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Investigating Plant Mineral Accumulation and Vigour Potential in Bottle Gourd (*Lagenaria siceraria*) through Crossbreeding Timing

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Abstract: Bottle gourd (Lagenaria siceraria) is a well-known cucurbit that possesses an active functional ingredient. A two-year field experiment was carried out at the Research Farm of Seed Science and Technology, CCS HAU, Hisar in a randomized block design during the *kharif* season and summer season. Five different crossing periods (CP), viz. CP1, CP2, CP3, CP4, and CP5, were considered to illustrate the effects of agro-climatic conditions on the quality and biochemical components of two bottle gourd parental lines and one hybrid, HBGH-35. The average mean temperature for the *kharif* season of 2017 was 31.7°C, and for the summer season, 40.1°C. Flowers were tagged weekly from the start of the crossing period until the end, and harvested separately at different times. The fruits harvested from different crossing periods under different environmental conditions influenced thebottle gourd's qualitative and biochemical traits and showed significant variations among the five crossing period environments. A positive significance and correlation were observed between weather variables and different biochemical traits. Henceforth, the F_4 crossing period at atemperature of 31.7°C had quality seed development, which may play an essential role in enhancing agricultural productivity and the national economy.

Keywords: bottle gourd; crossing periods; crude protein; quality; temperature; nutrition

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1. Introduction

The mineral content of seeds correlates with seedling quality. Seed development necessitates the accumulation of minerals and essential nutrients, especially during the early growth stages [1]. The primary factors influencing seedling development are the minerals accumulated during seed ontogeny; without these components, growth would be delayed.[2]. To comprehend the source-sink relationship's mechanism and to enhance the nutritional profiles of the crop, it is essential to recognize the interactions between macro- and micronutrients during different crossing periods of bottle gourd. [3] Seeds contain enough metabolic reserves to facilitate seedling establishment. Carbohydrates, lipids, and proteins interact synergistically to create alifen optimal environment for seed germination and seedling development. To develop bottle gourd genotypes with improved nutritional

and phytochemical compositions, micronutrient and macronutrient profiling of the germplasm is essential [4]. Prior to investigating the gene activity influencing the inheritance of nutritional and antinutritional traits in the parents and offspring, it is imperative to ascertain the ideal crossing times for desired genotypes..

Bottle gourd (*Lagenaria siceraria* (Molina) Standl) is an edible, useful, and medicinal vegetable crop that belongs to the Cucurbitaceae family. It is a cross-pollinated vegetable, and the amount of pollination ranges from 94 % to 99 %. The degree of cross-pollination also depends on factors such as flowering time, temperature, wind velocity and direction, planting design, insect population, and genotypic nature, ultimately determining the kernel quality [5–7]. In the cucurbits, signs of the reproductive phase begin with the start of flowering approximately six to seven weeks after the planting. As a result of the source-sink strength relationship, variation in seed lots can be observed [8]. Being indeterminate bottle gourd vines, it stands up to continuous dry and overcast weather at any stage like seed germination, emergence, seedling establishment, leaf canopy development, flowering, and seed maturation.

There are numerous abiotic factors, predominantly high and low temperatures, low light, moisture surpluses [9–11], and deficits that impose limitations on the growth, development, and, yield of different crops [12]. Cool nights are favorable during the fruiting period, but extreme or low temperatures may result in delayed growth and aborted fruiting sites. Temperature significantly affects leaf expansion, phenology, internodes elongation, partitioning of assimilates, and biomass production [13]. The variations in protein and lipid contents were attributed to several environmental factors [13,14]. The sowing date may affect light and temperature, representing the major ecological factors influencing crop growth and development [15].

Seed quality is a significant aspect of agricultural production and food security, especially during the growing uncertainty caused by climate change [16–18] and abiotic factors [19,20]. Hybrids hold the plant's entire genetic combination, making them a distribution platform for agricultural biotechnology and crop enhancement. Farmers constantly need high-quality sources to ensure efficient and effective plantations; thus, companies must sell high-quality seeds to maintain their competitive position in markets [21].

Quality is an essential aspect of crop production, and it demands specific quality and functionality during development [7]. The efficiency of hybrid seed production depends on multiple factors, such as selecting an appropriate agro-climate location, a suitable season, improved floral synchronization for enhanced seed setting through suitable crossing periods and supplementary pollination techniques, etc. [7]. The adaptation and distribution of bottle gourd are bi-hemispheric and it, therefore, grows well within tropical and temperate regions. Bottle gourd grows well in areas with rainfall of between 400 – 1 500 mm per year; however, moderate, rather than excessive soil water is desired for a good harvest. According to Grubben and Dento [22] bottle gourd grows well at temperatures (25 – 35°C). The optimum germination temperature is between 20 and 25°C. Temperatures below 15°C and above 35°C reduce the germination rate [23]. Agronomic practices that promote the production of more female flowers than male flowers could increase yields; however, Haque et al. [24] observed fewer seed sets due to the reduction of pollen. It is, therefore, important to determine the optimum ratio of male and female flowers to optimize the fruit and seed set. However, the effects of different environmental conditions on bottle-gourd seed properties are not well elucidated yet. Therefore, there is a need to study the impact of mineral accumulation in different fruit-setting periods at different temperatures. Thus, the present study aims to understand and elucidate the effect of pollination susceptibility and the impacts of the different crossing periods on the biochemical constituents of bottle gourd.

2. Materials and Methods

2.1. Plant Material and Experimental Setup

The research experiment was conducted at the experimental farm of Seed Science & Technology (29.1416° N, 75.7112° E) during the Kharif (2017) and summer season (2018) in a randomized block design. Seeds of bottle gourd parental lines, G-2 (Male Line) and Pusa Naveen (Female Line) were procured from the Department of Vegetable Science for the production of Hybrid HBGH-35. For the production of hybrid HBGH-35, seeds of the bottle gourd parental lines G-2 (male line) and Pusa Naveen (female line) were obtained from the Department of Vegetable Science. HBGH-35 is the first hybrid developed by the CCS HAU, Hisar, using the G2 male line from Gujarat regions and the female lines used as PUSA Naveen developed by IARI Pusa, both of which are popularly grown in the northern part of India. G2 is an early flowering line, and Pusa Naveen is a high-yielding line. To compare the potential of HBGH-35 concerning parental lines, these lines were selected.

Being an indeterminate crop, flowering in bottle gourd continues for around two months. There were ten vines per plot with a size of 6 x 3 meters and vine-to-vine spacing is 60 cm.

2.2. Pollination and Emasculation

The flowers (male and female) were emasculated one day before opening, and they were wrapped in a thin layer of cotton. On the next morning, pollen was collected from the male flower and dusted on the respective female flower, which was then wrapped with a thin layer of cotton until the fruit formation started. The pollinated female flower was tagged for further identification.

The emasculation and pollination work were done in both parental lines with the help of needles, scalpers, and forceps. The two glass cups, cotton, muslin cloth, plastic bag, camel brush, and scissors were used to collect pollen from male flowers and dust it on the stigmatic surface of emasculated female flowers for hybrid seed production. Manual emasculation and dusting were continued throughout the crossing period. Subsequently, pinch off all the floral buds emerging beyond 15 days after the crossing period to ensure better growth and production of crossed fruits (Figure 1).

At the start of the crossing period in hybrid and parental line seed production, the male flower was chosen in the parental line and bagged with paper bags the previous evening, pinched off the next morning, and kept carefully in a glass container.

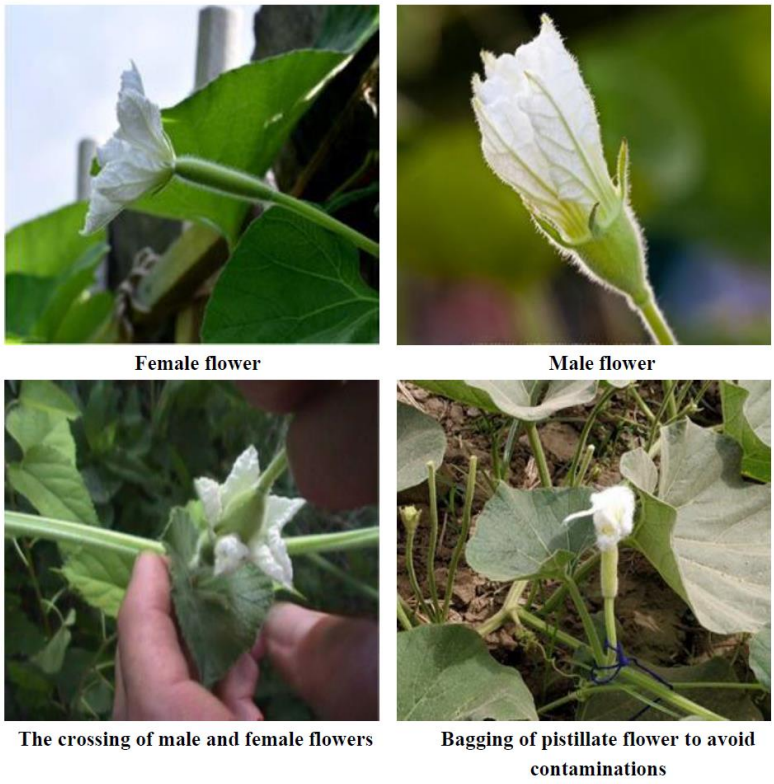

Figure 1. Stepwise emasculation and crossing procedures of bottle gourd male and female flowers.

2.3. Soil Profile

The concentrations of organic matter percent, total nitrogen, available phosphate, and rapidly available phosphate in the uppermost 20 – 30 cm soil were 0.49 %, 182 kg/ha, 18 kg/ha, and 285 kg/ha, respectively.

Table 1. Harvesting dates of different crossing periods**.**

Crossing periods	***Kharif* (2017)**	**Summer season (2018)**
CP-1 (first week of crossing period)	4 Sept to 10 Sept 2017	6 April to 22 April 2018
CP-2 (second week of crossing period)	11 Sept to 17 Sept 2017	23 April to 29 April 2018
CP-3 (third week of crossing period)	18 Sept to 24 Sept 2017	30 April to 6 May 2018
CP-4 (fourth week of crossing period)	25 Sept to 1 Oct 2017	7 May to 13 May 2018
CP-5 (fifth week of crossing period)	2 Oct- 8 Oct 2017	14 ay to 20 May 2018

2.4. Agro-meteorological Data

The harvested seeds of five crossing periods of all genotypes were studied for various biochemical parameters, under varying weather conditions. Agro-meteorological data were recorded at the agro-meteorological observatory during the seed development period of both seasons and converted to weekly data, which are presented in Table 2 and Figure 2.

Table 2. Meteorological data for 2017 (Kharif season) and 2018 (summer season) of bottle gourd growing seasons.

