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Research Article

ANALYSIS OF CARBON ISOTOPE DISCRIMINATION ON GAMMA RADIATED COWPEA VARIETIES (*Vigna unguiculata* L.)

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Abstract: Gamma irradiation can be useful for the alteration of one or a few physiological characters. The present programme was an attempt to study the carbon isotope discrimination and Biochemical observation on cowpea varieties treated with gamma radiation. Plant responses in terms of growth parameters, RWC were analyzed. The result indicated an improvement in growth performances of cowpea varieties under gamma radiation.

Keywords: Cowpea, Gamma Radiation, Carbon isotope discrimination ^{12}C and ^{13}C

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Introduction

Cowpea has many mechanisms of response and survival to drought. These include some physiological, biochemical and agro morphological responses. The understanding of the mechanism explaining the resistance of cowpea varieties to drought is of extreme importance for improving the production of this grain legume. The importance of these mechanisms in the breeding program for improving crops adaptation to drought was already reported [1-3]. The most sensitive growth stages of cowpea to drought were flowering and pod filling, with yield reduction from 35 to 69 % depending on the timing and length of the drought treatment. A soil water deficit during the vegetative stage had the least effect on crop yield [4]. The isotopes are unevenly distributed among and within different compound and this isotopic distribution can reveal information about the physical, chemical and, metabolic processes involved in carbon transformation. CO_2 must diffuse from the atmosphere tot eh chloroplast stroma. Since $^{12}CO_2$ diffuse faster than $^{13}CO_2$, several fractionation processes occur along this diffusion path, so that the CO_2 available at the sites of carboxylation is always significantly depleted in ^{13}C compared to the atmosphere [5].

Materials and Methods

The present study entitled was conducted in the research field on cowpea varieties at the Department of Plant Physiology, SHIATS, Allahabad. The experiment laid out in randomized block design (RBD) within five treatments, four verities, each replicated tree times. Experimental Materials four varieties of Cowpea (*Vigna unguiculata* L.) namely HR-1, BG-12, A Gomati, Khashi K were purchased from local market Allahabad, UP. Seeds treated by Gamma radiation in National Botanical Research Institute (NBRI) research institute of CSIR at Lucknow.

Plant height (cm)

Plants were selected randomly from each plot. The height of these plants was measured from the ground levels up to the tip of plant at 15 days intervals on 15, 30, 45 DAS. Average height was then calculated for each observation recorded.

Number of branches/ plants

From the selected plants number of branches was calculated on 15, 30 and 45 DAS.

Determination of Carotenoid

The carotenoid content in the leaves was estimated by weighing fresh leaves of 1 gm and this much quantity of leaves was taken in the test tubes and 15 ml acetone (80% acetone) was poured in the test tubes and was kept under refrigeration overnight at 4°C. Next to that the samples are homogenized at 3000rpm for about 15 minutes. 25 ml volume is made with acetone. The absorbances were recorded at D 480 and D 510.

Carotenoid (mg/g dry leaf) = 7.60 480-1.49D 510 x V / D x 1000 x W

Estimation of Relative water content (%)

0.5 g of leaf (FW) was taken and soaked in distilled water overnight to get them saturated. Then the leaf samples were weighed again to get the turgid weight (TW). The samples were then dried in an oven at 80°C for 24 h and weighed (DW). The relative water contents were measured as following:

RWC = (FW-DW)/ (TW-DW) x 100 each observation was recorded.

Quantification of $\Delta^{13}C$ composition in leaf samples

The carbon isotope Discrimination ($\Delta^{13}C$) estimation conducted with third fully expanded leaves that were developed during the experimental period were collected (5-6 for each genotype) Dried at 80°C for 3 days. Dried samples were powdered in a mortar and pestle. Care was taken to prevent any mixing of different samples by washing the pestle and mortar with alcohol after grinding each sample. One gram of powdered leaf sample was put in glass vial, properly labelled and it was conducted at GKVK, Bangalore for $\Delta^{13}C$ analysis [Fig-1].
Based on the fractionation (isotopic composition with respect to PDB), the ^{13}C discrimination ($\Delta^{13}C$) in the plant samples was computed as follows

Table-1 *Effect of Gamma Radiation on plant height (cm) of Cowpea varieties at different growth period*

