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# Biochemical Identification of Some Maize Genotypes

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**Abstract:** Laboratory experiments [Sodium Dodecyl Sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Randomly Amplified Polymorphic DNA (RAPD) PCR] were carried out during the two seasons of 2003 and 2004. The main objectives of this study were to estimate the level of polymorphism among six maize genotypes i.e. inbred lines Gemmeiza 2 (Gm 2), Sids 63 (Sd 63) and Sids 7 (Sd 7) as well as Single Cross 10 (SC 10), Single Cross 21 (SC 21) and Three-Ways Cross 321 (TWC 321) for rapid and accurate identification. Gemmeiza 2 (Gm 2) seeds characterized by proteins with molecular weight of 149.630, 110.644 and 69.362 kD. DNA pattern of Gm 2 using primers $B_{11}$, $B_{12}$, $B_{16}$, $B_{17}$ and $B_{18}$ produced 6, 4, 1, 11 and 10 bands, respectively. Sids 63 inbred line (Sd 63) had proteins with molecular weight of 166.518, 145.983, 128.639, 120.000, 78.366 and 72.526 kD. DNA pattern of Sd 63 using primers $B_{11}$, $B_{12}$, $B_{17}$, $B_{18}$ and $B_{20}$ produced 7, 13, 18, 19 and 9 bands, respectively. Sids 7 inbred line (Sd 7) had proteins with molecular weights of 228.169, 165.166, 136.830, 105.268 and 98.773 kD DNA pattern of Sd 7 using primers $B_{11}$, $B_{12}$, $B_{16}$, $B_{17}$ and $B_{18}$ produced 2, 9, 2, 5 and 4 bands, respectively. Single Cross 10 (SC 10) identified by proteins with molecular weights of 281.646, 258.554, 169.245, 142.449 and 121.572 kD. DNA pattern of SC 10 using primers $B_{11}$, $B_{12}$, $B_{14}$, $B_{17}$ and $B_{18}$ produced 5, 3, 1, 1 and 3 bands, respectively. Single Cross 21 (SC 21) was identified by proteins with molecular weights of 259.769, 173.383, 152.119, 134.686, 103.793, 100.656, 77.349 and 73.431 kD. DNA pattern of SC 21 using primers $B_{12}$, $B_{14}$, $B_{16}$, $B_{17}$ and $B_{18}$ produced 3, 1, 6, 9 and 5 bands, respectively. Three Ways Cross 321 (TWC 321) was characterized by proteins with molecular weights of 183.226, 155.934, 112.994 and 68.372 kD. DNA pattern of TWC 321 using primers $B_{11}$, $B_{12}$, $B_{14}$, $B_{17}$ and $B_{18}$ produced 5, 5, 6, 8 and 1 bands, respectively.

**Key words:** Egyptian maize genotypes, biochemical identification, PCR analysis, random primer

## INTRODUCTION

Maize (*Zea mays* L.) is one of the major cereal crops either in Egypt or all over the world. It is very essential for the human and animal feeding. National Maize Research Program, breeders and geneticists who are interested in corn improvement need conclusive information related to the identification of inbred lines, single cross and three-ways cross. The need for varietal identification or verification of varietal identity arises throughout the sequence of events from breeding, through variety release, pure-seed multiplication, varieties registration and sowing, seed quality control, processing of the harvested grain and marketing. The introduction of Plant Breeder's Rights has brought even more exacting requirements for genotype and distinctness testing in seed certification[1]. SDS Gel electrophoresis and isozymes can distinguish varieties within several species. They have been routinely used checking seed-lot purity in maize for the past 20 years. DNA-based technologies for checking seed and more recently developed methods that use the Polymerase Chain Reaction (PCR) can allow even more discriminative and faster identification of varieties. Biochemical identification raising genetic purity standards and enabling farmers and consumers better to utilize and benefit from increasing productive varieties that are bred from a more diverse base of genetic resources[2].

More recently, the use of specific proteins as markers of grain quality attributes, or other complex aspects of phenotype, takes gel electrophoresis beyond the function of genotype finger printing into the realm of selection for these attributes[1,3]. The breeder needs to be sure, that yield and quality evaluations have been performed on the same genotype. Electrophoretic identification can provide assurance that seed is true to label for sowing, but can also indicate the nature of off-type plants or strangers during propagation[4,5].

