Table 1. Replicated data fr F_2 progenies of sorghum cross CO 32 × Paiyur 2

Genotypes	SPY	SPY	SPY	MEAN SPY	TC	TC	TC	MEAN TC	AC	AC	AC	MEAN AC	TP	TP	TP	MEAN TP	TPC	TPC	TPC	MEAN TPC	TFC	TFC	TFC	MEAN TFC
III-1	43.20	44.50	45.00	44.20	66.20	65.50	65.50	65.73	35.00	36.20	36.70	35.95	3.50	3.40	3.40	3.42	26.00	26.80	26.80	26.54	8.50	8.20	8.60	8.43
III-4	48.70	50.00	50.70	49.80	60.50	59.80	59.50	59.92	39.80	40.50	40.90	40.39	5.10	4.90	4.90	4.97	24.00	24.50	24.80	24.43	11.60	11.70	11.70	11.67
III-16	39.00	38.50	38.30	38.60	65.10	64.00	64.70	64.61	29.00	28.00	28.30	28.45	3.70	3.50	3.80	3.65	22.00	21.50	21.70	21.72	5.80	5.60	5.70	5.70
III-28	51.00	52.80	53.10	52.30	63.80	64.20	64.00	64.01	37.50	38.50	38.30	38.11	4.00	4.20	3.90	4.02	19.00	19.60	19.80	19.47	9.00	8.80	9.00	8.94
III-46	39.50	40.00	40.80	40.10	74.20	73.50	73.70	73.80	39.10	40.00	40.00	39.70	6.60	6.50	6.60	6.57	8.00	8.30	8.50	8.27	6.40	6.80	6.70	6.63
III-50	45.00	46.30	46.10	45.80	64.20	64.80	64.80	64.61	23.50	24.80	23.80	24.02	8.00	8.20	8.00	8.08	12.00	12.50	12.40	12.31	7.50	7.60	7.60	7.57
III-53	46.20	47.00	47.50	46.90	58.20	59.30	59.20	58.90	38.00	39.20	39.20	38.80	2.60	2.80	2.70	2.71	20.00	20.80	20.90	20.57	10.70	10.90	10.80	10.81
III-55	57.50	58.90	59.10	58.50	72.50	73.00	72.80	72.78	44.00	45.50	45.00	44.82	2.90	3.10	2.90	2.97	30.00	29.80	29.90	29.89	8.60	8.90	8.70	8.72
III-56	50.00	50.60	51.50	50.70	20.10	20.20	20.00	20.12	18.50	19.60	19.00	19.02	4.00	4.10	4.10	4.08	43.00	43.90	43.90	43.62	11.00	11.50	11.40	11.31
III-67	53.00	53.70	54.10	53.60	64.70	65.00	65.10	64.92	45.00	46.20	45.60	45.61	8.20	8.50	8.30	8.34	10.00	10.40	10.50	10.29	9.50	10.00	10.10	9.87
III-85	56.00	57.20	57.50	56.90	64.10	63.50	63.80	63.80	28.20	29.50	28.70	28.80	10.20	10.80	10.40	10.48	10.20	10.80	10.60	10.52	8.20	7.80	8.20	8.08
III-115	41.00	41.50	42.60	41.70	30.20	30.80	31.80	30.94	14.50	15.80	15.20	15.16	6.40	6.70	6.80	6.62	13.00	13.50	13.20	13.22	7.30	7.80	7.60	7.57
III-140	55.50	56.80	56.60	56.30	98.80	99.40	99.20	99.14	55.00	56.30	55.60	55.63	11.10	10.90	10.90	11.05	13.00	13.40	13.10	13.18	8.40	8.30	8.40	8.36
III-149	42.00	43.10	43.30	42.80	38.70	39.50	39.40	39.20	37.00	38.20	37.80	37.66	9.20	9.60	9.30	9.37	9.50	10.30	9.90	9.92	6.00	6.50	6.10	6.20
III-167	47.00	48.10	48.60	47.90	72.50	73.20	73.20	72.98	49.00	50.50	49.90	49.82	4.00	4.50	4.40	4.31	14.00	14.70	14.40	14.37	11.10	11.40	11.20	11.24
III-171	44.70	45.80	46.00	45.50	56.80	57.50	57.50	57.43	27.50	29.00	28.40	28.29	9.70	10.10	9.90	9.91	3.50	3.70	3.60	3.61	3.30	3.50	3.40	3.41
III-174	49.80	50.60	50.80	50.40	54.90	55.50	55.30	55.22	36.20	37.40	37.30	36.98	3.60	3.90	3.80	3.77	14.00	15.00	14.50	14.51	7.00	7.60	7.50	7.35

