GENETIC DIVERGENCE IN TUNISIAN CASTOR BEAN GENOTYPES BASED ON TRAP MARKERS

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ABSTRACT
In the present study, the representatives of the genus Ricinus communis collected from 12 different parts of Tunisia were differentiated by the DNA fingerprinting patterns using 30 TRAP primers. The efficacy of the TRAP technique in this study is further supported by the obtained PIC values of the primers used in the analysis. PCR amplification of DNA using 30 primers for TRAP analysis produced 490 DNA fragments that could be scored in all 56 genotypes of Tunisian castor. The number of amplified fragments varied from 3 (TRAP 04 x arb 1, TRAP 22 x arb 3 and TRAP 23 x arb 3) to 13 (TRAP 56 x arb 2), and the amplicon size ranged from 100 to 1600 bp. Of the 490 amplified bands, 377 were polymorphic, with an average of 5.71 polymorphic bands per primer. To determine the level of polymorphism in the analysed group of Tunisian castor genotypes polymorphic information content (PIC) was calculated. The lowest values of polymorphic information content were recorded for TRAP 10 x arb 1 (0.555) and the highest PIC values were detected for TRAP 44 x arb 2 (0.961) with an average of 0.770. A dendrogram was constructed from a genetic distance matrix based on profiles of the 30 TRAP primers using the unweighted pair-group method with the arithmetic average (UPGMA). According to analysis, the collection of 56 Tunisian castor genotypes were clustered into five main clusters. Moreover, functional TRAP markers would be efficiently useful in genetic studies for castor genetic improvement.

Keywords: castor; DNA; dendrogram; PCR; PIC

INTRODUCTION
Castor bean (syn. castorbean, castor, castor-oil-plant), Ricinus communis L. (2n = 20, X = 10), is a species of flowering plant in the spurge family, Euphorbiaceae. It is an oilseed crop cultivated mainly in India, Mozambique, Brazil, and China (FAOSTAT, 2014). The seeds of castor bean have around 35 – 55% oil, and the commercial standard is 44%. The oil percentage of the seeds varies depending on the cultivation environment and the cultivar (Costa and Ramos, 2004). The hydroxylated fatty acid ricinoleic is approximately 80 – 90% of the total fatty acids, which gives castor bean oil (ricin oil) unique chemical and physical properties. Ricin oil is a renewable resource and raw material with various industrial applications (e.g., to manufacture paints, lubricants, cosmetics, pharmaceutical drugs, dyes, anilines, disinfectants, germicides, low-temperature lubricating oils, glues and adhesives, fungicide and insecticide bases, printing inks and varnishes, nylon and plastic), and more recently its use as biodiesel has been explored (Mutlu and Meier, 2010).

Now that some of these lineages have been developed, there is a need to study the genetic divergence among them. The genetic divergence among genotypes of any species can be evaluated with molecular markers, for example, target region amplification polymorphism (TRAP) markers. TRAP markers are functional markers that allow combining fixed and specific primers with arbitrary primers (Hu and Vick, 2003). These markers have high levels of polymorphism, which makes them a promising option for the genotypification of germplasm and identification of genes related to desirable agronomic characteristics. Besides, TRAP markers optimize the genetic gains in genetic improvement programs and are a valuable tool used by these programs to study genetic divergence (Agarwal et al., 2008).

Genetic diversity in castor bean was assessed by using both dominant and codominant molecular markers (random amplified polymorphic DNA, RAPD) (Reddy, Nadigatla and Mulpuri, 2015; Vivodík et al., 2015), inter-simple sequence repeats (ISSR) (Wang et al., 2013; Vasconcelos et al., 2016), start codon targeted (SCoT) (Kallamadi et al., 2015; Reddy, Nadigatla and Mulpuri, 2015; Reddy, Nadigatla and Mulpuri, 2015).
amplicon size ranged from 100 to 1600 bp. Of the 490 amplified bands, 377 were polymorphic, with an average of 5.71 polymorphic bands per primer. Results indicated the presence of wide genetic variability among different genotypes of Tunisian castor. To determine the level of polymorphism in the analysed group of Tunisian castor genotypes polymorphic information content (PIC) was calculated. The lowest values of polymorphic information content were recorded for TRAP 10 x arb 1 (0.555) and the highest PIC values were detected for TRAP 44 x arb 2 (0.961) with an average of 0.770.

