



# Characterization and Evaluation of New Sunflower Genotypes

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

The current investigation was planned to characterize the sunflower accessions based on DUS descriptors and to determine the level of genetic variability for sunflower genotypes. Thirty-three sunflower genotypes including checks were morphologically characterized using Protection of Plant Varieties and Farmers' Rights Authority (PPV& FRA) and other descriptors during *rab*2021-22 at the department of Genetics and Plant Breeding, ITM University, Gwalior, Madhya Pradesh, India. The analysis of variance revealed significant differences among the sunflower genotypes, suggesting genotypes had enough variability. Various morphological characters were studied which showed good extent of variation among genotypes. Large variation was recorded for strip colour and for ray floret colour differentiation which can utilize to identify the genotype at seed stage and at flowering stage and may serve as morphological markers to identify the accessions. Genotypes with black colored seed viz., GMU-837, GMU-934, GMU-78, and GMU-55 can be utilized for the development of breeding material and populations. All of the characteristics under study recorded larger phenotypic coefficient of variation (PCV) than genotypic coefficient of variation (GCV), which

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implies that environmental influence was more in their expression. High heritability along with genetic advance was observed for by plant height and days to maturity, which indicates selection of these traits at early generation would be effective.

**Keywords:** DUS descriptors; variability; evaluation; genetic advance; sunflower.

## 1. INTRODUCTION

Among various edible oilseeds crop sunflower is an important crop and its oil is consumed by large population in India. In 2017–18, a production level of 0.241 million tons at a productivity of 699 kg/ha was reported [1,2]. Characterization and evaluation of the working germplasm is a regular activity carried out by germplasm curator to unravel the variability present among the germplasm and to identify the important trait-specific accessions for breeding programme [3]. Once the trait specific working germplasm is identified which could be a donor source in the future breeding [4]. The DUS descriptors suggested by the Protection of Plant Varieties & Farmers Rights Authority (PPV&FRA) were useful in order to discriminate the genotypes morphologically and to group the accessions as per the trait description. The initial variation present in the working germplasm determines the success of breeding programme. Phenotypic selection based on performance may not always be sufficient. The reason is that genotypes may perform poorly in subsequent segregating generations, it is necessary to choose genotypes based on their genetic merit, i.e. based on their genetic value including heritability [5]. The amount of genetic gain expected from selection would be calculated using genetic variability and heritability calculations [6]. Variability and heritability are the parameters which can be used for selection to improve seed yield and its component attributes. Heritability estimates combined with genetic progress are usually more useful in projecting selection gain than heritability estimates alone [7]. Considering all of these aspects, the current investigation was planned to characterize the sunflower accessions based on DUS descriptors and to determine the level of genetic variability for sunflower genotypes.

## 2. MATERIALS AND METHODS

The field experiment was carried out at the crop research centre of department of Genetics and Plant Breeding, ITM University, Gwalior, Madhya Pradesh, India, geographically located at 26.22°N, 78.18°E and at an average elevation of about 197m above the mean sea level. Thirty

three germplasm accessions were collected from the ICAR-Indian Institute Oilseeds Research (IIOR) Rajendranagar, Hyderabad, India which included 30 sunflower germplasm accessions and three checks (DRSF-113, DRSF-108 (National checks), and Phule Bhaskar (Local check grown in Maharashtra). All the accessions were evaluated during *rabi* seasons of 2021-22 in a Randomized Block Design (RBD) with three replications, with spacing of 60 x 30 cm with a plot size of 3m x3m. For each genotype seed was seeded by dribbling and 2 seeds each hill were sown to ensure the germination. The experimental was subjected to standard agronomic practices. Eleven morphological characters were recorded as per DUS descriptors [8]. Extra character early vigor was recorded till 20 days after seedling emergence and recorded in three categories as poor, good and very good. For taking observations, a visual assessment was performed, and a group of plants or components was chosen based on the crop's stage and observations were gathered the percentage of aberrant plants or components in the total genotypes should not exceed 5% for recording observations. To record observations, five plants were picked at random from each entry and five plants from the check in each replication to record data on days to 50% flowering, days to maturity, head diameter (cm), seed filling (%), seed yield per plant (g), oil content (%), 100 g seed weight (g), hull content (%). The genotypic coefficient (GCV) and phenotypic coefficient of variation (PCV) were computed using Burton's [6] formula. As per the Sivasubramanian and Madhavamenon [9], PCV and GCV estimates were classed as low (0-10 percent), moderate (10-20 percent), and high (>20 percent). The broad notion of heritability ( $h^2$ ) was calculated using the formula proposed by Johnson et al. [10] and Hanson et al. [11].

