbo" had the highest mean value (3.50), the control sample produced from "okoiyawo" (OKCT) had 1.90 while the sample produced from "okoiyawo", fermented by *R. oligosporus* for 4 days had the least means value. Similarly, the mean value of "eba" ranged from 1.05 to 3.60. The "eba" sample produced from okoiyawo, fermented by *R. nigricans* (OKN6) for 6 days had the higher value while the sample obtained

Table 2. The description of sensory attributes of "eba" obtained from fermentation of cassava using some selected *Rhizopus species.*

Sample	Color	Smoothness	Flavour	Taste	Consistency	Moldability
ODL2	Creamy-white	Fine	Intense eba flavor	Strongly sour	Highly consistent	moldable
ODL4	Creamy-white	Coarse	Intense eba flavor	Strongly sour	Mildly consistent	moldable
ODL6	White	Fine	Mild eba flavor	Slightly sour	Highly consistent	moldable
ODN2	Creamy-white	Fine	Intense eba flavor	Slightly sour	Highly consistent	moldable
ODN4	Creamy-white	Fine	Intense eba flavor	Slightly sour	Highly consistent	moldable
ODN6	White	Fine	Intense eba flavor	Bland	Mildly consistent	moldable
ODZ2	Creamy-white	Fine	Intense eba flavor	Slightly sour	Mildly consistent	moldable
ODZ4	Creamy-white	Fine	Intense eba flavor	Slightly sour	Mildly consistent	moldable
ODZ6	White	Fine	Mild eba flavor	Slightly sour	Mildly consistent	moldable
ODCT	Creamy-white	Fine	Intense eba flavor	Strongly sour	Highly consistent	moldable
OKL2	Creamy-white	Fine	Mild eba flavor	Slightly sour	Mildly consistent	moldable
OKL4	Creamy-white	Fine	Mild eba flavor	Slightly sour	Mildly consistent	moldable
OKL6	Creamy-white	Coarse	Intense eba flavor	Strongly sour	Mildly consistent	moldable
OKN2	Creamy-white	Fine	Mild eba flavor	Slightly sour	Mildly consistent	moldable
OKN4	Brown	Fine	Mild eba flavor	Bland	Mildly consistent	moldable
OKN6	Creamy-white	Fine	Mild gari flavor	Slightly sour	Highly consistent	moldable
OKZ2	Creamy-white	Fine	Intense eba flavor	Slightly sour	Mildly consistent	moldable
OKZ4	Brown	Fine	Mild eba flavor	Strongly sour	Mildly consistent	moldable
OKZ6	Brown	Fine	Mild eba flavor	Slightly sour	Mildly consistent	moldable
OKCT	Creamy-white	Fine	Intense eba flavor	Slightly sour	Mildly consistent	moldable

from "okoiyawo" femented with *R. oligosporus* for 4 days (OKL4) had the least. The results showed that there was a significant difference in the color of the "gari" and "eba" samples. This may be due to the effects of processing conditions. According to Rosales-Soto et al., (2016), color changes in carbohydrate foods may be due to the chemical reaction between the amino acids and sugars present in the cassava mash, such as caramelization of carbohydrate, maillard reaction. Statistical results (Tables 4 and 5) showed that there was no significant ($p<0.05$) in the color of most of the "gari" samples fermented for 4 and 6 days, including the control sample, but there was a significant difference when compared with some of the samples fermented for 2 days. This suggests that "gari" color varied with the duration of fermentation (Brimer, 2015). According to Brimer (2015), browning occurs during garrification due to maillard reaction involving amino acids and sugars present in the cassava mash.

Effects on taste

Results of the percentage sensory attributes (Table 3) showed that the taste of about 45% of "gari" samples are strongly sour, 40% are slightly sour, while the remaining 15% are bland. Similarly, 30% of "eba" samples are strongly sour, 60% are slightly sour, while 10% are bland (Table 4). This may be due to the direct manifestations of various biochemical reactions that occur during fermentation. The fermentation processes were characterized by increased acid production (increase in T.T.A and decrease in pH) for all the cassava varieties. Acid production during cassava fermentation has been attributed to the activities of the microbial flora on the carbohydrates of the cassava root (Ogunnaike et al., 2015). Brimer (2015) reported that the rate of biochemical reactions such as cyanide hydrolysis, reducing sugar, and acid production during fermentation bring about the degree in the sourness of the taste of cassava based foods. The acceptability of "gari" is influenced by its sourness, which is related to the amount of lactic acid or length of fermentation (Karim et al., 2016).