Table 1 (Comtd.,). Replicated data fr F_2 progenies of sorghum cross CO 32 × Paiyur 2

Genotypes	TTC	TTC	TTC	MEAN TTC	TAC	TAC	TAC	MEAN TAC	Fe	Fe	Fe	MEAN Fe	Zn	Zn	Zn	MEAN Zn	DPPH	DPPH	DPPH	MEAN DPPH
III-1	16.99	17.35	17.47	17.23	2.87	3.14	3.18	3.01	953.51	1016.84	973.11	934.76	638.77	638.68	631.13	623.55	78.74	85.09	85.62	84.77
III-4	14.66	15.68	14.92	15.17	1.02	1.07	1.09	1.08	808.03	781.42	712.45	772.15	52.08	60.87	59.68	59.05	83.29	75.63	85.12	80.27
III-16	12.25	11.53	12.57	11.85	0.20	0.20	0.20	0.20	1053.87	1031.62	991.52	1067.07	96.96	90.43	106.68	95.60	70.29	87.27	72.72	76.13
III-28	12.43	13.43	12.15	12.43	1.41	1.28	1.14	1.23	1120.42	943.94	1057.95	1048.89	75.32	78.41	73.61	74.85	44.86	47.45	49.07	46.56
III-46	4.65	4.92	4.87	4.71	0.81	0.97	0.85	0.86	845.12	777.82	717.86	796.39	155.96	153.85	146.45	153.85	41.43	42.79	47.07	45.59
III-50	8.34	7.71	8.76	8.33	1.68	1.61	1.70	1.71	1028.75	1003.91	990.01	1013.71	136.15	128.55	137.44	140.35	76.20	74.37	68.45	72.25
III-53	12.48	11.47	13.76	12.92	0.83	0.84	0.90	0.86	981.34	954.16	1112.39	1004.40	760.90	809.14	811.45	764.23	81.08	77.64	80.65	74.77
III-55	17.84	18.33	18.43	18.84	0.52	0.57	0.53	0.53	876.99	947.94	851.82	858.00	90.29	91.74	87.63	90.75	86.24	88.66	78.13	88.56
III-56	16.77	16.58	16.76	16.88	1.01	1.00	1.06	0.98	847.26	816.91	846.03	859.75	212.27	210.50	201.20	200.43	86.07	85.15	82.73	84.77
III-67	7.04	6.96	6.86	6.91	2.18	2.02	1.99	1.99	1006.50	923.14	990.08	1007.48	660.32	587.98	664.20	652.85	50.10	49.70	46.74	49.82
III-85	5.05	5.23	4.97	5.05	1.02	0.99	0.90	0.98	1030.57	1031.82	1185.45	1094.84	332.31	308.71	281.07	308.75	78.70	92.62	74.08	77.93
III-115	7.72	7.96	7.28	7.35	2.31	2.19	2.22	2.14	787.04	776.40	854.82	787.30	137.67	156.67	143.88	140.70	38.77	31.09	33.78	34.14
III-140	4.61	4.73	5.01	4.75	0.16	0.16	0.16	0.15	774.23	862.98	793.68	792.35	99.26	93.02	103.29	98.10	45.34	43.43	41.14	41.17
III-149	5.53	5.53	5.41	5.39	0.08	0.07	0.07	0.08	940.21	857.53	1002.76	972.13	229.59	250.77	223.17	234.05	79.37	79.98	78.48	80.18
III-167	16.49	14.33	16.24	16.25	0.08	0.09	0.08	0.08	945.49	1041.17	1073.62	1011.01	120.24	111.56	112.93	115.20	76.22	76.05	79.51	74.70
III-171	1.58	1.58	1.38	1.53	1.39	1.27	1.21	1.23	995.02	1015.97	938.31	953.44	76.13	84.29	83.93	79.75	10.13	10.58	10.86	10.63
III-174	8.49	8.74	9.32	8.81	0.89	0.85	0.93	0.88	821.47	925.64	845.11	866.58	67.32	63.48	68.69	62.60	62.39	61.93	66.52	64.86