## 3. RESULTS AND DISCUSSION

Plant breeders are interested to understand genetic variation in germplasm based on quantitative and morphological traits. Evaluation of morphological traits does not require sophisticated techniques and expensive equipment's [3]. Morphological characterization was done using DUS descriptors to establish

diagnostic characteristics for each genotype. Based on DUS characters among 33 accessions, a wide range of variation for various traits is indicated. Abundant morphological variation was observed for early plant vigour it was observed that for six sunflower genotypes it was recorded as poor while good for 19 genotypes, and very good for 8 genotypes. Under the leaf related characters, there were greater variations for all the characters under study. Leaf margin/serration was fine for ten genotypes, medium serration for fourteen genotypes and coarse serration for nine genotypes. Disc floret color was yellow for twelve genotypes, purple for two genotypes, orange for nineteen genotypes. Good amount of variation was recorded for ray floret coloration with 13 genotypes as yellow, 10 as orange and pale yellow for rest of the genotypes. Usually black color seeds hybrids in sunflower are preferred by farmers in India [3]. Seed colour was black for eight genotypes, grey for 4 genotypes and brown for five genotypes. Genotypes with black colored seeds viz., GMU-

837, GMU-934, GMU-78, GMU-55 can be utilized for the development of breeding material and populations. All the checks (DRSF-108, DRSF-113, Phule Bhaskar) used in the present study also released variety TNAUCOSFV5 recorded black coloured seeds. Genotypes with black with white strips and light strips were for four genotypes each, grey with white strips for seven genotypes. In the present study we have recorded more variation in strips colours among different genotypes. Also, based on the strip colour and for ray floret colour differentiation can utilize to identify the genotype at seed stage and at flowering stage which may serve as morphological markers to identify the accessions. Earlier researchers who characterized sunflower accessions based on morphological traits were Virupakshappa and Sindagi, [12], Reddy and Reddy [13], and Dudhe et al. [3] and [14] supports to the present findings. Earlier large scale characterizations of 3273 sunflower accessions were characterized based on 33 DUS descriptor [15] which supports to our findings.

**Table 1. List of sunflower genotypes used in the study and their attributes**

| Sr. No | Acessions Number | Remark                         |
|--------|------------------|--------------------------------|
| 1      | GMU-78           | Early, dwarf                   |
| 2      | GMU-55           | High yield                     |
| 3      | GMU-1073         | High yield                     |
| 4      | GMU-463          | High yield, medium maturity    |
| 5      | GMU-190          | High yield, medium maturity    |
| 6      | GMU-1020         | High yield                     |
| 7      | GMU-934          | High yield                     |
| 8      | GMU-1021         | High yield                     |
| 9      | GMU-787          | High yield                     |
| 10     | GMU-59           | Early, medium maturity         |
| 11     | GMU-1079         | High yield                     |
| 12     | GMU-1096         | High yield                     |
| 13     | GMU-1026         | High yield                     |
| 14     | GMU-356          | High yield                     |
| 15     | GMU-177          | High yield                     |
| 16     | GMU-468          | High yield                     |
| 17     | GMU-231          | High yield                     |
| 18     | GMU-127          | High yield                     |
| 19     | GMU-1147         | High yield, medium to high oil |
| 20     | GMU-249          | High yield                     |
| 21     | GMU-1041         | High yield                     |
| 22     | GMU-486          | High yield                     |
| 23     | GMU-837          | High Yield                     |
| 24     | GMU-495          | Early, medium maturity         |
| 25     | GMU-383          | Early                          |
| 26     | GMU-1031         | High Yield                     |
| 27     | GMU-1037         | High Yield                     |
| 28     | GMU-687          | Medium to high yield           |
| 29     | GMU-1058         | High yield                     |
| 30     | TNAU COSFV5      | Released variety               |
| 31     | PHULE BHASKAR    | Local check ©                  |
| 32     | DRSF-108         | National check ©               |
| 33     | DRSF-113         | National check ©               |

