



A Nutritional analysis of selected heterotic hybrids of *Zea mays* L.

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ABSTRACT

The current experiment was designed to examine and evaluate the nutritional factors of 16 selected high yielding Maize (*Zea mays* L.) hybrids and their parental lines. A secondary objective of this study was to compare the nutritional factors of the hybrids with that of their parents. 71 genotypes (35 parents, F1 & F2 of 16 selected hybrids, 2 checks) were evaluated in two replications for their nutritional factor. The nutritional factors of hybrids was determined by estimating the content of Protein, Carbohydrates, Amino acids and Ascorbic acid. Lowry's method, Anthrone's method, Roe and Keuther method, Yemm et al., method was used to estimate the protein content, carbohydrate content, Ascorbic acid content and Amino acid content respectively. Among the select hybrids, four hybrids (SH1 (L29 x T3), SH8 (L24 x T3), SH13 (L11 x T5), SH15 (L24 x T5)) showed higher quantity of protein, carbohydrate and amino acids. Therefore, these four hybrids can be recommended to the farming community for production. This will help farmers to get good yielding maize with high nutritional factors. The rest of the hybrids can be recommended in their particular segment for nutritional benefits. We concluded that, high-nutrient and high-yielding heterotic maize hybrids were produced for cultivation and suggested to the farmers.

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INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal crops (Sleper and Poehlman, 2006) next to

wheat and rice. It is a member of Gramineae family with diploid chromosome number of $2n = 2x = 20$. Maize has its importance with human consumption (25%), cattle feed (12%), poultry feed (49%), raw material for the industrial products such as starch (12%) and brewery (1%). 1% of Maize is being used for seed production. It is a cheap form of starch when compared and a major energy source in animal feed (McKevith, 2004). Thus, it plays a major role in the world's agricultural economy. Corn can be used for consumption in different stages of growth (from baby corn to mature grain).

Public health and nutritional richness are key factors in any developed society. A lot of development was made for the genetic enhancement in the nutritional value of the crop. But still we can observe that malnutrition is a big problem. Worldwide around

200 million children under five years of age are found to be starved of proteins. Malnutrition leads to many health issues like stunted growth, susceptibility to infections and poor brain development.

Breeders should plan their breeding strategy for developing new hybrids depending on the future demand and market requirements (Dagade *et al.*, 2015). The nutritional quality of maize can be improved by selecting the essential amino acids through selection and breeding (Jaradat and Goldstein, 2014). Maize kernels with high methionine and lysine concentrations are important in poultry feed (Moore *et al.*, 2008; Adeyemo, 2012). Since Maize is the main feeding source for poultry industry, the above-mentioned methionine and lysine amino acid rich maize serves as good nutritional feed for poultry.

Plant breeders and geneticists have contributed much to improve the quality of plant proteins. They have identified natural mutations like Maize and barley with high-lysine content and bred in to elite genotypes (Bright *et al.*, 1983; Šramková *et al.*, 2008). However, the improvement in the plant nutritional quality was achieved by accident and not by design (Lindsay, 2002).

In maize Carbohydrate a pre-dominant biochemical component in terms of concentration. In Maize kernel, the percentage of carbohydrate is 72 to 75. Protein is the next largest biochemical in Corn, and it ranges from 8 to 11 % (Singh *et al.*, 2005; Orhun, 2013). Oil is the 3rd largest biochemical component in maize, and it ranges from 3 to 18 % (Ilyas *et al.*, 2014).

Selection of genetic resources for quality traits and assessment of relationships between agronomic and quality traits are two important things in a breeding strategy. (Seebauer *et al.*, 2010). In hybrids higher yield is linked with poor quality traits like protein concentration in comparison to the landraces and open-pollinated varieties of the past (Seebauer *et al.*, 2010). Further, open-pollinated landrace populations may contain traits related to nutrient quality that may be stabilized with classical breeding techniques (Jaradat *et al.*, 2010).

