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Effect of Elevated Atmospheric CO_2 on the Nutritional Composition of *Gossypium Hirsutim L.* Seed (Upland Cotton)

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ABSTRACT

Plants quality (both chemical and physiological) and the subsequent effects of CO_2 shifts in the atmosphere is a global challenge. Enhanced atmospheric CO_2 decreases the nutritive value of many plant foods. Cottonseed is an important ingredient in animal feed and possesses potential utility in human diets. The current study investigated the effects of CO_2 the on nutrient composition of cottonseed varieties commonly grown in Pakistan. The study investigated the effect of elevated of CO_2 on total yield, ginning out turn, seed index, seeds volume, proximate composition (g/100g), mineral concentrations (mg/kg^{-1}) and amino acids content (g/16gN) of 16 cotton cultivars grown at two partial pressures of CO_2; ambient [aCO_2 – 400µmol mol^{-1}] and elevated [eCO_2 – 800µmol mol^{-1}]. Results indicated an overall 25.51% increase in the total yield, G.O.T % increased by 5.73%, while seed index of the fuzzy seeds by increased 9.5% from the elevated CO_2 chamber. Total protein concentrations in whole seed, linted seed meal and delinted seed meals were significantly decreased with greater intra-varietal coefficient of variation. Accordingly, significant decline in the crude fiber and ash % were also observed. CO_2 enrichment significantly increased the percent oil content (13.33%) from the whole seed with higher intra varietal response variations. CO_2 enhancement resulted in significant decline in calcium, iron, Zinc Copper, Manganese. Most of the proteinogenic amino acids concentrations were reduced significantly. Reduction in the individual amino acids ranged from 13.26% - 1.28%. It is concluded that CO_2 elevation may improve total cotton yield but with altered nutrient composition of the cottonseed.

KEY WORDS: seed quality, proximate composition, mineral concentrations, amino acids.

1. INTRODUCTION

Carbon dioxide is an important gas and its atmospheric increase has occurred since industrial revolution affecting agricultural crops and other ecosystems. Atmospheric CO_2 is expected to rise further with an increasing atmospheric temperature [1]. The elevated CO_2 combined with other climate changes will affect crop yield and seed quality along with the changes in the chemical composition of the crops and seeds [2]. In spite of the differences in plants responses; elevated CO_2 will affect vegetation, C_3 crops such as soybean, barley, wheal, rice and C_4 crops such as sugar beet [3- 7]. As presumed crops might benefit from doubling of the atmospheric CO_2 leading to 30-40% greater crop yield however, the confounding effects of the CO_2 elevation on the nutrient composition shall not be over looked [8, 9].

The rise in the atmospheric CO_2 stimulate plants to store up more carbohydrates with a decrease in macromoles such as proteins and micro nutrients such as Zn, Fe, Mg, Ca [10]. Even a subtle change in the protein and other nutrients will affect human health, ecosystems and will pose a challenge to the breeders [11].

Belonging to the *Malvaceae* family and a tribe of *gossypieae*, *Gossypium* (cotton) grows naturally as a perennial shrub or tree. However, commercially it is being grown as an annual crop [12]. It is major cash crop known to be the source of world's most important textile fiber, the second best source of plant protein after soybean and the fifth oil producing plant [12].

Gossypium Hirsutum L. or upland cotton is Pakistan's main commercial crop grown on the 15% of the arable land and cultivated by 1.6 million farmers. Being the fourth largest producer globally, Pakistan stands third in the world for its spinning capacity, yarn export with life of millions of farmers and work force dependent on it [13]. Cottonseed a raw agricultural produce which was once wasted, after oil extraction, is now converted into feed for livestock, food for human, fertilizer and a variety of useful

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products [12]. Cottonseed meal is valued as a protein feed [14], a best source of most essential amino acids and is a rich source of minerals [15].

Response studies on climate change and their subsequent effects on crops physiology and nutritive value has always remained a neglected area in Pakistan. Keeping in view the magnitude of the problem of climate change and food insecurity the current study was designed to investigate the effects of CO_2 elevation the nutritional quality of both conventional and biotech cotton cultivars commonly grown in Pakistan.