**Table 2. Morphological characterization of sunflower genotypes**

| Sr. No | Morphological Character | Different Attributes  | Frequency | Germplasms  |
|--------|-------------------------|-----------------------|-----------|---|
| 1      | Early plant vigour      | Poor                  | 6         | GMU-837,GMU-485,GMU-383,GMU-1031,GMU-1037,GMU-687   |
|        |                         | Good                  | 19        | GMU-55,GMU-1073GMU-190,GMU-934,GMU-1021,GMU-59,GMU-1079,GMU-1096,GMU-356,GMU-1026,GMU-177GMU-468,GMU-127,GMU-1147,GMU-1041, DRSF-108, DRSF-113 Phule bhaskar,GMU-1058   |
|        |                         | Very good             | 8         | GMU-78,GMU-463,GM1020,GMU-787,GMU-231,GMU-249,GMU-486,TNAU COSFV-5  |
| 2      | Leaf shape              | Lanceolate            | 7         | GMU-78,GMU-1073,GMU-190,GMU-1020<br>GMU-934,GMU-249,GMU-1041  |
|        |                         | Triangle              | 13        | GMU-463,GMU-383GMU-1021,GMU-787,GMU-59,GMU-1079,GMU-1096,GMU-1026,GMU-356,GMU-177,GMU-468,GMU-231,GMU-1147  |
|        |                         | Cordated              | 5         | GMU-486,GMU-1031,GMU-1058,GMU-127, GMU-55   |
|        |                         | Rounded               | 8         | TNAUCOSVF5,GMU-687,GMU-1037,GMU-495,bGMU-837,bPHULE BHASKAR,DRSF-108,DRSF-113   |
|        |                         | Elongated             | 8         | GMU-55,GMU-1073,GMU-463,GMU-190,<br>GMU-1020,GMU-177GMU-468,GMU-231   |
| 3      | Ray floret              | Ovated                | 25        | GMU-78,GMU-934,GMU-1021,GMU-787,GMU-59,GMU-1096,GMU-1026,GMU-356,GMU-177,GMU-127,GMU-1147,GMU-249,GMU-1041,GMU-486,GMU-837,GMU-495,GMU-383,GMU-1031,GMU-1037,GMU-687,<br>GMU-1058,TNAU COSFV5, PhuleBhaskar, DRSF-113, DRSF-108 |
|        |                         | Rounded               | 0         | -Nil-   |
|        |                         | Pale yellow           | 10        | GMU-78,GMU-55,GMU-190,GMU-934,GMU-787,GMU-59,GMU-1079,GMU-1026,GMU-177, GMU-1073  |
| 4      | Ray floret colour       | Yellow                | 13        | GMU-463,GMU-1020GMU-1021,GMU-1096,GMU-356,GMU-468,GMU-231GMU-127GMU-1147,GMU-249GMU-1041,DRSF-113,DRSF-108  |
|        |                         | Orange                | 10        | GMU-486,GMU-837GMU-495,GMU-383,GMU-1031,GMU-1037,GMU-687,GMU-1058,Phule bhaskar,TNAUCOSFV5  |
|        |                         | Purple/Multi colour   | 0         | -Nil-   |
|        |                         | Concave               | 11        | GMU-78,GMU-55,GMU-190,GMU-1020,GMU-59,GMU-1079,GMU-1026,GMU-356,GMU-468,GMU-321,GMU-127   |
| 5.     | Head shape              | Flat                  | 6         | TNAUCOSFV5,GMU-1058, GMU-687,GMU-1031,GMU-495,GMU-1041  |
|        |                         | Convex                | 10        | GMU-1073,GMU-934,GMU-787,GMU-1147,GMU-249,GMU-1037,GMU-383,DRSF-108<br>DRSF-103,Phulebhaskar  |
|        |                         | Triangular/ Irregular | 6         | GMU-1096,GMU-177,GMU-1021,GMU-463,GMU-837,GMU-486   |
|        |                         | Inclined              | 9         | GMU-1073,GMU-190,GMU-463,GMU-1020,GMU-934,GMU-1021,GMU-787,GMU-59<br>GMU-1079   |
| 6.     | Head position           |                       |           |   |

