
Morphological Characterization, Quality, Yield and DNA Fingerprinting of Biofield Energy Treated Alphonso Mango (*Mangifera indica* L.)

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Abstract: Alphonso is the most delicious variety of mango (*Mangifera indica* L.) known for its excellent texture, taste, and richness with vitamins and minerals. The present study was attempted to evaluate the impact of Mr. Trivedi's biofield energy treatment on morphological characteristics, quality, yield and molecular assessment of mango. A plot of 16 acres lands used for this study with already grown mango trees. This plot was divided into two parts. One part was considered as control, while another part was subjected to Mr. Trivedi's biofield energy treatment without physically touching and referred as treated. The treated mango trees showed new straight leaves, without any distortion and infection, whereas the control trees showed very few, distorted, infected, and curly leaves. Moreover, the flowering pattern of control trees did not alter; it was on average 8 to 10 inches with more male flowers. However, the flowering pattern of treated trees was completely transformed into compact one being 4 to 5 inches in length and having more female flowers. Additionally, the weight of matured ripened mango was found on an average 275 gm, medium sized with 50% lesser pulp in the control fruits, while the fruits of biofield energy treated trees showed on average weight of 400 gm, large sized and having 75% higher pulp as compared to the control. Apart from morphology, the quality and nutritional components of mango fruits such as acidity content was increased by 65.63% in the treated sample. Vitamin C content in the treated Alphonso mango pulp was 43.75% higher than the pulp obtained from the control mango farm. The spongy tissue content in pulp of the matured ripened mangoes was decreased by 100% for two consecutive years as compared to the control. Moreover, the yield of flowers and fruits in the treated trees were increased about 95.45 and 47.37%, respectively as compared to the control. Besides, the DNA fingerprinting data using RAPD revealed that the treated sample did not show any true polymorphism as compared to the control. The overall results envisaged that the biofield energy treatment on the mango trees showed a significant improvement in the morphology, quality and overall productivity along with 100% reduction in the spongy tissue disorder. In conclusion, the biofield energy treatment could be used as an alternative way to increase the production of quality mangoes.

Keywords: Alphonso Mango, *Mangifera indica* L., Spongy Tissue Disorder, RAPD, Biofield Energy Treatment, Yield

1. Introduction

Mango (*Mangifera indica* L.) is the most popular tropical fruits in the Universe that belongs to the family of *Anacardiaceae*. It is known as the king of fruits. It is one of the most important fruit marketed in the world with global production exceeding 26 million tons in 2004 [1]. Mangoes are rich source of prebiotic dietary fibers, folate, and

vitamins such as A, C, B6, and B9, as per the United States Department of Agriculture (USDA) nutrient database. Among all the tropical fruits production, mangoes ranked as first position in the world. India is the first largest country for the production of mangoes in the world [2]. The mango fruits also contains various antioxidants, vitamins, phytonutrients, carotenoids, omega 3 and 6 fatty acids, polyphenols, amino acids, and dietary minerals such as potassium and copper. Owing to these properties, several literatures reported their

effectiveness in the inhibition of prostate and skin cancer [3-5]. It also protects serum oxidative stress in senile people due to its antioxidant properties [6]. Antiproliferative activity of mangoes inter and intra fruits (peel and flesh) has been reported on breast cancer cell line [7]. Banerjee *et al.* reported that due to presence of polyphenolics component it suppressed tumor growth in breast cancer xenografts in the mice model [8]. From the literature, it was found that mango extracts can prevent or arrest certain colon and breast cancer cells *in vitro* model [9].

The extract of *M. indica* has been reported for antiviral, antibacterial, analgesic, anti-inflammatory and immunomodulatory activities [10]. Complementary and alternative medicine (CAM) is increasing in biomedical health care sector. Among the alternative medicine, biofield therapies have claimed to modulate an individual's energy field for healing and wellbeing [11]. Any living body possesses some energy, and this energy can be transformed from one form to another. Human body has a tremendous resource of certain kind of energy, responsible for physical, emotional or mental activities. Human can achieve this kind of energy from food, water and light. These are the main resources of life [12]. The National Center for Complementary and Integrative Health (NCCIH), recommended the use of CAM therapies like biofield energy as an alternative in the healthcare field. About 36% of US citizens regularly use some form of CAM [13], in their daily activities. In the year 2002, Korotkov K measured the human energy field level during CAM therapy with the help of computerized gas discharge visualization (GDV) technique based on Kirlian effect. They claimed it is a first tool to visualize the distribution of human's fields, more easily, reproducibly, graphically and, very inexpensive [14, 15].