Months	*kharif* season (2017)							
	T_{max} (°C)	**T_{min} (°C)**	**RH (%) M**	**RH (%) E**	**BRI Sun (Hrs)**	**Pan Evap (mm)**	**Rainfall (mm)**	**Avg Ws (km/h)**
July	34.4	27.0	90.7	70.9	6.5	4.6	2.6	7.3
Aug	34.7	26.3	89.7	69.3	6.3	4.2	3.1	5.6
Sept	34.9	23.5	87.2	49.5	6.8	4.2	1.9	2.9
Oct	35.0	17.2	84.8	28.0	6.6	3.6	0.0	1.9
Nov	27.2	10.8	90.1	39.8	3.4	2.8	0.0	2.0
Dec	23.8	7.3	89.7	34.5	3.7	1.4	0.0	0.7
Mean	31.7	18.7	88.7	48.7	5.5	3.5	1.3	3.4
Summer season (2018)								
	T_{max} (°C)	T_{min} (°C)	RH (%) M	RH (%) E	BRI Sun (Hrs)	PAN Evap (mm)	Rainfall (mm)	Avg Ws (km/h)
Feb	28.02	12.80	92.60	48.00	6.48	2.80	0.00	2.84
March	30.92	12.18	82.03	35.87	6.89	3.65	0.00	2.70
April	36.65	19.53	60.33	33.40	7.26	6.41	0.47	5.59
May	40.63	23.73	56.74	28.71	7.05	8.61	0.00	7.05
June	39.58	27.75	71.70	46.87	4.38	8.88	1.96	8.77
July	35.02	26.74	88.00	69.32	4.95	4.76	5.11	5.57
Mean	35.13	20.45	75.23	43.69	6.17	5.85	1.26	5.42

*T_{max}: maximum temperature; T_{min}: minimum temperature; RH_m: morning relative humidity; RH_e: evening relative humidity; BRI: the bright sun; EVAP: evaporation, AVG WS: average wind speed.

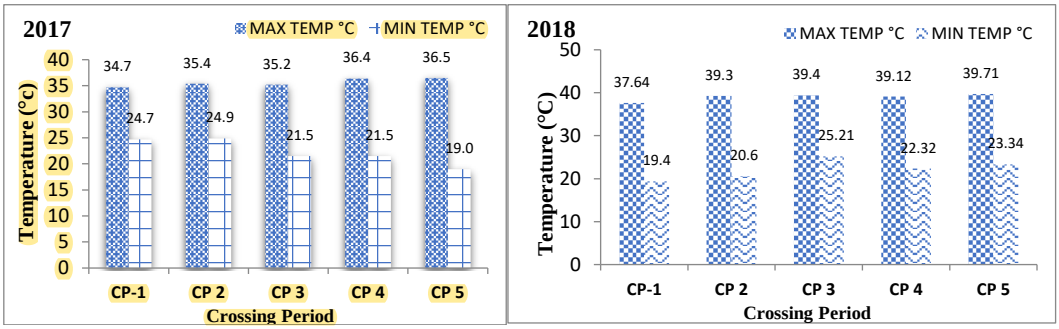

Figure 2. Average maximum and minimum temperature during different crossing periods in bottle gourd during Kharif 2017 and summer 2018.

2.5. Biochemical Analysis

The seeds were extracted from fruits set in each crossing period manually. After extraction, seeds are dried in the shade to lower the moisture content to 8 % and be stored for further biochemical analysis. The moisture content of the seed was measured using the hot oven method. In this method, the pre-weighed aluminum boxes along with seed material are placed in an oven maintained at 103°C. Seeds are dried at this temperature for 1 hr. The relative humidity of the ambient air in the laboratory must be less than 70 percent when the moisture determination is carried out.

The moisture content as a percentage by weight (fresh weight basis) is calculated to one decimal place, by using the following formulae:

$$\text{The percentage of seed moisture content (mc)} = \frac{M2-M3}{M2-M1} \times 100$$

M1 = Weighing container weight with cover in grams

M2 = Weighing container weight with cover and seeds before drying

M3 = After-drying weight of the weighing container with cover and seeds

Total soluble solids (°Brix) were determined by using a hand refractometer. The ash, moisture content, and crude fiber content in the seeds were determined by the method recommended by [25]. Total nitrogen (N) was estimated by the micro-Kjeldahl method, and crude protein was calculated by multiplying 4.27 by the N concentration [25]. The lipid content was determined by the Soxhlet method. Total carbohydrate was estimated by the described by [26]. Vitamin C was measured using the titration method of reduction of 2,6-dichlorophenol-indophenol (DCPIP) dye described in [27]. The energy value was calculated by multiplying the mean values of crude protein, lipid content, and total carbohydrate by the factors of 4, 9, and 4, respectively.

2.6. Measure of macro and micro-elements

Measurements of macro- and microelements in mature dried bottle gourd seeds were done using two methods: energy dispersive x-ray (EDX) analysis and atomic absorption spectrometry (AAS).

2.7. Energy dispersive x-ray analysis (EDX)

Three seeds from each selection were cryo-fractured in liquid nitrogen and split into halves using a scalpel blade. Seeds were mounted on stubs and secured using double-sided insulating carbon tape. Seed mineral composition was determined separately for the embryo, cotyledon, and seed coat regions.

2.8. Atomic absorption spectrometry

The contents of the elements were determined in the seed materials after incineration in a muffle furnace at 660°C. The resultant ash content was solubilized on porcelain crucibles using a 10 ml acid mixture (HNO_3 and HCl mixed in a 1:3 ratio) [28] using an atomic absorption spectrophotometer (Alpha 4 Model). A rapid sequential absorption spectrometer was used to evaluate potassium using flame atomic emission spectroscopy (FAES) (Varian AA280FS). Flame atomic absorption spectroscopy was used to determine the concentrations of Ca, Mg, Zn, Cu, Fe, and Mn (FAAS). Table 3 provides a summary of the constituents that were evaluated at each wavelength, recovery level, and relative standard deviation (RSD). Using a Varian Alpha UV-VIS spectrometer at 400 nm, the amount of phosphorus was measured (Spectronic Unicam, Berlin, Germany)[29].

Table 3. The relative standard deviations, and wavelength, recovery levelforelemental evaluation in bottle gourd seeds.

Element	Wavelength (λ)	Recovery level (%)	RSD (%)
K	590 nm	98.7	5.9
Ca	423 nm	99.8	7.9.8
Mg	285 nm	97.5	4.8
Zn	214 nm	98.2	6.4
Cu	325 nm	95.1	5.3
Fe	249 nm	98.3	7.5
Mn	280 nm	99.6	7.8

2.9. Measurement of germination axis

2.9.1. Standard Germination

One hundred seeds were taken in four replications from each treatment and placed uniformly between two sufficiently moist germination papers. Following that, they were rolled and placed in a germinator, where the temperature and relative humidity were kept at 25 1 °C and 95 1%, respectively. The final counts were made on the 14th day of the germination test for normal seedlings and expressed in percentages as per the ISTA protocol [30].

The germination percentage was worked out using the following formula:

$$\textbf{Germination}\ (\%) = \frac{\textbf{Number of normal seedlings}}{\textbf{The total number of seeds planted}} \boldsymbol{X}\ \mathbf{100}$$

2.9.2. Biomass accmulation

The plants were uprooted, and thoroughly washed with distilled water to eliminate the soil.To remove surface moisture, a paper towel was used. An electronic balance (Presica 105A) was used to measure fresh weights. Weighed fresh samples were heated in an air oven at 70 °C for 24 hours and then dried to a constant weight at 35 °C. The plant dry weight was equal to the sum of the shoot and root dry weights. The root-shoot ratio was calculated according to Agren and Ingestad [31].

$$\text{Root shoot ratio} = \frac{\text{Dry weight for roots}}{\text{Dry weight for the top of the plant}}$$

2.9.3. Root and shoot length

A two-factorial experiment was replicated three times to measure the germinant axis and provide experimental units. Sand particles were removed from seedlings that had developed through sand soil by uprooting and washing them. The lengths of the roots and shoots were measured using computer-assisted image analysis. A tabloid scanner (Hewlett Packard Scanjet 4c/t; Palo Alto, California) was used to capture digital photos at a scanning resolution of 250 dpi. Using image software analysis, the lengths of the roots and shoots were measured.

2.10. Statistical analysis

The data were presented as mean SD (three replicates)-way ANOVA was conducted to check the significance of the main effects (cultivars and temperature seasons) and their interaction on growth indices, followed by a post-hoc comparison (Tuckey's test) at a 5% level (P 0.05). Statistical analysis was performed using SPSS v25.0 software (SPSS for Windows, Chicago, IL, USA). The correlations between phytochemicals and weather variables were statistically evaluated and indicated by Pearson's coefficient indexes using two-tailed bivariate correlates analysis; p 0.05 was considered statistically significant.

3. Results and Discussion

3.1. Proximate Composition

Seed production in bottle gourds is a complicated process because seed development occurs within moist fruits for several weeks and the seeds are frequently kept wet before seed harvest. The moisture content varied significantly among the bottle gourd cultivars under different growing seasons ($p<0.05$) (Table 4). The male parental line (G2-line) had the highest moisture content (12.7%), followed by the female parental line with 12.8% at 31.7°C. Hybrid HBGH-35 retained its minimum moisture content (12.4%) at 31.7°C during the Kharif season, while at 40°C (summer season), it decreased by 12.2, 11.6, and 11.8%, respectively, during the 4th week of the crossing period (CP-4). Variation in seed moisture is due to the flesh of fruits from CP-1, which gets more time to dry out as compared to CP-5. Secondly, summers have more hot winds/temperatures, so fruits dry out faster as compared to the Kharif season. During the summer season, there was a sharp increase in moisture content, leading to poor seed quality and seed deterioration. The relatively low moisture content in bottle gourd is advantageous since the high moisture content is associated with increased bacterial action during storage and is a key determinant of seed quality [32]. The results of this study are very similar to those of [33] who stated that moisture content is very important in determining seed quality. The fruits were harvested from the hybrid and parental lines at maturity, when the colour turned from green to brown. Seeds were extracted manually by slicing the ripened fruits into small pieces, and seeds were separated by squeezing the fruit pulp. The extracted seeds were cleaned with fresh water

and dried to lower the moisture content. So, the effect of moisture content is generally low, as seed production in bottle gourds is a much more complicated process because seed development takes place within the moist fruits for several weeks and seeds are often held in a wet state before their final harvest.

The ash content represents the index of mineral elements present in the seed. This indicates that the whole seed may have a higher mineral content [34] and be of good quality. The ash content shows no significant variation ($p > 0.05$) among different crossing periods but within two different growing seasons (Table 4). Hybrid HBGH-35 had the highest ash content (5.8%), followed by Pusa Naveen (5.2%), while the minimum was in the male line (4.2%) in the Kharif season (31.7°C) during the 4th week of the crossing period. The ash content in whole seeds is generally higher than the 3.7% reported as the ash content of calabash seed [34,35]. Hassan et al. [34] observed that ash content represents an index of high minerals that improved the overall performance of seed quality.

Dietary fiber promotes food absorption and bowel movement through the small intestine [36]. The results presented in Table 4 depict crude fiber content in bottle gourd samples that varied significantly ($p < 0.05$), with Hybrid HBGH-35 having the highest value (48.3%), followed by Pusa Naveen (37.2%), while the G2-line seed fiber (30.3%) was the least at 31.7°C. With the increase in temperature, the crude protein declined and affected the overall seed fiber quality. Bottle gourd is a rich source of dietary fibers that expand food inside the colon wall, easing the passage of waste, and making it an effective anti-constipation agent. The crude fiber content of the bottle gourd seed is comparable with that of calabash (3.2 %) and watermelon (2.46%) [37].

