MULTIVARIATE ANALYSIS FOR DESIGNIING OKRA CROP IDEOTYPE WITH ENHANCED YIELDS AND STRATEGY FORMULATION: A CROP PHYSIOLOGY PERSPECTIVE

Abstract

A crop ideotype, representing an optimal crop type with higher yield and improved quality, requires minimal input per unit of dry matter produced. This concept aims to amalgamate all yield-contributing traits into a single plant genotype. Our study induced changes in okra plants using various growth regulators, known to positively influence growth, development, and yield. Through rigorous analysis, we identified key traits correlated with yield and proposed an ideal plant phenotype characterized by reduced plant height, leaf area index, and crude fiber content, alongside increased primary branches, chlorophyll content, and fruits plant^{-1}. This phenotype, with a slower fruit maturity rate, offers longer field-stand and shelf-life and accommodates more plants per unit area. Soil drenching with paclobutrazol at 400 ppm emerges as an effective technique to achieve this ideotype. Our findings provide valuable insights for crop breeders to develop cultivars with desired traits and offer crop physiologists strategies for optimizing plant architecture for enhanced yields. Overall, the proposed ideotype, attainable through simple farming practices, promises a rapid solution for yield maximization in okra cultivation.

Keywords: Okra; Crop ideotype; Plant growth regulators; Pearson correlation analysis; Principal component analysis

1. Introduction

While cereals and grains constitute the staple diet in India, vegetables play a crucial role in providing essential nutrients and minerals. Among these, okra stands out as a versatile crop with applications across various industries such as textiles and food fortification. Widely consumed in India, okra is valued for its high dietary fiber, folate, magnesium, manganese, potassium, vitamin K, vitamin C, vitamin B1, and B6 content. With an average nutritional value (ANV) of 3.21, it surpasses several other vegetables including cucurbits (excluding bitter gourd), eggplant, and tomato (Grubben, 1977). The increasing population necessitates a rapid escalation in vegetable production and productivity, a task not easily achieved solely through traditional crop improvement methods, which can be time-consuming. Plant growth regulators present a viable strategy for augmenting production by modulating plant physiological processes, aligning with the escalating demand for vegetable production.

A crop ideotype represents an ideal crop type that, when developed as a cultivar, is expected to exhibit higher yield and better quality. This ideotype requires minimal input per unit of dry matter produced (Donald, 1968). The term "ideotype" reflects a breeder's aim to combine all traits that contribute positively to yield into a single plant genotype (Saini et al., 2020). According to Saini et al. (2020), the concept of a plant ideotype involves modifying existing genotypes to create improved genotypes and redesigning the ideotype itself. From a crop physiologist's perspective, we induced changes in okra plants using various plant growth regulators. Numerous studies have demonstrated that various growth regulators positively influence the growth, development, yield, and quality of okra plants and fruits. These plant growth regulators enhance yield by modulating endogenous hormone levels, optimizing source-sink relationships, increasing water and nutrient uptake, and promoting biomass accumulation (Zhang et al., 2014).

Gauffreteau (2018) outlines the steps in ideotype breeding: (i) defining the variety specifications or breeding objectives; (ii) designing and constructing ideotypes by identifying single traits that contribute to yield; and (iii) evaluating their ability to meet specifications across multiple breeding cycles and environments. With our objective focused on maximizing yields, we initiated experiments with different plant growth regulators to identify traits contributing to yield. Designing an optimal plant type (crop ideotype) requires a genotype with multiple traits that enhance yield potential. Traits with significant variability, high correlation with yield, and positive inter-correlation among selected characteristics were used to develop the optimal plant type (Wirnas et al., 2021). Wirnas et al. (2021) used correlation and cluster analysis to study the correlation and variability of sorghum genotypes in a plant breeding context. In our research, we utilized Pearson correlation analysis and Principal Component Analysis (PCA) to evaluate the correlation and variability among plant traits. Given the lack of studies on designing ideotypes in okra, our findings will be valuable for crop breeders in developing cultivars with the proposed ideotype characteristics. Additionally, our results provide crop physiologists with insights into modifying plant architecture through various methods to enhance yield. The ideal traits induced through crop physiology-based approaches, such as the application of plant growth regulators, offer a rapid solution for achieving plants with features that favour yield maximization, which would be possible to attain with easy farm practices.