A dendrogram was constructed from a genetic distance matrix based on profiles of the 30 TRAP primers using the unweighted pair-group method with the arithmetic average (UPGMA). According to analysis, the collection of 56 Tunisian castor genotypes were clustered into five main clusters (Figure 1). Cluster 1 contained 2 unique genotypes of Tunisian castor (K-4 and BA-5) from different regions of Tunisia and cluster 2 was divided into two subclusters (2a and 2b). Subcluster 2a contained 7 genotypes of castor and subcluster 2b contained 4 genotypes of Tunisian castor. Cluster 3 contained 6 genotypes of Tunisian castor and cluster 4 was divided into 3 subclusters (4a, 4b, and 4c). Subcluster 4a contained 2 genotypes of Tunisian castor and subcluster 4b contained 6 genotypes of Tunisian castor and subcluster 4c contained 29 genotypes of Tunisian castor. (Figure 1).

TRAP markers were used by other authors (Miklas et al., 2006; Hu et al., 2007; Yu et al., 2007; Alwala et al., 2008; Kwon et al., 2010; Yue et al., 2010; Andru et al., 2011; Barakat et al., 2013; Cheng et al., 2013; Crotti-Franco et al., 2014; Kumar et al., 2014; Carmo et al., 2015; Feng et al., 2015; Luo et al., 2015; Dias Kanthack Junior et al., 2020).

Statistical analysis
A dendrogram based on hierarchical cluster analysis using the unweighted pair group method with arithmetic average (UPGMA) with the SPSS professional statistics version 17 software package was constructed. For the assessment of the polymorphism between genotypes ricin and usability RAPD markers in their differentiation, we used polymorphic information content (PIC) (Weber, 1990).
Zhang et al. (2013) assessment the genetic diversity and variation of Pinellia ternata collected from 43 populations in China using SRAP þ TRAP markers. A total of 13 SRAP primers in addition to 3 TRAP primer combinations yielded 292 bands in a total of which 286 were polymorphic (98.0%), with an average of 16 for each. The PIC value ranged from 0.88 to 0.95, with a mean polymorphic information content (PIC) of 0.92 over all the primers.

Luo et al. (2013) developed and characterized sequence tags (ESTs)-simple sequence repeats (SSRs) and targeted region amplified polymorphism (TRAP) markers to examine genetic relationships in the persimmon genus Diospyros gene pool. In total, we characterized 14 EST-SSR primer pairs and 36 TRAP primer combinations, which were amplified across 20 germplasms of 4 species in the genus Diospyros. Liu et al. (2016) study the genetic structure and genetic diversity among and within the 21 populations using target region amplified polymorphism (TRAP) and simple sequence repeat (SSR) markers. Sixteen pairs of TRAP primers generated a total of 398 fragments, of which 396 (99.50%) were polymorphic; fourteen pairs of SSR primers generated a total of 60 fragments, of which 59 (98.33%) were polymorphic. Al-Murish et al. (2013) study efficiency of SRAP, TRAP, and SSR primers in detecting genetic variation among 17 C. arabica genotypes collected from the different valleys of Yafea City, Yemen, and estimate genetic similarity coefficients among these genotypes and classify them according to genetic relationships. The results of the present study demonstrated the presence of genetic variation among coffee genotypes within and between valleys.

In this study, Liu et al. (2015) estimated the genetic relationships within P. aibuhitensis using Target Region Amplified Polymorphisms (TRAP) and Amplified Fragment Length Polymorphisms (AFLP) that were derived from related populations on the coasts of China.

