

Analysis of genetic divergence in safflower genotypes through morphological characters

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

Análisis de divergencia genética en genotipos de cártamo através de caracteres morfológicos

Received: 04/02/2021 | Reviewed: 04/11/2021 | Accept: 04/17/2021 | Published: 05/11/2021

Valvenarg Pereira da Silva

ORCID: <https://orcid.org/0000-0001-8450-3016>
Universidade do Estado de Mato Grosso, Brazil
E-mail: silvabiologo@hotmail.com

André Henrique Almissi Vital

ORCID: <https://orcid.org/0000-0002-9050-555X>
Universidade do Estado de Mato Grosso, Brazil
E-mail: vitalmotos_andre@hotmail.com

João Paulo Egues Lira

ORCID: <https://orcid.org/0000-0002-1487-2470>
Faculdade Estácio do Pantanal, Brazil
E-mail: jp_egues@hotmail.com

Juliana Parisotto Poletine

ORCID: <https://orcid.org/0000-0003-4740-7230>
Universidade Estadual de Maringá, Brazil
E-mail: jppoletine@uem.br

Lucas Henrique Pereira Morais

ORCID: <https://orcid.org/0000-0002-3251-5821>
Universidade do Estado de Mato Grosso, Brazil
E-mail: lucas.morais@unemat.br

Altacis Junior de Oliveira

ORCID: <https://orcid.org/0000-0002-6787-7160>
Universidade do Estado de Mato Grosso, Brazil
E-mail: altacismarquesfig@hotmail.com

Rafhael Felipin-Azevedo

ORCID: <https://orcid.org/0000-0002-4490-0823>
Universidade do Estado de Mato Grosso, Brazil
E-mail: rafaelfelipin@gmail.com

Marco Antonio Aparecido Barelli

ORCID: <https://orcid.org/0000-0002-6385-6733>
Universidade do Estado de Mato Grosso, Brazil
E-mail: mbarelli@unemat.br

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 Grossense 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.

Resumo

O aumento do consumo de energia no Brasil e no mundo gera uma crescente necessidade de se buscar energias renováveis e não poluidoras, como os biocombustíveis. O cártamo (*Carthamus tinctorius* L.) é uma planta com grande capacidade de produção de óleo e potencial para produção de biodiesel que apresenta alta produtividade e fácil adaptação edafoclimática. Pode ser uma opção de cultura econômica na rotação de culturas praticada pelos agricultores, no entanto ainda é necessário obter maiores informações técnicas sobre o seu cultivo e sobre as cultivares adaptadas e melhoradas. Dessa forma, a estimativa da divergência genética a partir de caracteres morfológicos utilizando técnicas multivariadas tornou-se uma alternativa comum entre os melhoristas. Neste sentido, o objetivo dessa pesquisa foi realizar análise da divergência genética entre os 49 genótipos de cártamo oriundos do banco de germoplasma do Instituto Mato-grossense de Algodão (IMA-MT), a partir de 13 descritores morfológicos preconizados pelo International Board for Plant Genetic Resources – IBPGR (1983) e Ministério Agricultura Pecuária e Abastecimento-MAPA (2013). A análise da divergência foi realizada por meio da matriz de dissimilaridade com base em variáveis multicategóricas e para a identificação dos genótipos mais similares foi utilizado os métodos de agrupamento de otimização de Tocher e o método hierárquico de agrupamento médio entre grupos (UPGMA). As estimativas dos coeficientes de dissimilaridade variaram de 0,00 a 0,46, indicando presença de diversidade genética entre os genótipos avaliados. O maior valor de dissimilaridade foi entre os genótipos 29 e 9, 42 e 1, 42 e 32, 47 e 9, sendo estes os mais divergentes geneticamente e assim materiais promissores para futuros cruzamentos genético. O dendrograma UPGMA e agrupamento de tocher foram parcialmente concordantes e eficazes no agrupamento dos genótipos de cártamo.

