

## CHARACTERIZATION OF TUNISIAN CASTOR BEAN GENOTYPES USING SDS-PAGE OF TOTAL SEED STORAGE PROTEINS

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### ABSTRACT

The objectives of this work, were to find out the level of genetic variability present in 56 Tunisian castor germplasm by using the electrophoretic profiles of total seed proteins with different molecular weights through SDS-PAGE. Fifty-six castor (*Ricinus communis* L.) genotypes were used in the present study. Seeds of castor were obtained from the University of Carthage, National Institute of Research in Rural Engineering, Waters and Forests (INRGRF), Regional Station of Gabès, Tunisia. Storage proteins were extracted from individual grains by the standard reference electrophoretic method by ISTA in the presence of sodium dodecyl sulfate (SDS-PAGE). Out of twenty-nine polypeptide bands, 5 (18%) were commonly present in all accessions and considered as monomorphic, while 24 (82%) showed variations and considered as polymorphic. The size of the protein bands obtained through SDS-PAGE ranged from 30 to 180 kDa. On the basis of banding profiles of proteins of different kDa, gel was divided into zones A, B and C. The major protein bands were lied in zones A and B, while minor bands were present in zones C. The dendrogram tree demonstrated the relationship among the 56 Tunisian castor genotypes according to the similarity index, using UPGMA cluster analysis. The dendrogram was divided into three main clusters. Similarly the present study of genetic variability in the seed storage polypeptide determined by SDS-PAGE technique proved that it is fruitful to identify genetic diversity among accessions of castor.

**Keywords:** castor; Tunisian genotypes; dendrogram; protein markers; SDS-PAGE

### INTRODUCTION

Castor bean (*Ricinus communis* L.), also known as castor oil bean, mole bean and wonder tree, is a member of Spurge family (*Euphorbiaceae*) which is originated from tropical Africa and is currently cultivated as an oilseed crop and also grown as an ornamental plant in many countries of Asia, Central and North America, Africa and Europe (Doan, 2004; Aslani et al., 2007).

Castor bean has recently been highly rated as a source of raw material (oil) for biodiesel production, because beyond its high oil content (25 – 55%), it is a culture of great social appeal in Brazil by intensive use of workmanship in the field and allows for intercropping with other crops as beans, groundnuts or maize (Madail et al., 2007; Lacerda et al., 2014). The castor bean contains 40% oil, 1 – 5% ricin and 0.3 – 0.8% ricinin (Johnson et al., 2005).

In mature castor seed, 90 – 95% of the total seed protein is in the endosperm. Castor seeds contain two toxins called ricin and *Ricinus communis* agglutinin (Hartley and Lord, 2004). Ricin is a ribosome inactivating protein that is manufactured in the endosperm. It is a small dipeptide molecule (approx. 65 kDa) containing both an A chain (-

32 kDa) and a B chain (~32 – 34 kDa) linked together by a disulphide bond (Kumar et al., 2004). The A chain of ricin is a ribosome-inactivating protein (Lord et al., 1994; Cheema et al., 2010).

The most common method used to determine the molecular size of a protein is SDS-PAGE analysis (Cheema et al., 2010; Malook et al., 2016). In this analysis, proteins are denatured using SDS and then, moved across a polyacrylamide matrix on an electric field, known as electrophoresis. So far, several investigations on the discrimination between crop genotypes using SDS-PAGE have been carried out by Yoon et al., (2010); Osman et al., (2013); Iqbal et al., (2014); Iqbal et al., (2014); Khan et al., (2014); AL-Huqail et al., (2015); Gregova et al., (2015); Kačmárová et al., (2016); Socha et al., (2016); Vivodík et al., (2018).

### Scientific hypothesis

The objectives were to find out the level of genetic variability present in 56 Tunisian castor germplasm by using the electrophoretic profiles of total seed proteins with different molecular weights through SDS-PAGE.

## MATERIAL AND METHODOLOGY

Fifty-six castor (*Ricinus communis* L.) genotypes were used in the present study. Seeds of castor were obtained from the University of Carthage, National Institute of Research in Rural Engineering, Waters and Forests (INRGRF), Regional Station of Gabès, Tunisia. The ricin genotypes were obtained from 12 regions of Tunisia: S-Souassi (5 genotypes), BT- Bouthay (4 genotypes), GH-Ghomrassen (5 genotypes), BA- Sidi bou ali (5 genotypes), MT- Matmata (4 genotypes), AG- Mateur (5 genotypes), N- Nefza (4 genotypes), MD- Mednine (5 genotypes), M-Mornag (5 genotypes), G- Gabes (4 genotypes), K- Kebili (5 genotypes), KJ- Ksar jedid (5 genotypes).