Laemmli[6] reported that seed protein electrophoresis becomes the technique of choice for laboratory assessment for the identification and characterization of different cultivars. He added that sodium dodecyle sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is

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considered a low cost, reproducible and rapid method for quantifying, comparing and characterizing proteins.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) reveals differences between lines and hybrids[7]. Poperelya *et al.*[8] suggested that electrophoretic patterns can be used to identity $F_1$ hybrids, as the patterns would have components from both parents and to determine the degree of hybridity in a seed lot. Wang *et al.*[9] PAGE system offer a promising approach for identifying cultivars and assessing maize genetic purity.

Gorinstein *et al.*[10] confirmed that electrophoretic patterns of the protein fractions are directly related to the genetic background of the protein and can be identified and used to certify the genetic makeup of wild cultivated, or newly derived cereals. Abdel-Tawab[11] found that protein system could be discriminate among all inbreds and to assign for each of them a unique fingerprint.

Ajmone-Marsan *et al.*[12] suggested that RAPD markers could be used for construction of genetic maps in maize and other polymorphic crops.

McDonald *et al.*[13] stated that the random amplified polymorphic DNA (RAPD) technique proved to be inexpensive, simple and fast, it avoids the use of growing plant tissue and can be applicable to a number of crops.

Sivolap *et al.*[14] found that analysis of the polymorphism of DNA amplification products enabled all the genotypes to be differentiated.

Moeller and Schaal[15] Guo *et al.*[16] and Shieh and Thseng[17] noticed that the RAPD analysis was a useful tool in determining the extent of genetic diversity among some maize inbred lines in the present case. EL-Hawary *et al.*[18] concluded that RAPD one of the DNA profiling techniques was used to identify inbred lines and the bands show different numbers for the investigated inbred lines and indicate differences in the molecular weights. Abdel-Tawab[11] concluded that the RAPD analysis gave unique markers that identified individual lines from each others and all of the inbred lines could be identified by those unique markers.

The aim of this investigation was to study the variability among studied maize genotypes using molecular methods of assessment to furnish standard uniform and definitive information of the characteristics of the genotypes under the environmental conditions of Dakahlia district.

## MATERIALS AND METHODS

Sodium Dodecyl Sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out under the laboratory condition of Seed Technology Research Department, Agriculture Research Center, Giza Governorate. The extracted protein grains samples of various genotypes were identified by SDS-PAGE according to the method of Laemmli[6] as follows:

### Reagents

**Monomer solution:** This stock solution was prepared by dissolving acrylamide (30 g), N, N-methylene bis acrylamide (0.8 g) in distilled water (100 mL). The insoluble material was removed by filtration under vacuum and the filtrate solution was kept at 4°C in a dark bottle as it is stable under these conditions.

**Sodium dodecyl sulphate (SDS):** Stock solution was prepared by dissolving SDS (10 g) in distilled water (100 mL). This solution was clear and stable at room temperature.

**Ammonium peroxide sulphate solution (APS 2%, w/v):** This solution was prepared by dissolving ammonium peroxide sulphate (0.2 g) in distilled water (10 mL) and kept at 4°C. This solution is unstable and freshly prepared just before use.

**Spacer gel buffer (1 M Tris, pH 6.8):** Tris (hydroxy methyl) methylamine (9 g) was dissolved in distilled water (50 mL) and the pH was adjusted to 6.8 by HCl solution (6 N), the volume was then completed to 100 mL by distilled water.

**Separating gel buffer (1.5 M Tris, pH 8.8):** Tris (hydroxy methyl) methylamine (27.23 g) was dissolved in distilled water (50 mL) and the pH was adjusted to 8.8 by HCl solution (6 N), the volume was completed to 100 mL by distilled water.

**Electrode buffer solution (running solution):** This buffer consisted of tris (6 g), glycine (28.8 g), SDS (2.09 g) and dissolved in distilled water (2 L). This stock solution was kept at 4°C.

**Commassie R-250 brilliant blue staining solution:** Staining solution was prepared as follows: Commassie brilliant blue R-250 (1 g), methanol (500 mL), glacial acetic acid (70 mL) and distilled water up to 1000 mL. This stock solution kept at room temperature in a dark bottle.

**Destaining solution:** This solution was prepared by mixing methanol (1.25 mL), glacial acetic acid (40 mL) and distilled water (700 mL).

**Sample buffer:** The sample buffer consisted of tris-HCl (1.25 mL, pH 6.8), SDS (2 mL, 10%), glycin (2 mL, 78%),

bromophenol blue (2 mL, 0.1%), mercaptoethanol (0.5 mL) and distilled water (2.25 mL).