Mr. Trivedi's unique biofield treatment (The Trivedi effect[®]) has been extensively contributed in scientific communities in the field of agricultural science [16-19], chemical science [20], etc. Due to the necessity of mango as the food resource, and to improve its overall productivity an effective measure need to be established. Under these circumstances, the present work was undertaken to evaluate the effect of biofield energy treatment on mangoes in relation to morphological characteristics of leaves, flowers and fruits, quality, and yield. The genetic analysis was performed using random amplified polymorphic DNA (RAPD).

2. Materials and Methods

All the trees were 32 years old at the time of treatment and had the spongy tissue disorder in mango fruits. An experiment on mango was designed at Wakavali mango orchard (16 acres lands), Dapoli, Maharashtra, India. The trees in this orchard were divided into two parts by an imaginary line. One part of the trees was selected for Mr. Trivedi's biofield energy treatment and the other part was selected as control trees. The treatment was given at the time when the flowering was completed and about 30% of the flowers were already converted into fruit. The control plot included 75 and the treated plot had 55 mango trees. Further,

the cultivation practices were the same as for the annual plants; *i.e.*, the control trees were given standard irrigation, fertilization, pesticides and fungicides, whereas the treated trees were given only irrigation. Five months later, the treatment was given to the mango trees, the matured fruits were harvested and analyzed for the presence of spongy tissue in the pulp, fresh weight of fruit, yield, quality assessment of the fruits (reducing and total sugar, vitamin C, acidity and TSS content), and general morphology and characteristics of flowers, fruits and leaves.

2.1. Biofield Energy Treatment Strategy

Mr. Trivedi provided the treatment to the plot assigned as treated through his inherent unique energy transmission process, while sitting on the ground close to the centre of the plot without physically touching a single tree. The treated plot was not provided any pesticides, fungicides or organic additives other than water to the treated trees, whereas for the control trees, all the measures were supplemented as usual. After that, the morphological characteristics like leaves, flowering, clustering, spongy tissue, fruit description, taste and aroma, fruit drop, and quality of mango ripe fruits were studied and compared with the control.

2.2. Morphological Characteristics of Mango Leaves, Flowers and Fruits

Control and treated mango trees were evaluated for the morphological features of leaves, flowers and fruits. The various identifiable parameters were observed in fruits such as clustering, spongy tissue, taste, aroma, fruit drop *etc.* and compared with the untreated trees' fruits.

2.3. Quality Studies of Alphonso Mango

Various essential nutritional parameters were recorded in support of quality of mango fruits such as total soluble solids (TSS) expressed as ([°]Brix), acidity (%), vitamin C (mg/100gm), reducing sugar (%) and total sugar (%) and were compared with the untreated tree's mango fruits. The soluble solids are primarily sugars; sucrose, fructose, and glucose, acidic content and minerals present in the mango juice. One degree of Brix is defined as 1 gram of sucrose in 100 grams of solution.

2.4. Measurement of the Yield and Ancillary Data of *Mangifera Indica* L.

The various yield parameters such as flowering percentage, yield kg/tree, and spongy tissue content were recorded in both control and treated samples for the period of two years.

2.5. Isolation of Plant Genomic Dna Using Ctab Method

Genomic DNA was isolated from mango leaves according to standard cetyl-trimethyl-ammonium bromide (CTAB) method from the leaves of mango trees [21]. Approximate 200 mg of plant tissues (leaves) were grinded to a fine paste

in approximately 500 μL of CTAB buffer. The mixture (CTAB/plant extract) was transferred to a microcentrifuge tube, and incubated for about 15 min at 55°C in a recirculating water bath. After incubation, the mixture was centrifuged at 12000 g for 5 min and the supernatant was transferred to a clean microcentrifuge tube. After mixing with chloroform and iso-amyl alcohol followed by centrifugation the aqueous layers were isolated which contains DNA. Then, ammonium acetate followed by chilled absolute ethanol were added to precipitate the DNA content and stored at -20°C. The RNase treatment was provided to remove any RNA material followed by washing with DNA free sterile solution. The quantity of genomic DNA was measured at 260 nm using spectrophotometer [22].

