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Changes in physical and biochemical properties of fenugreek (*Trigonella sp*. L.) leaf during different growth stages

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ABSTRACT

A field experiment was conducted during *rabi* season of 2011-12 NRC on Seed Spices, Tabiji, Ajmer. The treatment comprised of three varieties and three stages. The experiment was laid out in randomized block design with three replication. Plant height, fresh weight, dry weight, number of primary branches, leaf area, number of leaves, increased significantly with advancement of age. The maximum plant height, fresh weight, dry weight was recorded in Rmt-1 at 60 DAS and minimum in Pusa Kasuri at 30 DAS. Maximum leaf area and number of primary branches was recorded in Pusa Kasuri at 60 DAS and minimum in Am-1 at 30 DAS. Maximum number of leaves and relative water content was recorded in Pusa Kasuri and minimum in Rmt-1. Maximum chlorophyll a content was recorded in Pusa Kasuri at 45 DAS and minimum in Rmt-1 at 60 DAS. Maximum chlorophyll b, total chlorophyll and carotenoid content was recorded in Pusa Kasuri at 45 DAS and minimum in Am-1 at 60 DAS.

Key words : (*Trigonella sp*. L.), Physical, Biochemical properties, growth stages.

INTRODUCTION

Fenugreek (*Trigonella sp* L. 2n=2x=16) is an important leafy vegetables which is quite popular in India. It belongs to the family leguminaceae. There are two species of *Trigonella viz*. *T. foenum-graecum, T. corniculata.* In Hindi vernacular, the first one is known as common methi and later is known as kasuri methi. Both the species are used as a leafy vegetable though later is more scented. This crop is believed to be native of an area extending from Iran to northern India, but is now widely cultivated in China, north and east Africa, Ukraine and Greece (Petropoulos ,16 Marzougui *et al.* 13) In India, it is widely cultivated throughout the India for both leaf and seed purposes and covers an area of 1.031 lakh/ha with total production (seed) of 0.958 lakh tonnes. Fenugreek leaves are used as fresh vegetables or chopped as a flavouring agent. In other parts of Asia also, the young plants are used as potherbs and the seeds as a spice or as herbal medicine (Lust, 11 and Petropoulos,16). Fenugreek leaves and seeds have been used extensively to prepare extracts and powders for medicinal uses (Basch *et al.* 3). Fenugreek is reported to have anti-diabetic, anti-fertility, anticancer, anti-microbial, anti-parasitic and hypocholesterolaemic properties. (Al-Habori and Raman 2). Fenugreek has a high proportion of protein (approximately 20–30 %) as well as amino acid, 4-Hydroxyisoleucine, which has high potential for insulin stimulating activity. Its fresh tender leaves and pods are eaten as fried vegetable being rich in iron, calcium, protein and vitamins. Flavonoids and phenolic compounds are widely distributed in plants and have been reported to show multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic activity etc. They have also been suggested to be a potential iron chelator (Kumar *et al*. 10). The relationship between the antioxidative properties of food and health has been extensively investigated over the past decade. The quality of vegetables significantly differ at different growth stages as reported by Ranjan *et al.* (18), Ranjan *et al*. (17) in brinjal and Ekka *et al*. (7) in okra. But this information is limited in fenugreek leaves.

MATERIALS AND METHODS

The experiment was conducted during *rabi* season of 2011-12 at NRCSS, Tabiji Ajmer. The three treatment (Am-1, Rmt-1, Pusa Kasuri) comprised of three levels (30, 45, 60 DAS). The experiment was laid out in randomized block design with three replication. Physical parameters on plant height, fresh weight, dry weight, number of primary branches, leaf area, number of leaves and relative water content (RWC) were recorded at 30, 45, 60 DAS. Leaves were always collected from the mid section of either branches of seedling, in order to minimize age effects. Individual leaves were removed from the stem with tweezers. Leaves were then immediately weighed (fresh mass FM). The FM obtained from each sample was above the minimum 0.5 g recommended by Clausen and Kozlowski (6). In order to estimate the turgid mass (TM) leaves floated (except where noted) in distilled water

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inside a closed petri dish. During the imbibitions period, leaf sample were weighed periodically, after wiping the water from the leaf surface with tissue paper. At the end of imbibitions period, leaf samples were placed in a pre-heated oven (Catsky 4, Turner, 20) at 80^0 C, for 48hrs, in order to obtain the dry mass (DM). All mass measurements were made using an analytical scale, with precision of 0.0001g. Values of FM, TM and DM were used to calculate RWC, using the equation:

$$RWC\ (\%) = [(FM-DM)/ (TM-DM)] \times 100$$

Biochemical observations on chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content were recorded in 30, 45, 60 DAS. Photosynthetic pigments were estimated according to method of Hiscox *et al.,* (9) using Dimethyl sulfoxide (DMSO).

