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QUALITY OF GENOMIC DNA EXTRACTED FROM MAIZE (*Zea mays* L.) VARIETIES GROWN IN ABIA STATE, NIGERIA USING BY CETYLTRIMETHYLAMMONIUM METHOD

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ABSTRACT

A study was carried out at the laboratory of Plant Science and Biotechnology Department Michael Okpara University of Agriculture, Umudike, Abia State Nigeria to assess the quality of the genomic DNA extracted from 10 maize varieties grown in Abia State, Nigeria and to determine the suitability of extracted DNA for polymerase chain reaction. The grains of 10 maize (*Zea mays* L.) varieties namely Oba-98, SAMMAZ-28, SUWAN-1-SR-Y, BR9928-DMR-SR-Y, ART/98/SW-1-1, MDV-3, BR9943-DMR-SR-W, TZPB-SR-W, Oba super-2, SDM-2 provided by National Seed Company of Nigeria were used for the study. The result revealed that with the exception of Oba- 98, MDV-3 and TZPBSR-W, all the other varieties recorded values above 1.7 for A_{260}/A_{230} indicating that the DNA may not have been contaminated with polysaccharide. This indicates that maize seed yielded high quality DNA for PCR amplification.

Keyword: Maize, variety, quality, DNA extraction, polymorphism, amplification

1. INTRODUCTION

Maize (*Zea mays* L., $2n = 2x = 20$) belonging to the family Poaceae is one of the most important crops in the word and preferred staple food for more than 1 billion people in sub Saharan Africa and Latin America (Gupta *et al*., 2009). Maize is one of the most versatile emerging crops having wider adaptability. Apart from this, maize is an important industrial raw material and provides large opportunity for value addition. Beside this maize have many types like normal yellow and white grain, sweet corn, baby corn, popcorn, waxy corn, high amylase corn, high oil corn, quality protein maize, etc. Maize is the only food cereal crop that can be grown in diverse seasons, ecologies and uses. In Nigeria, maize is known and called by different vernacular names depending on locality like agbado, igbado or yangan (Yoruba); masara or dawar masara (Hausa); ogbado or oka (Ibo); apaapa (Ibira); oka (Bini and Isha); ibokpot or ibokpot union (Efik) and igumapa (Yala) (Abdulrahaman and Kolawole, 2006).

Genetic quality control of seed is very important especially in hybrid seed. Seed quality includes physical, physiological, genetic quality and pathology quality. Planting hybrid seeds that genetic quality is not true will decrease in productivity. In this regard it is necessary to develop techniques to identify and test the purity of hybrid thus genetic quality can be maintained. So far, the methods used to test the purity of hybrid is through observations of plants in the field (grow out test), but this requires time and substantial resources (Komori and Nitta 2004). In addition, the estimates of genetic purity based on morphological characters are sometimes difficult because these characters are influenced by the environment.

Extraction of large quantity and high quality DNA is often a limiting factor in genetic analysis of plant traits important to agriculture. However, extraction of enough DNA with satisfactory quality for molecular studies poses a challenge, most especially in developing nations. Due to

lack of the information, breeders have been using genetically similar parents extensively in a breeding program leading to a narrow genetic base (Xia *et al*., 2004; Rehman *et al.*, 2002). DNA marker is a tool to provide the exact information of the genotype. Molecular characterization of cultivars is also useful to evaluate potential genetic erosion (Manifesto *et al*., 2001). With the development of molecular biology, identification of varieties can be done with the help of molecular markers, either by DNA or protein. Molecular marker is an effective tool because it can detect genetic variation and is not influenced by the environment. A number of other similar studies have been conducted on maize (Pabendon, 2005), in rice (Yashitola *et al*., 2002), in soybean (Santoso, 2006) and in tomato hybrids (Bredemeijer *et al*. 2002; Liu *et al*. 2007). The aim of the extraction procedure is to isolate DNA of reasonable quantity, purity, integrity and quality to allow DNA amplification and is often the most time consuming step of a DNA-based detection method.