2.6. Random Amplified Polymorphic DNA (RAPD) Analysis

DNA concentration was considered about 25 ng/ μL using distilled deionized water for the polymerase chain reaction (PCR) experiment. The RAPD analysis was performed on the treated mango leaves using six RAPD primers, which were labelled as RPL 5A, RPL 7A, RPL 8A, RPL 16A, RPL 18A, and RPL 19A. The PCR mixture including 2.5 μL each of buffer, 4.0 mM each of dNTP, 2.5 μM each of primer, 5.0 μL (approximately 20 ng) of each genomic DNA, 2U each of *Thermus aquaticus* (Taq) polymerase, 1.5 μL of MgCl_2 and 9.5 μL of water in a total of 25 μL with the following PCR amplification protocol; initial denaturation at 94°C for 4 min, followed by 10 cycles of annealing at 94°C for 1 min, 35 cycles annealing at 35°C for 1 min, and extension at 72°C for 2 min, followed by 94°C and 38°C for 1 min and 72°C for 1.5 min. Final extension cycle was carried out at 72°C for 7 min. Amplified PCR products (12 μL of each) from control and treated samples were loaded on to 1.5% agarose gel and resolved by electrophoresis at 75 volts. Each fragment was estimated using 100 bp ladder (Genei™, Cat # RMBD19S). The gel was subsequently stained with ethidium bromide and viewed under UV-light [23]. Photographs were documented subsequently. The following formula was used for calculation of percentage of polymorphism. Percent polymorphism = $A/B \times 100$.

Where, A = number of polymorphic bands in treated plant; and B = number of polymorphic bands in control plant.

3. Results and Discussion

3.1. Morphological Characteristics of Mango Leaves, Flowers and Fruits

Based on the observed data, a very few new leaves were appeared in the untreated mango trees and they were distorted with curly appearance and mostly infected. Besides, in the case of biofield energy treated trees showed new leaves without any distortion, started appearing immediately within first 2 months after treatment at numerous places (Fig. 1). Even the leaves of treated trees were started erupting from the infected shoots and were free from any infections, while the control trees did not show such type of phenomenon.

Leaves were more in number in the treated plants as compared to the control plants. Apart from leaves, the control mango trees showed an average 8 to 10 inches long flowering pattern with more male flowers. However, the flowering pattern of treated plants was completely transformed into compact one being 4 to 5 inches in length and having more female flowers. Moreover, 50 to 80% of malformation was consistently noticed in the untreated trees (Fig. 2).



Figure 1. Representative photograph of mango leaves.



Figure 2. Flowering pattern of Alphonso mango (*Mangifera indica* L.).

Clusters of fruitlets were observed in large quantity giving an appearance of 'jhumka' in the control sample. However, no such types of cluster of fruit lets were observed at the tip of the panicles. The weight of natured ripened mango was found on average 275 gm, medium sized with 50% lesser pulp in the control, while the biofield energy treated trees showed on the average weight of 400 gm, large sized and having more than 75% higher pulp.

After maturation of mangoes, the spongy tissues content were observed in fruit at ripe stage approximately 80% fruits were affected and at 16 anna maturity level about 60% fruits were affected with spongy tissue in control fruit. However, in biofield treated sample at 16 anna maturity stage the spongy tissue disorder was completely eradicated. The representative photomicrograph related to spongy tissues of control and in the treated mangoes are shown in Fig. 3.



Figure 3. Representative photograph of mangoes with spongy tissue disorder.

