



## Heading Back and Pinching Affects the Biochemical and Physiological Parameters of Annual Moringa cv. PKM-1 Leaves

V. Divyabharathi<sup>1\*</sup>, V. Swaminathan<sup>2</sup>, P. Paramaguru<sup>3</sup>, K. Venkatesan<sup>4</sup>,  
T. Anitha<sup>5</sup> and T. Arumugam<sup>6</sup>

<sup>1</sup>Department of Vegetable Science, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam, Theni, India.

<sup>2</sup>Department of Horticulture, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai, India.

<sup>3</sup>Horticultural College and Research Institute for Women, Tamil Nadu Agricultural University, Tiruchirappalli, India.

<sup>4</sup>Department of Floriculture and Medicinal Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam, Theni, India.

<sup>5</sup>Department of Post-Harvest Technology, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam, Theni, India.

<sup>6</sup>Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam, Theni, India.

### Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

### Article Information

DOI: 10.9734/IRJPAC/2020/v21i2330312

#### Editor(s):

(1) Dr. Farzaneh Mohamadpour, University of Sistan and Baluchestan, Iran.

#### Reviewers:

(1) Janine Farias Menegaes, Federal University Of Santa Maria, Brazil.

(2) Maria Teresa Colinas-Leon, Universidad Autónoma Chapingo, Mexico.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/63454>

Original Research Article

Received 05 October 2020  
Accepted 10 December 2020  
Published 16 December 2020

### ABSTRACT

A trial was conducted to assess the physiological and biochemical changes in *M. oleifera* (L.) Millsp.cv. PKM 1 induced by heading back and pinching treatments. Chlorophyll content, nitrate reductase content, soluble protein content and total phenol content were assessed after the new shoot initiated from heading back and pruning treatments before the flower initiation. Height and

\*Corresponding author: E-mail: divi5594@gmail.com;

stage at which the apical growth was arrested significantly influenced chlorophyll, nitrate reductase, protein and phenol content in leaves. Heading back at 70 cm combined with pinching 100 days after heading back greatly influenced the physiological and biochemical factors except for soluble protein as it was increased by heading back at 30 cm.

**Keywords:** *Moringa oleifera* (L.) Millsp.; pruning, chlorophyll; nitrate reductase; soluble protein; phenols.

## 1. INTRODUCTION

*Moringa* (*Moringa oleifera* (L.) Millsp.), an indigenous vegetable of India is widely distributed in most tropical countries. *Moringa* is rightly termed as a 'miracle tree' of significant socio-economic importance due to several nutritional, pharmacological, and industrial applications. *Moringa* is adaptable in nature owing to its tuberous roots [1]. This tree as a whole is useful for nutraceuticals, medicinal, water purification, biodiesel production and functional food preparations [2]. For centuries *moringa* has been recognized for its contribution to traditional medicine. It plays a precise role in the maintenance of the cardiovascular system, blood-glucose level as well as a supplement of anti-oxidants and so on [3]. It is a rich source of alkaloids, flavonoids, phenolic compounds, vitamins, minerals, organic acids and phytosterols. As a reservoir of nutrients and nutraceuticals *moringa* can reduce malnutrition [4].

Canopy in a tree refers to its physical composition including stem, branches, shoots and leaves. The canopy density is determined by the number and size of the leaves, architecture of stem, branches and shoots. Canopy management of a tree deals with the development and maintenance of its structure concerning the size and shape for maximum productivity and quality [5]. The main objective of canopy management in a tree is to make the best use of the land and microclimate to increase productivity. Tree vigor, light, temperature and humidity play a vital role in the production and quality of fruits. The size, shape and volume of the canopy are affected by climate, planting density, rootstock, method of propagation, training, pruning, the regularity of bearing, soil type, nutrition, irrigation, intercrop, growth regulators used, diseases, pests, environmental pollution, etc., [6].

Canopy architecture is a natural expression of the genetic make-up of a tree. Genotypes vary in canopy size and shape. However, the size and

shape of the canopy may also be manipulated in several ways [7]. Heading back and pruning can be used, so that canopy structure can be modified to get a balanced structure with increased yield and quality. The removal of plant parts induces various biochemical and physiological changes. Heading back and pinching induced changes were studied in the annual *moringa* cv. PKM-1.

## 2. MATERIALS AND METHODS

The trial was conducted at the Department of Vegetable Science, Horticultural College and Research, Periyakulam, during 2018-19. The experiment was laid out in split-plot design with two replications. Seeds of annual *moringa* cv. PKM-1 were sown in an area of 0.63 acres with a spacing of 3 m x 3m. The main plot was imposed with heading back treatment and the sub-plot was with pinching treatments. The main-plot treatments include heading back at 30 cm ( $M_1$ ), 50 cm ( $M_2$ ) and 70 cm ( $M_3$ ) above the ground level whereas the sub-plot treatments were control (no pinching  $S_1$ ), pinching at 60 days ( $S_2$ ), 80 days ( $S_3$ ) and 100 days ( $S_4$ ) after heading back.

