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Evaluation of Vegetative Growth Parameters in Biofield Treated Bottle Gourd (*Lagenaria siceraria*) and Okra (*Abelmoschus esculentus*)

Mahendra Kumar Trivedi[1], Alice Branton[1], Dahryn Trivedi[1], Gopal Nayak[1], Mayank Gangwar[2], Snehasis Jana[2, *]

[1]Trivedi Global Inc., Henderson, USA
[2]Trivedi Science Research Laboratory Pvt. Ltd., Bhopal, Madhya Pradesh, India

Email address:
publication@trivedisrl.com (S. Jana)

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Abstract: The objective of the study was to assess the growth contributing characters of biofield treated bottle gourd (*Lagenaria siceraria*) and okra (*Abelmoschus esculentus*) seeds. The seeds of both crops were divided into two groups, one was kept aside and denoted as untreated, while the other group was subjected biofield energy treatment. The variabilities in growth contributing parameters were studied and compared with their control. Further the level of glutathione (GSH) in okra leaves, along with DNA fingerprinting in bottle gourd were analyzed using RAPD method. After germination, the plants of bottle gourd were reported to be strong and erect with better canopy as compared with the control. The vegetative growth of okra plants after biofield energy treatment was found to be stout with small canopy, strong steam, and more fruits per nodes, that contributed high yield as compared with the control. However, endogenous level of GSH in the leaves of okra was increased by 47.65% as compared to the untreated group, which may suggest an improved immunity of okra crops. Besides, the DNA fingerprinting data, showed polymorphism (42%) between treated and untreated samples of bottle gourd. The overall results suggest that the biofield energy treatment on bottle gourd and okra seeds, results an improved overall growth of plant and yield, which may enhance flowering and fruiting per plant. Study results conclude that the biofield energy treatment could be an alternate method to improve the crop yield in agricultural science.

Keywords: *Lagenaria siceraria*, *Abelmoschus esculentus*, Biofield Energy, Glutathione, DNA Fingerprinting, Polymorphism

1. Introduction

Some traditional medicines are derived from the vegetative plants, minerals and organic matter [1]. The calabash or bottle gourd (*Lagenaria siceraria*) is commonly known as long melon, *lauki, ghia* or *dudhi* in India. It is one of the first vegetative domesticated crop for human use. It can be used for many purposes such as food, medicine, etc. A total of six varieties of *Lagenaria* species are known, out of which *L. siceraria* is domesticated, while the rest are perennial, wild congeners, and dioecious in nature [2]. Bottle gourd is supposed to be originated from Africa and America [3], while it occurs in wild form in India and South Africa. However, most of the variability in terms of shape, size, and fruits are found in India [4]. It is considered as a part of complementary and alternative therapy against many diseases (*i.e.,* in case of high blood pressure, heart disease, liver function, nerve tonic, indigestion, ulcers, etc.) [5].

Abelmoschus esculentus is commonly known as okra or lady's finger, is an important vegetative crop throughout the tropics and subtropics region in warm temperate zones of the World. However, it is considered to be native to tropical Africa [6]. It is well distributed and cultivated in Indian subcontinent and are harvested as immature fruits and eaten as a vegetable. The content of oil in seeds is about 15%, and it is an important source of protein, minerals, vitamins and roughage [7]. However, its fresh tender pods were used to cure leucorrhea, constipation, spermatorrhea, jaundice, and diabetes. Its mucilage component is also used to treat different gastrointestinal disorders [8]. Although, the plants of okra are correlated with its benefits in few diseases, but most of the problems are associated with seed and soil borne pathogens, which finally results in a low final yield of the crops [9, 10], and different advanced methods have been adopted for the seeds to increase the yield [11]. Some alternate methods are required to improve the yield of vegetative crops, which could restrict the crop infections and lead to improve the yield.

