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Research Article

Identification of key yield determinants in sesame through multivariate analysis of F_2 and F_3 populations

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Abstract
The research was conducted to evaluate the F_2 and F_3 segregating populations of inter-varietal crosses in sesame for selecting superior segregants. The experiment involved F_2 generation of three superior crosses of sesame, namely; Thilak x Ayali 1, Thilathara x Ayali 2 and Thilak x Ayali 5. The seeds of the selected superior F_2 lines namely; TA1-5, TA5-2, TA5-4, TA5-5 and TA5-32 were advanced to F_3 generation, which was raised in a compact family block design. Principal Component Analysis was carried out to identify the key traits influencing yield and genetic divergence. In PC1, traits such as capsule length, capsule width, number of seeds per capsule, plant height, and seed yield were identified as critical determinants, while in PC2, crop duration traits like days to maturity and first flowering were observed to be critical. Segregants such as TA5-2, TA5-4, and TA5-32 stood out for their promising yield traits and genetic variability, demonstrating the practical utility of PCA in breeding efforts. Regression analysis of both F_2 and F_3 populations revealed that the number of capsules per plant was the most influential factor, with capsule width, number of seeds per capsule, and plant height also contributing substantially to yield variability.

Keywords: Sesame, F_2, F_3, Principal component analysis, Regression

INTRODUCTION

Sesamum (*Sesamum indicum* L.), known as the "Queen of oilseeds" due to its high-quality oil, has been cultivated for over 5,000 years. Sesame seeds are valued for its high oil content (~50%) and protein content (18–20%). As reported by NIFTEM (2022), nearly two-thirds of the sesame produced in India is processed for oil extraction, whereas the rest is primarily consumed as whole seeds for food purposes. Sesame is also integral in sweets, religious ceremonies, and industrial applications like pharmaceuticals and paints. Sesame breeding programs primarily aim to improve seed yield, oil quality and quantity, resistance to capsule shattering, seed retention, uniform maturity, and resilience to biotic and abiotic stresses. However, advancements in sesame breeding have been slower compared to crops like groundnut and sunflower. Selection for improved seed yield and yield components remains the key breeding strategy. The main yield-related traits in sesame include early and uniform maturity, reduced plant height, higher number of capsules per plant, number of branches per plant, number of seeds per capsule, and 1000-seed weight (Navaneetha *et al*., 2016). Advanced analytical tools like Principal Component Analysis (PCA) and standardized multiple linear regression play a vital role in sesame breeding by simplifying complex trait datasets and pinpointing the most influential yield factors. In this study, PCA was used to simplify the dataset and highlight the traits contributing most to variation in the F_2 and F_3 populations, while regression analysis helped to understand how these traits influenced seed yield. Together, these approaches were applied with the purpose of identifying the most reliable selection criteria for enhancing yield potential in the breeding material (Durge *et al*., 2022).

MATERIALS AND METHODS

The experimental material consisted of the F_2 segregating populations of three superior crosses, namely, Thilak x Ayali 1(TA1), Thilathara x Ayali 2 (TTA2) and Thilak x Ayali 5 (TA5), were sown during *rabi* 2023. Observations were recorded on the various biometric characters such as days to first flowering, number of primary branches, number of capsules per plant, capsule length (cm), capsule width(cm), number of seeds per capsules, days to maturity, plant height (cm) and seed yield per plant (g) of the F_2 populations and superior lines were identified and seeds collected after selfing. The seeds of selected F_2 lines namely, TA1-5, TA5-2, TA5-4, TA5-5 and TA5-32 were proceeded to F_3 generation in *kharif*, 2024. Seeds were sown in rows of 2.5 m length, at a spacing of 30 x 25 cm and plot size of 15 m^2 in compact family block design with four replications, each replication comprising 10 plants. Biometrical observations were recorded for the same set of nine characters as done in F_2 generation. The mean data were subjected to Principal Component Analysis (PCA) and Multiple linear regression analysis using GRAPES software, version 1.1.0 (Gopinath *et al*., 2020).

