International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 9, Issue 3, pp: 40-45, 2024
https://doi.org/10.55126/ijzab.2024.v09.i03.007 **http://www.ijzab.com**

Research Article

EFFECT OF AUXINS ON BIOCHEMICAL COMPOSITION OF APICAL CUTTINGS OF MULBERRY (*MORUS INDICA* L.) USING MINI CLONAL TECHNOLOGY

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Article History: Received 15th March 2024; Accepted 27th April 2024; Published 24th May 2024

ABSTRACT

A research study was carried out to examine the effects of auxin and rooting hormone on the biochemical composition of the mini clonal mulberry leaves raised using mini-clonal technique of (*Morus indica* L.) variety 'V1'.Two hormones, such as indole-3-butyric acid (IBA) and napthalene acetic acid (NAA), were used in the experiment. The hormones were replicated four times in each of the following concentration ranges: 1000, 2000, 3000, 4000, and 5000 ppm. Different hormone concentrations were applied to apical shoot cuttings before they were planted in the appropriate rooting medium in a greenhouse condition. The biochemical parameters analysed during the research study include leaf moisturecontent, nitrogen, phosphorous, potassium, chlorophyll-a, chlorophyll-b, carotenoid, total chlorophyll, crude protein, total carbohydrates, crude fat, total protein content present in mulberry leaves. The nutritive value of mulberry varieties depends on its biochemical constituents present in it. The results indicated that V1 mulberry mini clones have recorded better biochemical constituents and suitable for rearing of mulberry silkworms to get successful cocoon crop.

Keywords: Auxins, Apical cuttings, Biochemical, mulberry, Mini clonal technology.

INTRODUCTION

The deep-rooted mulberry tree (*Morus* spp.,) belongs to family Moraceae was propagated via stem cuttings, and the monophagous silkworm *Bombyx mori* L. feeds on its leaves to produce silk. Higher mulberry leaf quality increases the likelihood of high-quality cocoon crops (Ramamoorthy *et al.,* 2018). The number and quality of leaves that are gathered determine how many cocoons a silkworm will produce. Mini clonal plants has high vigour and survival under field conditions also produce good foliage with enhanced nutritional quality leaves (Parthiban *et al.,* 2021). The grade of mulberry leaves alone accounts for 38.20 percent of the production of high-quality cocoons (Sabhat *et al.,* 2011). Mulberry leaves fed to silkworms (*Bombyx mori* L.) have a major impact on their growth, development, and cocoon yield due to their high nutritional content. A vital component for improved management of silkworm rearing, in addition to biotic and abiotic factors and technology adoption, is the nutritional value of mulberry (*Morus* spp.) leaves. It is a known fact that different mulberry genotypes have different leaf qualities, which in turn accounts for variations in silkworm raising abilities. Research has shown that the amount of carbohydrates in the leaves has a significant impact on the amount of protein that larvae accumulate (Ohnuma *et al.,* 1997).When larvae are fed leaves with 3-4% sugar content during the first instar period and 4-5% sugar content during the second instar period, their growth is at its highest.

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Adding sucrose to a leaf that has little soluble carbohydrates yields good outcomes in terms of silk quantity and quality (Singh and Ninagi, 1995). Three stimulant factors—an attractant, a biting factor, and a swallowing factor are thought to be present in mulberry leaves, which provide them with excellent feeding value for silkworm larvae (David *et al.,* 1970).As leaves mature, their protein composition drops and their carbohydrate content increases, along with an increase in the components of fiber, fat, and ash. More acidity is seen in young leaves than in older ones. The ideal leaves to feed silkworm larvae are those that have reached their maximum size (Koul *et al.,* 1994).The state of silkworm development is largely dependent on the quality of the mulberry leaf, which is affected by a number of variables, including the variety of mulberry, the season, irrigation, temperature, sunshine hours, type and nature of soil profile, water table, pruning, leaf maturity, and leaf harvesting technique (Narayanan *et al.,* 1967).

The number and quality of leaves affect not only the growth and development of silkworms but also the production of cocoons and the quality of the raw silk. The proteins found in mulberry leaves provide up nearly 70% of the silk protein that silkworms generate (Miyashita, 1986). Sengupta *et al.,* (1972) have demonstrated that *B. mori* needs specific essential carbohydrates, proteins, amino acids, and vitamins for optimal development, survival, and silk gland growth. Rajabi *et al.* (2007) found that while the amount of vitamins in mulberry leaves varies depending on field practices, mulberry varieties, fertilizer usage, and environmental conditions, the vitamins are generally present in sufficient amounts to meet the minimum needs of silkworms.

