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*Original scientific article*

# EVALUATION OF IMPORTANT TRAITS AFFECTING YIELD IN SAFFLOWER (*Carthamus tinctorius* L.)

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The study assessed genetic diversity and trait relationships in 64 safflower genotypes obtained from the National Plant Gene-Bank of Iran. Employing an alpha lattice design with two replications, various agro-morphologic characteristics were measured. Analysis of pairwise association's unveiled positively significant interrelationships of yield performance with the other measured characteristics, except for 1000-seed weight. This suggested that these traits generally moved in the same direction, positively influencing seed yield. To gain a more nuanced understanding of trait interdependencies, sequential path analysis was employed. This analysis identified the capitula of each plant and the seeds of each capitulum as crucial first-order characteristics significantly impacting seed yield. All direct effects identified through sequential path analysis were deemed significant, emphasizing the robustness of the findings. The analysis further categorized characteristics as second or third order variables according to impacts on seed yield. Leaf area, plant's dry weight and 1000-seed weight were identified as second-order variables, indicating their indirect influence on seed yield. Additionally, height of plant, oil percentage, diameter of lateral capitulum and biological yield were categorized as third-order characteristics, further expanding the understanding of the complex trait relationships. The key takeaway from the study is the potential use of the capitula of each plant and the seeds of each capitulum as effective selection criteria for enhancing safflower yield performance. These findings provide valuable insights for safflower breeding programs, guiding the selection of genotypes with improved seed yield based on these identified influential traits.

*Keywords*: Multicollinearity, Path analysis, Resampling, Path analysis

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# INTRODUCTION

Selection is one of the foremost critical instruments in plant breeding programs and choice for a specific characteristic depends upon the degree of coordinate or roundabout impact of the characteristic on target characteristic such as seed yield. Seed yield of safflower as a quantitative characteristic, is to a great extent impacted by the non-genotypic effects like environmental and GE (genotype by environment) interaction and subsequently contains a low heritability (AFZAL *et al*., 2021). Thus, the reaction to direct determination of yield performance may be erratic, and breeders have to be look at the connections among diverse characteristics. Therefore, before starting selection, it gets to be fundamental to know the relative significance of the other characteristics in impacting the target characteristic within the wanted direction. In such circumstances, simple associations deficiently to depict the relations to choose on selection methodology in management of advancement program (COOPER *et al*., 2022).

The grasp of the effects' types (direct and indirect) of various characteristics, particularly in the context of safflower seed yield, which is influenced by various environmental factors and genotype × environment interactions, resulting in low heritability, is very important. The sequential path analysis is a valuable method for understanding the primary effects of predictor traits and obtaining a more accurate fit for various crops. However, it highlights a crucial point that the multi-collinearity of predictor traits was not assessed before organizing these traits into path orders. This consideration is crucial for ensuring the reliability and accuracy of the analysis. The rection to selection as directly form in the yield performance may be unpredictable, necessitating a comprehensive examination of relationships among different traits before initiating selection in breeding programs (COBB *et al*., 2019). The sequential path analysis is introduced as a beneficial approach for discerning the original effects of predictor traits and providing a better fit for any crop.

However, it is noted that the multi-collinearity attribute of predictor variable was not examined before organizing the traits into steps of path, a consideration that is essential for accurate analysis. The use of sequential path analysis has been successfully applied in other crops like wheat (JANMOHAMMADI *et al*., 2014) and rye (NAYEBI-AGHBOLAG *et al*., 2019), but its detailed application in safflower has not been explored despite past investigations on interrelationships coefficient and conventional path analysis in the literature.

The importance of testing for multi-collinearity among predictor traits before applying sequential path analysis is important. Multi-collinearity refers to the situation where used characteristics as predictors in analysis are highly associated, which can pose challenges in determining the independent contribution of each variable (FIROUZABADI *et al*., 2011). Sequential path analysis is credited with providing a better fit for various crops, offering a nuanced understanding of how different traits interact to influence the target variable. The text notes that this analytical approach has been successfully used in crops like maize and wheat, suggesting its effectiveness in uncovering complex trait relationships. The proposed of this research are outlined, emphasizing the estimation of correlations between different quantitative traits and the execution of both conventional and sequential path coefficient analyses. The goal is to understand the contributions of component traits to yield performance in safflower, which could prove valuable in genetic improvement of higher-yielding safflower cultivars.