2. MATERIALS AND METHODS

i. CO_2 Exposure and Experimental Conditions

The experiment was conducted during the growing season of Cotton 2013 at the Institute of Cotton Research, Ayub Agricultural Research Institute at Faisalabad, Pakistan. About sixteen varieties (8 Non-Bt :FH-682, FH-942, FH-1000, FH-87, CIM-496, FH-900, FH-901, and 8 Bt: A-1, FH-172A, FH-113, SITARA-009, CIM-598, MNH-886, FH-114) *of G. Hirsutum L* were grown in two separate acrylic glass chambers positioned on a leveled ground to receive maximum solar energy with the soil parameters given in Table-1. Each cotton variety was grown in 5 consecutive pots at two levels of Co_2 [ambient 400 umol^{-1}] and [elevated 800 umol^{-1}]. At ambient the greenhouse had no CO_2 injection while in the elevated/experimental greenhouse CO_2 was released from CO_2 cylinders through perforated plastic pipes. These plastic pipes were fixed in a manner of frame and were adjusted at the plants canopy level throughout the growth from germination until the final harvest. In both of the chambers the concentration of the Co_2 was monitored regularly by a gas analyzer with multi IR detectors (Max-42A Oldham, France). The concentrations of the Co_2 were regularly cross checked with a handy NIR sensor CTSI'S IAQ CALS^{TM} Indoor Air Quality Meters 7515, USA). Throughout this growth period temperature was maintained at 34^{o}C-39^{o}C.

Table-1: Soil Parameters Used in the Experiment

Parameters	Concentrations
Electrical conductivity (ds/m)	2.4
pH	8.16
Organic matter %	0.67%
Total nitrogen %	0.023%
Available P (mg/kg)	21.9
Available K (mg/kg)	208.9
Textural class	loamy clay
Soil moisture %	

ii. Seed sample preparation

Mature cotton bolls were handpicked and stored in separate plastic bags to avoid moisture absorption. Later all the samples were ginned in a lab ginning machine to separate fibers and seeds. Both fibers and seeds were stored in plastic bags and containers for further analyses. All the analyses were performed in triplicates for each parameters.

iii. Seed Quality Parameters

For physical fractions seeds were analyzed as whole linted seeds, linted seed cake, while delinted seed meal was recovered by treating the seeds with conc. sulfuric acid, washing thoroughly with water, drying in oven till constant weight, grinding and sieving through 0.2 mm sieve. Seed index was estimated as weight of hundred healthy seeds selected randomly, while volume was measured by putting 100 seeds in a 100 CC volumetric cylinder and immersing the seeds with ethanol from a 50CC burette till volume of 40 was achieved. The amount of ethanol displaced was estimated to be the volume of respective cottonseed samples. Percent dry matter was determined by weighing and placing the respective samples in lab oven at till constant weight were achieved.

iv. Proximate Analysis

Proximate composition of the Whole Linted Seeds (WLS), Linted Seed Meals (LSM), were obtained by grinding the linted seeds finely in a domestic heavy duty grinding machine (National Chopper and Grinder, 2002, Japan), while Delinted Seed Meals (DSM) were obtained by sieving the finely powdered acid delinted cotton seeds samples through a 0.2mm lab sieve. Analysis was performed as per AOAC procedures [16]. Results are presented as g/100g of samples.

v. Mineral analysis

Cotton samples were analyzed for one macro mineral Calcium (Ca), four micro minerals iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), and one heavy metal Cadmium (Cd) by AAS-Perkin Elmer, Aanalyst 700 atomic absorption spectrophotometer after digestion. About 0.5 mg of finely ground sample was digested with 10ml of acids mixtures (HNO3:HCL4 in the ratio of 5:1:1) at 80^{o}C. The digest was filtered with Whatman No.42 filter paper and was diluted with 50 ml of distilled water. Mineral concentrations (mg/kg-1) are expressed on a seed dry weight basis.

vi. Determination of Amino Acids (the statements in red colour are rephrased)

Chemicals and Reagents

Amino acid standard AAS-18-5m was purchased from Sigma Aldrich, Fluka, USA. Reagents and chemicals for HPLC and mobile phases were of reagent grades and were procured from Merck.

Instrumentation and Chromatographic Conditions

A gradient HPLC system (Agilent 1100 series, USA) equipped with fluorescent detector (G1315B), auto injectors, column compartment, quaternary pump and vacuum degasser was used. Separation of amino acids was achieved on XDB C18 column 2.1mm×15cm, 5µ particle size at 40ºC. Three mobile phases were employed to attain maximum resolution of the samples' components. Mobile phase-A consisted of 2.0N sodium citrate (P^H 3.20), $HClO_4$ and $Na_3C_6H_5O_3$-$2H_2O$. Mobile phase-B consisted of 0.6N sodium citrate (P^H -10), 02M H_3BO_3, NAOH, and $Na_3C_6H_5O_3$-$2H_2O$, Mobile phase-C contained 0.2M NaOH in distilled water. Derivatization of the reaction solutions-A consisted of 02.g sodium hypochlorite in a buffer solution of Na2Co3andH3BO3. Solution-B contained 0.4g OPA in 7ml ethanol, 0.5g N-Acetylecystein and 450 of the buffer solution. Samples were hydrolyzed with 6M Hcl overnight, reconstituted with 3ml Hcl, filtered through 0.22µm membrane filter and derivatized with reaction solutions. Samples were injected at a flow rate of 0.5ml/min for a run time of 25minutes at emission and excitation wavelengths of 350nm and 450nm respectively. Retention times were matched with the retention times of respective amino acids and quantified. The values obtained mg/kg of the sample were converted to g/16g N.