Table 4. Proximate composition analysis of bottle gourd seeds under two different growing seasons.

Seasons	Moisture (%)		Ash (%)		Crude fiber (%)	
	kharif	Summer	*kharif*	Summer	*kharif*	Summer
Hybrid HBGH-35						
CP-1	8.6±0.18 ^{e}	8.4±0.16 ^{e}	3.5±0.06^{d}	2.1±0.01 ^{d}	20.3±0.56 ^{a}	17.4±0.26 ^{a}
CP-2	9.4±0.16 ^{d}	9.2±0.17 ^{d}	3.9±0.01 ^{d}	2.7±0.06 ^{d}	26.6±0.46^{b}	20.2±0.49 ^{b}
CP-3	10.2±0.18 ^{b}	9.8±0.11 ^{b}	4.6±0.06^{b}	3.4±0.09 ^{b}	38.5±0.78 ^{d}	32.3±0.58 ^{d}
CP-4	12.4±0.19 ^{a}	11.8±0.10 ^{a}	5.8±0.07 ^{a}	4.9±0.04 ^{a}	48.3±0.69^{e}	42.6±0.47 ^{e}
CP-5	14.3±0.13 ^{c}	13.4±0.16 ^{c}	4.1±0.06 ^{c}	3.1±0.07 ^{c}	31.2±0.44 ^{c}	27.8±0.65 ^{c}
Pusa Naveen (♀)						
CP-1	8.4±0.12 ^{e}	8.2±0.23 ^{e}	2.5±0.06 ^{d}	1.8±0.04 ^{d}	20.1±0.36 ^{a}	16.7±0.32 ^{a}
CP-2	9.5±0.46 ^{d}	9.3±0.16 ^{d}	2.9±0.08 ^{d}	2.1±0.06 ^{d}	24.3±0.46 ^{b}	20.4±0.22 ^{b}
CP-3	10.8±0.18 ^{b}	9.8±0.15 ^{b}	3.9±0.04 ^{b}	3.4±0.09 ^{b}	31.2±0.25 ^{d}	26.9±0.36 ^{d}
CP-4	12.8±0.18 ^{a}	11.6±0.19 ^{a}	5.2±0.04 ^{a}	4.6±0.04 ^{a}	37.2±0.65 ^{e}	32.6±0.42 ^{e}
CP-5	14.6±0.17 ^{c}	13.7±0.16 ^{c}	3.5±0.03^{c}	2.1±0.03 ^{c}	27.4±0.33 ^{c}	23.1±0.87 ^{c}
G2-line (♂)						
CP-1	8.7±0.13 ^{e}	8.5±0.16^{e}	1.7±0.02 ^{d}	1.3±0.03 ^{d}	17.6±0.26 ^{a}	13.4±0.36 ^{a}
CP-2	9.7±0.14 ^{d}	9.5±0.12^{d}	2.1±0.05 ^{d}	1.7±0.06 ^{d}	19.6±0.46 ^{b}	16.3±0.54 ^{b}
CP-3	10.8±0.18 ^{b}	10.3±0.14^{b}	3.1±0.09 ^{b}	2.7±0.07 ^{b}	28.6±0.26 ^{d}	22.3±0.52 ^{d}
CP-4	12.7±0.16 ^{a}	12.2±0.19^{a}	4.2±0.08 ^{a}	3.8±0.08 ^{a}	30.3±0.54 ^{e}	27.6±0.41 ^{e}
CP-5	14.8±0.12 ^{c}	13.7±0.12^{c}	2.7±0.01 ^{c}	2.1±0.09 ^{c}	24.2±0.61 ^{c}	20.3±0.23 ^{c}

a-e Values with different superscripts in the same row are significantly different ($p < 0.05$) *CP-1: 4-10 Sept 2017 and 6-22 April 2018; CP-2: 11-17 Sept 2017 and 23-29 April 2018; CP-3: 18-24 Sept 2017 and 30 April -6 May 2018; CP-4: 25 Sept-1 Oct 2017 and 7-13 May 2018; CP-5: 2-8 Oct 2017 and 14-20 May 2018.

3.2. Biochemical constituents

Total soluble solids (TSS) measure the sugar content of seeds and play a significant role during seed production. The variation in TSS observed in fruits of exotic bottle gourd cultivars was significant (Table 5). The highest value of TSS (8.2 °Brix) was recorded in

Hybrid HBGH-35 at 31.7°C, while the values declined to 7.9 at 40°C in CP-4. The minimum value of TSS (4.1 to 4.7 °Brix) was recorded in the male line at 31.7°C and 40°C, respectively. The parental lines had TSS ranging from 6.2 to 7.6 °Brix (♀) and 6.0 to 6.5 °Brix (♂), respectively, during the 4th week of the crossing period. All the cultivars had TSS values with a bit of variation due to temperature fluctuations.

As illustrated in Table 5, crude protein varied significantly ($p < 0.05$) among different growing seasons and crossing periods. The order of concentration is Hybrid HBGH-35 > Pusa Naveen > G2-line. The seeds harvested in the 4th week of the crossing period with a temperature of 31.7°C had higher protein content (8.2-19.1%) while with the rise in temperature (40°C), the protein content decreased (6.2-17.6%), significantly due to the inactivation of enzymes involved in protein synthesis [38]. The protein forms the principal component of embryonic development; thus, this could be the reason for the higher protein concentration in Hybrid HBGH-35 (19.1%) at CP-4 and represents high-quality seeds [39]. Similar results are supported by [40] in soybean and in groundnut [41]. Lipids represent the energy content required during seed germination. A similar trend was observed for lipid content (Table 5). All the cultivars showed significant variations ($p < 0.05$) among them. However, the values are lower than the 41-46.8% reported in the same Lagenaria species [42].

Table 5. Biochemical analysis in bottle gourd seeds harvests at two different temperatures.

Seasons	TSS (°Brix)		Crude protein (%)		Lipid content (%)	
	***kharif* (31.7°C)**	**Summer (40°C)**	***kharif* (31.7°C)**	**Summer (40°C)**	***kharif* (31.7°C)**	**Summer (40°C)**
Hybrid HBGH-35						
CP-1	4.7±0.11^{a}	4.1±0.11^{a}	12.7±0.23^{e}	11.3±0.23^{e}	21.3±0.32^{e}	18.6±0.32^{e}
CP-2	5.6±0.07^{b}	4.9±0.10^{b}	13.3±0.32^{d}	12.5±0.14^{d}	24.7±0.26^{d}	20.4±0.21^{d}
CP-3	7.4±0.10^{d}	6.4±0.16^{d}	15.3±0.26^{b}	14.2±0.19^{b}	31.7.1±0.21^{b}	30.5±0.17^{b}
CP-4	8.2±0.16^{e}	7.9±0.13^{e}	19.1±0.21^{a}	17.6±0.21^{a}	37.4±0.27^{a}	35.2±0.16^{a}
CP-5	6.4±0.12^{c}	5.6±0.11^{c}	14.2±0.36^{c}	13.6±0.16^{c}	27.6±0.26^{c}	25.6±0.11^{c}
Pusa Naveen (♀)						
CP-1	3.4±0.12^{a}	3.0±0.13^{a}	9.1±0.26^{e}	8.6±0.09^{e}	15.6±0.26^{e}	12.3±0.23^{e}
CP-2	4.8±0.14^{b}	4.1±0.16^{b}	10.6±0.21^{d}	9.1±0.16^{d}	19.6±0.21^{d}	14.6±0.16^{d}
CP-3	6.7±0.13^{d}	5.7±0.15^{d}	14.3±0.19^{b}	11.3±0.15^{b}	25.1±0.16^{b}	22.1±0.17^{b}
CP-4	7.6±0.16^{e}	6.2±0.14^{e}	16.6±0.16^{a}	14.3±0.21^{a}	32.3±0.36^{a}	29.6±0.35^{a}
CP-5	5.7±0.12^{c}	5.1±0.13^{c}	12.3±0.21^{c}	10.6±0.36^{c}	22.6±0.23^{c}	19.6±0.26^{c}
G2-line (♂)						
CP-1	3.4±.16^{a}	2.9±0.10^{a}	8.2±0.11^{e}	6.2±0.06^{e}	11.6±0.18^{e}	9.1±0.11^{e}
CP-2	4.1±0.22^{b}	3.6±0.09^{b}	8.6±0.13^{d}	7.6±0.16^{d}	14.6±0.21^{d}	11.0±0.26^{d}
CP-3	5.4±0.19^{d}	4.9±0.16^{d}	10.3±0.23^{b}	9.6±0.19^{b}	23.6±0.26^{b}	20.6±0.24^{b}
CP-4	6.5±0.23^{e}	6.0±0.14^{e}	13.3±0.20^{a}	11.2±0.21^{a}	27.6±0.11^{a}	24.3±0.32^{a}
CP-5	4.7±0.11^{c}	4.1±0.17^{c}	9.6±0.16^{c}	8.6±0.19^{c}	20.3±0.19^{c}	17.6±0.17^{c}

a-e Values with different superscripts in the same row are significantly different ($p < 0.05$) *CP-1: 4-10 Sept 2017 and 6-22 April 2018; CP-2: 11-17 Sept 2017 and 23-29 April 2018; CP-3: 18-24 Sept 2017 and 30 April -6 May 2018; CP-4: 25 Sept-1 Oct 2017 and 7-13 May 2018; CP-5: 2-8 Oct 2017 and 14-20 May 2018; CP: crossing period.

3.3. Total carbohydrates and Ascorbic acid

Carbohydrates are a rich source of energy [43,44]. The carbohydrate content in Hybrid HBGH-35 retained its maximum value (22.1%), followed by Pusa Naveen (19.2%), while the G2-line seed fiber (15.5%) had the least (Table 6). Temperature significantly affects sugar accumulation thus, overall energy changes. At 31.7 °C, the seeds accumulated a high carbohydrate content (8.9-22.1 %) during the 4th week of the crossing period, while at 40°C, the concentration decreased (8.1-20.3%) significantly. The observed difference among the crossing periods indicates that the distribution of carbohydrates in seed tends

to concentrate more at lower temperatures (31.7°C). The observed trend was the same for the energy values expressed as kcal/100 g (Table 6) of the seeds harvested from different growing seasons and could be attributed to the high amount of crude protein and lipid content in the hybrid. The results are well in conformity with those observed in bottle gourd [45]; watermelon [46], oil bean seed [47], pumpkin seed [48], and African walnut [49], which confirmed that seeds possessing higher carbohydrates have a high germination rate and seed emergence.

Table 6. Total carbohydrates and antioxidants in bottle gourd seeds under different growing seasons.