Palavras-chave: *Carthamus tinctorius* L.; Dissimilaridade; Análise multivariada.

Resumen

El aumento en el consumo de energía en Brasil y en el mundo genera una creciente necesidad de buscar energía renovable y no contaminante, como los biocombustibles. El cártamo (*Carthamus tinctorius* L.) es una planta con una alta capacidad de producción de petróleo y potencial para la producción de biodiesel que tiene alta productividad y fácil adaptación edafoclimática. Puede ser una opción de cultura económica en la rotación de cultivos practicada por los agricultores, sin embargo, aún es necesario obtener más información técnica sobre su cultivo y sobre los cultivares adaptados y mejorados. Por lo tanto, la estimación de la divergencia genética de los caracteres morfológicos utilizando técnicas multivariadas se ha convertido en una alternativa común entre los criadores. En este sentido, el objetivo de esta investigación fue llevar a cabo un análisis de la divergencia genética entre los 49 genotipos de cártamo del banco de germoplasma del Instituto de Algodón Mato Grosso (IMA-MT), basado en 13 descriptores morfológicos recomendados por la Junta Internacional de Genética Vegetal Recursos - IBPGR (1983) y Ministerio de Agricultura, Ganadería y Abastecimiento-MAPA (2013). El análisis de divergencia se realizó utilizando la matriz de disimilitud basada en variables multicategoría y para identificar los genotipos más similares, se utilizaron los métodos de agrupación de optimización de Tocher y la agrupación jerárquica de medias entre grupos (UPGMA). Las estimaciones de los coeficientes de disparidad variaron de 0.00 a 0.46, lo que indica la presencia de diversidad genética entre los genotipos evaluados. El mayor valor de disimilitud fue entre los genotipos 29 y 9, 42 y 1, 42 y 32, 47 y 9, que son los materiales más genéticamente divergentes y, por lo tanto, prometedores para futuros cruces genéticos. El dendrograma UPGMA y la agrupación de tocher fueron parcialmente consistentes y efectivos en la agrupación de genotipos de cártamo.

Palabras clave: *Carthamus tinctorius* L.; Disimilitud; Analisis multivariable.

1. Introduction

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	¹ 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): is 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-horizontal 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.