**Preparation of samples:** Sample extract 400 μL equivalent to about 0.2 0.D per 10 μL was added to 400 μL sample buffer, thoroughly mixed and heated in a boiling water bath for 5 to 10 min, then centrifuged at 1000 rpm. for 5 min. The supernatant was kept at 4°C for analysis.

**Preparation of protein markers:** Three protein markers were used to characterize the proteins of the samples. These protein markers were:

- Protein testmishung 4 (SERVA) P4 were consisted of carbonhydrase 29.000 kD ovalbumine 45.000 kD, Bovin Serum Albumin (BSA) 67.000 kD and phosphorylase B 92.000 kD.
- Protein testmishung 5 (SERVA) P5 was comprised of trypsin inhibitors (lung) 6.500 kD, cytochrome c 12.000 kD, trypsin inhibitor (soybean) 21.000 kD and carbonhydrase 29.000 kD.
- Low molecular weight (pharmacia) [LMW] contained phosphorylase B 94.000 kD, bovin serum albumin (BSA) 67.000 kD, ovalbumine 43.000 kD, carbonic anhydrase 30.000 kD, trybsin inhibitor (soy bean) 20.000 kD and alfa-lactabumin 14.000 kD. All of the three protein markers were dissolved in the sample buffer and heated in the same manners of the sample extract.

**Application of samples:** A known sample of volume (20-30 μL) was applied to each slot (0.5X1.6X0.1 cm) and air bubbles were avoided in order to obtain sharp separation.

**Gel preparation:** The gel was prepared as quantitatively as show in Table 1. Two resolving gels were immediately poured into the space between glass plates to a height of 1.5 cm bellow bottom of comb (12 cm) and isopropanol was overland and left to polymerize for about 30 min. Two staking gels immediately poured over the two resolving gels. The gels were left for about 15 min to polymerize. The comb was removed after the upper buffer tank was filled with running buffer.

**Electrophoresis conditioning:** Low buffer tank was filled with running buffer and attached with upper buffer tank so that the gels were completely covered. The electrodes were connected to power supply and adjusted at 100 V until the bromophenol blue dye intered the resolving gel. The power was then increased to 250 V until the bromophenol blue reached the bottom of the resolving gel. The small triangle of one corner gel was cut so the orientation is not lost during staining.

Table 1: Composition of separating and stacking gels

| Stock solution | Separation gel (10%) | Stacking gel (5.6%) |
|---|---|---|
| Monomer solution | 10 mL | 1.67 mL |
| Resolving buffer (pH 8.8) | 3.75 mL | - |
| Stacking gel buffer (pH 6.8) | - | 2.5 mL |
| Distilled water | 14 mL | 5.19 mL |
| SDS (10%, w/v) | 300 μL | 100 μL |
| APS (10%, w/v) | 1.5 mL | 500 μL |
| TEMED | | |
| N,N,N,N-tetramethyl thylenediamine | 300 μL | 10 μL |
| Final volume | 30 mL | 10 mL |

**Detection of proteins**

**Gel staining:** The gels were placed in reclosable plastic bags containing staining solution.

**Gel destaining:** The gels were placed in plastic bags containing destaining solution and agitated gently on a shaker. The destaining solution was changed several times until the gel background was clear.

**Standard curve:** The protein migration (cm) was plotted against the protein lower molecular weight (LMW) protein to obtain standard curve of molecular weight.

**Scanning:** The scanning of polyacrylamide gel and analysis of the results was accomplished using color flat bed scanner (Epson GT 8000) connected with a computer and printer. The software used was scan Peck II. The estimation of molecular weight of different protein bands was automatically done by the aid of a protein marker.

RAPD markers assays are based on the PCR amplification of random locations in the plant genome. The DNA amplification protocol was performed as described by Williams *et al.*[19] with some modification.

**DNA isolation:** DNA isolation from plant tissues was done using DNA easy plant Mini kit (QIAGEN Hilden, Germany). Protocol for isolation of DNA was done as follows:

- Plant tissues were ground using liquid nitrogen to a fine powder using a mortar and pestle. Then the powder was transferred to an appropriately sized tube and the liquid nitrogen was allowed to evaporate.
- Then, 400 μL of buffer AP1 and 4 μL of RNase A stock solution (100 mg $mL^{-1}$) were added to a maximum of 100 mg of ground plant tissues and vortexed vigorously.
- Mixture was incubated for 10 min at 65°C and mixed 2-3 times during incubation by inverting tube.
- Then, 130 μL of buffer AP2 was added to the lysate, mixed and incubated for 5 min on ice.

