

Growth Attributes, Photosynthetic Pigments and Sensory Parameters in Selected Genotypes of Chickpea Cultivated as Microgreens under LED Light

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Abstract

Microgreens are pivotal for human health as these are rich in useful components which can be controlled by growing conditions (light, substrate, temperature, genetic composition and stage of crop harvest). Amongst these, intensity, quality and duration of light are key determining factors. The aim of the present study was to evaluate impact of Light Emitting Diodes providing 96% Photosynthetically Active Radiation on growth attributes, photosynthetic pigments and sensory parameters amongst chickpea genotypes cultivated as microgreens till 28th day. A consistent enhancement was depicted in shoot height, number of leaves, fresh and dry weight on per plant basis and the photosynthetic pigments. The genotype Pusa-3022 exhibited better performance for all growth parameters. The genotype Pusa-4005 showed higher total pigments and ratio of chlorophyll *a* to *b*, while, the proportion of total chlorophyll to carotenoids was maximum in Pusa-3062. The performance of sensory parameters was greatest on 14th day. Correlation data depicted inconsistent observations amongst some parameters. In view of the results obtained, chickpea microgreens can be considered as a potential functional food supplement till 14th day of cultivation.

Key words: Chickpea, Light-emitting diodes, Microgreens, Growth, Pigments, Sensory parameters

The rising demand for nutrient-dense functional foods has directed considerable attention toward microgreens, which are young, tender seedlings harvested shortly after germination. Among leguminous crops, chickpea (*Cicer arietinum* L.) holds significant potential due to its rich nutritional profile, including proteins, vitamins, minerals, and bioactive compounds. Cultivating chickpea as microgreens under controlled environments offers an opportunity to enhance both yield quality and nutritional value, particularly through the manipulation of light conditions.

Abiotic stresses have rendered nearly 5-10 million hectares of land unsuitable for crop cultivation every year (Goswami *et al.* [1]). To overcome the challenges of traditional agricultural practices, indoor farming under managed environmental conditions has gained popularity. The approach includes enhanced utilization efficiency of resources and round the year production with climate-resilient strategies. This allows regulation of intensity, quality and duration of supplemented light mimicking the wavelengths of natural sunlight with UV light limitation [2-3]. Such precise light management plays a crucial role in enhancing photosynthetic efficiency, plant growth, and the accumulation of essential phytochemicals in crops. Sunlight can be substituted with halogen and sodium (high pressure) lamps, fluorescent white lights and light-emitting diodes (LEDs) [4]. Light-emitting

diode (LED) technology has emerged as an efficient and customizable lighting source in controlled environment agriculture, enabling precise regulation of light intensity and spectral composition. Such control plays a critical role in influencing plant growth, photosynthetic activity, pigment accumulation, and sensory characteristics. However, limited studies have explored the genotype-specific responses of chickpea microgreens to LED light, particularly in relation to growth attributes, photosynthetic pigments, and consumer-relevant sensory parameters. LEDs are preferred as these can be articulated for particular wavelengths, enable fabrication of light quality and intensity with longer longevity and improved energy translation efficiency. Cultivation of microgreens under indoor conditions, necessitates the creation of ambient environmental conditions (light, temperature, and relative humidity). Besides this, genotype, temperature and relative humidity also govern morpho physiological attributes and sensory parameters in microgreens. Such studies have been conducted for cultivation of microgreens in distinct crops [5-6].

Hence, objective of the present study was to analyze the influence of artificial light emitting diodes on growth attributes, photosynthetic pigments and sensory parameters in chickpea microgreens at different stages of cultivation. Correlation study and principal components with interactions along with clustering patterns were also assessed.

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MATERIALS AND METHODS

Experimental design for cultivation of chickpea microgreens

The seeds of three chickpea genotypes namely, Pusa-3022, Pusa-3062 and Pusa-4005 were procured from Seed Production Unit, ICAR- IARI, New Delhi. Prior to sowing, seeds were thoroughly washed with water followed by 2-4 drops of cedeapol and 5 percent sodium hypochlorite (NaOCl). These were again washed and soaked in autoclaved distilled water overnight. A Randomized Block Design (RBD) was deployed in triplicates for cultivation as microgreens on uniform sand bed (4 cm thick) in trays (dimensions: 47 cm x 39 cm x 13cm; length x width x height) at Sharda School of Agricultural Sciences, Sharda University, Greater Noida, Uttar Pradesh. An ambient temperature of 25 °C / 23 °C ± 2 °C (day/night) with 30-50% humidity was maintained.