Apart from spongy tissues, the flesh was intruded with more fibers, along with a thin peel as usual in control fruit, while flesh was free from fibers with highest TSS and one of the best keeping qualities ever observed with a thick peel in the treated fruits. According to Pinto *et al.* the absence of fibers is a characteristic which is sought to mango consumers [24]. Disease and pest attack were observed upto 80% in case of untreated sample, whereas diseases and pests attack were absolutely free in the biofield treated sample. The mangoes obtained from biofield energy treated trees were found more delicious in taste and very good aromatic odour as compared to the control. Fruit drop phenomenon was very common even after immense control measures in control, whereas no fruit drop was observed throughout the season in the treated trees.

3.2. Quality Studies of Alphonso Mango

The parameters that affect the quality of mango fruits are shown in Table 1.

Table 1. Assessment of nutritional and quality parameters in Alphonso mango pulp (*Mangifera indica* L.).

Description	TSS (°Brix)	Acidity (%)	Vitamin C (mg/100 gm)	Reducing Sugar (%)	Total Sugar (%)
Control	16	0.32	38.4	6.5	28.03
Treated	16	0.53	55.2	4.6	20.87

TSS: Total soluble solids.

The TSS content was found as 16 °Brix in both control and treated groups. From the literature it was reported that greater than 14 °Brix of soluble solids indicates the good quality of mangoes. The TSS data were well matched with existed literature [25]. The present findings agreed with the results of the TSS content ranges between 16.25-27.65% Brix [26], and between 16.90 -28.26% Brix in genotype mango [27]. The acidity content in control Alphonso mango pulp was 0.32% and it was increased to 0.53% in the treated sample. There was about 65.63% increase in the acidity content in treated sample. In control sample vitamin C was found as 38.4 mg/100 gm and in the treated sample it was 55.2 mg/100 gm. Vitamin C in the treated Alphonso mango pulp content was 43.75% higher than the control mango farm. Vitamin C content has direct bearing with the immunity of the plant. Apart from these, the reducing sugar in control mangoes pulp was 6.5%, while it was reported 4.6% in treated pulp. Moreover, the content of total sugar was 28.03% in the control sample, whereas it was 20.87% in the biofield energy treated sample. Both the reducing and total sugar were reduced by 29.23% and 25.54%, respectively.

3.3. Measurement of the Yield and Ancillary Data of *Mangifera Indica* L.

In the treated plot of Alphonso mango, the biofield treatment effect was transmitted through out the following years. This was observed from the flowering and yield data recorded for the next year. The yield of flowers, fruits, and spongy tissue content within the pulp of control treated samples are shown in Table 2 and Fig. 4.

Table 2. Comparison of yield with respect of flowering, fruit and spongy tissue of Alphonso mango (*Mangifera indica* L.).

Year	Character	Control	Treated
Year 1	Flowering (%)	22	43
	Fruit (kg/tree)	19	28
	Spongy tissue (%)	23	0.0
Year 2	Flowering (%)	25	40
	Fruit (kg/tree)	20	25
	Spongy tissue (%)	17.5	0.0

The flowering was 95.45% and 60% more in the first and second year, respectively as compared to the control. Moreover, the fruit yield was increased by 47.37% and 25% in the first and second years, respectively as compared to the control. The spongy tissue content in pulp of matured ripened mangoes was decreased by 100% in two consecutive years as compared to the control (Fig. 4).

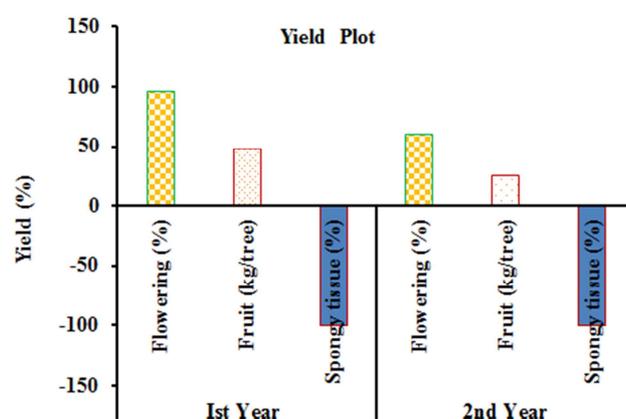


Figure 4. Percent yield of mango flowers, fruits and spongy tissues in the pulp.

