



Research Article

Effects of foliar application of melatonin on gas exchange and certain biochemical characteristics broccoli cv. Palam Samridhi

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Abstract

Considering the rich nutritional status and possibility of broccoli in improving the profitable yield, and wide role of Mel in regulating the plant physiological process, an investigation was carried out at the division of Basic Sciences and Humanities during 2017 to investigate the effect of foliar application of Mel on leaf photosynthetic and biochemical attributes broccoli. Thirty days old and uniform seedlings of broccoli cv. Palam Samridhi were transplanted in the field at a spacing of 45 × 45cm. Different concentrations of Mel, viz. 0, 20, 40, 60 and 80 ppm were sprayed on the plant foliage at 15 days after transplanting (DAT) replicating each treatment four times. Leaf gas exchange and biochemical attributes were tested following the standard procedures. The Results showed the lowest stipulated rate of photosynthesis (10.87 $\mu\text{mole}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$), stomatal conductance (301.44

mole $\text{H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{ses}^{-1}$) and leaf transpiration (1.14 mole $\text{H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{ses}^{-1}$) in untreated plants. Different doses of Mel significantly increased the values of these attributes and the highest values of photosynthesis (18.63 $\mu\text{mole}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$), stomatal conductance (324.37 $\text{mmole}\cdot\text{m}^{-2}\cdot\text{ses}^{-1}$) and leaf transpiration (3.23 $\text{mmole}\cdot\text{m}^{-2}\cdot\text{ses}^{-1}$) with Mel 60 ppm were recorded. The alterations in different biochemical attributes were also evident due to foliar application of Mel and maximum leaf sugar (77.0 and 85.9 $\mu\text{g/g}$), protein (56.9 and 77.3 $\mu\text{g/g}$), total phenols (260.1 and 339.9 $\text{mg}/100\text{g}$), antioxidants (142.8 and 159.9 $\text{mg GAE}/100\text{g DW}$) and MSI (94.89 and 97.43 percent) values with Mel 60ppm at 30 and 60DAT, respectively. Therefore, the present study signifies the useful effects of Mel in regulating the physio-biochemical properties of broccoli.

Keywords: Antioxidants, Broccoli, MDA, Melatonin, MSI, photosynthesis

INTRODUCTION

Broccoli (*Brassica oleracea* L.var *Italica* Plenck) is an important winter season exotic cole crop resembling cauliflower and grown for its edible head. It has large flower heads, usually green in color, arranged in a tree-like structure branching out from a thick-edible stalk. Leaves surround the mass of flower heads. It is fat-free, low in sodium and calories, high in vitamin C and good source of vitamin A, vitamin B₂ and calcium (Decoteau, 2000). Sprouting broccoli has about 130 times more vitamin A content than cauliflower and 22 times more than cabbage (Singh, 2007). It is a good source of carotenoids, glucosinolate and sulforaphane that are believed to be chemopreventive and associated with a decreased risk of human cancer (Higdon *et al.*, 2007). At present broccoli is cultivated as an important cash crop in various states of the country and gaining popularity among the consumers and vegetable growers due to its good organoleptic properties, high nutritive value and great lucrative returns. Yield and quality improvement of any crop plant can be made possible through regulating the growth and physiological processes, especially photosynthesis (Simkin *et al.*, 2019). The potential role of phytohormones in regulating the physiological processes, yield and quality of food crops, including vegetables have been well established. Melatonin (Mel) is a low molecular weight organic compound ($\text{C}_{13}\text{H}_{16}\text{N}_2\text{O}_2$) and has been regarded as nature's most versatile biological signal (Pandi-Perumal *et al.*, 2006). In higher plants, Mel was first discovered in 1995 (Dubbels *et al.*, 1995; Hattori *et al.*, 1995) and its presence has now been clearly demonstrated in all plant organs, including the root, stem, leaf, flower, fruit, and seed at concentrations usually ranging from picograms to micrograms per gram of tissue (Ramakrishna *et al.*, 2012). It has been reported to regulate various growth and physiological processes in plants with great potential for agricultural application (Nawaz *et al.*, 2016; Zhang *et al.*, 2017), especially under adverse environmental conditions.