The chlorophyll content, Nitrate reductase activity, soluble protein and phenol content of leaves were assessed at the vegetative stage after the completion of heading back and pinching treatments. The leaves were collected from five randomly selected trees in each replication and used for analysis. Chlorophyll 'a', 'b' and total chlorophyll content were estimated spectrophotometrically using the procedure described by Yoshida et al. [8]. The chlorophyll content was expressed in mg/g of leaf (on fresh weight basis). The nitrate reductase activity was estimated following the method proposed by Sinha and Nicholas [9]. The enzyme activity was expressed as  $\mu\text{g NO}_2^{-1} \text{g}^{-1} \text{h}^{-1}$  on a wet weight basis. The soluble protein content was estimated ensuring the methodology described by Lowry et al. [10]. The mean value was expressed as mg per gram on a net weight basis. The total phenol content was estimated using the

procedure proposed by Bray and Thorpe [11]. The mean was computed and expressed as  $\text{mg gram}^{-1}$ . The data obtained were statistically analysed as per the methods of Panse and Sukhatme [12] using agres statistical software.

### 3. RESULTS AND DISCUSSION

#### 3.1 Chlorophyll Content

The chlorophyll content is the most important physiological parameter which decides the photosynthetic activity. Chlorophyll 'a', chlorophyll 'b' and total chlorophyll content were

highest in heading back at 70 cm ( $M_3$ ), pinching at 100 days ( $S_4$ ) after heading back and they were statistically different from other treatments (Table 1.). In the interaction  $M_3S_4$  was significantly different from others except in total chlorophyll it was on par with  $M_3S_3$ . The removal of the growing point stimulates the photosynthetic activity of the leaves. This effect has resulted in the enlargement of mesophyll cell size, increase in chlorophyll content and lengthening stomal opening period by increasing leaf water content [13]. Pruning increases chlorophyll content according to the findings of Lal et al. [14] in guava; Hesami et al. [15] in tomato; Sharma and Singh [16] in mango.

**Table 1. Effect of heading back and pinching on chlorophyll content of leaves**

<b>Chlorophyll 'a' (<math>\text{mg g}^{-1}</math>)</b>					
<b>Treatments</b>	<b>S<sub>1</sub></b>	<b>S<sub>2</sub></b>	<b>S<sub>3</sub></b>	<b>S<sub>4</sub></b>	<b>Mean</b>
M1	1.53	1.73	1.72	1.67	1.66
M2	1.44	1.70	1.64	1.70	1.62
M3	1.56	1.67	1.78	1.86	1.72
MEAN	1.53	1.73	1.72	1.75	1.67
				<b>SE(d)</b>	<b>CD @ 5%</b>
M – Main plot				0.006	0.027
S – Sub plot				0.010	0.022
M @S				0.016	0.041
S @ M				0.017	0.038
<b>Chlorophyll 'b' (<math>\text{mg g}^{-1}</math>)</b>					
<b>Treatments</b>	<b>S<sub>1</sub></b>	<b>S<sub>2</sub></b>	<b>S<sub>3</sub></b>	<b>S<sub>4</sub></b>	<b>Mean</b>
M1	0.55	0.54	0.51	0.47	0.52
M2	0.48	0.50	0.39	0.52	0.47
M3	0.49	0.46	0.57	0.66	0.54
MEAN	0.51	0.50	0.49	0.55	0.51
				<b>SE(d)</b>	<b>CD @ 5%</b>
M – Main plot				0.009	0.038
S – Sub plot				0.008	0.018
M @S				0.015	0.044
S @ M				0.013	0.031
<b>Total Chlorophyll (<math>\text{mg g}^{-1}</math>)</b>					
<b>Treatments</b>	<b>S<sub>1</sub></b>	<b>S<sub>2</sub></b>	<b>S<sub>3</sub></b>	<b>S<sub>4</sub></b>	<b>Mean</b>
M1	1.96	2.19	2.13	2.02	2.07
M2	1.90	2.07	1.93	2.14	2.01
M3	1.90	1.99	2.27	2.30	2.11
MEAN	1.92	2.08	2.11	2.15	2.07
				<b>SE(d)</b>	<b>CD @ 5%</b>
M – Main plot				0.005	0.022
S – Sub plot				0.014	0.033
M @S				0.022	0.053
S @ M				0.025	0.056

**Table 2. Effect of heading back and pinching on Nitrate reductase ( $\text{NO}_2^{-1}\text{g}^{-1}\text{h}^{-1}$ ) content of leaves**

Treatments	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean
M1	6.51	6.15	7.45	9.45	7.39
M2	8.79	8.27	7.36	8.27	8.17
M3	6.51	7.99	9.89	9.59	8.50
MEAN	7.27	7.47	8.23	9.10	8.02
				<b>SE(d)</b>	<b>CD @ 5%</b>
M – Main plot				0.026	0.111
S – Sub plot				0.029	0.066
M @S				0.051	0.142
S @ M				0.051	0.115