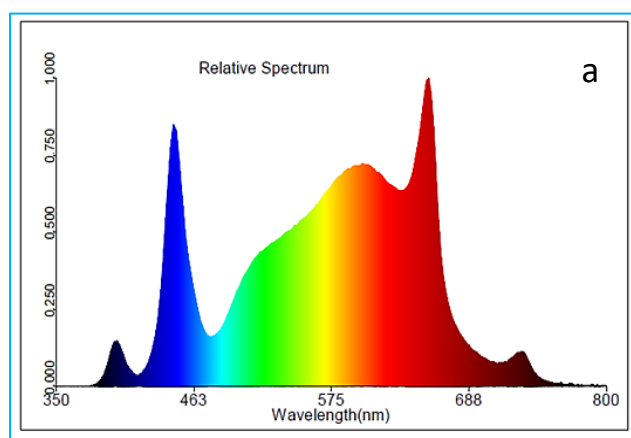


Fig 1 (a) Relative spectrum of LED lights utilized for cultivation of chickpea microgreens; (b) Cultivation of chickpea microgreens under artificial illumination utilizing Light Emitting Diodes (LED)

Estimation of growth attributes

Growth attributes were measured in terms of shoot height, number of leaves with fresh and dry weight of stem, leaves and shoot on per plant basis. The fresh weights were recorded using analytical balance and the dry weights were taken after drying at 105 °C for 3 hours in an oven.

Determination of photosynthetic pigments

The optimized methodology by Martínez-Ispizua *et al.* [7] (2022) was followed for determination of chlorophylls and carotenoids. 100 milligram fresh leaves were macerated in 80% acetone and centrifuged at 6000 rpm. The supernatant was collected and final volume was made to 10 ml. The concentrations of chlorophyll (*a*, *b* and total) and carotenoids were expressed as milligram/g fresh weight basis and were computed using the equations given below:

$$\text{Chlorophyll } a = \frac{(12.7 \times \text{Abs. at } 663\text{nm} - 2.69 \times \text{Abs. at } 645\text{nm}) \times \text{Volume}}{1000 \times \text{Fresh weight}}$$

$$\text{Chlorophyll } b = \frac{(22.9 \times \text{Abs. at } 645\text{nm} - 4.68 \times \text{Abs. at } 663\text{nm}) \times \text{Volume}}{1000 \times \text{Fresh weight}}$$

$$\text{Total chlorophyll} = \text{Abs. at } 652 \times \frac{1000}{34.5} \times \frac{\text{Volume}}{1000 \times \text{Fresh weight}}$$

$$\text{Carotenoids} = \frac{\text{Abs. at } 480\text{nm} + [(0.114 \times \text{Abs. at } 663\text{nm}) - (0.638 \times \text{Abs. at } 645\text{nm})] \times \text{Volume}}{1000 \times \text{Fresh weight}}$$

Sensory parameters

Sensory parameters were analyzed by same group of 20 semi-trained, individuals from Sharda University to assess the consumer acceptability of microgreens at different days of

Seeds were evenly sown, covered with moist sand up to 2 cm height which was regularly drenched using Reverse Osmosis water. After an initial three days dark period, 16 h photoperiod and 8 h dark period was maintained with the help of LED (light-emitting diode) lamps (XE series, Fluortronix, Noida, Uttar Pradesh). These provided light in the combination of 3500K (warm white and green: 39%), 660nm (red: 38% - 118.25 $\mu\text{mole/m}^2/\text{s}$), 450nm (blue: 20% - 44 $\mu\text{mole/m}^2/\text{s}$), 730nm (far-red:3%) and UV - A (1%) radiation at photosynthetic photon flux density (PPFD) of 275 $\mu\text{mole/m}^2/\text{s}$. The complete spectrum emitted 96% of Photosynthetically Active Radiation (PAR) (Fig 1 a-b). Uniform light intensity was regularly maintained using a lux meter. For estimation of growth attributes, photosynthetic pigments and sensory parameters, chickpea microgreens were harvested on 7th, 14th, 21st and 28th day of cultivation.

cultivation. Nine-point hedonic scale was used for ranking of samples as 9 (Like extremely) to 1 (Dislike extremely) for sensory parameters of colour and appearance, taste, aroma, mouth-feel and overall acceptability.

Statistical analysis

Three replications were utilized and the results were expressed as average with standard deviation (mean \pm SD). One Way ANOVA was used for analysis of variance and the differences were calibrated on basis of Tukey's test (post-hoc) at 95% probability, using SPSS version 22 (IBM). Significant differences between values were denoted as alphabets above bars. The correlation study amongst pigments and sensory parameters was performed using Pearson's Correlation Coefficient at $p < 0.05$. Principal Components (PC) were extracted and analyzed. Correlation plots and biplots depicting interactions between scores (genotypes) and loadings (parameters) were constructed with Origin Pro 2026 software.

RESULTS AND DISCUSSION

Growth attributes

Comparative shoot height (cm/plant) exhibited a gradual and consistent increase from 7th day onwards till 28th day amongst genotypes of chickpea. Similarly, the number of leaves / plant also exhibited a consistent increase (4, 8, 14 and 20) till the end of growth stage. The cumulative mean calibrated through the growth period varied as 14.50 cm/plant in P-3022, 13.74 cm/plant in P-3062 and 12.04 cm/plant in P-4005. Number of leaves was highest in P-4005 (13) followed by the number in P-3022 (12) and P-3062 (9).