RESULTS AND DISCUSSION

Principal Component Analysis (PCA) is a statistical method that simplifies complex data by reducing its dimensions while pinpointing the main features that drive genetic divergence. In the present study, PCA was utilized to analyze biometric observations recorded from F_2 and F_3 populations of sesame, focusing on identifying traits critical for yield and selecting superior genotypes. Analysis of F_2 performance resulted in generation of nine principal components (PCs), with the first two PCs having eigenvalues >1 and cumulatively accounting for 88.83% of the variance (**table 1**). The first PC (PC1) explained 71.28% of the variance, primarily influenced by traits such as capsule length, number of seeds per capsule, capsule width, plant height, and seed yield per plant. The second PC (PC2) contributed 17.56% of the variance and was strongly associated with days to maturity and days to first flowering. This indicates that PC1 traits were vital for yield, while PC2 was related to crop duration. Similar findings on the influence of these traits have been reported by Ismaila and Usman (2014), Baraki *et al*. (2015) and Singh *et al*. (2018). **Fig. 1** visualizes the cumulative variance explained, showing a sharp decline after PC2, confirming that these two components are sufficient for effective genotype evaluation. The per cent contributions of nine characters towards the first two principal components (**table 2)**, indicate that traits such as capsule length, number of seeds per capsule, capsule width, plant height, number of primary branches, number of capsules per plant, and seed yield per plant contributed strongly to PC1 (Tanwar and Bisen, 2017; Sasipriya *et al*., 2022). Days to first flowering and days to maturity were the dominant contributors for PC2, guiding selection for phenological traits (Mukhthambica *et al*., 2023). Visual

Table 1. Principal components showing the Eigen values, proportion of variation and cumulative percentage of variance in F_2 population of sesame

Principal Components	Eigenvalue	Percentage of variance	Cumulative percentage of variance
PC1	6.415*	71.275	71.275
PC2	1.58*	17.557	88.832
PC3	0.535	5.946	94.778
PC4	0.213	2.364	97.142
PC5	0.176	1.953	99.095
PC6	0.057	0.63	99.726
PC7	0.012	0.137	99.863
PC8	0.009	0.101	99.964
PC9	0.003	0.036	100

Table 2. Percentage contribution of variables on PCA in F_2 segregating population of sesame

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Days to first flowering	**4.752**	**43.234**	1.329	0	0.152	0.001	2.314	47.337
Number of primary branches	**12.63**	**0.418**	1.742	75.704	6.289	3.024	0	0.192
Number of capsules per plant	**12.29**	**4.229**	22.781	2.524	8.788	0.095	3.939	1.515
Capsule length	**13.858**	**0.753**	10.574	0.078	0.059	74.511	0.138	0.004
Capsule width	**13.621**	**0.37**	16.905	8.553	0.43	9.255	44.212	1.556
Number of seeds per capsule	**13.684**	**0.333**	16.009	8.821	0.064	12.123	42.765	1.167
Days to maturity	**4.29**	**45.238**	0.913	0.265	0.006	0.068	2.348	45.998
Plant height	**12.623**	**1.282**	5.446	1.399	78.112	0.921	0.132	0.032
Seed yield per plant	**12.253**	**4.143**	24.302	2.656	6.101	0.001	4.151	2.199