2. MATERIALS AND METHOD

2.1 EXTRACTION OF DNA USING CTAB METHOD

Total genomic DNA extracted from 10 varieties of maize grains was used for DNA isolation following Cetyltrimethylammonium (CTAB) method, (George and Regalado, 2004). Maize seeds were soaked in water for 1 hour, it was later grinded and 600 µl of 2X CTAB buffer was added to the grinded maize and it was incubated at 65 ^{0}C for 20 minutes. The sample was removed from the incubator and allowed to cool to room temperature and chloroform was added, the sample was mixed by gently inversion of the tube several times. Thereafter, the sample was spun at 14,000 rpm for 15 mins and the supernatant was transferred into a new eppendorf tube and equal volume of cold Isopropanol was added to precipitate the DNA. The sample was kept in the freezer for 1hr and later spun at 14,000 rpm for 10mins and the supernatant was discarded and the pellet was washed with 70 % ethanol later the sample was air dried for 30mins on the bench. The pellet was re-suspended in 100µl of sterile distilled water. DNA concentration of the samples was measured on spectrophotometer at 260 nm and 280 nm and the genomic purity were determined.

2.2 TEST FOR DNA QUALITY

The concentration and quality of DNA extracted was first determined using NanodropND-100 spectrophotometer. Each DNA extract was measured 10 times and averaged by placing a drop of the solution free from air bubbles on the eye sensor of the machine. The DNA concentration was measured directly in ng/µl. DNA quality was measured as DNA/RNA ratioA_{260}/A_{280} while DNA purity was measured as DNA/protein ratio A_{260}/A_{230}. The size of the extracted DNA was evaluated by mixing 5 µl of undiluted DNA solution together with 2 µl of loading dye and these

was analyzed on 1 % w/v agarose gel stained with ethidium bromide. The gel was run at 90 V for 30 minutes in 1 % running buffer.

2.3 DNA ELECTROPHORESIS

Agarose gel electrophoresis was used to determine the quality and integrity of the DNA by size fractionation on 1.0 % agarose gels. Agarose gels were prepared by dissolving and boiling 1.0 g agarose in 100 ml 0.5 X TBE buffer solutions. The gels were allowed to cool down to about 45 ^{0} C and 10 µl of 5 mg/ml ethidium bromide was added, mixed together before pouring it into an electrophoresis chamber set with the combs inserted. After the gel has solidified, 3 µl of the DNA with 5µl sterile distilled water and 2 µl of 6X loading dye was mixed together and loaded in the well created. Electrophoresis was done at 80 V for 2 hours. The integrity of the DNA was visualized and photographed on UV light source.

