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Biochemical variations over different environments of *Macrotyloma uniflorum*

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ABSTRACT

Macrotyloma uniflorum is a slender herb grown in drought prone areas and consumed mainly by the poor people. Plants have inherent capacity to produce several secondary metabolites which serve to cure several diseases. The plant is used to cure kidney stones, piles, bronchitis, leucoderma, asthma etc. In the present world there is a population explosion. The demand of food is also increasing day by day. This legume can be used as a good source of protein, carbohydrates and antioxidants. This experiment was designed to study the locational variations in biochemical properties of the plant in two different regions in the state of West Bengal. Fifteen accessions of Macrotyloma uniflorum seeds were assessed for difference in protein, carbohydrate, phenol, β-carotene and lycopene content.

The main aim of this study is to investigate the variability in biochemical parameters arising due to different environmental conditions.

Keywords: *Drought tolerant, secondary metabolites, population explosion, assessed, environmental conditions*

INTRODUCTION

Sustainable agriculture is essential to meet the increasing demands of food across the world. In 2007 it was estimated that around three billion people residing in rural areas depend on agriculture as their main source of income. Sustainable agriculture encompasses global, economic, social and environmental issues unlike conventional agriculture[1]. All these factors can immensely affect agriculture[2] . Agricultural productivity is essential to be maintained not only in terms of quantity but also quality as it is directly proportional to human health, which is one of the major concerns for any country[3,4] .

Legumes are good sources of cheap and widely available proteins for human consumption. Legumes serve as staple food of many people in the world[5]. Legume seeds have an average of twice as much protein as cereals and the nutritive value of the proteins are usually high[6]. Legumes seeds are of prime importance in human and animal nutrition due to their high protein content[7]. *Macrotyloma uniflorum* previously known as *Dolichos biflorus* is one such legume commonly known as horse gram. It is generally consumed by the poor people of the society so also called as Poor Man's Pulse. Horse gram is native to the old world tropics. It is a twining annual leaves are 3-foliolate; leaflets are 2.5-5 cm broadly lanceolate or oblong in shape. Flowers are 1-3 in the axils of the leaves; corolla yellow, 1.3-2 cm long. Pods are about 5 cm long, scimitar-shaped, compressed, recurved. Seeds are small, 3-6 mm, flattened, shining and seed colour ranges from light red, brown, black or mottled[8] . It shows very good drought tolerance. Environmental stresses or adverse growth conditions such as drought, salinity, chilling, freezing, and high temperatures affect plants regularly. Disruption of plant water management caused by drought, salinity or low temperature is a major yield decreasing factor[9].

Plants have developed means to avoid such stress. There are about 25 known species of horse gram most of which are located in Africa[10,11].

The aims and objects of this paper are to present the content of antioxidants (phenol, β-carotene, lycopene) as well as soluble proteins, and soluble carbohydrates of different accessions of horse gram grown in two varied agroclimatic regions of West Bengal .

MATERIALS AND METHODS

Fifteen genotypes viz., IC 89032, IC 203201, IC 139506, IC 561040, IC 267941, IC 385389, IC 49552, IC 392329, IC 320970, IC 145247, IC 9624, IC 24842, IC 341296, IC 22827 and IC 139523 were considered for this study. The seeds were brought from National Bureau of Plant Genetic Resources (NBPGR), New Delhi. The seeds were sown in two different locations of varied agroclimatic regions one at Gagnabad, Adra , Purulia (location 1) and the other at the Crop Research Farm, Golapbag, Burdwan (location 2) in the state of West Bengal. The climate of location-1 was comparatively adverse than location-2.

(i) Estimation Of Total Phenol

Seed samples of fifteen accessions each measuring 100 mg from both the locations were extracted in 5-10 ml of 80% ethyl alcohol and allowed to boil for 5-10 minutes in hot water bath and then cooled. The tissues were crushed in a mortar and pestle and passed through double layered cloth. The tissues were extracted again by the same way and filtered through Whatman no. 1 filter paper. The concentration of phenol in the seed extracts was estimated following the method of Mahadevan and Sridhar (1982) using Folin-Ciocalteau reagent. Catechol was used as the standard.

(ii) Estimation Of β-Carotene

The method of Nagata and Yamashita (1992) was used to determine the content of β-carotene in the plant sample. The dried methanolic extract (100 mg) was vigorously shaken with 10 ml of acetone-hexane mixture (4:6) for 1 minute and filtered through Whatman no. 4 filter paper. The absorbance of the filtrate was measured at 453, 505, 645 and 663 nm in Vis Spectrophotometer (Systronics-117). The following formula was used to estimate the β-carotene content of the seed sample (mg/100ml) = $0.216\ A_{663}$ - $0.304\ A_{505}$ + $0.452\ A_{453}$

The readings were taken in triplicate and the results were mean values $\pm$ standard deviations and expressed as mg of β-carotene/g of extract.