	15 DAS						30 DAS						45 DAS					
	Plant height (cm)						Plant height (cm)						Plant height (cm)					
	T_0	T_1	T_2	T_3	T_4	Var. Mean	T_0	T_1	T_2	T_3	T_4	Var. Mean	T_0	T_1	T_2	T_3	T_4	Var. Mean
V_1	10.8	7.5	9.6	8.7	7.1	8.74	30.6	26.5	23.5	22.5	18.2	24.2	69.5	57	45.6	39.8	31.6	48.7
V_2	16.5	12.8	10	11.3	12.4	12.6	28.6	25.4	22.3	22.2	20.5	23.8	66	51.6	48.5	40	31.8	42.4
V_3	10.6	7.1	10.4	8.7	7.5	8.86	30.6	26.5	23.4	22.4	18.3	24.2	68.5	57	45.6	39.8	31.9	48.5
V_4	10.1	7.2	10	8.8	7.5	8.72	30.4	26.4	23.5	22.3	18.6	24.2	70.5	55	44.6	39.8	32.9	48.5
Treatment Mean	12	8.6	10	9.3	8.6	9.73	30.05	26.2	23.1	22.3	18.9	24.1	68.6	55.1	47.5	39.8	24.1	47
	plant height (cm)		Varieties		Interaction		plant height (cm)		Varieties		Interaction		plant height (cm)		Varieties		Interaction	
S. Ed. (±)	0.15		0.17		0.34		0.37		0.41		0.83		0.76		0.85		1.70	
C. D. (P = 0.01)	0.58		0.65		1.31		0.76		1.60		1.23		2.92		3.26		6.53	

Table-2 *Effect of gamma radiation on total chlorophyll a (mg/g) and carotenoid (mg/g) content in different varieties of cowpea*

Varieties	Total Chlorophyll (mg/g) FW						Carotenoid (mg/g) FW					
	T0	T1	T2	T3	T4	Var. mean	T0	T1	T2	T3	T4	Var. mean
V1	2.19	1.58	1.43	1.34	1.17	1.542	3.95	3.14	3.07	2.41	2.2	2.954
V2	2.12	2.01	1.66	1.48	1.33	1.72	3.29	3.06	3.02	2.07	1.42	2.572
V3	3.19	2.86	2.71	2.57	2.49	2.764	3.14	2.33	2.19	2.17	1.92	2.35
V4	2.13	1.96	1.7	1.45	1.21	1.69	5.03	2.41	1.84	1.83	1.44	2.51
Treatment mean	2.4075	2.1025	1.875	1.71	1.55	1.929	3.8525	2.735	2.53	2.12	1.745	2.5965
	Total Chlorophyll		Varieties		Interaction		Carotenoid		Varieties		Interaction	
S. Ed. (±)	0.03		0.03		0.06		0.04		0.04		0.09	
C. D. (P = 0.01)	0.11		0.13		0.06		0.16		0.17		0.35	

Table-3 *Effect of Gamma Radiation on number of branches of Cowpea varieties at different growth periods*

Varieties	15 DAS						30 DAS						45 DAS					
	No of branches						No of branches						No of branches					
	T_0	T_1	T_2	T_3	T_4	Var. Mean	T_0	T_1	T_2	T_3	T_4	Var. Mean	T_0	T_1	T_2	T_3	T_4	Var. Mean
V_1	3	2	2	2	0	1.8	6	4	2	3	2	3.4	12	8	5	6	4	7
V_2	3	2	3	2	0	2	6	6	4	4	2	4.4	10	7	5	4	3	5.8
V_3	4	3	2	3	2	2.8	8	6	4	4	4	5.2	12	7	5	4	3	6.2
V_4	3	2	2	3	2	2.4	7	6	4	4	2	4.6	10	7	4	4	3	5.6
Treatment Mean	3.25	2.25	2.25	2.5	1	2.25	6.75	5.5	3.5	3.75	2.5	4.4	11	7.25	4.75	4.5	3.25	6.15
	No of branches		Varieties		Interaction		No of branches		Varieties		Interaction		No of branches		Varieties		Interaction	
S. Ed. (±)	0.03		0.04		0.08		0.07		0.08		0.16		0.10		0.11		0.23	
C. D. (P = 0.01)	0.14		0.16		0.32		0.27		0.3		0.61		0.39		0.44		0.88	

Statistical analysis

The experiment used RBD with three treatments and each treatment was analysed with three replications. Statistical analysis was performed using ANOVA.P values d ≤ 0.05 were considered as significant.