To meet the ever-increasing demand of maize for the human population as well as the food-processing industry, hybrid varieties are the only solution, as their yields are, in general, much higher than those of the traditional varieties. Therefore, there is an urgent need for developing corn hybrids that are suited to different climatic conditions.

To estimate the quality of a grain, we need to analyze the quantity of protein, carbohydrate, starch and oil

in it. The main aim of the present experiment was to identify hybrids with high nutritional factor for cultivation and promising maize germ plasm with quality traits for further maize research and breeding program.

MATERIALS AND METHODS

Present study was carried out in the Department of Biotechnology, Karpagam Academy of Higher Education, Coimbatore (Tamil Nadu). Thirty-five genetically diverse Maize inbred lines, F₁, F₂ seeds of 16. Select high yielding maize hybrids and F₁, F₂ seed of two check varieties were used as material for this experiment (Table 1 & Table 2). To get F₂ seeds, the F₁ seeds of the select hybrids were sown and shelved, 10 plants were selected from each entry for this Shelving program. The parental lines, F₁ hybrids and F₂ seed were used in lab studies for the estimation of their nutritional quality. The seeds were crushed into powder form for the analysis. Four nutritional factors, i.e. total content of carbohydrate (mg/g), total content of protein (mg/g), Ascorbic acid content (mg/g), and total content of Amino acids (mg/g). Grains from five random plants of each parental lines, F₁ and F₂ seeds of selected hybrids and check varieties were used for this experiment. The observation results were analyzed by statistical analysis using mean values of replications.

METHODOLOGY

In the present study, the estimation of carbohydrates, proteins, Amino acids and Ascorbic acids was done. In seventy one maize genotypes (Table 1 & Table 2). Lowry's method was followed for the estimation of protein content (Waterborg and Matthews, 1984), Anthrone method for the estimation of carbohydrates, Roe and Kuether (1943) for the estimation of Ascorbic acid content and Yemm *et al.* (1955) for the estimation of amino acid content.

Estimation of protein content

For the estimation of protein, Maize kernels were crushed in to powder. Lowry's method was followed for this experiment (Lowry *et al.*, 1957). Bovine Serum Albumin was used as standard protein. The working standards of BSA were prepared by taking 0, 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml & 1ml in test tubes. Zero was used as blank. The total volume of each test tube was made up to 1 ml with distilled water. To all the test tubes 4.5 ml Reagent-I was added and the tubes were kept for incubation for 10 minutes. Then 0.5 ml of reagent-II was added and left for incu-

Table 1: Maize germ plasm used for the experiment

S.No.	Code	Line number	S.No.	Code	Line number
1	T1	TM-0019	19	L14	TM-0123
2	T2	TM-0034	20	L15	TM-0126
3	T3	TM-0068	21	L16	TM-0296
4	T4	TM-0072	22	L17	TM-0297
5	T5	TM-0125	23	L18	TM0298
6	L1	TM-0003	24	L19	TM-0299
7	L2	TM-0020	25	L20	TM-0300
8	L3	TM-0030	26	L21	TM-0301
9	L4	TM-0031	27	L22	TM-0302
10	L5	TM-0032	28	L23	TM-0304
11	L6	TM-0064	29	L24	TM-0305
12	L7	TM-0065	30	L25	TM-0306
13	L8	TM-0067	31	L26	TM-0307
14	L9	TM-0071	32	L27	TM-0308
15	L10	TM-0103	33	L28	TM-0309
16	L11	TM-0106	34	L29	TM-0310
17	L12	TM-0111	35	L30	TM-0311
18	L13	TM-0118			