(iii) Estimation Of Lycopene

The same method was used for lycopene estimation as that was used for the estimation of β-carotene. The following formula was used to calculate the concentration of lycopene in the seed sample (mg/100ml) = $-\ 0.0458\ A_{663}$ + $0.372\ A_{505}$ - $0.086\ A_{453}$

The readings were taken in triplicate and the results were mean values $\pm$ standard deviations and expressed as mg of lycopene/g of extract.

(iv) Estimation Of Protein

Seed samples weighing 1 g were grinded in chilled mortar and pestle with phosphate buffer (0.1 M, pH 6.8). Then samples were centrifuged at 5000 rpm for 15 minutes and the respective supernatants were collected for protein estimation. TCA (10%) measuring 5 ml was added to each sample (supernatant) and boiled for 3 minutes in water bath. Centrifugation was carried out for the second time at 5000 rpm for 15 minutes. The supernatants were collected and 5 ml of NaOH (0.1N) was added to each. To 1 ml of the homogenate, 5 ml of the alkaline solution (5% Na_2CO_3 and 0.5% $CuSO_4$ in 1% potassium sodium tartrate) was added to each tube including the blank, mixed well and allowed to stand for 10 min.Then 0.5ml of Folin-Ciocalteau Reagent was added to each tube, mixed well and incubated at room temp. in the dark for 30min till blue colour was developed. The method of Lowry et al. (1951) was used to determine the concentration of protein in the plant extract. The O.D. was measured at 650 nm and BSA (1 mg/ml) was used as standard.

(v) Estimation of Soluble Carbohydrate

100 mg of the seeds were homogenised in ethanol and volume made up to 10 ml. Then the samples were centrifuged at 5000 g for 15 minutes. 2 ml of the supernatant was taken and to each 3 ml of anthrone was added. Then O.D. was taken at 640 nm using UV-visible spectrophotometer (Shimadzu-1601). Soluble carbohydrate was estimated following the anthrone method as described by Sadasivam and Manickam (2008). D-glucose at the concentration of 100 µg/ml was used to prepare the standard curve. The amount of carbohydrate present in the sample was expressed in g/100g.

RESULTS AND DISCUSSIONS

The biochemical analyses of the seed samples of the two locations were done according to the procedures mentioned above. The O.D. values were taken in triplicate and the results were expressed as the mean values ± standard errors.

It has been found from the ANOVA calculations (Table- 3 and 4) that location-1 gave the highest MSS values against soluble carbohydrate and β-carotene. The values of protein and lycopene also gave the standard values . The σ^2g and σ^2p values were highest in location-1 than location-2 for all the components except protein. Similarly, the co-efficients of variances differed to a little extent in between two locations for protein composition than the other biochemical compositions. More adaptive tendency of the population grown in location-1 (drought prone) with respect to the biochemical composition than that of the population grown in location-2 was observed.

The accessions IC 392329 in location-1 (2.03g/100g) and IC 267941 in location-2 (1.971g/100g) exhibited highest phenol content. IC 24842 in location-1 (1.003g/100g) and IC 22827 in location-2 (0.827g/100g) had the minimum content of phenol among all the accessions. In both the locations IC 49552 had the maximum β-carotene content (69.893 mg/100g in location 1 whereas 63.574 mg/100g respectively) whereas IC 341296 (16.479 mg/100g in location 1 and 16.362 in location 2 respectively) had the lowest. The β-carotene content of the accessions IC 89032, IC 267941, IC 49552, IC 392329, IC 22827 and IC 139523 showed marked variation ; the accessions IC 320970, IC 139506, IC 9624 and IC 24842 differed slightly whereas the rest of the accessions had almost same concentrations over both the locations. The lycopene content of IC 89032 was the highest (18.182 mg/100g in location 1 and 16.796 mg/100g in location 2) and was lowest in IC 22827 (2.451 mg/100g in location 1 and 1.626 mg/100g in location 2). High variations in the lycopene content over both the locations were observed in the accessions IC 89032, IC 139506, and IC 341296. Others differed slightly. IC 267941 had the highest protein content in location 1 (27.821 g/100g) contrastingly IC 49552 was observed to have the highest protein content in location 2 (28.569g/100g). IC 49552 and IC 24842 showed marked difference in their content of protein between the two locations. IC 267941 with soluble carbohydrate concentration of 32.325 g/100g and 27.341 g/100g in location 1 and 2 respectively proved to be the better source of carbohydrate among the 15 accessions. IC 320970, IC 392329, IC 49552, IC 267941 and IC 89032 showed greater variations in their soluble carbohydrate contents.