Storage proteins were extracted from individual grains by the standard reference electrophoretic method by ISTA in the presence of sodium dodecyl sulfate (SDS-PAGE) (Wrigley, 1992). Storage proteins were extracted from individually ground seeds using extracting using a buffer composed of 6.25 mL Tris (1.0 mol.L<sup>-1</sup>, pH = 6.8), 10 mL glycerol, 12.05 mL H<sub>2</sub>O and 2.0 g SDS, diluted with mercaptoethanol and H<sub>2</sub>O in a 17:3:40 (v/v) proportion. The buffer was added to flour in a 1:25 (w/v) proportion. Extraction was performed at room temperature overnight and heating in boiled water for 5 minutes, centrifugation at 5000 x g for 5 min. 10 µL of extracts were applied to the sample wells. The gel (1.0 mm thick) consists of two parts: stacking gel (3.5% acrylamide, pH = 6.8 acrylamide) and resolution gel (10% acrylamide, pH = 6.8). Staining of gels was performed in a solution of Coomassie Brilliant Blue R250 dissolved in acetic acid and methanol solution. Gel was scanned with densitometer GS 800 (Bio-Rad) and evaluated with Quantity One-1D Analysis Software.

### Statistic analysis

A dendrogram was constructed based on hierarchical cluster analysis using the unweighted pair group method with arithmetic average (UPGMA).

## RESULTS AND DISCUSSION

The number of total scorable protein bands was twenty-nine as a result of SDS-PAGE technique but those that were not consistent in reproducibility and showed occasional variation in sharpness and density were not considered. Based on these bands 56 accessions of castor were screened. Out of twenty-nine polypeptide bands, 5 (18%) were commonly present in all accessions and considered as monomorphic, while 24 (82%) showed variations and considered as polymorphic. The size of the protein bands obtained through SDS-PAGE ranged from 30 to 180 kDa.

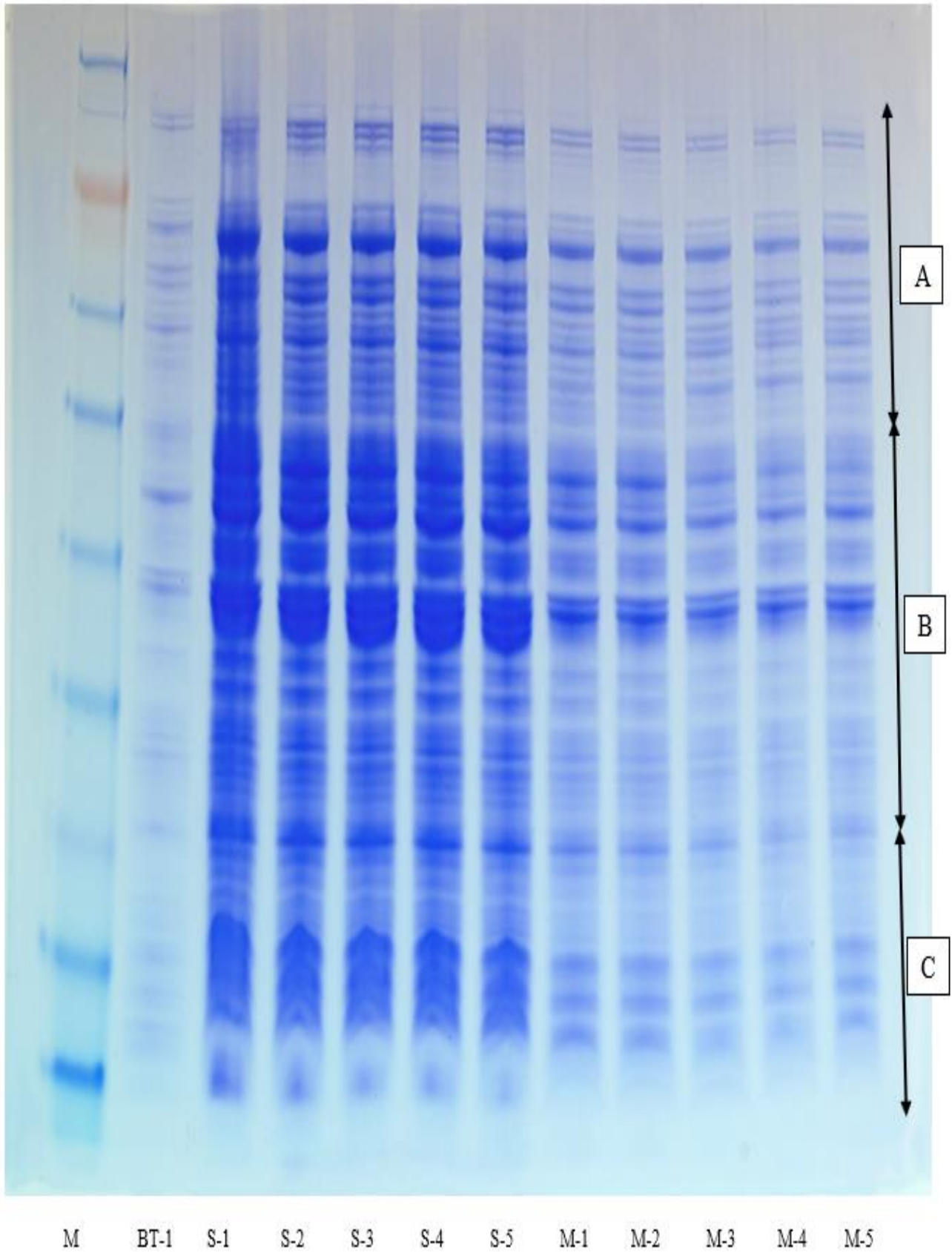
On the basis of banding profiles of proteins of different kDa, gel was divided into zones A, B and C (Figure 1). The major protein bands were lied in zones A and B, while minor bands were present in zones C. It was noted that different accessions of castor showed more diversity in seed storage proteins in minor bands in comparison to major bands. In zone A out of 13 protein bands, 3 were monomorphic and 10 were polymorphic. In zone B out of 10 protein bands, 2 was monomorphic and 8 was polymorphic and in zone C out of 6 protein bands, 1 were

