A Preliminary Investigation of the diversity of Some *Sorghum bicolor* L. Moench Varieties

By:

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Dedication

I dedicate this project to my dear father who has always been there for me when I needed him, and to all of his efforts to bring me the way I am today, I am thankful to him and I will always be.
Contents

Dedication .................................................................................................................. I
Table of contents ...................................................................................................... II
Abstract ..................................................................................................................... IV
Abstract in Arabic ..................................................................................................... v

Chapter One

1. Introduction ........................................................................................................... 1

Chapter Two

2. Literature Review .................................................................................................. 3
2.1. Nutritional Value .................................................................................................. 4
2.2. Uses of Sorghum .................................................................................................. 4
2.3. Cultivation ........................................................................................................... 6
2.4. Nuclear DNA content in the genus Sorghum ................................................... 6

Chapter Three

Materials and Methods

3. Plant Material ......................................................................................................... 8
3.1. Sorghum bicolor Plant Material ........................................................................... 8
3.1.1. Growth of the three varieties ........................................................................ 8
3.1.2. Collection of the Seedlings for Molecular Biology Studies ....................... 8
3.2. Molecular Characterization .................................................. 8
3.2.1. DNA Extraction and Purification ........................................ 8
3.2.2. Reagents ........................................................................... 9
3.2.3. Equipments ....................................................................... 9
3.3. Modified CTAB Protocol for DNA isolation from plant .............. 9
3.4. Quality of the Extracted DNA .................................................. 10
3.5. Preparation of Agarose Gel for Electrophoresis ......................... 10
3.6. Loading of DNA Samples onto the Agarose .............................. 10
3.7. Screening the Agarose gel ...................................................... 11

Chapter Four

4. Results and Discussion ........................................................... 12
4.1. Germination of the Sorghum specimens .................................. 14
4.2. DNA Extraction Results ....................................................... 15

5. References ............................................................................... 17
Abstract

In this research the characteristics of the three acquired accessions Ras elgirid (HSD 5958), Wad Ahmed (HSD 6478) and Fatarita baladi (HSD 6478) are studied. The three selected sorghum lines are successfully germinated in about three weeks. The cultivar Wad Ahmed demonstrated more vigorous growth than both of the other two cultivars; Fatarita and Ras Al Girid. This is manifested in the number of seeds that germinated for each type which averaged 4/pot for Wad Ahmed, 3/pot for Fatarita and one/pot for Ras Algirid Plate (1).

DNA is successfully extracted from all of the three samples (Fatarita – WD Ahmed – Ras Algirid) using CTAB method and is detected in each case using Gel Electrophoresis. The ideal conditions of performing gel electrophoresis to determine variations at the molecular level should have included restriction of the genomic DNA of each variety and inclusion of a suitable molecular weight marker to determine polymorphism with respect to restricted fragments and determination of the molecular weight of the genome of each variety. This has not been performed due to lack of the consumable material and inability to procure them from other sources.
اسم المستخلص

في هذا البحث، تم دراسة ثلاث عينات (فرئته، ود. أحمد، ورأس القرد)، الثلاث عينات تم اختيارها بناءً على نتائج في فترة ثلاث أسابيع. ود. أحمد كان أكثر العينات من حيث العادات والذين تم اختيارهم سابقًا فرئته ورأس القرد، وهذا مقارنةً مع البذور النامية في كل عينة، والتي كانت متوسطتها (4) بذور نامية. لود أحمد، 3 بذور نامية للفرئته وبدرة واحدة نامية لرأس القرد. تم استخلاص الـ DNA من كل من الثلاث عينات (فرئته ود. أحمد، ورأس القرد) باستخدام الـ Gel Electrophoresis وتحقيق منه بواسطة CTAB Method.