Bold values represent principal components with eigen values >

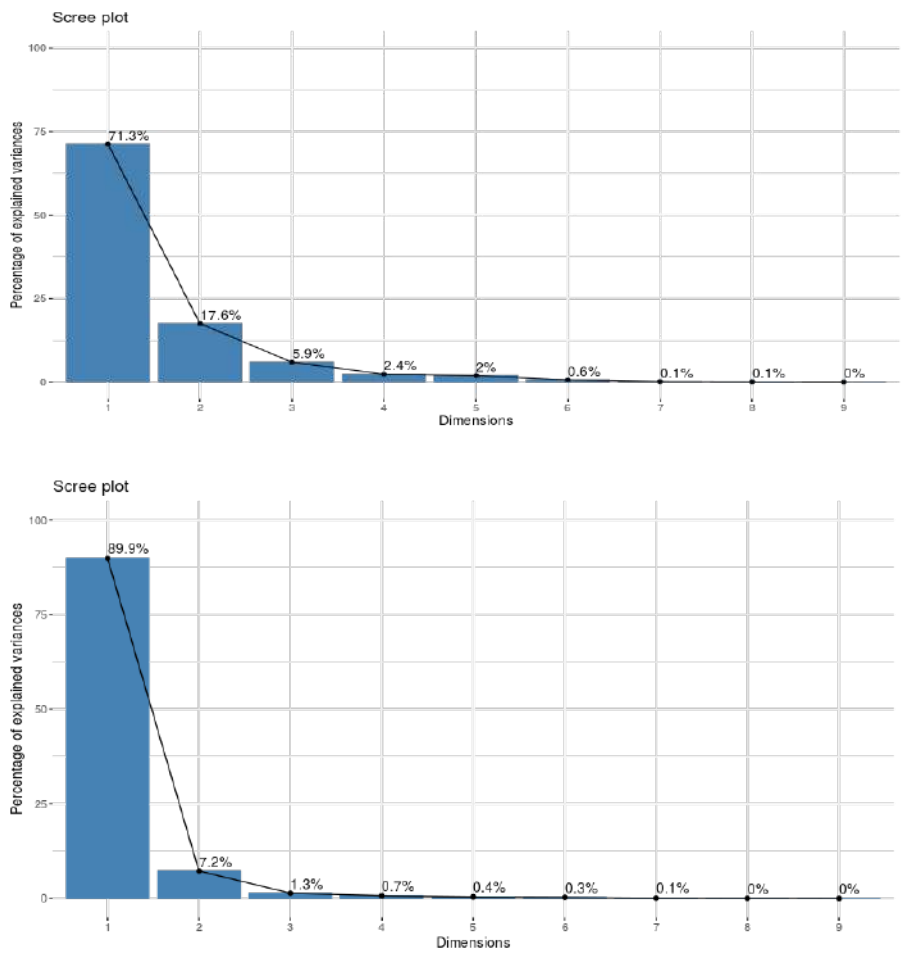

Fig. 1. Scree plot indicating eigen values and percentage variation in F_2 and F_3 populations of sesame

representation of each trait's contribution, are depicted in **fig. 2**. Factor loading of various traits in relation to the principal components is reflected in the **table 3**. Loadings with absolute scores greater than 0.300 are considered to contribute profusely to genetic divergence. High absolute loadings (>0.300) in PC1 highlight yield-related traits, while PC2 shows high loadings for flowering and maturity traits (Shim *et al*., 2016). High PC scores indicated superior segregants in terms of the traits associated with the respective components (**table 4**). For instance, segregants TA1-5, TA5-2, TA5-4, TA5-5, and TA5-32 exhibited high PC scores, indicating high yield potential (Wang *et al*., 2017; Singh *et al*., 2018). The distribution of genotypes in the first two principal components is presented in **Fig. 3**. The plot shows how the genotypes cluster based on trait similarities, which in turn helps in identifying elite genotypes with desirable trait combinations. **Fig. 4** further explains the biplot quadrants, indicating that the first quadrant includes high-yielding segregants, the second quadrant contains longer-duration segregants, and the third and fourth quadrants correspond to early-maturing individuals (Mukhthambica *et al*., 2023).

The results of PCA of the F_3 population revealed that the first principal component (PC1) accounted for the maximum variance (89.94 %) with an eigenvalue >1. PC1 was primarily influenced by traits such as plant height, days to first flowering, number of seeds per capsule, and seed yield per plant (**table 7**). The second component contributed 7.234%, bringing the cumulative variance to 97.169% and visually depicts the cumulative variance, showing that the contribution of components beyond PC1 is minimal, indicating that selection based on PC1 alone is most effective (**Fig. 1**). The contribution of individual traits toward PC1 was examined, with plant height, days to first flowering, number of seeds per capsule, number of capsules per plant, days to maturity, capsule width, capsule length, and seed yield per plant emerging as major contributors (**table 8**) and visually highlights these contributions (**Fig. 2**), making it easier to prioritize traits for selection of segregants. These traits collectively contributed to genetic divergence, as confirmed by high factor loadings exceeding 0.30(**table 9**). These findings are in accordance with findings of Dash *et al*. (2020), Gupta *et al*. (2021), and Durge *et al*. (2022), which emphasized the significant role of yield-related parameters in sesame.

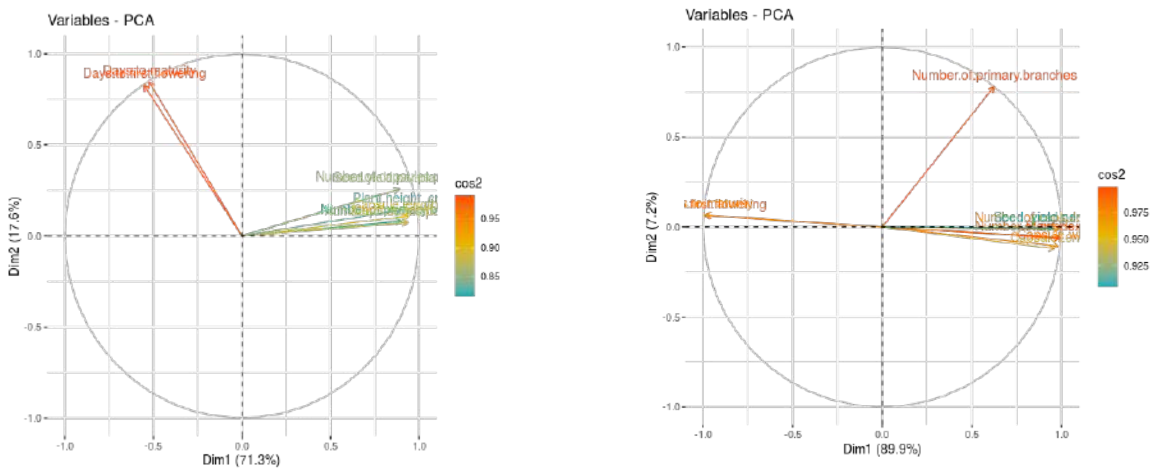

Fig 2. Projection of yield related characters on the first two PCs in F_2 and F_3 populations of sesame