Mel is reported to involve in the regulation of numerous physiological processes in plants such as growth, photosynthesis, rhizogenesis, chlorophyll stability and water balance (Ye *et al.*, 2016; Zhang *et al.*, 2017; Wei *et al.*, 2015; Stephen *et al.*, 2018), and also functions as

an anti-stress agent against a variety of abiotic stresses by protecting plants against reactive oxygen species (ROS) (Reiter *et al.*, 2015; Nawaz *et al.*, 2016). It also functions as a plant regulator, with growth-promoting effects similar to those of indole acetic acid (IAA) (Hernandez and Arnao, 2008). Therefore, considering the potential scope of broccoli in improving the profitable yield and wide role of Mel in regulating the growth, physiological process and yield of crops, the present investigation was carried out to investigate the effect of Mel application on leaf gas exchange and physio-biochemical attributes in broccoli.

MATERIALS AND METHODS

The investigation was carried out at the Division of Basic Sciences and Humanities, Faculty of Horticulture, SKUAST-Kashmir during the year 2017. One month old uniform and healthy seedling of broccoli cv. Palam Samridhi was collected from the nursery, Division of Vegetable Science (SKUAST-K) and transplanted at a spacing of $45 \times 45\text{cm}$ in a well prepared field. Different concentrations of Mel viz 0, 20, 40, 60 and 80ppm were sprayed through hand spray on the foliage of plants at 15 days after transplanting (DAT). One plot constituted one sample unit and each treatment consisted of four plots with 6 plants per plot. Net photosynthetic rate, stomatal conductance and transpiration rate of broccoli leaves were measured at 60 DAT using TPS-2 photosynthesis measurement system. The TPS-2 is a high precision non-dispersive infrared gas analyzer used for the measurement of leaf photosynthesis, transpiration as well as stomatal conductance which operates on the 'open system principle' that includes an infrared source, a gold plated and highly polished sample cell and detector. The leaf was placed in a sealed enclosure with a window for illumination referred as the leaf cuvette. A measured flow of air was passed through the cuvette. The $\text{CO}_2/\text{H}_2\text{O}$ concentration of the air entering and leaving were measured. To measure the concentration of $\text{CO}_2/\text{H}_2\text{O}$ TPS-2 uses a single CO_2 and water sensor and alternatively switches the reference and analysis air. From the rate flow of air and the change in concentration of $\text{CO}_2/\text{H}_2\text{O}$, the assimilation and transpiration rate as well as stomatal resistance were calculated. Biochemical analysis of the leaf was done at 30 and 60

DAT using standard procedures. For total soluble sugar 100mg of dried sample was hydrolysed with 5.0 ml of 2.5N HCl by keeping it in a boiling water bath for 3 hours. After cooling at room temperature the content was neutralized with solid sodium bicarbonate until effervescence ceases and volume was made to 100 ml. Centrifuged the content at 500g for 10 minutes and supernatant was collected. The amount of total soluble sugars was estimated by phenol sulphuric acid method (Dubois *et al.*, 1956) colorimetrically. The extraction of protein was done by grinding 0.2g of sample with a 0.1M phosphate buffer (ph 7.5) in a mortar and pestle. Centrifuged it and used the supernatant for protein estimation (Bradford 1976). Bovine Serum albumin (BSA) was used as protein standard. Total phenol was determined by Spectrophotometric measurement of blue coloured complex by the reaction of phenols with phosphomolybdic acid in Folion Ciocalteur in alkaline medium (Bray and Thorpe, 1954). The total anti-oxidant capacity of the plant leaf extracts was measured by the method described by Prieto *et al.* (1999). The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acid pH. Malondialdehyde (MDA) content generated as a product of lipid peroxidation in the leaves of broccoli was estimated in different treatments according to Cakmak and Horst (1991). Leaf sample of 0.12 g were ground in 1.2 ml 0.1% (w/v) trichloroacetic acid (TCA), then centrifuged at 12000 g for 10 min. Then 0.3 ml 0.5% (w/v) thiobarbituric acid (TBA) was added to 0.3 ml of the supernatant. The resulting mixture was boiled at 100°C for 20 min. The reaction was stopped by placing the reaction tubes in an ice bucket. The absorbance values were measured at 532, 600 and 450nm. The interference of soluble sugars in the samples at A532 and A450 was corrected by subtraction. The MDA content ($\mu\text{mol g}^{-1}$ FW) was calculated as per the equation below :

$$\text{MDA } (\mu \text{ mol/g FW}) = 6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450} \dots\dots\dots \text{Eq. 1}$$