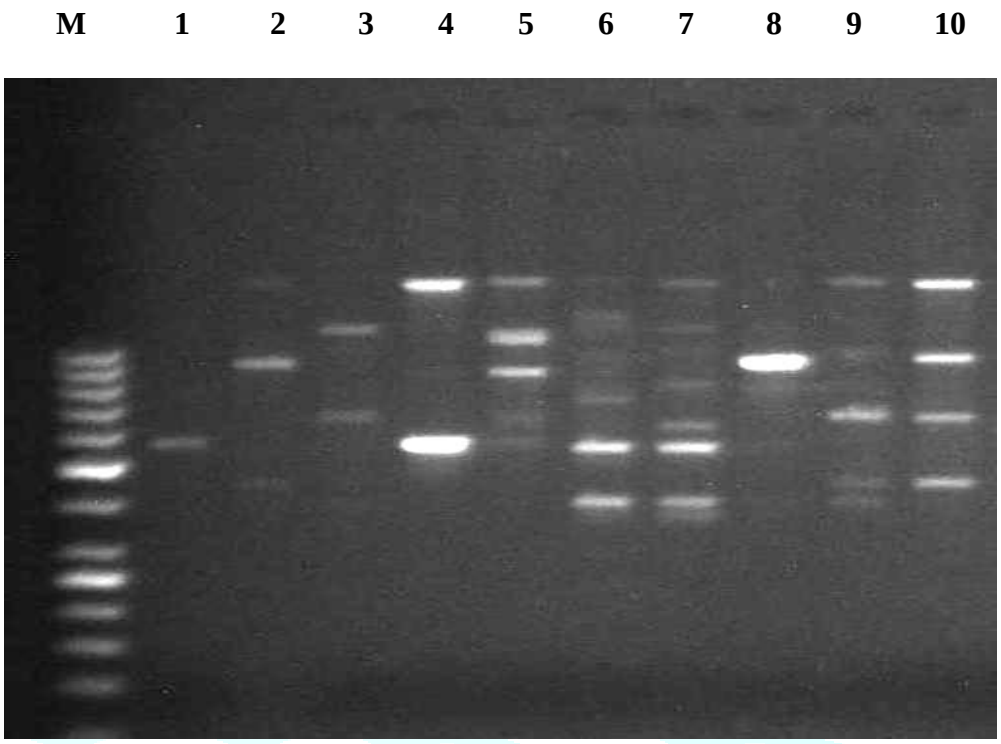

Plate 2.1: RAPD analysis carried out with primer OPB-02 on genomic DNA extracted from seed of 10 maize varieties.(1) SAMMAZ-28 (2) OBASUPER-2 (3) OBA-98(4) MDV-3 (5) TZPB-SR-W (6) ART/98/SW1-1 (7) SUWAN-1-SR-Y (8) BR-9928-DMR-SRY (9) BR-9943-DMR-SRW (10) SDM-2

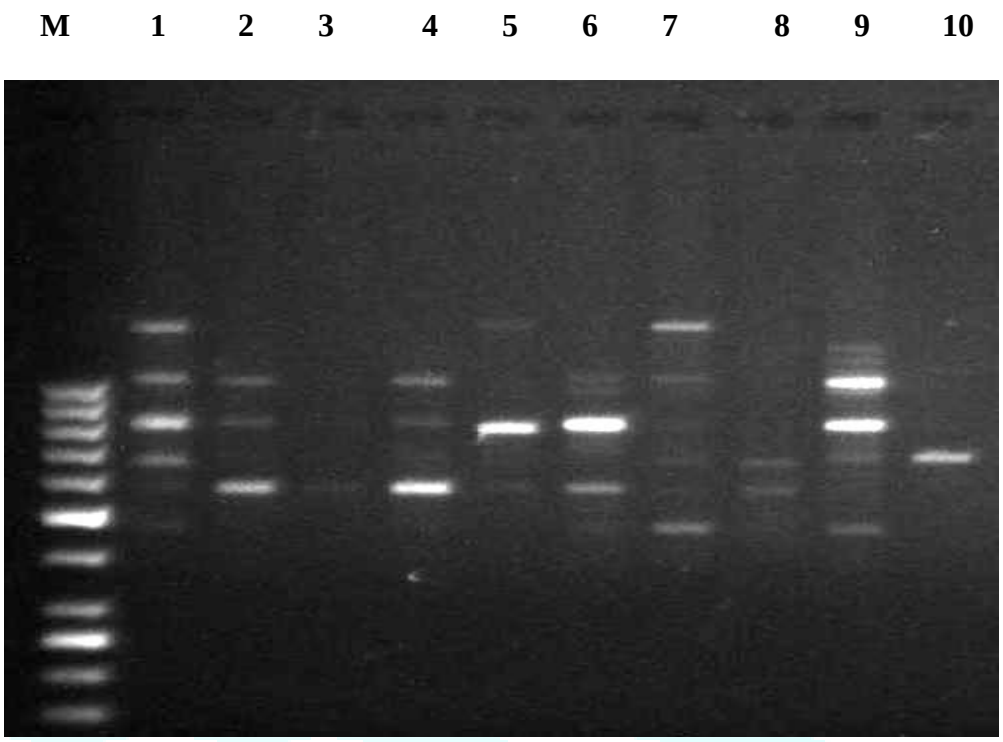

Plate 2.2: Gel Electrophoresis using RAPD analysis carried out with primer OPB-08 on genomic DNA extracted from seed of 10 maize varieties.(1) SAMMAZ-28 (2) OBASUPER-2 (3) OBA-98(4) MDV-3 (5) TZPB-SR-W (6) ART/98/SW1-1 (7) SUWAN-1-SR-Y (8) BR-9928-DMR-SRY (9) BR-9943-DMR-SRW (10) SDM-2