Table 3. PCA for nine yield and related traits of F_2 segregating population in sesame

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Days to first flowering	0.218	0.658	-0.115	0.002	-0.039	0.003	-0.152	0.688	0.094
Number of primary branches	-0.355	0.065	-0.132	-0.87	0.251	0.174	-0.002	0.044	-0.001
Number of capsules per plant	-0.351	0.206	0.477	0.159	0.296	-0.031	-0.198	-0.123	0.662
Capsule length	-0.372	0.087	-0.325	0.028	-0.024	-0.863	0.037	-0.006	-0.016
Capsule width cm.	-0.369	0.061	-0.411	0.292	0.066	0.304	-0.665	-0.125	-0.226
Number of seeds per capsule	-0.37	0.058	-0.4	0.297	0.025	0.348	0.654	0.108	0.224
Days to maturity	0.207	0.673	-0.096	-0.051	-0.008	0.026	0.153	-0.678	-0.094
Plant height	-0.355	0.113	0.233	-0.118	-0.884	0.096	-0.036	-0.018	0.023
Seed yield per plant	-0.35	0.204	0.493	0.163	0.247	0.004	0.204	0.148	-0.665

Table 4. PC scores from standardised variables of selected F_2 plants

Genotypes	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
TA1-5	4.445	0.902	-0.806	-0.654	0.714	0.194	0.411	-0.158	-0.180
TA5-2	6.375	0.520	0.529	0.393	0.722	-0.519	0.290	-0.135	-0.003
TA5-4	6.440*	0.279	0.561	0.186	0.525	-0.009	0.846	-0.150	0.037
TA5-5	6.377	0.174	0.425	0.375	0.780	-0.353	-0.067	0.046	-0.052
TA5-32	6.202	0.040	0.653	0.281	0.496	-0.200	0.345	-0.129	0.106

Table 5. Variables selected by multiple linear regression model in F_2 population of sesame

Parameter	Estimate	Std. Error	T value	P-value
Intercept	-3.364	1.898	-1.772	0.078
Days to first flowering	0.035	0.023	1.510	0.133
Number of primary branches	-0.018	0.079	-0.224	0.823
Number of capsules per plant	0.150	0.002	72.966	0.000*
Capsule length	-0.811	0.833	-0.973	0.332
Capsule width	-15.513	3.047	-5.091	0.000*
Number of seeds per capsule	0.346	0.067	5.204	0.000*
Days to maturity	-0.037	0.024	-1.526	0.129
Plant height	0.011	0.003	3.212	0.002*

Regression coefficients with P-value <0.05 are significant.
R squared – 0.993; Adj. R squared – 0.993; Residual Standard error – 0.568

Fig 3. Individual PCA in F_2 and F_3 populations of sesame

Table 6. Variables selected by multiple linear regression model after stepwise regression with backward elimination in F_2 population of sesame

Parameter	Estimate	Std. Error	T value	P-value
Intercept	-5.655	1.283	-4.407	0.000
Number of capsules per plant	0.149	0.002	75.384	0.000*
Capsule width	-16.007	2.925	-5.473	0.000*
Number of seeds per capsule	0.329	0.066	5.009	0.000*
Plant height	0.010	0.003	3.078	0.0024*

Regression coefficients with P-value <0.05 are significant.

R squared – 0.993; Adj R squared – 0.993; Residual Standard error – 0.568

Table 7. PCs showing the eigen values, proportion of variation and cumulative percentage of variance in F_3 population of sesame

Principal Components	Eigenvalue	Percentage of variance	Cumulative percentage of variance
PC1	8.094*	89.935	89.935
PC2	0.651	7.234	97.169
PC3	0.12	1.332	98.501
PC4	0.06	0.672	99.173
PC5	0.035	0.394	99.567
PC6	0.025	0.276	99.843
PC7	0.008	0.093	99.935
PC8	0.004	0.045	99.981
PC9	0.002	0.019	100