monomorphic whereas 5 polymorphic. By considering these facts zone A and B were more polymorphic.

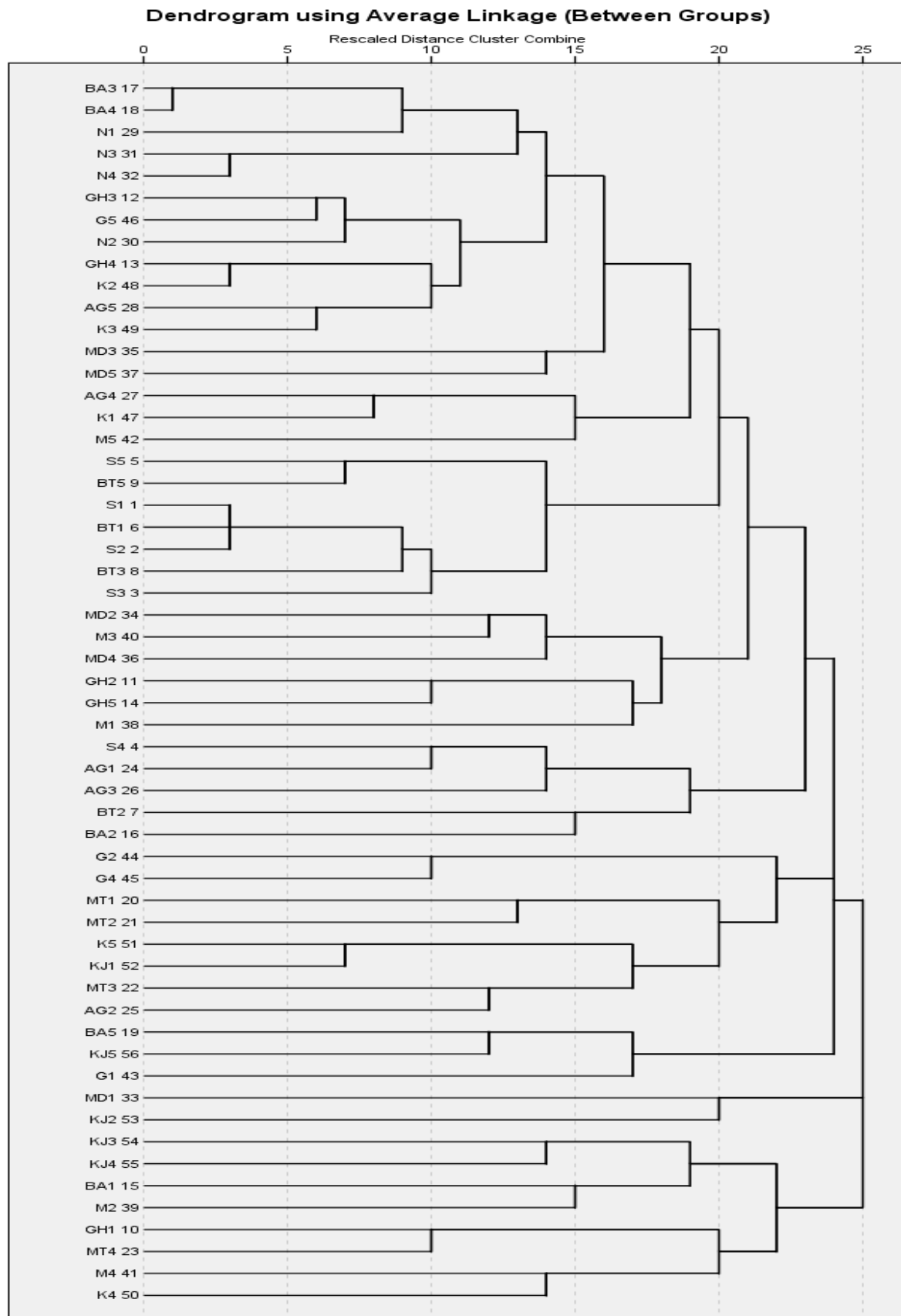
The dendrogram tree (Figure 2) demonstrated the relationship among the 56 Tunisian castor genotypes according to the similarity index, using UPGMA cluster analysis. The dendrogram was divided into three main clusters. The first one contained eight genotypes from castor, while the second cluster contained the two genotypes of Tunisian castor (MD 1 and KJ 2). Cluster 3 was divided into 3 subclusters – 3A, 3B and 3C. Subcluster 3A contained three genotypes (BA 5, KJ 5 and G 1), subcluster 3B contained eight genotypes of Tunisian castor and subcluster 3C contained 35 Tunisian castor genotypes (Figure 2).