## MATERIALS AND METHODS

The study involved 64 genotypes were prepared from the National Plant Gene-Bank of (Table 1).

*Table 1 The name and flower color of studied safflower genotypes.*

| No. | Name | Origin | Flower color | No. | Name | Origin | Flower color |
|---|---|---|---|---|---|---|---|
| 1 | Unnamed 2 | - | Yellow | 33 | Esfahan | Iran | Yellow-Red |
| 2 | Unnamed 3 | - | Yellow | 34 | Esfahan Kuseh | Iran | Red |
| 3 | Zarghan | Iran | Red | 35 | Kurdistan 6 | Iran | Yellow |
| 4 | 73-14-34 | - | Yellow | 36 | $C_{114}$ | Iran | Yellow |
| 5 | Dincer | Turkey | Yellow-Red | 37 | 21 | - | Red |
| 6 | Unnamed 5 | - | Yellow | 38 | 324-S6-697 | - | Yellow |
| 7 | $C_{111}$ | Iran | Yellow-Red | 39 | $C_{411}$ | Iran | Orenge |
| 8 | Unnamed 1 | - | Yellow | 40 | 60 | - | Yellow |
| 9 | 27/N | - |  | 41 | Kurdistan 7 | Iran | Orenge |
| 10 | $C_{444}$ | Iran | Yellow | 42 | S-541 | USA | Yellow |
| 11 | PI- 405985 | Iran | Yellow | 43 | 40 | - | Yellow |
| 12 | 46 | - | Yellow | 44 | PI- 253384 | Palestine | Yellow |
| 13 | PI- 258417 | Portugal | Yellow-Red | 45 | Kurdistan 3 | Iran | Red |
| 14 | Kurdistan 9 | Iran | Red | 46 | 301055 | Turkey | Yellow |
| 15 | 330 | - | Yellow | 47 | 38 | - | Orenge |
| 16 | Kurdistan 5 | Iran | Red | 48 | IL | Iran | Red |
| 17 | 376 | - | Yellow | 49 | PI- 506426 | China | Orenge |
| 18 | $C_{4110}$ | Iran | Red | 50 | 44 | - | Yellow |
| 19 | Yinice | Turkey | Yellow | 51 | 62 | - | Yellow |
| 20 | Syrian | Syria | Red | 52 | 17 | - | Yellow |
| 21 | Kurdistan 4 | Iran | Red | 53 | PI- 250537 | Egypt | Yellow |
| 22 | 508 | - | Yellow | 54 | Kurdistan 2 | Iran | Red |
| 23 | Syprus Bregon | Cyprus | Yellow | 55 | CW-4440 | USA | Yellow |
| 24 | LRV-51-51 | Iran | Yellow | 56 | 168-S6-58/41 | - | Orenge |
| 25 | Kino-76 | Mexico | Yellow-Red | 57 | Kurdistan 1 | Iran | Red |
| 26 | Gila | USA | Yellow-Red | 58 | Hartman | USA | Yellow |
| 27 | PI-250190 | Pakistan | Yellow-Red | 59 | Unnamed 4 | - | Yellow |
| 28 | 47 | - | Red | 60 | Kurdistan 8 | Iran | Yellow |
| 29 | PI-98844 | France | Yellow-Red | 61 | Sina | Iran | Yellow |
| 30 | Marand | Iran | Yellow | 62 | LRV-55-295 | - | Yellow |
| 31 | PI-537636-S | USA | Yellow | 63 | Arak | Iran | Yellow |
| 32 | PI-537636 | USA | Yellow | 64 | 307-S6-697 | - | Yellow |