Table 8. Percentage contribution of variables on PC in F_3 population of sesame

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Days to first flowering	**12.197**	0.638	0.408	3.463	4.26	0.574	44.637	1.547
Number of primary branches	**4.811**	93.556	1.112	0.113	0.03	0.306	0.001	0.071
Number of capsules per plant	**12.072**	0.018	2.226	1.772	0.389	73.662	5.665	3.404
Capsule length	**11.392**	2.229	19.695	64.863	1.428	0.144	0.202	0.045
Capsule width	**11.938**	1.766	2.788	3.41	37.24	7.891	5.335	29.561
Number of seeds per capsule	**12.171**	0.524	1.838	2.719	13.463	0.002	1.532	65.119
Days to maturity	**11.987**	0.686	0.123	8.221	42.362	12.764	23.751	0.006
Plant height	**12.243**	0.576	0.401	3.324	0.109	0.238	18.853	0.133
Seed yield per plant	**11.189**	0.007	71.41	12.116	0.718	4.419	0.023	0.115

Bold values represent principal components with eigen values >1

Table 9. PCA for nine yield and yield related traits of F_3 segregating population in sesame

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Days to first flowering	0.349	-0.08	0.064	-0.186	0.206	-0.076	0.668	-0.124	-0.568
Number of primary branches	-0.219	-0.967	-0.105	-0.034	0.017	0.055	0.004	0.027	-0.002
Number of capsules per plant	-0.347	0.013	0.149	0.133	-0.062	-0.858	0.238	0.184	0.089
Capsule length	-0.338	0.149	-0.444	-0.805	-0.12	-0.038	0.045	0.021	-0.005
Capsule width	-0.346	0.133	-0.167	0.185	0.61	0.281	0.231	0.544	0.027
Number of seeds per capsule	-0.349	0.072	-0.136	0.165	0.367	-0.005	0.124	-0.807	0.162
Days to maturity	0.346	-0.083	0.035	-0.287	0.651	-0.357	-0.487	0.008	0.032
Plant height	-0.35	0.076	-0.063	0.182	-0.033	-0.049	-0.434	-0.036	-0.801
Seed yield per plant	-0.335	0.008	0.845	-0.348	0.085	0.21	-0.015	-0.034	-0.006

Table 10. PC scores from standardised variables of selected F_3 plants

Genotypes	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
TA1-5-1	3.723	1.179	0.125	-0.136	0.040	-0.198	0.026	0.066	0.020
TA5-2-2	5.441	1.839	0.908	-0.072	0.123	-0.150	0.005	-0.048	0.042
TA5-4-7	7.756	1.395	0.910	-0.060	-0.054	1.249	-0.354	-0.128	-0.114
TA5-5-1	3.816	0.185	-0.199	0.132	-0.320	0.009	0.126	0.005	0.017
TA5-32-25	8.785*	1.231	1.708	-0.260	-0.020	0.975	-0.250	0.086	-0.071

Fig 4. Two dimensional biplot of PCA in F_2 and F_3 populations of sesame

The distribution of segregants in the first two PCs is illustrated in **Fig. 3**, showing how individuals cluster according to trait similarities and enabling the identification of superior segregants for selection. Selected F_3 progenies, including TA1-5-1, TA5-2-2, TA5-4-7, and TA5-32-25, demonstrated high PC scores (**Table 10**) and variability for yield-related traits. These genotypes were positioned in the first quadrant of the biplot (**Fig. 4**), indicating their potential for high yield. The observed influence of traits such as capsule length, number of seeds per capsule, and plant height aligns with studies by Shim *et al*. (2016), Aristya *et al*. (2016), and Hassen and Endale (2023).

A comparative analysis of the PCA between F_2 and F_3 segregating populations revealed significant differences with respect to the PC components. In the F_2 population, the variance among the genotypes were primarily influenced by yield related traits such as capsule length, number of seeds per capsule, capsule width, plant height, and seed yield per plant. The characters, plant height, days to first flowering, number of seeds per capsule and seed yield per plant contributed to maximum variance in the F_3 population. The study suggests higher scope for selection for yield related characters from the segregating generations.

Regression analysis: A linear regression model was applied in the F_2 and F_3 populations, where seed yield per plant was the dependent variable and agronomic traits served as independent variables. The fitted model

has R^2 value of 99.3% in the F_2 population. These results indicate that the R-Squared statistic explains 99.3% of the variability in seed yield. The adjusted R-squared statistic, which is more suitable for comparing models with different numbers of independent variables was also 99.3%. The computed standard error indicates that the residuals exhibit a standard deviation of 0.568. As the P-value exceeds 0.05, there is no evidence of serial autocorrelation in the residuals at the 95% confidence level. **Table 5** depicts the outcomes of fitting a multiple linear regression model to characterize the average relationship between seed yield and eight independent variables. The prediction equation derived was:
Seed yield per plant (Y) = -3.364 + 0.035 X_1 - 0.018 X_2 + 0.150 X_3 - 0.811 X_4 - 15.513 X_5 + 0.346 X_6 - 0.037 X_7 + 0.011 X_8