Table 2: Selected high yielding Maize hybrids used for the experiment

S.No.	F1 (select & Superior from 150 hybrids)	Cross combination	Cross code	Sample used	Sample used
1	SH-1	TM-00310 X TM-00068	L29 X T3	F1	F2seed
2	SH-2	TM-00310 X TM-00125	L29 X T5	F1	F2seed
3	SH-3	TM-00020 X TM-00068	L2 X T3	F1	F2 seed
4	SH-4	TM-00032 X TM-00068	L5 X T3	F1	F2 seed
5	SH-5	TM-00302 X TM-00068	L22 X T3	F1	F2 seed
6	SH-6	TM-00106 X TM-00068	L11 X T3	F1	F2 seed
7	SH-7	TM-00311 X TM-00068	L30 X T3	F1	F2 seed
8	SH-8	TM-00305 X TM-00068	L24 X T3	F1	F2 seed
9	SH-9	TM-00299 X TM-00068	L19 X T3	F1	F2 seed
10	SH-10	TM-00020 X TM-00125	L2 X T5	F1	F2 seed
11	SH-11	TM-00032 X TM-00125	L5 X T5	F1	F2 seed
12	SH-12	TM-00302 X TM-00125	L22 X T5	F1	F2 seed
13	SH-13	TM-00106 X TM-00125	L11 X T5	F1	F2 seed
14	SH-14	TM-00311 X TM-00125	L30 X T5	F1	F2 seed
15	SH-15	TM-00305 X TM-00125	L24 X T5	F1	F2 seed
16	SH-16	TM-00299 X TM-00125	L19 X T5	F1	F2 seed
17	CHECK-I	30V 92		F1	F2 seed
18	CHECK-II	NK-6240		F1	F2 seed

Table 3: Parental lines - Protein, Carbohydrate, Ascorbic acid & Amino acids estimation

Sample ID	Protein mg/g	Carbohydrate mg/g	Ascorbic acid mg/g	Amino acids mg/g
T1	21.883	133.775	273.516	101.845
T2	20.144	85.691	141.013	118.097
T3	21.795	126.427	209.454	99.070
T4	15.407	125.079	218.716	120.427
T5	20.281	157.869	133.317	95.233
L1	9.823	92.700	327.698	77.939
L2	6.154	27.466	387.525	85.573
L3	6.520	143.937	198.936	64.940
L4	7.182	138.489	200.264	76.957
L5	10.372	83.144	379.093	86.211
L6	5.583	111.603	348.695	36.848
L7	6.201	91.896	321.063	112.473
L8	7.537	125.665	270.394	81.828
L9	10.310	54.552	275.01	102.018
L10	6.513	94.679	378.449	62.678
L11	4.576	113.548	223.82	47.711
L12	9.969	90.692	209.876	62.341
L13	7.901	95.312	236.632	77.537
L14	8.322	56.493	259.722	72.715
L15	4.167	119.254	129.237	97.314
L16	5.635	157.151	133.224	126.147
L17	3.909	68.158	235.52	132.387
L18	8.372	71.918	268.447	160.307
L19	7.183	132.673	150.564	82.588
L20	11.004	139.635	380.169	123.617
L21	5.856	135.935	271.764	127.142
L22	7.496	112.704	193.691	90.830
L23	3.499	83.185	240.846	156.378
L24	10.868	72.993	273.763	135.948
L25	15.903	164.269	396.108	101.584
L26	7.240	96.745	361.573	106.538
L27	11.259	130.638	332.468	137.166
L28	6.161	142.207	235.92	123.342
L29	3.920	138.481	291.237	132.710
L30	14.137	146.652	214.554	147.480

bation for 30 minutes. Then the OD values were recorded at 660 nm. A standard graph was prepared by plotting the standard concentrations on the X-axis and OD values on the Y-axis (Figure 1). From standard graph, the protein content was estimated in the given sample.

