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Water Absorption and the Genetic Relationship of Four Different Types of Acha (*Digitaria exilis*) Grains Using PCR

M. F. Istifanus[1*], E. B. Agbo[2] and A. F. Umar[2]

[1]Department of Microbiology, Plateau State University, Bokkos, Plateau State Nigeria
[2]Department of Biological sciences, Abubakar Tafawa-Balewa University Bauchi, Bauchi State, Nigeria

**Corresponding author*

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A B S T R A C T

The genomic DNA extraction of four different Acha types based on colour (yellow, brown, cream and white) was carried out using five (5) set of microsatellite primers. The PCR result revealed the genetic relationship and difference between the Acha types, where two of the primers did not amplify the grain, whereas three did. The water absorption behaviors of the grains were also determined using two water temperatures 35^{o}c and 50^{0}c. There was an initial rapid rate of water absorption within the first hour which later reduces and become steady from 6 hours up to the 24thhours of steeping. The summary of the analysis reveals that yellow, brown and white colour types are not significantly different at p-value confidence level.

Introduction

Cereal grain which could be used to produce variety of dishes requires preliminary preparation, such as soaking and washing in water, fermentation and milling to produce fine flour and grits. The treatment used depends on the product to be produced, (Addo *et al.,* 2006). During the soaking of food materials water is progressively being absorbed the extent of absorption depends primarily on the soaking water temperature, time and physio chemical properties of the food material. Soaking is the most common preliminary process applied to Acha grain during the production of various Acha based food product like masa, kunun-zaki, pap etc. (Shittu *et al.,* 2004). Acha is of different varieties, the report of CIRAD, (2004) says there are over 300 *digitaria species* which are sometimes grown as fodder, only three or four are sometimes grown as cereals.

In a similar study carried out by (Istifanus and Agbo, 2016), fourteen (14) different varieties of Acha grain were identified from four selected local Governments of Bauchi and Plateau states. Due to the variations that exist in Acha grain, the need for genetic characterization is very important which will reveal a lot of information on the taxonomy, cytogenetic and compatibility of the grain. It will also help in improving the agronomic and quality characteristic of the grain through

higher yielding accession. (Kwon-Ndung and Dachi, 2007).

This work is therefore, aimed at studying the genetic relationship of four different types of Acha grain using PCR, and to also optimized the water absorption behaviour of the grains.

Materials and Method

Optimisation of Maximum Moisture Uptake

About 10g of each of the grains was soaked in 50ml of distilled water in a 100ml beaker. Weight gain during soaking at 35^{O}C and 50^{O}C was determined. The grains were kept, at room temperature (35^{O}C) and in a water bath (sodeteg, TE7, France and Labovolt 81015) at 50^{O}C. Weight of soaked grains were taken at an interval of 1 to 6-hour period and at 24h periods. The soak water was drained off the grains by the use of a sieve; the free water was allowed to drain from the grain. Water absorbed by the grains with respect to soaking time was determined by subtracting the original weight of grains from the weight of the water – absorbed grains (Seyhan-Gurtas*et al.*, 2001). Soaking of grains in water continued until they stopped absorbing water (i.e the moisture absorption capacity was reached). Total solid of the soaking water for each beaker containing the 10g for each Acha grain was determined at each interval of time, by evaporating off the water with a laboratory oven (prolabo, 53921) at 110^{O}C until dryness. Total solids of the soak water were determined and added to the weights gained during soaking to obtained correct weight without solid loss. (Tagawa *et al.*, 2003).

After which the analysis of variance was carried out using the repeated measure longitudinal analysis method, it is being used when analysing two variables with time. It was used to determine the difference in water absorption rate of the four (4) types of Acha grain used.

DNA Extraction

The DNA extraction of the four types of Acha samples was done using the simple sequence repeat or microsatellites marker techniques (SSRs) by the protocol described by Yin *et al.*, 2011, but were scaled down so that extraction was done in a 1.5ml tube. The samples identify are 1.CM, 2. YL, 3.WE and 4.BNthe protocols were as follows: -

Acha grains were grind into power and transferred immediately into a 1.5ml centrifuge tubes

To each 10mg sample, 500ul of extraction buffer, was added, the contents were mixed very well and were incubated at 65^{O}C for 20minutes with occasional swirling.