Comparative fresh as well as dry weight of stem enhanced with the growth from 7th day till 28th day of cultivation. The calibrated cumulative mean for fresh weight of stem was 185.9 milligram/plant, while, the dry weight was 23.8 milligram/plant. The fresh and dry weight of stem was maximum in P-3022 (247.4; 35.5) and minimum in P-3062 (134.5; 13.7) as milligram/plant. When fresh and dry weight (FW; DW) of leaves were compared on milligram/plant basis, these enhanced consistently from 7th day onwards (110.4 FW, 14.4 DW) till 28th day (462.9 FW, 96.2 DW). Calibrated cumulative mean for leaves showed that the genotype P-3022 performed the best (314.1 milligram/plant, FW and 64.7 milligram/plant, DW). Genotype P-3062 depicted the lowest

fresh (219.2 milligram / plant) and dry weight (37.4 milligram/plant). The mean fresh weight of shoot varied as 248.5 milligram/ plant (7th day), 306.3 milligram/plant (14th day), 512.2 milligram/plant (21st day) and 737.9 milligram/plant (28th day). The mean dry weight of shoot was lowest (22.2) on 7th day and increased to a highest of 141.2 at 28th day as milligram/ plant. The observations recorded clearly indicated an enhancement in the stem, leaves and shoot weights with the growth duration. Mean values depicted highest fresh weight (561.5 milligram/plant) and dry weight (100.2 milligram/plant) of shoot by P-3022, whereas, genotype P-3062 showed the lowest values (353.7 milligram/ plant, FW; 51.1 milligram/ plant, DW) (Fig 2 a-b, Fig 3 a-d).

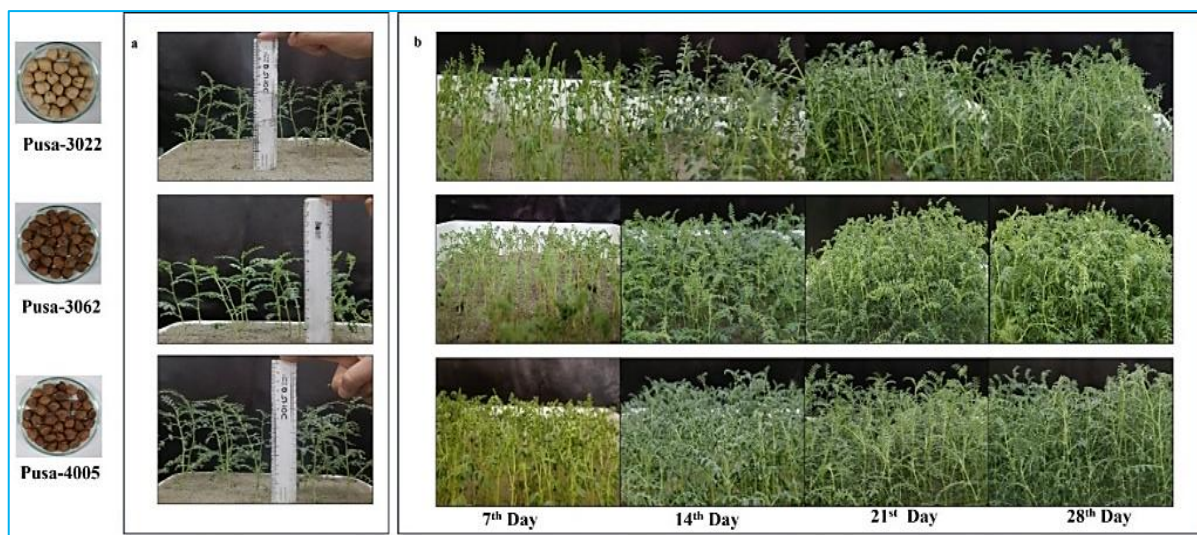


Fig 2 (a) Variation in shoot height at different days of growth amongst chickpea genotypes; (b) Gradual enhancement in shoot biomass