- Lysate was applied to the QiA shredder spin column sitting in a 2 mL collection tube and centrifuged for 2 min at 1400 rpm.
- Flow-through fraction from step 5 was transferred to a new tube without disturbing the cell-debris pellet typically, 450 µL of lysate was recovered.
- Then, 0.5 volume of buffer AP3 and 1 volume of ethanol (96-100%) were added to the cleared lysate and mixed by pipetting.
- Then, 650 µL of the mixture from step 7 was applied through DNeasy Mini spin column sitting in a 2 mL collection tube. Then, centrifuged for 1 min at 8000 rpm and flow-through was then discarded.
- DNeasy column was then placed in a new 2 mL collection tube. Then, 500 µL of buffer AW was added onto the DNeasy column and centrifuged for 1 min at 8000 rpm. Flow-through was discarded and the collection tube in step 10 was reused.
- Then, 500 µL of buffer AW was added to DNeasy column and centrifuged for 2 min at 14000 rpm to dry the column membrane.
- DNeasy column was then transferred to a 1-5 mL microfuge tube and µL of preheated (65°C) buffer AE was pipetted directly onto the DNeasy column membrane. Then, incubated for 5 min at room temperature and centrifuged for 1 min at room temperature and centrifuged for 1 min at 8000 rpm to elute the purified DNA from the DNeasy spin column.
- Elution (step 11) was repeated once as described. A new microfuge tube can be used for first elute. Alternatively the microfuge tube can be reused for the second elution step to combine the elutes.

**Randomly Amplified Polymorphic DNA (RAPD):** RAPD-PCR reactions were conducted using 7 arbitrary 10-mer primers. Their names and sequences are shown in Table 2.

The reaction conditions according to Williams *et al.*[19] were optimized and mixtures (30 µL total volume) consisted as follows :

| | |
|---|---|
| dNTPs (2.5 mM) | 0.2 mM |
| $MgCl_2$ (25 mM) | 1.5 mM |
| 10 X buffer | 3.0 µL |
| Primer (10 µM) | 0.2 µM |
| Template DNA (50 ng $\mu L^{-1}$) | 2.0 µL |
| Taq (5 µ $\mu L^{-1}$) | 0.3 µL |
| d $H_2O$ | up to 30 µL |

Amplification was carried out in a PTC-200 thermal cycler (MJ Research, Watertown, USA) programmed for 40 cycles as follows: 94°C/4 min (1 cycle); 94°C/30 sec: 36°C/1 min; 72°C/2 min (40 cycles); 72°C/ 10 min (1 cycle) and 4°C (infinitive).

Table 2: Primer names and sequences from 5 to 3 direction

| Primer name | Sequence (5 to 3) |
|---|---|
| $B_{11}$ | GTAGACCCGT |
| $B_{12}$ | CCTTGACGCA |
| $B_{14}$ | TCCGCTCTGG |
| $B_{16}$ | TTTGCCCGGA |
| $B_{17}$ | AGGGAACGAG |
| $B_{18}$ | CCACAGCAGT |
| $B_{20}$ | GGACCCTTAC |

**Gel electrophoresis:** Agarose (1.2%) was used for resolving the PCR products. One kb DNA ladder was used as standard DNA. Molecular weights in kb of the resulted 10 bands are: 12, 10, 8, 6, 4, 3, 2, 1.6, 1 and 0.5.

**TBE buffer (10 X):**

| | |
|---|---|
| Tris | 108.00 g |
| Boric acid | 55.00 g |
| EDTA | 7.44 g |
| d $H_2O$ | p to 1 L |

**Gel preparation:**

| | |
|---|---|
| Agarose | 1.2 g |
| TBE buffer (1 X) | 100 mL |
| Ethidium bromide (10 µg/µL) | 1.5 µL |

**Sample preparation:**

| | |
|---|---|
| PCR-product | 15 µL |
| Loading buffer (6 X) | 3.0 µL |

**Loading buffer (6 X):**

| | |
|---|---|
| Bromophenol blue | 0.25 g |
| Xylene cyanol | 0.25 g |
| Glycerol (30%) | 100 mL |

The gels were run at 100 V for about 2 h. After electrophoresis, the RAPD patterns were visualized with an UV transilluminator. RAPD markers were scored from the gels as DNA fragments present in one lane and absent in the other(s). The gels were photographed using image analyzer device.