After which samples were allowed to cool at room temperature, thereafter, 300ul volume of chloroform was added, which were incubated on ice for 5munites.

Next they were centrifuged at 8,000rmp for 20munites at 4^{O}C; the supernatants were carefully pipette off to a new free tube.

Steps 3 and 4 were repeated.

150ul volume of 8m LiCl was added to each of the tubes mixed gently and was incubated at 80^{O}C for 2hrs or overnight as desired.

After the 2hrs or overnight, the tubes were centrifuged at 8,000rmp for 20munites at 4^{O}C.

The solutions were then transferred to DNase-free tubes, and then 1ml volume of ethanol was added and incubated on ice for 30munites. They were centrifuged again, but this time at 10,000rmp for 10minutes. The pellet was

washed with 75% ethanol one to two times; air dried the pellet for 10minutes and then dissolved the pellet in 100ul DNase – free water. Now we have our extracted DNA.

DNA Amplification Process

The genomic DNA extracted from four different types of Acha grains (CM, YL, WE and BN) was amplified using five sets of microsatellite primers developed by (Barnaud *et al.*, 2016) as previously described (Table 1). In this process a very small amount of each of the samples of extracted DNA were added to a PCR cocktail for amplification in a thermo cycler.

Table.1 Microsatellite Primers Used and their Reaction Mix Per Sample

PRIMER SEQUENCE	BASE PAIR
De-03F; TTAAGACCATTTGGATTAGAGAA De-03R; CTTAAACGCCCAATCTTTAG	111
De-05F; AAGCCTTGCGTTCTATCTTA De-05R; TTAATATGATGCTACCCCTCA	219
De-23F; CGTGGACTAACGTATCAAGAA De-23R; ACTCCCTCTCCCCAATCT	168
De-30F; GTGCTAGGTGGAGCGAGA De-30R; CGTGAGCAGGTTCTCCAG	131
De-37F; TGAACAAATTCCTCTTGCTC De-37R; TGGCAATGTTCCATAAAGA	198

The reaction mix per sample is presented below:

REAGENT	**1X (ul)**
Nuclease free water	12.0
10 XPCR buffer	2.0
Dntp MIX (10Mm)	0.2
Magnesium Chloride (25mM)	0.8
Forward primer (4uM)	1.0
Reverse primer (4uM)	1.0
GoTaq Polymerase ()	0.5
	18.0
DNA template	2.0
Total reaction Volume	**20.0**

Source: Barnaud *et al.*, 2016.

This is a "magic" step that has revolutionized molecular biology. We started with almost no DNA and wind up with enough that we can see it on a gel. Various "cocktail" recipes existed, they contain the thermophilic bacterial enzyme Taq Polymerase (essential), the dNTP mix (nucleotides that will allow massive replication of the targeted DNA), magnesium chloride, and the fluorescently labelleddNTPs (these will bind to the specially added M13 or T3 tail and light up under the laser and make bands of DNA alleles show up on the gel)

The cycling program for the amplification which was performed on a 9600PCR system (Applied Biosystems) is given below: -

Initial denaturation	94° C for 5 min	
Denaturation	94° C for 30 sec	
Annealing	58° C for 1 min 30 sec	35 cycles
Extension	72° C for 1 min 30 sec	
Final extension	72° C for 10 min	

The PCR products were then cooled at +4o C until used for electrophoresis.

Agarose Gel Preparation

1.5g of agarose powder was dissolved in 100ml of TBE buffer (0.5X) and melted in a microwave for 3munites to give a 1.5% agarose gel. 5ul of ethidium bromide was added to the cooking gel before pouring into the gel casting tray. The tray was already fitted with a comb. Once the gel is set, it was transferred into an electro phoretic tank containing 0.5XTBE buffer. 10ul of the amplified product was mixed with 2ul of loading dye and was loaded into the wells. A 50bp molecular weight marker was also added. Each set of the reactions had a non-template control (negative control).