The trial took place in the research field of University of Maragheh, Iran, with coordinates 37°23′N 46°14′E. The mean altitude was 1477 m, and the soil was characterized as clay loam with a pH of 8.0 and in the field (Table 2), 40 kg ha$^{-1}$ of phosphorus pentoxide and 30 kg ha$^{-1}$ of N were used in early season, with an additional 20 kg ha$^{-1}$ of N before the onset of stem elongation. Weed control was carried out manually, and in the spring of 2020. An 8 × 8 alpha lattice design with two repetitions was performed. Genotypes were sown in five rows, covering a 4 m length, and with a spacing of 50 cm between rows. Random samples were gotten from each plot to determine various parameters, including plant height (PH), main capitulum diameter (MCD), lateral capitulum diameter (LCD), number of capitula per plant (NCP), number of seeds per capitulum (NSC), leaf area (LA), and dry weight of the plant (DWP). The 1000-seed weight (TSW) and oil percent (OP) were recorded on a random section of seeds gathered from genotypes. A 4.5 $m^2$ (1.5 × 3.0 $m^2$) area from the middle three rows was harvested and biological yield (BY) and seed yield (SY) were determined. Using biological yield, oil content, and seed yield, the harvest index (HI) and oil yield (OY) were computed. To measure seed oil, approximately 45 to 50 grams of healthy seeds from each sample were selected. After peeling, the seeds were powdered using a mill. The seed powder was then subjected to extraction using the Soxhlet method, employing a temperature of 45°C and diethyl ether (dry) as the solvent. The solvent in the extracted oil was separated using an oven under vacuum conditions at a temperature of 45°C. Path analysis was conducted to categorize the measured traits into various paths regarding their portions to the observed variability in seed yield performances and low multi-collinearity. The magnitudes of standard error of each path were calculated via bootstrap analysis.

*Table 2. Properties and available nutrients of the studied farm soil*

| Soil properties | | Available nutrients | |
|---|---|---|---|
| Soil texture | Sandy loam | K (mg/l) | 360 |
| Total nitrogen (mg/l) | 0.6 | Zn (mg/l) | 0.14 |
| $CaCO_3$ (Equivalent to mg/l) | 9.75 | Mn (mg/l) | 1.53 |
| Organic carbon (mg/l) | 0.31 | Fe (mg/l) | 3.12 |
| Water EC (ds/m) | 0.229 | Cu (mg/l) | 1.65 |
| Soil EC (ds/m) | 1.06 | Mg (mg/l) | 5.34 |

## RESULTS AND DISCUSSIONS

To ascertain the most accurate assessment of direct and indirect influences on yield performance, we employed correlation analyses and conducted path coefficient analyses. The outcomes of the simple correlation coefficients (Table 3.) revealed strong positive associations among yield performance and all the assessed traits, with the exception of TSW. Likewise, in alignment with SADIGHFARD *et al*. (2022), our findings indicated a positively strong association between yield performance and factors such as plant height, number of capitula per plant, capitulum diameter, and number of seeds per capitulum. However, a noteworthy negative correlation was seen between seed performance and 1000-seed weight. AHMADZADEH *et al*. (2012) unearthed compelling evidence showcasing a substantial positive interconnection between yield performance and several key parameters. Specifically, there were robust associations identified with plant height, the number of seeds per capitulum, dry weight of the plant, biological yield, and oil yield. Conversely, a significant negative correlation was established among yield performance and the weight of 1000 seeds. Our current investigation, as delineated in Table 3, accentuates the intricate relationships existing between all the examined traits and LA, DWP, and PH. Strikingly, these correlations were consistently positive and statistically significant, excluding the trait TSW. Impressively, these findings harmonize closely with the outcomes elucidated by BALJANI *et al*. (2015), further fortifying the robustness of our results through congruence with prior safflower research.