Table 2. Replicated data for F_3 progenies of sorghum cross CO 32 × Paiyur 2

Genotypes	SPY	SPY	SPY	MEAN SPY	TC	TC	TC	MEAN TC	AC	AC	AC	MEAN AC	TP	TP	TP	MEAN TP	TPC	TPC	TPC	MEAN TPC	TFC	TFC	TFC	MEAN TFC
III-1	41.6	42.6	43.6	42.6	70.21	71.34	74.8	72.12	40.92	41.3	42.4	41.54	5.73	5.21	6.1	5.68	30.4	30.2	31.2	30.60	10.2	10.6	10.8	10.53
III-4	49.2	50.2	51.2	50.2	60.3	61.39	60.4	60.70	41.3	42.4	41.8	41.83	5.3	5.2	5.6	5.37	26.2	24.3	28.2	26.23	12.1	12.3	12.4	12.27
III-16	37.4	38.4	39.4	38.4	68.3	64.6	67.3	66.73	25.4	28.2	24.7	26.10	4.2	4.4	4.8	4.47	22.1	21	23.4	22.17	10.2	10.8	11.2	10.73
III-28	46.1	47.1	48.1	47.1	65.1	64.3	66.2	65.20	40.2	40.4	42.4	41.00	4.8	4.6	4.5	4.63	20.2	24.2	22.4	22.27	12.4	12.6	12.3	12.43
III-46	43.7	44.7	45.7	44.7	75.2	76.3	77.8	76.43	45.3	46.4	48.2	46.63	7.1	7.3	6.8	7.07	10.2	11.4	10.8	10.80	10.4	11.2	10.8	10.80
III-50	41.6	42.6	43.6	42.6	68.2	69.3	65.2	67.57	28.2	26.4	27.3	27.30	7.3	7.6	7.8	7.57	13.5	14.2	14.6	14.10	8.2	8.1	8.7	8.33
III-53	47.3	48.3	49.3	48.3	65.3	64.2	65.4	64.97	40.2	42.3	44.8	42.43	3.1	3.4	3.8	3.43	25.4	26.2	28.4	26.67	12.2	12.3	12.5	12.33
III-55	58.4	59.4	60.4	59.4	78.3	78.2	76.3	77.60	42.3	43.1	40.4	41.93	3.5	3.2	3.1	3.27	35.4	34.2	36.8	35.47	10.3	10.4	10.7	10.47
III-56	45.2	46.2	47.2	46.2	35.2	36.3	38.4	36.63	25.8	26.3	27.4	26.50	4.5	4.8	5.1	4.80	40.1	42.1	40.3	40.83	12.1	12.6	12.8	12.50
III-67	46.9	47.9	48.9	47.9	70.2	72.1	74.3	72.20	46.3	48.2	47.2	47.23	8.61	8.72	8.53	8.62	11.2	11.9	12.3	11.80	10.5	10.6	10.1	10.40
III-85	54.9	55.9	56.9	55.9	66.2	68.2	67.3	67.23	30.1	32.3	31.4	31.27	12.1	10.4	12.5	11.67	15.2	15.1	15.5	15.27	14.4	14.8	14.6	14.60
III-115	45.5	46.5	47.5	46.5	42.2	44.5	45.7	44.13	18.2	21.21	20.8	20.07	7.3	8.1	8.2	7.87	18.1	18.5	20.2	18.93	8.1	8.5	8.9	8.50
III-140	58.8	59.8	60.8	59.8	60.2	61.3	63.4	61.63	60.2	61.3	64.4	61.97	10.1	11.3	11.4	10.93	10.2	10.4	10.3	10.30	9.1	10.4	10.8	10.10
III-149	48.2	49.2	50.2	49.2	42.4	41.3	44.3	42.67	40.2	41.3	44.4	41.97	10.8	9.6	10.5	10.30	10.2	11.1	10.8	10.70	8.3	8.2	8.4	8.30
III-167	52.7	53.7	54.7	53.7	75.3	76.4	78.2	76.63	50.2	51.3	51.4	50.97	5.2	5.6	5.8	5.53	18.2	20.4	21.2	19.93	15.3	14.6	14.1	14.67
III-171	51.6	52.6	53.6	52.6	65.2	65.6	68.4	66.40	30.2	32.4	31.4	31.33	12.1	12.8	12.6	12.50	8.2	8.3	8.8	8.43	7.8	7.5	7.6	7.63
III-174	53.3	54.3	55.3	54.3	60.2	61.4	62.3	61.30	40.1	41.2	41.8	41.03	4.1	4.2	4.5	4.27	16.2	18.1	18.5	17.60	10.1	10.2	10.6	10.30