It was noted that the characters, namely, days to first flowering, number of primary branches, capsule length and days to maturity were not significant (P value > 0.05) (**table 6**). Hence, these variables were removed. Using stepwise regression with a backward elimination approach, a linear regression model was fitted to characterize the relationship between seed yield per plant and four independent variables. According to this analysis, the prediction equation was:
Seed yield per plant (Y) = -5.655 + 0.149 X_3 – 16.007 X_5 + 0.329 X_6 + 0.010 X_8 (R squared = 99.3 %), where Y, X_3, X_5, X_6 and X_8 represent seed yield per plant, number of capsules per plant, capsule width, number of seeds per capsule and plant height respectively.

The analysis revealed significant contribution of number of capsules per plant, capsule width, number of seeds per capsule and plant height on seed yield per plant (**Fig. 5**).

In F_3 population, the fitted model for regression analysis has R^2 value of 90.30 % which indicates that 90.30% of the variability in seed yield was explained. The adjusted R^2 value, which is better suited for comparing models with different numbers of independent variables, stood at 89.9%. Additionally, the standard error of the estimate reveals that the residuals' standard deviation is 1.271. Given that the P-value is greater than 0.05, there is no sign of serial autocorrelation in the residuals at the 95.0% confidence level. The results of fitting a multiple linear regression model to describe the average relationship between seed yield and eight independent variables is furnished in **table 11**. The prediction equation derived was:
Seed yield per plant (Y) = 50.215 - 0.184 X_1 + 0.095 X_2 + 0.134 X_3 - 2.024 X_4 + 21.535 X_5 -0.746 X_6 - 0.069 X_7 - 0.026 X_8

It was noted that the characters, namely, days to first flowering, number of primary branches, capsule length, capsule width, number of seeds per capsule, plant height and days to maturity were not significant (P values > 0.05). Stepwise regression, with backward elimination, did not exhibit a major deviation in R^2 value (**table 12**). Hence the prediction equation model that elucidates the relationship between seed yield per plant and seven independent variables is not changed. Thie character, number of capsules per plant only had an estimate of P value < 0.05, suggesting its contribution to seed yield per plant.

The trait number of capsules per plant was identified as the most important factor explaining the total variation in seed yield through regression analysis (**Fig. 5**). This observation was supported by Parimala and Mathur (2006), who reported its significant influence on seed yield in sesame. El-Mohsen *et al*. (2008) also highlighted the critical role of the number of capsules per plant in determining yield. Atalou *et al*. (2014) confirmed that segregants with a higher number of capsules per plant contributed substantially to overall seed yield. Aristya *et al*. (2016) further emphasized the importance of this trait for selection in sesame breeding programs.

In addition, plant height was found to be an important determinant of seed yield, as reported by Narayanaswamy *et al*. (2012). Parimala and Mathur (2006) also noted the significant contribution of number of seeds per capsule to seed yield through step-wise regression analysis. These findings collectively indicate that the number of capsules per plant, plant height, and number of seeds per capsule are key traits for improving yield in sesame.

This study underscores the effectiveness of PCA in identifying critical traits and selecting superior genotypes in sesame. For the F_2 population, the high correlation of PC1 with traits such as capsule length, number of seeds per capsule, and seed yield suggests that these parameters play a crucial role in enhancing yield potential, while in the F_3 population, yield-related parameters including plant height, days to first flowering, and seed yield per plant. dominated PC1. Genotypes such as TA5-2, TA5-4, and TA5-32 were identified as promising candidates for selection due to their high PC scores and desirable traits. These findings align with prior research and reinforce the utility of PCA in sesame breeding programs. The regression analysis conducted in this study underscores the significant relationships between agronomic traits and seed yield per plant in sesame. In both the F_2 and F_3 populations, the number of capsules per plant was identified as the key determinant of seed yield. Combining PCA and regression analyses, it is clear that yield-related traits, particularly number of capsules per plant, plant height, number of seeds per capsule, and capsule width, are consistently critical for high seed yield across F2 and F3 segregants. PCA identifies these traits as primary contributors to genetic divergence, while regression confirms their direct quantitative impact on seed yield. Therefore, selection based on these traits will efficiently improve productivity in sesame breeding programs.