Biofield energy treatment is one of the alternative approaches recently reported to alter the agricultural crops crop yield [12, 13]. Biofield therapy (the electromagnetic field) is one type of energy medicine, which comes under the category of complementary and alternate medicine (CAM). Use of energy medicine is prescribed and included under CAM category by National Institutes of Health/National Center for Complementary and Integrative Health (NIH/NCCIM) [14]. It involves the use of human biofield, which will regulate and concentrate the bioenergy and transmit it into any living or non-living object. Thus, the human has the ability to harness the energy from the environment and can transmit it into useful way. Now, a days, biofield therapies has been widely practiced and reported with several benefits in medical science [15-17]. Mr. Mahendra Kumar Trivedi, is one of the well-known biofield therapy practitioner, and possesses a unique biofield energy. His unique biofield energy treatment is popularly known as The Trivedi Effect®. The results of biofield treatment have been reported in the field of agricultural science that improves the yields and growth of vegetative plants [18, 19].

Inspired by the previous results of biofield treatment, and importance of vegetative crops, the present study was designed to evaluate the effect of biofield energy treatment on bottle gourd and okra seeds. Genetic variability parameters of bottle gourd (DNA fingerprinting) using standard molecular method were performed, while level of glutathione (GSH) in okra leaves after biofield treatment was studied.

2. Materials and Methods

Bottle gourd (*Lageneria siceraria*) and okra (*Abelmoschus esculentus*) were selected for present study for its economic importance. Nirmal-200 variety of bottle gourd was procured from Nirmal Seeds, Jalgoan, Maharashtra, India, while F1 hybrid okra variety was purchased from Syngenta India Ltd, Pune, Maharashtra, India. Each variety of the seeds was divided into two parts, one part was considered as control, as untreated. The other part was coded as treated and subjected to Mr. Trivedi's biofield energy treatment. Both variety of seeds were cultivated in Shahapur agricultural land in Maharashtra for growth analysis. Further, the cultivation practices were the same as for all the plants; *i.e.,* the control plants were given standard irrigation, fertilization, pesticides and fungicides, whereas the treated plants were given only irrigation. The sampling was done for laboratory analysis at three developmental stages, *i.e.,* pre-flowering, flowering and post-flowering. Further, the level of GSH was estimated in control and treated okra crop leaves. The random amplified polymorphic DNA (RAPD) analysis was done in bottle gourd using Ultrapure Genomic DNA Prep Kit; Cat KT 83 (Bangalore Genei, India).

2.1. Biofield Treatment Strategy

The treated groups of seeds were subjected to Mr. Trivedi's biofield energy treatment under standard laboratory conditions. Mr. Trivedi provided the unique biofield treatment through his energy transmission process to the treated group of both seeds without touch. The treated samples were assessed for the growth germination of seedlings, leaves, length of plant, and rate of infections. Variability in different growth contributing parameters of control and treated crops were compared [18].

2.2. Analysis of Growth and Related Parameters of Crops

Control and treated seeds of bottle gourd and okra were cultivated under similar conditions. Vegetative growth of the crops with respect to plant height, canopy, shape of leaves, flowering conditions, infection rate, etc. were analyzed and compared with respect to the control groups [19].

2.3. Estimation of Glutathione in Okra Leaves

For the extraction of GSH, approximate 5 gm of okra leaves were crushed and mixed with 5 mL of 80% ice-cold methanol (as a solvent). Further, the extract was sonicated for 10 minutes, and 1 mL of 5% trichloroacetic acid (TCA) was added to the extract. This sample was used for the analysis of GSH content. The GSH levels were estimated as per reported method of Moron *et al.* and TCA was taken as blank [20].

2.4. DNA Fingerprinting in Bottle Gourd

2.4.1. Isolation of Plant Genomic DNA Using CTAB Method

After germination when the plants were reached the appropriate stage, leaves disc were harvested from each plant. Genomic DNA was isolated according to standard cetyl-trimethyl-ammonium bromide (CTAB) method [21]. Approximately 200 mg of plant tissues (seeds) 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 55ºC in a recirculating water bath. After incubation, the mixture was centrifuged at 12000g 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 the DNA. Then, ammonium acetate followed by chilled absolute ethanol were added, to precipitate the DNA content and stored at -20ºC. 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].