The statistical results showed that the taste of "gari" and "eba" samples ranged from 1.10 to 2.87 and 1.15 to 2.80, respectively (Tables 5 and 6). "Gari" produced from "odongbo" fermented by *R. oryzae* for 6 days (ODZ6) had the highest value while the least value cut across various samples (OKZ4, OKL6, ODZ4 and ODZ2). Similarly, the sample produced from "odongbo" fermented by *R. oryzae* for 6 days (ODZ6) also had the highest value out of all the "eba" samples while the sample produced from "okoiyawo" fermented by *R. oryzae* for 4 days (ODZ4) had the least. The result of the statistical analysis showed that there was no significant difference ($p<0.05$) in the taste of the most of the samples fermented with the selected *Rhizopus* species for 4 and 6 days but there was a significant difference when compared with the samples fermented for 2 days and the control samples. It also showed that there was no significant difference in the taste of the samples fermented with *R. oligosporus*

Table 3. The percentage of "gari" samples with similar sensory attributes.

Attribute	No. of samples	Percentage
Colour		
White	3/20	15
Creamy-white	14/20	70
Yellow	Nil	0
Brown	3/20	15
Smoothness		
Fine	6/20	30
Coarse	14/20	70
Small Lumps	Nil	0
Big Lumps	Nil	0
Flavour		
Intense gari flavour	3/20	15
Mild gari flavor	17/20	85
Taste		
Strongly sour	9/20	45
Slightly sour	8/20	40
Bland	3/20	100

and *R. nigricans* but there was a significant difference when compared with the samples fermented with *R. oryzae* and the control samples. There was a significant difference in the taste of gari/ eba samples produced from "odongbo" and their control sample as well as the samples produced from "okoiyawo" and their control sample. This suggests that acid production may be dependent not on the cassava variety but on the microbial flora and the processing conditions (Ehirim, 2018). Olaoye et al., (2015) also reported that the taste of "gari" depends on the degree of fermentation. Long fermentation (generally more than three days) results in an unacceptable sour taste.

Effects on aroma

Tables 5 and 6 showed the statistical results of sensory attributes of "gari" and "eba". "Gari" aroma ranged from 1.13 to 2.00 while that of "eba" ranged from 1.30 to 2.00. Of all the "gari" samples, the sample produced from "okoiyawo", fermented by *R. oligosporus* for 4 days (OKL4) had highest mean value for flavor while the control sample produced from "okoiyawo" (OKCT) had the least. Similarly, the sample produced from "okoiyawo" as well as "odongbo", fermented by *R. oligosporus* for 2 days (OKL2 and ODL2) had the highest mean value out of all the "eba" samples while the control sample produced from "okoiyawo" had the least.

The result showed that there was a significant difference ($p<0.05$) between the flavor of the control sample of "gari" and "eba" produced from "odongbo" and other "odongbo" sample fermented with *Rhizopus* species. Similarly, there was a significant difference in the aroma of "okoiyawo" control sample and other "okoiyawo" samples fermented with *Rhizopus* species. This may be as a result of the biochemical reactions that occur in the cassava mash when fermented with *Rhizopus* species. The significant difference in peculiar aroma of "gari" may be due to the variation in the quantity of aldehydes and esters produced during fermentation. Intense "gari" aroma may suggest that the quantity of the aldehydes and esters is adequate in the fermented cassava mash while mild "gari" aroma may connote that the quantity of aldehydes and esters is less. In addition, garrifying process may produce entirely new flavor other than the natural flavor of foods (Sineenart et al., 2018).

Effects on smoothness

The result of the percentage sensory attributes of "gari" and "eba" are shown in Tables 3 and 4. This showed that 70% of "gari" samples are coarse while 30% are fine. After turning the "gari" samples into "eba", the result shows that 90% are fine while 10% are coarse. This may be due to the effects of some properties of starches in hot water. When a suspension of starch granules in water is heated, the granules swell due to water uptake and gelatinize, this increase the viscosity of the suspension

Table 4. The percentage of “eba” samples with similar sensory attributes.

Attribute	No. of sample	Percentage (%)
Colour		
White	3/20	15
Creamy-white	14/20	70
Yellow	Nil	0
Brown	3/20	15
Smoothness		
Fine	18/20	90
Coarse	2/20	10
Flavour		
Intense eba flavor	11/20	55
Mild eba flavor	9/20	45
Taste		
Strongly sour	6/20	30
Slightly sour	12/20	60
Bland	2/20	10
Consistency		
Highly consistent	5/20	25
Mildly consistent	15/20	75
Not consistent	Nil	0
Moldability		
Mouldable	20/20	100
Not Mouldable	0/20	0

and finally, a paste is formed which on cooling can form a gel and stiffens (Ehirim, 2018).

