Analysis of genetic divergence in safflower genotypes through morphological characters

Análise da divergência genética em genótipos de cártamo através de caracteres morfológicos

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Abstract
The increase in energy consumption in Brazil and in the world generates an increasing need to seek renewable and non-polluting energy, such as biofuels. Safflower (Carthamus tinctorius L.) is a plant with a great oil production capacity and potential for biodiesel production that presents high productivity and easy edaphoclimatic adaptation. It can be an economic culture option in crop rotation practiced by farmers, however it is still necessary to obtain more technical information about its cultivation and about the adapted and improved cultivars. Thus, the estimate of genetic divergence from morphological characters using multivariate techniques has become a common alternative among breeders. In this sense, the objective of this research was to carry out an analysis of the genetic divergence among the 49 safflower genotypes from the germplasm bank of the Instituto Mato Grossoense do Algodão (IMA-MT), based on 13 morphological descriptors recommended by the International Board for Plant Genetic Resources - IBPGR (1983) and Ministério da Agricultura, Pecuária e Abastecimento - MAPA (2013). The divergence analysis was performed by means of the dissimilarity matrix based on multi-categorical variables and to identify the most similar genotypes, Tocher's optimization grouping methods and the hierarchical average grouping method between groups were used. The estimates of the dissimilarity coefficients ranged from 0.00 to 0.46, indicating the presence of genetic diversity among the evaluated genotypes. The highest dissimilarity value was between genotypes 29 and 9, 42 and 1, 42 and 32, 47 and 9, which are the most genetically divergent and thus promising materials for future genetic crosses. The UPGMA dendrogram and tocher grouping were partially consistent and effective in grouping safflower genotypes.

Keywords: Carthamus tinctorius L.; Dissimilarity; Multivariate analysis.
Safflower (Carthamus tinctorius L.) is an oil plant belonging to the botanical family Asteraceae, has several branches on the stem that can form yellow, red, white or orange chapters in each chapter has about 15 to 30 seeds (Khan et al., 2009). It has an average cycle of 110 to 150 days, with ample resistance to water stress with pivoting roots that can reach up to three meters in depth, withstands high temperatures, soils with high salt content and a low relative humidity of the air (Weiss, 2000; Santos and Silva 2015; Sá et al., 2020).

It is a multi-purpose culture, being cultivated in more than 60 countries, presenting several purposes, in which its flowers can be used as fabric tissue (Weiss, 1983), is vastly utilized in Traditional Medicine for various medical conditions, namely dysmenorrhea, amenorrhea, post partum abdominal pain and mass, trauma and pain of joints (Delshad et al., 2018), safflower seed oil can be used to make paints, varnishes, enamels, soaps and biodiesel (Moura et al., 2015), it also serves as a potential raw material for use in compression ignition engines (Yesilyurt, et al., 2020). Safflower is reported to be anticoagulant, antioxidant and neuroprotective (Hiramatsu et al., 1998).
For efficiency in safflower breeding programs, the characterization and identification of genetic diversity is essential, as according to Borém and Miranda (2013), one of the main needs of breeders is the identification of plants that have superior genes in a segregating progeny. And according to Cruz et al., (2014), active germplasm banks have basic raw material for the development of new cultivars, as they retain maximum variability, and therefore deserve research attention.

The study of this genetic diversity in safflower is carried out mainly by means of multivariate techniques to be quantified by multivariate procedures, such as the generalized Mahalanobis distance, average Euclidean, matrix of dissimilarities with multicategorical variables, canonical variables, main components, among others (Lira et al., 2021). The choice of the method varies depending on the statistical design, the ease of analysis and interpretation of the results and the way of obtaining the data (Cruz et al., 2014). These studies are usually complemented by agglomerative and hierarchical methods of clustering, such as the Tocher’s method (Rao, 1952) and average distance (UPGMA).

It is notable that safflower has a wide genetic diversity in different regions of the world (Knowles, 1989). However, there is still a scarcity of research that evaluated the genetic divergence of safflower based on morphological characteristics. Therefore, in order to add valuable information to safflower breeding programs, the present research involves an investigation covering 49 different safflower genotypes from different agroclimatic zones and from several countries in the world using a variety of morphological characteristics.

2. Methodology

Were evaluated 49 safflower genotypes from the germplasm bank of the Instituto Mato-grossense de Algodão (IMA-MT), located in the county of Primavera do Leste, state of the Mato Grosso, Brazil and, assigned to the reference collection of the Laboratory of Genetic Resources & Biotechnology (LRG&B) of the State University of Mato Grosso (UNEMAT), Campus of the Cáceres (Table 1).