Table 2 (Comtd.). Replicated data for F_3 progenies of sorghum cross CO 32 × Paiyur 2

Genotypes	TTC	TTC	TTC	MEAN TTC	TAC	TAC	TAC	MEAN TAC	Fe	Fe	Fe	MEAN Fe	Zn	Zn	Zn	MEAN Zn	DPPH	DPPH	DPPH	MEAN DPPH
III-1	20.2	20.4	20.8	20.47	4.1	4.3	4.1	4.17	950.1	951.2	956.4	952.57	650.2	656.4	653.1	653.23	88.2	86.4	86.1	86.90
III-4	16.2	16.6	16.8	16.53	2.1	2.5	2.6	2.40	780.1	793.2	796.4	789.90	83.2	81.4	80.6	81.73	82.4	83.1	84.2	83.23
III-16	12.2	12.1	12.4	12.23	1.1	1.3	1.5	1.30	1124.1	1106.2	1123.2	1117.83	110.3	114.2	112.1	112.20	80.1	81.4	82.6	81.37
III-28	14.4	14.6	14.8	14.60	2.4	2.8	2.2	2.47	1081.3	1056.3	1066.4	1068.00	80.4	83.4	84.1	82.63	50.4	51.2	52.4	51.33
III-46	5.3	5.4	5.2	5.30	1.5	1.6	1.8	1.63	800.2	810.2	814.3	808.23	181.3	183.4	181.1	181.93	52.4	51.3	50.6	51.43
III-50	10.1	10.2	10.3	10.20	3.81	3.21	3.56	3.53	1124.2	1021.2	1041.5	1062.30	163.2	164.2	166.1	164.50	78.4	76.4	74.3	76.37
III-53	14.3	14.4	14.7	14.47	1.4	1.5	1.6	1.50	1015.7	1016.2	1018.4	1016.77	780.1	784.2	783.7	782.67	80.1	81.3	80.6	80.67
III-55	20.4	20.2	20.3	20.30	2.2	2.4	2.6	2.40	900.3	912.4	916.4	909.70	120.1	124.2	126.8	123.70	90.4	92.3	94.6	92.43
III-56	18.1	18.4	18.6	18.37	1.1	1.2	0.4	0.90	864.2	862.4	880.2	868.93	210.2	214.3	215.6	213.37	88.3	86.4	88.2	87.63
III-67	8.2	8.3	8.5	8.33	2.6	2.1	2.3	2.33	1018.2	1026.4	1031.4	1025.33	700.4	715.5	716.4	710.77	53.2	52.1	54.6	53.30
III-85	6.1	6.3	6.4	6.27	1.3	1.8	1.5	1.53	1121.4	1134.6	1128.2	1128.07	312.1	314.4	318.8	315.10	80.4	82.1	80.3	80.93
III-115	10.2	10.1	10.4	10.23	0.2	0.3	0.2	0.23	801.3	805.6	808.9	805.27	150.4	151.3	154.2	151.97	40.4	41.4	42.4	41.40
III-140	6.2	6.4	6.5	6.37	0.5	0.1	0.23	0.28	802.4	809.2	806.4	806.00	100.2	101.4	103.2	101.60	50.4	53.2	52.1	51.90
III-149	7.1	7.8	7.5	7.47	0.1	0.18	0.16	0.15	1012.1	1014.3	1016.2	1014.20	250.3	253.2	251.2	251.57	82.1	84.3	84.5	83.63
III-167	18.1	18.4	18.6	18.37	0.2	0.3	0.21	0.24	1024.3	1026.8	1023.1	1024.73	120.3	121.4	124.6	122.10	81.3	80.4	80.6	80.77
III-171	2.4	2.8	2.6	2.60	0.4	0.6	0.9	0.63	1016.4	1014.3	1012.4	1014.37	84.3	80.2	81.4	81.97	12.4	12.6	14.3	13.10
III-174	10.4	10.2	10.1	10.23	1.6	1.4	1.2	1.40	890.2	891.4	873.2	884.93	70.3	73.4	74.3	72.67	80.2	81.3	84.2	81.90