2. Materials and methods

The current study was conducted on the okra cultivar Arka Anamika, derived from an interspecific cross between Abelmoschus esculentus (IIHR 20-31) and Abelmoschus manihot spp. tetraphyllus, developed through the backcross method. The experiment took place at the Western block of the Horticulture Farm of Pandit Jawaharlal Nehru College of Agriculture and Research Institute in Karaikal, located between 10°49' and 11°01' North latitude and 79°52' East longitude, at an altitude of 4 meters above mean sea level. The study was arranged in a Randomized Block Design with ten treatments, each replicated three times.

The plant growth regulators used included gibberellic acid (GA_3) at 50 ppm (Cryzib 90% a.i.), Naphthalene acetic acid (NAA) at 200 ppm (Planofix 4.5% SL), Triacontanol (TRIA) at 1 ppm (Vipul 0.1% EW), Brassinosteroid (BR) at 0.1 and 0.2 ppm (Double 0.04%), Cycocel (CCC) at 400 ppm (Lihocin 50% SL), and Paclobutrazol (PBZ) at 400 ppm (Cultar 23% a.i.), all applied in their commercially available formulations. Except for Paclobutrazol, which was soil-drenched at 7 and 14 days after sowing and again at 15 and 30 days after sowing, all growth regulators were applied as foliar sprays 30 and 45 days after sowing. Each plant received 10 ml of 400 ppm Paclobutrazol solution.

The plant traits recorded from tagged plants included the days to first flowering, node of first flower, plant height at initial and final harvest, number of primary branches at first flowering and final harvest, internode length, stem diameter, leaf area plant^{-1}, leaf area index, relative water content, total chlorophyll content, fruit length, fruit girth, fruit weight, number of fruits plant^{-1}, and yield plant^{-1}. The distance between the fifth and sixth nodes on the main stem was measured 75 days after sowing and averaged in centimeters (Venkadeswaran, 2013). Leaf area plant^{-1} at 60 DAS was estimated using a leaf area meter (Systronics Leaf Area Meter - 211). The leaf area index was calculated following Williams' method (1946). Relative water content (RWC) was determined from the fourth fully developed mature leaf from the tip (Slatyer, 1967). Total chlorophyll content was spectrophotometrically analyzed from the same leaf, using the standard procedure (Sadasivam and Manickam, 1996). The Pearson correlation analysis and principal component analysis were performed in RStudio using the "factoextra" and "factoMiner" packages (version 4.3.3) (Kassambara and Mundt, 2020; Le et al., 2008; RStudio Team, 2020).

3. Results and discussion

The section provides nuance understanding on the role of plant architecture on growth and yield of okra plants, through Pearson correlation analysis. Through analysing the relationship among the plant traits, a crop ideotype for okra is proposed to maximize fruit yield and a technique to attain such a crop ideotype is also strategized and validated with a principal component analysis. All the plant growth regulators proved significant effect on all the plant traits, except for node of first flower and fruit weight, as reported in our earlier work (Jyothsna et al., 2022).

3.1. Identifying traits relationships and crop ideotype features

The Pearson correlation analysis revealed the relationships among the plant traits studied. Table 1 presents the correlation coefficients (r matrix) for these traits, while Fig. 1 illustrates the significance of these correlations at $P<0.05$. Yield plant^{-1} exhibited a strong positive correlation with the number of fruits plant^{-1} (r = 0.93), followed by stem diameter (r = 0.62) and fruit girth (r = 0.55), although the latter two correlations were not statistically significant. This indicates that the number of fruits plant^{-1} is the primary contributor to yield plant^{-1}. Therefore, any plant growth regulator that can increase the number of fruits plant^{-1} could potentially enhance fruit yield. The number of fruits plant^{-1} showed the highest correlation with fruit yield plant^{-1}, followed by fruit girth (r = 0.61), although fruit girth was not significantly correlated with the number of fruits plant^{-1}.