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**Table 1** Characterization of the fixed primers (target region amplification polymorphisms, TRAPs) used to genotype 56 lineages of Tunisian castor bean.

| TRAP Primer | Sequence (5' – 3') |
|-------------|--------------------|
| 1. TRAP 01  | CCACATCCACGCACCTTTTG |
| 2. TRAP 02  | TGTTGAGGCTTGTGAGATTTC |
| 3. TRAP 03  | TGTCTGAGGCAAGATAC |
| 4. TRAP 04  | TGTCCTATTTTGGCCACG |
| 5. TRAP 15  | CCGTGATCTTGTGTTGAG |
| 6. TRAP 16  | TTACACTTGGCGACTTCC |
| 7. TRAP 10  | CCGGTTGCCAACAGTACTAG |
| 8. TRAP 11  | GGCGGAGCTATCTGTTGAA |
| 9. TRAP 22  | CAGCTGTGCCTGAGCAGCT |
| 10. TRAP 23 | AGCAAGCAGCAGCTACAG |
| 11. TRAP 24 | GTCCAGAAGCAAGCCACCT |
| 12. TRAP 25 | CCACCAAAACCCACGAGT |
| 13. TRAP 19 | AATTCCGACAGCTACAG |
| 14. TRAP 30 | CTTTCAAGTGGCCACGTC |
| 15. TRAP 31 | CCACCAATGAAACCACTAG |
| 16. TRAP 32 | TGCCGCTCTCTTCCTTCCT |
| 17. TRAP 35 | CCTCATCAGTGTTGCTGCT |
| 18. TRAP 27 | CGAAAATCTTCCTGCTTCT |
| 19. TRAP 28 | GCCACATCTCTACACAG |
| 20. TRAP 37 | GCTCAGCAGCTGACTCAT |
| 21. TRAP 39 | GCCACCAAAACCTCCACCT |
| 22. TRAP 40 | CTTCCACCACAGCCTACAC |
| 23. TRAP 44 | TCCTGTCAATGCTGAACC |
| 24. TRAP 46 | CCAGTCCAGTGTGTTGCT |
| 25. TRAP 49 | TCTTGTCCTAATGCTGAAC |
| 26. TRAP 51 | CCACCGAGAGAGCATACCA |
| 27. TRAP 52 | GTGGCAAATGTCTCAGAGT |
| 28. TRAP 53 | TACACTTCCGCTGGTTGGA |
| 29. TRAP 55 | TGATGAAACACCTTGGTGAA |
| 30. TRAP 56 | CTTTGCGCTTACACACTGC |

**Table 2** Arbitrary primers used to genotype the 56 lineages of Tunisian castor bean.

| Arbitrary primers | Nucleotide sequence (3' – 5') |
|-------------------|-----------------------------|
| 1. arb 1          | GACTGCGGTACGAATTTGAC        |
| 2. arb 2          | GACTGCGGTACGAATTTGGA        |
| 3. arb 3          | GACTGCGGTACGAATTTGCA        |
| 4. arb 4          | GACTGCGGTACGAATTTGACC       |
| 5. arb 5          | GACTGCGGTACGAATTTGCC        |
| 6. arb 6          | GACTGCGGTACGAATTTGACC       |
### Table 3

Combinations of target region amplification polymorphism (TRAP) primers selected to analyze the polymorphism in 56 lineages of Tunisian castor bean.