3.4. Random Amplified Polymorphic DNA (RAPD) Analysis

The polymorphic DNA is responsible for giving the information about genetic marker due to its selective neutral nucleotide sequence and distinct genomes pattern [28, 29]. Here, RAPD was used as a DNA fingerprinting technique for evaluation of mango leaves. The control and treated samples were evaluated based on their various RAPD patterns. It is very simple to detect, because there is no need of DNA sequence information or synthesis of specific primers. It is a preferred tool being used nowadays to correlate the genetic similarity or mutations between species. The simplicity and wide field acceptability of RAPD technique due to short nucleotide primers, which were unrelated to known DNA sequences of the target organism [30]. The DNA fingerprinting by RAPD method was performed using six primers in the control and treated samples. The RAPD patterns of treated sample did not show any polymorphic bands as compared to the control. The bands scored of primers RPL 5A, RPL 7A, RPL 8A, RPL 16A, RPL 18A, RPL 19A were observed as 17, 15, 12, 14, 19, and 16, respectively. However, all the bands were common in control and treated samples; no band was unique. The level of true polymorphism between control and four treated samples was evaluated at 0% with all the six primers.

According to the laws of nature, two to three months before the flowering starts, the tree itself designs the program of flowering period, fruiting period and the final product in terms of quality and quantity. So if any treatment is given during or after flowering, it will not so effective because of the pre-determined program of a tree. But after Mr. Mahendra Trivedi's biofield energy treatment, on the plot was completely transformed even though they had already started flowering. In the treated trees, Trivedi's biofield energy not only altered the flowering pattern but also gave rise to complete eradication of spongy tissue malady by 100% in two consecutive years. Such enhanced growth patterns after biofield treatment also resulted into increased vitamin C level by 43.75% and decreased sugar content approximately by 30%. Throughout the experiment, neither any disease nor pest's attack was observed in the treated trees, no airborne infection too. Looking at the above improved parameters, it is expected that Mr. Trivedi's biofield energy treatment have caused these changes only by altering the DNA of the trees. On the contrary, after performing the DNA fingerprinting on leaves, no polymorphism was detected between control and The Trivedi Effect® sample. Based on RAPD data, it is assumed that Mr. Trivedi's unique biofield energy has altered either the biochemical or physiological pathways without causing any change at DNA level as the possibility of DNA getting altered has been ruled out. This is a unique phenomenon in the field of existing laws of present sciences. In the treated plot of Alphonso mango, The Trivedi effect® was transmitted through out the following years. This was observed from the flowering and yield data recorded for the second year. Biofield energy treatment could be responsible to improve the morphology of leaves, flowering pattern, fruits weight, quality and production yield. Based on these results, it is expected that biofield energy treatment has the scope to be an alternative approach for improvement in the morphological features, quality, immunity and overall productivity of the mangoes.

4. Conclusions

Overall data suggest that the biofield energy treated Alphonso mango trees showed better morphological characteristics with respect to leaves, flowering pattern and fruits as compared to the control. Moreover, the quality and nutritional parameters were also improved in the treated sample. For example, acidity content and vitamin C were increased by 65.63% and 43.75%, respectively and sugar was decreased by about 29% as compared to the control. Besides, the spongy tissue content was absolutely diminished in the treated trees as compared to the control. Moreover, the yield of flowers and fruits in the treated plants were increased about 95.45 and 47.37%, respectively as compared to the control. No polymorphism was detected between control and four treated samples. In conclusion, the present investigation demonstrates that Mr. Trivedi's unique biofield energy treatment could be utilized as an alternate therapeutic approach concurrent with other existing therapy to improve the productivity of mango by reducing the spongy tissue

content in pulp in the field of agriculture.

Abbreviations

USDA: United States department of agriculture; PCR: Polymerase chain reaction; NCCIH: National center for complementary and integrative health; CAM: Complementary and alternative medicine; RAPD: Random amplified polymorphic DNA; GDV: Gas discharge visualization; CTAB: Cetyl-trimethyl-ammonium bromide; TSS: Total soluble solids

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