Apart from yield and attributes, other traits that expressed significant correlations are detailed below. The number of primary branches plant^{-1}, both at the time of first flowering and final harvest, showed significant and high negative correlation with the plant height, at both initial and final harvests. The intermodal length was found to be positively associated with the plant height at initial and final harvests (r = 0.88 and 0.90, respectively). The internodal length showed significant negative correlation with the number of primary branches at final harvest. Since the number of primary branches plant^{-1} showed higher correlation, yet non-significant, the plant height control in okra plants, through reducing intermodal length and thereby the plant height, would essentially maximize the number of fruits plant^{-1} and ultimately the fruit yield plant^{-1}, through increased number of primary branches plant^{-1}. Our previous report states that modified plant architecture, suitably a shorter plant with optimal height would be an ideal phenotype for yield maximization (Jyothsna et al., 2022). This is again supported by the Pearson correlation analysis. The shortest plant height was observed with the application of paclobutrazol at 400 ppm, administered as a soil drench on the 7th and 14th days after sowing (DAS). This effect is likely due to paclobutrazol's inhibitory impact on gibberellic acid activity in the plant, resulting in shorter internodes and reduced plant height. Paclobutrazol is known to be an effective inhibitor of GA_3 biosynthesis, preventing the oxidation process that converts kaurene to kaurenoic acid in the GA_3 biosynthetic pathway. Specifically, it interacts with kaurene oxidase (a cytochrome P-450 oxidase) to inhibit the oxidation of kaurene, kaurenal, and kaurenol (Megbo, 2010). Similar findings on plant height reduction by paclobutrazol have been reported by Benjawan et al. (2007) and Megbo (2010) in okra. Additionally, paclobutrazol at 400 ppm, when applied as a soil drench on the 7th and 14th DAS, outperformed other plant growth regulators by producing the highest number of primary branches at both first flowering and final harvest stages. This aligns with the findings of Rai et al. (2003) in bottle gourd. Besides its anti-gibberellin effects, paclobutrazol also promotes cytokinin biosynthesis, possibly by redirecting reactions within the cytokinin biosynthetic pathway due to the shared intermediate precursor (Adil et al., 2011). The increase in cytokinin synthesis induced by paclobutrazol likely contributed to the formation of lateral branches by releasing axillary buds from apical dominance (Rai et al., 2003).

Furthermore, the plant height and interNodal length showed significant negative correlation with relative water content, which indicates lower water contents and a slower cellular water transport in taller plants than in shorter plants. This again supports that the shorter plants have favourable features for yield maximization. Interestingly, the relative water content is negatively associated with the number of seeds fruit^{-1}. Therefore, the plant growth regulators with potential to reduce plant height are desirable not only in commercial crop production but also in seed production of okra. This down-trend of number of seeds fruit^{-1} and relative water content might be due to the reduced source-to-sink flower of assimilates, which hampered the seed development. The relative water content also showed a negative correlation with the fruit weight, yet the correlation was non-significant. It is also essential to note that the fruit weight didn't significantly affect the yield plant^{-1}, regardless of the plant growth regulator treatment, indicating that number of fruits plant^{-1} as a key trait for increasing the yield plant^{-1}. This insignificant role of fruit weight on yield plant^{-1} is an unlikely phenomenon in okra, whereas the same traits were reported to be strongly associated with one another in other fruit vegetables (Paulino et al., 2007; Ara et al., 2009; de Souza et al., 2012; Diaz-Perez et al., 2015, Oniya et al., 2020; Damnjanović et al., 2022; Kadium and Svyantek, 2023; Ouma et al., 2024).