Where,

A_{532} , A_{600} and A_{450} represent the absorbance of the mixture at 532, 600, and 450 nm, respectively.

The leaf membrane stability index (MSI) was determined according to the method of Premachandra *et al.* (1990) as modified by Sairam (1994). Leaf discs (100 mg) were thoroughly washed in running tap water followed by washing with double distilled water. Thereafter, the discs were heated in 10 ml of double distilled water at 40°C for 30 minutes. Then electrical conductivity (C_1) was recorded by EC (Electrical Conductivity) meter. Subsequently, the same samples were placed in a boiling water bath (100°C) for 10 minutes and their electrical conductivity was also recorded (C_2). The MSI was calculated using equation below):

$$\text{MSI } (\%) = 1 - C_1/C_2 \times 100 \dots\dots\dots \text{Eq. 2}$$

The data recorded on various variables were statistically analysed by using technique of analysis of variance and significance was determined as given by Panse and Sukhatme (1967).

RESULTS AND DISCUSSION

The process of carbon dioxide fixation (photosynthesis) and partitioning is believed to be a major determinant of crop yield. Data presented in table 1 revealed that untreated plants showed lowest stipulated rate of photosynthesis ($10.87 \mu\text{mole.m}^{-2}.\text{sec}^{-1}$), stomatal conductance ($301.44 \text{ mmole.m}^{-2}.\text{ses}^{-1}$) and leaf transpiration ($1.14 \text{ mmole.m}^{-2}.\text{ses}^{-1}$) which was found to increase significantly due to foliar application of Mel. The increasing dose of Mel spray resulted in a corresponding increase in these attributes and got saturated at Mel application dose of 60ppm with a maximum rate of photosynthesis ($18.63 \mu\text{mole.m}^{-2}.\text{sec}^{-1}$), stomatal conductance ($324.37 \text{ mmole.m}^{-2}.\text{ses}^{-1}$) and leaf transpiration ($3.23 \text{ mmole.m}^{-2}.\text{ses}^{-1}$). However, the rate of photosynthesis was found to drop to $15.08 \mu \text{ mole CO}_2 \text{ m}^{-2}/\text{sec}^{-1}$ at Mel 80ppm. Earlier studies also indicated that graded concentrations of Mel spray increased the photosynthetic attributes in tomato up to a certain level and then showed a decrease indicating the need for an optimum level (XioLong *et al.*, 2017). Earlier workers have also communicated an increased rate of photosynthesis due to the application of Mel (Ye *et al.*, 2016; Zhang *et al.*, 2017) which may be attributed to an increase in the amount of photosynthetic pigments in the leaf (Mansha Gul *et al.*, 2018; Stephen *et al.*, 2018). It has been suggested (Lazar *et al.*, 2013) that increased photosynthetic efficiency due to external application of Mel was the result of increased quantum yield of photochemistry of photosystem II. Increased stomatal conductance due to application of Mel has also been reported in earlier research (Ye *et al.*, 2016). There have been a positive relationship of photosynthetic rate with stomatal conductance as well as leaf transpiration (Ancu *et al.*, 2014).