## RESULTS AND DISCUSSION

**Protein identification:** Results of SDS-PAGE patterns of seed protein fraction of the studied maize genotypes (Table 3 and Fig. 1) indicated distinct differences between them the banding patterns were 14, 18, 18, 20, 20 and 14 bands of major proteins of Gm 2, Sd. 63, Sd 7, SC 10, SC 21 and TWC 321, respectively. Gm 2 seeds characterized

Table 3: SDS polyacrylamide gel electrophoresis (SDS-PAGE) of identified genotypes

| Protein MW (kD) | Gm 2 | Sd 63 | Sd 7 | SC 10 | SC 21 | TWC 321 |
|---|---|---|---|---|---|---|
| 281.646 | | | | + | | |
| 259.769 | | | | | + | |
| 258.554 | | | | + | | |
| 228.169 | | | + | | | |
| 220.877 | | | | + | + | + |
| 199.000 | | | + | + | | |
| 196.120 | + | + | | | + | |
| 183.226 | | | | | | + |
| 173.383 | | | | | + | |
| 169.245 | | | | + | | |
| 166.518 | | + | | | | |
| 165.166 | | | + | | | |
| 155.934 | | | | | | + |
| 152.119 | | | | | + | |
| 149.630 | + | | | | | |
| 145.983 | | + | | | | |
| 142.449 | | | | + | | |
| 136.830 | | | + | | | |
| 134.686 | | | | | + | |
| 128.639 | | + | | | | |
| 121.572 | | | | + | | |
| 120.000 | | + | | | | |
| 119.513 | + | | + | | | |
| 118.539 | | | | | + | + |
| 114.426 | | | + | + | | |
| 113.470 | | + | | | + | |
| 112.994 | | | | | | + |
| 110.644 | + | | | | | |
| 107.010 | | + | | | + | |
| 105.268 | | | + | | | |
| 104.526 | | + | | + | | |
| 103.793 | | | | | + | |
| 102.369 | + | | + | + | | + |
| 100.656 | | | | | + | |
| 98.773 | | | + | | | |
| 96.430 | | | + | + | + | + |
| 95.859 | + | + | | | | |
| 93.446 | | | + | + | | |
| 92.429 | | + | | | + | |
| 91.539 | + | | | + | | |
| 86.782 | + | + | + | + | + | + |
| 85.625 | + | + | + | + | + | + |
| 84.536 | + | + | + | + | + | + |
| 83.446 | + | + | + | + | + | + |
| 82.650 | | + | | | + | |
| 80.247 | + | | | + | | |
| 79.332 | | | + | | | + |
| 78.366 | | + | | | | |
| 77.349 | | | | | + | |
| 74.530 | + | | + | + | | + |
| 73.431 | | | | | + | |
| 72.526 | | + | | | | |
| 69.362 | + | | | | | |
| 68.372 | | | | | | + |
| 59.212 | | + | + | + | | |
| No. of total bands | 14 | 18 | 18 | 20 | 20 | 14 |
| No. of distinguish bands | 3 | 6 | 5 | 5 | 8 | 4 |

by proteins with molecular weights of 149.630, 110.644 and 69.362 kD Sd 63 grains had proteins with molecular weights of 166.518, 145.983, 128.639, 120.000, 78.366 and 72.526 kD Sd 7 seeds characterized by proteins

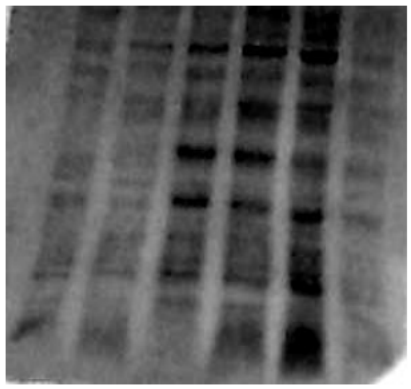

Fig. 1: SDS polyacrylamide gel electrophoresis (SDS-PAGE) of identified genotypes.

with molecular weights of 228.169, 165.166, 136.830, 105.268 and 98.773 kD SC 10 seeds distinguished by proteins with molecular weights of 281.646, 258.554, 169.245, 142.449 and 121.572 kD SC 21 seeds contained proteins with molecular weights of 259.769, 173.383, 152.119, 134.686, 103.793, 100.656, 77.349 and 73.431 kD TWC 321 seeds had proteins with molecular weights of 183.226, 155.934, 112.994 and 68.372 kD. These results were in line with those reported by Koranyi[7], Poperelya *et al.*[8], Wang *et al.*[9], Gorinstein *et al.*[10] and Abdel-Tawab[11].