Sequencing

The amplified products (gel) were run through the sequencer at 150 volts for 40 minutes until all the alleles have had times to run by the laser, to separate the products.

The process which illuminates the fluorescent nucleotides and makes bands light up on the gel. The sequencer generates an analogue image which was viewed on an ultraviolet transilluminator and captured using a camera.

The major variables in optimization include: temperature (the primer sequence will have a predicted melting temperature but what actually works may be higher or lower), the PCR-programmed times for denaturing, annealing and extending steps Magnesium chloride concentrations.

Results and Discussion

Maximum moisture optimization

The result in Table 2 shows variation in the water absorption characteristics of the four Acha types. This was due to their difference, because water absorption behavior of each food material is unique due to their physiochemical properties (Shittu *et al.*, 2004).

The water absorption at 50^0c was more rapid than at 35^0c, figure 1. Generally, more water was absorbed at 50^0c for all Achatypes; this was as a result of the increase in temperature, because temperature appears to accelerate the rate of water absorption of food materials. This is in agreement with previous works that says an increase in temperature of the soaking medium increases water uptake by various seed which subsequently results in a reduction of the soaking time (Sopade and Obekpa, 1990; Shittu *et al.*, 2004; Addo *et al.*, 2006).

Table.2 Moisture uptake of Four Different Types of Acha Based on Time Temperature

	Optimization of Moisture Uptake condition			
	Water absorption at 0 time		Water absorption after 1 hour	
	35^{O}C	50^{O}C	35^{O}C	50^{O}C
YL	49.20g	50.05g	52.39g	53.29g
BN	47.45g	49.10g	51.02g	52.15g
CM	46.05g	49.02g	50.12g	51.20g
WE	48.15g	48.53g	51.69g	52.50g
After 2 hours of steeping			**After 4 hours of steeping**	
YL	53.10g	53.91g	54.05g	54.75g
BN	52.35g	53.76g	53.85g	54.60g
CM	51.09g	52.25g	52.56g	53.45g
WE	52.22g	53.45g	53.22g	54.05g
After 6 hours of steeping			**After 24 hours of steeping**	
YL	55.56g	55.57g	55.59g	55.59g
BN	55.80g	55.81g	55.83g	55.83g
CM	54.44g	54.46g	54.48g	54.48g
WE	54.69g	54.70g	54.71g	54.71g

Key: YL = Yellow BN = Brown CM = Cream WE = White

Table.3 The Summary of the Moisture Uptake of the Four Colour Types of Acha Grain

Parameters	**YL**	**BN**	**CM**	**WE**
Water Absorption ml/g	54.3±0.28^{a}	54.1±0.135^{a}	52.9±0.18^{b}	53.6±0.134^{a}

Values are means of triplicate determination + Standard Deviation
Means with same superscript in each row are not significantly different from each another (LSD, $p<0.05$).
Key: YL = Yellow BN = Brown CM = Cream WE = White

Fig.1 The mean and standard deviation of the soaked grain based on time and temperature