Detailed procedure for biochemical analysis

Protocol followed for total carbohydrate estimation

Step 1: 50 mg of the samples of the selected genotypes were weighed and placed into respective boiling tube. Then they were hydrolysed by keeping it in a boiling water bath for 1-2 hr with 5 mL of 2.5 N-HCl and then cooled to room temperature. The neutralization was performed by adding solid sodium carbonate until the effervescence ceased. The volume was adjusted to 100 mL, and the mixture was centrifuged.

Step 2: The supernatants were collected, and a 0.5 mL aliquot were taken for analysis. The volume in all the sample tubes was adjusted to 1 mL by adding distilled water. Then, 4 mL of anthrone reagent was added. The heat generated by the exothermic reaction was cooled down by placing the tubes under running water, and the developed green to dark green color was read at 630 nm.

Protocol followed for estimation of soluble protein

Step 1: Extraction of protein from sample was carried out with phosphate buffer (pH = 6.5) used for the enzyme assay. A quantity of 500 mg of the fine seed powder was weighed and thoroughly ground with a pestle and mortar in 5-10 mL of the buffer. The content was then centrifuged, and the supernatant was used for protein estimation.

Step 2: 0.1 mL of the sample extract was pipetted out into each test tube. The volume was then adjusted to 1 mL in all the test tubes. To 1 mL of this protein sample, 5 mL of reagent C was added, mixed well and was kept at room temperature for 10 min. Then, 0.5 mL of reagent D was added and the content was mixed immediately on the vortex mixture. After 30 min, absorbance was read at 660 nm.

Table1. Reagent used for estimation of soluble protein

S.No	Chemical	Preparation
1.	**Reagent A**	2 % Sodium Carbonate in 0.1 N Sodium Hydroxide
2.	**Reagent B**	0.5% Copper Sulphate in 1% potassium sodium tartarate
3.	**Reagent C**	Mix 50 ml of a and 1ml of B prior to use.
4.	**Reagent D (FC Reagent)**	Folin's Phenol Reagent is mixed with distilled water in the ratio 1:2.
5.	**Stock Solution**	Dissolve 50mg BSA in 50ml of distilled water in a volumetric flask (concentration 1 µg /ml).
6.	**Working Standard**	The Stock Solution of 10 ml is distilled to 50 ml with distilled water in a standard flask (concentration 200 µg/ml).

Protocol followed for estimation of amylose content

Sample preparation: 50 mg of powdered sorghum seed sample of each selected genotype was transferred to a 25 mL of volumetric flask followed by addition of 2 mL of 80 % ethanol and 5 mL of 1 N NaOH. All the test tubes were then placed in water bath for 15 min to boil. The contents were then cooled down to room temperature and the volume was made up to 25 mL with distilled water.