لتحديد الاختلافات على المستوى الجزيئي لم تتوفر اجراء الـ Gel Electrophoresis، السطحية لأجراء الـ Gel Electrophoresis، أن يتضمن التحديد للـ DNA لكل عينة، وأن تتضمن محدد للوزن الجزيئي لقياس التعدد الكلي من نواة العينة، وتحديد الوزن الجزيئي للجينوم لكل عينة. يتم انجاز هذا العمل بحصة لعدم توفر المواد الاستهلاكية واستخدام المقترح على استراء هذه المواد من مصادر أخرى.
Chapter One

1. Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is a very important crop in the Sudan serving as a primary source of food, beverage, and total livelihood for millions of people in the country. Phenotypic diversity among landraces was high, as expressed by the large range of variation for mean quantitative traits (Grenier *et al.* 2004). Landraces from Gezira-Gedarif tended to be shorter in stature, earlier in maturity and less sensitive to changes in photoperiod. They also had long, narrow and compact panicles that may result from adaptation to low rainfall and early adoption of mechanized farming practices. Collections from Kassala showed a higher frequency of landraces with kernels that were more difficult to thresh. Landraces from Blue Nile tended to have greater agronomic eliteness with higher proportion of landraces with white kernels, poorly covered and that were easy to thresh.

Molecular genetics techniques have grown to the state where diversity can be studied at the level of a Kingdom down to monoclonal variation among parts of an individual, and is used additionally to classification. There are different techniques used to study variation including random amplification of polymorphic DNA (RAPD), inter simple sequence repeat (ISSR) and amplified fragment length polymorphism (AFLP) (Etelka, 2006). Restriction fragment length polymorphism (RFLP), DNA barcodes (Kress, 2005), Single nucleotide polymorphisms (SNPs) (Beatriz *et al.*, 2005) and a fuzzy system augmented by a learning process based on evolutionary computation (GFS) (Eiben and Smith, 2003) were developed to determine genetic relatedness between individuals. Application of any of the aforementioned techniques requires characterization of the organisms under study.
using basic taxonomic characteristics including morphological characteristics and chemotaxonomic characteristics.

The aim of the present investigation is to explore the genetic relatedness in *Sorghum bicolor* between a hybrid; WD Ahmed, and two land races which are Fatarita and Ras El Girid to determine if there is any genetic basis to the apparent morphological variation between these three species or that the morphological variations are only manifestations of their interactions with their different variable habitats.

1. *WD Ahmed*
2. *Fatarita*
3. *Ras Al Girid*

this will be achieved by:

1. Using molecular techniques to differentiate between the three samples which include:

1- DNA Extraction and purification.

2- Gel Electrophoresis.

3- RNase Procedure.
Chapter Two

2. Literature Review

Sorghum bicolor belongs to the tribe Andropogoneae of family Poaceae, is the fifth important cereal of the world. It is widely grown in the semi-arid areas of the tropics and subtropics. The wild species of sorghum represent a potential diverse source of germplasm for sorghum breeding programmers. Sorghum has 25 species that have been taxonomically classified into five subgenera which are: 1- Eusorghum 2- Parasorghum 3- Chaetosorghum 4- Heterosorghum and 5- Stiposorghum (Garber, 1950). These five sections have chromosome numbers ranging from $2n=10$ (S. leiocladum), $2n=20$ (S. bicolor) to $2n=40$ (S. laxiflorum). Variation is within *Sorghum bicolor* is resolved into five basic races (bicolor, guinea, caudatum, kafir, and durra) and all combinations of their hybrid derivatives, for a total of 15 races (Harlan and de Wet, 1972). Intermediate races are designated, for example, as kafir-caudatum, durra-bicolor, etc while subraces are the commonly used agronomic groups (Fatarita, kaura, sorgo, sudangrass, etc.) familiar to sorghum workers. None of these requires a formal Latin name.

*Sorghum bicolor* ranks fifth in importance among the world’s grain crops being well adapted to areas with too little rain fall for growing other food and feed grains (Chittenden *et al.*, 1993). It is a staple food crop in parts of Africa and Asia and a significant feed crop in the U.S. It is also a grass species cultivated for its edible grain. Sorghum was originated in northern Africa, and is now cultivated widely in tropical and subtropical regions. It is typically an annual, but some cultivars are perennial. It grows in clumps
that may reach over 4 meters high. The grain is small, ranging from 3 to 4 mm in diameter.