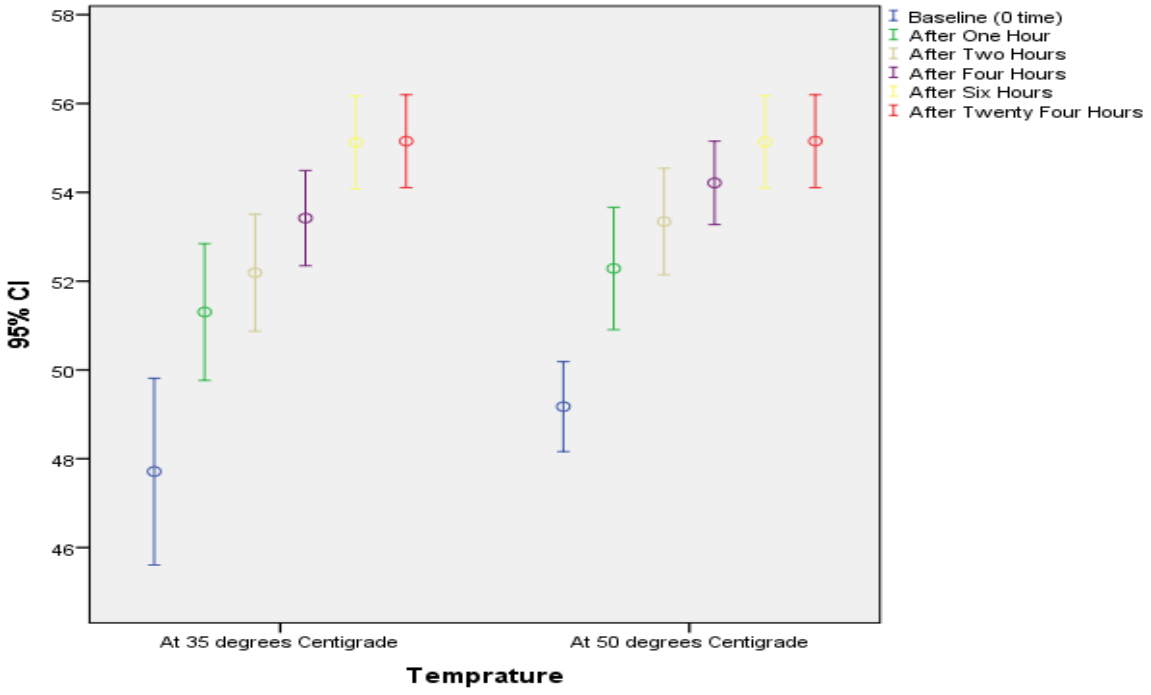
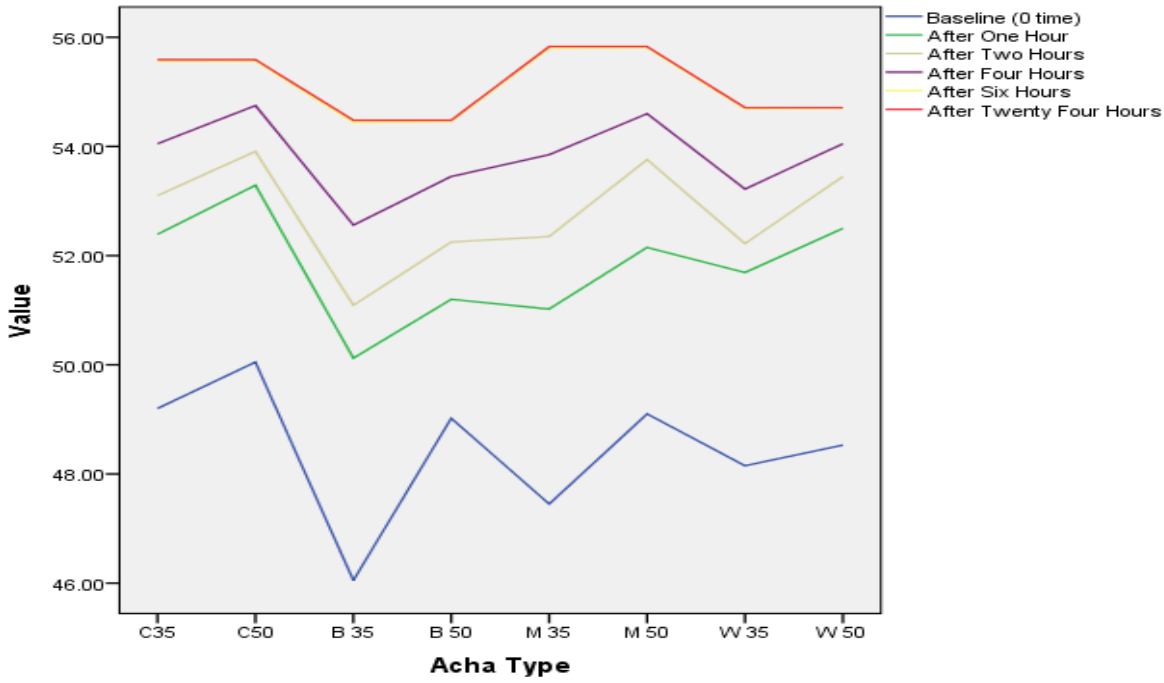