2.4.2. Random Amplified Polymorphic DNA (RAPD) Analysis

DNA concentration was considered about 25 ng/µL using distilled deionized water for polymerase chain reaction (PCR) experiment. The RAPD analysis was performed on the treated mustard seeds using five RAPD primers, which were label as RPL 4A, RPL 5A, RPL 6A, RPL 13A, 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 7 min, followed by 8 cycles of annealing at 94°C for 45 sec, annealing at 35ºC for 1 min, and extension at 72ºC for 1.5 min. Further 35 Final extension cycle was carried out at 72ºC 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 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.

$$\text{Percent polymorphism} = A/B \times 100$$

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

3. Results and Discussion

3.1. Effect of Biofield Treatment on Different Growth Contributing Parameters of Bottle Gourd

The results of growth attributes of bottle gourd in control and biofield treated seeds after germination were evaluated and compared. Control or untreated seeds of crops after germination showed larger and thicker leaves, with presence of yellow spots in majority of the crops (Fig. 1a). The stems of the bottle gourd plants were soft and tender. The crop or fruits of plants were largely infected with diseases and showed signs of attack by insects. Much of the fruits in control group were deformed or infected with pest (Fig. 1c).

Bottle gourd plants generally grow as climbers, but, after receiving of Mr. Trivedi's biofield energy treatment, it was reported for the first time that most of the plants were germinated and grew like other plants; stands erect for approximately 45 days, and then converted into climbers. The stems of the biofield treated plants were strong, that may be the possible reason that plants were erect for a longer duration as compared with the control. The shape and size of leaves were completely altered with better canopy as compared with the control. The leaves were thinner and all of the leaves were pointing towards the sun shows the good sign of growth. The color of the leaves were bright green, uniform and completely different from normal bottle gourd plants (Fig. 1b). As compared to the control group, there were more hairs on the leaves in biofield treated plants, which showed a good defense mechanism against insect attack. The crop of treated plants was absolutely disease free as compared with the control (Fig. 1d).

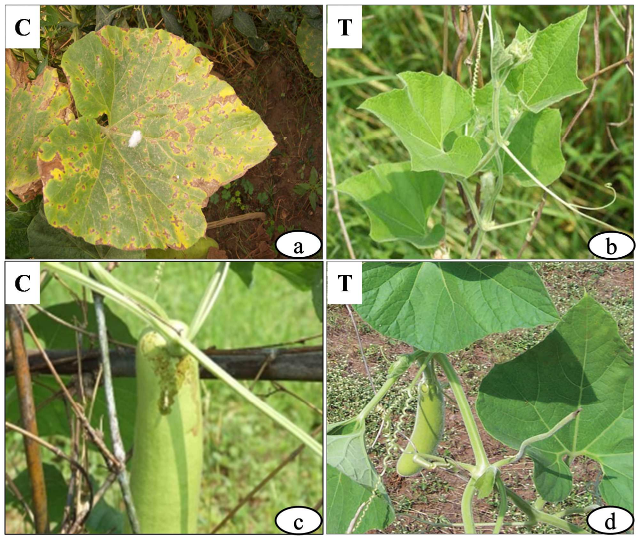

Figure 1. *Effect of biofield energy treatment on bottle gourd (a) leaf of control plants were reported as highly infected (b) biofield treated plant leaves were free from any kind of disease or pest attack, (c) control plants fruit showed infected and unhealthy bottle gourd resulted in less vegetative yield, (d) biofield treated seeds showed healthy bottle gourd and quite free from infections with high yield. C: Control; T: Treated.*

According to Aladjadjiyan, 2010, most of the seeds are having paramagnetic behavior. While exposing the seeds with external magnetic fields, behaviors of seed particles orient themselves in the direction of the applied external field. The impact of the external magnetic field was reported with increased seeds energy and ultimately improved final yield [24]. Biofield treatment on bottle gourd seeds might improve the internal field of the seeds after treatment, and resulted in improved agronomical characteristics of the crop as compared with the control.

3.2. Effect of Biofield Treatment on Different Growth Contributing Parameters of Okra

The vegetative growth and canopy of the plants in control and treated group were analyzed and compared. The plants in control group were reported with more height and a large canopy. The leaves were very broad in size with rough surfaces and many were diseased with yellow dots, even the veins were yellowish in color and the color of leaves were not uniform in the untreated crops (Fig. 2a). The stem was analyzed as tenderer and easily bendable, due to which, many plants fell down, as they could not bear their own weight that contributed poor crop yield in the control group. The gap between the internodes of okra control plants was larger, that depicted less fruiting which causes the overall less yield (Fig. 2b). The stalk of the fruit in control was tough for plucking. The skin was thin, and, when cut, the fruit was found to have more seeds than the treated plants (Fig. 2c). The shape and color of the fruits were not uniform, while the fruit had less hair. The control fruits were of usual stickiness, and the crop had the fair incidence of infection.

