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Molecular Analysis of Biofield Treated Eggplant and Watermelon Crops

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Abstract

Eggplant and watermelon, as one of the important vegetative crops have grown worldwide. The aim of the present study was to analyze the overall growth of the two inbreed crops varieties after the biofield energy treatment. The plots were selected for the study, and divided into two parts, control and treated. The control plots were left as untreated, while the treated plots were exposed with Mr. Trivedi's biofield energy treatment. Both the crops were cultivated in different fields and were analyzed for the growth contributing parameters as compared with their respective control. To study the genetic variability in both plants after biofield energy treatment, DNA fingerprinting was performed using RAPD method. The eggplants were reported to have uniform colored, glossy, and greener leaves, which are bigger in size. The canopy of the eggplant was larger with early fruiting, while the fruits have uniform shape and the texture as compared with the control. However, the watermelon plants after the biofield treatment showed higher survival rate, with larger canopy, bright and dark green leaves compared with the untreated plants. The percentage of true polymorphism observed between control and treated samples of eggplant and watermelon seed samples were an average value of 18% and 17%, respectively. Overall, the data suggest that Mr. Trivedi's biofield energy treatment has the ability to alter the plant growth rate, and can be utilized in better way as compared with the existing agricultural crop improvement techniques to improve the overall crop yield.

Keywords: *Solanum melongena; Citrullus lanatus*; Biofield energy; Plant growth attributes; DNA Fingerprinting; Polymorphism

Introduction

The eggplant (*Solanum melongena* L.) is considered as one of the most important fruit vegetable crops all over the World [1]. In South Asia, Southeast Asia and South Africa, eggplant is commonly known as brinjal of family *Solanaceae*. The fruit grown are utilized for vegetables, which contributes all the essential nutrients in our diet [2-4]. The yield of eggplant fruit is dependent on several factors such as its flowering rate (anthesis), pest attack, and diseases infections, soil nutrient status, its fertility, and application of fertilizers [5]. Eggplant is considered as heavy feeder, which occupies the ground for long time, so at least two dressings for fertilizers are required [6]. The low level of soil fertility was linked with the poor prevailing climatic conditions, that results in low final yield.

Watermelon (*Citrullus lanatus*) belongs to the family of *Cucurbitaceae* [7], grown as a cash crop. It is mainly grown for its edible fruit that is a special kind of berry named as pepo. This plant is originally from Southern Africa, while its center of origin is between Kalahari and Sahara deserts in Africa [8]. These areas has been regarded as the point of diversification to other parts of the World [7]. For better nutrient status, the soil fertility factors must meet the criteria for better yield of fruit crop. Some methods has been prescribed for better yield of soil is to boost it with the use of organic materials, like animal waste, poultry manure, and use of compost or with the use of inorganic fertilizers [9]. This crop is considered as heavy feeder of nitrogen that required a high application of NPK fertilizers before sowing, followed by nitrogenous fertilizers till flowering stage [10]. The most important source of nitrogen is the inorganic fertilizers, which yield the vigorous vegetative growth, dark green leaves, and high photosynthetic rates. It was reported that extensive use of fertilizers will delay the ripening, reduce fruit setting and its number [11]. Therefore, some alternative approach besides the use of fertilizers, which could improve crop yield, overall plant growth, and its vegetative growth.

Phenotypic characters are based on the genetic identification, which affect the morphological characters of plant. DNA polymorphism identification is independent of environmental conditions using different molecular markers. Molecular markers of randomly amplified polymorphic DNAs (RAPD) analysis shows variation in the genome, which might expressed or not, while morphological markers reflect variation in expressed regions [12]. Using RAPD analysis, maximum genetic relatedness among plant genome can be identified, due to their simplicity, speed and low-cost [13].

Apart from these traditional approaches to improve the crop yield, recent research suggest that treatment of seeds with electric and magnetic field can improve the growth and yield of agricultural crops [14-16]. National Center for Complementary and Alternative Medicine (NCCAM) recommended the use of energy treatment as an alternative integrative medicine to promote human wellness [17]. Biofield is a type of electromagnetic field that permeates and surrounds the living organisms. Scientifically, it can be defined as biologically produced electromagnetic and subtle energy field within the organism. The objects always receive the energy and responding to the useful way that is called biofield energy treatment. Mr. Trivedi's unique biofield treatment is known as The Trivedi Effect®. Mr. Trivedi having the unique biofield energy, which has been reported in several research areas [18-21]. On the basis, of present literatures, present study was designed to evaluate the biofield treatment on selected plots (control and treated) for the seeds of eggplant and watermelon crop. Genetic variability parameters of both the crops were studied using RAPD (DNA fingerprinting).