Seasons	TCC (%)		Vitamin C (mg/100g)		Energy values (kcal/100g)	
	***kharif* (31.7°C)**	**Summer (40°C)**	***kharif* (31.7°C)**	**Summer (40°C)**	***kharif* (31.7°C)**	**Summer (40°C)**
Hybrid HBGH-35						
CP-1	10.3±0.23^{a}	9.4±0.12^{a}	13.1±0.23^{e}	12.4±0.16^{e}	196.2±23.6^{a}	154.3±26.6^{a}
CP-2	12.6±0.15^{b}	10.3±0.16^{b}	14.2±0.32^{d}	13.7±0.23^{d}	247.3±23.6^{b}	202.5±41.2^{b}
CP-3	19.5±0.16^{d}	17.9±0.26^{d}	15.8±0.24^{b}	15.2±0.26^{b}	385.6±35.6^{d}	320.1±52.6^{d}
CP-4	22.1±0.25^{e}	20.3±0.41^{e}	16.5±0.16^{a}	16.0±0.16^{a}	456.3±37.6^{e}	412.3±38.6^{e}
CP-5	15.6±0.14^{c}	13.6±0.16^{c}	14.9±0.21^{c}	14.2±0.32^{c}	310.3±41.3^{c}	287.3±23.6^{c}
Pusa Naveen (♀)						
CP-1	9.5±0.12^{a}	8.4±0.10^{a}	8.7±0.23^{e}	7.8±0.12^{e}	142.3±32.6^{a}	121.3±26.6^{a}
CP-2	10.6±0.23^{b}	9.2±0.09^{b}	9.5±0.16^{d}	8.0±0.16^{d}	185.6±42.6^{b}	154.2±22.6^{b}
CP-3	17.6±0.19^{d}	13.8±0.16^{d}	11.6±0.18^{b}	9.2±0.21^{b}	274.6±23.3^{d}	254.1±28.6^{d}
CP-4	19.2±0.21^{e}	16.5±0.35^{e}	14.6±0.19^{a}	12.1±0.19^{a}	385.6±56.4^{e}	365.6±38.4^{e}
CP-5	13.2±0.16^{c}	11.7±0.16^{c}	10.3±0.21^{c}	8.6±0.16^{c}	212.6±23.6^{c}	174.8±21.3^{c}
G2-line (♂)						
CP-1	8.9±0.23^{a}	8.1±0.12^{a}	7.4±0.13^{e}	6.8±0.10^{e}	132.6±23.6^{a}	102.3±36.2^{a}
CP-2	9.6±0.16^{b}	8.7±0.16^{b}	8.6±0.16^{d}	7.5±0.26^{d}	142.3±32.6^{b}	112.5±24.6^{b}
CP-3	13.2±0.19^{d}	10.6±0.25^{d}	10.6±0.23^{b}	8.7±0.21^{b}	232.3±21.4^{d}	206.3±35.2^{d}
CP-4	15.5±0.11^{e}	12.3±0.16^{e}	12.6±0.26^{a}	10.6±0.16^{a}	287.6±23.6^{e}	255.2±29.6^{e}
CP-5	11.6±0.17^{c}	9.4±0.18^{c}	9.4±0.27^{c}	7.9±0.06^{c}	184.2±21.2^{c}	149.6±20.4^{c}

a-e Values with different superscripts in the same row are significantly different ($p < 0.05$). *CP-1: 4-10 Sept 2017 and 6-22 April 2018; CP-2: 11-17 Sept 2017 and 23-29 April 2018; CP-3: 18-24 Sept 2017 and 30 April -6 May 2018; CP-4: 25 Sept-1 Oct 2017 and 7-13 May 2018; CP-5: 2-8 Oct 2017 and 14-20 May 2018; CP: crossing period.

The data presented in Table 6 for vitamin C content in bottle gourd cultivars exhibited significant differences under different environmental conditions. Hybrid HBGH-35 had the highest vitamin C content (16.5 mg/100 g) followed by Pusa Naveen (14.6 mg/100 g) while the G2-line had a minimum (12.6 mg/100 g) during the 4th week of the crossing period at 31.7°C. The fruits harvested during the summer season (40°C) accumulated lower concentrations (6.8-16.0%) of vitamin C in all cultivars. The hybrid HBGH-35 represented adaptability to the agro-climatic conditions of the study site. The results are well confirmed by several researchers [50–54], who reported that temperature plays a crucial role in the accumulation of vitamin C. A higher vitamin C accumulation indicates a higher antioxidant potential of the seed [55].

3.4. Analysis of Energy dispersive x-ray

An elemental composition comparable with the different minerals observed in the scanning electron microscope (SEM) was revealed by the energy dispersive x-ray (EDX) analysis of bottle gourd parental and hybrid lines. The key components that were found were largely consistent. Results for EDX are semi-qualitative estimations based on conventional analysis and theoretical intensity adjustments.[56]. It is clear from Table 7 that P, K, and Mg are abundant in each of the regions under investigation, with the embryonic region having the highest concentration. It has been reported that phytin globoids are

prevalent in the periplasm of embryonic cells in legume seeds, and also that P, Mg, and K accumulate in the globoids.. Calcium was scarcely found in the embryonic tissues of bottle gourd seeds. Similar observations were previously reported [57,58]. In this investigation, we showed that the seed coat had a higher amount of calcium than the embryonic and cotyledon sections. The results given here suggest that Ca(I) forms Ca-pectin complexes with the carboxyl of pectin molecules inside the cell wall. Zn, Cu, and Mn are abundant in the embryonic area, and they are also found in large concentrations in the cotyledon tissue of bottle gourds and in their seed coatings. In the seed embryonic region, the percentages of Zn, Cu, and Mn associated with the percentage of P, suggested a potential relationship with phytate. Iwai et al. [59] reported that there are some specific distribution patterns of minerals in seeds dependent upon cell type. However, this report was based on single spot analysis, which involves analyzing areas of the size of the electron beam.

3.5. Macro-elements

The P, K, and Mg mineral compositions of seeds from different parental lines of bottle gourds did not differ significantly ($p > 0.05$) (Table 7). The ratio of K, P, and Mg in the seed coat area of the bottle gourd lines was relatively low than the embryonic region composition.The hybrid HBGH35 and Pusa Naveen lines had higher mean P, K, and Mg compositions than the G2 line. The function of phytate synthesis in seeds is to deposit phosphates and cations for the sufficient period during germination [54]. According to the results of the present study, seeds may be a significant source of nutrients for catering the requirements of seedling growth.[60] High seed P content improves plant establishment and increases dry matter accumulation. This is a corollary of faster initial root growth, which gives seedlings earlier access to growth-limiting resources (water and mineral elements). According to Grant et al. [61], seed P reserves sustain a maximal growth of seedlings for several weeks after germination, until the plant has three or more leaves and a substantial root system.

Indole acetic acid (IAA)-induced cell wall weakening and solute aggregation inside the cell to establish internal osmotic potential are essential for cell expansion.[62]. K+ buildup lowers the water potential and maintains the pH of growing cells. Since a stoichiometric K^+ inflow electrochemically counterbalances the IAA-stimulated H+ outflow, IAA-induced elongation quickly decreases and eventually stops when K+ is absent. A direct correlation between seed K content and shoot and root elongation may thus appear.

Table 7. Mmacro-nutrients analysis of bottle gourd parental lines by EDX analysis.

	Phosphorus (%)			**Potassium (%)**			**Magnesium (%)**		
Crossing periods	**Hybrid**	**Pusa Naveen**	**G2**	**Hybrid**	**Pusa Naveen**	G2	Hybrid	Pusa Naveen	G2
CP1	53.10	13.24	2.30	28.78	7.46	0.84	16.12	4.03	1.07
CP2	52.73	14.30	2.29	29.38	6.76	0.85	15.73	3.93	1.05
CP3	53.03	13.60	2.297	29.22	6.51	0.87	15.70	3.93	1.05
CP4	53.13	13.50	2.3	29.11	6.92	1.12	16.35	4.09	1.09
Mean	52.1	14.01	3.29	30.12	7.11	0.92	16.01	4.2	1.06
P (0.05)	0.92	0.89	0.95	0.93	0.68	0.20	0.42	0.41	0.39
l.s.d	1.48	2.65	0.06	2.14	1.81	0.32	1.00	0.25	0.06
cv %	1.50	13.30	1.50	2.90	14.00	17.60	2.30	2.30	2.30

3.6. Micro-nutrients Analysis

Between the parental lines, calcium levels varied significantly (p 0.05). Hybrid HBGH-35 has a seed coat region with a calcium concentration of 2.14% and a G2 embryonic area with a calcium level of 0.45 % (Table 8). HBGH-35 had the highest level of zinc in the embryo area (1.43 %), whereas G2 had the lowest proportion in the seed coat region (0.08 %). In terms of copper, the embryonic area of HBGH-35 recorded the greatest

percentage (0.39 %), whereas the seed coat region of G2 recorded the lowest percentage (0.03 %). Significant variations were seen in the manganese percentage, with HBGH-35 having the largest amount in the embryo area (0.31 %) and G2 having the lowest amount in the seed coat region (0.02 %).

Table 8. The micro-nutrients analysis of bottle gourd parental lines by EDX analysis.

Region	Parental lines	Ca (%)	Zn (%)	Cu (%)	Mn (%)
Embryo	CP1	1.01^{a}	1.52^{a}	0.373^{a}	0.29^{a}
	CP2	0.45^{d}	0.24^{d}	0.10^{d}	0.06^{d}
	CP3	0.74^{c}	0.45^{c}	0.30^{c}	0.10^{c}
	CP4	0.82^{b}	0.80^{b}	0.34^{b}	0.16^{b}
	l.s.d (P¼0.05)	0.16	0.16	0.01	0.041
Cotyledon	CP1	1.060^{a}	0.810^{a}	0.19^{b}	0.077^{a}
	CP2	0.507^{c}	0.131^{d}	0.08^{d}	0.075^{c}
	CP3	0.793^{b}	0.245^{c}	0.124^{c}	0.032^{ab}
	CP4	0.873^{b}	0.426^{b}	0.218^{a}	0.054^{a}
	l.s.d (P¼0.05)	0.085	0.091	0.014	0.027
Seed Coat	CP1	2.02^{a}	0.603^{a}	0.171^{a}	0.055^{a}
	CP2	0.74^{d}	0.094^{d}	0.99^{c}	0.01^{c}
	CP3	0.95^{c}	0.183^{c}	0.016^{d}	0.041^{b}
	CP4	1.301^{b}	0.320^{b}	1.59^{b}	0.038^{b}
	l.s.d (P¼0.05)	0.085	0.065	0.019	0.027

Means were separated using Duncan's test. Means with the same letters within a column are not significantly different and means with different letters within a column are significantly different.

3.7. . Seed macro- and Microelement Composition

The results of the mineral contents present in bottle gourd seeds are shown in Table 9. Sodium (Na) is an element associated with potassium concentration, which is responsible for maintaining body fluid homeostasis [63]. Potassium (K) is the most abundant element in bottle gourd (Table 9). All the cultivars showed significant variation ($p < 0.05$) in their potassium content, with the highest amount found in hybrid HBGH-35 (3015.2 mg/100 g) at the 4th week of the crossing period, while the minimum was found in the male line (2054.2 mg/100 g). The high amount of potassium is noted as a characteristic feature of most plant foods [44,64–66]. At 31.7°C, the concentration of sodium also varied significantly ($p < 0.05$) within the cultivars, with the 4th week of crossing period seeds having the higher amount (1042.6-1412.2 mg/100 g). At 40°C, these elements were reduced significantly (1365.3-1011.3 mg/100 g) (Table 9). A high potassium content in relation to sodium content is thought to be beneficial to hypertensive individuals [67]. Moreover, the recommended dietary allowance (RDA) for Na and K is 200 and 500 mg, respectively [68], indicating that bottle gourd seeds may be regarded as a rich source of these minerals for the body.