The mucilage content was significantly and positively correlated with the chlorophyll content (r = 0.68) and fruit length (r = 0.66). Notably, the fruit crude fibre content showed significant positive correlation with plant height and negative correlation with the number of primary branches plant^{-1}. This indicates that a phenotype with reduced plant height and increased number of primary branches plant^{-1}, not only increase the fruit and seed yield plant^{-1}, but also has slower maturing fruits, enabling fruits with longer shelf-life. This trait is particularly beneficial in okra, where fruits mature quickly, often leading to unmarketable fibrous fruits.

The leaf area plant^{-1} and leaf area index didn't show any significant correlation with other plant traits, except for crude fibre contents i.e. higher leaf area and leaf area index (plant with larger leaves) produced fruits that mature rapidly. Our previous report states the same, revealing the the paclobutrazol treatments showed the lowest leaf area plant^{-1}, leaf area index and crude fibre content (Jyothsna et al., 2022). It is intriguing that plants with the lowest leaf area index exhibited the highest fruit yield. This inverse relationship can be explained by the interplay of factors such as leaf characteristics, intercepted radiation, and radiation use efficiency. Leaf characteristics are crucial for understanding plant growth as they determine the amount of intercepted radiation under specific radiation conditions. The optimal display of leaves within a canopy is a key determinant of the efficiency of photosynthetic conversion of available light into assimilates. In the current study, higher plant yield coincided with the lowest leaf area index, achieved by the application of paclobutrazol. This suggests that paclobutrazol modified plant architecture to facilitate greater light interception, leading to increased photosynthetic assimilate accumulation. Leaf angle is a significant trait related to light interception and photosynthesis, and paclobutrazol is known to decrease the leaf angle (Dwyer et al., 1995), making leaves more erect. Plants with erect leaves are more efficient in light interception, capturing more light for photosynthesis and carbon gain, and allowing for denser plantings, all contributing to increased yield (Zhang et al., 2014). Additionally, reduced petiole length and increased leaf angle minimize shading effects.

Based on these findings, we propose an okra crop ideotype with relatively smaller leaves, which may be more favorable for longer field-stand and post-harvest shelf-life of okra fruits, reducing labor costs associated with frequent harvesting. However, further investigation is required in this aspect. The paclobutrazol-altered plant architecture includes smaller, erect leaves with a narrow leaf angle, short petioles, short branches, and a narrow plant canopy, which facilitates the accommodation of more plants per unit area, thereby maximizing yield.

3.2. Strategies for attaining the proposed crop ideotype

The interaction of plant growth regulators and okra plant traits were revealed through a principal component analysis. The analysis also provided the direction and magnitude of association among the traits and the plant growth regulator treatments. It helps us to identify the plant growth regulator treatments with similar effects. It ultimately dissects the variation in the plant traits as induced by the plant growth regulator treatments.

The Fig. 2 shows the results of the principal component analysis. The PCs with eigen value >1 are selected. In our investigation, the first five PCs are considered (Fig. 2a). These five PCs contributed 92.65% of the total variability induced by the plant growth regulator treatments (Fig. 2b). The highest correlation in PC1 was observed with plant height at final harvest ($r = 0.96$), followed by intermodal length ($r = 0.87$) and plant height at initial harvest ($r = 0.85$). In PC2, the highest correlation was seen with fruit length ($r = 0.85$) and chlorophyll content ($r = 0.79$) (Fig. 2c). The Fig. 2d shows the factor loadings of plant trait under different principal components. The highest factor loadings in PC1 were that of number of primary branches at final harvest (0.31) and number of primary branches at initial harvest (0.30), indicating the trait's utility on improving the yield.

The traits that showed the highest contribution to the total variation were captured in PC1 (Fig. 2e). The highest scores were seen for plant height at final harvest (11.57), plant height at initial harvest (10.09), number of primary branches at final harvest (9.86), number of primary branches at first flowering (9.45), intermodal length (9.63), crude fibre content (8.63) and relative water content (8.17). All these traits are crucial in determining plant architecture, as well as fruit and seed yield, and are associated with slower fruit maturity. This indicates that these traits can be modified under the influence of an appropriate plant growth regulator to produce a desirable plant phenotype. This approach offers an alternative to long-term breeding efforts, enabling any high-yielding cultivar to be altered with this ideal phenotype to maximize yields and profits.