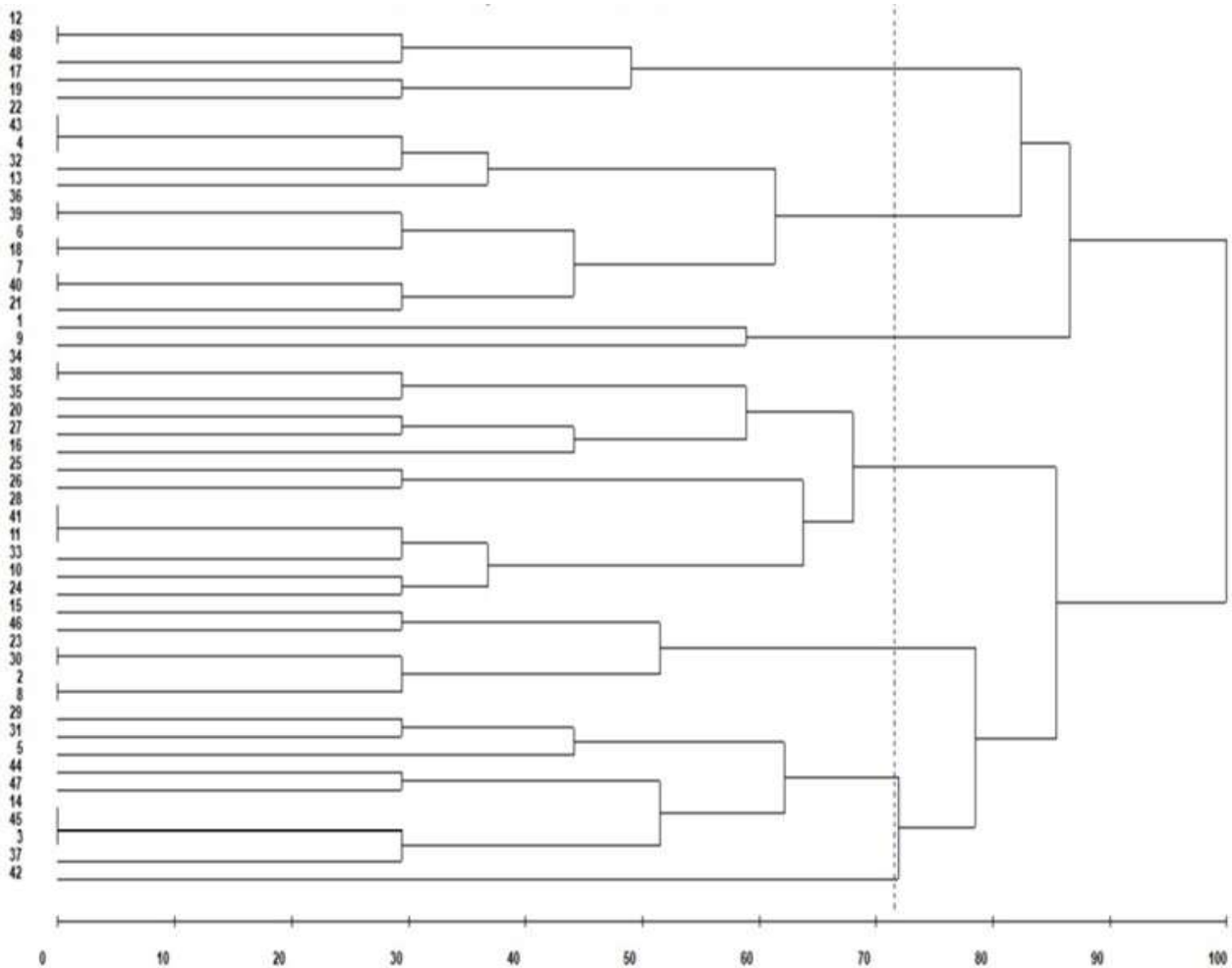
Using the UPGMA grouping method considering the cut at 72% of genetic distance, significant by the statistical program, presented the formation of seven groups (Figure 1). Group I formed by 10.20% of the genotypes, with genotypes 12 and 19 being the most divergent within this group, where the genotypes 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.



Source: Authors.

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.

Acknowledgments

This study was partially financed by State Research Support Foundation of Mato Grosso (FAPEMAT). Authors also thank Mato Grosso Institute of Cotton, Mato Grosso State for germplasm imports.