Vii. Statistical Analysis

Data obtained from all the triplicates assessments were analyzed for mean and standard deviation, Intra-varietal differences in the cultivars' responses were assessed by coefficient of variations, and test of significance ($P \leq 0.05$) for cultivars responses were obtained through an Analysis of Variance. Data was analyzed using IBM SPSS 20 package (SPSS Inc..USA, 2011).

3. RESULTS

i. Effect of CO_2 Elevation on Yield, Seed Quality and Seeds Physical Fractions.

The results obtained for total crop yield (Table-2) indicated that CO_2 elevation significantly ($P \leq 0.007$) increased the crop yield by 25.51. The percent linters as Ginning out Turn increased by 5.73% while seed index (weight (g) of 100 seeds) for the linted/fuzzy seeds showed significant (0.034) 9.50% gain and acid delinted seeds showed insignificant but positive changes (2.37%) respectively. The seed index and seed volume of delinted seeds did increased appreciably ($P \leq 0.05$). The physical fraction (expressed as percentages of fractions recovered from the weights of the cotton seeds) indicated insignificant positive effects of CO_2 on seed % linters, seed kernel/meal, and hull (data not shown here).

Table – 2: Effects of CO_2 Enhancement on the Seeds' Quality Parameters

Varieties	Main effects	Total Yield (gm)	G.O.T%	Seed Index linted/fuzzy seeds	Seed Index Delinted seeds	Seed Volume
FH-682 (NBt)	[a Co2]	66	37.9	10.0±0.11	8.44±0.06	7.1±0.02
	[e Co2]	73	39.0	10.2±0.65	8.59±0.09	7.2±0.19
	% Change	+9.58	+2.90	+2.00	+1.78	+1.4
FH-942 (NBt)	[a Co2]	71	41.0	8.80±0.91	8.70±0.25	6.5±0.06
	[e Co2]	85	42.5	10.70±0.58	8.94±0.04	6.7±0.02
	% Change	+16.70	+3.65	+8.50	+2.76	+2.9
FH-1000 (NBt)	[a Co2]	55	38.5	9.5±0.81	9.32±1.30	6.3±0.17
	[e Co2]	65	39.0	10.32±0.59	9.44±0.81	6.5±0.09
	% Change	+18.19	+1.29	+8.74	+1.29	+3.17
FH-87 (NBt)	[a Co2]	73	39.2	10.0±0.51	8.18±0.91	7.2±0.02
	[e Co2]	83	41.0	10.3±0.29	8.15±1.17	7.3±0.07
	% Change	+13.69	+4.59	+3.00	+0.04	+1.38
FH-634 (NBt)	[a Co2]	65	37.6	10.3±0.31	8.90±0.92	7.4±0.11
	[e Co2]	71	38.0	10.6±0.58	8.89±0.14	7.4±0.02
	% Change	+9.23	+1.06	+2.92	-0.11	0.00
CIM-496 (NBt)	[a Co2]	43	37.8	9.5±0.19	8.34±0.71	7.3±0.08
	[e Co2]	63	39.0	10.8±0.45	8.35±0.38	7.4±0.16
	% Change	+31.74	+3.53	+13.69	+0.012	+1.38
FH-900 (NBt)	[a Co2]	43	38.0	8.4±0.48	7.460±0.71	7.3±0.03
	[e Co2]	53	39.4	10.3±0.57	7.33.56	7.3±0.02
	% Change	+18.86	+3.68	+22.62	-1.78	0.00
FH-901 (NBt)	[a Co2]	61	40.0	10.2±0.37	8.42±0.29	7.2±0.04
	[e Co2]	75	41.0	10.8±0.11	8.66±1.02	7.3±0.02
	% Change	+22.95	+2.5	+5.89	+2.86	+1.38
A-1 (Bt)	[a Co2]	54	38.7	11.07±0.28	10.76±0.47	7.2±0.44
	[e Co2]	68	40.0	10.76±0.51	10.07±0.63	7.1±0.42
	% Change	+25.92	+3.35	+6.24	-6.41	-1.38
FH-142 (Bt)	[a Co2]	42	37.9	8.2±0.41	8.28±0.03	7.5±0.11
	[e Co2]	58	38.0	10.4±0.65	8.47±0.35	7.3±0.48
	% Change	+38.09	+0.26	+26.83	+2.30	-2.66
FH-172A (Bt)	[a Co2]	68	38.5	10.0±0.23	8.46±0.33	6.2±0.02
	[e Co2]	71	39.0	10.49±0.14	8.49±0.29	6.2±0.02