Fig. 3 illustrates the interaction between various plant growth regulators and plant traits. The PCA biplot indicates that plants subjected to control conditions and water sprays exhibited inferior performance compared to those treated with any plant growth regulator, despite the application of the recommended fertilizer dose.The biplot also shows the correlation among the plant traits. The negative correlation between the yield traits; and leaf area plant^{-1}, leaf area index and crude fibre is notable. The yield plant^{-1} is highly and positively correlatied with number of fruit plant^{-1}, fruit girth, chlorophyll content and number of primary branches. It is mentioned above that larger leaf plant^{-1} and leaf area index is not userful for yield maximization. The PCA biplot shows that the chlrophyll content of the leaves contributes more to the yield than the leaf size (leaf area plant^{-1} and leaf area index). The paclobutrazol treatments scored the highest for the yield plant^{-1} and associated traits. Amid the two paclobutrazol treatments paclobutrazol 400 ppm at 7 and 14 DAS, as soil drench, was superior to the soil drenching of paclobutrazol 400 ppm at 15 and 30 DAS. The highest number of fruits plant^{-1} was found with the Paclobutrazol 400 ppm soil drenched on 7th and 14th DAS. Similarly, Almeida et al. (2016) also observed an increased number of fruits plant^{-1} in common bean. This preference for a higher number of fruits plant^{-1} could be attributed to shortened internodes, potentially leading to an increased number of productive nodes (Narkar, 2016), resulting in a higher number of fruits plant^{-1}. Additionally, the increased number of branches may have contributed to the highest number of fruits plant^{-1}. Paclobutrazol may delay plant senescence by enhancing photosynthetic efficiency, thereby sustaining the activity of enzymes such as RuBisCo and phosphoenol carboxylase during senescence (Rai et al., 2003). The higher yield observed under paclobutrazol application could also be attributed to rapid carbohydrate production and its accelerated accumulation in plant organs, necessitating rapid assimilate unloading (Benjawan et al., 2007).

As indicated earlier, the biplot highlights a negative correlation between relative water content and both the number of seeds fruit^{-1} and plant height. To enhance seed yield in commercial okra seed production fields, a simple strategy involves applying two foliar sprays of either 1 ppm triacontanol or 50 ppm GA_3 at 30 and 45 DAS. Particularly, the latter demonstrated superiority over the former in terms of increased the number of seeds fruit^{-1}. Additionally, it is noteworthy that seed yield correlates with plant height, while fruit yield correlates with the number of primary branches plant^{-1}. Application of 0.2 ppm brassinosteroid is recommended to increase fruit weight; however, fruit girth appears to be more influential in achieving higher yields than fruit weight.

Conclusion and future prospects

The multivariate analyses, namely Pearson correlation analysis and principal component analysis, were conducted on plant data collected from okra plants subjected to various plant growth regulator treatments. The Pearson correlation analysis revealed insights into the implications of plant architecture on yield maximization. Plant height and the number of primary branches plant^{-1} exhibited a negative correlation, with the former negatively associated and the latter positively associated with the number of fruits plant^{-1}, a key determinant of fruit yield plant^{-1}. Taller plants tend to have lower relative water content due to the increased energy required for water and nutrient transport, particularly hindered by mucilage, which may impede transport to growing fruits. Relative water content displayed negative correlations with most studied traits, particularly with the number of seeds fruit^{-1}, crucial for seed multiplication. Higher chlorophyll content, rather than greater leaf area plant^{-1} and leaf area index, is necessary for higher yield and slower fruit maturation. Fruits maturing rapidly, indicated by higher crude fiber content, result in unmarketable fruits. Hence, a slower increase in crude fiber allows growers to harvest fruits at their convenience, albeit at slightly reduced frequency, reducing labor costs. This regulation can mitigate surplus production during peak fruiting seasons and stabilize produce prices in markets.

