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Effect of Biofield Energy Treatment on Chlorophyll Content, Pathological Study, and Molecular Analysis of Cashew Plant (*Anacardium occidentale* L.)

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Abstract: In the world scenario, India occupies a premier position contributing to about 43 per cent production of the cashew nut (*Anacardium occidentale* L.) along with export and processing. The aim is to study the impact of biofield energy treatment on selected farms for cashew farming. The control and biofield treated farms were divided as control and treated farms, and Mr. Trivedi provided the biofield energy treatment to the treated farms. Further, the plants and fruits were analyzed for overall growth of plants, chlorophyll content, productivity, pathological study, and shelf life using UN specifications for International Trade, biophoton emission study, and DNA fingerprinting using RAPD method. No chemicals, fertilizers, were used on the treated plot, although regular practices were followed on control farms such as fertilizers, pesticides and fungicides due to the high incidence of disease and the requirement of nutritional supplements in the region. The analysis showed that biofield treated farm plants have thicker and stronger branches with more secondary and tertiary branches, flowering pattern, and canopy of plants was improved than trees of the same variety along with height of the plants, as compared with the control. The results showed that chlorophyll a and b content in biofield treated lands plants were increased by approximately 30% and 93% respectively, while total chlorophyll content by 45% as compared with the control. The pathological examination showed the presence of fungi namely *Colletotrichum gloeosporioides* and *Botryodiplodia theobromae* in control, which were absent in treated plants. Biophoton study suggested that the cashew fruits were bigger in size with high density, strength, and vitality as compared with the control. The shelf life analysis reflected that the biofield treated cashews showed sweet taste, and can be stored for longer duration due to less moisture, and altered minerals content, such as high iodine, and low *p*-anisidine level. RAPD analysis showed a high level of polymorphism among control and treated samples, while level of true polymorphism among V4 variety of cashew was ranges from 0 to 100%, and in V7 variety, it ranged from 25 to 91% using different set of RAPD primers. Overall, study results suggest that Mr. Trivedi's biofield energy treatment on land planted with cashew could be an alternative approach to improve the overall growth of plant, and fruit yield.

Keywords: *Anacardium occidentale* L., Biofield Energy Treatment, Chlorophyll a, b, Biophoton Emission, Shelf-Life, Fungi

1. Introduction

The cashew nut (*Anacardium occidentale* L.) belongs to the family of *Anacardiaceae*, and is the most important cash crop. The family *Anacardiacea* consist of approximately eight species, most of them are native of Brazil [1]. The cashew tree is of multipurpose use, such as for food, income, and the wood is used for the construction of boats and ferries [2]. Apart from these importance, cashew resins are used in the production of insect repellants and natural insecticides. Cashew fruits and its different parts are used for medicinal values to cure various ailments [3]. The characteristic property of cashew tree is sprawling broad-leafed evergreen, easily adapted to dry, poor sandy locations, and drought resistant conditions. Besides, these tree grow best on well drained sandy soils with pH 4.5 to

6.5 [4], with favorable temperature approximately between 15 to 35°C and rainfall over 400 mm. The perfect flowers of cashew tree are born on the same inflorescence and this will decide the cashew productivity. Trees having more perfect flowers, will bear more fruits that leads to high productivity [5]. Cashew production directly depends upon the planting material, susceptibility of pest and disease attack, and lack of proper management [6]. Among these factors, disease attack is very common, and anthracnose is the main disease caused by *Colletotrichum gloeosporioides* (Penz.) Sacc., a very common fruit plant pathogen which affects the productivity of banana, avocado, papaya, citrus, guava, mango, and passion fruit cultivation [7]. This is a type of group species and highly genetically variable pathogen. To improve the productivity of cashew, desirable genetic species or sophisticated chemical treatment to get rid of disease and infections. So, some alternative and safe measures are required to improve the growth of cashew plants and fruit production for longer duration. Biofield energy treatment, is an alternative approach recently reported in agricultural plants to improve the productivity and growth of plants [8, 9].

The biofield energy is a form of electromagnetic field exerted by the human body [10] that was generated through some internal processes in the human body. It involves a very low intensity stimuli/energy absorbed by different biomolecules, due to changes in the movements of component parts in human body. Therefore, the human not only radiate but also absorb some frequencies, which can be channelized in some useful way, known as biofield energy treatment [11]. Energy medicine, is a kind of biofield energy recommended by National Center for Complementary and Alternative Medicine (NCCAM) as one complementary and alternate medicine (CAM) [12]. The unique biofield energy of Mr. Mahendra Kumar Trivedi has been well recognized, and has the ability to alter the characteristics of living and non-living things. Mr. Trivedi's unique biofield treatment is also termed as The Trivedi Effect®, which has been studied in the field of agricultural science research [13], biotechnology [14], microbiology [15], *etc*. After considering the significant effects of biofield treatment, and factors affecting the productivity of cashew, the present study evaluates the impact of The Trivedi Effect® on land selected for cashew production with respect to their growth related attributes, chlorophyll content, DNA fingerprinting analysis, shelf life study, and other productivity parameters.