Procedure

100 mg of fresh leaf portion was kept into a test tube containing 5 ml of dimethyl sulfoxide (DMSO). The test tube was then placed in an oven at 60^o C for about 2 h or more (if required) to facilitate the extraction of the pigments. After 2 h and at attaining the room temperature, absorbance was recorded at 645, 663, 638 and 480 nm on a computer aided spectrophotometer (Spectrophotometer -119 Lab India UV 3000) running a multiple wavelengths programme. DMSO was used as blank. Calculations for different pigments were made according to Welburn (1994).

$$\text{Chl 'a' (g/ml)} = 12.19\ A_{665} - 3.45\ A_{645}$$

$$\text{Chl 'b' (g/ml)} = 21.99\ A_{645} - 5.32\ A_{665}$$

Quantity of all these pigments were calculated in mg g^{-1} tissue dry weight and expressed as moles g^{-1} tissue dry weight by using the following relationship.

moles of chl 'a' = mg chl 'a' × 1.119

moles of chl 'b' = mg chl 'b' × 1.102

moles of carotenoides = mg carotenoids x 1.809

moles of total chlorophyll = chl 'a' (moles) + chl 'b' (moles)

Statistical analysis of data was done by statistical procedure prescribed by Panse and Sukhatme (15).

RESULTS AND DISCUSSION

Physical parameters

Physical parameters like plant height, fresh weight and dry weight increases with the age of plant. The average plant height, fresh weight and dry weight were the maximum at 60 DAS. Variety and stage both were significant with respect to plant height, fresh weight and dry weight. Interaction between variety and stage was also found significant. As per interaction of variety and stage the maximum plant height, fresh weight and dry weight was found in variety Rmt-1 at 60 DAS and minimum was recorded in Pusa Kasuri at 30 DAS (Table 1). The variation in plant height at different stage may be attributed to genotypic differences as reported by Chandra *et al.*(5) in fenugreek. The genotypic variation was also observed with respect to fresh weight of the plant. McCormick, (14) also observed significant phenotypic variation in terms of plant morphology, plant phenology, and yield in fenugreek. They found biomass per plant varied from 1.8 to 16.6 g in different genotypes. The initial increase in dry weight was mainly due to enlargement of the cells. These results corroborate with those reported by Gowda *et al.,* (8) in fenugreek found was highest dry weight (16.12 g). A progressive increase was observed with leaf area and number of primary branches in fenugreek varieties. Maximum number of leaves, leaf area and number of primary branches was recorded in Pusa Kasuri at 60 DAS and minimum in Am-1 at 30 DAS. At some point photosynthesis is great enough to produce more sugar than is needed for plant growth. This results in an increase in the reserve carbohydrates and its utilization for growth and development of plant viz plant height, leaf area, number of branches etc. The variation in number of branches at different stage may be attributed to genotypic differences as reported by Chandra *et al.,* (5) in fenugreek. The maximum relative water content was rerecorded in variety Pusa Kasuri at 45DAS and minimum in variety Am-1 at 60DAS. These results corroborate with those reported by Singh *et al.,* (19) in fenugreek in which the highest relative water content (84.80%) was observed in fenugreek.

Biochemical parameters

Maximum chlorophyll a content was recorded in Pusa Kasuri at 45 DAS and minimum in Rmt-1 at 60 DAS. Maximum chlorophyll b, total chlorophyll and carotenoid content was recorded in Pusa Kasuri at 45 DAS and minimum in Am-1 at 60 DAS. Chlorophyll is known to influence the photosynthetic rate and in turn influence growth and development of plants. Chlorophyll 'a' and chlorophyll 'b' and total chlorophyll content of leaf increase up to 45 DAS and after that decrease with increase growth stages. During vegetative stage sugar is synthesized in photosynthesis and breaks down during respiration by plants. Increase in total chlorophyll content up to 45 DAS cope with increased carbohydrate content requirement of plants. After 45 DAS sugars carbohydrate transports towards sink (immature pod) and degradation of chlorophyll molecules may be due to increased activity of chlorophylase enzyme activity. The results are supported by Abdouli *et al*.,(1). Carotenoids have showed similar trends to cholorophyll molecules.

Total protein and chlorophyll peaked at different times during leaf growth, declined at a steady rate during maturity, and then declined faster in the senescence phase (Makrides and Goldthwaite,12).

