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Assessment of biochemical and nutritional parameters to successive pickings in upland cotton (*Gossypium hirsutum* L.) genotypes

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Abstract
The present investigation assessed thirty upland cotton (*Gossypium hirsutum* L.) genotypes for key biochemical parameters (sugar, phenol, gossypol, oil and crude protein) and nutritional components (iron, zinc and calcium) across first and second pickings under field conditions at CCS HAU, Hisar. Significant genotypic variability and picking-wise differences were observed for all traits, except zinc content. Sugar content ranged from 3.12-5.67% in the first picking and 3.14-5.63% in the second. Phenol content varied between 0.81-1.16% and 0.80-1.23% in the first and second pickings, respectively, with genotype H 1528 consistently showing the highest phenol content, and H 1553 the lowest. Gossypol content ranged from 0.33-0.57% in the first picking and 0.31-0.58% in the second, with H 1593 recording the maximum and H 1559-1 the minimum value. Oil content varied from 10.63-16.00% in the first picking and 10.43-15.37% in the second. For nutritional traits, iron content ranged from 98.86-109.47 mg/kg (first picking) and 98.17-106.59 mg/kg (second picking); zinc content from 47.80-57.28 mg/kg and 48.20-55.18 mg/kg; and calcium content from 0.90-1.56 g/kg and 0.88-1.67 g/kg, respectively. These findings underscore the importance of genotype selection and harvest timing in optimizing seed quality traits in cotton.

Keywords: *Gossypium hirsutum*, biochemical traits, nutrient profiling, gossypol content, oil content, cotton genotypes

Introduction
Cotton is an important commercial crop of India. It is an important fiber yielding crop of global importance, which is grown in tropical and subtropical regions of more than 80 countries of the world. It provides 65% of the raw material for textile industry, lint, oil and protein rich meal from its seed (Kaliyaperumal *et al.*, 2013) [12]. It is regarded as the "king" of fibre crops belonging to the Malvaceae family. The genus *Gossypium* contains forty-nine species, of which only four are cultivated and have spinnable lint; the remaining forty-five are wild species with short seed fuzz. The four species of *Gossypium* that yields lint, sometimes known as real cotton (Hu *et al.* 2019) [11]. India holds a significant position in the worldwide cotton industry due to several distinct features. These include having the largest cultivated area, growing all four domesticated species, supplying a substantial amount of tetraploid cotton, producing a notable quantity of extra-long staple cotton, potential exclusivity in hybrid cotton cultivation, being the birthplace of old-world cotton, and encountering diverse agro-climatic conditions for cotton cultivation. In India, an area of about 130.00 lakh ha is occupied by cotton with a production of 520.00 lakh bales and 400 kg/ha productivity. In 2023-24, it covered an area of 6.47 lakh ha in Haryana with a production of 17.20 lakh bales and 452 kg/ha productivity (Anonymous 2023) [1].
In the northern region, cotton is cultivated in extreme temperature conditions. The timing of harvesting the cotton is highly important to consider as the quality of the seeds and their biochemical characteristics can vary at different stages of picking. The mixing of immature and mature bolls during picking can negatively impact the quality of the lint and the germination of the seeds. The studies by Soomro *et al.* (2004) [20] have emphasized this point, additionally, the quality of the seeds is influenced by the timing of the harvest (Khatun *et al.* 2009) [13].

The ideal time for harvesting is when the crop reaches physiological maturity, as this is when all seed quality factors are optimal. Conversely, early harvesting can significantly reduce both seed yield and quality. Seed deterioration due to post-maturation is a major issue in seed production, as noted by Caldwell (1972) [6]. The intricate nature of seed cotton yield is determined by its various characteristics. These features are interconnected, so it is essential to understand how each trait interacts with others and their individual components simultaneously (Chaudhari *et al.* 2017) [8]. The productivity, quality, as well as resistance of the cotton plant, can be influenced by biochemical features that are critical to its production. The quality of cotton fibre is one of its most significant biological traits. Cotton fibre quality is determined by its length, strength, and fineness, every single one of which is impacted by biochemical activities occurring within the plant. Secondary metabolites contain insecticidal, antibacterial, antifertility, and poisonous effects (Benbouza *et al.* 2002) [4]. Biochemical features have a significant impact on the susceptibility of cotton to pests and diseases as well. Phenol is naturally insecticidal; gossypol has contraceptive properties and cotton fibre is composed of cellulose. These secondary metabolites have proven to be valuable in enhancing crop defence mechanisms and adding value to fibre products. To effectively improve yield and quality, it is required to comprehend the link between yield and its component elements (Saraswat *et al.* 2022) [17]. Overall, knowledge of cotton's biochemical properties is essential for increasing cotton production, improving the quality of the fibre, and developing new varieties that are more resistant to diseases and pests. Thus, the current study was conducted to estimate the biochemical and nutritional parameters in seeds of upland cotton genotypes.