| Sr. No | Morphological Character | Different Attributes    | Frequency | Germplasms   |
|--------|-------------------------|-------------------------|-----------|--|
| 7.     | Leaf colour             | Vertical                | 8         | GMU-78,GMU-55,GMU-1096,GMU-1026,GMU-177,GMU-468,GMU-356,TNAUCOSFV5   |
|        |                         | Half turned down        | 16        | GMU-231,GMU-127,GMU-1147,GMU-249,GMU-1041,GMU-486GMU-837,GMU-495,GMU-383,GMU-1031,GMU-1037,GMU-687,GMU-1058,DRSF-103,DRSF-118, PhuleBhaskar                                |
|        |                         | Turned Head             | 0         | -Nil-  |
|        |                         | Light green             | 11        | TNAUCOSFV5,GMU-1073,GMU-190,GMU-1020,GMU-787,GMU-59,GMU-1079,GMU-1026,GMU-356,GMU-468,GMU-78   |
|        |                         | Dark green              | 8         | GMU-1058,GMU-687GMU-1037,GMU-1031, GMU-383,GMU-495,GMU-486,GMU-249   |
| 8.     | Leaf serration          | Green                   | 12        | GMU-55,GMU-463,GMU-1096,GMU-177 GMU-231,GMU-127,GMU-1041,GMU-837,DRSF-108,DRSF-113,Phule Bhaskar, GMU-59   |
|        |                         | Fine                    | 10        | GMU-1026,GMU-356GMU-687,GMU-1058,GMU-127,GMU-1147,GMU-249,GMU-1041,GMU-486,GMU-837   |
|        |                         | Medium                  | 14        | GMU-78,GMU-55GMU-1073,GMU-463,GMU-190, GMU-1020GMU-934,GMU-1021,GMU-787,GMU-59GMU-1079,GMU-1096,GMU-177,GMU-468  |
|        |                         | Coarse                  | 9         | GMU-231,GMU-495,GMU-383,GMU-1031,GMU-1037, PhuleBhaskar, TNAUCOSFV5, DRSF-108,DRSF-113   |
| 9      | Disc floret colour      | Yellow                  | 12        | GMU-78,GMU-177,GMU-231,GMU-1020,GMU-934,GMU-249,GMU-1041,GMU-59,GMU-1079,GMU-1096,GMU-55,GMU-468   |
|        |                         | Purple                  | 2         | GMU-463GMU-190   |
|        |                         | Orange                  | 19        | GMU-1021,GMU-787,GMU-1031GMU-495,GMU-468,GMU-1026,GMU-383,GMU-1037,GMU-687, GMU-1058,GMU-837,GMU-356,GMU-1147, GMU-486,GMU-127,DRSF-113,DRSF-108, TNAUCOSFV5,Phule Bhaskar |
| 10     | Seed colour             | Black                   | 8         | GMU-837,GMU-934,GMU-78,GMU-55, DRSF-108,DRSF-113,TNAUCOSFV5,Phule Bhaskar  |
|        |                         | Black with white strips | 4         | GMU-787,GMU-383,GMU-127,GMU-463  |
|        |                         | Black with light strips | 4         | GMU-1041,GMU-59,GMU-468,GMU-1073   |
|        |                         | Brown                   | 5         | GMU-249,GMU-177,GMU-1031,GMU-190,GMU-837   |
|        |                         | Grey                    | 4         | GMU-1037,GMU-1058,GMU-231GMU-1010  |
|        |                         | Grey with white strips  | 7         | GMU-1147,GMU-687,GMU-1021,GMU-356, GMU-1020,GMU-1015,GMU-495   |