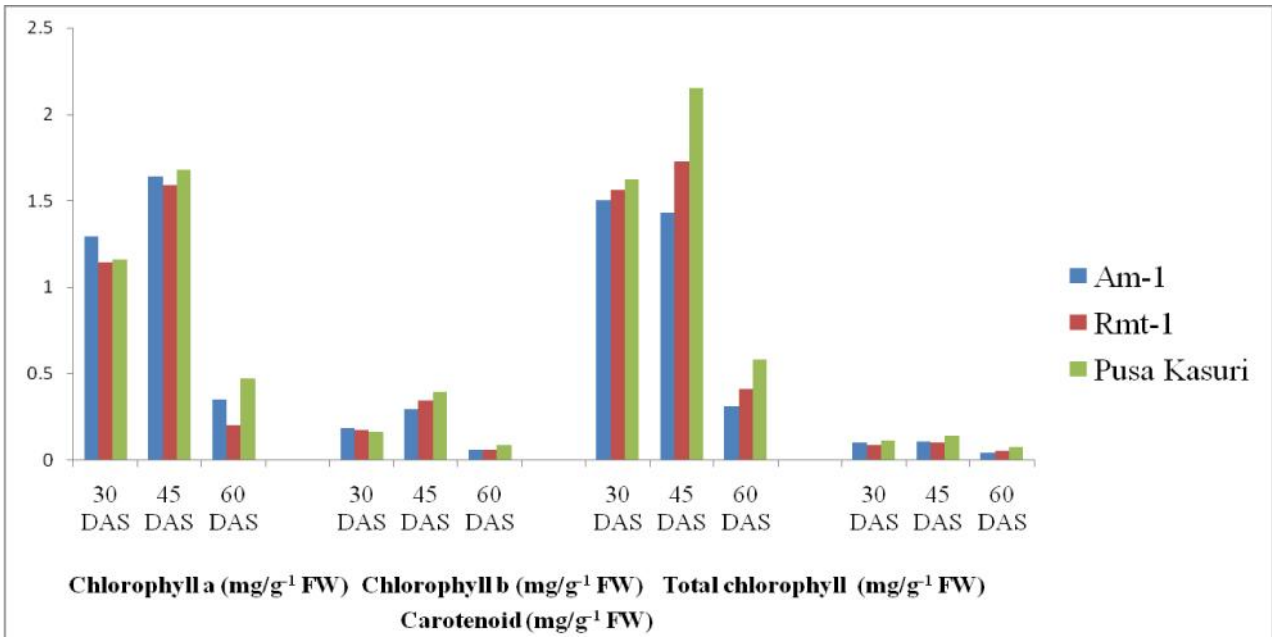
Table 1. Physical changes such as (Plant height (cm), Fresh weight (g), Dry weight (g), No. of leaves) in fenugreek during different growth stages

Variety	Plant height (cm)			Fresh weight (g)			Dry weight (g)			No. of leaves		
	30DAS	45DAS	60 DAS	30DAS	45DAS	60 DAS	30DAS	45DAS	60 DAS	30DAS	45DAS	60 DAS
Am-1	9.00	14.13	41.47	0.95	2.91	14.07	0.13	0.59	3.20	5.20	19.07	66.33
Rmt-1	11.93	15.8	45.30	1.33	4.33	16.2	0.17	0.82	3.33	6.87	21.33	56.53
Pusa Kasuri	6.67	9.90	18.87	0.46	2.11	11.17	0.07	0.37	2.07	7.53	15.93	79.00
CV	11.71			6.97			18.23			10.76		
CD	$CD_{0.05}$	SEm±		$CD_{0.05}$	SEm±		$CD_{0.05}$	SEm±		$CD_{0.05}$	SEm±	
Variety	2.25	1.06		0.41	0.2		0.22	0.1		3.31	1.57	
Stage	2.25	1.06		0.41	0.2		0.22	0.1		3.31	1.57	
Interaction	3.89	1.84		0.72	0.34		0.38	0.18		5.74	2.71	

Table 2. Physical changes such as (Leaf area (cm^2), Number of primary branches, Relative water content (%)) in fenugreek during different growth stages

Variety	Leaf area (cm^2)			Number of primary branches			Relative water content (%)		
	30DAS	45DAS	60 DAS	30DAS	45DAS	60 DAS	30DAS	45DAS	60 DAS
Am-1	7.75	9.34	11.06	3.67	4.2	7.8	84.54	85.78	73.79
Rmt-1	9.48	10.25	12.14	4.13	4.6	7.67	85.55	81.86	75.64
Pusa Kasuri	8.48	10.96	21.41	3	5	8.47	83.41	89.75	77.6
CV	15.95			11.61			2.70		
CD	$CD_{0.05}$		SEm±	$CD_{0.05}$		SEm±	$CD_{0.05}$		SEm±
Variety	1.78		0.84	0.63		0.3	2.21		1.04
Stage	1.78		0.84	0.63		0.3	2.21		1.04
Interaction	3.09		1.46	1.08		0.51	3.83		1.8

Fig. 1 Biochemical changes such as (Chlorophyll a(mg/g^{-1} FW), Chlorophyll b(mg/g^{-1} FW), Total chlorophyll (mg/g^{-1} FW), Carotenoid(mg/g^{-1} FW)) in fenugreek leaves during different growth stages



CONCLUSION

There is significant difference among fenugreek varieties with respect to various physical and biochemical characters at different growth stages.

The most ideal stage of harvesting of fenugreek leaves when the leaves attained sufficient colour and at the same time quality is at peak i.e. the leaf area etc. At this stage of growth the content of chlorophyll also remain high.

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