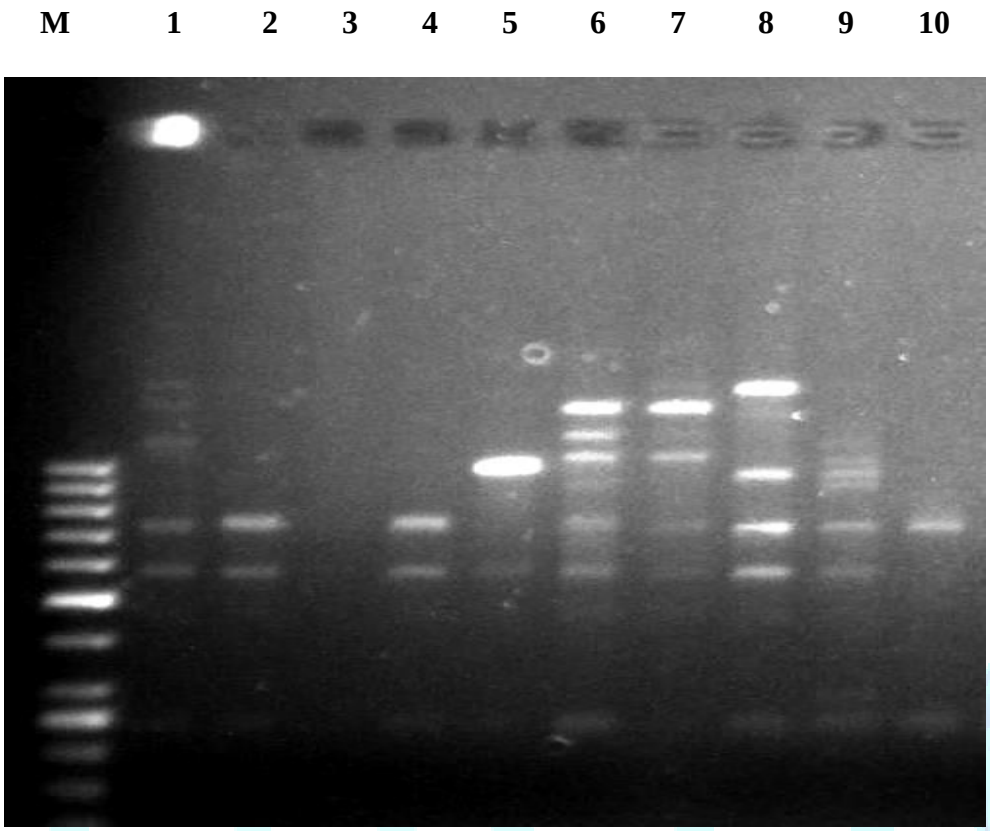

Plate 2.3: RAPD analysis carried out with primer OPB-16 on genomic DNA extracted from seed of 10 maize varieties. (1) SAMMAZ-28 (2) OBASUPER-2 (3) OBA-98(4) MDV-3 (5) TZPB-SR-W (6) ART/98/SW1-1 (7) SUWAN-1-SR-Y (8) BR-9928-DMR-SRY (9) BR-9943-DMR-SRW (10) SDM-2