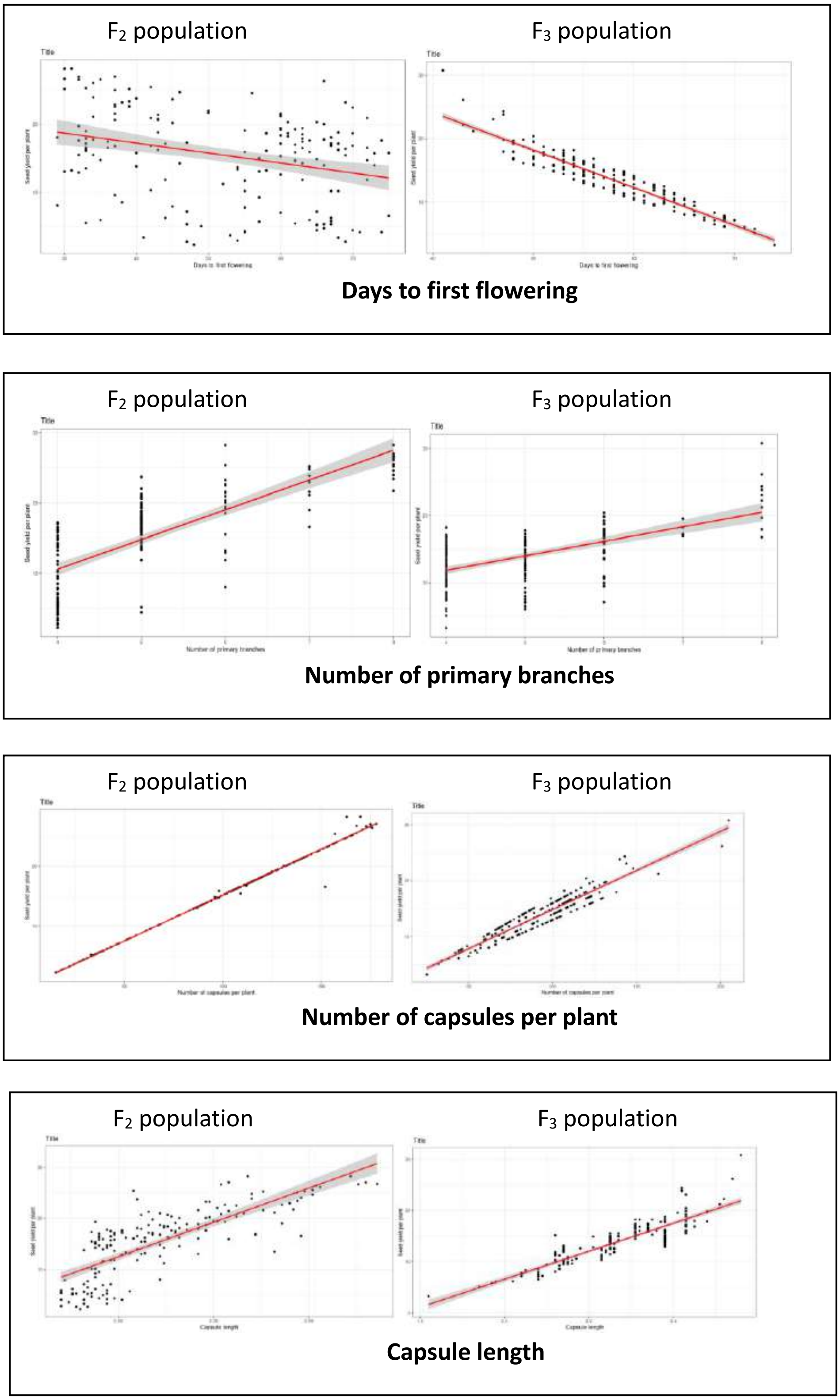

Fig 5. Multiple linear regression of seed yield per plant in F_2 and F_3 populations of sesame