Table 1 : Mean values for the biochemical parameters of location 1

Accessions	Total phenol (g/100g)	β-carotene (mg/100g)	Lycopene (mg/100g)	Total protein (g/100g)	Soluble carbohydrate (g/100g)
IC 89032	1.388 ±0.007	37.616 ± 0.046	18.182 ± 0.059	23.331 ± 0.060	27.535 ± 0.079
IC 203201	1.416 ±0.004	43.507 ± 0.215	17.074 ± 0.082	25.170 ± 0.090	27.432 ± 0.074
IC 139506	1.619 ±0.004	36.233 ± 0.108	15.388 ± 0.083	24.685 ± 0.175	24.992 ± 0.046
IC 561040	1.351± 0.009	20.176 ± 0.042	8.544 ± 0.077	24.412 ± 0.111	25.517 ± 0.113
IC 267941	1.270± 0.014	55.263 ± 0.038	11.225 ± 0.088	27.821 ± 0.134	32.325 ± 0.124
IC 385389	1.164± 0.019	34.205 ± 0.657	6.825 ± 0.043	22.662 ± 0.101	23.564 ± 0.094
IC 49552	1.422± 0.009	69.893 ± 0.137	8.939 ± 0.081	26.183 ± 0.149	28.182 ± 0.120
IC 392329	2.030± 0.020	41.799 ± 0.075	4.514 ± 0.049	24.586 ± 0.097	26.297 ± 0.083
IC 320970	1.884± 0.041	29.756 ± 0.142	10.628 ± 0.076	23.281 ± 0.264	30.731 ± 0.079
IC 145247	1.910± 0.013	26.591 ± 0.085	9.484 ± 0.049	22.610 ± 0.103	22.938 ± 0.058
IC 9624	1.387± 0.019	23.271 ± 0.093	12.169 ± 0.129	23.163 ± 0.097	27.202 ± 0.090
IC 24842	1.003± 0.044	24.577 ± 0.019	5.787 ± 0.037	26.806 ± 0.128	27.254 ± 0.063
IC 341296	1.132± 0.028	16.479 ± 0.036	9.056 ± 0.097	21.637 ± 0.162	22.718 ± 0.036
IC 22827	1.074± 0.055	48.888 ± 0.089	2.451 ± 0.128	22.437 ± 0.118	23.118 ± 0.073
IC 139523	1.690± 0.049	37.266 ± 0.050	10.657 ± 0.034	23.601 ± 0.153	24.503 ± 0.049

Table 2 : Mean values for the biochemical parameters of location 2

Accessions	Total phenol (g/100g)	β-carotene (mg/100g)	Lycopene (mg/100g)	Total protein (g/100g)	Soluble carbohydrate (g/100g)
IC 89032	1.242±0.033	35.532 ± 0.064	16.796 ± 0.164	24.557 ± 0.125	25.739 ± 0.121
IC 203201	1.385± 0.009	43.174 ± 0.082	16.697 ± 0.082	25.810 ± 0.097	26.100 ± 0.072
IC 139506	1.549± 0.005	35.439 ± 0.034	13.385 ± 0.048	24.793 ± 0.129	24.160 ± 0.093
IC 561040	1.241± 0.022	20.072 ± 0.049	8.313 ± 0.098	25.133 ± 0.147	24.643 ± 0.036
IC 267941	1.971± 0.033	53.206 ± 0.021	10.626 ± 0.052	27.192 ± 0.046	27.341 ± 0.075
IC 385389	1.181± 0.043	34.348 ± 0.055	6.358 ± 0.025	22.339 ± 0.123	23.243 ± 0.063
IC 49552	1.345± 0.024	63.574 ± 0.062	8.099 ± 0.052	28.569 ± 0.130	25.622 ± 0.070
IC 392329	1.842± 0.023	39.691 ± 0.098	4.284 ± 0.038	24.793 ± 0.096	24.498 ± 0.322
IC 320970	1.601± 0.021	28.813 ± 0.043	10.090 ± 0.142	24.266 ± 0.148	27.319 ± 0.069
IC 145247	1.547± 0.024	26.404 ± 0.051	8.028 ± 0.130	22.644 ± 0.080	22.704 ± 0.085
IC 9624	1.081± 0.034	22.838 ± 0.088	11.727 ± 0.025	24.689 ± 0.076	26.738 ± 0.067
IC 24842	0.851± 0.020	23.565 ± 0.019	5.295 ± 0.046	28.553 ± 0.149	26.507 ± 0.093
IC 341296	0.932± 0.018	16.362 ± 0.047	7.875 ± 0.032	22.161 ± 0.107	22.302 ± 0.151
IC 22827	0.827± 0.021	44.656 ± 0.050	1.626 ± 0.079	22.286 ± 0.086	23.460 ± 0.246
IC 139523	1.349± 0.020	32.927 ± 0.492	10.110 ± 0.044	23.777 ± 0.128	23.802 ± 0.043