*Table 3. Pearson's correlation coefficient between studied traits of 64 safflower genotypes*

| | LA | DWP | PH | MCD | LCD | NCP | NSC | TSW | TB | SY | OP | HI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DWP | 0.84* | | | | | | | | | | | |
| PH | 0.45 | 0.27 | | | | | | | | | | |
| MCD | 0.81 | 0.74 | 0.49 | | | | | | | | | |
| LCD | 0.84 | 0.79 | 0.25 | 0.72 | | | | | | | | |
| NCP | 0.82 | 0.81 | 0.41 | 0.69 | 0.75 | | | | | | | |
| NSC | 0.84 | 0.63 | 0.51 | 0.69 | 0.65 | 0.59 | | | | | | |
| TSW | -0.83 | -0.79 | -0.53 | -0.74 | -0.69 | -0.87 | -0.72 | | | | | |
| TB | 0.65 | 0.55 | 0.65 | 0.68 | 0.41 | 0.71 | 0.61 | -0.74 | | | | |
| SY | 0.86 | 0.64 | 0.58 | 0.75 | 0.73 | 0.73 | 0.87 | -0.79 | 0.67 | | | |
| OP | 0.77 | 0.77 | 0.32 | 0.70 | 0.58 | 0.70 | 0.64 | -0.72 | 0.72 | 0.61 | | |
| HI | 0.78 | 0.55 | 0.46 | 0.63 | 0.72 | 0.60 | 0.83 | -0.69 | 0.42 | 0.95 | 0.47 | |
| OY | 0.88 | 0.69 | 0.46 | 0.73 | 0.77 | 0.70 | 0.88 | -0.76 | 0.53 | 0.97 | 0.65 | 0.97 |

*Critical vales of correlation $P<0.05$ and $P<0.01$ (D.F. 62) are 0.25 and 0.32, respectively

For trait abbreviations refer to text

These associations offer valuable insights for the selection of diverse genotypes with desirable characteristics, such as those exhibiting high seed yield or a high percentage of oil content. Notably, a statistically significant and positive correlation was discerned between MCD, LCD, and NCP, and various other safflower traits, excluding TSW as indicated in Table 3. Remarkably, these findings align closely with the investigation performed by NASERIRAD *et al.* (2013), underscoring the consistent influence of morphological traits on various yield components. The results highlight the significant impact of morphological traits on key yield components, emphasizing the importance of considering changes in yield components and interrelations with other morphological traits, such as the number of capitula per plant, when selecting safflower genotypes. Additionally, NSC, HI, OP, and OY demonstrated positively strong association with other characteristics, except for TSW, as detailed in Table 3. Consequently, these traits emerge as crucial factors for enhancing oil yield in safflower, aligning with the findings of HAMZA and ABDALLA (2015), who similarly highlighted the positive correlation between the capitula of each plant and the seeds of each capitulum with oil yield.

In conducting a comprehensive assessment of the impact of various traits on safflower seed yield, a sophisticated approach was employed—conventional path analysis. This analytical method not only delves into the correlation coefficients but also disentangles them into distinct components, shedding light on the direct and indirect influences of the measured characteristics. The intricate web of relationships among these variables was explored, providing nuanced insights into their contributions. In particular, the investigation into the direct influences of characteristics on safflower yield preference involved treating characteristics as first-order traits. To ensure the robustness of our analysis, indices of multi-collinearity, including Tolerance and VIF, were meticulously considered and integrated into the examination. The detailed results of these analyses are meticulously presented in Table 4, offering a comprehensive view of the interplay among the variables. However, the pursuit of clarity led us to a notable revelation—when all characteristics were examined as first-orders, a scenario of heightened multicollinearity emerged. This phenomenon was especially pronounced for traits with significant direct effects, such as HI with a VIF of 176.2, OY with a VIF of 241.2, LA with a VIF of 14.5, and OP with a VIF of 13.9. Recognizing the potential distortion introduced by multicollinearity, these traits were judiciously excluded as first-order variables. This strategic adjustment aimed at enhancing the precision of our analysis and ensuring a more nuanced understanding of the intricate relationships at play in the context of safflower yield performance. The direct effects, conducted through sequential path analysis (Table 5), took into account characteristics organized into first, second, and third-order traits concerning safflower yield performance. To enhance the clarity of interrelationships among different traits, multicollinearity analysis was applied, revealing a noteworthy reduction in VIF values (Table 5).