Estimation of carbohydrate content

The carbohydrate content can be estimated by polysaccharide hydrolysis into simple sugars (monosaccharides) by acid treatment. 100 mg of

the maize kernel powder was taken in a test tube and kept in hot water for 3 hours with 5ml of 2.5 N-HCl and cooled to room temperature; then the tube was neutralized using solid sodium carbonate until the effervescence stopped. The volume was made to 100 ml using distilled water and kept for centrifugation. 1 ml of aliquots was prepared with the supernatant formed in the test tube. Using working standard 0, 0.2, 0.4, 0.6, 0.8 and 1ml of standard samples were prepared. '0' was used as blank. Then using distilled water volumes were

Table 4: F₁ hybrids - Protein, Carbohydrate, Ascorbic acid & Amino acids estimation

SAMPLE ID	Protein mg/g	Carbohydrate mg/g	Ascorbic acid mg/g	Amino acids mg/g
F1-SH1	11.368	95.376	55.308	70.348
F1-SH2	14.379	110.066	35.307	95.165
F1-SH3	20.405	49.533	55.233	94.162
F1-SH4	13.300	54.903	100.626	61.860
F1-SH5	6.460	62.956	59.900	78.476
F1-SH6	7.514	51.729	85.786	68.850
F1-SH7	5.569	86.398	89.149	76.269
F1-SH8	14.050	99.670	32.618	82.769
F1-SH9	4.689	66.467	69.141	72.591
F1-SH10	13.082	96.137	58.983	95.265
F1-SH11	15.713	69.559	56.026	83.734
F1-SH12	11.432	37.555	43.498	102.125
F1-SH13	8.036	37.404	74.962	79.859
F1-SH14	3.511	124.322	71.336	87.259
F1-SH15	16.287	34.401	71.799	55.273
F1-SH16	10.685	108.844	101.77	106.753
F1-CH1	2.900	166.760	89.520	96.034
F1-CH2	8.600	122.216	110.641	145.368

*CH = Check variety and SH = Selected hybrid based on high yield

*F₁ = Hybrid seed use for planting**Table 5: F₂ hybrid - Protein, Carbohydrate, Ascorbic acid & Amino acids estimation**

Sample ID	Protein mg/g	Carbohydrate mg/g	Ascorbic acid mg/g	Amino acids mg/g
F2 -SH1	23.174	176.077	177.546	123.064
F2 -SH2	22.905	134.794	210.412	130.483
F2 -SH3	30.002	123.282	194.041	122.254
F2 -SH4	14.709	141.237	156.282	154.184
F2 -SH5	20.150	198.915	179.36	114.835
F2 -SH6	6.038	176.021	139.601	137.706
F2 -SH7	8.807	130.035	161.454	126.186
F2 -SH8	16.667	174.523	112.145	131.222
F2 -SH9	13.936	145.910	207.456	101.508
F2 -SH10	19.229	121.059	183.470	122.326
F2 -SH11	12.334	128.804	162.051	128.006
F2 -SH12	20.977	159.036	181.245	90.181
F2 -SH13	18.658	157.236	144.145	137.577
F2 -SH14	17.839	125.367	192.575	121.404
F2 -SH15	19.108	181.369	177.464	132.485
F2 -SH16	15.670	129.923	113.631	134.655
F2 -CH-I	7.444	150.543	174.146	119.044
F2 -CH-II	9.329	145.277	274.165	110.797