MATERIALS AND METHODS

To characterize the nutritional quality of mini clonal propagated mulberry leaves: Collection of samples

Two hormones, such as indole-3-butyric acid (IBA) and napthalene acetic acid (NAA), were used in the experiment at nursery level. The hormones were replicated four times in each of the following concentration ranges: 1000, 2000, 3000, 4000, and 5000 ppm. Different hormone concentrations were applied to apical shoot cuttings before they were planted in the appropriate rooting medium in a greenhouse. Samples of mini clonal mulberry leaves were taken at 90 DAP from the five best-performing treatments, and they were then shade-dried for examination. Using a mixer grinder, the dried leaves were ground into a powder and put in an airtight container. Four replications per treatment were used to study and the data were statistically analysed. The Department of Seriulture laboratory at Forest College and Research Institute, Mettupalayam, were all the experiments done.

Leaf moisture content

The fresh leaf weight and dry leaf weight of mulberry leaves were used to calculate the moisture content, which was then expressed as a percentage (Sujathamma and Dandin, 2000).

$$\text{Moisture content in leaf (\%)} = \frac{\text{Fresh wg of leaves - Dry wg of leaves}}{\text{Fresh wg of mulberry leaves}} \times 100$$

Chlorophyll content

Fresh leaves from the middle of the best-performing mini clones were taken for each replication in order to measure the amount of chlorophyll. Using a spectrophotometer at wave lengths of 645 nm and 663 nm, the chlorophyll a, b, and total chlorophyll content in leaves were measured according to the method proposed by Cock *et al.* (1976). The results were computed as follows:

$$\text{Chlorophyll a} = (12.7 \times OD^{@\,663nm}) - (2.69 \times OD^{@\,645nm}) \times V/W \times 1000$$

$$\text{Chlorophyll b} = (2.69 \times OD^{@\,645nm}) - (4.68 \times OD^{@\,663nm}) \times V/W \times 1000$$

$$\text{Total Chlorophyll} = OD^{@652nm} \times 1000/34.5 \times V/W \times 1000$$

Where,

OD - Optical Density @ particular absorbance

V - Final volume of supernatant liquid

W - Weight of leaf sample taken for study

Carotenoid content

The following formula was used to calculate the amount of carotenoids in mulberry leaf extract, which was then represented in mg/g of fresh leaf weight.

$$\text{Carotenoid} = (7.6 \times OD^{@480nm}) - (1.49 \times OD^{@510nm}) \times V/W \times 1000$$

Total carbohydrate

As advised by Yemm and Willis (1954), the total carbohydrate content of the leaf sample was calculated, with the findings reported as milligrams per gram of fresh leaf weight.

Crude protein

A formula for calculating the crude protein composition of mulberry leaf samples was proposed by Jones and Breese (1931). It involves multiplying the 'N' content (%) by a factor of 6.25.

Total protein

Total protein level in mulberry leaf sample was calculated by following the process prescribed by Lowry *et al.* (1951) and given in terms of mg/g of fresh leaf weight.

Estimation of crude fat

Dried leaf sample was taken and crushed and two gram of sample was taken in a paper thimble and connected to the

Soxhlet extractor. Then, 300 ml of petroleum ether was poured in the flask and refluxed for 12 hours with a heating mantle. The flask was cooled in a desiccator and the weight was taken. Crude fat was determined by using the formula,

$$\text{Crude fat (\%)} = \frac{\text{Wg of flask with fat} - \text{Wg of empty flask}}{\text{Wg of original sample}} \times 100$$

Analysis of major nutrients in mulberry mini-clone leaf sample

Total nitrogen

Total nitrogen composition in mulberry sample was determined by micro-kjeldahl method as prescribed by Humphries (1956) and expressed in percentage.

Total phosphorus

To determine total phosphorous level in the mulberry sample, a process suggested by Jackson (1973) was followed and unit was given in terms of per cent.

Total potassium

The potassium levels in plant sample was estimated as recommended by Jackson (1973) and given in terms of percentage.