*Table 4. Regression slope coefficient and direct effects of first-order predictor variables on the seed yield of 64 safflower genotypes and two common measures of collinearity in conventional path analysis.*

| Traits | Slope | Direct effect | Tolerance | VIF* |
|---|---|---|---|---|
| LA | 0.076 | 0.015 | 0.069 | 14.5 |
| DWP | -0.960 | -0.010 | 0.095 | 10.5 |
| PH | -1.518 | -0.016 | 0.416 | 2.4 |
| MCD | 90.959 | 0.033 | 0.216 | 4.6 |
| LCD | 60.004 | 0.020 | 0.163 | 6.1 |
| NCP | 0.516 | 0.004 | 0.099 | 10.1 |
| NSC | -2.734 | -0.026 | 0.118 | 8.5 |
| TSW | 11.431 | 0.053 | 0.089 | 11.2 |
| TB | 0.167 | 0.371 | 0.147 | 6.8 |
| OP | -31.694 | -0.122 | 0.072 | 13.9 |
| HI | 64.263 | 0.476 | 0.006 | 176.2 |
| OY | 0.020 | 0.410 | 0.004 | 242.1 |

*VIF: variance inflation factor. For trait abbreviations refer to text

The utilization of a stepwise regression model in our current research played a pivotal role in minimizing collinearity measures for all the measured traits. This strategic approach facilitated the identification of the real portion of every predictor trait in the context of paths. The discernible advantages of the sequential procedure over conventional model became evident, notably in its ability to mitigate collinearity issues and pinpoint the genuine portion of each variable in the paths. This methodology aligns with the findings in other crops, such as rapeseed (SABAGHNIA *et al*., 2010) and wheat (JANMOHAMMADI *et al*., 2014), underscoring its effectiveness in achieving favorable and reliable results across diverse agricultural contexts. In the realm of these analyses, breeders typically pursue not only a unique point estimator but also an understanding of its variability, coupled with a confidence interval for the statistical parameter. The bootstrap method, serving as a resampling tool, proves invaluable by providing approximations of the statistics’ distribution and magnitude of standard error. Through the examination of paths derived from bootstrapping, a striking alignment with the observed values was discerned (Table 6). The notably low errors and bias in these estimates underscored the robustness of this method employed in our study. Significance tests conducted through bootstrap resampling, although not explicitly shown in the provided data, demonstrated the meaningfully of direct effects.

*Table 5. Measures of collinearity values (tolerance and variance inflation factor, VIF) for predictor variables in conventional path analysis (CPA, all predictor variables as first-order variables) and sequential path analysis (SPA, predictors grouped into first-, second-, and third-order variables).*

| Predictor variable | Response variable | Tolerance |  | VIF |  |
|---|---|---|---|---|---|
|  |  | CPA | SPA | CPA | SPA |
| NSC | SY | 0.118 | 0.646 | 8.5 | 1.5 |
| NCP |  | 0.099 | 0.646 | 10.1 | 1.5 |
| LA | NSC | 0.069 | 0.288 | 14.5 | 3.5 |
| DWP |  | 0.095 | 0.288 | 10.5 | 3.5 |
| DWP | NCP | 0.095 | 0.379 | 10.5 | 2.6 |
| TSW |  | 0.089 | 0.379 | 11.2 | 2.6 |
| PH | LA | 0.416 | 0.893 | 2.4 | 1.1 |
| OP |  | 0.072 | 0.638 | 13.9 | 1.6 |
| LCD |  | 0.163 | 0.663 | 6.1 | 1.5 |
| OP | DWP | 0.072 | 0.669 | 13.9 | 1.5 |
| LCD |  | 0.163 | 0.669 | 6.1 | 1.5 |
| LCD | TSW | 0.163 | 0.834 | 6.1 | 1.2 |
| TB |  | 0.147 | 0.834 | 6.8 | 1.2 |

Furthermore, the coefficient of determination (0.83) emerged as a pivotal metric, offering insight into the cumulative influence of NSC and NCP traits, treated as first-order variables, on the total variability observed in safflower seed yield (Table 6). This comprehensive evaluation, supported by the bootstrap method, enhances the reliability and depth of our understanding of the interplay between traits and their impact on safflower seed yield. Within the realm of NSC and NCP traits, it's noteworthy that NSC exhibited a notably greater direct effect (0.68) on seed yield compared to NCP (0.32). The amount of indirect influence on NSC, via NCP, was low but positive (0.19), while the indirect effect on NCP, via NSC, was positively high (0.41) as presented in Table 7. This suggests that NSC plays a more influential role in directly impacting seed yield, while NCP contributes significantly to seed yield indirectly through its positive influence on NSC. For a more visual representation and enhanced grasp of the association of various traits and their respective contributions to yiel performance, the sequential model (Fig. 1) serves as a valuable resource.