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Materials and Methods

Eggplant (*Solanum melongena*) and watermelon (*Citrullus lanatus*) were selected for the present study. Both the plants were selected from inbreed variety for all the experimental parameters**.** The biofield treated plot size for eggplant was 64 × 8 feet, while the control plot size was 47 × 12 feet. The treated plot size for watermelon was 64 × 16 feet, while the control plot size was 35 × 25 feet. Both the plots were have same number of plants, and were compared with respect to respective control. The control plots were left untreated, while the treated plots of eggplant and watermelon was subjected to Mr. Trivedi's biofield energy treatment. The seeds from each crop were cultivated for analysis. However, the control plants were given standard cultivation parameters such as proper irrigation, fertilizers, pesticides and fungicides; while the treated plots were given only irrigation, without any supportive measure. DNA fingerprinting of both the plants were performed using RAPD techniques using Ultrapure Genomic DNA Prep Kit; Cat KT 83 (Bangalore Genei, India) to study the genetic relationship before and after treatment.

Biofield treatment strategy

The treated plots were subjected to Mr. Trivedi's biofield energy treatment. Mr. Trivedi provided the unique biofield treatment through his energy transmission process to the selected treated plots of both the crops. The plant samples of treated plots were assessed for the growth attributes with respect to control. Variability in different growth contributing parameters and genetic relatedness using RAPD of control and treated crops were compared [18].

Analysis of growth and related parameters of crops

The seeds of eggplant and watermelon were cultivated under similar conditions. The vegetative growth of the crops with respect to plant canopy, the shape of leaves, flowering conditions, infection rate, etc. were analyzed and compared with respect to the plants of control plots [22].

DNA fingerprinting isolation of plant genomic DNA using CTAB method

The leaves disc of both plants were harvested after germination, as it reached the appropriate stage. The genomic DNA from both plant leaves was isolated according to the standard cetyl-trimethyl-ammonium bromide (CTAB) method [23]. Approximately 200 mg of plant tissues 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 contain the 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].

Random amplified polymorphic DNA (RAPD) analysis

The RAPD analysis was performed on the each treated plot plants using RAPD primers, which were label as RPL 6A, RPL 13A, RPL 16A, RPL 18A, and RPL 19A for eggplant, while RPL 2A, RPL 7A, RPL 12A, RPL 14A, RPL 18A, and RPL 23A for watermelon. The DNA concentration was considered about 25 ng/µL using distilled deionized water for polymerase chain reaction (PCR) experiment. 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. The PCR cycle condition for eggplant and watermelon includes initial denaturation at 94°C for 5 min, followed by 40 cycles of annealing at 94°C for 1 min, annealing at 36°C for 1 min, and extension at 72°C for 2 min, while final extension was carried out at 72°C for 10 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 watermelon sample was analyzed with help another ladder of 500 bp ladder (Genei™; Cat # RMBD13S). The gel was subsequently stained with ethidium bromide and viewed under UV-light [24]. Photographs were documented subsequently. The following formula was used for calculation of the percentage of polymorphism.

Percent polymorphism = A/B × 100

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

Results and Discussion

Effect of biofield treatment on growth contributing parameters of eggplant

The eggplant crop in control plots showed the survival as less than 60-65%. The growth of eggplants was much less in the control group as well. The eggplants had a small canopy in control plot. The leaves were small in size, and their color was light green in eggplants of control plot. The fruit of eggplant in control plot did not have uniform shape and most of the fruit was diseased even after being sprayed with pesticides and fungicides.

On the other hand, the biofield treated plot for eggplants showed the leaves were thick, glossy, more green in color and bigger in size. The canopy of the eggplant in biofield treated plot was also larger as compared with the control crop. The budding was early, that suggest early fruiting as compared with the control. In biofield treated plots, all eggplants fruits had uniform shape and the texture as compared with the control. Further, no disease in the treated plants were observed as compared with the control (Figure 1).

Research study suggest that both mineral fertilizers and organic manures have their own roles in soil fertility management, however none can completely provide all the nutrients and conditions, which may enhance the growth of eggplant [25]. Biofield treatment on soil selected for eggplant crops, showed enhanced growth in the absence of chemical fertilizers, and suggested the alternate method to improve the crop yield.