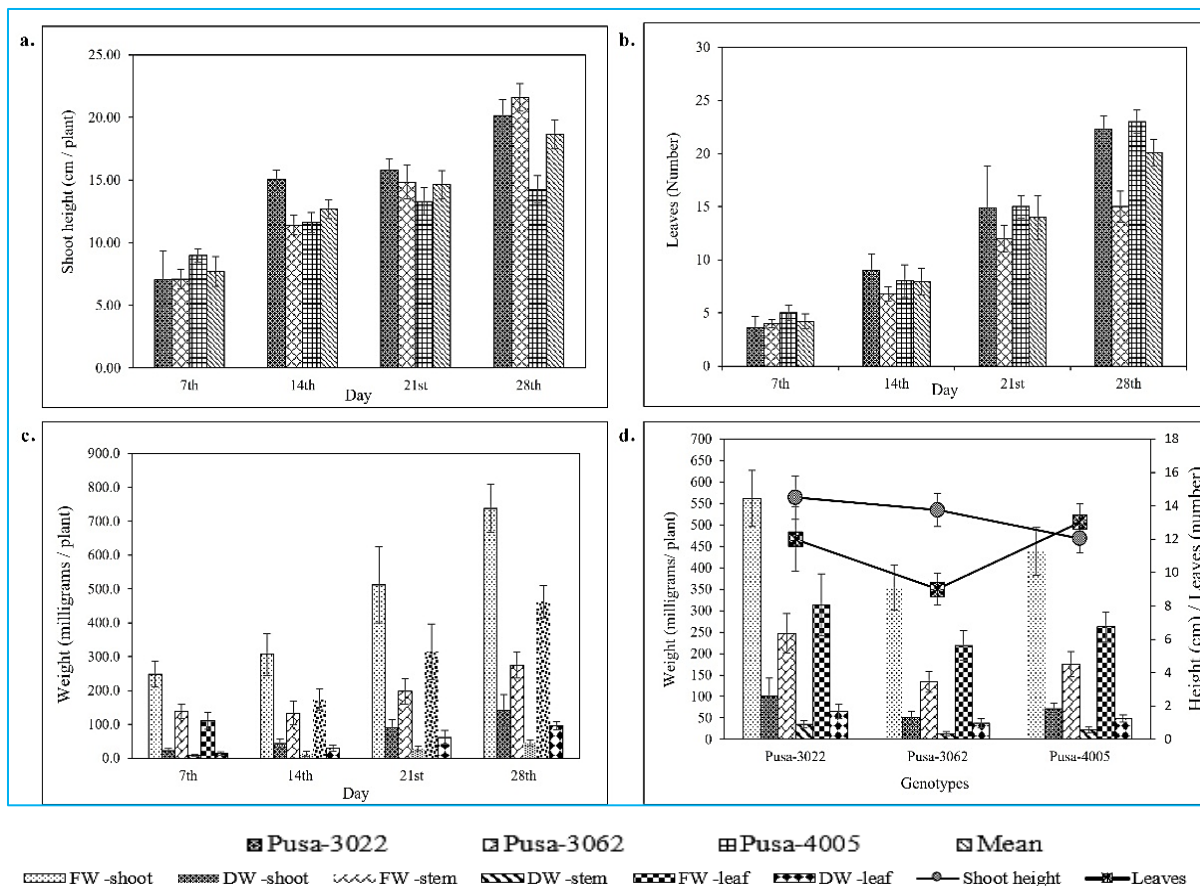


Fig 3 (a) Comparative shoot height at different days of growth, (b) Comparative number of leaves at different days of growth, (c) Comparative fresh and dry weight of stem, leaves and shoot at different days of growth, (d) Comparative fresh and dry weight of stem, leaves and shoot, shoot height and number of leaves amongst chickpea genotypes

Photosynthetic pigments

Comparative total chlorophyll, chlorophyll *a* and chlorophyll *b* when expressed as milligram/ g fresh weight basis showed a consistent enhancement from 7th day onwards till 28th day, however, the carotenoid content depicted a slow increase. When cumulative means were calculated, the total chlorophyll, chlorophyll *a* as well as chlorophyll *b* were maximum in P-4005 and minimum in P-3062. Accordingly, chlorophyll *a* / *b* demonstrated similar pattern, i.e. highest in P-4005 (3.44) and lowest in P-3062 (2.77). On the other hand, carotenoid content was maximum (0.10) in P-3022 and minimum in P-4005 (0.08) calibrated as milligram /g on fresh weight basis. The ratio of

total chlorophyll to carotenoid was very high in all genotypes and the ratios recorded were 49.9 (Pusa-4005) followed by 38.8 (Pusa-3022) and 38.7 (Pusa-3062). The percent contribution of chlorophyll *a* to total chlorophyll varied from a highest of 72% (Pusa-3022) to the lowest of 66% (Pusa-4005), while chlorophyll *b* exhibited varying contribution from a maximum of 25% (Pusa-3062) to the minimum of 21% (Pusa-4005) and the percent contribution by carotenoids was only 2% to 3% (Table 1, Fig 4 a-b). Overall, these findings indicate genotype-specific variations in pigment composition, with P-4005 showing superior chlorophyll accumulation while P-3022 exhibited relatively higher carotenoid content.

Table 1 Comparative cumulative mean for chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, total pigments (milligram/g), ratio of chlorophyll a and b; and ratio of total chlorophyll and carotenoids amongst chickpea genotypes

Genotype	Chl <i>a</i>	Chl <i>b</i>	Total Chl	Carot.	Total pigments	Chl <i>a</i> / <i>b</i>	Total Chl/ Carot
Pusa-3022	2.82 ± 0.08	0.86 ± 0.04	3.81 ± 0.14	0.10 ± 0.01	3.91 ± 0.15	3.35 ± 0.10	39.9 ± 2.73
Pusa-3062	2.27 ± 0.09	0.82 ± 0.06	3.26 ± 0.17	0.08 ± 0.00	3.34 ± 0.17	2.77 ± 0.13	51.3 ± 2.53
Pusa-4005	2.95 ± 0.17	0.95 ± 0.06	4.36 ± 0.24	0.09 ± 0.01	4.45 ± 0.25	3.44 ± 0.08	46.2 ± 1.11