Fig-1 Isotope Ratio Mass Spectrometer for Carbon Isotope Discrimination ($\Delta^{13}C$)

Results and Discussion

Alteration in growth performance of cowpea under the treatment of gamma ration by analyzing the growth parameters.

The Data presented in [Table-1] shows the effect of gamma radiation on plant height (cm) of different cowpea varieties. The results showed that there was a significant difference in plant height (cm) among all the treatments. The results observed for plant height is summarized.

There was a significant difference in plant height (cm) in all the varieties under different treatments at 15 DAS. The maximum plant height was observed in variety BG-12 in control T_o (16.5 cm), whereas the minimum was observed in variety HR-1 in T4 (7.1 cm) at 40 Gy of gamma radiation, under all the treatments.

At 30 DAS, the data showed that there was a significant difference in plant height (cm) in all the varieties under different treatments. The maximum plant height was observed in variety Ankur Gomati in control T_o (30.6 cm), whereas the minimum was observed in variety HR-1 in T4 (18.2 cm) at 40 Gy of gamma radiation, under all the treatments.

There was a significant difference in plant height (cm) in all the varieties under different treatments at 45 DAS is presented in [Table-1]. The maximum plant height was observed in variety Khashi kanchan in control T_o (70.5 cm), whereas the minimum was observed in variety HR-1 in T4 (31.6 cm) at 40 Gy of gamma radiation, under all the treatments. Gamma irradiation can be useful for the alteration of one or a few physiological characters [6]. The Data presented in [Table-2] shows the effect of gamma radiation on number of branches of different cowpea varieties. The results showed that there was a significant difference in number of leaves among all the treatments. The results observed for number of branches is summarized. At 15 DAS, the data showed that there was a significant difference in number of branches in all the varieties under different treatments. The maximum number of branches was observed in variety Ankur Gomti in control T_o (4), whereas the minimum was observed in variety Khashi Kanchan in T4 (2) at 40 Gy of gamma radiation, under all the treatments. At 30 DAS, there was a significant difference in plant number of leaves in all the varieties under different treatments. The maximum number of branches was observed in variety number of leaves in HR-1 and BG-12 in control T_o (6), whereas the minimum was observed in variety Khashi kanchan, HR-1 and BG-12 in T4 (2) at 40 Gy of gamma radiation, under all the treatments. There was a significant difference in number of branches in all the varieties under different treatments at 45 DAS is presented in [Table-2]. The maximum number of branches was observed in variety HR-1 and Ankur gomti in control T_o (12), whereas the minimum was observed in variety Khashi kanchan, BG-12 and Ankur Gomti in T4 (3.00) at 40 Gy of gamma radiation, under all the treatments. The Data presented in [Table-3] shows the effect of gamma radiation on total chlorophyll a (mg/g) and carotenoid (mg/g) content of different cowpea varieties. The results showed that there was a significant difference in total chlorophyll a (mg/g) and carotenoid (mg/g) content among all the treatments. The results observed for chlorophyll content is summarized.

There was a significant difference in total chlorophyll a (mg/g) content in all the varieties under different treatments. The maximum Chlorophyll (a) content was observed in variety Ankur Gomati in control T_o (3.19 mg/g), whereas the minimum was observed in variety HR-1 in T4 (1.17 mg/g) at 30 Gy of gamma radiation, under all the treatments. The data presented in [Table-3] showed that there was a significant difference in carotenoid (mg/g) content in all the varieties under different treatments. The maximum carotenoid (mg/g) content was observed in variety Khashi kanchan in control T_o (5.03 mg/g), whereas the minimum was observed in variety BG-12 in T4 (1.42 mg/g) at 40 Gy of gamma radiation, under all the treatments. Gamma irradiation induces various physiological and biochemical alterations in plants. The irradiation of plants with high dosages of gamma rays disturbs the hormone balance, leaf gas-exchange, water exchange and enzyme activity. The Data presented in [Table-4] shows the effect of gamma radiation on Relative water content (%) of different cowpea varieties. The results showed that there was a significant difference in Relative water content (%) among all the treatments. There was a significant difference in Relative water content (%) in all the varieties under different treatments. The maximum Relative water content was observed in variety HR-1 in control T_o (0.63%), whereas the minimum was observed in variety Khashi kanchan in T4 (0.26%) at 30 Gy of gamma radiation, under all the treatments.