	% Change	+4.22	+1.29	+4.90	+0.35	0.00
FH-113 (Bt)	[a Co2]	83	39.7	9.5±0.62	8.35±1.19	6.3±0.21
	[e Co2]	87	41.0	12.4±0.25	8.58±0.43	6.5±0.37
	% Change	+4.82	+2.51	+30.53	+2.59	+3.17
Sitara-009 (Bt)	[a Co2]	63	38.6	9.8±0.31	8.48±0.62	6.3±0.05
	[e Co2]	71	39.0	10.2±0.15	8.57±0.02	6.3±0.02
	% Change	+12.69	+1.04	+3.50	+1.10	0.00
CIM-598 (Bt)	[a Co2]	62	39.1	10.0±0.19	8.21±0.50	6.3±0.06
	[e Co2]	71	39.5	10.8±0.25	8.22±0.02	6.2±0.27
	% Change	+14.52	+1.02	+7.41	-0.13	-1.58
MNH-886 (Bt)	[a Co2]	50	38.0	10.02±0.23	8.38±1.02	7.2±0.28
	[e Co2]	59	38.2	10.3±0.14	8.61±0.56	7.2±0.76
	% Change	+18.0	+0.53	+2.80	+2.75	0.00
FH-114 (Bt)	[a Co2]	62	38.2	9.5±0.53	8.6±0.27	6.5±0.14
	[e Co2]	69	39.0	10.9±0.41	9.1±0.71	6.8±0.25
	% Change	+11.29	+2.09	+14.75	+5.82	+4.41
Mean	[a Co2]	54.87	38.66	9.72±1.53	8.1±2.47	6.41±1.0
	[e Co2]	68.875	40.87	10.59±2.96	9.9±2.40	6.51±1.91
	% change	+25.51	+5.73	+9.50	+2.37	+1.69
	P level	0.007	0.116	0.134	0.18	0.33

ii. Effect of CO_2 Elevation on Proximate Composition.

Proximate composition of whole linted seed, linted seed meal and acid delinted seed meals of the cotton varieties showed considerable intra-varietal variations to CO_2 elevation. The mean effects (Table – 3) showed consistent decline in the crude protein at the elevated CO_2 concentration. The mean percent decline (11.09% and 1.16%) were highly significant ($P < 0.009$, 0.000, 0.000) in WLS, DSM, and LSM. The highest mean decline were 23.20% in the whole seed, 18.48% in the LSM to 17.78% from in DSM [eCO_2]. Crude fat (η-hexane extracted) varied considerably among the varieties however the net effect of CO_2 elevation was 13.32% increase in the percent oil of the whole seed. Similar trend were also observed in the LSM and DSM. Ash % throughout the seed and seed products (LSM and DSM) declined significantly at elevated CO_2 concentrations. (13.37%, 4.89%, 14.02% respectively).

Table – 3: Macronutrient Content (g/100g) of the Cotton Cultivars Grown Under Two Levels of CO_2 Concentrations

Parameters	Main Effects	Mean	Minimum	Maximum	SD	P level
Crude Protein%						
1. WLS	[aCO_2]	22.3829	20.07	27.35	1.63708	0.009
	[eCO_2]	22.2275	19.49	25.36	1.93090	
2. LSM	[aCO_2]	24.9356	20.95	29.89	2.79339	0.039
	[eCO_2]	26.8675	25.05	28.64	1.10074	
3. DSM	[aCO_2]	40.4131	26.80	46.10	4.73029	0.000
	[eCO_2]	44.8900	35.16	48.66	3.67301	
Crude Fiber%						
1. WLS	[aCO_2]	22.2081	18.13	26.83	2.08582	0.017
	[eCO_2]	23.8563	19.72	27.94	2.17655	
2. LSM	[aCO_2]	17.7844	16.06	19.84	1.09173	0.047
	[eCO_2]	18.4815	17.04	19.98	.88500	
3. DSM	[aCO_2]	3.7925	3.02	4.86	.54232	.003
	[eCO_2]	3.9781	3.02	5.51	.69357	
Crude Fat%						
1. WLS	[aCO_2]	23.8767	17.27	27.66	3.34231	0.032
	[eCO_2]	27.0563[a,b]	20.21	32.32	3.41336	
2. LSM	[aCO_2]	2.4663	1.60	2.99	0.38709	ns
	[eCO_2]	2.8656	2.00	3.90	0.53726	
3. DSM	[aCO_2]	5.6973	5.03	7.00	0.53231	0.041
	[eCO_2]	6.1681	3.18	7.11	0.99539	
Ash%						
1. WLS	[aCO_2]	4.3450	3.37	4.96	.36876	.017
	[eCO_2]	3.50	2.43	5.31	.78695	
2. LSM	[aCO_2]	3.89	5.96	4.9719	.57092	.000
	[eCO_2]	3.07	5.73	4.0806	.79482	
3. DSM	[aCO_2]	5.92	7.64	7.1512	.47416	.003
	[eCO_2]	5.09	7.19	6.2800	.63992	

WLS, Whole linted seed, LSM, Linted seed meal, DSM Decorticated seed meal Significance at P≤0.05 level

iii. Effect of CO_2 Elevation of Elemental Concentration (mg/Kg^{-1})