Increasing demand for limited fresh water supplies, coupled with global warming trends, indicate that dry land crops such as sorghum will become increasingly important to agriculture in both arid regions and more moderate climates.

2.1. Nutritional value

The chemical composition of sorghum is quite variable. In the most widely grown commercial hybrids protein content may range from 10 to 13 %, depending on the cultivar and on the soil and climatic conditions. Sorghum and millets are predominantly starchy and the protein content is nearly equal among these grains and is comparable to that of wheat and maize (FAO, 1995). Sorghum and millets and other cereals are estimated to provide - per 100 g edible portion and 12 percent moisture- 329 kcal energy provided by 70.7 g carbohydrates, 6.3 g dietary fiber, 3.10 g fat and 10.4 g protein. Sorghum and millets contain neither vitamin A nor vitamin C however; sorghum germ is rich in B-complex vitamins. The protein content of the grain is also significantly and inversely correlated with its weight and starch content.

2.2. Uses of sorghum

In the semi-arid tropics worldwide, sorghum is cultivated by farmers on a subsistence level and consumed as food by humans. In Sudan, sorghum is used by humans as stable food and as a beverage and is also used in poultry and animal feeding. However, a nutritional limitation to its use is the poor digestibility of
sorghum protein when wet cooked (Duodu, *et al.*, 2003). Sorghum use is generally declining in urban cities while its use is still prevailing in rural areas. However, demand for sorghum for human consumption is decreasing with enhanced socioeconomic status of population in general and easy availability of preferred cereals in sufficient quantities at affordable prices (Sheoran *et al.*, 2000). Ethanol demand is increasing drastically in the present time due to its blending in automotive fuels, which is desirable for getting clean exhaust and fuel sufficiency. Suresh *et al.*, 1998 reported that more ethanol was produced from the damaged sorghum (2·90% v/v) than damaged rice (2·09% v/v) under optimal fermentation conditions of simultaneous saccharification and fermentation (SSF) of damaged grains using *Aspergillus niger* (NCIM 1248) and *Saccharomyces cerevisiae* VSJJ1. (Prasad *et al.*, 2007) reported on the high potential of Sweet sorghum as a raw material for fuel-grade ethanol production. Ethanol production from sorghum has many advantages over other grain resources due to sorghum rapid growth rate, early maturity, greater water use efficiency, limited fertilizer requirement, high total value and wide adoptability. Besides its use as feed, fodder, beverages and energy production trials were made to use of sorghum grain water extracts as herbicides. (Cheema and Khaliq, 2000) reported that water extract of mature *Sorghum bicolor* (L. Moench) plants obtained after soaking in water for 24 h could be used as a spray as a natural herbicide for wheat crop where it controlled up to 35–49% weeds and increased wheat yield by 10–21%. Sorghum grain water extract (sorgaah) was used as a natural weed inhibitor in spring mungbean (Cheema, *et al.*, 2001). The authors reported An inhibition of 44.11, 28 and 43% in total weed dry weight was noticed by three sorgaah sprays, one hand weeding and pendimethalin treatment, respectively as well as enhanced increase in grain yield.
of mungbean by 18%, 10% and 13 by application of hand weeding and pendimethalin treatment respectively.

2.3. Cultivation:
Sorghum grows well in arid soils and withstands prolonged droughts. Drought-stress is a major constraint to sorghum productivity worldwide however; sorghum is one of the most drought tolerant grain crops and is an excellent model for evaluating mechanisms of drought tolerance (Kebede et al., 2001). It has adapted to a wide range of soils, from the deep sands of the Goz in Kordofan state, Sudan to the heavy black cracking clays of the Gedaref, Sudan. The best characterized form of drought tolerance during crop growth is the non-senescence or "stay-green" trait (FAO, 2011). Good drainage, however, is necessary the great advantage of sorghum is that it can become dormant under adverse conditions and can resume growth after relatively severe drought. Shoot removal lowers its capacity to withstand drought. Early drought stops growth before floral initiation and the plant remains vegetative; it will resume leaf production and flower when conditions again become favorable for growth. Late drought stops leaf development but not floral initiation (Whiteman and Wilson, 1965).