| Combinations         | Total fragments | Polymorphism fragments | PIC    |
|----------------------|-----------------|------------------------|--------|
| TRAP 01 x arb 1      | 8               | 6                      | 0.896  |
| TRAP 02 x arb 1      | 10              | 8                      | 0.689  |
| TRAP 03 x arb 1      | 6               | 6                      | 0.874  |
| TRAP 04 x arb 1      | 3               | 2                      | 0.755  |
| TRAP 15 x arb 1      | 8               | 8                      | 0.854  |
| TRAP 16 x arb 1      | 9               | 6                      | 0.668  |
| TRAP 10 x arb 1      | 11              | 5                      | 0.555  |
| TRAP 11 x arb 1      | 4               | 4                      | 0.899  |
| TRAP 22 x arb 1      | 5               | 4                      | 0.877  |
| TRAP 23 x arb 1      | 8               | 7                      | 0.788  |
| TRAP 24 x arb 1      | 12              | 8                      | 0.786  |
| TRAP 25 x arb 1      | 6               | 5                      | 0.854  |
| TRAP 19 x arb 1      | 5               | 5                      | 0.869  |
| TRAP 30 x arb 1      | 9               | 6                      | 0.789  |
| TRAP 31 x arb 1      | 9               | 5                      | 0.745  |
| TRAP 32 x arb 2      | 7               | 4                      | 0.658  |
| TRAP 35 x arb 2      | 7               | 7                      | 0.780  |
| TRAP 27 x arb 2      | 4               | 3                      | 0.754  |
| TRAP 28 x arb 2      | 8               | 6                      | 0.666  |
| TRAP 37 x arb 2      | 11              | 7                      | 0.731  |
| TRAP 39 x arb 2      | 12              | 9                      | 0.591  |
| TRAP 40 x arb 2      | 6               | 6                      | 0.781  |
| TRAP 44 x arb 2      | 5               | 4                      | 0.961  |
| TRAP 46 x arb 2      | 9               | 8                      | 0.812  |
| TRAP 49 x arb 2      | 8               | 8                      | 0.739  |
| TRAP 51 x arb 2      | 9               | 6                      | 0.630  |
| TRAP 52 xarb 2       | 4               | 3                      | 0.891  |
| TRAP 53 x arb 2      | 6               | 5                      | 0.709  |
| TRAP 55 x arb 2      | 12              | 11                     | 0.900  |
| TRAP 56 x arb 2      | 13              | 10                     | 0.810  |
| TRAP 01 x arb 3      | 11              | 9                      | 0.712  |
| TRAP 02 x arb 3      | 9               | 9                      | 0.890  |
| TRAP 03 x arb 3      | 6               | 5                      | 0.731  |
| TRAP 04 x arb 3      | 8               | 8                      | 0.912  |
| TRAP 15 x arb 3      | 7               | 4                      | 0.611  |
| TRAP 16 x arb 3      | 8               | 6                      | 0.723  |
| TRAP 10 x arb 3      | 9               | 5                      | 0.600  |
| TRAP 11 x arb 3      | 6               | 4                      | 0.599  |
| TRAP 22 x arb 3      | 3               | 3                      | 0.896  |
| TRAP 23 x arb 3      | 3               | 3                      | 0.879  |
| TRAP 24 x arb 3      | 8               | 8                      | 0.911  |
| TRAP 25 x arb 3      | 4               | 3                      | 0.823  |
| TRAP 19 x arb 3      | 4               | 3                      | 0.818  |
| TRAP 30 x arb 3      | 5               | 5                      | 0.910  |
| TRAP 31 x arb 3      | 5               | 5                      | 0.901  |
| TRAP 32 x arb 4      | 4               | 3                      | 0.801  |
| TRAP 35 x arb 4      | 6               | 5                      | 0.780  |
| TRAP 27 x arb 4      | 8               | 6                      | 0.699  |
| TRAP 28 x arb 4      | 9               | 6                      | 0.689  |
| TRAP 37 x arb 4      | 7               | 4                      | 0.609  |
| TRAP 39 x arb 4      | 7               | 7                      | 0.839  |
| TRAP 40 x arb 4      | 7               | 7                      | 0.798  |
| TRAP 44 x arb 4      | 8               | 5                      | 0.698  |
| TRAP 46 x arb 4      | 11              | 8                      | 0.801  |
| TRAP 49 x arb 4      | 10              | 6                      | 0.639  |
| TRAP 51 x arb 4      | 11              | 7                      | 0.709  |
| TRAP 52 x arb 4      | 9               | 6                      | 0.806  |
| TRAP 53 x arb 4      | 6               | 6                      | 0.809  |
| TRAP 55 x arb 4      | 5               | 5                      | 0.796  |
| TRAP 56 x arb 4      | 4               | 3                      | 0.908  |
| TRAP 01 x arb 5      | 4               | 3                      | 0.869  |
| TRAP 02 x arb 5      | 8               | 5                      | 0.703  |
| TRAP 03 x arb 5      | 7               | 6                      | 0.666  |
| TRAP 04 x arb 6      | 9               | 5                      | 0.599  |
| TRAP 15 x arb 6      | 10              | 5                      | 0.669  |
| TRAP 16 x arb 6      | 10              | 6                      | 0.759  |

**Averages:** 7.42, 5.71, 0.770

**Note:** PIC = polymorphism information content
**Figure 1** Dendrogram of 56 Tunisian castor genotypes prepared based on 30 TRAP markers.