Phosphorous (P) and calcium (Ca) are associated with each other and are required for the growth and maintenance of teeth, bones, and muscles [69]. The level of Ca in bottle gourd cultivars is generally low and shows no significant variations ($p > 0.05$) among the different growing seasons (Table 9). The lowest concentration of Ca was found in the male line (1.3 mg/100 g) followed by the female line (1.6 mg/100 g) and the hybrid (1.89 mg/100 g). While P content was high in hybrids with a value of 1250.3 mg/100g at the 4th week of the crossing period at 31.7°C, there were no significant differences ($p > 0.05$). In general, the P content of bottle gourd seeds was reported to be higher than conventional seeds and nuts (11-350 mg/100 g) [40,70,71]. The RDA of Ca and P for an adult is 810 mg/day [50]. This confirms that bottle-gourd seeds are good sources of phosphorous [72–74].

The magnesium (Mg) displayed significant variations ($p < 0.05$) between the analyzed samples, with hybrid (570.6 mg/100 g) having the highest value, followed by Pusa Naveen (485.3 mg/100 g) seeds, and then G2-line (449.6 mg/100 g) at 31.7°C. The amount of Mg in

bottle gourd seeds is higher than that in groundnut, shea nut, cotton seed, and calabash seed [70,75]. Hence, bottle gourd seeds may thus be a rich source of Mg, assuming a total absorption by the body and ignoring the effect of anti-nutritional factors [76]. Iron (Fe) is the most abundant microelement in crop seeds. A similar trend was observed for Fe content, with no significant differences ($p > 0.05$). The results are well supported by the values of [64–66,77].

Table 9. Mineral composition of hybrid bottle gourd under different growing seasons.

Season	*Kharif* season (2017-18)					Summer season (2018-19)				
Crossing periods	**CP-1**	**CP-2**	**CP-3**	**CP-4**	**CP-5**	**CP-1**	**CP-2**	**CP-3**	**CP-4**	**CP-5**
Hybrid HBGH-35										
Sodium	1042.6±1.2^{a}	1154.6±1.6^{b}	131.74.3±1.6^{d}	1412.2±1.5^{e}	1265.3±1.3^{c}	1011.3±1.3^{a}	1069.3±2.1^{b}	1302.1±2.0^{d}	1365.3±1.8^{e}	1223.5±1.9^{c}
Potassium	2054.2±4.8^{a}	2145.3±5.3^{b}	2654.2±2.3^{d}	3015.2±6.6^{e}	2436.0±3.2^{c}	2001.3±2.1^{a}	2086.3±2.6^{b}	2436.2±3.2^{d}	2865.2±3.6^{e}	231.75.1±4.1^{c}
Calcium	1.89±0.23^{e}	2.69±0.16^{d}	3.65±0.18^{b}	3.80±0.16^{a}	3.23±0.15^{c}	1.48±0.21^{e}	2.17±0.23^{d}	3.44±0.32	3.65±0.23	3.02±0.21
Phosphorus	976.6±4.2^{a}	1065.3±2.3^{b}	1224.3±6.3^{d}	1250.3±2.3^{e}	1165.3±4.2^{c}	923.4±3.1^{a}	1001.3±4.2^{b}	1214.2±2.6^{d}	1235.6±3.3^{e}	1141.0±2.4^{c}
Magnesium	300.6±2.2^{a}	385.6±2.3^{b}	541.3±4.5^{d}	570.6±6.3^{e}	475.2±5.6^{c}	275.3±4.3^{a}	325.6±3.6^{b}	514.3±3.2^{d}	540.2±3.9^{e}	451.3±2.2^{c}
Iron	31.2±1.2^{e}	35.3±1.6^{d}	54.2±1.5^{b}	65.3±2.6^{a}	48.2±2.2^{c}	21.6±1.3^{e}	28.3±1.9^{d}	47.6±3.2^{b}	55.3±4.3^{a}	40.2±2.9^{c}
Copper	9.6±0.23^{e}	11.4±0.26^{d}	13.2±0.45^{b}	15.2±0.56^{a}	12.4±0.36^{c}	8.3±0.12^{e}	10.6±0.12^{d}	12.6±0.16^{b}	14.2±0.19^{a}	11.0±0.13^{c}
Zinc	0.10±0.03^{a}	0.11±0.09^{b}	0.16±0.02^{d}	0.20±0.11^{e}	0.13±0.1^{c}	0.07±0.06^{a}	0.09±0.02^{b}	0.14±0.03^{d}	0.18±0.06^{e}	0.11±0.04^{c}
Manganese	16.5±0.32^{e}	21.6±0.16^{d}	23.3±0.12^{b}	26.4±0.16^{a}	20.3±0.18^{c}	13.5±0.15^{e}	18.6±0.12^{d}	21.3±0.16^{b}	24.3±0.12^{a}	18.6±0.16^{c}
Pusa Naveen (♀)										
Sodium	982.6±1.6^{a}	1089.6±1.3^{b}	1289.6±1.2^{d}	1324.6±1.1^{e}	1212.4±1.4^{c}	946.2±1.5^{a}	1021.3±1.6^{b}	1264.2±2.3^{d}	1289.3±2.1^{e}	1186.6±1.7^{c}
Potassium	1965.3±4.5^{a}	2084.3±4.6^{b}	2464.2±3.2^{d}	2865.3±2.6^{e}	2246.2±2.9^{c}	1856.3±3.6^{a}	1956.3±4.5^{b}	2365.3±6.5^{d}	2689.6±3.2^{e}	2236.5±2.9^{c}
Calcium	1.6±0.36^{e}	2.3±0.36^{d}	3.2±0.45^{b}	3.5±0.55^{a}	2.7±0.16^{c}	1.3±0.23^{e}	1.6±0.12^{d}	2.8±0.13^{b}	3.2±0.23^{a}	2.2±0.12^{c}
Phosphorus	932.3±4.2^{a}	985.6±3.2^{b}	1056.3±2.1^{d}	1145.3±4.3^{e}	1041.3±4.6^{c}	865.3±3.6^{a}	984.3±3.2^{b}	1149.6±2.3^{d}	1132.6±2.6^{e}	1065.8±3.6^{c}
Magnesium	265.3±4.2^{a}	289.6±3.6^{b}	463.2±3.1^{d}	485.3±4.2^{e}	421.6±3.6^{c}	232.4±5.2^{a}	284.6±3.6^{b}	451.2±2.3^{d}	487.6±4.3^{e}	412.0±3.6^{c}
Iron	21.6±1.6^{e}	27.6±2.3^{d}	47.2±2.6^{b}	51.2±2.1^{a}	36.4±1.6^{c}	18.9±1.9^{e}	21.6±1.7^{d}	40.6±2.2^{b}	38.6±2.6^{a}	28.6±2.1^{c}
Copper	8.6±0.10^{e}	9.5±0.09^{d}	10.6±10.16^{b}	12.5±0.18^{a}	10.1±0.16^{c}	7.6±0.15^{e}	8.2±0.14^{d}	9.1±0.21^{b}	10.6±0.12^{a}	9.3±0.08^{c}
Zinc	0.07±0.03^{a}	0.09±0.02^{b}	0.14±0.02^{d}	0.18±0.04^{e}	0.11±0.09^{c}	0.04±0.06^{a}	0.06±0.09^{b}	0.11±0.07^{d}	0.15±0.06^{e}	0.09±0.04^{c}
Manganese	13.6±0.10^{e}	17.4±0.11^{d}	20.6±0.16^{b}	23.1±0.17^{a}	18.6±0.14^{c}	10.2±0.03^{e}	13.2±0.06^{d}	17.4±0.10^{b}	20.7±0.13^{a}	15.6±0.10^{c}
G2-line (♂)										
Sodium	921.3±1.2^{a}	1021.3±1.3^{b}	1242.2±1.5^{d}	1265.3±1.2^{e}	1176.3±2.3^{c}	895.3±1.3^{a}	983.2±1.1^{b}	1232.6±2.6^{d}	1254.2±2.1^{e}	1124.3±2.0^{c}
Potassium	1945.2±5.2^{a}	2021.3±4.2^{b}	2364.3±3.1^{d}	2445.3±3.6^{e}	2145.3±2.9^{c}	1745.2±2.1^{a}	1846.5±2.1^{b}	2246.2±3.5^{d}	2564.3±4.1^{e}	2146.3±2.6^{c}
Calcium	1.3±.23^{e}	1.8±0.21^{d}	2.6±0.12^{b}	3.2±0.16^{a}	2.2±0.18^{c}	1.0±0.16^{e}	1.3±0.19^{d}	2.2±0.22^{b}	3.0±0.23^{a}	1.9±0.19^{c}
Phosphorus	881.3±4.6^{a}	945.2±3.2^{b}	1022.3±2.3^{d}	1121.3±4.3^{e}	1005.3±5.6^{c}	832.6±4.3^{a}	947.2±6.3^{b}	1084.3±5.4^{d}	1064.3±3.2^{e}	1011.3±4.2^{c}
Magnesium	232.6±4.2^{a}	231.7.3±3.2^{b}	411.6±5.1^{d}	449.6±2.6^{e}	359.6±3.6^{c}	384.3±4.6^{a}	220.6±3.8^{b}	402.6±4.9^{d}	412.5±4.6^{e}	375.6±3.8^{c}
Iron	19.6±2.1^{e}	21.3±1.6^{d}	37.4±1.4^{b}	44.2±1.2^{a}	31.6±1.3^{c}	15.1±1.8^{e}	18.7±2.1^{d}	33.4±2.4^{b}	31.6±1.9^{a}	23.4±1.7^{c}

Copper	7.2±0.09 [e]	8.3±0.10 [d]	9.1±0.12 [b]	10.4±0.16 [a]	9.4±0.11 [c]	6.2±0.06 [e]	7.4±0.10 [d]	8.3±0.09 [b]	8.9±0.13 [a]	8.5±0.15 [c]
Zinc	0.05±0.01 [a]	0.06±0.02 [b]	0.09±0.06 [d]	0.15±0.04 [e]	0.08±0.04 [c]	0.03±0.01 [a]	0.04±0.02 [b]	0.08±.0.03 [d]	0.11±0.09 [e]	0.07±0.04 [c]
Manganese	10.6±0.13 [e]	15.9±0.10 [d]	18.6±0.11 [b]	20.3±0.13 [a]	14.6±0.06 [c]	8.9±0.02 [e]	10.2±0.04 [d]	13.2±0.04 [b]	17.6±0.12 [a]	11.6±0.10 [c]

a-e Values with different superscripts in the same row are significantly different ($p < 0.05$); *CP-1: 4-10 Sept 2017 and 6-22 April 2018; CP-2: 11-17 Sept 2017 and 23-29 April 2018; CP-3: 18-24 Sept 2017 and 30 April -6 May 2018; CP-4: 25 Sept-1 Oct 2017 and 7-13 May 2018; CP-5: 2-8 Oct 2017 and 14-20 May 2018; CP: crossing period.