Chl - Chlorophyll; Carot - Carotenoids

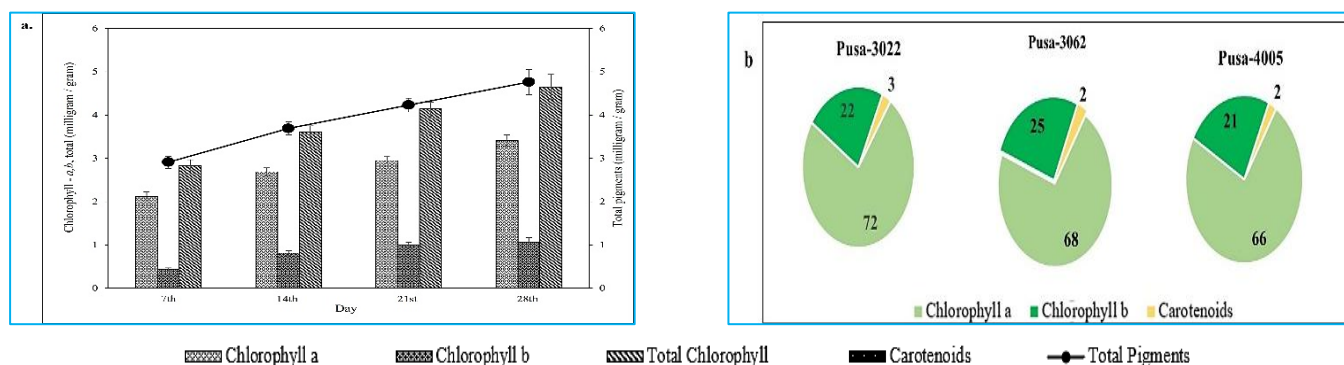


Fig 4 (a) Comparative chlorophyll *a*, chlorophyll *b*, total chlorophyll and total pigments at different days of growth, (b) Comparative percent contribution of chlorophyll *a*, chlorophyll *b* and carotenoids to total pigments amongst selected chickpea genotypes

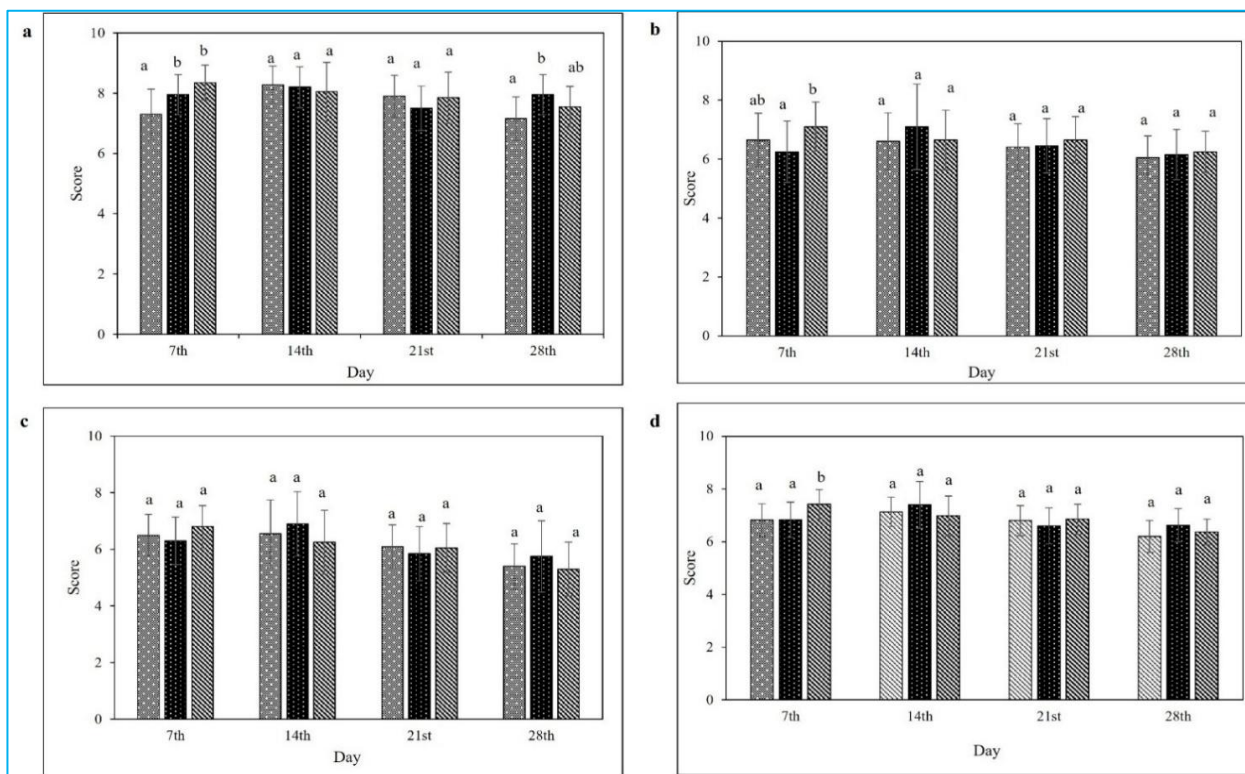


Fig 5 Comparative sensory parameters at different days of growth, (a) Colour and appearance (b) Taste (c) Mouth feel and (d) Overall acceptability

■ Pusa-3022

■ Pusa-3062

■ Pusa-4005

Sensory parameters

Observations on sensory parameters of colour and appearance, taste, mouth-feel and overall acceptability were recorded. As, no aroma was detected in chickpea microgreens, this parameter was not ranked on hedonic 9- point scale. Mean values for each of these were highest at 14th day (8.18; 6.78; 6.57; 7.17) and showed a decline thereafter. The calibrated cumulative mean varied as 7.84 (colour and appearance), 6.53 (taste), 6.15 (mouthfeel) and 6.84 for overall acceptability.