Materials and Methods

Experimental site and planting materials

The experiments were conducted during *Kharif* seasons of 2023 at research farm of the Cotton Section, Department of Genetics & Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University (CCSHAU), Hisar. The experimental site is situated in a semi-arid sub-tropical zone between 29°10'N latitude and 75°46'E longitude, at a height of 215 meters above mean sea level. In *Kharif* 2023 season, experiments were laid out in a randomized block design with three replications. A total of thirty genotypes of upland cotton (Table 1) with pure seeds sourced from CCSHAU, Hisar, were sown in four rows of 5.1 m length with a spacing of 67.5 x 30 cm. Standard package of practices were followed during the entire cropping period (CCSHAU, 2024) [7].

Evaluation of biochemical parameters in cotton seeds

The seed cotton from opened bolls was picked separately and ginned. Five biochemical parameters i.e. sugar content, phenol content, protein content, gossypol content, oil content and three nutrient parameters viz., iron content, zinc content and calcium content were estimated in the seeds of all the genotypes at two picking. These experiments were performed at Biochemistry Laboratory of Cotton Section, Central Laboratory and Post-Graduate Laboratory, Department of Agronomy, CCSHAU, Hisar using the standard procedures for sugar content (Dubois *et al.*, 1956) [10], phenol content (Bray and Thorpe, 1954) [5], protein content (AOAC, 1990) [2], gossypol content (Bell, 1967) [3] and oil content (AOAC, 1990) [2], while the nutrient parameters *viz.*, iron content, zinc content and calcium content were estimated using atomic absorption spectroscopy (AAS).

Statistical Analysis

The experimental data for various factors were statistically analysed by the methods of analysis of variance (ANOVA) as described by Panse and Sukhatme (1985) [14] (Table 2). The mean values for every character were obtained for statistical analysis. The significance between pickings and each parameter was studied through independent t-test and were analysed through critical differences (CD) (Cochran and Cox, 1959; Sheoran, 2006) [9, 18]. In the laboratory experiments completely randomized design (CRD) and in field experiments randomized block design (RBD) were followed.

Results and Discussions

Biochemical and nutritional parameters

Cotton stands as the most widely utilized fibre crop globally, and it holds a significant position as a cash crop. The timing of cotton picking plays a critical role in determining seed quality. Typically, growers pick cotton three times throughout the entire growing period. However, adverse weather conditions, temperature fluctuations, and high humidity levels can lead to a change in biochemical and nutritional parameters. Keeping these factors in mind, the current study was aimed to assess biochemical and nutritional parameters in cotton genotypes after first and second picking. Five biochemical i.e., oil content (%), sugar content (%), phenol content (%), gossypol content (%) and crude protein content (%) including three nutrient parameters-iron content (mg/kg), zinc content (mg/kg) and calcium content (g/kg) varied significantly at Ist and 2nd picking in different cotton genotypes. Sugar content in case of first and second pickings, varied from 3.12 to 5.67% and 3.14 to 5.63%, respectively with mean values of 4.70% and 4.74%, respectively for both the pickings. The maximum sugar content (5.67% and 5.63%) was recorded for genotype H 1531 in both pickings and the minimum sugar content (3.12%) was recorded for genotype H 1588 in first picking and (3.14%) was recorded for genotype H 1480 for second picking. Phenol content during first picking, varied from 0.81 to 1.16% with a mean value of 0.96%, while in case of second picking, phenol content varied from 0.80 to 1.23% with a mean value of 0.99%. The maximum phenol content (1.16 and 1.23%) was recorded for genotype H 1528 in both pickings and the minimum phenol content (0.81 and 0.80%) was recorded for genotype H 1553 in both pickings.