Copper (Cu) is an essential trace element and an integral part of ceruloplasmin (a Cu protein) and cytochrome (an electron carrier). It plays a vital role in oxygen and energy metabolism [39,78]. Hybrid (570.6 mg/100 g) had the highest value (15.2 mg/100 g) followed by Pusa Naveen (12.5 mg/100 g) seeds then G2-line (10.4 mg/100 g) at 31.7°C (Table 9). Iron (Fe) plays an important role in the haemoglobin synthesis and oxygen-carrying capacity of the body. Among the samples analyzed, hybrid HBGH-35 (65.3 mg/100 g) accumulated a higher amount of Fe, followed by Pusa Naveen (51.2 mg/100 g) at 31.7°C (Table 9). There is a significant difference in Fe content in the two growing seasons, i.e., 19.6-65.3 mg/100 g to 15.1-55.3 mg/100 g at 31.7°C and 40°C, respectively. High temperatures disrupt Fe homeostasis and inactivate enzymes. The values obtained in the present study seem high compared with the pride of Barbados [67] and calabash seeds [75].

The temperature was directly influenced by zinc (Zn) and manganese (Mn) accumulation in bottle-gourd cultivars. Manganese plays a structural role in the chloroplast membrane and is a cofactor for DNA, RNA, and fatty acid synthesis [79], while Zn is required for growth, tissue repair, and normal functioning of the immune system [80]. At 31.7°C, hybrids retained their maximum Zn (0.20 mg/100g) and Mn (26.4 mg/100g) contents during the 4th week of the crossing period, while at 40°C, the levels decreased by 0.18 mg/100g and 24.3 mg/100g, respectively, in all cultivars. The differences in the accumulation of minerals between cultivars might be due to their genetic makeup and also to their comparative responses to variations in agro-climatic conditions.

3.8. . Correlation analysis between weather variables and biochemical components

Correlation studies provide a better understanding of the association of different traits that may be useful to breeders in selecting cultivars possessing groups of desired agronomical traits [81,82]. The correlation analysis of biochemical constituents with weather variables showed significant variation among the five different crossing periods (Table 10). Protein content correlated significantly with minimum temperature and morning and evening relative humidity. Crude fiber (r = 0.956**), TSS (r = 0.990**), and total carbohydrate content (r = 0.813*) had a highly positive correlation with minimum temperature. Vitamin C accumulation (r = 0.967**) had a positive and significant correlation with minimum temperature. With the temperature rise, the moisture content also increased, as shown by a positive correlation (r = 0.827*) with maximum temperature, thus justifying the present study results. Similar results were reported in previous studies [83–86]. The introduction of high-yield cultivars depends upon the agro-climatic conditions and phenological traits and choosing a small number of important traits with a positive correlation [44,87]. Henceforth, the results showed that all the biochemical components were significantly positively correlated to minimum temperature, and morning and evening relative humidity, suggesting their relationship with temperature and solar radiation.

Table 10. Pearson's correlation coefficient analysis between weather variables and biochemical constituents.

	T_{max}	T_{min}	RH_m	RH_e	Moisture	Lipid	CP	CF	TSS	TCC	Vit. C
T_{max}	1	.793	.521	.696	.827*	.784*	.424	.457	.491	.470	.501
T_{min}		1	.208	.810*	.410	-.405	.816*	.956**	.990**	.813*	.967**
RH_m			1	.736	.961**	.950**	.960**	.959**	.924**	.932**	.907**
RH_e				1	.842*	.844*	.861*	.879*	.883*	.879*	.875*
Moisture					1	.923**	.993**	.968**	.925**	.921**	.913**
Lipid						1	.923**	.988**	.994**	.997**	.986**
CP							1	.964**	.919**	.923**	.900**
CF								1	.990**	.989**	.983**
TSS									1	.998**	.997**
TCC										1	.991**
Vit. C											1

*Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed); # Tmax: maximum temperature; Tmin: minimum temperature; RHm: morning relative humidity; RHe: evening relative humidity; CP: crude protein; CF: crude fibre; TSS: total soluble solids; TCC: total carbohydrate content; Vit. C: vitamin C.

3.9. Seed vigour

3.9.1. Germination indices

The germination was observed to be higher (95.84%) in the seeds harvested from the Kharif crop as compared to the summer crop (94.11%) (Table 11). Among the three genotypes studied, HBGH-35 had the highest standard germination rate (95.60%), followed by Pusa Naveen (94.93%), and G2 had the lowest (94.40%). Among the crossing periods, the lowest germination percentage (94.22%) was recorded in the seeds produced from crossing period CP1. A significant increase in germination percentage was observed from CP1 up to the CP4 crossing period, after which a significant decline was recorded in CP5. The maximum germination was recorded in the seeds of the CP4 treatment (95.94%), followed by the CP3 (95.17%).

Table 11. The germination percent of bottle gourd lines under different crossing periods.

	Season (S)							
Genotype ➡ Crossing Period ↓	**Kharif 2017**				**Summer 2018**			
	HBGH-35	**Pusa Naveen (♀)**	**G-2 (♂-)**	**Mean**	**HBGH-35**	**Pusa Naveen (♀)**	**G-2 (♂-)**	**Mean**
CP_1	96.00 (78.49)	95.00 (77.05)	94.67 (76.63)	95.22 (77.39)	94.00 (75.79)	93.00 (74.63)	92.67 (74.27)	**93.22 (74.90)**
CP_2	96.33 (78.95)	95.33 (77.51)	95.00 (77.09)	95.56 (77.85)	94.33 (76.21)	94.00 (75.79)	93.00 (74.63)	**93.78 (75.54)**
CP_3	97.00 (80.09)	96.00 (78.43)	95.33 (77.51)	96.11 (78.68)	94.67 (76.63)	94.33 (76.21)	93.67 (75.40)	**94.22 (76.08)**
CP_4	97.67 (81.22)	96.33 (78.95)	96.00 (78.43)	96.67 (79.54)	95.33 (77.51)	95.67 (77.97)	94.67 (76.63)	**95.22 (77.37)**
CP_5	96.00 (78.43)	95.67 (77.97)	95.33 (77.51)	95.67 (77.97)	94.67 (76.63)	94.00 (75.79)	93.67 (75.40)	**94.11 (75.94)**
Mean	**96.60 (79.44)**	**95.68 (77.98)**	**95.27 (77.43)**	**95.54 (78.28)**	**94.60 (76.55)**	**94.20 (76.08)**	**93.53 (75.27)**	

3.9.2. Growth characteristics

The HBGH-35 and G2 parental lines' hybrid root length, shoot length, root dry mass, and shoot dry mass were all substantially different ($p < 0.01$) from one another (Figure 3). At CP4, HBGH-35 and G2 had the greatest and lowest RL values, respectively (53.4 mm) and (27 mm) (Figure 3a). At CP1 and CP3, HBGH-35 obtained the highest SL readings of

32 mm and 39.05 mm, respectively. At both sampling intervals, G2 had the lowest SL (Figure 3b). At CP4, HBGH-35 and G2 had the greatest (0.52 mg plant-1) and lowest (0.24 mg plant^{-1}) root biomass measurements, respectively. RDM values were considerably elevated at CP4, with HBGH-35 recording the lowest biomass at 0.78 mg plant-1 and G2 recording the greatest biomass at 2.15 mg plant^{-1} (Figure 3c). After CP1 and CP4, respectively, the shoot dry mass varied from 0.34 to 0.7 mg plant-1 and from 2.71 to 5.20 mg plant^{-1}, considerably varying between treatments. The SDM for HBGH-35 was the greatest, while the SDM for G2 was the lowest (Figure 3d).

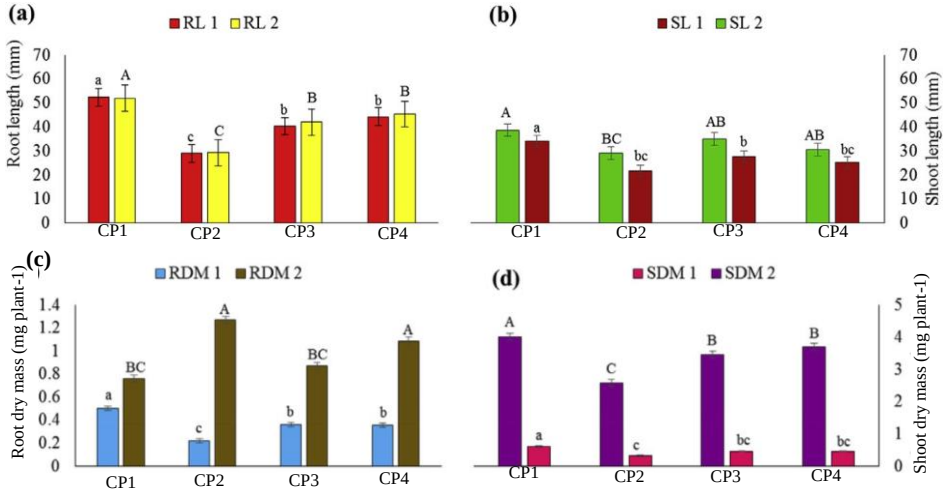

Figure 3. Growth response bottle gourd parental lines at CP1 and CP4. (a) Root length (RL); (b) Shoot length (SL); (c) Root dry mass (RDM) and (d) Shoot dry mass (SDM). RL 1 (root length of HBGH35), RL 2 (root length of G2 line).

5. Conclusions

Despite having a small influence on the overall mineral makeup of a mature plant, the minerals found in seeds have a considerable impact on the nutrient availability in early seedlings. Relatively high mineral reserves in the seed enable quicker seedling establishment, which in turn results in plants with greater vigour capability and radical emergence efficiency. As a possible source of seedling vigour in soils deficient in nutrients, our research findings indicate that dark-colored hybrid HBGH-35 seeds at the fourth week of the crossing period (CP4) merit additional investigation.Seed composition and mineral contents of bottle gourds differed under crossing periods, which could be due to variations in the adaptation mechanisms of plants to high temperatures. Protein and fibre contents increased at CP4, while the fat content decreased. The variation in biochemical constituents suggested that their accumulation is strongly influenced by temperature and seedling crossing period. Thus, the 4th week of the crossing period during the Kharif season, with a temperature of 31.7 °C, was favorable for high-quality seed production in bottle gourd. Unfortunately, published mineral element values for bottle gourd lines are not available. Therefore, this work can be considered a pilot study in order to generate data for elemental composition in bottle gourd seedlings for future comparisons. Noteworthy, rising global average temperatures, within the context of climate change, may impact crop yield, changing the nutritional properties of seeds and grains. This response can increase the nutrient requirements in a future scenario of global food scarcity, which needs to be considered in upcoming agronomic studies.