Interestingly, these findings contrast with those of AHMADZADEH *et al*. (2012), who identified the strongest direct effects of PH (Plant Height), TSW (Thousand Seed Weight), and DWP (Dry Weight of Plant) on seed yield. Additionally, KHALILI *et al*. (2013) highlighted the strongest direct effects of NCP and capitulum diameter on yield performance. In contrast, the conclusions drawn by SADIGHFARD *et al*. (2022) align more closely with your study, emphasizing that NSC and NCP traits exert the most substantial influence on seed yield. These variations in findings underscore the complexity of genotype-trait interactions and the context-specific nature of such relationships in different studies and environments. Specifically, LA and DWP traits exhibited positive influences on NSC, collectively elucidating more than 73% of the seen variability, as detailed in Table 6. Moreover, DWP had a favorable impact, while TSW exerted a negative influence on the NCP trait, jointly explaining over 80% of the total observed variability in this context. Also, the results unveiled intricate relationships. PH, OP, and LCD positively influenced LA, accounting for approximately 86% of the variability. Additionally, OP and LCD affected DWP, contributing to roughly 76% of the observed variation (Table 6). Furthermore, LCD and TB negatively impacted TSW, explaining around 73% of the observed variation.

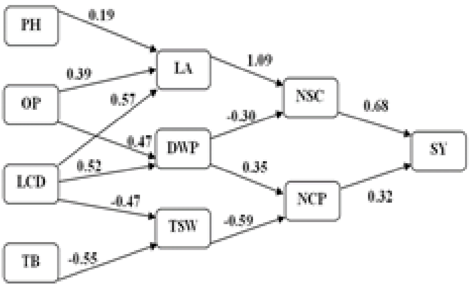

Fig. 1 Sequential path analysis diagram illustrating the interrelationships among various traits contributing to seed yield of safflower. For trait abbreviations refer to text.

*Table 6. Estimation of standard error values of path coefficients using bootstrap analysis.*

| Predictor variable | Response variable | Adjusted $R^2$ | Direct effect | Bootstrap | | |
|---|---|---|---|---|---|---|
| | | | | Mean | Bias | SE |
| NSC | SY | 0.83 | 0.68 | 0.68 | 0.0018 | 0.0571 |
| NCP | | | 0.32 | 0.32 | -0.0024 | 0.0696 |
| LA | NSC | 0.73 | 1.09 | 1.09 | -0.0018 | 0.1105 |
| DWP | | | -0.30 | -0.29 | 0.0038 | 0.1006 |
| DWP | NCP | 0.80 | 0.35 | 0.35 | 0.0072 | 0.0767 |
| TSW | | | -0.59 | -0.59 | 0.0049 | 0.0819 |
| PH | LA | 0.86 | 0.19 | 0.19 | 0.0051 | 0.0465 |
| OP | | | 0.39 | 0.39 | 0.0033 | 0.0594 |
| LCD | | | 0.57 | 0.56 | -0.0071 | 0.0505 |
| OP | DWP | 0.76 | 0.47 | 0.47 | 0.0044 | 0.0822 |
| LCD | | | 0.52 | 0.50 | -0.0187 | 0.1143 |
| LCD | TSW | 0.73 | -0.47 | -0.46 | 0.0042 | 0.0656 |
| TB | | | -0.55 | -0.55 | 0.0010 | 0.0675 |

