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*Full Length Research Paper*

# Genetic polymorphism in exotic safflower (*Carthamus tinctorious* L.) using RAPD markers

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**Safflower is a drought tolerant annual oil crop and this gives it an advantage over the other crops in the drier parts of Kenya. It is valued worldwide as a source of high quality vegetable oil. In the past, characterization of safflower using molecular markers has been limited. The objective of this study was to evaluate the degree of polymorphism in 36 safflower accessions using RAPDs. Sixty-one amplification products were scored using 14 random 10 mer primers and binary matrices subjected to statistical analyses using NTSYS. A resemblance matrix was developed using SMC, which was used with the UPGMA to compute cluster analysis and PCA. Eight groups were formed at a similarity coefficient of 0.79. Cluster two had 14 accessions originating from India, USA, Australia and Bangladesh while cluster three had 9 accessions from India, USA and Mexico. Proportionally accessions from India were highest in cluster one and two. The differences between pairs of accessions were basically related to the number of RAPD fragments shared. Four Indian accessions PI 214150, PI 199910, W6 16821 and PI 248359 clustered together. However, Girna also from India formed an independent cluster. SMC among accessions ranged from 0.37 (PI 248359 and PI 262419) to 0.98 (PI 560177 and T65). The last two accessions may be genetically related since they constituted the nearest to a complete match for all markers. Accessions from different countries tended to group together though random scattering often occurred. Using PCA the first three components explained 44% of the total variation. The results indicate genetic polymorphism between the safflower accessions under study.**

**Key words**: Amplification, PCA, RAPDs, simple matching coefficients, electrophoresis.

## INTRODUCTION

Safflower (*Carthamus tinctorius* L. 2n = 2 x = 24, family Asteraceae) has a strong central branched stem and varying number of branches. Typically the plants are, herbaceous and thistle-like usually with many long sharp spines on the leaves and bracts (Helm et al., 1991). Globally, over 60 countries grow safflower, on about 1.2 million hectares with annual production of 0.79 million tons (Singhal, 1999). India is the largest producer of safflower flower (68%, 0.2 million tons) in the world with highest acreage (60%, 0.43 million hectares) and production is mainly for the domestic vegetable oil market (Johnson and Marter, 1993). Safflower has a wide range of related species. Within the genus of *Carthamus*, there are more than 20 species divided into 4 sections (Knowles, 1988). Section one (2n = 20) has (*oxyantha* and *palaestinu),* section two (2n = 24) has (*tinctorius, alexandrius, glaucus, syriacus* and *tenuis*) section three (*lanatus* 2n = 44) while section four (*baeticus* 2n = 64). The first two sections are diploids, the third is a tetraploid and the fourth section consists of hexaploid species (Khidir, 1969).

Safflower is a drought tolerant annual oil crop and this gives it an advantage over the other crops in the drier parts of Kenya. It is valued worldwide as a source of high quality vegetable oil. In the past, safflower germplasm was characterized entirely on the basis of morphological traits, abiotic stresses and (or) biochemical characters

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**Abbreviations: UPGMA**, Unweighted pair group method with arithmetic mean; **NSTYS,** numerical taxonomy and multivariate analysis system; **PCA,** principal component analysis**; SDW,** sterile distilled water; **SMCs,** simple matching coefficients; **Rpm,** revolutions per min. **UV**, utra violet.

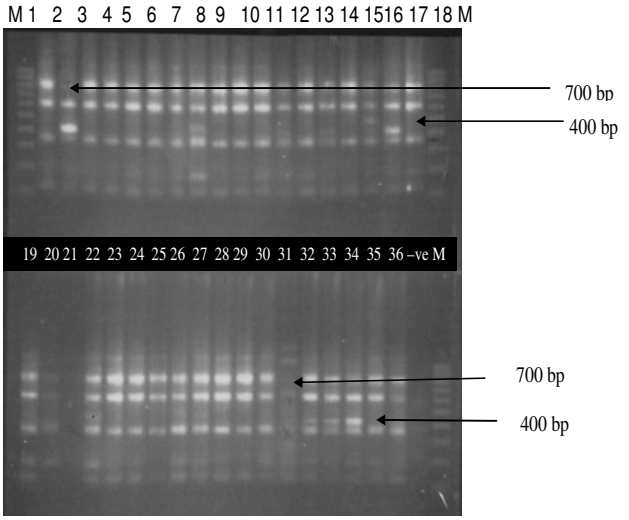


**Figure 1.** RAPD markers of 36 safflower accessions amplified with primer AB4 – 19 (sequence 5´-GGTGCACGTT- 3´) M = 100 bp ladder, lane numbers represent the accessions numbers while arrows indicate polymorphic markers