RESULTS AND DISCUSSION

IBA @ 3000 ppm treated mulberry leaves grown in Soil: Coir pith: FYM medium showed significantly higher chlorophyll a (2.47 mg/g), chlorophyll b (0.91 mg/g) and total chlorophyll (2.93 mg/g) content compared to control. A higher level of photosynthetic activity may be the cause of leaves' higher chlorophyll concentration. The amount of chlorophyll a was two to three times that of chlorophyll b. The current results are consistent with those of Hadimani *et al.* (2019), who reported that the V1 variety had the highest levels of chlorophyll a (1.55 mg/g), chlorophyll b (0.73 mg/g), and total chlorophyll (2.27 mg/g) followed by the S-36 variety. Manjula and Kumari (2017) also reported similar results on the V1 variety, with a total chlorophyll of 3.06 mg/g. One of the key factors in evaluating the quality of a leaf is its moisture content (Bharathi *et al.*, 2022). It is mostly determined by the mulberry variety's root proliferation and the amount of soil moisture that is accessible (Rahmathulla *et al.,* 2006). Regarding leaf moisture, there was no apparent alteration under the current study's process. In this investigation, moisture content was measured at 77.61 percent at 3000 ppm using IBA, compared to 76.00 percent in the control. These may result from the rooting media's inclusion of FYM and coir pith, which combine to hold the most water. Similarly, Babu *et al.* (2013) discovered that the mulberry leaf produced by organic cultivation has no detrimental effect on leaf moisture.

The protein concentration of all treatments varied significantly, averaging 26.04 mg/g, which is less than the control's 28.00 mg/g. For the development of silk quality and productivity, protein content is crucial. The maturity level of clonal leaves may be the cause of the reduced protein content. In comparison to the control (17.23 mg/g), IBA at 3000 ppm showed a greater carbohydrate content of 18.41 mg/g. The main dietary source for silkworm larvae is carbohydrates. Proteins and carbohydrates have a significant relationship. The presence of a sufficient amount of organic manure in the growing media may be the cause of the elevated carbohydrates, as it provides vital nutrients for plant growth. In mature leaves, the use of carbohydrates increases as the protein level declines. Umesha and Sannappa (2014) came to similar conclusions, stating that mulberry leaves' biochemical and mineral nutritional content was improved by FYM combined with other organic manures. Application of FYM @ 10 Mt/ha, according to Ram *et al.* (2017), had a positive effect on crude protein (22.40 %), moisture content (76.78 %), nitrogen (3.58 %), phosphorus (0.35 %), and total protein (24.78 mg/g). Chowdhury *et al.* (2013) supported this, reporting that the nutritional quality of mulberry leaves was enhanced by the combined application of coir pith and FYM.

The nitrogen content of the leaves determines the crude protein content. The current study's crude protein content at 3000 ppm in IBA was 24.43 percent, which is less than that of the control sample (24.56 percent). It varied from 15.00 to 25.00 percent in this investigation. Similar findings were reported by Ramachandra *et al.* (2008) and Srivastava *et al.* (2006) in *M. alba* (15.31 – 30.91 %). The energy source that is crude fat is 2.25 times higher in calories than the caloric value of proteins or carbohydrates in diet. Crude fat levels in the current investigation ranged from 7.41 to 4.23%. These results are consistent with those of Liang *et al.* (2012), who discovered that Chinese cultivars had crude fat values ranging from 1.23 to 2.23 percent. Anusuya (2019), who claimed that the Thaibeelad type contains high crude fat (10.80%) in its leaves, provided supporting for this. For plants, nitrogen is an essential component. In the meantime, it plays a crucial role in both internal and exterior metabolic processes in many plant structures. In the current investigation, the control sample nitrogen content (3.93%) was higher than that of the mini clonal leaves' treated with IBA (3.91%) at 3000 ppm. Comparably, IBA at 3000 ppm recorded potassium content of 1.77 percent, followed by 1.76 percent in the control, while phosphorus content of 0.34 percent in the control and 0.33 percent in IBA at 3000 ppm were also statistically non-significant.

The presence of organic manures like FYM in the soil increases nitrogen uptake, which results in an increase in NPK content in mulberry leaves. This demonstrated how the introduction of organic manure promoted the growth of advantageous bacteria in growth media in a synergistic manner. This was supported by Vijaya *et al.* (2009), who observed improved macro NPK content in mulberry leaves with application of 100% recommended dose of fertilizers

+ foliar nutrition (2%) and Rajanna *et al.* (2000), who confirmed the recommended application of FYM+NPK recorded higher macro nutrients in mulberry leaf followed by sheep manure + recommended FYM.