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References

1. Punia, H.; Madan, S.; Malik, A.; Sethi, S.K. STABILITY ANALYSIS FOR QUALITY ATTRIBUTES IN DURUM WHEAT (TRITICUM DURUM L.) GENOTYPES. *Bangladesh J Bot* **2019**, *48*, 967–972.
2. Punia, H.; Tokas, J.; Malik, A.; Sangwan, S. Characterization of Phenolic Compounds and Antioxidant Activity in Sorghum [Sorghum Bicolor (L.) Moench] Grains. *Cereal Res Commun* 1–11.
3. Ogunbusola, E.M. Nutritional and Antinutritional Composition of Calabash and Bottle Gourd Seed Flours (Var Lagenaria Siceraria). *Journal of Culinary Science & Technology* **2018**, *16*, 326–335.
4. Mkhize, P.; Mashilo, J.; Shimelis, H. Progress on Genetic Improvement and Analysis of Bottle Gourd [Lagenaria Siceraria (Molina) Standl.] for Agronomic Traits, Nutrient Compositions, and Stress Tolerance: A Review. *Front. Sustain. Food Syst. 5: 683635. doi: 10.3389/fsufs* **2021**.
5. Kaul, M.L.H. Reproductive Biology in Tomato. In *Genetic improvement of tomato*; Springer, 1991; pp. 39–50.
6. Singh, S.P.; Gill, S.S. Fruiting Behaviour Studies for Hybrid Seed Production and Estimation of Vertical and Horizontal Fruiting Intervals in Cotton (Gossypium Spp.). *SEED RESEARCH-NEW DELHI-* **2003**, *31*, 33–38.
7. Priya, K.; Deshpande, V.K.; Masuthi, D.K.; Haleshkumar, H.B. Influence of Growth Regulators and Crossing Period on Flowering, Seed Yield and Quality of Chilli Hybrid HCH-9646. *International Journal of Agricultural Sciences* **2012**, *8*, 1–4.
8. Domínguez, F.; Cejudo, F.J. Programmed Cell Death (PCD): An Essential Process of Cereal Seed Development and Germination. *Front Plant Sci* **2014**, *5*, 366.
9. Jan, S.; Alyemeni, M.N.; Wijaya, L.; Alam, P.; Siddique, K.H.; Ahmad, P. Interactive Effect of 24-Epibrassinolide and Silicon Alleviates Cadmium Stress via the Modulation of Antioxidant Defense and Glyoxalase Systems and Macronutrient Content in Pisum Sativum L. Seedlings. *BMC Plant Biol* **2018**, *18*, 1–18.
10. Anjum, N.A. Calcium and Potassium Supplementation Enhanced Growth, Osmolytes, Secondary Metabolite Production, and Enzymatic Antioxidant Machinery in Cadmium-Exposed Chickpea (Cicer Arietinum L.). *Front Plant Sci* **2016**.
11. Ahmad, P.; Sarwat, M.; Bhat, N.A.; Wani, M.R.; Kazi, A.G.; Tran, L.-S.P. Alleviation of Cadmium Toxicity in Brassica Juncea L.(Czern. & Coss.) by Calcium Application Involves Various Physiological and Biochemical Strategies. *PLoS One* **2015**, *10*, e0114571.
12. Sankaranarayanan, K.; Praharaj, C.S.; Nalayini, P.; Bandyopadhyay, K.K.; Gopalakrishnan, N. Climate Change and Its Impact on Cotton (Gossypium Sp.). *Indian Journal of Agricultural Sciences* **2010**, *80*, 561–575.
13. Singh, M.; Singh, T.H.; Chahal, G.S. Genetic Analysis of Some Seed Quality Characters in Upland Cotton (Gossypium Hirsutum L.). *Theoretical and applied genetics* **1985**, *71*, 126–128.
14. Ye, Z.; Lu, Z.; Zhu, J. Genetic Analysis for Developmental Behavior of Some Seed Quality Traits in Upland Cotton (Gossypum Hirsutum L.). *Euphytica* **2003**, *129*, 183–191.
15. Chen, J.; Lv, F.; Liu, J.; Ma, Y.; Wang, Y.; Chen, B.; Meng, Y.; Zhou, Z. Effects of Different Planting Dates and Low Light on Cotton Fibre Length Formation. *Acta Physiol Plant* **2014**, *36*, 2581–2595.

16. Handa, N.; Kohli, S.K.; Sharma, A.; Thukral, A.K.; Bhardwaj, R.; Abd_Allah, E.F.; Alqarawi, A.A.; Ahmad, P. Selenium Modulates Dynamics of Antioxidative Defence Expression, Photosynthetic Attributes and Secondary Metabolites to Mitigate Chromium Toxicity in Brassica Juncea L. Plants. *Environ Exp Bot* **2019**, *161*, 180–192.
17. Alyemeni, M.N.; Ahanger, M.A.; Wijaya, L.; Alam, P.; Bhardwaj, R.; Ahmad, P. Selenium Mitigates Cadmium-Induced Oxidative Stress in Tomato (Solanum Lycopersicum L.) Plants by Modulating Chlorophyll Fluorescence, Osmolyte Accumulation, and Antioxidant System. *Protoplasma* **2018**, *255*, 459–469.
18. Ahmad, P.; Abdel Latef, A.A.; Abd_Allah, E.F.; Hashem, A.; Sarwat, M.; Anjum, N.A.; Gucel, S. Calcium and Potassium Supplementation Enhanced Growth, Osmolyte Secondary Metabolite Production, and Enzymatic Antioxidant Machinery in Cadmium-Exposed Chickpea (Cicer Arietinum L.). *Front Plant Sci* **2016**, *7*, 513.
19. Punia, H.; Tokas, J.; Bhadu, S.; Mohanty, A.K.; Rawat, P.; Malik, A. Proteome Dynamics and Transcriptome Profiling in Sorghum [Sorghum Bicolor (L.) Moench] under Salt Stress. *3 Biotech* **2020**, *10*, 1–10.
20. Punia, H.; Tokas, J.; Malik, A.; Rani, A.; Gupta, P.; Kumari, A.; Mor, V.S.; Bhuker, A.; Kumar, S. Solar Radiation and Nitrogen Use Efficiency for Sustainable Agriculture. In *Resources use efficiency in agriculture*; Springer, 2020; pp. 177–212.
21. Yamasaki, Y.; Gao, F.; Jordan, M.C.; Ayele, B.T. Seed Maturation Associated Transcriptional Programs and Regulatory Networks Underlying Genotypic Difference in Seed Dormancy and Size/Weight in Wheat (Triticum Aestivum L.). *BMC Plant Biol* **2017**, *17*, 1–18.
22. Grubben, G.J.H.; Denton, O.A. Plant Resources of Tropical Africa 2. Vegetables. *Plant resources of tropical Africa 2. Vegetables.* **2004**.
23. Sivaraj, N.; Pandravada, S.R. Morphological Diversity for Fruit Characters in Bottle Gourd Germplasm from Tribal Pockets of Telangana Region of Andhra Pradesh, India. *Asian Agrihist* **2005**, *9*, 305–310.
24. Haque, M.M.; Hasanuzzaman, M.; Rahman, M.L. Effect of Light Intensity on the Morpho-Physiology and Yield of Bottle Gourd (Lagenaria Vulgaris). *Academic Journal of Plant Sciences* **2009**, *2*, 158–161.
25. International, A. Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis. *J AOAC Int* **1995**, *78*, 143A-160A.
26. Dubois, M.; Gilles, K.A.; Hamilton, J.K.; Rebers, P.A. t; Smith, F. Colorimetric Method for Determination of Sugars and Related Substances. *Anal Chem* **1956**, *28*, 350–356.
27. Baloch, A.; Ahmad, F. Development of Iodimetric Redox Method for Routine Estimation of Ascorbic Acid from Fresh Fruit and Vegetables. *J. Chem. soc. Pak* **2013**, *35*, 783.
28. Hassan, L.G.; Umar, K.J. Proximate and Mineral Composition of Seeds and Pulp of African Locust Bean (Parkia Biglobosa L.). *Nigerian Journal of basic and applied sciences* **2004**, *13*, 15–27.
29. Sharifuddin, H.A.H.; Fauziah, I.; Zaharah, A.R. Technique of Soil Testing and Plant Analysis and Their Utilization for Crop Production in Malaysia. *Commun Soil Sci Plant Anal* **1990**, *21*, 1959–1978.
30. Standardization, I.O. for *ISO/IEC 17025: 1999: General Requirements for the Competence of Testing and Calibration Laboratories*; International Organization for Standardization, 1999;
31. Ågren, G.I.; Ingestad, T. Root: Shoot Ratio as a Balance between Nitrogen Productivity and Photosynthesis. *Plant Cell Environ* **1987**, *10*, 579–586.
32. Akintayo, E.T. Characteristics and Composition of Parkia Biglobbossa and Jatropha Curcas Oils and Cakes. *Bioresour Technol* **2004**, *92*, 307–310.
33. Badifu, G.I.O. Effect of Processing on Proximate Composition, Antinutritional and Toxic Contents of Kernels from Cucurbitaceae Species Grown in Nigeria. *Journal of food composition and analysis* **2001**, *14*, 153–161.
34. Hassan, L.G.; Sani, N.A.; Dangoggo, S.M.; Ladan, M.J. Nutritional Value of Bottle Gourd (Lagenaria Siceraria) Seeds. *Global Journal of Pure and Applied Sciences* **2008**, *14*, 301–306.
35. Osagie, A.U.; Eka, O.U. Nutritional Quality of Plant Foods. **1998**.
36. Punia, H.; Tokas, J.; Malik, A.; Kharor, N.; Yashveer, S. Deciphering Biochemical Responses, Metabolome Analysis and Key Genes Controlling Sorghum [Sorghum Bicolor (L.) Moench] Ion Transport in Responses to Salt Stress. **2021**.