Examination of the data presented in table 2 revealed that both leaf sugar ($39.5 \mu\text{g/g}$) as well as protein ($43.3 \mu\text{g/g}$) contents were minimum in untreated plants compared to Mel-treated plants and leaf sampling at 30DAT showed lower values of sugar ($55.8 \mu\text{g/g}$) as well as protein ($43.1 \mu\text{g/g}$) contents compared to the higher values of leaf sugar ($65.3 \mu\text{g/g}$) and protein ($66.7 \mu\text{g/g}$) contents at 60DAT. Comparison of foliar applied doses revealed that 60ppm of Mel was most efficient dose that produced 81.5 and 67.1 $\mu\text{g/g}$ of sugar and protein contents. The role of Mel in improving the leaf soluble sugar contents may be attributed to its effect on enhanced photosynthesis rate (Ye *et al.*, 2016; Zhang *et al.*, 2017), which might have resulted in accumulation of photosynthates in the form of soluble

Table 1. Effect of foliar application of Mel (Mel) on leaf photosynthetic attributes and transpiration in broccoli.

Treatments	Net Photosynthetic Rate ($\mu\text{mole.m}^{-2}.\text{sec}^{-1}$)	Stomatal conductance ($\text{mmole.m}^{-2}.\text{ses}^{-1}$)	Transpiration rate ($\text{mmole.m}^{-2}.\text{ses}^{-1}$)
Mel 0ppm	10.87	301.44	1.14
Mel 20ppm	12.87	312.32	1.92
Mel 40ppm	14.43	317.28	2.12
Mel 60ppm	18.63	324.37	3.23
Mel 80ppm	15.08	315.68	2.40
CD ($p \leq 0.05$)	1.52	3.98	0.98

Table 2. Effect of foliar application of Mel (Mel) on leaf sugar soluble protein contents in broccoli.

Treatment	Sugar content ($\mu\text{g/g}$)			Soluble Protein content ($\mu\text{g/g}$)		
	30 DAT	60 DAT	Mean	30 DAT	60 DAT	Mean
Mel 0ppm	33.6	45.3	39.5	31.4	55.1	43.3
Mel 20ppm	47.7	54.4	51.1	39.5	61.5	50.5
Mel 40ppm	59.2	68.7	64.0	43.7	68.2	56.
Mel 60ppm	77.0	85.9	81.5	56.9	77.3	67.1
Mel 80ppm	61.3	72.1	66.7	44.0	71.3	57.7
Mean	55.8	65.3	60.5	43.1	66.7	54.9
CD ($p \leq 0.05$)	8.1	11.2	-	5.6	6.0	-

Table 3. Effect of foliar application of Mel on leaf phenol and total antioxidant capacity in broccoli.

Treatment	Phenols ($\text{mg}/100\text{g DW}$)			Total antioxidant ($\text{mg GAE}/100\text{g DW}$)		
	30 DAT	60 DAT	Mean	30 DAT	60 DAT	Mean
Mel 0ppm	218.4	294.4	256.4	109.2	128.8	119
Mel 20ppm	239.1	313.3	276.2	117.6	135.2	126.4
Mel 40ppm	255	327.5	291.3	124.1	148.6	136.4
Mel 60ppm	260.1	339.9	300	142.8	159.9	151.4
Mel 80ppm	254.6	337.5	296.1	131.5	140.1	135.8
Mean	245.4	322.5	-	125.0	142.5	-
CD ($p \leq 0.05$)	6.4	7.1	-	11.9	10.3	-

sugar. Zhao *et al.* (2015) reported that exogenous applications of Mel regulated carbohydrate metabolism in *Arabidopsis thaliana* by modulating the sugar metabolism-related genes during plant development. Wei *et al.* (2014) suggested that Mel probably achieved its growth promotional roles in soybean through enhancement of genes involved in carbohydrate metabolism. Improved leaf protein content due to the application exogenous application of Mel has also been reported in the literature (Zhang *et al.*, 2017; Liang *et al.*, 2018).

Present study revealed that total phenol (218.4mg/100gDW; 294.4mg/100gDW) and antioxidant

(109.2 mg GAE /100g DW; 128.8 mg GAE /100g DW) contents (Table 3) obtained at 30 and 60 DAT, respectively under control were increased significantly due to foliar application of Mel. The highest values of total phenol (300.0mg/100gDW) and antioxidants (151.4mg GAE /100g DW) were recorded with 60ppm of Mel. However, these values were found higher (322.5 mg/100g DW; 142.5 mg GAE /100g DW) at 60DAP compared to lower values (245.4 mg/100g DW; 125.0 mg GAE /100g DW) at 30DAT. Earlier studies verified the increased level of total phenols due to exogenous application of Mel in *Vigna radiate* (Szafranska *et al.*,

Table 4. Effect of foliar application of Mel (Mel) on Malondialdehyde (MDA) Content and Membrane stability index (MSI) in broccoli.