Effect of biofield treatment on growth contributing parameters of watermelon

In the control plot, the survival rate of the watermelon plants was less than 60 to 65%. The canopy was small, and the color of the leaves were pale green in plants of control plot. Many of the plants were reported as diseased and even the fruits were infected at an early stage, with small fruit size in plants of control plot.

The biofield energy treatment on plots with watermelon plants showed high survival rate i.e., more than 99%. The canopy of watermelon plants was much larger in treated plots than in the control plot plants.

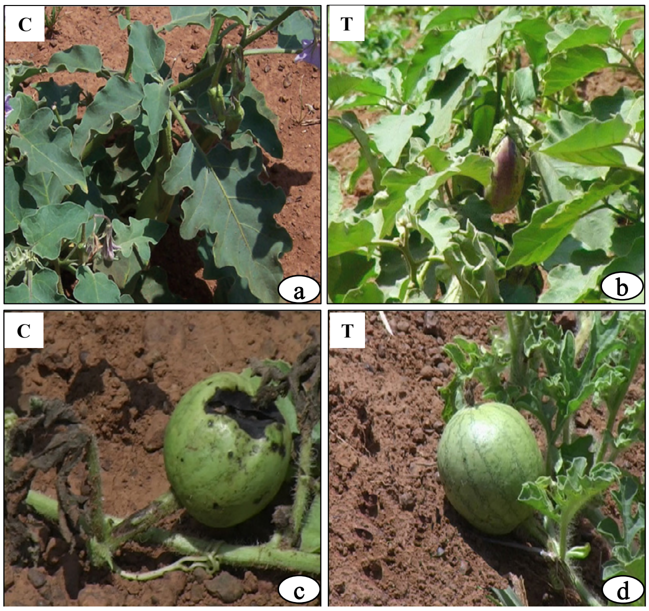

Figure 1: The Trivedi Effect® on eggplant and watermelon.
(a) leaf of control eggplant was reported with less growth and infection,
(b) biofield treated leaves and flowers of eggplant are healthy and infection free,
(c) control watermelon plants showed infection in fruits and leafs, while
(d) biofield treated watermelon showed leaves were free from any kind of disease with healthy growth and fruits in high yield.
C: Control; T: Treated

The color of the leaves was bright and dark green. The watermelons were bigger in size, and the texture of the fruit was different. The treated watermelon plants were absolutely free from disease. Although the growth of watermelon plants has been reported to show vast variation due to different seasons such as effect of light, heat, temperature, etc. [26] After biofield treatment, the growth characters were reported with huge change as compared with the control in similar conditions, so results suggest that biofield treatment might alter some basic physiological character of plants responsible for the overall growth (Figure 1).

Therefore, it can be suggested that biofield treatment on land could be a new approach to improve the yield of eggplant and watermelon as compared with the control. Mahajan et al. reported that on exposure of plant seeds to electric and magnetic field, seeds become polarized and can retain the change in polarization. However, the polarized seeds when come in contact with water, significant interaction takes place between water dipole and seed dipoles, which results in better water uptake. This phenomenon might be responsible for better yield of crops [15]. Our experimental results suggest better growth of plants that might be due to the higher water retention in biofield treated land plants may be due to better dipole interaction.

The different environmental factors somehow contribute to the growth of the plant. A report on the effect of magnetic field on plant seeds with respect to the growth of the plant was measured, and suggest the improved growth of roots and shoots [27]. Further, the effect was also reported to have improved level of photosynthesis, stomatal conductance and chlorophyll content after magnetic field treatment under stress conditions [28]. Biofield energy treatment is a type of complementary and alternative energy medicine, which involves low-level energy field interactions. Overall results assumed that the biofield energy might provide energy to the plant that change the paramagnetic behaviors of the tested plants, which might help in improved growth of eggplant and watermelon.

RAPD analysis of eggplant and watermelon

DNA fingerprinting using RAPD molecular markers have been widely accepted technique to study the changes in vegetable crops [29]. Using different RAPD markers, important information for genetic diversity can be evaluated for different species of plants. Besides genetic diversity, population genetics study, pedigree analysis and taxonomic discrimination can also be correlated [24]. However, RAPD is considered as a powerful tool to evaluate the differences between inter- and intra-population of plants [30]. Biofield energy treatment was reported with high genetic variability among species using RAPD fingerprinting [18]. However, the effect was also reported in case of biofield treated ginseng, blueberry [31], and lettuce, tomato [32] with an improved overall agronomical characteristics.