To assess genetic divergence, safflower genotypes were grown in a greenhouse in plastic pots of five liters with substrate with the seeds sown at a depth of around 3 cm, with the sampling unit two pots for each genotype. Sowing was initially carried out with four seeds, and after 15 days thinning was carried out, leaving only two plants per pot. Fertilization was performed at the time of sowing, of 6.5g of the compound NPK in formulation 4-14-8 evenly distributed in the pot. After 15 days of cultivation, weekly fertilization with urea and potassium chloride was started.
Table 1. Safflower genotypes from the active collection of the Genetic Resources & Biotechnology laboratory (LRG&B) of the State University of Mato Grosso (UNEMAT), Campus of the Cáceres, state of Mato Grosso, Brazil.

| Order | PI    | Source       | Order | PI    | Source       |
|-------|-------|--------------|-------|-------|--------------|
| 1     | 193473| Ethiopia     | 26    | 304438| Iran         |
| 2     | 195895| Morocco      | 27    | 305161| India        |
| 3     | 237539| Turkey       | 28    | 305198| India        |
| 4     | 248385| India        | 29    | 305207| India        |
| 5     | 248620| Pakistan     | 30    | 305209| India        |
| 6     | 248808| India        | 31    | 305540| Kazakhstan   |
| 7     | 248828| India        | 32    | 306832| India        |
| 8     | 248839| India        | 33    | 306833| India        |
| 9     | 248852| India        | 34    | 306838| India        |
| 10    | 250083| Egypt        | 35    | 306844| India        |
| 11    | 250188| Pakistan     | 36    | 306866| India        |
| 12    | 250190| Pakistan     | 37    | 343783| Iran         |
| 13    | 250203| Pakistan     | 38    | 343930| Ethiopia     |
| 14    | 250204| Pakistan     | 39    | 367833| Argentina    |
| 15    | 250840| Iran         | 40    | 369842| Armênia      |
| 16    | 250922| Iran         | 41    | 369845| Tajikistan   |
| 17    | 251978| Turkey       | 42    | 369849| Rússia       |
| 18    | 253540| Hungary      | 43    | 369854| Uzbekistan   |
| 19    | 253899| Syria        | 44    | 392029| Turkey       |
| 20    | 259996| Pakistan     | 45    | 392030| Turkey       |
| 21    | 259997| Pakistan     | 46    | 392031| Turkey       |
| 22    | 262443| Spain        | 47    | 393500| Iran         |
| 23    | 262447| Kazakhstan   | 48    | 401474| Bangladesh   |
| 24    | 262450| India        | 49    | 401475| Bangladesh   |
| 25    | 279344| Japan        |       |       |              |

¹Plant introduction. Source: Authors.

For the analysis of the genetic divergence among the 49 safflower genotypes, 13 morphological descriptors recommended by the International Board for Plant Genetic Resources – IBPGR (1983) and Ministério Agricultura Pecuária e Abastecimento-MAPA (2013), of which:

Angle of Branches (AB): determined with the aid of a graduated protractor (semicircular instrument used to measure angle), measuring from zero to one hundred and eighty degrees. The instrument is positioned between the branch and the main stem, thus having the value of the angle of the branches in degrees (°), where, 0-No branches (0°); 3-Rushed (15° to 20°); 5-Intermediate (20° to 60°); 7 – Spreading out (60° to 90°) and 9-Falling (>90°).

Growth Habit (GH): determined by the growth of the branches and the flowering of the plant, classified as determined or undetermined of size 1-erect; 2-erect to semi-erect; 3-semiereto; 4-semi-horitontal and 5-horizontal.

Bracts Wrapping the Head (BWH): determined visually through (1) absence or (2) presence of bracts.

Flower Color (FC): determined visually, where: 1 - white; 2 - Yellow; 3 - Orange and 4 - Red.

Leaf Margins (LM): visually determined, where: 1 - Whole Margin; 2 - Serrated or slightly jagged margin and 3 - Very jagged.

Leaf Shape (LS): visually determined, being: 1 - Ovada; 2 - Oblong; 3 - Lanceolate and 4 - Linear.
Leaf Color (LC): visually determined, where: 1 - Light Green; 2 - Dark Green; 3 - Gray and 4 - Other colors.

Chapter Shape (CS): visually determined, where: 1 - Conical; 2 - Oval and 3 - Flat.

Leaf Hairiness (LH): determined visually through: 1 - Absence or 2 - Presence of hair.

Thorns (T): determined visually through: 1 Absence or 2 - Presence of thorns.

Seed Color (SC): visually determined, where: 1 - White; 2 - Cream; 3 - Brown; 4 - Black; 5 - Gray and 6 - Others.

Seed Shape (SS): visually determined, where: 1 - Oval; 2 - Conical and 3 - Crescent.

Pappus (P): determined visually through: 1 - Absence or 2 - Presence of pappus.

The analysis of the divergence between the genotypes was carried out by obtaining the dissimilarity matrix based on multi-categorical variables and to identify the most similar accesses, the Tocher optimization grouping methods and the hierarchical mean grouping method between groups were used (UPGMA), using the Genes computational resource (CRUZ, 2013).

3. Results and Discussion

Based on the evaluated morphological variables, the dissimilarity matrix of the 49 safflower genotypes was generated, in which the estimates of the dissimilarity coefficients ranged from 0.00 to 0.46, this variation is indicative of the presence of genetic diversity between the evaluated genotypes. The smallest genetic distances found occurred between genotypes 8 and 2, 14 and 3, 18 and 6, 22 and 4, 28 and 11, 30 and 23, 38 and 34, 39 and 36, 40 and 7, 41 and 11, 41 and 28, 43 and 4, 43 and 22, 45 and 3, 45 and 14, 49 and 12, both with a dissimilarity coefficient of 0.00 indicating the genotypes are similar for the variables evaluated. This fact is due to the fact that the genotypes shared AB, BWH, LM, LS, FC, CS, LH, SS and SC as common characteristics.