**Polymerase Chain Reaction (PCR):** As shown from Table 4 and Fig. 2a, primer 1 produced a maximum of 6 bands at the molecular sizes which ranged between 1437.3 to 3142.9 bp. Primer 2 gave a total of 4 bands ranged from 836.6 to 2228.8 bp. Primer 3 exhibited one band at the molecular size of 2695.5 bp. Primer 4 produced a total of 11 bands at the molecular sizes which ranged between 824.5 to 3283.9 bp. Primer 5 gave a maximum of 10 bands at the molecular sizes which ranged between 867.8 to 3611.3 bp.

The results clearly indicated that primer 1 exhibited a maximum of 7 bands at the molecular sizes that ranged between 1500 to 4341 bp. Primer 2 produced a total of 13 bands ranged from 1027 to 4353 bp. Primer 3 gave a total of 18 bands at the molecular sizes which ranged between 1500 to 7040 bp. Primer 4 produced a maximum of 19 bands at the molecular sizes that ranged from 1422 to 7217 bp. Primer 5 resulted a total of 9 bands at the molecular sizes which ranged between 1739 to 6102 bp (Table 5 and Fig. 2b).

The results showed that primer 1 gave a total of 2 bands at the molecular sizes which were 864 and 2056 bp. Primer 2 produced a maximum of 9 bands ranged from 693 to 5415 bp. Primer 3 exhibited a maximum of 2 bands which were 2530 and 3769 bp. Primer 4 gave a total of 5 bands at the molecular sizes that ranged

Table 4: DNA pattern of Gm 2 inbred line using 5 primers

| Molecular weight (bp) | Number of primer 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1 | 3142.9 | 2228.8 | 2695.5 | 3283.9 | 3611.3 |
| 2 | 2816.4 | 1639.5 | | 2900.0 | 2900.0 |
| 3 | 2041.6 | 1223.7 | | 2637.0 | 2656.4 |
| 4 | 1738.2 | 836.6 | | 2415.5 | 2363.1 |
| 5 | 1580.6 | | | 1911.6 | 2133.2 |
| 6 | 1437.3 | | | 1675.8 | 1816.2 |
| 7 | | | | 1479.9 | 1615.7 |
| 8 | | | | 1395.8 | 1375.6 |
| 9 | | | | 1197.1 | 1065.0 |
| 10 | | | | 1088.6 | 867.8 |
| 11 | | | | 824.5 | |
| Total | 6 | 4 | 1 | 11 | 10 |

Sequences of primers used

| No. of primer | Primer name | Sequence |
|---|---|---|
| 1 | $B_{11}$ | GTAGACCCGT |
| 2 | $B_{12}$ | CCTTGACGCA |
| 3 | $B_{16}$ | TTTGCCCGGA |
| 4 | $B_{17}$ | AGGGAACGAG |
| 5 | $B_{18}$ | CCACAGCAGT |

Table 5: DNA pattern of Sd. 63 inbred line using 5 primers

| Molecular weight (bp) | Number of primer 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1 | 4341 | 4353 | 7040 | 7217 | 6102 |
| 2 | 3944 | 4116 | 6458 | 6814 | 4710 |
| 3 | 3024 | 3627 | 5262 | 5696 | 4161 |
| 4 | 2413 | 3124 | 4675 | 4908 | 4013 |
| 5 | 2152 | 2837 | 4450 | 4687 | 3737 |
| 6 | 1879 | 2631 | 4205 | 4450 | 3558 |
| 7 | 1500 | 2239 | 4052 | 4192 | 2957 |
| 8 | | 1912 | 3833 | 3917 | 2533 |
| 9 | | 1656 | 3640 | 3695 | 1739 |
| 10 | | 1519 | 3477 | 3384 | |
| 11 | | 1453 | 3265 | 3111 | |
| 12 | | 1231 | 2674 | 2815 | |
| 13 | | 1027 | 2533 | 2631 | |
| 14 | | | 2381 | 2468 | |
| 15 | | | 2207 | 2250 | |
| 16 | | | 2033 | 2076 | |
| 17 | | | 1771 | 1836 | |
| 18 | | | 1500 | 1656 | |
| 19 | | | | 1422 | |
| Total | 7 | 13 | 18 | 19 | 9 |