Table-4 *Estimation of Relative water content (%) in different cowpea varieties*

Varieties	Relative water content (%)					
	T0	T1	T2	T3	T4	Var. mean
V1	0.63	0.56	0.56	0.43	0.41	0.51
V2	0.62	0.53	0.46	0.44	0.41	0.49
V3	0.51	0.49	0.48	0.46	0.42	0.47
V4	0.61	0.58	0.44	0.41	0.26	0.46
Treatment mean	0.59	0.54	0.48	0.43	0.375	0.48
	Relative water content		Varieties		Interaction	
S. Ed. (±)	0.007		0.008		0.016	
C. D. (P = 0.01)	0.028		0.032		0.064	

These effects include changes in the plant cellular structure and metabolism. The effect of gamma irradiation on soluble protein content of bean callus culture, it had been reported that at high irradiation dosage [7]. Showed that gamma irradiation, did not induce significant loss in water soluble components such as total soluble proteins, minerals, nitrogenous constituents, and sugars. Carotenoid is a large class of isoprenoid molecules, which are de novo synthesized by all photosynthetic and many non-photosynthetic organisms. The statistical analysis showed $\Delta^{13}C$ in [Table-5] and [Fig-2] showed significant but adverse effect in $\Delta^{13}C$ due to application of Gamma radiation.

Table-5 *$\Delta^{13}C$ Carbon Isotope Discrimination in cowpea variety with IRMS.*

Varieties	$\Delta^{13}C$ Carbon Isotope Discrimination (per mil)					
	T0	T1	T2	T3	T4	Var. mean
V1	21	19.5	19.6	19.2	18.7	19.60
V2	20.7	20.4	20.2	19.5	18.3	19.82
V3	23.8	21.1	20.8	20.5	20.2	21.28
V4	21.5	20.3	19.8	19.2	18.8	19.92
Treatment mean	21.75	20.32	20.1	19.6	19.0	20.15
	$\Delta^{13}C$ Carbon Isotope Discrimination (per mil)		Varieties		Interaction	
S. Ed. (±)	0.30		0.34		0.69	
C. D. (P = 0.01)	1.18		1.32		0.89	

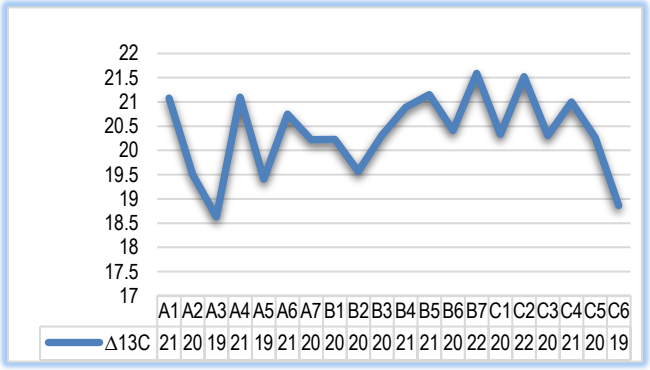

Fig-2 Grouping of High WUE varieties analyzed by $\Delta^{13}C$ with IRMS to showing the variations in physiological traits among four Cowpea varieties

The results showed significant difference in $\Delta^{13}C$ among the treatments, maximum number of Carbon Isotope was found in variety Khashi kanchan - B7 (21.6 per mil). And minimum was found in HR-1 -A3 (18.6 per mil) among four Cowpea varieties under all the treatments. Carbon isotope discrimination during photosynthesis ($\Delta^{13}C$) has been well established, as a time averaged surrogate for WUE [8]. Several others have successfully screened large number of genotypes for variations in WUE by this approach [9-13].

Conclusion

The present study on the basis of observation it was concluded that Khashi kanchan and HR-1 maximum growth, among all the varieties and whereas BG-12 and Aankur Gomati were gamma radiation sensitive variety. The morpho-physiological biochemical parameters in 4 cowpea varieties such as height of plant, branches, Relative water content, $\Delta^{13}C$ – Carbon Isotope Discrimination showed significant decrease with the increase in gamma radiation treatment. From the agricultural point of view, the use of novel approaches combining genetic, physiological, biochemical, and molecular techniques should provide exciting results in the development of water use efficient cowpea varieties in the near future.

Application of research: Understanding response of cowpea varieties in terms of growth and development under for High water use Efficiency in Gamma Radiated Cowpea varieties (*Vigna unguiculata* L.)

Research Category: Plant Physiology

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Study area / Sample Collection: National Botanical Research Institute, IRMS

Cultivar / Variety / Breed name: HR-1, BG-12, A Gomati, Khashi K

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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