The highest dissimilarity value found was between genotypes 29 and 9, 42 and 1, 42 and 32, 47 and 9, these being the most genetically divergent with a dissimilarity value of 0.46. This dissimilarity value between these genotypes is due to the fact that they diverged for the variables GH, FC, LM, LS, SS and P.

Regarding Tocher's optimization method, a method based on the formation of groups whose distances within groups are shorter than the distances between groups, it was possible to observe the formation of nine distinct groups (Table 2).
Table 2. Groups of safflower genotypes with similarity patterns by the Tocher’s method, evaluated in the county of Cáceres, state of Mato Grosso, Brazil.

| Groups | Genotypes          | %   |
|--------|--------------------|-----|
| I      | 2, 8, 5, 46, 23, 30, 20, 15  | 16.32 |
| II     | 3, 14, 45, 7, 40, 10, 29, 37, 44, 21, 31, 36, 39  | 26.56 |
| III    | 4, 22, 43, 13, 32, 19, 6, 18  | 16.32 |
| IV     | 11, 28, 41, 24, 27, 33, 16, 26, 34, 38  | 20.40 |
| V      | 12, 49, 17, 48  | 8.16 |
| VI     | 1, 9  | 4.08 |
| VII    | 42, 47  | 4.08 |
| VIII   | 25  | 2.04 |
| IX     | 35  | 2.04 |
| Total  | 49  | 100 |

Source: Authors.

It can be seen that group I and III were constituted by eight genotypes each representing 16.32% of the total, with genotype 2 and 15 being the most divergent within group I because they differ for GH and LM characteristics, and genotypes 4 and 18 the most divergent within group III for diverging for FC and LS characteristic. Group II formed by 13 genotypes represents 26.53% of the total, with genotypes 3 and 39 being the most divergent within this group, diverging for FC and P.

Group IV formed by 10 genotypes, representing 20.40% of the total, with genotypes 11 and 38 being the most divergent in the group, diverging for GH and LS. Group V consists of 3 genotypes 12 and 48 the most divergent, disagreeing for the GH characteristic. Group VI and VII gathered only two genotypes, diverging only for the LS features in group VI and for ESP in group VII. In a study on Gerhardt's genetic dissimilarity (2014), evaluating the genetic divergence between safflower genotype in the city of Botucatu, state of the São Paulo, Brazil, using 16 genotypes in the study, when grouping by the Tocher method, found 6 different groups, further stating that the possible crosses of these cultivars between individuals in the same group decrease the possibility of obtaining superior genotypes.

Groups I and X were formed by a single genotype, suggesting that they are more divergent from the others analyzed. According to Barros et al., (2005) groups formed by only one individual, point in the direction that this individual is more divergent in relation to the others, possibly the individualization of the genotype 35 is due to the absence of T, diverging from the other materials and the 25 by the LS.

Using the UPGMA grouping method considering the cut at 72% of genetic distance, significant by the statistical program, presented the formation of seven groups (Figure 1). Group I formed by 10.20% of the genotypes, with genotypes 12 and 19 being the most divergent within this group, where the genotypes differed between GH and LS variables. Group II formed by 24.48% of the genotypes, where genotype 22 and 21 are the most divergent within this group evaluated, differing only for variable LS. Group III presented 4.08% of the genotypes presenting only genotypes 1 and 9.

Group IV and VI formed the largest groups, with 28 and 27 %, respectively, of the total genotypes, where genotype 34 and 24 are the most divergent within group IV, diverging for GH and LS characteristic, and 46 and 37 within group VI, diverging
for GH, LM and LS variables. Group V presented 12.24% of the total genotypes, with genotypes 15 and 8 being the most divergent in the group, diverging for GH and LM.

Group VII presented only genotype 42, totaling 2.04% of the genotypes, showing divergence from the others, since it formed an exclusive group and remained isolated from the other accessions in the dendrogram. Faria et al. (2012), emphasizes that genotypes in an isolated group can be explored in breeding programs.

**Figure 1.** Dendrogram obtained by the UPGMA hierarchical clustering method, and the respective groups, based on the morphological descriptors of the 49 evaluated safflower genotypes.

Comparing the grouping methods used, it is observed that both were partially concordant between the Tocher´s and UPGMA methods. According to Buttow et al. (2010) the differences between the Tocher´s method and UPGMA are due to the way in which each method calculates the genetic variability or because the genotypes are grouped in groups with extreme, maximum and minimum values, by the optimization method. Through the analyzes carried out, the genotypes present genetic diversity.

### 4. Conclusion

The use of multivariate analysis via morphological variables was efficient in discriminating the genetic divergence of the safflower genotypes evaluated in the present study.
The UPGMA dendrogram and tocher grouping were partially consistent and effective in grouping safflower genotypes.

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