| Sr. No | Morphological Character | Different Attributes | Frequency | Germplasms  |
|--------|-------------------------|----------------------|-----------|---|
| 11     | Seed coated strips      | Present              | 11        | GMU-1147,GMU-687,GMU-1021,GMU-356,GMU-1020,GMU-1041,GMU-59,GMU-468,GMU-1015,GMU-1073,GMU-495  |
|        |                         | Absent               | 22        | GMU-78,GMU-55,GMU-463,GMU-190,GMU-934, GMU-787,GMU-177,GMU-1079,GMU-1096,GMU-1026,,GMU-231,GMU-127,GMU-249,GMU-486,GMU-837,GMU-383, GMU-1037,GMU-1058, TNAUCOSFV5,DRSF-108<br>DRSF-113, PhuleBhaskar  |
| 12     | Strips colour           | White                | 1         | GMU-59  |
|        |                         | Grey                 | 3         | GMU-1021,GMU-1041,GMU-468,  |
|        |                         | Brown                | 3         | GMU-356,GMU-1020,GMU-687  |
|        |                         | Black                | 3         | GMU-1147,GMU-1073,GMU-495   |
|        |                         | Absent               | 23        | GMU-78,GMU-55,GMU-463,GMU-190,GMU-934,GMU-787,GMU-1079,GMU-1096,GMU-1026,GMU-177,GMU-231,GMU-127,GMU-249,GMU-486,GMU-837,GMU-383,GMU-1037,GMU-687,GMU-1058,TNAUCOSFV5,PhuleBhaskar, DRSF-108,DRSF-113 |

Analysis of variance revealed significant differences among the genotypes for all the characters. A wide range of variability was exhibited by most of the traits under study (Table 3). The wide range of variation noticed in all the characters would offer scope of selection for improvement of desirable types. Similar observations were recorded by Dudhe et al. [14] which supports the present findings.

The estimates of PCV were moderate in case of seed yield per plant (19.13%), hulling% (15.73%), oil content (12.02%), 100 seed weight (11.28%) (Table 4). The estimates of GCV were moderate in case of seed yield per plant (16.20%). All of the characteristics had a larger phenotypic coefficient of variation (PCV) than genotypic coefficient of variation (GCV), which implies that environmental variables influenced their expression. The large difference in phenotypic and genotypic coefficients of variation suggested their sensitivity to environmental changes, whereas the smaller difference indicated less environmental interference in the expression of these characteristics. The qualities with the highest phenotypic and genotypic coefficients of variation are economically important, and they can be improved by selection. The high estimate of genetic advance as percent of mean was observed for the seed yield per plant (28.26%) followed by days to 50% flowering (15.10 %), hulling (14.66%), plant height (14.02%), days to maturity (13.85%). The genetic advance as percent of mean was moderate number of 100 g seed weight (10.88%) and volume of weight (8.01%). High heritability was associated with high genetic advance as percent of mean for plant height, while high heritability coupled with moderate genetic advance as percent of mean was observed for 100g seed weight. Moderate

heritability along with low genetic advance as percent of mean was expressed by remaining characters.

Variability is an essential pre-requisite for any crop development effort, especially for traits where improvement is needed. The overall variance was divided into three categories: phenotypic, genotypic, and environmental factors. The magnitude of PCV was higher than the GCV for all the ten characters under study. A narrow gap between PCV and GCV were observed for all the characters, suggesting non-significant influence of the environment on these traits with additive gene effect. Similar findings were also reported by Dudhe et al. [3] and Makane et al. [16]. The highest heritability was recorded for Days to 50% flowering followed by plant height, days to maturity, seed yield per plant. The moderate value for heritability was recorded for volume weight followed by 100 seed weight, hulling, seed filling per cent. The low value for heritability oil content, head diameter, our results are in agreement with the earlier findings of Virupakshappa and Sindagi (1988), Gangappa [17], Suma and Virupakshappa [18], Patil et al. [19] and Reddy and Reddy [13]. The result showed that highest value for genetic advance was observed for plant height followed by days to maturity which may implies the presence of additive genes in the trait and further suggest reliable crop improvement through selection of such traits. The low estimate of genetic advance was observed for seed yield per plant, days to 50% flowering, hulling, volume of weight, seed filling per cent and oil content while least genetic advance was recorded for 100 seed weight head diameter. Similar results were reported by Sultana et al. [20] and Reddy and Reddy [13].