Effect on plants genetic characters from control and biofield treated plots were compared and analyzed for their epidemiological relatedness and genetic characteristics. Genetic similarity or mutations between the two groups were analyzed using RAPD. Both the samples required short nucleotide random primers, which were unrelated to known DNA sequences of the target genome.

Random amplified polymorphic-DNA fragment patterns of control and treated eggplant samples were generated using five RAPD primers, with 100 base pair DNA ladder. The results of DNA polymorphism in control and treated samples are presented in Figure 2. The DNA profiles of treated group were compared with their respective control.

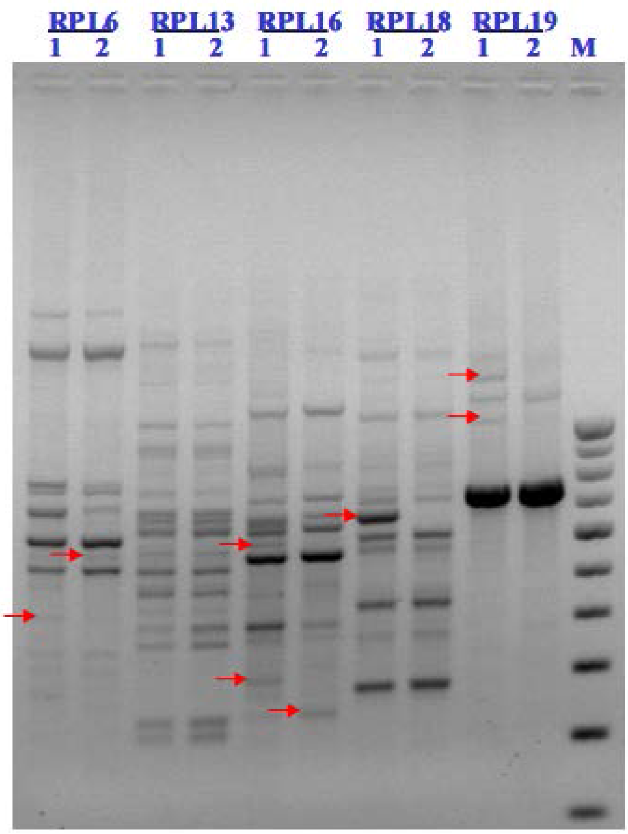

Figure 2: Random amplified polymorphic-DNA fragment patterns of eggplants of biofield treated plots generated using five RAPD primers, RPL 6A, RPL 13A, RPL 16A, RPL 18A, and RPL 19A. M: 100 bp DNA Ladder; Lane 1: Control; Lane 2: Treated.

The polymorphic bands observed using different primers in control and treated samples were marked by arrows. The results of RAPD patterns in biofield treated eggplant showed some unique, common and dissimilar bands as compared with the control. The DNA polymorphism analyzed by RAPD analysis, showed different banding pattern in terms of total number of bands, and common, and unique bands, which are summarized in Table 1. The percentage of polymorphism between samples was varied in all the primers, and were ranged from 0 to 40% between control and treated samples. However, level of polymorphism was maximum using the primer RPL 19A and minimum with RPL 18A, while RPL 13A primers did not show any level of polymorphism.

On the other hand, watermelon also showed high level for polymorphism using five primers with respect to the control. Different banding pattern was observed using RAPD DNA polymorphism in terms of total number of bands, and common, and unique bands, which are summarized in Table 2. The polymorphic bands observed using six different primers in control and treated samples of watermelon were marked by arrows in Figure 3. The level of polymorphism percentage in watermelon samples were varied in all the primers, and were ranged from 8 to 100% between control and treated samples. However, level of polymorphism was detected as 7%, 16%, 18%, 12%, and 33% using the primer RPL 2A, RPL 7A, RPL 12A, RPL 18A, and RPL 23A respectively. Highest level of polymorphism was detected using primer RPL 23A, 33%, while RPL 14A did not shown any polymorphism.

RAPD analysis using different primers explains the relevant degree of genetic diversity among the tested samples. Overall, RAPD showed that polymorphism was detected between control and treated samples. The percentage of true polymorphism observed between control and treated samples of eggplant and watermelon sample was an average value of 18% and 17%, respectively.