Hence, we propose that a plant ideotype characterized by reduced plant height, leaf area index, and crude fiber content, along with increased number of primary branches, chlorophyll content, and number of fruits plant^{-1}, would yield higher yields compared to taller plants. Additionally, the suggested ideal phenotype for okra includes a slower fruit maturity rate, facilitating longer field-stand and post-harvest shelf-life. The shorter stature of plants would also accommodate more plants in a given unit area. We advocate the strategy of soil drenching with paclobutrazol at 400 ppm, either at 7 and 14 days after sowing (DAS) or at 15 and 30 DAS, as an ideal technique to achieve the proposed crop ideotype with enhanced yields. This is feasible as all plant architectural traits influencing yield are highly modifiable by plant growth regulators.

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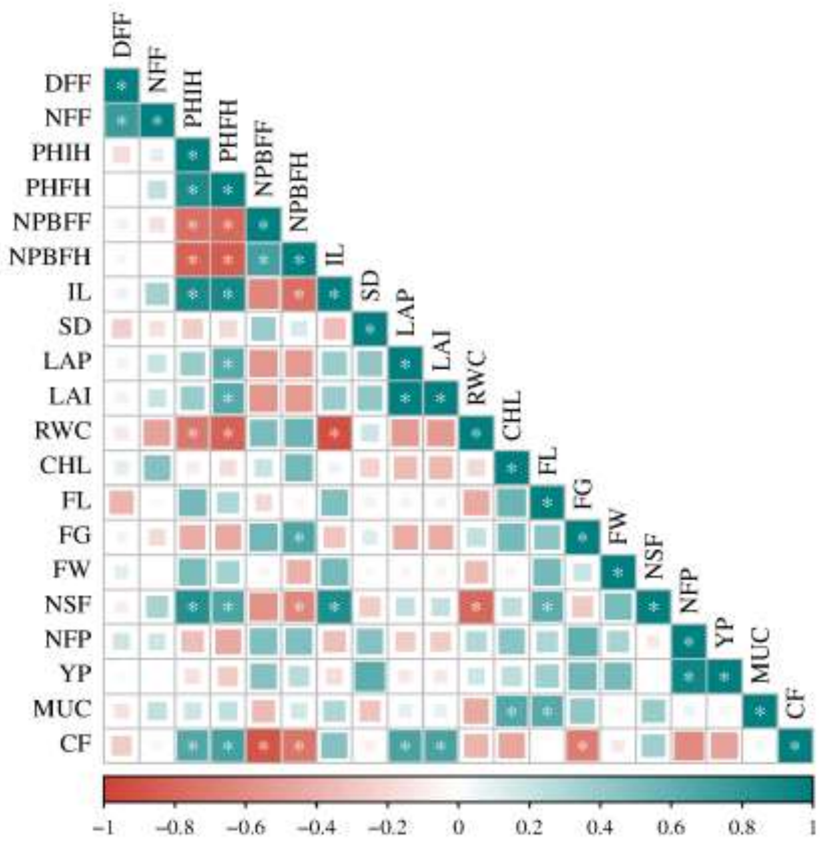
Table 1. Pearson correlation coefficient matrix for the plant traits of okra under the effects of different plant growth regulators