2.4. Nuclear DNA content in the genus Sorghum:
Nuclear DNA content is apparently important to the evolution and adaptation of plant species (Price, 1976). DNA content affect cellular properties including nuclear volume, cell volume, the duration of mitosis and meiosis and minimum generation time and may influence ecological adaptation and distribution. Geographic and ecological parameters
including latitude, moisture availability and temperature, and growth form have been correlated with nuclear DNA content. (Bennett 1998). Annual and perennial species of a genus often differ in DNA content. The mean DNA content of perennials is greater than that of annuals, also the latest results shows that the DNA content of Sorghum is lower than previously reported and that there is no significant variation in DNA content between the 2n=20 Sorghum accessions. (Bennett 1972)

Sorghum is an important staple food throughout semiarid Asian and African regions (Ahmed et al., 2000). Studying the genetic variation of sorghum germplasm collections from Sudan attracts special interest for several reasons. Beyond the economic importance of the crop Sudan is within the geographical range where sorghum is believed to be domesticated for the first time (Mannet et al., 1983) and where the largest genetic variation for both cultivated and wild sorghum is found (Doggett, 1988).
3. Plant material:

3.1. *Sorghum bicolor* plant material:

*Sorghum bicolor* seeds were kindly supplied by the Plant Genetic Resources Unit-Agricultural Research Corporation (Dr Tahir Ibrahim, Genetic Resources Department, Agricultural Research Corporation Wad Medani).

3.1.1 Growth of the three Varieties:

The seeds were sown in alluvial soil in three pots each pot with five seeds for each used sorghum type on the 23rd, of January, 2012 At 9:00 am. Each growth experiment was replicated three times. Germination was carried out in the botany department green house at the conditions of sixteen hours of sunshine, day after day watering in a chicken wire enclosure for protection from trampling and wandering monkeys.

3.1.2 Collection of seedlings for molecular biology studies:

After three weeks from the sowing date; the 12th February, 2012 the seedlings for each type were carefully rooted from the alluvial damp soil and care was taken to collect whole seedlings. Each type of the collected plants was carefully washed to remove soil particles and the healthiest seedling from each type was then used for molecular biology studies.

3.2. Molecular Characterization

3.2.1. DNA Extraction and Purification
3.2.2. Reagents:
CTAB, chloroform, isooamyl alcohol, isopropanol, Tris HCl, EDTA, Na Cl, Agarose gel, ethanol alcohol, distilled water, Na OH, ethidium bromide, ammonium acetate, bromophenol blue, HCl.

3.2.3. Equipments:
Measuring cylinder, automatic pipette, flasks, autoclave, microwave, mortar, electrophoresis kit, water bath, micro centrifuge, sensitive balance, falcon tubes, and rack.

3.3 Modified CTAB Protocol for DNA Isolation from plant:
Genomic DNA was extracted, separately, from the fresh leaves of the selected seedling for *Wad Ahmed, Fatarita* and *Ras Elgirid* using modified CTAB method (Porebski et al., 1997). DNA was extracted from fresh leaves by grinding 0.2g of fresh tissue treated with dry ice and transferring the ground tissue, in each case, into a 1.5 ml Falcon tube containing (500μl) of pre-heated (60 °C) CTAB buffer using a Perfect automatic pipette. Isoamylalcohol chloroform mixture (500μl, 1:24 v: v) was added to each tube. The solutions were mixed gently for 5 minutes at room temperature to make a homogenous mixture. The cell debris was removed by centrifugation at 8000 rpm for 15 min and the clear supernatant (containing DNA) was transferred to a new sterile tube for each specimen. The nucleic acids in the aqueous phase were precipitated by adding an equal volume of deep cooled isopropanol (4 °C). The contents for each specimen were mixed gently and collected by centrifugation at 8000 rpm for 5 min. The supernatant was decanted and the pellet was re-suspended in 200 μl of 70% ethanol and was then centrifuged at 8000 rpm for 5 min. The remaining ethanol trace was removed by leaving the pellet to dry at room temperature. The pellet was dissolved in (100μl) TE buffer (10 mM Tris, 1 mM EDTA, pH 8), and stored at -20 °C for further use.
3.4. Quality of the extracted DNA:

The quality of the extracted DNA was refined by ridding each extract from carried over RNA during extraction. This was performed using RNase Procedure (Miller et al., 1999). The RNA in each crude extract was digested by adding 150 ml of an RNase solution (10 mg of RNase per ml, 100 mM Tris HCl, 10 mM EDTA [pH 8]) and incubating the preparation for 1 h at 37°C. This method is used to get rid of all kinds of RNA whether it belongs to a plant or any other creature, and that is done by using RnaSE enzyme which breaks down every RNA molecule around.

3.5. Preparation of Agarose Gel for Electrophoresis

The extracted DNA samples were quantified, in each case, using gel electrophoresis. Agarose gel was prepared by dissolving 0.8g of agarose in 50 ml 1xTBE (Tris base-Boric Acid- EDTA). The solution was boiled for 3 minutes until it became clear then 3 µl of ethidium bromide were added to it when it sufficiently warm. The gel was then poured into the gel tray; the combs were then placed to make loading wells after the gel cools.

3.6. Loading of DNA samples onto the Agarose gel:

Five micro liters of the DNA, from each plant material was mixed with 3 µl bromophenol blue dye to mark the running front of electrophoresis. The gel was submerged in 1xTBE buffer and then the mixed DNA extract and bromophenol blue from each plant material carefully loaded into a separate well in the submerged
agarose gel. Electric power was then connected and electrophoresis was performed for 30 minutes at 40 V.

3.7. Screening the Agarose gel:

After thirty minutes of electrophoresing, the electric power was disconnected and the agarose gel was removed from the electrophoresis chamber and was then observed under UV light. Agarose gel running pattern was captured by a digital camera. The USB of the camera was then connected to a computer and the image was viewed.
Chapter Four
4. Results and Discussion

The characteristics of the three acquired accessions Ras elgirid (HSD 5958), Wad Ahmed (HSD 6478) and Fatarita baladi (HSD 6478) are hereby cited (Dr Tahir Ibrahim, Genetic Resources Department, Agricultural Research corporation Wad Medani, Pers. Comm.)

Variety Ras Elgirid (HSD 5958) is a cultivated sorghum collected from Bados in the South of Kordofan State (latitude 12° 30' North, longitude 29° 40' East and altitude -unknown). Ras Elgirid has plant height of 282 cm, number of leaves 21, days to 50% flowering 100, 100 seed weight 3.1 gm and grain number per panicle 1667.

Variety Wad Ahmed (HSD 6478) is a cultivated sorghum collected from Shurac in the White Nile state. (Latitude 12° 36' North, longitude 32° 46' East and altitude 1284 feet). Wad Ahmed has plant height of 143 cm, number of leaves 18, days to 50% flowering 83, 100 seed weight 3 gm and grain number per panicle 2366.