37. Badifu, G.I.O.; Ogunsua, A.O. Chemical Composition of Kernels from Some Species OfCucurbitaceae Grown in Nigeria. *Plant Foods for Human Nutrition* **1991**, *41*, 35–44.
38. Punia, H.; Tokas, J.; Malik, A.; Bajguz, A.; El-Sheikh, M.A.; Ahmad, P. Ascorbate–Glutathione Oxidant Scavengers, Metabolome Analysis and Adaptation Mechanisms of Ion Exclusion in Sorghum under Salt Stress. *Int J Mol Sci* **2021**, *22*, 13249.
39. Copeland, L.O.; McDonald, M.F. *Principles of Seed Science and Technology*; Springer Science & Business Media, 2012; ISBN 1461516196.
40. Enujiugha, V.N.; Akanbi, C.T. Compositional Changes in African Oil Bean (Pentaclethra Macrophylla Benth) Seeds during Thermal Processing. *Pakistan journal of nutrition* **2005**, *4*, 27–31.
41. Kadiri, M.O. Protein Contents and Amino Acids of Some Leguminous Seeds. *Nig. J. Sci. and Technol* **1988**, *4*, 42–46.
42. Madaan, T.R.; Lal, B.M. Some Studies on the Chemical Composition of Cucurbit Kernels and Their Seed-coats. *Plant Foods for Human Nutrition* **1984**, *34*, 81–86.
43. Punia, H.; Tokas, J.; Bhadu, S.; Mohanty, A.K.; Rawat, P.; Malik, A.; Satpal Proteome Dynamics and Transcriptome Profiling in Sorghum [Sorghum Bicolor (L.) Moench] under Salt Stress. *3 Biotech* **2020**, *10*, 412, doi:10.1007/s13205-020-02392-1.
44. Punia, H.; Tokas, J.; Malik, A.; Singh, S.; Phogat, D.S.; Bhuker, A.; Mor, V.S.; Rani, A.; Sheokand, R.N. Discerning Morpho-Physiological and Quality Traits Contributing to Salinity Tolerance Acquisition in Sorghum [Sorghum Bicolor (L.) Moench]. *South African Journal of Botany* **2021**, *140*, 409–418.
45. Hassan, L.G.; Sani, N.A.; Dangoggo, S.M.; Ladan, M.J. Nutritional Value of Bottle Gourd (Lagenaria Siceraria) Seeds. *Global Journal of Pure and Applied Sciences* **2008**, *14*, 301–306.
46. Oyolu, C. A Quantitative and Qualitative Study of Seed Types in Egusi (Colocynthis Citrullus L.). **1977**.
47. Osagie, A.U.; Okoye, W.I.; Oluwayose, B.O.; Dawodu, O.A. Chemical Quality Parameters and Fatty Acid Composition of Oils of Some Underexploited Tropical Seeds. *Nig. J. Applied Sci* **1986**, *4*, 151–162.
48. Farinu, G.O. Chemical Composition of Some Plant Products of the Savanna Forest Zone of Nigeria. *Food Chem* **1986**, *22*, 315–320.
49. Ogunsua, A.O.; Adebona, M.B. Chemical Composition of Tetracarpidium Conophorum (Conophor Nut). *Food Chem* **1983**, *10*, 173–177.
50. Iqbal, M.; Usman, K.; Arif, M.; Jatoi, S.A.; Munir, M.; Khan, I. Evaluation of Bottle Gourd Genotypes for Yield and Quality Traits. *Sarhad Journal of Agriculture* **2019**, *35*.
51. Sharma, D.R.; Choudhary, M.R.; Jakhar, M.L.; Dadheech, S. Heterosis in Bottle Gourd (Lagenaria Siceraria (Mol.) Standl.). *Int. J. Life Sci* **2012**, *1*, 212–216.
52. Singh, B.; Singh, S.K. Evaluation Trial of Bottle Gourd. *Asian Journal of Horticulture* **2014**, *9*, 116–119.
53. Janaranjani, K.G.; Kanthaswamy, V. Correlation Studies and Path Analysis in Bottle Gourd. *Journal of Horticulture* **2015**, *2*, 1–4.
54. Bakhsh, K.; Ahmad, B.; Hassan, S.; Gill, Z.A. An Analysis of Technical Efficiency of Growing Bitter Gourd in Pakistan Punjab. *Pakistan Journal of Agricultural Sciences (Pakistan)* **2007**.
55. Iqbal, M.; Usman, K.; Arif, M.; Jatoi, S.A.; Munir, M.; Khan, I. Evaluation of Bottle Gourd Genotypes for Yield and Quality Traits. *Sarhad Journal of Agriculture* **2019**, *35*.
56. Mandizvo, T.; Odindo, A.O. Seed Mineral Reserves and Vigour of Bambara Groundnut (Vigna Subterranea L.) Landraces Differing in Seed Coat Colour. *Heliyon* **2019**, *5*, e01635.
57. Kyriacou, B.; Moore, K.L.; Paterson, D.; de Jonge, M.D.; Howard, D.L.; Stangoulis, J.; Tester, M.; Lombi, E.; Johnson, A.A.T. Localization of Iron in Rice Grain Using Synchrotron X-Ray Fluorescence Microscopy and High Resolution Secondary Ion Mass Spectrometry. *J Cereal Sci* **2014**, *59*, 173–180.
58. Lombi, E.; Smith, E.; Hansen, T.H.; Paterson, D.; de Jonge, M.D.; Howard, D.L.; Persson, D.P.; Husted, S.; Ryan, C.; Schjoerring, J.K. Megapixel Imaging of (Micro) Nutrients in Mature Barley Grains. *J Exp Bot* **2011**, *62*, 273–282.
59. Iwai, T.; Takahashi, M.; Oda, K.; Terada, Y.; Yoshida, K.T. Dynamic Changes in the Distribution of Minerals in Relation to Phytic Acid Accumulation during Rice Seed Development. *Plant Physiol* **2012**, *160*, 2007–2014.

60. Brinch-Pedersen, H.; Sørensen, L.D.; Holm, P.B. Engineering Crop Plants: Getting a Handle on Phosphate. *Trends Plant Sci* **2002**, *7*, 118–125.
61. Grant, C.A.; Flaten, D.N.; Tomasiewicz, D.J.; Sheppard, S.C. The Importance of Early Season Phosphorus Nutrition. *Canadian journal of plant science* **2001**, *81*, 211–224.
62. Zhao, M.; Han, Y.; Feng, Y.; Li, F.; Wang, W. Expansins Are Involved in Cell Growth Mediated by Abscisic Acid and Indole-3-Acetic Acid under Drought Stress in Wheat. *Plant Cell Rep* **2012**, *31*, 671–685.
63. Omole, J.O. The Chemical Composition of Caesalpinia Pulcherima. *Nigerian Journal of Animal Production* **2003**, *30*, 15–19.
64. Tokas, J.; Punia, H.; Malik, A.; Sangwan, S.; Devi, S.; Malik, S. Growth Performance, Nutritional Status, Forage Yield and Photosynthetic Use Efficiency of Sorghum [Sorghum Bicolor (L.) Moench] under Salt Stress. *Range Management and Agroforestry* **2021**, *42*, 59–70.
65. Malik, A.; Mor, V.S.; Tokas, J.; Punia, H.; Malik, S.; Malik, K.; Sangwan, S.; Tomar, S.; Singh, P.; Singh, N. Biostimulant-Treated Seedlings under Sustainable Agriculture: A Global Perspective Facing Climate Change. *Agronomy* **2020**, *11*, 14.
66. Punia, H.; Tokas, J.; Malik, A.; Sangwan, S.; Rani, A.; Yashveer, S.; Alansi, S.; Hashim, M.J.; El-Sheikh, M.A. Genome-Wide Transcriptome Profiling, Characterization, and Functional Identification of NAC Transcription Factors in Sorghum under Salt Stress. *Antioxidants* **2021**, *10*, 1605.
67. Olaofe, O.; Ogunlade, I.; Jibuah, N. Proximate, Mineral Composition and Functional Properties of Pride of Barbados (Caesalpinia Pulcherrima) Seed Flour. In Proceedings of the Proceedings of the 27th International Conference of the Chemical Society of Nigeria; 2004; pp. 130–135.
68. Thangadurai, D.; Viswanathan, M.B.; Ramesh, N. Nutritional Potential of Biochemical Components in Galactia Longifolia Benth.(Fabaceae). *Food/Nahrung* **2001**, *45*, 97–100.
69. Turan, M.; Kordali, S.; Zengin, H.; Dursun, A.; Sezen, Y. Macro and Micro Mineral Content of Some Wild Edible Leaves Consumed in Eastern Anatolia. *Acta Agriculturae Scandinavica, Section B-Plant Soil Science* **2003**, *53*, 129–137.
70. Almustapha, D.A.; Bilbis, L.S.; Rades, J.M.; Abubakar, M.K. Chemical Composition Or Some Oil Seed Grown in Northern Nigeria. **1995**.
71. Yusuf, A.A. Physico-Chemical Properties of Almond Seed Protein Concentrate. *Italian journal of food science* **2003**, *15*, 531–540.
72. Malik, A.; Punia, H.; Singh, N.; Singh, P. Bionanomaterials-Mediated Seed Priming for Sustainable Agricultural Production. In *Bionanotechnology: Emerging Applications of Bionanomaterials*; Elsevier, 2022; pp. 77–99.
73. Punia, H.; Tokas, J.; Malik, A.; Yashveer, S. Reconnoitering Bionanomaterials for Mitigation of Abiotic Stress in Plants. In *Bionanotechnology: Emerging Applications of Bionanomaterials*; Elsevier, 2022; pp. 101–126.
74. Srivastava, D.; Khan, N.A.; Shamim, M.; Yadav, P.; Pandey, P.; Singh, K.N. Assessment of the Genetic Diversity in Bottle Gourd (Lagenaria Siceraria [Molina] Standl.) Genotypes Using SDS-PAGE and RAPD Markers. *National Academy Science Letters* **2014**, *37*, 155–161.
75. Dunu, D.J.; Eka, O.U.; Ifon, E.T.; Essien, E.U. Chemical Evaluation of the Nutritive Value of Fruit of Calabash Plants (Lagenaria Scierania). *Nigerian Journal of Science* **1986**, *20*, 47–50.
76. Thangadurai, D.; Viswanathan, M.B.; Ramesh, N. Nutritional Potential of Biochemical Components in Galactia Longifolia Benth.(Fabaceae). *Food/Nahrung* **2001**, *45*, 97–100.
77. Tokas, J.; Kumari, P.; Thakral, N.K. EVALUATION OF FORAGE SORGHUM [SORGHUM BICOLOR (L .) MOENCH] GENOTYPES FOR QUALITY AND YIELD. **2017**, *43*, 235–238.
78. Adeyeye, E.I. Determination of the Chemical Composition of the Nutritionally Valuable Parts of Male and Female Common West African Fresh Water Crab Sudananautes Africanus Africanus. *Int J Food Sci Nutr* **2002**, *53*, 189–196.
79. Ayaz, F.A.; Glew, R.H.; Millson, M.; Huang, H.S.; Chuang, L.T.; Sanz, C.; Hayırlıoglu-Ayaz, S. Nutrient Contents of Kale (Brassica Oleraceae L. Var. Acephala DC.). *Food Chem* **2006**, *96*, 572–579.
80. Ortiz-Ospina, E.; Roser, M. World Population Growth. *OurWorldInData. org* **2016**.

81. Himani, B.S.; Tokas, J. Variation in Structural Carbohydrates of Forage Sorghum [Sorghum Bicolor (L.) Moench] under Saline Conditions. *Forage Res* **2019**, *45*, 123–127.
82. HIMANI, S.; MIDATHALA, R.; MUNJAL, R.; Sethi, S.K. Physico-Chemical and Correlation Studies in Durum Wheat (Triticum Durum L. Em Thell.) Genotypes. *Advn. Life Sci.* **2016**, *5*, 6109–6115.
83. Liu, J.; Ma, Y.; Lv, F.; Chen, J.; Zhou, Z.; Wang, Y.; Abudurezike, A.; Oosterhuis, D.M. Changes of Sucrose Metabolism in Leaf Subtending to Cotton Boll under Cool Temperature Due to Late Planting. *Field Crops Res* **2013**, *144*, 200–211.
84. Sunayana; Sangwan, R.S.; Khichar, M.L.; Singh, M. Weather Parameters Influencing Biochemical Constituents in Asiatic Cotton (Gossypium Arboreum L.) under Different Environmental Conditions. *JOURNAL OF AGROMETEOROLOGY* **2019**, *21*, 160–166.
85. Li, W.; Meng, Y.L.; Chen, B.L.; Wang, Y.H.; Zhou, Z.G. Effects of Climatic Factors on Fat and Total Protein Content in Cottonseeds. *Acta Ecol Sin. 2009b* **2009**, *29*, 1832–1839.
86. Zhu, L.; Zhou, Z.; Zhao, W.; Meng, Y.; Chen, B. Effects of Plant Densities on Cottonseed Biomass, Fat and Protein Contents. *Acta Agronomica Sinica* **2010**, *36*, 2162–2169.
87. Nawaz, R.; Inamullah, H.A.; Uddin, S.; Iqbal, M.S.; Gürsoy, S.; Kutbay, H.G.; Abdallah, F. Agromorphological Studies of Local Wheat Varieties for Variability and Their Association with Yield Related Traits. *Pak. J. Bot* **2013**, *45*, 1701–1706.