For trait abbreviations refer to text

These findings highlight the complexity of trait interactions and their cascading effects across different orders of variables, offering a nuanced understanding of the factors influencing the observed variations in the studied traits. Such insights are crucial for refining breeding strategies and optimizing trait selection for the desired outcomes in safflower cultivation. The success of safflower cultivation hinges on the substantial improvement in both seed yield and oil percentage achieved through breeding efforts. Essential traits such as branches of plant and 1000-seed weight play pivotal roles in influencing seed yield, as emphasized in prior research by YASSEIN *et al*. (2020). In our study, plant height and oil percentage were identified as third-order variables with positive effects, underscoring their importance in contributing to the overall improvement of safflower performance. While some traits did not exhibit positive effects or were not measured in this study, the focus on key factors such as NCP and NSC as they relate to seed yield holds significant promise for indirect selection. The efficiency of utilizing these traits as selection criteria, however, is contingent upon their heritability and genetic association with overall crop performance. Given that safflower seed yield stands as a primary breeding goal, selection efforts must prioritize this objective to ensure meaningful progress. The recognition of the significance of NCP and NSC as yield component traits aligns with findings from previous studies, such as those by NASERIRAD *et al.* (2013), lending additional support to the observations made in this study regarding the crucial role of these traits in influencing safflower seed yield. This reinforces the importance of considering these traits in breeding programs aimed at enhancing safflower productivity and quality.

*Table 7. Direct and indirect effects for the predictor variables in sequential path analysis (grouped into first, second and third order variables).*

**SY**

| | NSC | NCP |
|---|---|---|
| NSC | 0.68 | 0.19 |
| NCP | 0.41 | 0.32 |

**NSC**

| | LA | DWP |
|---|---|---|
| LA | 1.09 | -0.25 |
| DWP | 0.92 | -0.30 |

**NCP**

| | DWP | TSW |
|---|---|---|
| DWP | 0.35 | 0.47 |
| TSW | -0.27 | -0.59 |

**LA**

| | PH | OP | LCD |
|---|---|---|---|
| PH | 0.19 | 0.12 | 0.14 |
| OP | 0.06 | 0.39 | 0.33 |
| LCD | 0.05 | 0.22 | 0.57 |

**DWP**

| | OP | LCD |
|---|---|---|
| OP | 0.47 | 0.30 |
| LCD | 0.27 | 0.52 |

**TSW**

| | LCD | TB |
|---|---|---|
| LCD | -0.47 | -0.22 |
| TB | -0.19 | -0.55 |

Oil percentage stands out as an economically pivotal trait in safflower, wielding a significant influence on its success, especially in new cultivation areas. The noteworthy and positive correlations observed between seed yield and oil percentage, coupled with higher oil yield values, resonate well with previous reports, as documented by ADA (2013). This alignment with prior research underscores the reliability and consistency of the observed relationships, emphasizing the economic importance of oil percentage in safflower crops. Looking towards future breeding and selection endeavors, a profound understanding of the variation in crop yield and its components, as well as the intricate ways in which these components collectively shape overall yield formation, becomes paramount. Path analysis emerges as a valuable tool in this regard, not only for pinpointing the most influential traits directly impacting yield but also for unraveling the intricate network of indirect effects through interrelated traits, as elucidated by SABAGHNIA *et al*. (2010). Crucially, path analysis aids in the identification of yield component compensation phenomena. This phenomenon occurs when two or more yield components influencing overall yield, or any other yield component, exert opposing effects. In safflower, it becomes apparent that the number of capitula per plant, number of seeds per capitulum, and 1000-seed weight emerge as three crucial yield component factors. Interestingly, TSW indicates a compensation phenomenon due to its negative association with the other two yield components. This nuanced insight enhances our understanding of the dynamic interactions among these identified traits, providing valuable information for strategic breeding efforts and trait selection in safflower cultivation.