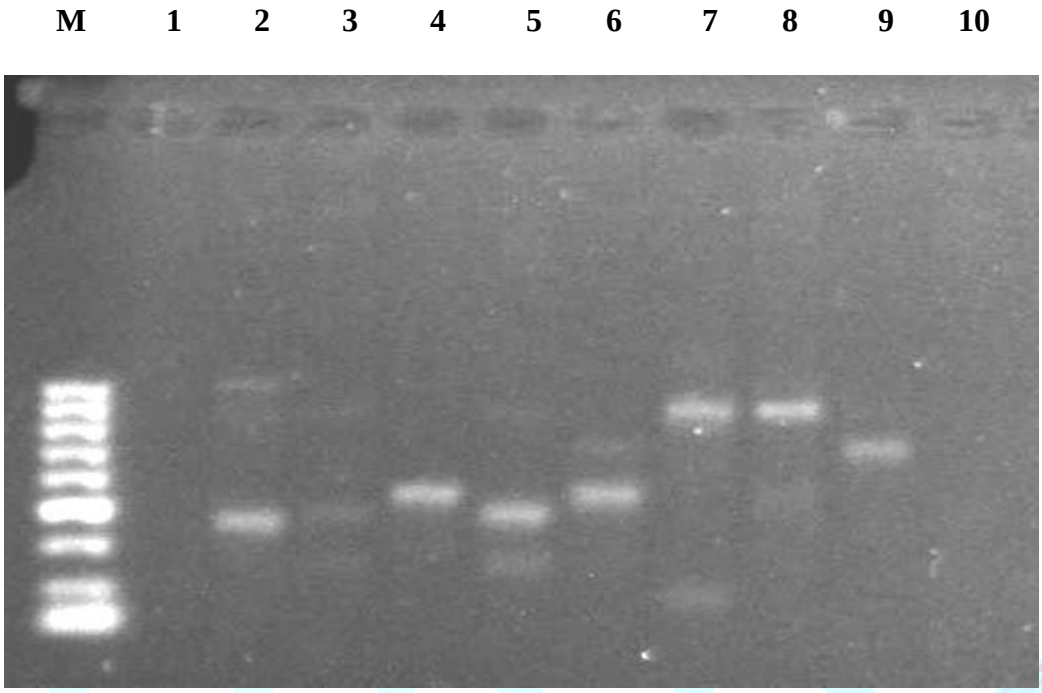

Plate 2.4: RAPD analysis carried out with primer OPH-02 on genomic DNA extracted from seed of 10 maize varieties. (1) SAMMAZ-28 (2) OBASUPER-2 (3) OBA-98(4) MDV-3 (5) TZPB-SR-W (6) ART/98/SW1-1 (7) SUWAN-1-SR-Y (8) BR-9928-DMR-SRY (9) BR-9943-DMR-SRW (10) SDM-2