Variables	DFF	NFF	PHIH	PHFH	NPBFF	NPBFH	IL	SD	LAP	LAI	RWC	CHL	FL	FG	FW	NSF	NFP	YP	MUC	CF
DFF	1.00																			
NFF	0.77	1.00																		
PHIH	-0.18	0.10	1.00																	
PHFH	-0.02	0.23	0.89	1.00																
NPBFF	0.07	-0.16	-0.75	-0.78	1.00															
NPBFH	0.04	-0.03	-0.83	-0.84	0.73	1.00														
IL	0.07	0.35	0.88	0.90	-0.62	-0.75	1.00													
SD	-0.24	-0.15	-0.26	-0.20	0.38	0.16	-0.34	1.00												
LAP	-0.07	0.21	0.39	0.66	-0.54	-0.53	0.40	0.43	1.00											
LAI	-0.07	0.21	0.39	0.66	-0.54	-0.53	0.40	0.43	1.00	1.00										
RWC	-0.11	-0.49	-0.72	-0.82	0.52	0.57	-0.93	0.19	-0.53	-0.53	1.00									
CHL	0.12	0.46	-0.10	-0.17	0.20	0.53	0.07	-0.24	-0.36	-0.36	-0.20	1.00								
FL	-0.40	-0.04	0.52	0.31	-0.18	-0.04	0.48	-0.08	-0.06	-0.06	-0.45	0.57	1.00							
FG	-0.07	-0.18	-0.44	-0.45	0.54	0.70	-0.31	0.13	-0.42	-0.42	0.23	0.53	0.44	1.00						
FW	0.11	0.00	0.52	0.35	-0.06	-0.41	0.53	0.03	-0.06	-0.06	-0.35	-0.05	0.52	0.20	1.00					
NSF	-0.09	0.33	0.84	0.69	-0.56	-0.64	0.87	-0.27	0.23	0.23	-0.79	0.25	0.64	-0.30	0.50	1.00				
NFP	0.18	0.19	-0.32	-0.44	0.50	0.49	-0.33	0.47	-0.26	-0.26	0.30	0.46	0.31	0.61	0.33	-0.10	1.00			
YP	0.03	0.03	-0.14	-0.27	0.47	0.28	-0.18	0.62	-0.11	-0.11	0.18	0.25	0.38	0.55	0.53	-0.01	0.93	1.00		
MUC	-0.14	0.23	0.19	0.23	-0.35	0.16	0.32	-0.50	0.10	0.10	-0.44	0.68	0.66	0.43	-0.04	0.38	0.07	-0.06	1.00	
CF	-0.26	-0.04	0.67	0.74	-0.88	-0.74	0.46	-0.08	0.71	0.71	-0.39	-0.48	-0.01	-0.70	-0.10	0.36	-0.61	-0.50	0.06	1.00
	1.00									0										-1.00



"*" denotes the significance of the correlation coefficient at $P_{0.05}$

Fig. 1. Correlation matrix showing the significant correlation among the plant traits