Procedure: 0.5 mL aliquot sample of each genotype was pipetted out into separate test tube and the volume was made to 2.5 mL with distilled water followed by addition of 2-3 drops of phenolphthalein indicator. Then, 0.1 N hydrochloric acid (HCl) was added incrementally, drop by drop, until the pink color just vanished. Following that step, 0.1 mL of iodine reagent was incorporated, and the final volume was adjusted to 10 mL with water. The optical density (OD) was measured at

Table 2. Reagent used for estimation of amylose content

S.No	Chemical	Preparation
1.	**1N Sodium Hydroxide**	Dissolve 4 g of NaOH in 100 ml of distilled water.
2.	**Phenolphthalein indicator**	0.5% Copper Sulphate in 1% potassium sodium tartarate
3.	**0.1 N HCl**	Take 2.1 ml of concentrated HCl with a pipet, and dilute to 250 ml with distilled water in a 250 ml volumetric flask
4.	**Iodine reagent**	Dissolve 1 g of iodine and 10 g KCl in water and then make up to 500 ml.
5.	**Stock Solution**	Dissolve 20mg of amylose in little amount of distilled water and make up the volume to 20 ml in a standard flask.
6.	**Working Standard**	The Stock Solution of 10 ml is distilled to100 ml with distilled water in a standard flask (concentration 100 µg/ml).

Protocol followed for polyphenol estimation

Sample preparation - Preparation of the methanolic extract as mentioned above with 1 % acidified methanol

Methanolic extraction of polyphenols by maceration (MAE) using methanol as solvent

Before the extraction of polyphenols samples were defatted twice with hexane and were dried in the fume hood overnight. Further, extraction was carried out by maceration by using 1 % acidified methanol (acidified with HCl). For maceration extraction, 30 mL (v/v) of solvent was added to 1 g of defatted sample and kept for 2 h at low speed in orbital shaker and samples were centrifuged at 2790 g for 10 min. This process was repeated twice to the residue by addition of another 30 mL of solvent. After the extraction process, the extracts were evaporated to reduce the volume to 10-15 mL using a rotary flash evaporator. The extract then obtained were transferred to falcon tube and final volume was made to 15 mL. The extracts were then stored at −20 °C till further analysis.

Procedure followed for the polyphenol estimation: In separate test tubes, 0.1 mL of the individual sample extracts were pipetted out, and the volume was adjusted to 3.5 mL. Subsequently, each sample was mixed with 0.5 mL of the Folin-Ciocalteu reagent, followed by the addition of 2 mL of % Na_2CO_3 after a five-minute interval. The mixture was thoroughly blended. The test tubes were immersed in a water bath for one minute, and subsequently, the absorbance of each sample was measured using a UV-Vis Spectrophotometer at 660 nm.

Table 3. Reagent used for the polyphenol estimation

S.No	Reagent	Preparation
1.	80 % Ethanol	80 ml of ethanol in 100 ml of distilled water
2.	Folin-Ciocalteu reagent	Dissolve 10 g of sodium tungstate and 2.5 g of sodium molybdate in 70 ml of water. Add 5 ml of 85% phosphoric acid and 10 ml of concentrated hydrochloric acid. Reflux for 10 hours. Add 15 g of lithium sulfate, 5 ml of water, and 1 drop of bromine. Reflux for 15 minutes. Cool to room temperature and bring to 100 ml with water. Commercially available, dilute it in 1:2 ratio using distilled water.
3.	20 % Na_2CO_3	20g of Na_2CO_3 dissolved in 100 ml of distilled water
4.	Gallic acid stock solution	100 mg catechol in 100 ml of ethanol
5.	Working standard	Dilute the stock 10 times

Protocol followed for flavonoid estimation

Sample preparation: The sample was extracted by the common methanolic extraction

Procedure followed for the flavonoid estimation: For standard, extract volumes ranging from 0.1 mL to 0.5 mL of the quercetin working standard solutions (i.e., 100 µg to 500 µg) were transferred into a series of test tubes. For sample, 0.2 mL of the extracts were dispensed into their respectively labelled test tubes. The volume was then adjusted to 3 mL using distilled water across all the test tubes, including both the blank and the standards. Following this, 1.5 mL of methanol, 0.1 mL of $AlCl_3$, and 0.1 mL of potassium acetate solution were added to each test tube, and thorough mixing ensued. The solutions in all the test tubes were filtered through Whatman filter paper before measuring the optical density at 415 nm with the help of UV-Vis Spectrophotometer.