The effects of CO_2 enhancement on the mean elemental composition is presented in Table-4. The decline of 68.75 percent samples was observed in calcium (Fig-1). The data showed iron being the most vulnerable nutrient affected (Fig-2) by CO_2 elevation. Decline in iron was observed in all of the varieties at CO_2 800µmol mol^{-1} with the highest decline of 16.66% - 9.75%. The percent decline in the Zinc (Fig-3) was also observed in 68.75% varieties with the decline of (26.99% FH-113) being the most significant. Intra varietal and mean difference in Copper showed 15.2% decline in FH-142 and

16.4% increase in Cu in FH-113 (Fig-4). Manganese was the least affected element (Fig-5) where decline though occurred but was non-significant. An insignificant ($P < 0.109$) uptake of Cadmium was also observed in some of the cotton varieties grown at 800µmol mol^{-1}(Fig-6)

Table – 4: Mean Micronutrient content (mg/kg of dry weight) of cotton varieties under the elevated CO_2 Canopy Environment

Minerals	Main Effects	Mean	Minimum	Maximum	SD	P level
Calcium						
	[aCO₂]	0.1450	0.02	0.19	.04561	ns
	[eCO₂]	0.1406	0.02	0.19	.04407	
Iron						
	[aCO₂]	140.9400	103.30	186.72	24.63889	0.000
	[eCO₂]	128.6307	96.09	166.67	21.17520	
Zinc						
	[aCO₂]	27.8413	21.31	35.86	4.48116	0.023
	[eCO₂]	26.1660	21.72	37.72	4.31339	
Copper						
	[aCO₂]	157.1333	132.00	178.00	16.30016	0.040
	[eCO₂]	153.3333	130.00	174.00	10.30719	
Manganese						
	[aCO₂]	24.1253	18.83	30.30	3.44461	0.233
	[eCO₂]	23.9693	18.67	30.33	3.51173	
Cadmium						
	[aCO₂]	0.0049	0.00	0.02	.00557	ns
	[eCO₂]	0.0107	0.00	0.03	.01216	

*ns: non-significant at P<0.05

Fig-1: Ca

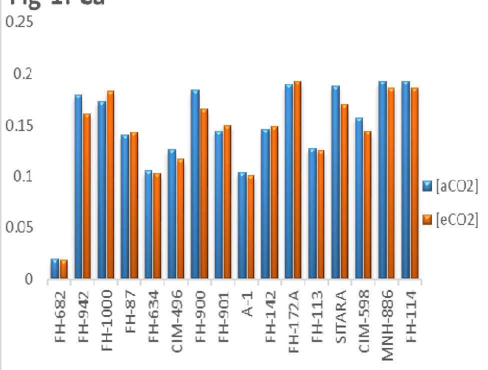


Fig-2: Fe

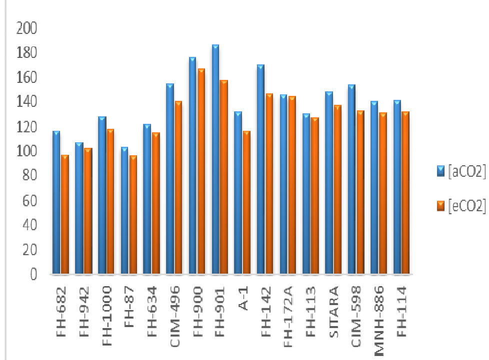


Fig-3: Zn

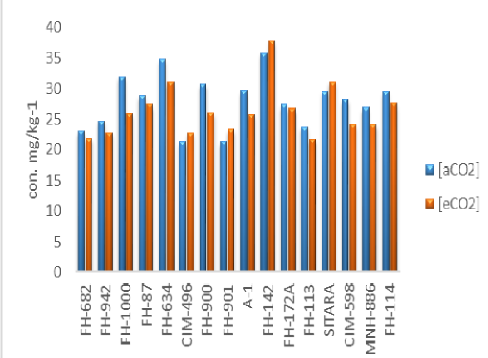
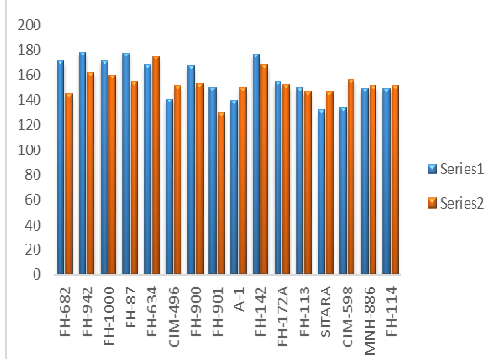
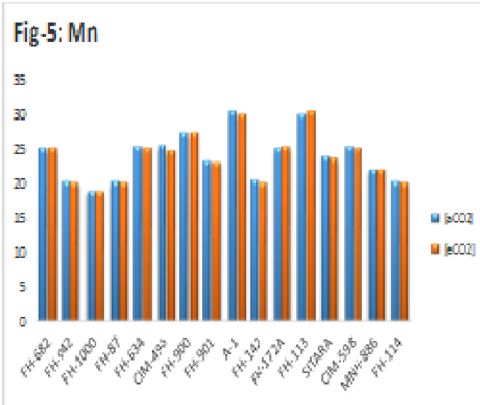
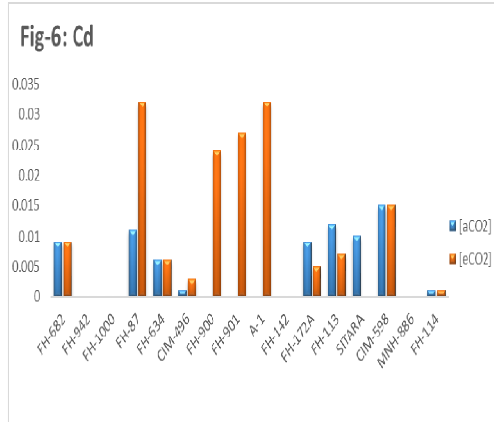