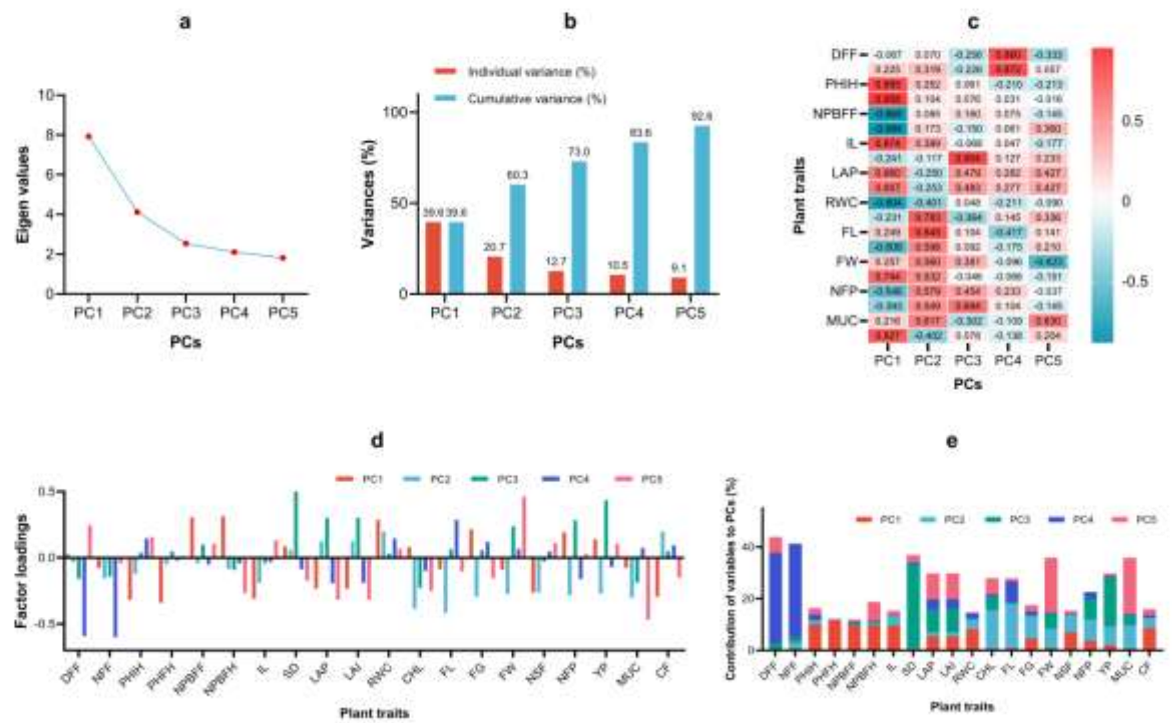


Fig. 2. PCA results showing the phenotypic variability in the okra plant traits under the influence of different plant growth regulators. a) Eigenvalue of PCs; b) Variances of PCs; c) Correlation between PCs and plant traits; d) Factor loadings of plant traits under PCs; e) Contribution of plant traits to PCs

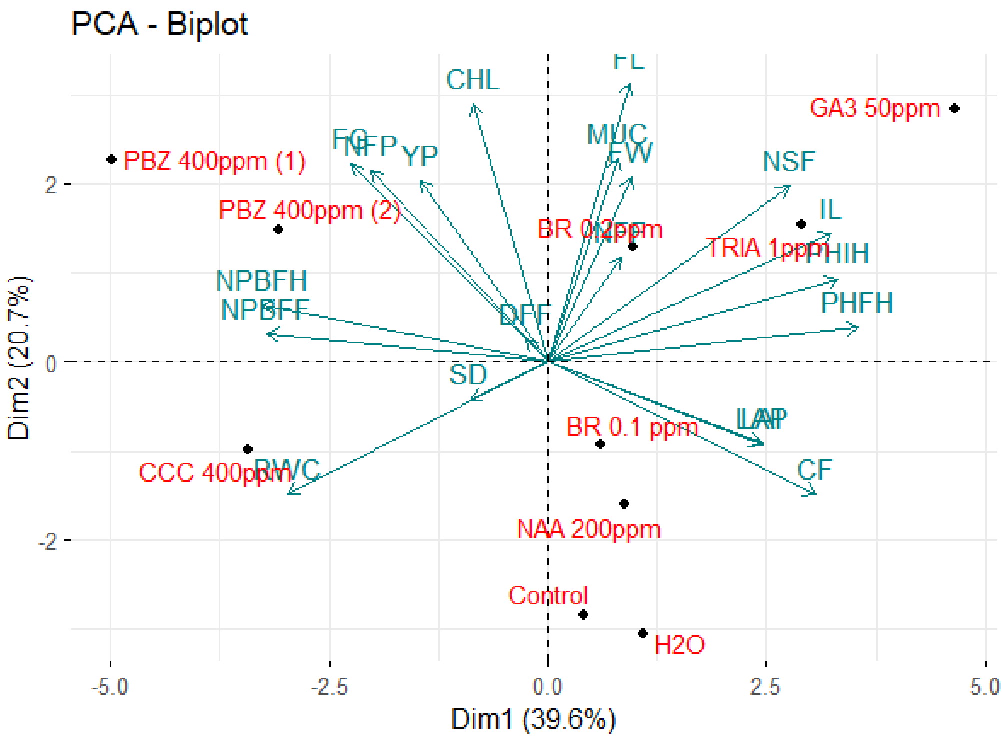


Fig. 3. PCA biplot showing plant growth regulators – plant traits interactions in okra