Tables 5 and 6 showed the statistical analysis of “gari” and “eba” samples. There was no significant difference in the smoothness of “gari” samples produced from “odongbo” and the control sample but some of the samples produced from “okoiyawo” (OKL4, OKL6 and OKCT) are significantly different when compared with those produced from “odongbo”. There was a significant difference between the control sample and other samples produced from “okoiyawo”. Similarly, there was no significant difference in the smoothness of all the “eba” samples produced from “odongbo” and but there was a significant difference when compared with some of the samples produced from “okoiyawo” (OKCT and OKN2). This suggests that cassava variety play a vital role in determining the fineness of the sample or not. This may be attributed to the moisture content in the cultivars and the biochemical reaction that occur in the cassava mash during processing and fermentation (Brimer, 2015). Molds require lesser moisture content for growth while bacteria flourish well in plentiful moisture content. The smoothness of “gari” can also be affected by the efficiency of grating and the method of garrifying (Ehirim, 2018). According to him, when the grating is efficient, the particle sizes should be small. If the initial garrifying temperature is too high, the product becomes lumpy and forms large agglomerates.

Effects on moldability

The result of the moldability of the “eba” samples obtained from the “gari” samples produced from the fermentation of cassava using some selected *Rhizopus* species is shown in Table 6. This shows that there was no significant difference ($p<0.05$) between the samples produced from “odongbo” as well as “okoiyawo”. The result of the percentage of “eba” samples on moldability showed that 100% are moldable. This may be due to effective fermentation and garrification. Garrification process causes starch in “gari” to gelatinize. The method and duration of garrifying as well as the temperature of the boiling water used in turning “gari” to “eba” can affect

Table 5. Evaluation of sensory attributes of "gari" samples produced from fermentation cassava using some selected *Rhizopus species*.

Sample code	Color	Smoothness	Flavour	Taste
ODL2	1.13^{g}	1.27^{c}	1.87^{abcde}	2.00^{c}
ODL4	1.93^{ef}	2.00^{ab}	1.73^{ef}	2.00^{c}
ODL6	1.93^{ef}	1.97^{ab}	1.83^{bcdef}	1.6^{e}
ODN2	3.43^{a}	1.97^{ab}	1.97^{ab}	2.00^{c}
ODN4	2.00^{def}	1.97^{ab}	1.87^{abcde}	1.90^{cd}
ODN6	1.93^{ef}	2.00^{ab}	1.77^{def}	1.20^{fg}
ODZ2	1.97^{ef}	2.03^{a}	1.83^{bcdef}	1.10^{g}
ODZ4	1.97^{ef}	1.97^{ab}	1.70^{f}	1.10g
ODZ6	1.07^{g}	1.16^{cd}	1.93^{abc}	2.87^{a}
ODCT	3.50^{a}	2.00^{ab}	1.93^{abc}	2.80^{ab}
OKL2	2.23^{bcd}	1.27^{c}	1.90^{abcd}	1.93^{cd}
OKL4	1.00^{g}	1.23^{cd}	2.00^{a}	2.63^{b}
OKL6	1.87^{f}	1.23^{cd}	1.33^{g}	1.10^{g}
OKN2	2.27^{bc}	1.93^{ab}	1.80^{cdef}	1.23^{fg}
OKN4	1.93^{ef}	1.93^{ab}	1.77^{def}	1.13^{g}
OKN6	3.30^{a}	2.00^{ab}	2.00^{a}	1.93^{cd}
OKZ2	2.40^{b}	1.90^{b}	1.83^{bcdef}	1.80^{d}
OKZ4	1.97^{ef}	2.03^{a}	1.80^{cdef}	1.10^{g}
OKZ6	2.13^{cde}	2.00^{ab}	1.80^{cdef}	1.33^{f}
OKCT	190^{ef}	1.13^{d}	1.13^{h}	1.20^{fg}

Means with the same superscript along the column are not significantly different are 5% probability.

Table 6. Evaluation of sensory attributes of "eba" samples produced from fermentation cassava using some selected *Rhizopus species*.