Fig-4: Cu







iv. Effect of CO_2 Enrichment of Amino Acids

The results obtained for the amino acids composition grown at two levels of CO_2 concentration indicated (Table-5 and Figures 7-14) high variability among individual amino acids from the elevated chambers. Cotton cultivars responses the elevated CO_2 showed a mean 10.25% decline in threonine, 11.6% in serine, 13.6% glutamine, 12.6% alanine, 10.0% tyrosine and 7.14% in glycine. A 12.23% increase in valine, 3.96% leucine, and 0.66 in aspartic acid. The 16.0% increase in the arginine was due to high variability from control in four samples (FH-113, FH-114, 172A, FH-1000). The multivariate linear variance ($P\leq 0.05$) showed insignificant impact of the decline of crude protein along elevated CO_2 indicating amino acid responses to CO_2 elevation were independent of the decline in crude protein (data not shown here).

Table-5: Mean Amino Acids Composition (g/16 g N) of the Cotton Cultivars grown under Elevated CO_2 levels

	Means			Cof.Var (%)		Minimum		Maximum		P-Values
Amino acids	**[aCO₂]**	**[eCO₂]**	**% Change**	**[aCO₂]**	**[eCO₂]**	**[aCO₂]**	**[eCO₂]**	**[aCO₂]**	**[eCO₂]**	
Aspartic acid	7.51±1.75	7.450.99±0.85	+0.66	8.7	18.45	3.15	5.68	8.90	8.79	0.09
Threonine	3.90±1.78	3.50±0.83	-10.25	7.8	27.42	2.66	2.00	10.38	5.95	0.002
Serine	3.28±2.12	2.71±0.83	-11.6	8.51	33.45	0.97	1.45	10.27	3.83	0.000
Glutamine	14.00±4.53	12.36±2.38	-13.26	8.75	28.06	2.02	10.37	17.46	17.61	0.007
Glycine	4.22 3.26	3.9±0.42	-7.14	8.9	28.79	2.37	2.92	16.30	4.40	ns
Alanine	3.56± 2.16	3.11±0.35	-12.6	7.74	37.7	1.20	2.44	11.12	3.94	ns
Valine	3.67±0.91	3.25±0.57	+12.23	7.98	47.83	0.98	2.46	4.39	4.68	0.051
Methionine	1.26±0.48	1.10±.51	-8.3	8.37	48.16	0.00	0.00	1.86	1.89	0.032
Isoleucine	2.37±0.67	2.34±0.67	-1.28	7.97	55.98	0.59	1.59	3.24	3.92	0.067
Leucine	3.79±1.20	3.64±1.45	3.96	7.63	37.47	1.75	0.00	5.84	5.79	ns
Tyrosine	2.03±0.91	1.8±0.63	-10.0	8.31	68.56	.88	0.58	4.11	2.81	ns
Phenylalanine	3.28±0.42	3.07±0.63	-5.46	6.61	35.68	2.41	2.20	4.13	3.98	0.024
Histidine	1.55±0.31	1.48±.48	-4.53	9.07	28.07	0.99	0.87	2.10	2.26	0.016
Lysine	3.89±1.23	3.66±1.02	-5.9	7.8	37.59	1.51	2.07	6.61	5.42	0.049
Arginine	9.48±3.52	11.09±2.31	+16.0	7.75	56.78	.83	6.99	16.24	17.94	0.000