*CH = Check variety and SH = Selected hybrid based on high yield

*F₂ = Outcome of F₁ seed planted

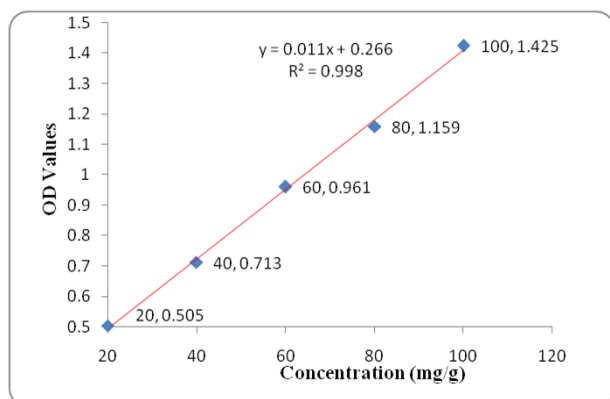


Figure 1: Standard graph for estimation of protein content

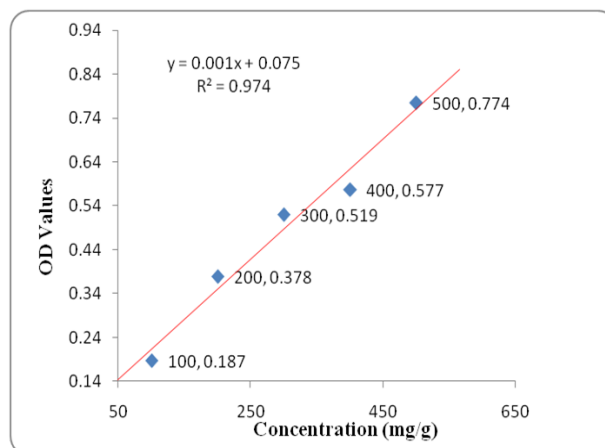


Figure 4: Standard graph for estimation of Amino acid content

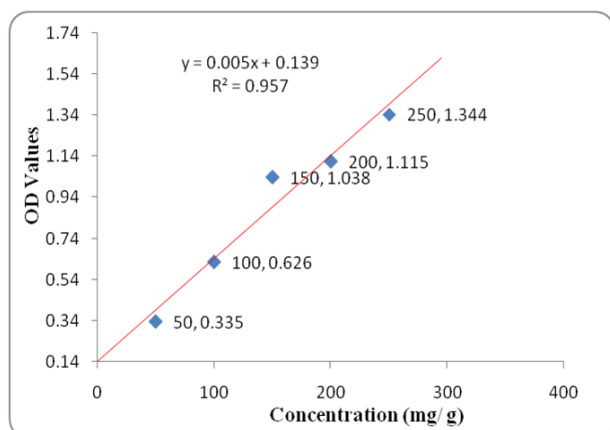


Figure 2: Standard graph for estimation of carbohydrate content

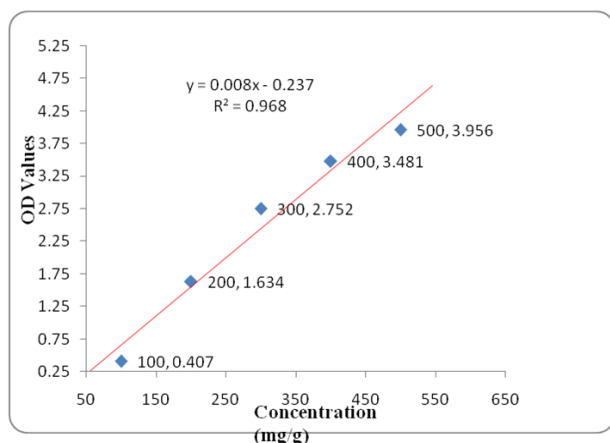


Figure 3: Standard graph for estimation of Ascorbic acid content

made up to 1ml in all the tubes. 4ml of Anthrone's reagent was added to the tubes. The test tubes were kept in hot water for eight minutes and cooled rapidly. The solution's colour turned to dark green. The intensity of the solution can be read by noting OD values at 630 nm. The standard graph was plotted using concentration on the X-axis and OD values on the Y-axis (Figure 2). Using the standard graph, the carbohydrate content was estimated in the given sample.