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).

**Figure 2** Electrophoretic profile, on 2% agarose gel, obtained from the amplification of the genomic DNA of 25 lineages of *Ricinus communis* L.

Note: (lanes 1-25) using TRAP 01 x arb 3 primer. Lane M: 100-bp molecular weight marker.
Among the TRAPs, 449 bands were observed in total, 439 of which (97.77%) were polymorphic between the *P. aibuhitensis* populations and were shared between at least four individuals. The Qinzhou (QZ) population had the highest PPB (78.62%), and the Dalian (DL) population had the lowest (60.13%). The objective of study *Farias da Silva et al. (2016)* was to analyze the genetic diversity of the clonal germplasm of the guarana plant using Target Region Amplification Polymorphism (TRAP) and Sequence-Related Amplification Polymorphism (SRAP) markers. Sixty clones of the guarana plant were analyzed; 18 were cultivars, eight were similar clones according to morpho-agronomic traits, and 34 were clones of a different origin. *Singh et al. (2017)* study the genetic variations among the twenty-five sugarcane genotypes employing functional molecular (TRAP) markers. Genetic diversity exists among sugarcane germplasm was exploited to identify promising genotypes bearing enviable agronomic traits (sucrose content and multiple disease resistance). Genetically diversified genotype could be exploited as proven parents in sugarcane hybridization programs to establish a promising cross. *Mirajkar et al. (2017)* study molecular marker profile using 57 markers, comprising of 27 TRAP and 30 SRAP markers in the gamma ray-induced sugarcane mutants. Collectively these markers produced 260 PCR amplicons among which 147 were polymorphic (56.54%). The TRAP marker-based analysis showed that the mutants AKTS-01 and AKTS-16 were more diverse (GS = 94 and 92%, respectively) than the rest of the mutants. In the study of *Fabriki-Ourang and Yousefi-Azarkhanian (2018)* target region amplification polymorphism (TRAP) and conserved region amplification polymorphism (CoRAP) markers were used for genetic diversity and relationship analysis of 25 Salvia ecotypes/species. Twelve TRAP and CoRAP primer combinations (four arbitrary primers and three fixed primers from *Salvia miltiorrhiza* expressed sequence tag sequences) amplified 180 loci, of which all were polymorphic. *Srivong et al. (2019)* study 17 sugarcane genotypes from Hawaii and Thailand using 12 target region amplification polymorphism (TRAP) markers and partial Sai nucleotide polymorphism. A total of 275 fragments were produced, of which 273 (99.27%) were polymorphic. The polymorphic information content (PIC) ranged from 0.912 – 0.959 with an average value of 0.938. Genetic similarity (GS) by Dice’s similarity coefficient ranged from 0.19 – 0.81 with a mean of 0.44.

**CONCLUSION**

PCR amplification of DNA using 30 primers for TRAP analysis produced 490 DNA fragments that could be scored in all 56 genotypes of Tunisian castor. The number of amplified fragments varied from 3 (TRAP 04 x ar 1, TRAP 22 x ar 3 and TRAP 23 x ar 5) to 13 (TRAP 56 x ar 2), and the amplicon size ranged from 100 to 1600 bp. Of the 490 amplified bands, 377 were polymorphic, with an average of 5.71 polymorphic bands per primer. To determine the level of polymorphism in the analysed group of Tunisian castor genotypes polymorphic information content (PIC) was calculated. A dendrogram was constructed from a genetic distance matrix based on profiles of the 30 TRAP primers using the unweighted pair-group method with the arithmetic average (UPGMA).

TRAP markers could be used to select elite parent genotypes, analyzing genetic variation, utilization of genotype potential for trait improvement for adaptation to stress environment. It is therefore suggested that a focused breeding scheme should be adopted while analyzing genome diversity for parent selection to gain maximum value and practical impact on breeding program. TRAP markers exhibited remarkable discriminatory power for genetic diversity analysis.

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