Similar results were detected by other authors (Cheema et al., 2010; Arslan et al., 2012; Lacerda et al., 2014; Riaz and Farrukh, 2014; Schieltz et al., 2015; Brandon et al., 2016; Malook et al., 2016; Rao et al., 2017) and these results presented a high level of polymorphism of Tunisian castor genotypes detected by SDS-PAGE.

The objective of Lacerda et al. (2014) was to study the parameters of an extraction process of protein from castor bean cake by solubilisation in alkaline medium. Initially, the castor bean cake was ground, sieved and submitted to chemical analyses in order to determine its composition. The present work of Cheema et al. (2010) was conducted to see the feasibility of electrophoresis for intra-specific characterization of castor bean on the basis of their total seed storage proteins. To facilitate the analysis of castor (*Ricinus communis* L.) seed fractions and germplasm for ricin content, Brandon et al. (2016) investigated the use of enzyme-linked immunosorbent assay (ELISA) methods to differentiate between ricin toxin and the related *Ricinus communis* agglutinin (RCA). Schieltz et al. (2015) used liquid chromatography and MRM-MS to determine rRNA N-glycosidase activity for each cultivar and the overall activity in these cultivars was compared to a purified ricin standard. Riaz and Farrukh (2014) analyzed sixteen different medicinal castor oil samples for contamination with ricin toxin. The classical and gel filtration methods for extraction of purified ricin were adopted. In the present study Malook et al. (2016) observed the biochemical and molecular characterization of castor bean (*Ricinus communis* L.) collected from different climatic zones of Pakistan (Lahore, Peshawar, Rawalpindi, Dera Ismail Khan, Swat and Kohat). The protein banding pattern of all 6 accessions was found same and no specific variation was noticed among the proteins of high molecular weight. Arslan et al. (2012) study the genetic diversity of wild castor bean genotypes collected from the eastern Mediterranean region of Turkey was evaluated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of seed storage proteins. Five distinct groups were identified from the cluster analysis of the castor bean genotypes studied at 0.80 coefficient level. Rao et al. (2017) analyzed six varieties, nine hybrids of castor and their parents on the basis of electrophoresis of total soluble seed proteins.



**Figure 1** Protein profile showing total seed storage proteins in Tunisian castor genotypes as a result of SDS-PAGE.



**Figure 2** Dendrogram of 56 Tunisian castor genotypes prepared based on protein marker. Note: S- Souassi (5 genotypes), BT- Bouthay (4 genotypes), GH- Ghomrassen (5 genotypes), BA- Sidi bou ali (5 genotypes), MT- Matmata (4 genotypes), AG- Mateur (5 genotypes), N- Nefza (4 genotypes), MD- Mednine (5 genotypes), M- Mornag (5 genotypes), G- Gabes (4 genotypes), K- Kebili (5 genotypes), KJ- Ksar jedid (5 genotypes).

## CONCLUSION

Seeds of castor were obtained from the University of Carthage, National Institute of Research in Rural Engineering, Waters and Forests (INRGREF), Regional Station of Gabès, Tunisia. The dendrogram was divided into three main clusters. The first one contained eight genotypes from castor, while the second cluster contained the two genotypes of Tunisian castor (MD 1 and KJ 2). Cluster 3 was divided into 3 subclusters – 3A, 3B and 3C. Result from this study show that protein markers are powerful and efficient in characterising and identifying of castor genotypes in addition to their usefulness in phylogenetic studies.

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