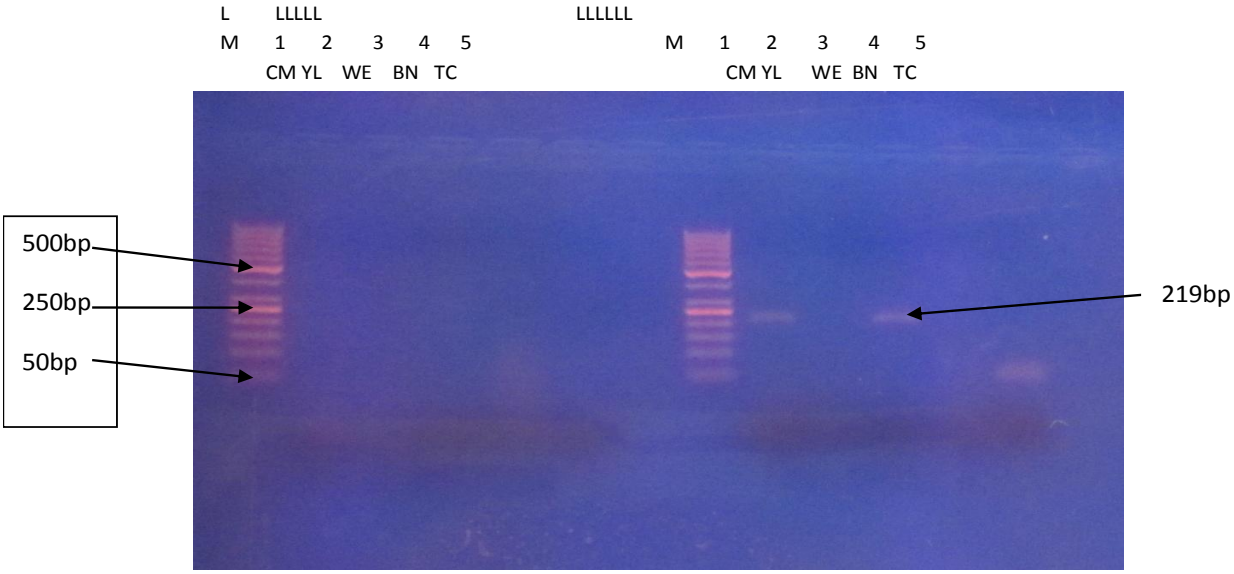