Sample	Color	Smoothness	Flavour	Taste	Consistency	Moldability
ODL2	1.25^{e}	1.10^{de}	2.00^{a}	2.00^{cde}	1.20^{hi}	1^{a}
ODL4	2.00^{cd}	1.20^{cde}	1.35^{ef}	1.85^{cdef}	1.60^{defg}	1^{a}
ODL6	2.00^{cd}	1.30^{bcd}	1.40^{def}	1.70^{fg}	1.60^{defg}	1^{a}
ODN2	1.80^{d}	1.00^{e}	1.90^{ab}	2.40^{b}	1.75^{abcde}	1^{a}
ODN4	1.80^{d}	1.20^{cde}	1.60^{cd}	2.05^{cd}	1.70b^{cdef}	1^{a}
ODN6	1.80^{d}	1.10^{de}	1.30^{f}	1.30^{hi}	1.00^{i}	1^{a}
ODZ2	2.00^{cd}	1.20^{cde}	1.30^{f}	1.20^{i}	1.25^{h}	1^{a}
ODZ4	1.95^{cd}	1.20^{cde}	1.40^{def}	1.50^{gh}	1.85^{abc}	1^{a}
ODZ6	1.30^{e}	1.00^{e}	1.30^{f}	2.80^{a}	1.80^{abcd}	1^{a}
ODCT	2.40^{b}	1.00^{e}	1.55^{cde}	2.50^{b}	1.90^{ab}	1^{a}
OKL2	2.20^{bc}	1.20^{cde}	2.00^{a}	2.00^{cde}	1.40^{gh}	1^{a}
OKL4	1.05^{e}	1.20^{cde}	1.70^{bc}	2.10^{c}	1.60^{defg}	1 a
OKL6	1.90^{d}	1.15^{cde}	1.75^{bc}	1.90^{cdef}	1.95^{a}	1^{a}
OKN2	2.20^{bc}	1.30^{bcd}	1.70b^{c}	1.90^{cdef}	1.65^{cdef}	1^{a}
OKN4	2.00^{cd}	1.30^{bcd}	1.40^{def}	1.80^{def}	1.40^{gh}	1^{a}
OKN6	3.60^{a}	1.35^{bc}	1.60^{cd}	1.75^{efg}	1.50$_{fg}$	1^{a}
OKZ2	2.20^{bc}	1.50^{ab}	1.35^{ef}	1.20^{i}	1.65^{cdef}	1^{a}
OKZ4	2.00^{cd}	1.45^{b}	1.40^{def}	1.15^{i}	1.55^{efg}	1^{a}
OKZ6	1.90^{d}	1.35^{bc}	1.35^{ef}	1.80^{def}	1.65^{cdef}	1^{a}
OKCT	1.95^{cd}	1.70^{a}	1.30^{f}	1.50^{gh}	1.55^{efg}	1^{a}

Means with the same superscript along the column are not significantly different at 5% probability.

the moldability (Ehirim, 2018).

Effects on consistency

Table 4 showed that percentage of “eba” that are consistent and not consistent. 25% of the samples were highly consistent while 75% were mildly consistent. The consistency of the samples ranged from 1.00 to 1.95 (Table 6). The control sample (OKCT) produced from “okoiyawo” had 1.55 while that of “odongbo” (ODCT) had 1.90. The sample produced from “okoiyawo” fermented with *Rhizopus oligosporus* 6 days (OKL6) had the highest mean value for consistency while the sample produced from “odongbo” fermented with *R. nigricans* for 6 days (ODN6) had the least.

The results of the statistical analysis showed that there was a significant difference ($p<0.05$) in consistency of the samples produced from “okoiyawo” when compared with that of “odongbo”. There was a significant difference in the consistency of the control sample and other samples produced from “odongbo” as well as “okoiyawo” control sample and the remaining samples produced from “okoiyawo”. In addition, there was a significant difference in consistency when comparing the samples that was fermented with *R. oligosporus* with ones fermented with *R. oryzae* and *R. nigricans*. This suggests that the variation in consistency may be due to the effect of cassava varieties, fermentation process and garrifying process. According to Oluwafemi and Udeh (2016), when starch granules is heated, some of the intermolecular hydrogen bonds are disrupted and swelling is noticeable. Further heating causes more loosening of the network, allowing additional water to enter and enlarge the granules.

Conclusion

The study showed that the sensory properties of “gari” and “eba” could be influenced by the process variables used. *Rhizopus* species effectively ferment cassava mash within two days and produce sour “gari”. The method and duration of garrifying further influence the smoothness of “gari”. Moderate garrifying temperature with consistent stirring of the cake gives fine “gari” but when the initial garrifying temperature is too high, the product become lumpy and forms agglomerates. It is, therefore, concluded that two days fermentation of cassava from “odongbo” are of good quality in term of color, taste, flavor, and smoothness compared with samples produced from “okoiyawo”. The sensory evaluation of the samples showed that the creamy-white is the most dominant color. A peculiar flavor, fine, and sour “gari”, consistent and moldable “eba” was also obtained.

Recommendation

Further research should be carried out on preference test of sensory evaluation to determine consumers’ acceptability of “gari” and “eba” produced from fermentation of cassava using some *Rhizopus* species.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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