Genotypes Pusa-3062 and Pusa-4005 depicted similar pattern for colour and appearance. On the other hand, Pusa-4005 and Pusa -3062 performed best for taste and mouth-feel. However, overall acceptability was highest for genotype Pusa -4005 (6.91) and decreased in Pusa-3062 (6.87) and Pusa-3022 (6.74) (Fig 5 a-d). Overall, the results indicate that the 14th day of growth is optimal for achieving superior sensory quality in chickpea microgreens, with genotype Pusa-4005 exhibiting the highest overall consumer acceptability.

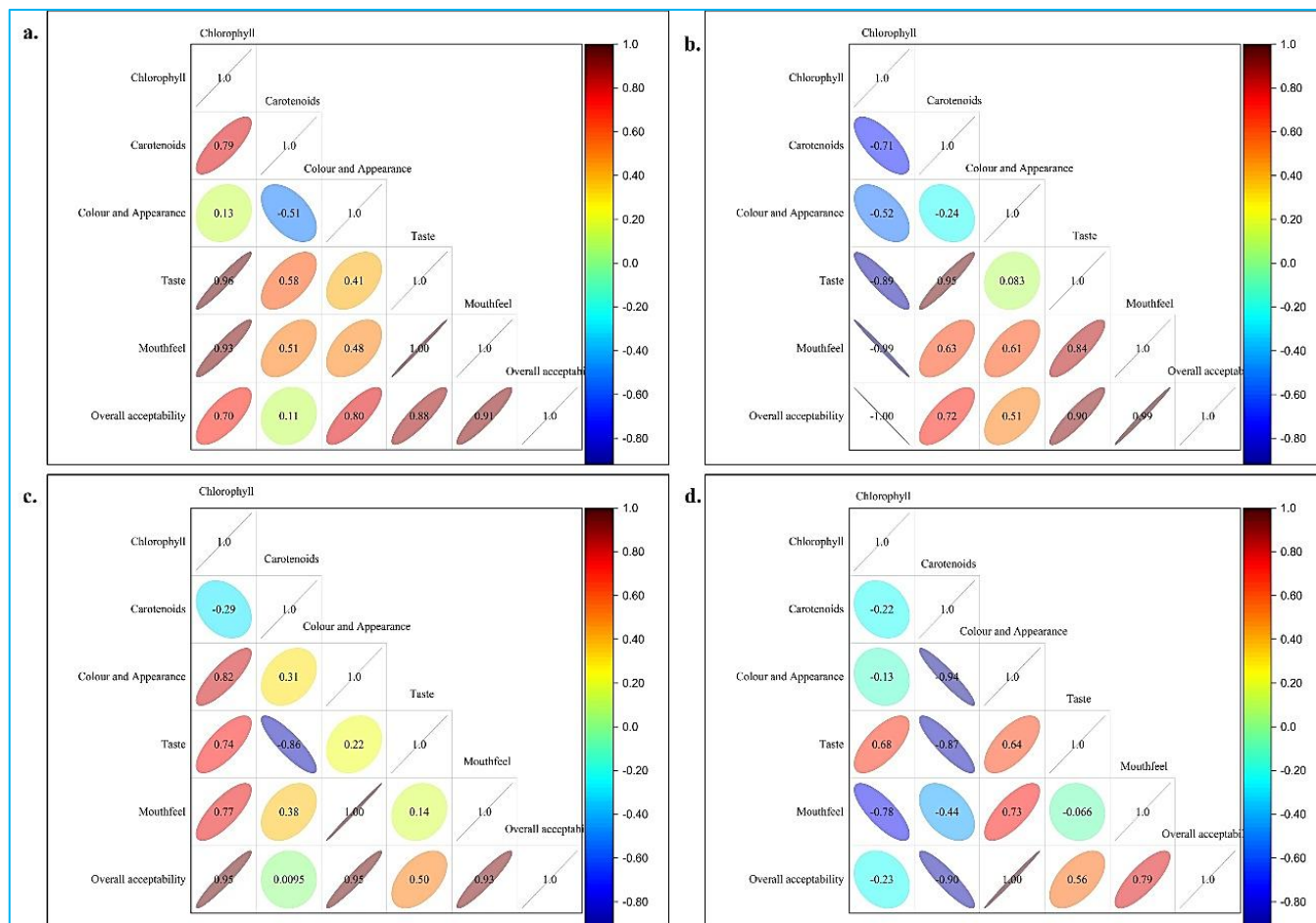


Fig 6 Correlation plot depicting degree of correlation between photosynthetic pigments, and sensory parameters at different days of growth ($p < 0.05$) (a) 7th day, (b) 14th day, (c) 21st day and (d) 28th day

The colour gradient on right corresponds to correlation coefficient, where red depicts a strong positive correlation ($r = +1$), while, dark blue depicts a strong negative correlation ($r = -1$). The size of ellipse depicts the strength of correlation i.e. lesser the width of ellipse, stronger the correlation and vice-versa.