References

- Barros, A. M., Faleiro, F. G., Karia, C. T., Shiratsuch, I. L. S., Andrade, R. P. & Lopes, G. K. B. (2005). Variabilidade genética e ecológica de *Stylosanthes macrocephala* determinadas por RAPD e SIG. *Pesquisa Agropecuária Brasileira*, 40(9), 899-909.
- Borém, A. & Miranda, G. V. (2013). Melhoramento de plantas. (6a ed.), Editora UFV. 523p.
- Büttow, M. V., Barbieri, R.L., Neitzke, R. S., Heiden, G. & Carvalho, F. I. F. D. (2010). Diversidade genética entre acessos de pimentas e pimentões da Embrapa Clima Temperado. *Ciência Rural*, 40(6), 1264-1269.
- Cruz, C. D., Carneiro, P. C. S. & Regazzi, A. J. (2014). Modelos biométricos aplicados ao melhoramento genético. (3a ed.), UFV, 668p.
- Cruz, C.D. (2013). GENES - a software package for analysis in experimental statistics and quantitative genetics. *Acta Scientiarum*, 35(3), 271-276.
- Delshad, E., Yousefi, M., Sasaneezha, D. P., Rakhshandeh, H. & Ayati, Z. (2018). Medical uses of *Carthamus tinctorius* L. (Safflower): a comprehensive review from Traditional Medicine to Modern Medicine. *Electron Physician*, 10(4), 6672-6681.
- Faria, P. N., Cecon, P. R., DA Silva, A. R., Finger, F. L., Silva, F., Cruz, C. D. & Sávio, F. L. (2012). Métodos de agrupamento em estudo de divergência genética de pimentas. *Horticultura Brasileira*, 30(3), 428-432.
- Gerhardt, I. F. S. (2014). Divergência genética entre acessos de cártamo (*Carthamus tinctorius* L.). 28 f. Dissertação (Mestrado) - Curso de Agronomia, Universidade Estadual Paulista Júlio de Mesquita Filho, Botucatu, SP.
- Hiramatsu, M., Komatsu, M., Xu, Y. & Kasahara, Y. (1998). In vitro and in vivo study of antioxidant action in food plant (*Carthamus tinctorius* L.). *Pathophysiology*, 5(79).
- IBPGR- International Board for Plant Genetic Resources. (2020). Safflower descriptors. <https://www.bioversityinternational.org/index.php?id=244&tx_news_pi1%5Bnews%5D=251&cHash=0a34293e359ab49c97ad47c744af5f8b Roma1983>.
- Khan, M. A., Von Witzke-Ehbrecht, S., Maass, B. L. & Becker, H. C. (2009). Relationships among diferente geographical groups, agro-morphology, fatty acid composition and RAPD marker diversity in safflower (*Carthamus tinctorius*). *Genetic Resources and Crop Evolution*, 56(1), 19-30.
- Knowles, P.F. (1969). Centers of plant diversity and conservation of crop germplasm: Safflower. *Economic Botany*, 21, 156-162.
- Lira, J. P. E., Barelli, M. A. A., Silva, V. P., Felipin-Azevedo, R., Santos, D. T., Galbiati, C., Sá, R. O. & Poletine, J. P. (2021). Safflower genetic diversity based on agronomic characteristics in Mato Grosso state, Brazil, for a crop improvement program. *Genetics and Molecular Research*, 20(1), 1-12.
- MAPA –Ministério da Agricultura, Pecuária e Abastecimento. Instruções para execução dos ensaios de distinguibilidade, homogeneidade e estabilidade de cultivares de cártamo (*Carthamus tinctorius* L.). <https://sogi8.sogi.com.br/Arquivo/Modulo113.MRID109/Registro191701/9176a8a12cc184e854c5e0d7c67c7e2f.pdf>.
- Moura, P. C. S., Bortolheiro, F. P. A. P., Guimarães, T. M., Leal, D. P. V. & Silva, M. A. (2015). Características gerais e ecofisiologia do cártamo (*Carthamus tinctorius*). *Journal of Agronomic Sciences*, 4, 136-150.
- Rao, R.C. (1952). Advanced statistical methods in biometric research. John Willey and Sons, 1952. 390p.
- Sá, E. L. C., Poletine, J. P., Brondani, S. T., Barelli, A. A. B., Silva, V. P. (2020). Parâmetros genéticos e dissimilaridade entre acessos de cártamo. *Journal of Agronomic Sciences*, 4 (nº especial), 269-286.
- Santos, R. F. & Silva, M. A. (2015). *Carthamus tinctorius* L.: Uma alternativa de cultivo para o Brasil. *Acta Iguazu*, 4(1), 26-35.
- Weiss, E.A. (2000). Oil Seed Crops. Blackwell Science Ltd, Oxford, UK.
- Weiss, E.A. (1983). Oil seed Crops. Safflower. Longman Group Limited, Longman House, p. 216-281.
- Yesilyurt, M. K., Cesur, C., Aslan, V. & Yilbasi, Z. (2020). The production of biodiesel from safflower (*Carthamus tinctorius* L.) oil as a potential feedstock and its usage in compression ignition engine: A comprehensive review. *Renewable and Sustainable Energy Reviews*, v.19.