The gossypol content in first picking, varied from 0.33 to 0.57% with a mean value of 0.40%. The maximum gossypol content (0.57%) was recorded for genotype H 1593 and the minimum gossypol content (0.33%) was recorded for genotype H 1574. In case of second picking, gossypol content varied from 0.31 to 0.58% with a mean value of 0.39%. The maximum gossypol content (0.58%) was recorded for genotype H 1593 and the minimum gossypol content (0.31%) was recorded for genotype H 1559-1. However, oil content in first picking, varied from 10.63 to 16.00% with a mean value of 12.69%. The maximum oil content (16.00%) was recorded for genotype H 1535 and the minimum oil content (10.63%) was recorded for genotype H

1607. In case of second picking, oil content varied from 10.43 to 15.37% with a mean value of 12.44%. The maximum oil content (15.37%) was recorded for genotype H 1574 and the minimum oil content (10.43%) was recorded for genotype H 1588. In case of crude protein content, during first picking, crude protein content varied from 16.36 to 22.75% with a mean value of 20.31%. The maximum crude protein content (22.75%) was recorded for genotype H 1622 and the minimum crude protein content (16.36%) was recorded for genotype H 1621. In case of second picking, crude protein content varied from 16.55 to 22.50% with a mean value of 20.54%. The maximum crude protein content (22.50%) was recorded for genotype H 1551 and minimum crude protein content (16.55%) was recorded for genotype H 1480.

Nutritional content such as iron content in first picking, varied from 98.86 to 109.47 mg/kg with a mean value of 104.76 mg/kg (Table 3). The maximum iron content (109.47 mg/kg) was recorded for genotype H 1639 and the minimum iron content (98.86 mg/kg) was recorded for genotype H 1553. In case of second picking, iron content varied from 98.17 to 106.59 mg/kg with a mean value of 102.63mg/kg. The maximum iron content (106.59 mg/kg) was recorded for genotype H 1618 and the minimum iron content (98.17 mg/kg) was recorded for genotype H 1593. Zinc content in case of first picking, varied from 47.80 to 57.28 mg/kg with a mean value of 52.51 mg/kg. The maximum zinc content (57.28 mg/kg) was recorded for genotype H 1611 and the minimum zinc content (47.80 mg/kg) was recorded for genotype H 1588. In case of second picking, zinc content varied from 48.20 to 55.18 mg/kg with a mean value of 52.03 mg/kg. The maximum zinc content (55.18 mg/kg) was recorded for genotype H 1600 and the minimum zinc content (48.20 mg/kg) was recorded for genotype H 1584. In case of first picking, calcium content varied from 0.90 to 1.56 g/kg with a mean value of 1.14 g/kg. The maximum calcium content (1.56 g/kg) was recorded for genotype H 1593 and the minimum calcium content (0.90 g/kg) was recorded for genotype H 1518. In case of second picking, calcium content varied from 0.88 to 1.67 g/kg with a mean value of 1.67 g/kg. The maximum calcium content (1.67 g/kg) was recorded for genotype H 1621 and the minimum calcium content (0.88 g/kg) was recorded for genotype H 1578.

Study of both pickings revealed that sugar content, oil content and crude protein content increased significantly in 1st picking while, phenol content and gossypol content increased significantly in 2nd picking. High sugar, oil and crude protein content in 1st picking may be due to the reason that these biochemical constituents are essential in the early stages of growth particularly during flower formation and boll formation stage while high phenol and gossypol content in 2nd picking may be a result of increased adult plant resistance mechanism. These results were in accordance with the results of Pinki *et al.* (2018) [15] and Saraswat *et al.* (2022) [17]. Similarly, Ramani *et al.* (2017) [8] recorded high protein content found in genotype 0821-B4-11-7, low in genotype IAN-1327. However, high phenol content was observed in genotype C-1622, low in genotype Demeter 111(1). In case of gossypol, high content was found in genotype C-1622, low in genotype Bar-12/13. Other similar study conducted by Sonika *et al.* (2017) [19] concluded the function of biochemical traits such as such as total sugar, phenols, gossypol, tannin, crude protein contents, and the enzymatic activities of peroxidase and polyphenol oxidases of *Gossypium hirsutum* L., for resistance to cotton leaf curl disease (CLCuD). In present study, both pickings revealed that iron content increased significantly in 1st picking while calcium content increased significantly in 2nd picking. Zinc content was found to have no pickings effect.

Table 1: Details of cotton genotypes grown during *Kharif* 2023

Sr. No.	Name of genotype	Pedigree
1	H 1480	F 2228 × H 1117
2	H 1518	H 1360
3	H 1528	F 2228 × H 1117
4	H 1529	H 1117 × PIL 8
5	H 1531	HS 6 × Badnawar
6	H 1535	F 2228 × H 1117
7	H 1547	H 1226 × RS 875
8	H 1551	F 2276
9	H 1553	F 2228 × H 1226
10	H 1559	GM 39
11	H 1564	H 1226 × RS 875
12	H 1566	LH 2108 × G 1236
13	H 1574	F 2164 × H 1236
14	H 1578	H 974 × J 34
15	H 1584	H 1226 × RS 875
16	H 1588	H 777 × AC 134
17	H 1591	Khakhi Kapas
18	H 1593	GM 37
19	H 1594	H 1117 × CSH 171
20	H 1600	H 1480 × H 1316
21	H 1603	GCA 90
22	H 1607	F 2228 × H 1117
23	H 1609	H 1508 × H 202
24	H 1610	H 1523 × H 202
25	H 1611	H 1523 × H 210
26	H 1613	H 1226 × PIL 8
27	H 1618	LH 2107 × H 1117
28	H 1621	H 1491 × H 202
29	H 1622	H 1491 × H 210
30	H 1639	-