Table1.Chlorophyll and carotenoid estimation of hormone treated miniclonal leaves in Soil: Coirpith: FYM medium.

Treatments	Chlorophyll a (mg/g)	Chlorophyll a (mg/g)	Total chlorophyll (mg/g)	Carotenoid (mg/g)
T_1-IBA@2000	1.91^{d}	0.77^{d}	2.06^{d}	0.93^{d}
T_2-NAA@4000	2.03^{c}	0.83^{c}	2.33^{c}	1.02^{c}
T_3-IBA@3000	2.47^{a}	0.91^{a}	2.93^{a}	1.38^{a}
T_4-IBA@4000	1.56^{e}	0.60^{e}	1.97^{e}	0.77^{e}
T_5-NAA@3000	1.33^{f}	0.52^{f}	1.85^{f}	0.70^{f}
Control – V1 Nursery leaf	2.33^{b}	0.86^{b}	2.78^{b}	1.31^{b}
SE(d)	0.204	0.010	0.032	0.009
CD@ 0.05 %	0.042	0.021	0.069	0.019

Each value is the mean of four replications Means followed by common letter(s) are not significantly different by DMRT (P =0.05).

Table2.Macronutrient analysis of hormone treated miniclonal leaves in Soil: Coirpith: FYM medium.

Treatments	Nitrogen (%)	Phosphorous (%)	Potassium (%)
T_1-IBA@2000	2.87^{b}	0.26^{bc}	1.33^{d}
T_2-NAA@4000	2.94^{b}	0.28^{b}	1.46^{b}
T_3-IBA@3000	3.91^{a}	0.33^{a}	1.77^{a}
T_4-IBA@4000	2.60^{c}	0.26^{c}	1.38^{c}
T_5-NAA@3000	2.54^{c}	0.25^{c}	1.31^{d}
Control – V1 Nursery leaf	3.93^{a}	0.34^{a}	1.76^{a}
SE(d)	0.033	0.004	0.019
CD@ 0.05 %	0.071	0.01	0.041

Each value is the mean of four replications Means followed by common letter(s) are not significantly different by DMRT (P =0.05).

Table 3.Proximatecompositionof hormone treated miniclonal leaves in Soil: Coirpith:FYM medium.

Treatments	Moisture content (%)	Total protein (mg/g)	Carbhohydrates (mg/g)	Crude protein (%)	Crude fat (%)
T1-IBA@2000	74.99b	24.30c	15.11c	17.93b	4.96c
T2-NAA@4000	75.03b	25.66b	15.03c	18.37b	5.07b
T3-IBA@3000	77.61a	26.04b	18.41a	24.43a	7.41a
T4-IBA@4000	72.31c	23.31d	13.78d	16.25c	4.81d
T5-NAA@3000	71.50c	20.66e	13.21e	15.87c	4.23e
Control – V1 Nursery leaf	76.00b	28.00a	17.23b	24.56a	7.38a
SE(d)	0.748	0.306	0.129	0.269	0.031
CD@ 0.05 %	1.572	0.642	0.271	0.565	0.065

Each value is the mean of four replications Means followed by common letter(s) are not significantly different by DMRT (P =0.05).

CONCLUSION

In mulberry, mini cuttings treated with IBA @ 3000 ppm raised under Soil: Coirpith: FYM medium propagated successfully through miniclonal technology. After 90 DAP, mini clonal leaves analysed for various biochemical parameters revealed that the leaves grown in IBA @ 3000 ppm under Soil: Coir pith: FYM medium registered significantly higher chlorophyll a (1.91 mg/g), chlorophyll b(0.77mg/g),totalchlorophyll(2.06mg/g)andcarotenoidconte nt(0.93mg/g). Proximate composites namely moisture content (74.99%) and carbohydrate(15.11%) were significantly higher in IBA @3000 ppm compared to other treatments whereas total protein content of 26.04 mg/g was recorded in IBA 3000ppm was next only to control. In IBA 3000 ppm, crude protein (24.43 mg/g) and crude fat (7.41 mg/g) were almost on par to the control. Major nutrients

(N, P, and K) in mulberry mini clonal leaves were examined. IBA treated at 3000 parts per million leaves was on par to control in many parameters.

ACKNOWLEDGMENT

The authors express sincere thanks to the Head of the Directorate of Open and Distance Learning, Tamil Nadu Agricultural University. Coimbatore for the facilities provided to carry out this research work.

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