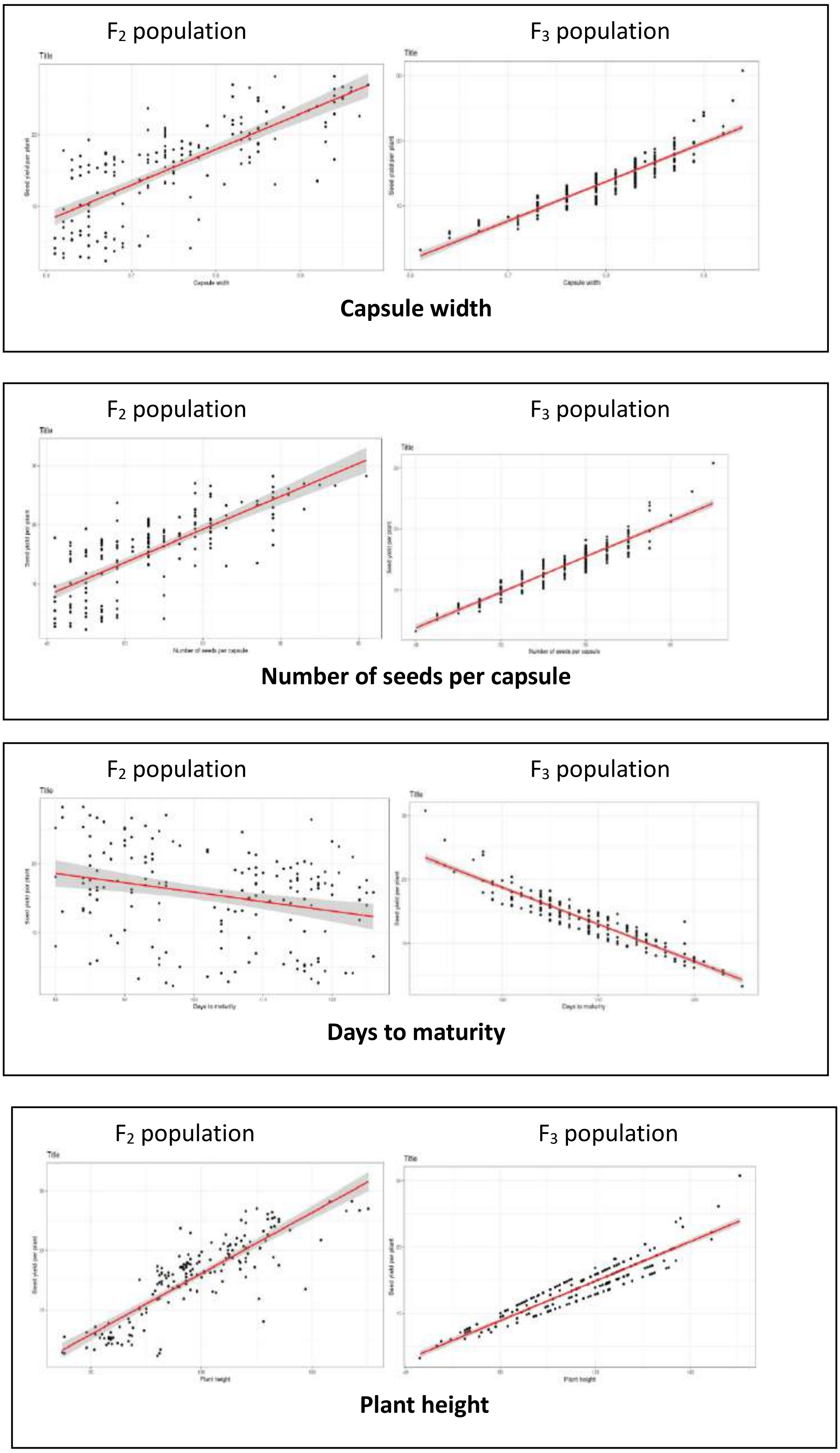

Fig 5. Multiple linear regression of seed yield per plant in F_2 and F_3 populations of sesame

Table 11. Variables selected by multiple linear regression in F_3 population of sesame

Parameter	Estimate	Std. Error	T value	P-value
Intercept	50.215	22.480	2.234	0.027
Days to first flowering	-0.184	0.223	-0.825	0.411
Number of primary branches	0.095	0.114	0.829	0.408
Number of capsules per plant	0.134	0.021	6.275	0.000*
Capsule length	-2.024	2.528	-0.801	0.424
Capsule width	21.535	14.133	1.524	0.129
Number of seeds per capsule	-0.746	0.473	-1.578	0.116
Days to maturity	-0.069	0.097	-0.710	0.479
Plant height	-0.026	0.071	-0.369	0.712

Regression coefficients with P-value <0.05 are significant.
R squared – 0.903; Adj R squared – 0.899; Residual Standard error – 1.271

Table 12. Variables selected by multiple linear regression after stepwise regression with backward elimination according to the model used in F_3 population of sesame

Parameter	Estimate	Std. Error	T value	P-value
Intercept	41.755	20.223	2.065	0.040
Days to first flowering	-0.173	0.107	-1.608	0.109
Number of primary branches	0.105	0.113	0.929	0.354
Number of capsules per plant	0.132	0.019	6.820	0.000*
Capsule width	19.951	13.872	1.438	0.152
Number of seeds per capsule	-0.852	0.446	-1.912	0.057

Regression coefficients with P-value <0.05 are significant.
R squared – 0.902; Adj R squared – 0.900; Residual Standard error – 1.266

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