On the other hand, the growth of the biofield treated plants was stout, with average plant height and had a small canopy (Fig. 2d). So, it can be concluded that biofield treated okra crops were strong and may contribute to high yield as compared with the control. The shape of the leaves were different from the control group of plants. There was less vegetative growth, and the leaves were not broad in size, rather the leaves were narrow with even green coloring and smooth surfaces. The stem was very strong and sturdy and it was not easily bendable. The color of the flowers was quite different from the control group. The internode gap was much smaller as compared to the control, and almost every node produced fruit, yielding more crop than the control plants. The stalk of the fruit was tender and it could be easily plucked (Fig. 2e). There were fewer seeds in the fruit, and the skin of the fruit was thick (Fig. 2f). The shape of the fruit was very uniform in all the treated plants. There was more hair on the fruit, which may help to prevent the insect attack. The taste of the fruit was exceptionally smooth and crisp, similar to that of cucumber. The fruit was only 10% as sticky as compared to the control group. The treated crops were reported as disease free as compared with the control. Plant population, growth, and canopy of the plant are the major yield contributing parameters, which overall contributes the final yield of okra.

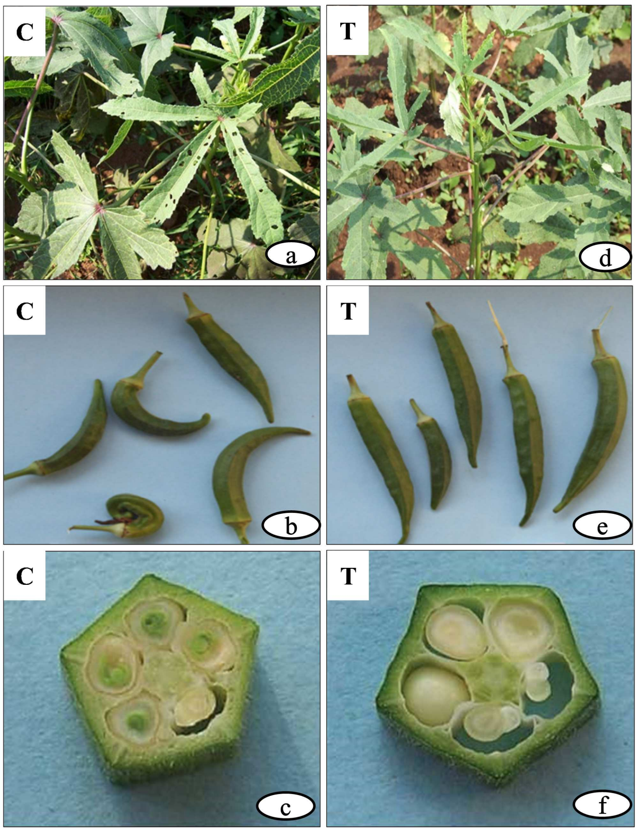

Figure 2. *Effect of biofield energy treatment on okra (a) leaf of control plants were reported with high pest attack that leads to infection (b) control seeds of okra results in infected fruits with curved shape fruit (c) cross section of control showed more seeds (d) biofield treated group showed healthy leaves free from infection (e) biofield treated okra fruits are long, fresh, and free from infection, less vegetative yield (f) cross section of biofield treated okra fruit showed less seeds with high yield. C: Control; T: Treated.*

Okra is the multipurpose crop because of various uses of the fresh leaves, buds, flowers, pods, stems, and seeds [25]. Further, okra contains important oils and rich source of protein, which made it more important for medicinal properties such as antioxidant and different pharmacological activities [26].