Fig.2 Comparing the water absorption of the four colour types of Acha based on time and temperature



Key: C – creamAcha B – brown Acha W- white Acha M – milk Acha

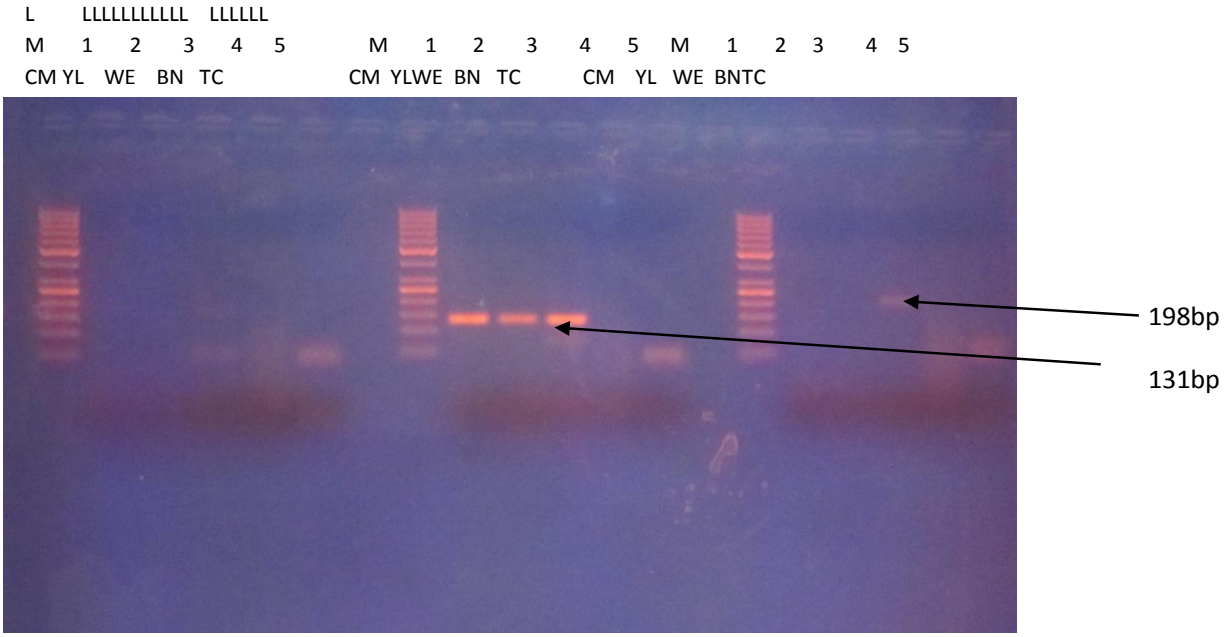
Fig.3 Gel picture showing the amplification of four Acha types using primers De03 and De05



KEY

L – Lane	LM - Lane M = 50bp	bp – Base pair	
YL–Yellow	CM – cream	WE - white	BN – brown
TC - template control	De03 – primer 111bp	De05 – primer 219bp	

Fig.4 Gel picture showing the amplification of four Acha types using primers De23, De30 and De37



KEY

L – Lane	LM - Lane M = 50bp	bp – Base pair	
YL–Yellow	CM – cream	WE - white	BN – brown
TC - template control	De23 – primer 168bp	De30 – primer 131bp	De37 – primer 198bp

Water absorption of the grains for the two temperatures used followed an exponential pattern. There was an initial rapid rate within the first 1hour, which later reduced and become steady from 6hours up to 24thhours of steeping Figure 2. The significant difference seen in the water absorption rate from 1-6hours was due to the fact that during the soaking of food materials water is progressively being absorbed, (Addo *et al.*, 2006). While between 6 and 24thhours there was no significant difference in the four Acha types, and that was why the sixth hour was swallowed up in the 24thhours in the graph. It was so because the grain has reached its moisture absorption capacity. This accurate description of the moisture absorbed by cereal during soaking is relative to soaking time and temperature which are essential in order to achieve optimum water content of the described process, (Seyhan-Gurtas *et al.*, 2001).

The summary of the analysis in table3 showed that the yellow, brown and white colour types are not significantly different at p-value confidence level

PCR Result

Out of the five (5) set of micro satellite primers used to test the four Acha grain types, two did not amply the grain, while two presented a clear multilocus pattern and one has a unique amplification site, figure 3 and 4. A maximum of three alleles were found per locus. This reveals a clear genetic relationship and difference between the Acha types, meaning not all Acha types are the same they are of different varieties, evethough they have some similarities. This agrees with the report of CIRAD (2004), which says there are over 300 digitaria species which are sometimes grown as fodder only three or four are sometimes grown as cereals. Similarly Istifanus and Agbo (2016), also identified fourteen different varieties of Acha grain which differ from each other in their physioco-chemical attributes.

The study reveals that temperatures help to accelerate the rate of water absorption of food materials, as the water absorption at 50^{0}c was more rapid than at 35^{0}c. All the four types of Acha grain stopped absorbing water between 6th and 24th hours because they have reached their water absorption capacity.

The findings has enable us have the understanding that Acha grain differs from each other which was seen in the water absorption behavior and the genetic variation using PCR.

Recommendations

There is need for more genetic characterization and the sequencing of the different types of Acha grain, which will reveal a lot of information on the taxonomy, cytogenetics and compatibility of the grain.

In view of the above, a collaborative effort from the government and academia in Nigeria is necessary to tackle the problem.

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