Principal components analysis (PCA)

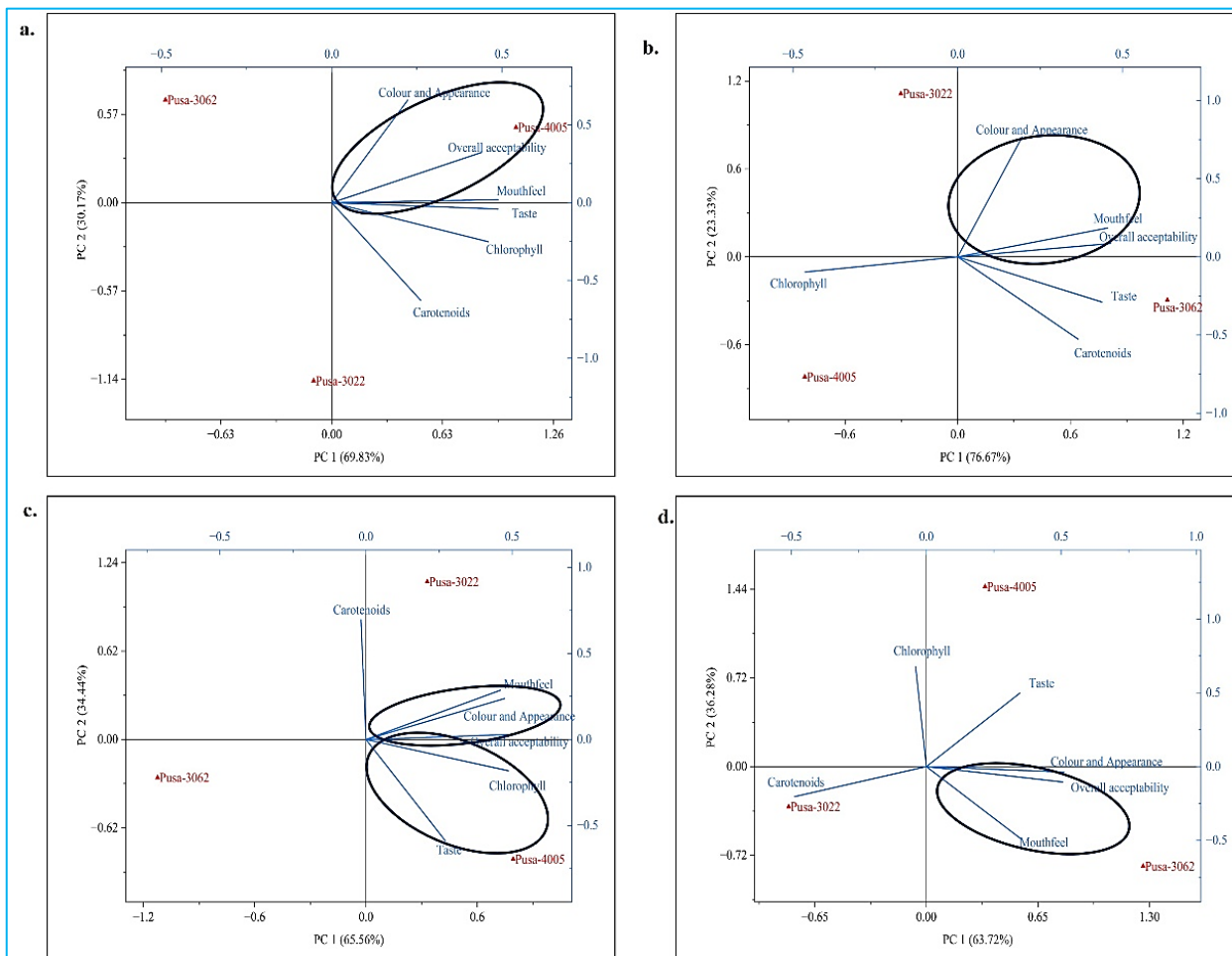
PCA and the biplots constructed distinctly aided in understanding the variability in association with interactions amongst the studied parameters and their relations with each of the components.

The first principal component (PC1) explained majority of variations with growth stages, i.e. 69.83% (7th day), 76.67% (14th day), 65.56% (21st day) and 63.72% (28th day). Second principal component (PC2) depicted variance as 30.17%, 23.33%, 34.44% and 36.28% on different days of growth. Thus, both the components accounted for a cumulative 100% variance for the selected parameters. On 7th and 14th day, loadings were observed to form a distinct cluster in region of positive PC1, while, on 21st day, two distinct clusters were depicted, one in

region of high PC1 and PC2 values. On 28th day, a single cluster was observed in region of positive PC2.

Further, genotype Pusa-3022 was located in quadrant corresponding to negative PC1 and PC2 values on 7th and 28th day, and region of positive PC2 on 14th and 21st day of growth. Pusa-3062 was located in region of positive PC2 on 7th day, positive PC1 and negative PC2 on 14th and 28th day. Pusa-4005 was depicted in quadrants corresponding to positive PC1 and PC2 on 7th and 28th day of growth. Contrary to this, it was in region of negative values of both PCs on 14th day and in quadrant IV (positive PC1) on 21st day. Pusa-4005 depicted association with sensory parameters on 7th day, Pusa -3062 on 28th day, and Pusa-3022 on 21st day. On 28th day, Pusa-3022 exhibited an association with carotenoids.