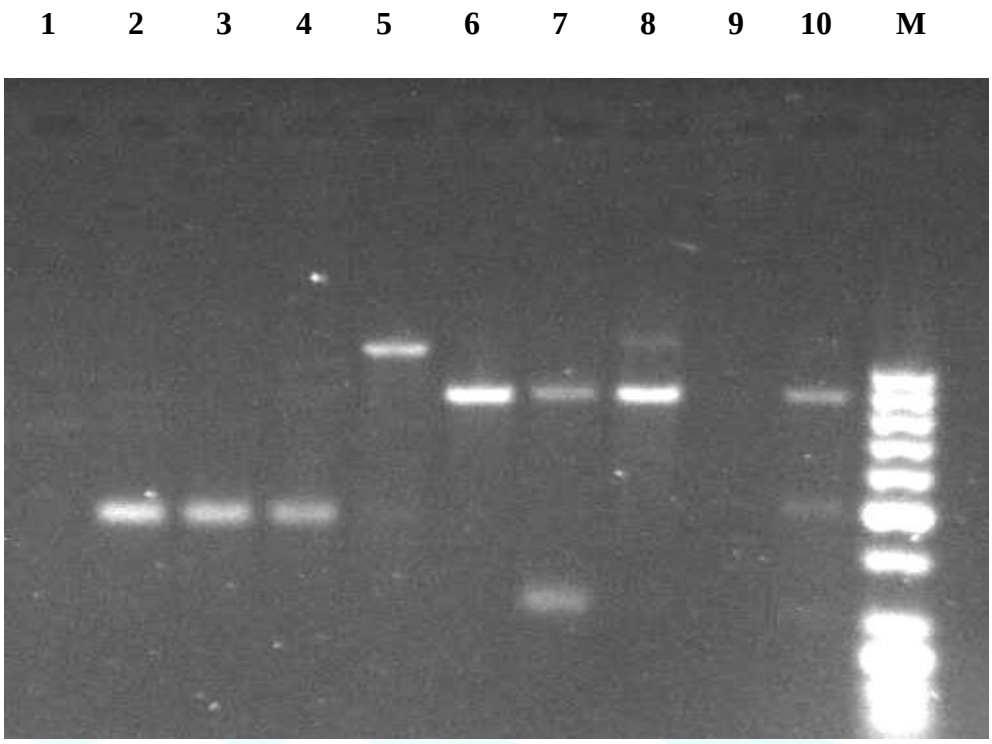

Plate 2.5: RAPD analysis carried out with primer OPH-12 on genomic DNA extracted from seed of 10 maize varieties.(1) SAMMAZ-28 (2) OBASUPER-2 (3) OBA-98(4) MDV-3 (5) TZPB-SR-W (6) ART/98/SW1-1 (7) SUWAN-1-SR-Y (8) BR-9928-DMR-SRY (9) BR-9943-DMR-SRW (10) SDM-2

3. RESULT AND DISCUSSION

3.1 RESULT

The genetic purity of maize seeds has received much attention. High quality DNA extractions are a prerequisite for genetic studies of a variety of plants including maize. DNA extracted is intended for further molecular analyses requiring high purity in order to obtain reliable and consistent results. For PCR analysis, DNA suitability is usually assessed measuring absorbance at 230, 260 and 280 nm. Mean squares from ANOVA showed that there were significant differences in the concentration, quality and purity of DNA of maize varieties. The concentration of DNA extracted from TZPB-SR-W variety

(260.18 mg/100g) was significantly higher (P < 0.05) than the other varieties with SDM-2 (235.16 mg/100g) having the least value.