Treatment	MDA (μ mol MDA/g FW)		Mean	MSI (%)		Mean
	30 DAT	60 DAT		30 DAT	60 DAT	
Mel 0ppm	0.37	0.31	0.34	72.58 (8.52)	79.32 (8.91)	75.95
Mel 20ppm	0.31	0.28	0.30	78.67 (8.87)	85.11 (9.23)	81.89
Mel 40ppm	0.29	0.26	0.28	81.66 (9.04)	90.65 (9.52)	86.12
Mel 60ppm	0.26	0.19	0.23	94.89 (9.74)	97.43 (9.87)	96.16
Mel 80ppm	0.25	0.20	0.23	85.69 (9.26)	92.33 (9.61)	89.01
Mean	0.25	0.2	0.27	85.69	92.33	85.83
CD ($p \leq 0.05$)	0.03	0.01	-	0.31	0.11	-

Figures in parenthesis are square root transformed data for the purpose of statistical analysis

2014). Mel has been reported to have antioxidant potential itself (Arnao and Hernandez, 2015) and its exogenous application was found to enhance the total antioxidant capacity in several studies (Gao *et al.*, 2018; Huang *et al.*, 2019).

Results with regard to MDA and MSI (Table 4) indicated that untreated plants showed highest MDA content (0.37 & 0.31 μ mol MDA/g FW) both at 30 and 60DAT whereas foliar spray of Mel significantly reduced the leaf MDA content and minimum values at 30 and 60DAT were recorded with Mel 60ppm (0.26 & 0.19 μ mol MDA/g FW). However, in contrast to MDA content the electrolyte analysis of the leaves showed lowest MSI (72.58 and 79.32%) with zero ppm of Mel and highest MSI (94.89 and 97.43%) with 60ppm of Mel, respectively at 30 and 60 DAT. In general, leaves sampled at 30 DAT showed higher values of MDA contents (0.25 mol MDA/g FW) coupled with the lower values MSI (85.69%) as compared to MDA value of 0.2 mol MDA/g FW and MSI value of 92.33 % of leaves sampled at 60DAT. The study supports the earlier views that as a powerful antioxidant Mel in addition to direct scavenging of ROS generated as a by-product of metabolic reactions also improve the plant's antioxidant system (Yu *et al.*, 2018). Improved level of antioxidants caused by exogenous application of Mel have also been reported by earlier workers in different crops (Gao *et al.*, 2018; Huang *et al.*, 2019; Li *et al.*, 2018). Our results are also concordant with previous reports that Mel facilitated the maintenance of membrane functions by protecting the fatty acid peroxidation (Li *et al.*, 2012; Zhang *et al.*, 2014). Further, the depression in MDA content due to Mel treatment in broccoli showed the reduction of lipid peroxidation leading to improved membrane integrity. Our results were also in agreement with those of Jiang *et al.* (2016) who stated that the application of Mel in maize resulted in a decreased contents of electrolyte leakage and malondialdehyde content compared with untreated plants.

Conclusion

Foliar application of Mel (@ 60 ppm at 15 days after transplanting resulted in significant improvement of gas exchange parameter including photosynthesis. Significant alterations in leaf sugar, protein, phenols, antioxidants and MSI were also evident due to foliar applications of Mel @ 60ppm. Further, leaves sampled at 30 DAT showed relatively lower values of these attributes compared to the leaves sampled at 60DAT, probably because of the altered source and sink ratio at the time of head development. However, in contrast to antioxidant and MSI, leaf MDA content showed the least value at 60ppm compared to other treatments, including control. It was also higher at 30 days of sampling compared to 60 days of sampling. Therefore, the present study suggested that Mel's judicious use can help improve the physiological and biochemical attributes of plant that may result in an improved head yield and quality of broccoli.

Conflict of interest

The authors declare that they have no conflict of interest.

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