Variety Fatarita baladi (HSD 6478) is a cultivated sorghum collected from Kowannis in the Blue Nile state; (latitude 12° 6' North, longitude 33° 48' East and altitude 1585 feet). Fatarita baladi has plant height of 98 cm, number of leaves 8, days to 50% flowering 62, 100 seed weight 29 gm and grain number per panicle 643. The characteristics of the three varieties are summarized in table 1
<table>
<thead>
<tr>
<th>Accession No.</th>
<th>Variety name</th>
<th>HSD 5958</th>
<th>HSD 5661</th>
<th>HSD 6478</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ras el girid</td>
<td>Cultivated</td>
<td>Cultivated</td>
<td>Cultivated</td>
</tr>
<tr>
<td>Collection site</td>
<td>Kowarnis</td>
<td>Bados</td>
<td>Shurac</td>
<td></td>
</tr>
<tr>
<td>Latitude Degree</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Latitude Minute</td>
<td>3</td>
<td>6</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Latitude Direction</td>
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<td>N</td>
<td></td>
</tr>
<tr>
<td>Longitude Degree</td>
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<td>33</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Longitude Minute</td>
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<td>48</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>Longitude Direction</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td></td>
</tr>
<tr>
<td>Altitude (feet)</td>
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<td>1284</td>
<td></td>
</tr>
<tr>
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<td>Damazin</td>
<td></td>
<td></td>
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<tr>
<td>Locality</td>
<td>El Dalang</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Region / State</td>
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<td>Blue Nile</td>
<td>White Nile</td>
<td></td>
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<tr>
<td>Plant height (cm)</td>
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</tr>
<tr>
<td>No. of leaves</td>
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<td>Days to 50% flowering</td>
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<td>83</td>
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</tr>
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<td></td>
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<td>3</td>
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<td>Grain number/ panicle</td>
<td>1667</td>
<td>643</td>
<td>2366</td>
<td></td>
</tr>
</tbody>
</table>

Table (1) Characteristics of the three sorghum bicolor accessions; Ras el girid, Fatarita baladi and Wad Ahmed (Dr Tahir Ibrahim Pers. Comm.)
4.1. Germination of the sorghum Specimens.

The three selected sorghum lines successfully germinated in about three weeks. The potted seedlings of Wad Ahmed, Fatrita and Ras Al Girid are displayed in figures 1, 2 and 3 respectively.

The cultivar Wad Ahmed demonstrated more vigorous growth than both of the other two cultivars; Fatrita and Ras Al Girid. This was manifested in the number of seeds that germinated for each type which averaged 4/pot for Wad Ahmed, 3/pot for Fatrita and one/pot for Ras Al Girid Plate (1).

![Wad Ahmed, Fatrita, Ras Al Girid](image)

Plate (1). Shows the seedlings of Wad Ahmed, Fatrita and Ras Al Girid

The variation in germination performance between the three varities may be attributed to variations in soil type and other edaphic factors to which each variety is adapted. On the other hand genetic variation could not be ruled out as a factor that affects germination performance. This could only be tested if each of the
varieties was tested in comparison with the others with provision of the same local conditions at which the variety grows.

4.2. DNA extraction results:
DNA was successfully extracted from all of the three samples (Fatarita – WD Ahmed – Ras Algirid) using CTAB method and was detected in each case using Gel Electrophoresis.

Gel electrophoresis was performed on genomic DNA of the three varieties (Plate 2).

Plate 2. Gel electrophoresis of genomic DNA extract from the three sorghum bicolor varieties.

The ideal conditions of performing gel electrophoresis to determine variations at the molecular level should have included restriction of the genomic DNA of each variety and inclusion of a suitable molecular weight marker to determine
polymorphism with respect to restricted fragments and determination of the molecular weight of the genome of each variety. This has not been performed due to lack of the consumable material and inability to procure them from other sources. Nevertheless, Lane 1 (Wad Ahmed) shows one dense band and three other minor bands while each of the other two varities; Ras elgrid and Fatarita baladi, show a very dense band similar to that of (Wad Ahmed) which did not migrate from the loading well. The three bands displayed by Wad Ahmed could be randomly broken fragments from the total genomic DNA. In this case we would have expected similar bands generated by each of the other varieties; Ras elgrid and Fatarita since the same method of extraction was used to extract DNA from each of the three varieties. Since such bands were not observed it is most probable that the three observed bands for Wad Ahmed indicate some sort of genomic variation between this variety and the other two varities. The three cultivars; Ras Elgrid, Fatarita Baladi and Wad Ahmed belong to the Mugud group (Abu Assar et al., 2005).
5. References


Miller, D. N., Bryant, J. E. Madsen, E. L and Ghiorse, W. C. 1999. _Evaluation and Optimization of DNA Extraction and Purification_.