**Table 3. Analysis of variance for different characters**

| Sr. No | Characters               | Replication (D. f=2) | Treatment (D. f=32) | Error (D. f=64) |
|--------|--------------------------|----------------------|---------------------|-----------------|
| 1      | Plant height (cm)        | 13.5                 | 382.53**            | 23.8            |
| 2      | Days to 50% flowering    | 4.58                 | 59.05**             | 0.9             |
| 3      | Days to maturity         | 25.18                | 176.65**            | 20.48           |
| 4      | Head diameter(cm)        | 0.00                 | 0.47**              | 0.12            |
| 5      | Seed filling (%)         | 11.27                | 40.72**             | 15.36           |
| 6      | Seed yield per plant (g) | 11.76                | 100.57**            | 11.65           |
| 7      | Oil content (%)          | 37.83                | 21.75**             | 10.57           |
| 8      | 100 g seed weight (g)    | 0.01                 | 0.48**              | 0.12            |
| 9      | Hull content (%)         | 10.67                | 40.88**             | 15.26           |
| 10     | Volume weight(g/100 ml)  | 58.05                | 31.27**             | 8.37            |

\*\* 5% Significant level

**Table 4. Variability parameters for different traits under study**

| S. No | Character                | Range       | Mean  | Genotypic Coefficient Variation (GCV %) | Phenotypic Coefficient Variation (PCV%) | Heritability (%) | Genetic advance | Genetic advance as percent of mean |
|-------|--------------------------|-------------|-------|---|---|------------------|-----------------|------------------------------------|
| 1     | Plant height (cm)        | 116.5-162.1 | 146.8 | 7.4                                     | 8.1                                     | 83.4             | 20.5            | 14.0                               |
| 2     | Days to 50% flowering    | 48.3- 63.5  | 58.5  | 7.4                                     | 7.6                                     | 95.5             | 4.1             | 15.1                               |
| 3     | Days to maturity         | 17.2- 23.0  | 21.4  | 4.0                                     | 8.6                                     | 22.2             | 0.8             | 39.5                               |
| 4     | Head diameter (cm)       | 3.5 - 5.4   | 4.3   | 7.7                                     | 11.2                                    | 46.8             | 0.4             | 10.8                               |
| 5     | Seed filling (%)         | 27.0- 36.9  | 31.4  | 6.1                                     | 12.0                                    | 25.9             | 2.0             | 6.4                                |
| 6     | Seed yield per plant (g) | 77.1- 105.1 | 90.8  | 7.9                                     | 9.3                                     | 71.7             | 12.5            | 13.8                               |
| 7     | Oil content (%)          | 17.6- 44.9  | 33.   | 16.                                     | 19.1                                    | 71.7             | 9.4             | 28.2                               |
| 8     | 100 g seed weight (g)    | 21.1- 36.8  | 28.1  | 10.5                                    | 15.7                                    | 45.2             | 4.1             | 14.6                               |
| 9     | Hull content (%)         | 67.4- 80.5  | 74.0  | 3.94                                    | 6.5                                     | 35.8             | 3.6             | 4.8                                |
| 10    | Volume weight (g/100 ml) | 42.5- 57.1  | 49.4  | 5.6                                     | 8.1                                     | 47.9             | 3.9             | 8.0                                |

#### 4. CONCLUSION

We have recorded large amount of morphological variation through the present finding based on DUS descriptors and can be utilized to distinguish the genotype from others. Strip colour and for ray floret colour differentiation can utilize to identify the genotype at seed stage and at flowering stage which may serve as morphological markers. A narrow gap between PCV and GCV were observed for all the characters, suggesting non-significant influence of the environment on these traits with additive gene effect i.e. mechanism of quantitative inheritance such that the combined effects of genetic alleles at two or more gene loci are equal to the sum of their individual effects may operate.

#### FUTURE WORK

The present investigation was carried out for identifying of promising germplasms lines by study on "Evaluation and characterization of sunflower (*Helianthus annuus* L.) germplasms" accessions. The results obtained through assessment of genetic variability for 10 characters in 33 germplasms lines showed desirable variability. Estimates of genotypic and phenotypic coefficient of variations indicated significant variability for the traits plant height, oil content, seed filling percentage, 100-seed weight, seed yield per plant indicate good scope for the genetic improvement of these traits. Correlation study for most of the character showed same direction at genotypes and phenotypes level. Genotypic estimates are higher then phenotypic estimates and significant positive correlation was observed for plant height and seed filling, oil content, days to 50% flowering, head diameter and 100 seed weight, days to maturity and hulling while seed field per plant did not show any type of correlation with the characters, The characters showing positive correlation may helpful for selection of parents in hybridization program.

From my study genetic parameters characters which plant height, seed filling%, Oil content%, 100 seed weight and this also seed yield per plant can be selected for genetic improving of this germplasm.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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