**Table 3. Effect of heading back and pinching on soluble protein (mg 100 g<sup>-1</sup>) content of leaves**

Treatments	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean
M1	11.07	12.96	12.13	11.71	11.97
M2	11.28	11.30	12.32	12.39	11.82
M3	10.91	11.69	12.13	12.79	11.88
MEAN	11.09	11.98	12.19	12.30	11.89
				<b>SE(d)</b>	<b>CD @ 5%</b>
M – Main plot				0.009	0.039
S – Sub plot				0.022	0.050
M @S				0.035	0.083
S @ M				0.039	0.087

**Table 4. Effect of heading back and pinching on Phenol (mg g<sup>-1</sup>) content of leaves**

Treatments	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	Mean
M1	0.160	0.260	0.160	0.151	0.183
M2	0.201	0.211	0.279	0.229	0.230
M3	0.239	0.279	0.251	0.211	0.245
MEAN	0.200	0.250	0.230	0.197	0.219
				<b>SE(d)</b>	<b>CD @ 5%</b>
M – Main plot				0.006	0.024
S – Sub plot				0.009	0.020
M @S				0.015	0.038
S @ M				0.016	0.035

### 3.2 Nitrate Reductase

Nitrate reductase plays a significant role in the reduction of nitrate to nitrite and in triggering root architecture remodeling. Nitrate assimilation is greatly influenced by light perception [17]. In the present study, NR-ase was found to be higher in heading back at 70 cm (M<sub>3</sub>) and pinching at 100 days (S<sub>4</sub>) after heading back also in the combination of pinching at 80 days (S<sub>3</sub>) after heading back at 70 cm (Table 2.). Those treatments were significantly different from other treatments. This might be because, well-established lateral branches in headed back and pinched trees promotes increased perception of light by phytochromes. Also, the

level of auxin and cytokinin decides the NR-ase activity in crops. Soares et al. [18] reported that increased cytokinin synthesis promoted nitrate reductase activity in soybean. Increased auxin content also promotes nitrate reductase enzyme production according to Anbarasu [19] in annual moringa and Joshi et al. [20].

### 3.3 Soluble Protein Content

In the present study heading back at 30 cm (M<sub>1</sub>), pinching at 100 days (S<sub>4</sub>) after heading back and in the combination of heading back at 30 cm (M<sub>1</sub>) with pinching at 60 days (S<sub>2</sub>) after heading back showed the highest soluble protein content and those treatments were significantly different from

other treatments (Table 3). Photosynthetic efficiency of a crop can be indexed with RUBP carboxylase activity. RUBP carboxylase activity is measured considering the amount of protein in plants [21,22]. RUBISCO protein is the most abundant protein in leaves. Fifty percent of the leaf protein goes to RUBISCO. The level of nitrogen in plants also determines the availability of soluble protein in leaves. Pinching mainly affects nitro genre distribution resulting from the removal of nitrogen demanding shoot tops. This accumulation of nitrogen increased RUBISCO protein synthesis (Yamashita, 1998). The results are in line up with the findings of Devi [23] in annual moringa cv. PKM 2 and Kalicharan [24] in annual moringa cv. PKM 1.

### 3.4 Total Phenols

Heading back at 70 cm ( $M_3$ ), pinching at 60 days ( $S_2$ ) after heading back and the interaction effect of heading back at 70 cm ( $M_3$ ) with pinching at 60 days ( $S_2$ ) on annual moringa cv. PKM-1 showed the highest phenol content in leaves (Table 4.). Interactions such as  $M_2S_3$ (heading back at 50 cm with pinching at 80 days),  $M_3S_2$  (heading back at 70 cm with pinching at 60 days)and  $M_1S_2$ (heading back at 30 cm with pinching at 60 days)were statistically on par with  $M_3S_2$ . Phenolic compounds directly influence the metabolism, growth and development in plants [25]. They act as physiologically active compounds, attractants, feeding deterrents, stress protecting agents and resistance to pathogens [26]. The phenolic content in plants may be influenced by environmental stresses and cultivational techniques. The pruned trees had a higher amount of IAA and lower IAA oxidase which may favour phenol content accumulation [27,28]. The intensity of pruning also decides the amount of phenol accumulation. The wound created by pruning cut activates the defense mechanism of a plant by which phenolic content along with polyphenol oxidase will be increased in trees [29]. A similar finding was also observed by Sanjay et al. [30] in mango.

### 4. CONCLUSION

Less intensive heading back ( $M_3$ ) and pruning ( $S_3$  and  $S_4$ ) positively influenced the growth and development of moringa by promoting physiological parameters such as chlorophyll content and nitrate reductase, whereas intensive heading back at 30 cm above the ground level favours the protein content. Hence heading back

at 30 cm and 70 cm followed by pinching at 100 days can be adopted to increase the quality of moringa cv. PKM-1 leaves.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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The peer review history for this paper can be accessed here:  
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