Table 2: Analysis of variance (ANOVA) for biochemical traits in thirty cotton genotypes (1st picking and 2nd picking)

Picking	Source of variation	DF	Mean sum of squares							
			SC	PC	GC	OC	CPC	IC	ZC	CC
1st picking	Replication	2	0.1	0.07	0.02	0.69	2.02	43.18	7.87	0.54
	Genotype	29	1.58**	0.03*	0.02*	26.99**	35.43**	21.76**	20.36**	0.92**
	Error	58	0.16	0.05	0.01	2.15	1.16	12.19	17.55	0.58
2nd picking	Replication	2	0.19	0.12	0.08	0.56	2.05	48.39	9.14	0.75
	Genotype	29	2.69**	0.07*	0.03*	28.13**	25.46**	21.92**	17.52**	0.63**
	Error	58	0.22	0.04	0.01	1.56	1.04	18.77	18.5	0.85

** Significant at 1% level of significance, *Significant at 5% level of significance,

DF: Degree of freedom, SC: Sugar content (%), PC: Phenol content (%), GC: Gossypol content (%), OC: Oil content (%), CPC: Crude protein content (%), IC: Iron content (mg/kg), ZC: Zinc content (mg/kg), CC: Calcium content (g/kg)

Table 4: Mean performance of biochemical traits of 30 cotton genotypes (1st picking and 2nd picking)