*ns: non-significant at $P<0.05$

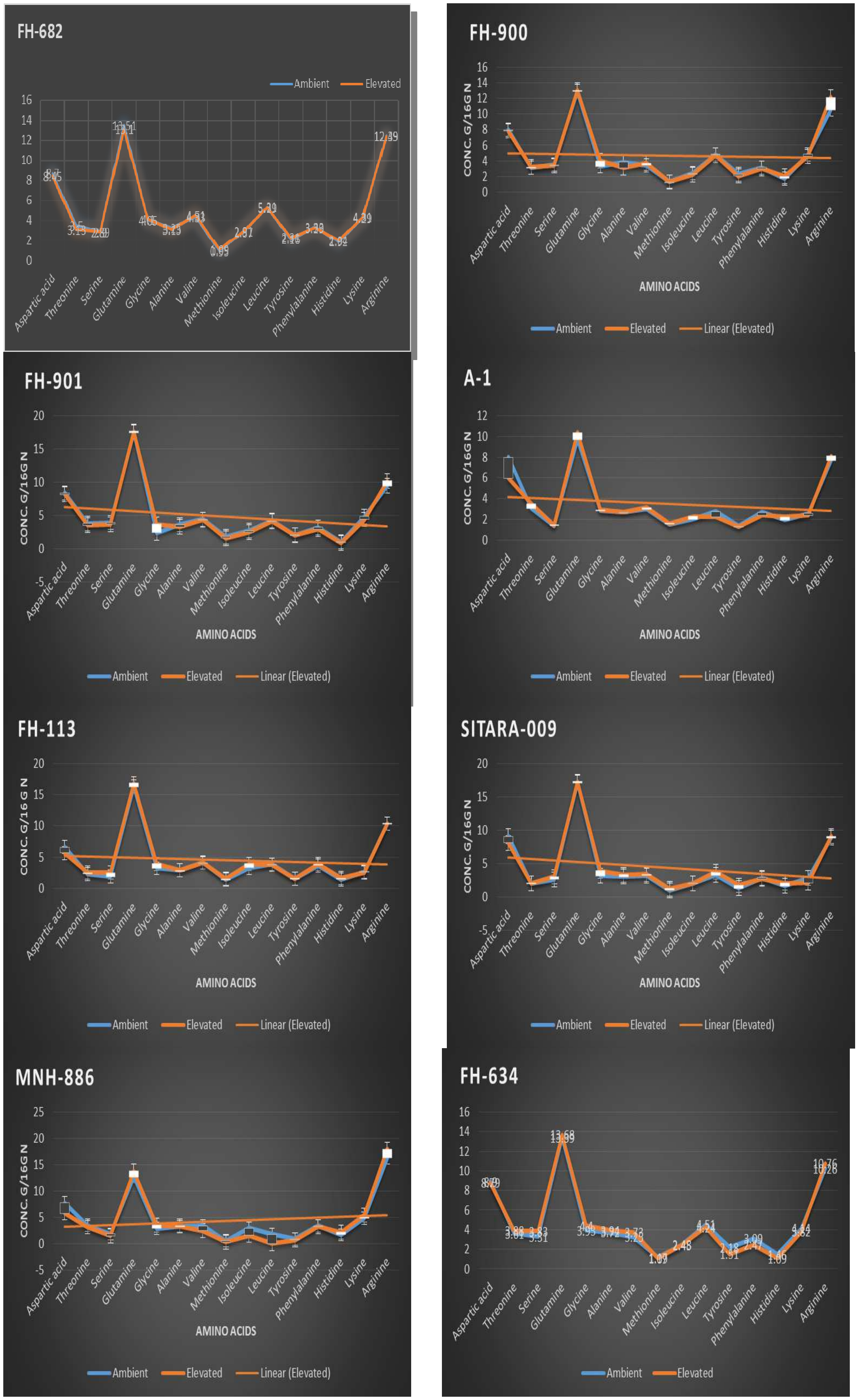


Fig-7-14: Comparison of Amino Acids at two levels of Atmospheric CO_2 in some representative Cotton Genotypres

4. DISCUSSION

The CO_2 induced shifts in the environment is becoming a global challenge that needs to be tackled at all levels as far plants quality in general and nutritional quality in particular is concerned. The seed quality parameter being important from the breeders' point of view indicated a significant ($P < 0.007$) mean 25.51% increase in the total yield of the cotton cultivars. These results are in conformity with the findings of Reddy et al. [17] and Kimball et al. [19] who reported increase of up to 63% yield of the cotton under elevated CO_2. Intra-varietal increase in the yield was up 38.09-to-31.74% in our study. The G.O.T (%) indicated an overall increase however both linter percent and seed index were low. The current study showed little changes in the seed index (weight of 100 seeds) and are similar to other findings such Kimball [19] and Rawson [20] observed little changes in the seed weight (seed index) of wheat and grains. These authors attributed this little increase in the seed weight to the lowered availability of soil N under the elevated carbon dioxide concentrations, delayed anthesis and a slower transition from vegetative to reproductive phase by the cultivars, and accumulation of more carbohydrates by the seeds at the expense of protein. The decline in the seed weight of few genotypes in the current study are as per another study and have been attributed to the accelerated growth rate and reduced seed filling duration under elevated CO_2 [19]. According to Hogy and Fangmeier this little gain in the seed weight might also be attributed to the microcosm environment of the pots in which the cultivars were grown or due to the fact that cotton being C_3 plant will be least affected by the climatic factors [22]. The insignificant differences in the seed volume (a component of seed quality measure seed size along with seed surface area and seed diameter will not be affected under CO_2 elevation. Seed volume plays an important role in the establishment of earlier life stages such as seed germination and early stage survival of the seedlings and is an important component of the cotton breeding selection index [22, 23]. The current findings, therefore, confirm that under the effects of CO_2 elevation the seed vigor characteristics such as seed index and seed volume will not be affected drastically [24]. The insignificant but positive increase in the percent seed Kernel and percent lint (% of lint removed during delinting) might be attributed to the translocation of carbohydrates that can cause an increase in the seed weight and cause reduction in the nitrogen concentrations [21, 25].