Sequences of primers used

| No. of primer | Primer name | Sequence |
|---|---|---|
| 1 | $B_{11}$ | GTAGACCCGT |
| 2 | $B_{12}$ | CCTTGACGCA |
| 3 | $B_{17}$ | AGGGAACGAG |
| 4 | $B_{18}$ | CCACAGCAGT |
| 5 | $B_{20}$ | GGACCCTTAC |

between 481 to 3131 bp. Primer 5 produced a total of 4 bands at the molecular sizes which ranged between 1844 to 3769 bp (Table 6 and Fig. 2c)

Primer 1 exhibited a maximum of 5 bands at the molecular sizes which ranged between 599.1 and 2577.6 bp. Primer 2 produced a total of 3 bands ranged from 796.3 to 1869.3 bp. Primer 3 and primer 4 gave one band at 796.3 and 2114.6 bp, respectively. Primer 5

Table 6: DNA pattern of Sd 7 inbred line using 5 primers

| Molecular weight (bp) | Number of primer 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1 | 2056 | 5415 | 3769 | 3131 | 3769 |
| 2 | 864 | 3682 | 2530 | 2428 | 3033 |
| 3 | | 2056 | 1801 | 2428 | |
| 4 | | 1938 | 844 | 1844 | |
| 5 | | 1671 | 481 | | |
| 6 | | 1460 | | | |
| 7 | | 1025 | | | |
| 8 | | 851 | | | |
| 9 | | 693 | | | |
| Total | 2 | 9 | 2 | 5 | 4 |

Sequences of primers used

| No. of primer | Primer name | Sequence |
|---|---|---|
| 1 | $B_{11}$ | GTAGACCCGT |
| 2 | $B_{12}$ | CCTTGACGCA |
| 3 | $B_{16}$ | TTTGCCCGGA |
| 4 | $B_{17}$ | AGGGAACGAG |
| 5 | $B_{18}$ | CCACAGCAGT |

Table 7: DNA pattern of SC 10 using 5 primers

| Molecular weight (bp) | Number of primer 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1 | 2577.6 | 1869.3 | 796.3 | 2114.6 | 2324.9 |
| 2 | 1460.9 | 1197.1 | | | 1531.8 |
| 3 | 1038.4 | 796.3 | | | 1291.5 |
| 4 | 917.9 | | | | |
| 5 | 599.1 | | | | |
| Total | 5 | 3 | 1 | 1 | 3 |

Sequences of primers used

| No. of primer | Primer name | Sequence |
|---|---|---|
| 1 | $B_{11}$ | GTAGACCCGT |
| 2 | $B_{12}$ | CCTTGACGCA |
| 3 | $B_{14}$ | TCCGCTCTGG |
| 4 | $B_{17}$ | AGGGAACGAG |
| 5 | $B_{18}$ | CCACAGCAGT |

produced a total of 3 bands at the molecular sizes which ranged between 1291.5 to 2324.9 bp (Table 7 and Fig. 2d).

The results showed that primer 1 gave a total of 3 bands at the molecular sizes which ranged between 2566 to 4236 bp. Primer 2 produced only one band at the molecular sizes of 2911 bp. Primer 3 exhibited a maximum of 6 bands at the molecular sizes that ranged between 2550 to 6736 bp. Primer 4 resulted in a total of 9 bands at the molecular sizes which ranged between 2112 to 6525 bp. Primer 5 gave a total of 5 bands at the molecular sizes ranged between 3378 to 5784 bp (Table 8 and Fig. 2e).

It is obvious from Table 9 and Fig. 2f that primer 1 gave a total of 5 bands at the molecular sizes which ranged between 197.21 to 656.09 bp. Primer 2 produced a maximum of 5 bands at the molecular sizes that ranged from 447.52 to 1783.80 bp. Primer 3 exhibited a maximum of 6 bands ranged from 82.57 to 1345.91 bp. Primer 4 gave a total of 8 bands at the molecular sizes which ranged between 127.61 to 1738.71 bp. Primer 5 produced one