Sensory analysis of taste, mouth feel and overall acceptability was strongly related to PC1 on 7th and 14th day, while, on 21st and 28th day, mouth feel, colour and appearance, and overall acceptability depicted a strong relation to PC1. Colour and appearance showed a strong relation with PC2 up to 14th day, and similar observation was shown for taste at last two stages (Fig 7 a-d).



(a) 7th day, (b) 14th day, (c) 21st day, (d) 28th day

Fig 7 Principal Component Analysis and biplots for photosynthetic pigments and sensory parameters at different days of growth

The present study utilized LED lights comprising combination of different wavelengths with effective photosynthetic active radiations for cultivation of chickpea genotypes as microgreens. As per literature surveyed, such study has not been reported earlier in microgreens. The work involved assessment of growth attributes, photosynthetic pigments, and sensory parameters at 7th till 28th day of cultivation of chickpea microgreens grown under LED lights. In our experiment, shoot height depicted an enhancement with growth and a variability was exhibited amongst the genotypes. Fresh and dry weights of stem, leaves and shoot indicated an enhancement with the growth duration. Shoot comprising stem and leaves forms the edible part of microgreens; therefore, their fresh weight was an important attribute for harvest consideration according to the consumer's preference. Shoot height was comparable to reports on turnip and amaranth microgreens under blue light and fresh weight per plant showed 3-fold amplification in turnip microgreens due to blue light, while, dry biomass decreased below 4.3% under red light in both the plant species [8].

Blue light is known to have a significant role in biomass production though red light reports a pivotal role in photomorphogenetic response stimulating elongation of shoot [9-10]. The sole blue or red light promoted longer shoots than combination of both, however, fresh yield was higher in kale and mustard when grown under combined application in a proportion of 1:3 with maximum shoot dry weight in mustard [11]. Highest fresh weight was reported in broccoli (red + far red light) and cabbage microgreens (red light) after 13 days of sowing in contrast to radish microgreens under red, blue and far-red treatment at 10 days. However, dry weight was highest

under red light for broccoli and red, blue and far-red light in cabbage and radish microgreens at similar growth stage [12]. Results on length of hypocotyls in seven-day old broccoli highlighted the effect of light treatments with red and blue light (5:1) promoting fresh weight on per plant basis [13]. Blue light influences hypocotyl length by Phot 1 mediated shade avoidance response along with CRY1 regulating effect of gibberellic acid on stem elongation [14]. On the other hand, red and far-red light perception regulates morphogenesis via co-ordinated action of type I (phyA) and type II (phyB-E) phytochromes which have a regulatory effect on germination and hypocotyl length [15]. Supplementation with ultraviolet wavelength reports a significant increase in length of hypocotyl in basil and pak choi microgreens [16-17].

Pigments (chlorophyll *a*, *b*, total and carotenoids) exhibited an increasing pattern with the growth of microgreens with slight variability amongst the genotypes. Blue and red are photosynthetically active wavelengths regulating synthesis of pigments [18]. Though green light is considered photosynthetically ineffective, it can efficiently penetrate deeper than red and blue, thus, may improve photosynthetic efficiency of leaves [19]. Wide spectrum LED light at 250 $\mu\text{mole/m}^2/\text{s}$ and combined supplementation with red and blue light enhanced accumulation of photosynthates [20]. Cultivation of amaranth microgreens under red, blue and green LED lights resulted in highest chlorophyll (*a*, *b*, total), total carotenoids [21]. Pronounced effect of blue light has been reported on chlorophyll (*a*, total) and carotenoids (30% increase) in amaranth microgreens as compared to white and red light, whereas, in turnip, no effect of light treatment was observed.

CONCLUSION

The outcome of the present study highlights the impact of artificial light source (LEDs) as an optimized combinations of different wavelengths on growth parameters, pigments, and sensory attributes in three chickpea genotypes cultivated as microgreens till 28th day. The supplemented UV wavelength in the spectrum of light used supported the limitation faced during indoor farming as most artificial lights lack UV wavelength essential for synthesis of secondary metabolites. Growth parameters including shoot height, number of leaves, fresh and dry weights of stem, leaves and shoot on per plant basis showed an enhancement till 28th day. Pigments (chlorophyll *a*, *b* and total; carotenoids) showed gradual enhancement. A statistically significant correlation amongst the studied parameters was established at each growth stage. Results revealed that the microgreens can be conveniently grown till 28th day under artificial light conditions indoors for an easy availability and access to the consumers. Future studies may be designed to understand the modulation in light perceiving photo-receptors, their level of expression, downstream regulation and interplay under LED light supplementation.

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Author contribution

TS: Experimentation, data finalization and analysis, tabulations, preparations of figures and alignments, writing the original draft; AG: Planning, supervision and coordination, DWD: Data curation, review and editing, finalization of manuscript.

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Data availability: The data that support the findings of this study can be obtained from the corresponding author upon reasonable request.

Declaration: Conflict of interest: The authors have no conflict of interest.

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