The findings of the current study showed significant decline in the crude protein content of the cotton seed genotypes. These results in agreement with the findings of other similar studies [25, 26, and 18] who reported significant decline in total nitrogen and crude protein in grain seeds. Hocking and Meyer [27] attributed this marked reduction in protein synthesis to the limited size of amino acid pool in the leaves which consequently cause lowered levels of nitrogen and protein in the grain seeds. As demonstrated by Pikki et al., enhancement of atmospheric CO_2 tend to increase the protein yield mainly due to the overall increase in the total crop yield but cause reduced grain protein and N inside the grains [28]. Results of our study also confirm the findings of a previous work [18] where a 3.9-14% decline in the protein content of grains was reported and that reduction in seed N and total protein was postulated to be dependent upon the exposure systems and rooting volume. Similar significant reduction in the total protein and increase in total yield of rice grown under CO_2 enrichment were also observed by many other studies [25, 28, and 29]. The current study observed significant decline in the crude fiber content of cotton seeds. This reduction in crude fiber might have contributed to the insignificant percent increase in the kernel/meal weight or would have been the results of increased macro nutrients in the ceel matrix as reported by Uprety et al. that seed index is strongly correlated with increase in yield of percent oil and carbohydrate content of seeds [30].The decline in the crude fiber content was attributed to the CO_2 induced changes in C/N ratio [2]. This reduction, as reported by Clandinin and Robblee will minimize the liginin content of the seed meal and will adversely affect protein and amino acid digestibility of the feed in monogastric animals [31]. Since crude fiber is mainly cellulose and hemi-cellulose; an overall reduction will result in the reduced nutrients value and lowered metabolic energy of the cottonseed meals. We found significant increase in the percent oil content in our study which is in agreement with the findings of other studies [29, 32] which reported significant increases in the oil contents of brassica and soybean respectively. As suggested this increase in the seed oil content might have been possible at the expense of protein due to the participation of additional CO_2 in the formation of abundant Malonyl CoA under elevated CO_2 which possibly play a role in the biosynthesis of fatty acids [29].

The mean decline across all the five minerals assessed in our study are similar to the findings of another study which reported declined concentrations of minerals in herbaceous plants, crops, foliar and edible tissues [9]. Similar decline trends in Ca, Zn but much lower concentrations of Mn concentrations were also found in another study [33]. It is suggested that increase in the atmospheric CO_2 is the possible cause of changes in the mineral content of plants. This overall decline in mineral concentrations under CO_2 enrichment has been attributed to the larger root systems which appear to be unable to gather proportionally more nutrients [35]. In another study, however, such similar decline in Fe, Zn, Ca were attributed to the dilution effect induced by the increase in carbohydrate content in seeds [36].

The significant declines in majority of the proteinogenic amino acids were more obvious in the current study. This decline, with greater coefficient of variation in the samples from the elevated CO_2 chamber, was uniform throughout the essential and non-essential amino acids. These similar decreased concentration of most of the amino acids are similar to the findings of other studies performed on soybean and oil seed rape under elevated CO_2 atmosphere [9, 33]. This reduction in amino acid will have negative impacts on the synthesis of nutrients and nutritive value of cotton seeds both as a food and feed.

CONCLUSION

Our research showed that elevated atmospheric CO_2 concentrations resulted in an overall declines in the protein, crude fiber, ash, minerals and amino acid concentrations in the cotton cultivars though elevated CO_2 increased the total oil yield. This is the first report from Pakistan where cotton dominate agricultural sector. Any negative change due to climate will subsequently affect economy of this country. It is unclear, however, whether the combined effects of temperature rise as a consequence of CO_2 elevation, drought and other climatic stress and improved fertilization will offset the decline in the nutrient content or will further worsen the situation. It seems appropriate here to conclude that current responses of the cotton cultivars are the possible range of plants performance under CO_2 enhancement alone.

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