The traits selected for path analysis in this study proved to be sufficient in explaining the variation in the dataset of 64 safflower genotypes. Notably, NSC and NCP emerged as the variables with the most significant direct and indirect effects on seed yield, making substantial contributions. As a result, these traits should be prioritized in genetic improvement programs for safflower, aiming to develop more productive genotypes. The utilization of selection indexes, taking into account these variables, is recommended to enhance gains in seed yield. We found positive association among yield performance and other traits, with NSC and NCP as first-order variables, LA, DWP, and TSW as second-order variables, and PH, OP, LCD, and TB as third-order variables. This layered understanding underscores the intricate web of relationships influencing safflower seed yield. In conclusion, the importance of NSC and NCP traits for selection in safflower genetic improvement, particularly in the context of improving yield performance under semi-arid conditions, is evident. These findings contribute valuable insights to the ongoing efforts aimed at enhancing the productivity and resilience of safflower crops.

This research text emphasized the importance of assessing predictor traits before organizing them into path orders and the sequential path analysis has been applied successfully in safflower which is not only identifies the most crucial traits affecting seed yield directly but also reveals how these traits influence yield indirectly through other traits. Finally, the findings suggest that the NSC and NCP traits play a significant role in yield which emphases the importance of incorporating these traits into breeding programs for genetic improvement to achieve more productive safflower genotypes. The capitulum of each plant, seeds of each capitulum and 1000-seed weight are reported as important seed yield components of safflower (SADIGHFARD *et al*., 2022), while we found only first two components and 1000-seed weight was not related. These components are crucial determinants of safflower seed yield whereas NSC and NCP directly contribute to the total seed production. Breeders and researchers often analyze these components to understand the factors influencing safflower productivity and to develop strategies for improving seed yield. Also, the compensation phenomenon in yield components, where several components may act inversely in their effects can be exist in safflower It can be concluded that by evaluation of traits' associations and sequential analysis which provide some information for associations of seed yield with other traits. Finally, the investigation outlines the nuances of applying sequential path analysis in safflower, underscores the importance of specific traits, and highlights the implications for safflower breeding programs. It emphasizes the need for a detailed understanding of trait relationships to guide effective breeding strategies. Path analysis in safflower is a valuable tool for crop improvement, offering a systematic approach to unravel the intricate relationships among traits. Scientific studies utilizing this technique contribute essential knowledge for sustainable safflower breeding programs.

Data availability statements

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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# PROCENA VAŽNIH OSOBINA KOJE UTIČU NA PRINOS *Carthamus tinctorius* L.

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## Izvod

Studija je procenila genetsku raznolikost i odnose između osobina kod 64 genotipa *Carthamus tinctorius*. dobijenih iz Nacionalne banke biljnih gena Irana. Koristeći alfa lattice dizajn sa dva ponavljanja, merene su različite agromorfološke karakteristike. Analiza parnih asocijacija otkrila je pozitivno značajne međusobne odnose prinosa sa ostalim merenim karakteristikama, osim za težinu 1000 semena. Ovo sugeriše da su se ove osobine generalno kretale u istom smeru, pozitivno utičući na prinos semena. Da bi se steklo nijansiranije razumevanja međuzavisnosti osobina, korišćena je sekvencijalna analiza putanje. Ova analiza je identifikovala kapitulu svake biljke i seme svake kapitule kao ključne karakteristike prvog reda koje značajno utiču na prinos semena. Svi direktni efekti identifikovani putem sekvencijalne analize putanje ocenjeni su kao značajni, što naglašava robusnost nalaza. Analiza je dalje kategorisala karakteristike kao varijable drugog ili trećeg reda prema uticajima na prinos semena. Površina lista, suva težina biljke i težina 1000 semena identifikovane su kao varijable drugog reda, što ukazuje na njihov indirektni uticaj na prinos semena. Pored toga, visina biljke, procenat ulja, prečnik bočnog kapituluma i biološki prinos kategorisani su kao karakteristike trećeg reda, što dodatno proširuje razumevanje složenih odnosa između osobina. Ključni zaključak iz studije je potencijalna upotreba kapituluma svake biljke i semena svakog kapituluma kao efikasnih kriterijuma selekcije za poboljšanje prinosa *Carthamus tinctorius*. Ovi nalazi pružaju vredne uvide za programe oplemenjivanja *Carthamus tinctorius*, vodeći selekciju genotipova sa poboljšanim prinosom semena na osnovu ovih identifikovanih osobina.

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