Genotype	I st picking								2nd picking							
	SC	PC	GC	OC	CPC	IC	ZC	CC	SC	PC	GC	OC	CPC	IC	ZC	CC
H 1480	3.99	1.08	0.36	13.50	16.69	99.07	48.88	1.33	3.14	1.11	0.45	12.27	16.55	100.81	51.70	1.23
H 1518	4.98	0.97	0.37	15.77	20.67	106.97	54.34	0.90	5.18	0.87	0.36	14.87	20.33	105.00	52.11	1.07
H 1528	5.46	1.16	0.41	15.20	21.35	106.80	49.69	1.07	5.41	1.23	0.37	14.40	21.67	105.60	50.30	1.23
H 1529	5.47	1.01	0.40	11.50	20.44	106.67	52.48	1.43	5.30	0.95	0.32	11.10	19.71	102.54	51.03	1.26
H 1531	5.67	0.82	0.41	12.53	21.52	105.03	52.62	1.23	5.63	0.87	0.37	11.53	21.88	98.48	50.24	0.93
H 1535	3.22	1.06	0.52	16.00	21.75	105.33	53.88	1.37	3.86	0.96	0.54	13.47	21.44	105.00	51.58	1.25
H 1547	4.20	0.95	0.35	10.90	19.88	103.73	52.62	1.50	4.62	0.86	0.38	12.43	20.23	99.60	53.06	1.53
H 1551	4.82	0.91	0.35	12.70	22.58	102.33	50.96	1.35	4.67	0.80	0.34	11.60	22.50	102.90	51.94	1.54
H 1553	5.29	0.81	0.52	10.83	20.56	98.86	52.13	1.23	3.35	0.80	0.47	10.57	20.29	99.16	50.19	1.26
H 1559	4.27	0.92	0.37	12.37	20.38	107.67	54.14	1.23	4.41	0.87	0.31	14.60	19.54	101.57	52.28	1.00
H 1564	5.47	1.09	0.38	13.97	21.54	103.72	52.69	1.40	5.33	1.00	0.36	12.97	20.92	101.98	53.66	1.20
H 1566	4.72	1.00	0.36	12.07	20.52	106.52	52.54	1.37	4.84	1.10	0.33	11.53	20.69	100.98	52.71	1.20
H 1574	5.00	0.94	0.33	12.47	20.00	107.00	54.26	1.33	5.53	1.06	0.34	15.37	21.02	105.94	51.65	1.33
H 1578	3.54	0.86	0.38	10.77	21.83	104.87	53.18	0.93	3.21	0.95	0.44	10.53	20.69	101.40	52.33	0.88
H 1584	4.77	1.02	0.48	11.60	21.06	104.90	50.06	1.27	4.87	0.93	0.40	10.57	19.52	100.87	48.20	1.00
H 1588	3.12	0.97	0.41	11.70	20.79	100.52	47.80	1.03	5.32	1.10	0.44	10.43	22.02	105.23	50.10	0.96
H 1591	5.08	0.94	0.40	12.07	22.40	105.50	53.34	1.25	4.87	1.02	0.36	11.50	21.00	101.79	52.20	1.43
H 1593	5.00	0.96	0.57	10.87	20.06	104.28	48.67	1.56	5.27	0.83	0.58	13.33	20.79	98.17	50.26	1.30
H 1594	4.41	0.88	0.50	11.57	21.83	107.37	53.05	1.26	4.85	0.88	0.51	11.33	20.48	105.20	53.97	1.50
H 1600	3.79	0.89	0.34	12.83	19.94	108.07	53.39	0.93	3.88	0.97	0.41	14.73	20.71	104.88	55.18	1.00
H 1603	4.26	0.94	0.34	10.87	20.23	104.33	53.18	1.52	4.56	1.01	0.34	11.43	21.85	103.30	54.15	1.40
H 1607	4.48	0.99	0.41	10.63	18.25	104.47	52.10	1.37	4.38	1.09	0.36	13.87	20.67	106.05	53.10	1.50
H 1609	3.87	1.14	0.36	12.30	20.00	104.70	52.84	1.17	3.75	1.19	0.34	10.73	20.27	103.78	50.19	1.07
H 1610	4.90	1.02	0.39	14.77	20.85	104.48	49.13	1.17	5.00	1.09	0.48	11.67	21.35	104.07	50.90	1.26
H 1611	5.03	0.96	0.42	10.90	20.56	105.77	57.28	1.24	5.17	0.91	0.37	10.53	22.25	104.64	55.00	1.46
H 1613	4.69	0.82	0.39	15.83	18.88	106.29	51.41	1.43	4.41	0.90	0.34	14.70	18.44	105.58	51.42	1.23
H 1618	5.37	0.92	0.37	12.27	21.63	103.77	52.48	1.25	5.16	1.04	0.41	10.80	20.85	106.59	50.98	1.45
H 1621	5.31	1.08	0.42	11.27	16.36	104.97	54.86	1.33	5.44	1.15	0.38	11.13	16.90	106.32	54.77	1.67
H 1622	5.32	0.87	0.36	15.30	22.75	105.10	57.09	1.43	5.27	0.89	0.31	14.07	21.44	105.25	54.79	1.55
H 1639	5.55	1.02	0.35	15.33	19.00	109.47	55.29	1.20	5.56	1.08	0.38	15.07	20.27	106.17	52.51	1.33
Mean	4.70	0.96	0.40	12.69	20.31	104.76	52.51	1.14	4.74	0.99	0.39	12.44	20.54	102.63	52.03	1.67
Min.	3.12	0.81	0.33	10.63	16.36	98.86	47.80	0.90	3.14	0.80	0.31	10.43	16.55	98.17	48.20	0.88
Max.	5.67	1.16	0.57	16.00	22.75	109.47	57.28	1.56	5.63	1.23	0.58	15.37	22.50	106.59	55.18	1.56
SE	0.21	0.04	0.02	1.20	0.52	2.28	2.44	0.05	0.34	0.03	0.04	1.15	0.58	1.82	2.14	0.04
CD (5%)	0.62	0.02	0.05	1.50	0.67	2.56	2.74	0.23	0.82	0.09	0.03	1.45	0.47	1.86	2.34	0.25
CV (%)	4.76	4.50	5.45	3.40	4.43	3.80	5.06	2.96	4.66	4.25	5.15	3.62	4.23	3.55	5.26	2.36

SC: Sugar content (%), PC: Phenol content (%), GC: Gossypol content (%), OC: Oil content (%), CPC: Crude protein content (%), IC: Iron content (mg/kg), ZC: Zinc content (mg/kg), CC: Calcium content (g/kg)

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Declarations

Conflict of interest

The authors have no conflicts of interest.

Authors' contributions

The idea was conceived by AJ. The layout and experiments were carried out by AJ and SM. SM wrote the original draft, statistically analysed the data. The final draft was read and approved by all the authors.

Declaration of competing interest

The authors declare no competing financial or non-financial interests. The research work was supported as part of a M.Sc. program and institutional resources. No additional funding was received for the revision or publication of this manuscript.

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