3.3. Estimation of Glutathione Level in Okra Leaves

Effect of Mr. Trivedi's biofield energy treatment on okra seeds showed 0.512 mM GSH concentration in the control group plants leaves, while 0.756 mM in treated group. Overall, 47.65% increased level of GSH was reported after biofield treatment as compared with the control. GSH is one of the important chemical entities in plants involved for detoxification of reactive oxygen species (ROS). Different studies have been reported that are in support of the role of GSH in oxidative stress. Stress can be induced due to exposure to chemical oxidants [27], gaseous pollutants [28], high air temperatures, and water stress [29]. The plant GSH system, as an essential component of the cellular anti-oxidative defense system is regarded as stress marker in drought tolerance conditions, which control and manage the ROS level. Biofield treatment increases the level of GSH, which suggested that the treated plants might have higher immunity power than the control group plants, and can resist in environmental stress condition.

Abelmoschus esculentus fruit is commonly used as vegetable, but reported for different medicinal properties such as antidiabetic [30], hypolipidemic [31], *in vitro* antioxidant properties [32], and many more. Oxidative stress plays a considerable role in the pathogenesis and progression of diabetes because of increased free radical and reduced antioxidant defense. It was reported that okra peel and seed powder have significant *in vivo* antioxidant property in diabetic rats [33]. Our experimental results showed enhanced GSH level in biofield treated seeds, hence it is presumed that biofield treated okra seeds might be rich in its biological activity as an antidiabetic effect, along with better crop yield.

3.4. DNA Fingerprinting of Biofield Treated Bottle Gourd by RAPD Analysis

Biofield energy treated bottle gourd was analyzed and compared with the control for their epidemiological relatedness and genetic characteristics. Genetic similarity or mutations between the biofield treated and the control group was analyzed using RAPD. It required a short nucleotide random primers, which were unrelated to known DNA sequences of the target genome. DNA polymorphism can be efficiently detected using PCR primers and identify inter-strain variations among plant species after biofield treatment. The degree of relatedness and genetic mapping can be correlated between similar or different treated samples [34].

Random amplified polymorphic-DNA fragment patterns of control and treated bottle gourd samples were generated using five RAPD primers, and 100 base pair DNA ladder. The results of DNA polymorphism in control and treated using five primers are presented in Fig. 3. The DNA profiles of treated group were compared with their respective control. The polymorphic bands observed using five different primers in control and treated samples were marked by arrows. The RAPD patterns using five designed primers in biofield treated bottle gourd sample showed some unique, common and dissimilar bands as compared with the control. 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 were varied in all the five primers, while level of polymorphism using five primers were ranged from 12 to 88% between control and treated samples. However, level of polymorphism between control and treated groups using RPL 4A, RPL 5A, RPL 6A, RPL 13A, and RPL 19A was found to be 30, 40, 88, 42, and 12%, respectively. The highest change in DNA sequence was observed with RPL 6A primer; however minimum polymorphism was detected with RPL 19A primer in treated group as compared to the control. RAPD also explains the relevant degree of genetic diversity. Overall, RAPD showed that polymorphism was detected between control and treated samples. The percentage of true polymorphism observed between control and treated samples of bottle gourd seed sample was an average value of 42%.

Table 1. *DNA polymorphism of bottle gourd analyzed after biofield treatment using random amplified polymorphic DNA (RAPD) analysis.*

S. No.	Primer	Band Scored	Common bands in control and treated	Unique band	
				Control	Treated
1.	RPL 4A	12	7	2	1
2.	RPL 5A	6	4	1	1
3.	RPL 6A	13	4	5	3
4.	RPL 13A	9	5	-	3
5.	RPL 19A	9	8	-	1

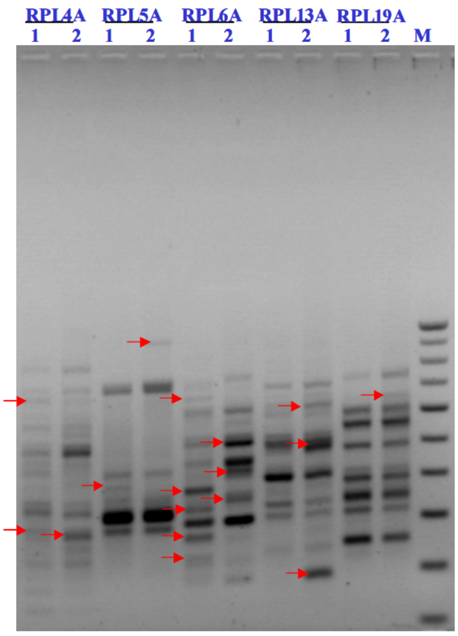

Figure 3. *Random amplified polymorphic-DNA fragment patterns of biofield treated bottle gourd generated using eight RAPD primers, RPL 4A, RPL 5A, RPL 6A, RPL 13A, and RPL 19A. M: 100 bp DNA Ladder; Lane 1: Control; Lane 2: Treated.*

However, this technique has the potential to detect polymorphism throughout the entire genome. After biofield treatment, higher number of polymorphic bands indicated that the genotypes selected in bottle gourd possess a higher degree of polymorphism. However, wide level of genetic variability has been reported in bottle gourd plant [35].