Table 4. Reagent used for flavonoid estimation

S.No	Reagent	Preparation
1.	1% acidified methanol	1ml of 100 % methanol in 99 ml of distilled water
2.	1% Aluminium chloride	Dissolve 1 g of $AlCl_3$ in 100 ml of distilled water
3.	1M Potassium acetate	Dissolve 98.15 g of CH_3COOK in 100 ml of distilled water
4.	Quercetin stock sol.	100 mg of quercetin in 100 ml of methanol
5.	Working standard	10 ml of stock solution diluted to 100 ml using methanol

Protocol followed for tannin estimation

Sample preparation: The sample was extracted by the common methanolic extraction

Procedure followed for the tannin estimation: For standard, extract volumes ranging from 0.1 mL to 0.5 mL of the tannic acid working standard solutions (i.e., 5 µg to 25 µg) were transferred into a series of test tubes. For sample, 0.1 mL of the extracts were pipetted out into their respectively labelled test tubes. The final volume was made upto 7.5 mL by adding distilled water to all the test tubes including the standards. The blank was prepared with 7.5 mL of distilled water. Add 0.5 mL of Folin-Denis reagent followed by 1.0 mL of sodium carbonate solution. To all the test tubes 0.5 mL of Folin-Denis reagent was added followed by addition 1.0 mL of sodium carbonate solution. The test tubes were shaken well and the absorbance was noted at 700 nm after 30 min of incubation (in dark) using spectrophotometer.

Table 5. Reagent used for tannin estimation

S. No	Reagent	Preparation
1.	Folin- Denis reagent	100 g sodium tungstate + 20 g phosphomolybdic acid dissolved in 750 ml distilled water to which 50 ml phosphoric acid was added. After reflux of 2 hours, the volume was made 1000 ml with distilled water
2.	Sodium Carbonate solution	350 g sodium carbonate dissolved in one litre of distilled water at 70° - 80° c. The solution was filtered through glass wool and allowing it to stand overnight.
3.	Standard tannic acid solution	100 mg tannic acid in 100 ml of distilled water.
4.	Working standard solution	Dilute 5 ml of the stock solution to 100 ml with distilled water (1ml contains 50 µg of tannic acid).

Protocol followed for anthocyanin estimation

Sample preparation: The sample was extracted by the common methanolic extraction.

Procedure followed for the anthocyanin estimation: 1 mL of each sample was pipetted out in their respectively numbered test tubes in two replicates. Each set of replicates were then added with 3 mL of pH 1.0 buffer and pH 4.5 buffer respectively. Multiwavelength absorbance were then recorded for all the test tubes at two OD values i.e., 515 nm and 700 nm, respectively.

Reagents used for Anthocyanin estimation

1. pH 1.0 buffer (potassium chloride, 0.025M): weigh 1.86 g of KCl into a beaker and add distilled water to 980 mL. Measure the pH and adjust the pH to 1.0 (±0.05) with HCl/NaOH. Transfer to a 1 L volumetric flask and dilute to volume with distilled water.

2. pH 4.5 buffer (sodium acetate −0.4M): weigh 54.43 g of $CH_3CO_2Na{\cdot}3H_2O$ in a beaker, add distilled water to 960 mL. Measure the pH and adjust pH to 4.5 (+0.05) with HCl/NaOH. Transfer to a 1 L volumetric flask and dilute to volume with distilled water.

Protocol followed for the estimation of micro-nutrient (Fe and Zn):

Acid digestion: 200 mg of the powdered sample from each genotype were taken in a series of 100 mL conical flasks. Then, 25 mL of triacid mixture was added to each flask. The mouth of the conical flask was then plugged in with cotton and left undisturbed overnight for digestion. After digestion the conical flasks were placed in a sand bath (next day) for 1-2 hr till a clear solution appeared. The clear solution thus obtained was diluted to a final volume of 100 mL with distilled water. The diluted samples were used to estimate the micronutrients with the help of AAS. Iron and zinc in acid digested samples were determined by Atomic Absorption Spectrophotometer.

Protocol followed for total A.O activity:

0.1 mL of each sample extract was pipetted out in their respectively numbered test tubes and volume was made up to 1 mL with methanol. It was followed by addition of 1 mL of 0.02 mM of DPPH solution. All the samples were then incubated in dark for 30 min. It was followed by measuring the absorbance at 517 nm using UV-Vis spectrophotometer. Distilled water was used as blank and methanol added with DPPH acted as control.