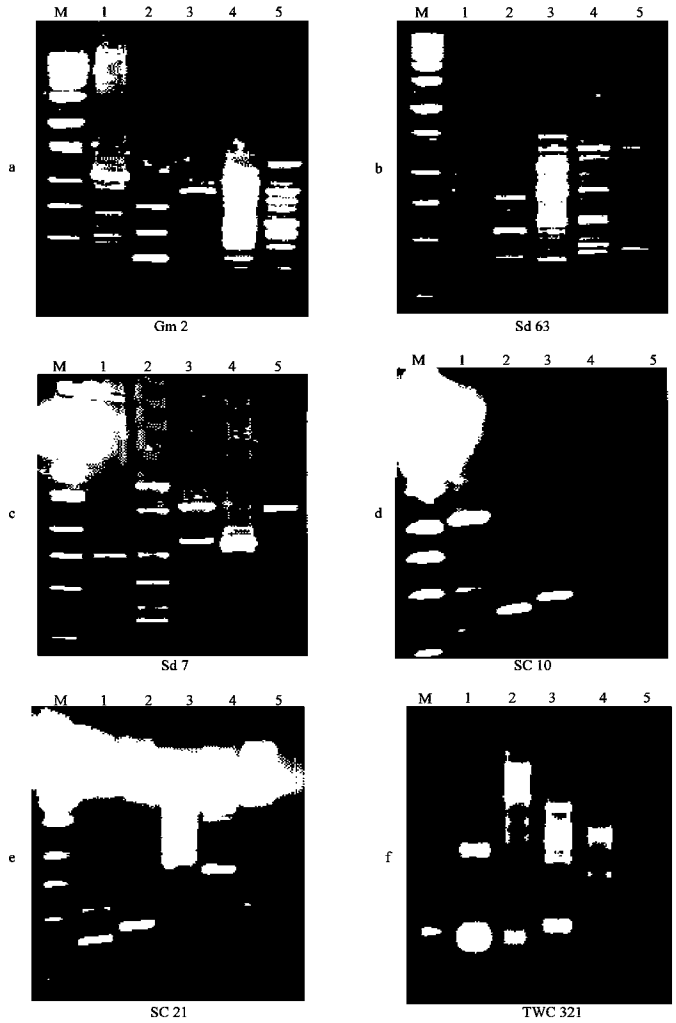


Fig. 2 a-f : DNA Pattern of Gm2, Sd63, Sd7, SC10, SC21 and TWC321 genotypes, using 5 primers

Table 8: DNA pattern of SC 21 using 5 primers

| Molecular weight (bp) | Number of primer 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1 | 4236 | 2911 | 6736 | 6525 | 5784 |
| 2 | 3226 | | 5722 | 6095 | 5203 |
| 3 | 2566 | | 5300 | 5632 | 4573 |
| 4 | | | 4857 | 5355 | 3695 |
| 5 | | | 3915 | 4480 | 3378 |
| 6 | | | 2550 | 3789 | |
| 7 | | | | 2729 | |
| 8 | | | | 2542 | |
| 9 | | | | 2112 | |
| Total | 3 | 1 | 6 | 9 | 5 |

Sequences of primers used

| No of primer | Primer name | Sequence |
|---|---|---|
| 1 | $B_{12}$ | CCTTGACGCA |
| 2 | $B_{14}$ | TCCGCTCTGG |
| 3 | $B_{16}$ | TTTGCCCGGA |
| 4 | $B_{17}$ | AGGGAACGAG |
| 5 | $B_{18}$ | CCACAGCAGT |

Table 9: DNA pattern of TWC 321 using 5 primers

| Molecular weight (bp) | Number of primer 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1 | 656.09 | 1783.80 | 1345.91 | 1738.71 | 709.56 |
| 2 | 508.65 | 1345.91 | 827.40 | 1154.22 | |
| 3 | 364.62 | 1041.85 | 425.18 | 940.42 | |
| 4 | 248.33 | 640.48 | 312.69 | 578.12 | |
| 5 | 197.21 | 447.52 | 178.01 | 483.25 | |
| 6 | | | 82.57 | 393.74 | |
| 7 | | | | 235.93 | |
| 8 | | | | 127.61 | |
| Total | 5 | 5 | 6 | 8 | 1 |

Sequences of primers used

| No of primer | Primer name | Sequence |
|---|---|---|
| 1 | $B_{11}$ | GTAGACCCGT |
| 2 | $B_{12}$ | CCTTGACGCA |
| 3 | $B_{14}$ | TCCGCTCTGG |
| 4 | $B_{17}$ | AGGGAACGAG |
| 5 | $B_{18}$ | CCACAGCAGT |

band at the molecular size which was 709.65 bp. Moeller and Schaal[15] Guo *et al.*[16], EL-Hawary *et al.*[18] and Abdel-Tawab[11] confirmed these results.

It could be concluded that PAGE-patterns and RAPD markers can be identified and determine genetic diversity among maize genotypes which in turn support quality control and keep the purity of the superior maize genotypes under multiplication.

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