Estimation of Ascorbic acid content

100 mg of sample was weighed and the volume was made to 1 ml using 4% TCA. The tube was kept for centrifugation at 2000 rpm for 10 mins. The clear supernatant was collected and the volumes were made into 2.0 ml using 4% TCA. To the test tube 0.5 ml of DNPH reagent was added, then two drops of 10% thiourea solution added. The test tubes were kept for incubation for 3 hours at 37°C. The osazones were dissolved in 2.5 ml of 85% H₂SO₄ in cold, with no appreciable rise in temperature. Then samples were incubated for 30 minutes at room temperature, colour intensity was read at 540 nm (Roe and Kuether, 1943). The standard graph was plotted using concentrations on the X-axis and OD values on the Y-axis (Figure 3). Using the standard graph, the Ascorbic acid content was estimated in the given sample.

Estimation of Amino acid content

Sample extract was dissolved in 100 mg/ml concentration of 85% ethanol, and 1ml of Ninhydrin solution added (0.8 stannous chloride dissolved in 500 ml of 0.2 M citrate buffer [pH 5.0]). Then the solution was added with 2g of Ninhydrin in 500ml methanol. The volume was made into 2ml with distilled water. The tube was kept in a boiling hot water for 20min. Then 5ml of the diluent solvent

(water and n-propanol mixture in equal volumes) was added. After 15 min the intensity of purple colour was read by using spectrophotometer at 570 nm (Yemm *et al.*, 1955). The standard graph was plotted using concentration on the X-axis and OD values on the Y-axis (Figure 4). Using the standard graph, the Amino acid content in the given sample was estimated.

RESULTS AND DISCUSSION

The observations were noted for the estimation of nutritional factors namely Carbohydrate content (mg/g), Protein content (mg/g), Ascorbic acid concentration (mg/g), and Amino acid content (mg/g) (Tables 3, 4 and 5). Five random plants were selected from each entry for taking observations. The same procedure was followed for parents, F₁ and F₂ seeds of select hybrids and check varieties. The observations were studied in three replications, and mean values were used for analysis. Since F₂ seed is used as food, feed and for industrial purpose, the study was well-focused on the analysis of F₂ seed of 16 select high yielding hybrids to compare with two popular Maize checks in the market.

The nutritional factors of Maize can be determined by the presence of protein, carbohydrate, Ascorbic acid and the content of amino acids. Here in our experiment, we have chosen two Maize hybrids (NK 6240&30 V 92) as checks, which are popular in the market. We compared our 16 high yielding selected hybrids for nutritional quality with check hybrids. Since we use the outcome of F₁ seed for consumption, we checked the nutritional factors of F₂ seeds. In the study, it has been observed that the F₂ seed has more total protein and carbohydrate content than its parents lines. In Ascorbic acid content we found that all parental lines in the present study are dominant over their F₁ and F₂ seed.

In case of protein content, the F₂ samples range from 8.6 to 30.0 mg/g, whereas check-I and check-II have values of 7.44 and 9.32 mg/g. 14 hybrids have shown significant superiority over checks in case of protein content. They are SH3 (30.00 mg/g), SH1 (23.17 mg/g), SH2 (22.90 mg/g), SH12 (20.97 mg/g), SH5 (20.15 mg/g), SH10 (19.23 mg/g), SH15 (19.11 mg/g), SH13 (18.66 mg/g), SH 14 (17.84 mg/g), SH 8 (16.67 mg/g), SH16 (15.67 mg/g), SH4 (14.71 mg/g), SH9 (13.94 mg/g), SH11 (12.33 mg/g). Since these hybrids are already proven in the field for their yield they are now qualified for their high quantity of proteins.

The carbohydrate content of F₂ ranges from 122.2 mg/g to 176.07 mg/g, whereas check show 150.54 mg/g (CH-I) and 145.28 mg/g (CH-II). In case of car-

bohydrate content F₂ has shown superiority over parents and their F₁. Eight hybrids have shown superiority over best check in case of carbohydrate content, i.e. SH5 (198.92 mg/g), SH15 (181.37 mg/g), SH1 (176.08 mg/g), SH6 (175.02 mg/g), SH8 (174.52 mg/g), SH12 (159.04 mg/g), and SH13 (157.24 mg/g). These hybrids may prove themselves to be good carbohydrate sources.