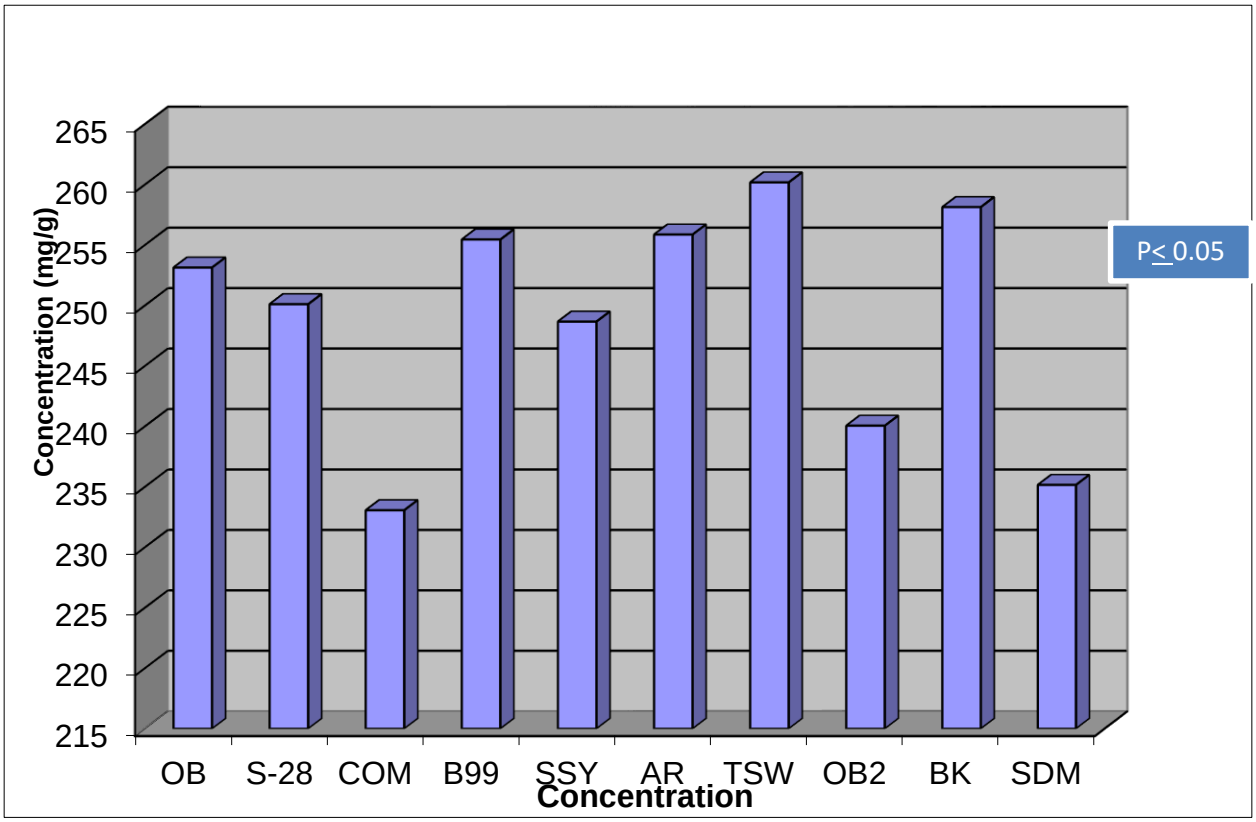

Fig. 3.1: composition of DNA concentration

OB =Oba 98, S-28= SAMMAZ -28. COM2 = MDV-3, B99 = BR99 43-DMR-SRW, SSY =SUWAN-1-SR-Y, AR= ART/98/SW1-1, TSW= TZPB-SR-W, OB2= Oba Super -2, BK=BR 9928DMR-SRY, SDM= SDM-2

The concentration of DNA extracted from TZPB-SR-W variety (260.18 mg/100g) was significantly higher (P < 0.05) than the other varieties with SDM-2 (235.16 mg/100g) having the least value.