Which do not necessarily reflect genetic diversity (Fernandez-Martinez et al., 1993). The environmental has a strong influence on morphological traits (especially quantitative traits).Studies have also shown that there are not sufficient numbers of morphological markers to provide detailed coverage of most genomes (Shawla, 2002). Isozymes directly relate to genes, but their inherently low level of polymorphism among closely related cultivars, constrain their use in genetic linkage analyses and marker-assisted selection (Tanksley and Orton, 1983). Hence selection of genotypes based on molecular markers would be highly reliable and cost effective. Yazdi-Samadi et al. (2001) and Amini et al. (2007) used RAPD markers to detect genetic diversity in safflower accessions. Sehgal and Raina (2005) characterized 14 Indian safflower cultivars using RAPD, SSR and AFLP. AFLP markers were found to be the most efficient since two primer pairs were sufficient to genotype the cultivars. Using two marker systems RAPD and ISSR Yadla (2004) has reported that RAPD markers could be used to assess the intra varietal variation while ISSR markers could be useful tool in phylogetic analysis. Johnson et al. (2007) have found AFLP markers to be useful for distinguishing safflower variation within and among different geographical regions. This shows limited work has been reported in safflower on molecular methods. Hence the objective of this study was to evaluate genetic diversity in introduced safflower accessions using RAPDs and its application to germplasm identification and classification. Hypothesis for the study was that the “introduced safflower accessions are not genetically related”.

## MATERIALS AND METHODS

The study was conducted on 36 exotic safflower accessions, which were supplied from India, Bangladesh, China, Australia, USA, Mexico and FAO. DNA isolation was accomplished using a modified Cetyltrimethylammonium Bromide (CTAB) method (Hulbert and Bennetzen, 1991). The DNA was quantified on a spectro-photometer at a wavelength of 260 and 280 nm. While intactness and quantity was checked by running samples along some uncut unmethylated lambda (λ) DNA standards (20, 50 and 100 ng). The gel was stained in ethidium bromide (10 µg/ml) and visualized on an UV transilluminator. Out of 24 random 10-mer oligonucleotide primers obtained from Operon Technologies Inc. (USA), 14 primers that amplified clear and reproducible band profiles were selected. Each 10 µl PCR optimized reaction mixture contained 1X PCR buffer, 2.5 mM $Mgcl_2$, 100 nM dNTPs (-dATP, dCTP, dGTP and dTTP, Sigma Chemicals); 0.5 units of Taq DNA Polymerase (from Biolabs in UK), 200 nM Primer, 0.5 µl DNA template (10 ng), and 6.9 µl SDW. Amplification of DNA reactions were performed in a DNA thermocycler machine (Mastercycler) with a heated lid (93°C) programmed as follows; one hot start cycle of 93°C for 5 min (for strand separation), 40 cycles of 93°C for 1 min (DNA denaturation), 42°C for 1.5 min (annealing), 72°C for 1 min (DNA polymerization) and a final extension cycle of 72°C for 10 min. The samples were then maintained at 4°C. The PCR products were electrophoresed on 1.5% agarose gels stained in ethidium bromide and visualized on a UV light transilluminator. Band profiles were manually scored on two independent occasions and compiled into a rectangular binary matrix (not shown). Positive amplification were treated as separate characters and scored for the presence (1) or absence (0) of bands (Figure 1) Only intensely stained unambiguous bands were scored. The binary matrix file was transposed and the code "99" inserted for missing values before statistical analysis.

## RESULTS AND DISCUSSION

A total of 61 amplification products were scored with an average frequency of 4.4 bands per primer. The binary matrices were subjected to statistical analyses using NTSYS (Rohlf, 1992). The calculation of SMCs was based on the presence or absence of RAPD fragments from paired samples given by $SM_{jk} = (a + d)/ (a + b + c + d)$. Where a, b, c, d are matches for each pair of acessions until a 36 x 61 similarity matrix was developed. The distance coefficient was the proportion of unmatched markers between a given pair of entries and this has been suggested as an appropriate estimator of relatedness under the assumption that the presence or absence of a discrete character in two or more genotypes results from the same genetic changes (Skrotch et al., 1992). The similarity matrix was then used to cluster the data using UPGMA algorithm and PCA. SMCs were generated using the NTSYS software (Table 2). The matrix values estimated the number of RAPD fragments shared (or not shared) between two accessions.