RAPD molecular markers are widely accepted as the important tools for crop improvement in vegetable crops [36]. Using different RAPD markers, important information for genetic diversity can be evaluated for different plant species samples. Further, analysis of population genetics, pedigree analysis and taxonomic discrimination can be easily studied and compared [23]. Several research reports have shown that DNA fingerprinting using RAPD method is a powerful tool for evaluation of discriminating differences at inter- and intra-population among several organisms including plants [37]. Plants phenotypic plasticity was reported to be higher than animals in different environmental conditions, while their changes in DNA can easily be monitored phenotypically. Biofield energy treatment might be a new approach which can alter the genetic variability of the plant, and results in high crop yield as compared with the control. Biofield treatment has been reported with increased yield and plant growth with high immunity. However, content of chlorophyll was reported as consistently higher with genetic variability among species using RAPD fingerprinting [19]. The biofield treatment on ginseng, blueberry [12]; and lettuce, tomato were reported [13] with an improved overall agronomical characteristics of crops.

Biofield treatment on bottle gourd and okra results in improved different growth contributing characteristics as compared with the control. GSH level in okra leaves and genetic variability on bottle gourd were reported after biofield treatment. This suggests that Mr. Trivedi's biofield energy treatment might have the capability to interact with the plant genome, and could improve the final yield of crop with higher immunity. According to Naz *et al.* magnetic field treatment before sowing of okra seeds were reported with enhanced germination, growth, and final yield of crop [38]. Another study conducted by Anand *et al.* shown enhanced seedling growth, leaf water status, photosynthesis, and an improved growth rate under the influence of magnetic field before sowing the seeds of maize (*Zea mays* L.) [39]. Electromagnetic field therapy now considered as a novel tool to enhance the germination and seedling vigor of many important vegetative crops such as onion (*Allium cepa* L.) and rice (*Oryza sativa* L.) seeds [40]. Human biofield energy involves the use of low electromagnetic field, which was harnessed by the biofield practitioner from the environment and transmitted in the living or non-living material [41]. Thus, the improved growth attributes in bottle gourd and okra, after biofield treatment might be due to increased photosynthetic rate, which can enhance flower and fruits per plant [42]. It can also be suggested that biofield energy treatment might activate the ions, polarization of dipoles in the living cells, possibly the biochemical, physiological, and metabolic alterations in the crops. Therefore, biofield energy treatment, might be considered as an alternative approach in agricultural science to improve the germination rate, growth, increased level of important phytohormones, and overall yield of bottle gourd and okra crops.

4. Conclusions

The study findings conclude that the biofield energy treatment on bottle gourd and okra showed an improved growth contributing characteristics such as germination, vegetative growth, plant canopy, green, brighter and healthy fruits and leaves as compared to their respective control. Biofield treated okra crops were strong and may contribute to high yield due to more hairs on the fruit, which may help to prevent the insect attack as compared with the control. Further, 47.65% increased level of GSH was reported after biofield treatment, which suggested that crops might be more resistant towards infections and can also contribute better medicinal properties as compared with the control okra. Although, the percentage of true polymorphism was also observed between control and treated bottle gourd seed sample on an average value of 42% using RAPD analysis. Overall, it can be concluded that biofield treatment could be a better future aspect in agricultural research in terms of vegetative growth, overall yield, and their related parameters.

Abbreviations

ROS: Reactive oxygen species; GSH: Glutathione; RAPD: Random Amplified Polymorphic DNA; PCR: Polymerase chain reaction; NIH: National Institutes of Health; NCCIM: National Center for Complementary and Integrative Health; CAM: Complementary and Alternative Medicine.

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