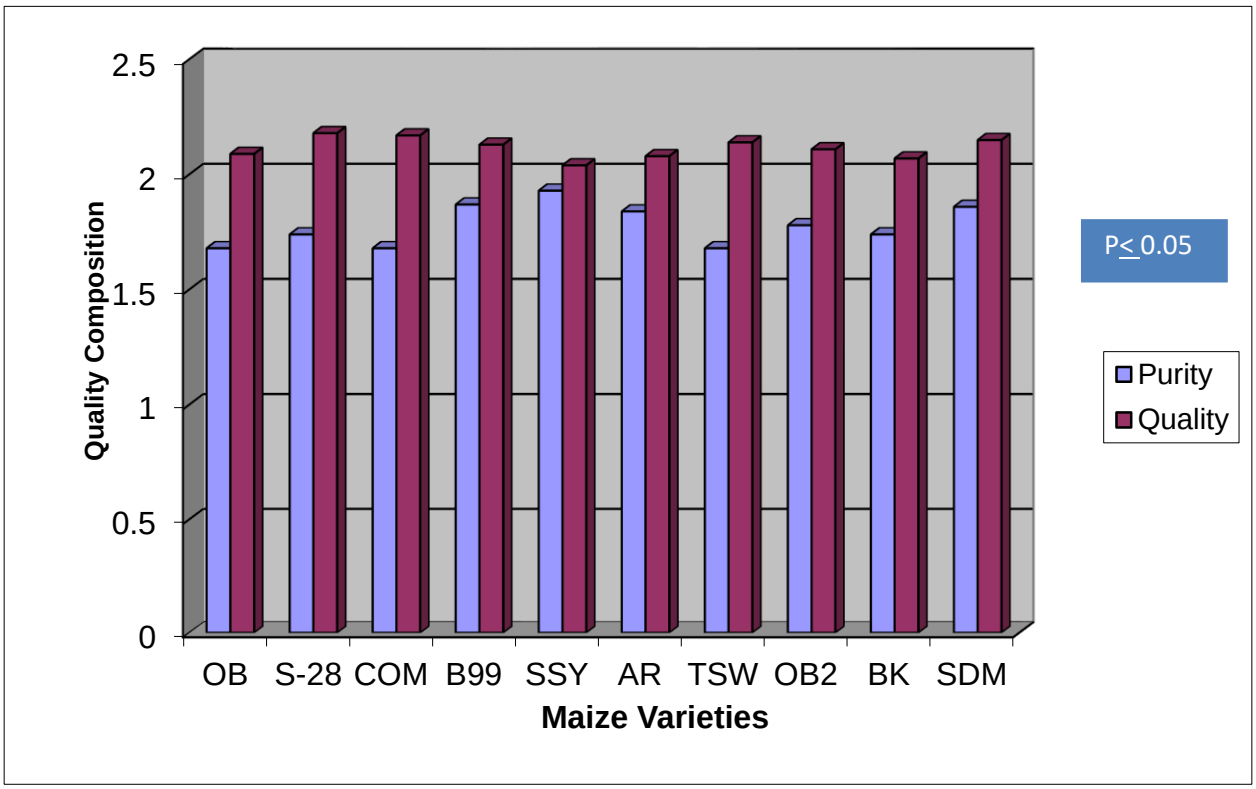

Fig. 3.2: composition of DNA quality and purity

OB =Oba 98, S-28= SAMMAZ -28. COM2 = MDV-3, B99 = BR99 43-DMR-SRW, SSY =SUWAN-1-SR-Y, AR= ART/98/SW1-1, TSW= TZPB-SR-W, OB2= Oba Super -2, BK=BR 9928DMR-SRY, SDM=SDM-2

DNA purity was significantly higher ($P<0.05$) in SUWAN-1-SR-Y variety (1.93) followed by BR9943-DMR-SRW (1.87) and SDM-2 (1.86) respectively while varieties Oba-98, MDV-3 and TZPB-SR-W with no significant differences had the least value (1.68) for purity. The quality of DNA was significantly higher ($P< 0.05$) in SAMMAZ-28 (2.18), MDV- 3 (2.17) and SDM-2 (2.15), followed by TZPB SR-W (2.14) while SUWAN-1-SR-Y had the least value (2.04).

3.2 DISCUSSION

A high A_{260}/A_{280} value is an indication of RNA contamination while a low A_{260}/A_{280} ratio indicates DNA contamination with proteins (Meyer, 2003). According to CRL (2007) a ratio of A_{260}/A_{280} greater than 1.7 indicates that sample is free from protein contamination while a ratio of A_{260}/A_{230} greater than 1.7 indicates that the sample is free from polysaccharide. A value below 1.7 is associated with the presence of carbohydrate and phenolic compounds that may interfere with PCR (Corbisier *et al*., 2007; Demeke *et al*., 2009; Gryson, 2010). However, with the exception of Oba- 98, MDV-3 and TZPBSR-W, all the other varieties recorded values above 1.7 for A_{260}/A_{230} indicating that the DNA may not have been contaminated with polysaccharide. Therefore, this result indicates that DNA extracted from all the varieties were not contaminated with RNA since DNA quality (A_{260}/A_{280}) recorded in most varieties is greater than 1.7 and this indicates that maize seed is capable of yielding high quality DNA.

The results obtained were similar to other reports concluding that methods yielding high DNA concentration might contain impurities that hinder proper amplification, as compared to those yielding a lower amount of DNA compromising yield in order to produce a more purified preparation (Terry *et al.*, 2002; Corbisier *et al.*, 2007). This is in agreement with the work of (Di Bernardo *et al*. (2007) and Smith *et al*. (2005) that CTAB method provided high DNA yield. Also, Wu *et al*. (2006) succeeded in using a rapid and cheap DNA extraction method to extract high quality genomic DNA from grains of two F1 maize hybrids and their parental lines for PCR.

CONCLUSION

The result obtained from this study established with the exception of Oba- 98, MDV-3 and TZPBSR-W, all the other varieties recorded values above 1.7 for A_{260}/A_{230} indicating that the DNA may not have been contaminated with polysaccharide. These results revealed that the extraction method applied to the maize grains produced DNA of suitable quantity, purity and quality for PCR amplification.

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