SMCs ranged from 0.37 (for accessions PI 262419 and PI 248359) and 0.39 (for accessions PI 537598 and PI 248359) that is, least genetic similarity to 0.98 (for accession PI T65), greatest genetic similarity. The latter two accessions (Oleic Leed and T65 originated from USA and India respectively). There is a strong indication that they may be genetically related since they constitute the nearest to a complete match for all markers (high degree of homology). However, similarities in accessions can also arise due to convergent evolution, selection, or shar-

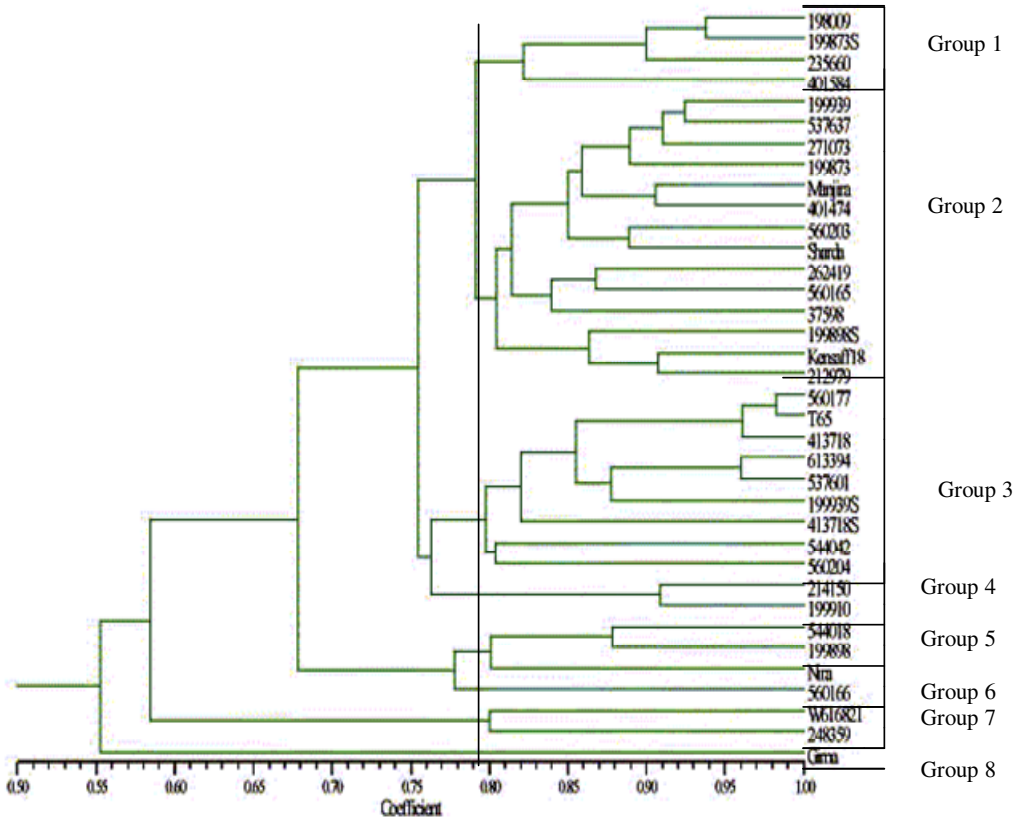


**Figure 2.** Dendrogram of 36 safflower accessions based on RAPD analysis of 61 markers using the simple matching coefficient and UPGMA clustering.

ing of a common parentage. This is consistent with earlier findings by Johnson et al. (2007) that molecular markers could be used for identification of duplicate accessions. Using RAPDs, breeders can select related or unrelated parental germplasm to maximize variability in the Kenyan breeding programme. Mating dissimilar accessions is likely to result into heterosis the magnitude of which deends on genetic distance. The RAPD results also suggest that some accessions from the same country were clustered together, other accessions were scattered with accessions obtained from different countries. Amini et al. (2007) have reported similar results in a genetic diversity study involving sixteen breeding lines and four safflower introductions. A small proportion of polymorphic primers would indicate that the germplasm is reasonably homogeneous. This observation among the safflower accessions is not unexpected due to movement of breeding material across the world into different programmes.

## Cluster analysis

The UPGMA cluster analysis showed that the 36 accessions were grouped into eight marker-based groups (Figure 2). Cluster 2 had the largest number of accessions (14) originating from India, USA, Australia and Bangladesh. Cluster 3 had the second largest number of accessions from India, USA and Mexico. But proportionally accessions from India were represented at a higher level in both cluster 1 and 2. The dispersion into the various groups appeared to be at random though a few accessions formed distinct clusters (Figure 2). Since India is a secondary Centre of diversity for safflower, this helps to explain why accessions from there were found in almost every cluster due to movement of safflower from the Centre of origin.