Ascorbic acid is known as the source of vitamin - C present in maize, and serves as one of the nutritional parameters. Parents have shown superiority over F₁ and F₂ in case of Ascorbic acid content. Among Parents, it ranges from 32.62 to 396.11 mg/g. These findings correlate with the observations of Seebauer *et al.* (2010). The amount of quality traits has been reduced in hybrids compared to the wild varieties, land races and open-pollinated varieties. (Seebauer *et al.*, 2010). So, we can use the parental lines with good ascorbic acid content for the breeding program with good combining-ability lines in order to increase the traits that contribute to the quality parameters in the hybrids.

The Amino acid content in F₂ ranges from 36.85 to 160.31 mg/g. 13 hybrids have shown superiority over best check in the total Amino acid content, i.e. SH4 (154.18 mg/g), SH6 (137.71 mg/g), SH13 (137.58 mg/g), SH16 (134.66 mg/g), SH15 (132.49 mg/g), SH8 (131.22 mg/g), SH2 (130.48 mg/g), SH11 (128.00 mg/g), SH7 (126.19 mg/g), SH1 (123.06 mg/g), SH10 (122.33 mg/g), SH3 (122.25 mg/g), and SH 14 (121.4 mg/g).

Further, in periods of famine or economic stress, maize may be the only food available. Modified maize with increased protein content will help in the recovery of malnourished children and other victims of protein deficiency. When selecting a grain for yield alone, the genetic gain is always superior to one with multiple characters or traits. Adding protein content to yield as a selection criterion slows down the breeding progress because of its low heritability (Rosales *et al.*, 2011).

CONCLUSIONS

The conclusion and recommendations pertain to genotype selection for high-yielding and nutrient-rich maize hybrids for cultivation. Since the sixteen Maize hybrids were already proven for their yield, the present study was focused on finding hybrids that can add nutritional factors in addition to yield. Improvement in terms of quality and quantity is the only solution to meet the increasing demand for food and to overcome malnutrition.

The 14 hybrids, namely SH3 (30.0 mg/g), SH1

(23.17 mg/g), SH2 (22.90 mg/g), SH12 (20.97 mg/g), SH5 (20.15 mg/g), SH 10 (19.23 mg/g), SH15 (19.11 mg/g), SH13 (18.66 mg/g), SH14 (17.84 mg/g), SH8 (16.67 mg/g), SH16 (15.67 mg/g), SH4 (14.71 mg/g), SH9 (13.94 mg/g), and SH11(12.33 mg/g) can be recommended for cultivation and farmer adaptation based on their protein content.

Seven hybrids i.e. SH5 (198.92 mg/g), SH 15 (181.37 mg/g), SH1 (176.08 mg/g), SH6 (175.02 mg/g), SH8 (174.52 mg/g), SH12 (159.04 mg/g), and SH13 (157.24 mg/g) have shown superiority over checks in terms of carbohydrate content.

Thirteen hybrids have shown superiority in case of Amino acid content. They are SH4 (154.18 mg/g), SH6 (137.71 mg/g), SH13 (137.58 mg/g), SH16 (134.66 mg/g), SH15 (132.49 mg/g), SH8 (131.22 mg/g), SH2 (130.48 mg/g), SH11 (128.00 mg/g), SH7 (126.19 mg/g), SH1 (123.06 mg/g), SH10 (122.33 mg/g), SH3 (122.25 mg/g), and SH 14 (121.4 mg/g).

From the above findings it can be concluded that all the hybrids may not have all the nutritive factors traits. The four hybrids i.e. SH1, SH8, SH13, and SH15 have shown superiority in maximum quality parameters (Protein, carbohydrate, Amino acid content) associated with high yield. Therefore, we can recommend these four Maize hybrids for cultivation. This will help farmers to get good yield and offer the much-needed nutritional quality.

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