Table 3 : Combined ANOVA for Location 1

Character	S.V.	df	SS	MSS	F
Protein	R	3	0.29	0.097	1.311^{ns}
	V	14	171.155	12.225	165.203^{**}
	E	42	3.115	0.074	
Soluble Carbohydrate	R	3	0.062	0.021	0.75^{ns}
	V	14	445.254	31.804	1135.857^{**}
	E	42	1.17	0.028	
β carotene	R	3	0.046	0.015	0.227^{ns}
	V	14	11317.782	808.413	12248.682^{**}
	E	42	2.76	0.066	
Lycopene	R	3	0.055	0.018	0.692^{ns}
	V	14	1089.173	77.798	2992.231^{**}
	E	42	1.081	0.026	
Phenol	R	3	0.006	0.002	0.667^{ns}
	V	14	5.596	0.400	133.333^{**}
	E	42	0.131	0.003	

Table 4 : Combined ANOVA for Location 2

Character	S.V.	df	SS	MSS	F
Protein	R	3	0.105	0.035	0.648^{ns}
	V	14	242.097	17.293	320.241^{**}
	E	42	2.275	0.054	
Soluble Carbohydrate	R	3	0.197	0.066	1.245^{ns}
	V	14	160.650	11.475	216.509^{**}
	E	42	2.212	0.053	
β carotene	R	3	0.389	0.130	1.757^{ns}
	V	14	9179.919	655.709	8860.932^{**}
	E	42	3.093	0.074	
Lycopene	R	3	0.090	0.030	1.111^{ns}
	V	14	1005.306	71.808	2659.556^{**}
	E	42	1.136	0.027	
Phenol	R	3	0.028	0.009	4.5^{**}
	V	14	4.914	0.351	175.5^{**}
	E	42	0.086	0.002	

Table 5 : Components of variances (Location 1)

Components	σ^2g	σ^2p	σ^2e	CV
Protein	3.038	3.112	0.074	1.126
Soluble carbohydrate	7.944	7.972	0.028	0.637
β carotene	202.087	202.153	0.066	0.705
Lycopene	19.443	19.469	0.026	1.603
Phenol	0.099	0.102	0.003	3.842

Table 6 : Components of variances (Location 2)

Components	σ^2g	σ^2p	σ^2e	CV
Protein	4.310	4.364	0.054	0.938
Soluble carbohydrate	2.856	2.909	0.053	0.924
β carotene	163.909	163.983	0.074	0.784
Lycopene	17.945	17.972	0.027	1.769
Phenol	0.087	0.089	0.002	3.539

Table 7 : Co-efficients of variances (Location 1)

Characters	GCV	PCV	h^2
Protein	7.215	7.302	0.976
Soluble carbohydrate	10.722	10.741	0.996
β carotene	39.017	39.023	0.999
Lycopene	43.823	43.852	0.998
Phenol	21.727	22.064	0.970

Table 8 : Co-efficients of variances (Location 2)

Characters	GCV	PCV	h^2
Protein	8.381	8.433	0.988
Soluble carbohydrate	6.782	6.845	0.982
β carotene	36.888	36.896	0.999
Lycopene	45.614	45.648	0.998
Phenol	23.374	23.641	0.978

CONCLUSION

Seeds of horse gram contain 23.6% protein which is much higher in content than whole egg protein (7-13%). But characteristically, like other legumes, horse gram cannot match the essential amino acid composition of an egg protein (Kadam and Salunkhe, 1985; Satwadhar et al. 1981). The protein content in horse gram can increase to a certain limit as an adaptive mechanism against drought stress conditions (Khandpal et al. 1981; Bharadwaj and Yadav 2012 b). Carbohydrate metabolism is affected due to stress. Increased sugar content acts as osmoregulant at lower temperatures. (Korner C. 1999). All the metabolic constituents investigated varied in their components of variances as well as co-efficients of variances between the two locations. Plants growing in semi-arid or drought prone areas have several morphological and physiological characteristics to acclimatize in stressful conditions (Kramer, P., 1983 and Tanaka-Oda, A.et al ., 2010). Plants activate various metabolic and defence systems to survive when subjected to environmental stresses such as drought, temperature, salinity. Narrow difference between PCV and GCV in both the locations implied its relative resistance to environmental alteration.

In this context the differences in the biochemical compositions among the two locations arises due to the acclimatization of the crop to the varied environments.

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