The 4 Indian accessions PI 214150, PI 199910, W6 16821 and PI 248359 (group 4 and group 7) clustered together and diverged from the other accessions. Probably they have a common ancestor or could be an indicator of duplicates. However, similarities in accessions can also arise due to convergent evolution, selection, or sharing of a common parentage. Nicese et al. (1998) observed similar results in walnut and concluded that the RAPD assay can be useful in breeding programmes for identification of new cultivars as well as assessment of the genetic similarity among different genotypes. However, group 8 (Girna) formed an independent cluster and appeared to be most distantly related to all others (Figure 2). This accession originated from India and may have resulted from similar selection pressures at different pla-

**Table 1.** Eigen value, explained variance and cumulated variance in the PCoA using characters used to classify 36 safflower accessions using RAPDs.

| Principal coordinate | Eigen value | Explained variance (%) | Cumulative variance (%) |
|---|---|---|---|
| **1** | 1.93621729 | 20.6867 | 20.6867 |
| **2** | 1.26839267 | 13.5516 | 34.2383 |
| **3** | 0.95635288 | 10.2178 | 44.4561 |

**Table 2.** SMCs for some of the exotic safflower accessions studied.

| PI No/Name | PI 235660 | PI 99873S | PI 262419 | PI 560177 | PI 537598 |
|---|---|---|---|---|---|
| PI 199939 | | 0.90 | | | |
| PI 271073 | | 0.90 | | | 0.39 |
| PI 401584 | | 0.90 | | | |
| PI 238359 | | | 0.37 | | |
| Kensaff 18 | 0.75 | | | | |
| PI 560203 | 0.75 | | | | |
| PI 212979 | 0.75 | | | | |
| T65 | | | | 0.80 | |
| PI 544042 | | | | 0.80 | |
| PI 199898 | | | | 0.80 | |
| Nira | | | | 0.80 | |

ces in an effort to develop uniform genotypes leading to similar forms with a different genetic background. Apparently the 5 accessions constitute an independent genetic pool, which could be of interest to safflower breeders. The results of this study agree with those reported by Amini et al. (2007) where cluster analysis based on RAPD markers and 54% coefficient of similarity divided the safflower genotypes into 5 distinct groups.

## Principal component analysis (PCA)

The PCA indicated that the first 3 components accounted for 44% of the total variation (Table 1). Since molecular markers reveal neutral sites of variation at the DNA sequence level, this implies that most of the variation measured in the morphological study (Mahasi et al., 2006) was due to the environmental effects. The was similarity among markers (Table 2) for some accession pairs e.g. 99873S had a 0.90 genetic similarity with accession PI 199939, PI 211073 and PI 401584. While PI 413718 and PI 560177 and PI 413718 and T 65 had a genetic similarity of 0.96 between them. Several other accessions can be identified in the present study, which had genetic similarities e.g. 199898S with PI 401474, Sharda and PI 560165 at 0.75 genetic similarity (Table 2). This helps to explain the observation by Knowles (1989) that safflower has moved from the Centre of origin in the Middle East to other regions like India and accessions from the same areas would be expected to share at least some common genetic structure in the cause of germplasm movement across countries admixtures between accessions can occur.

## Conclusions

It is evident from this study that the RAPD assay can be useful in safflower breeding programmes, for identification of new cultivars as well as assessment of the genetic similarity. Germplasm characterization from diverse world sources with large number of loci and more markers/different markers like AFLP and ISSR may provide guidance in enriching the present safflower collections in Kenya. During inter-mating accessions with greater genetic distance may provide unique genetic combination and useful variation for breeding. The results of the present study confirm that polymorphism based on RAPDs was useful in revealing genetic relatedness between the safflower accessions under study. Therefore any meaningful effort for construction of genetic relatedness trees can best be done using molecular markers as opposed to agronomic traits which are subject to environmental effects. Markers are useful for genotyping accessions and other factors but plant breeders still need the agronomic data to compliment molecular information in order to understand variation among accessions. Though some accessions from the same country were grouped together, some accessions grouped randomly with those introduced from different countries. The RAPD dendrogram revealed that the closer the geographical locations the closer the genetic relationships. The results of this study, have disapproved the hypothesis that “the introduced safflower accessions are not genetically related” as, reveal-ed by the RAPD groupings.

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