However, RAPD is a tool which will detect the potential of polymorphism throughout the entire tested genome. Biofield treated plot plants eggplant and watermelon showed varied number of polymorphic bands that indicated that the genotypes selected possess a higher degree of polymorphism as compared with the control. Molecular analyses and genetic diversity of eggplant and watermelon have been reported using RAPD analysis. Mr. Trivedi's biofield energy treatment on plots showed different level of polymorphism in eggplant and watermelon that suggested that biofield energy treatment might have the capability to alter the genetic character of plants, which might be useful in terms of productivity.

S.No.	Primer	Primer Sequence	Band Scored	Common bands	Unique band	
					Control	Treated
1.	RPL 6A	TGGACCGGTG	11	8	1	1
2.	RPL 13A	CCTACGTCAG	16	16	-	-
3.	RPL 16A	AGGCGGGAAC	14	8	2	1
4.	RPL 18A	GAACGGACTC	11	10	1	-
5.	RPL 19A	CACACTCCAG	5	2	2	-

Table 1: DNA polymorphism of eggplant analyzed after biofield treatment using random amplified polymorphic DNA (RAPD) analysis.

S.No.	Primer	Primer Sequence	Band Scored	Common bands	Unique band	
					Control	Treated
1.	RPL 2A	CAGGCCCTTC	19	18	1	-
2.	RPL 7A	GTGATCGCAG	19	17	3	-
3.	RPL 12A	AGGACTGCCA	18	16	1	1
4.	RPL 14A	ACGGATCCTG	13	13	-	-
5.	RPL 18A	GAACGGACTC	13	13	-	1
6.	RPL 23A	CAGCACCCAC	14	14	1	3

Table 2: DNA polymorphism of watermelon analyzed after biofield treatment in plot using random amplified polymorphic DNA (RAPD) analysis.

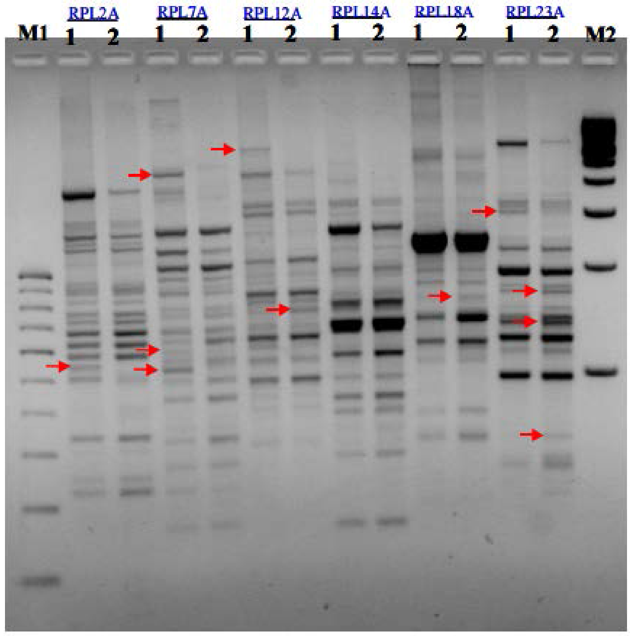

Figure 3: Random amplified polymorphic-DNA fragment patterns of watermelon in biofield treated plot generated using six RAPD primers, RPL 2A, RPL 7A, RPL 12A, RPL 14A, RPL 18A, and RPL 23A. M1: 100 bp, M2: 500 bp DNA Ladder; Lane 1: Control; Lane 2: Treated.

Conclusions

In summary, biofield energy treatment on the eggplant and watermelon showed improved growth characteristics such as fruits, leaves and free from pest attack. The canopy of plant and fruits of eggplant and watermelon was reported as large compared to their respective control. Biofield treated eggplant and watermelon plants showed strong and uniform colored leaves, with high survival rate, which suggest higher immunity of plant as compared with the control. Further, the watermelons were bigger in size, and the texture of the fruit was different from untreated fruits. It is assumed that after biofield treatment, the polarization of seeds might be affected that changed the interaction between water and seed during germination. Besides, the percentage of true polymorphism observed between control and treated samples of eggplant and watermelon seed sample was an average value of 18% and 17%, respectively. Overall, the experimental results suggested that Mr. Trivedi's biofield energy treatment might be used to improve the overall crop productivity with the capability to alter at genetic level.

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