2. Materials and Methods

2.1. Study Area

Cashew crop of about 106 acres of untilled land in Vaibhavwadi, Maharasthra, India, was selected for the study. Two farms (farm 1 and farm 2) were selected for the study, which contained two varieties of cashew for plantation, and that locations was regarded as quite inappropriate for any professional farming. Cashew saplings of variety V4 and V7 were selected and purchased from the Agricultural University, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth (BSKKV), Dapoli, Maharashtra-India.

2.2. Study Design and Biofield Treatment

Selected farms were divided into two parts, *i.e.* control and treated. Control section of land was planted with V4 and V7 variety of cashew saplings (untreated land), while similar variety of cashew was planted in the biofield treated land. Mr. Trivedi provided the biofield energy treatment, and exposed the land to his unique thought energy transmission process. Further, the saplings were cultivated on the biofield energy treated land. After four years, samples of cashew plants were collected from control and treated farm for experimental analysis and, to study the effect of biofield energy treatment on cashew and its trees. The collected samples were examined for its chlorophyll content, pathological study, biophoton emission, and the shelf life of plants.

2.3. Growth Attributes of Cashew Plants

Cashew plants on control and biofield treated farms were allowed to germinate under similar experimental conditions, with application of fertilizers in control farm only. Overall plant height, primary and secondary branches, flowering pattern, leaves, *etc.* of the control and treated farm plants were analyzed and compared [16].

2.4. Determination of Chlorophyll Content

Samples were collected and studied in laboratory of Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Maharashtra, India by using standard method described by Arnon 1949. Ten samples were taken for each observation and approximately, 50 mg of fresh leaf tissues were weighed accurately; chlorophyll was extracted by crushing the cashew leaf and suspended in test tubes containing 10 mL of dimethyl sulphoxide (DMSO). Test tubes were incubated at 60 to 65°C for 4 hour in a hot air oven. The supernatant was decanted and the chlorophyll extract was transferred to a cuvette and the absorbance was read in a spectrophotometer at 645 and 663 nm on spectronic-20. Chlorophyll a, b, total chlorophyll and chlorophyll a/b ratio were calculated by using formulae given by Arnon [17].

2.5. Pathological Examination

Collected samples from the farm of both the varieties (V4 and V7) of cashews were collected from ten different locations and studied for plant pathological analysis such as presence of fungi, and chemicals used in study area. The pathological tests of collected samples were studied in the laboratory of Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth University, Dapoli, Maharashtra, India.

2.6. Biophoton Emission Study

The cashew nuts were collected from control and treated plots for further studied for bioluminescence, which was

supposed to be correlated to the vitality of the living material. The cashew nuts were of two types, namely n and b. The n type cashew nuts were purchased from the market *i.e.* control, while the b type cashew nuts were obtained from the trees from biofield treated farms by Mahendra Trivedi. The cashew nuts of b type were bigger in size, so that only 4 of b type and 6 of n type could go in a cuvette. As a result, cuvettes contained different masses. The cashew nuts of each group were kept in quartz cuvette, and it does not emit any appreciable visible light after exposure. The measurements were repeated after 24 hours. The two samples were put at the same place after the first measurement. Initially, the sample was exposed of white light illumination for 10 secs, for decay photon signal, which was measured by detecting photons in intervals of 0.05 to 100 sec. The signal strength and signal shapes was denoted as a good indicators of health, vitality and other physiological factors of a living material. The strength of the signal was determined by the counts in the first interval of 0.05 sec, and referred as NB_1. The shape is determined by four parameters and was denoted by,

$$N(t) = B_0 + B_1/ (t+t_0) + B_2/ (t+t_0)^2$$

Where N (t) is the number of photons detected at time t. The parameters B_0, B_1, B_2 and t_0 characterize a live system, and kinetics of plant sample [18].

2.7. Shelf-Life Study

The collected samples were studied for the shelf life as per the standards of UN quality recommendation for international trade, and typical recommended values of moisture content, magnesium, free fatty acids, peroxide values, iodine, and *p*-anisidine values [19].

2.8. DNA Fingerprinting

2.8.1. Isolation of Plant Genomic DNA Using Nucleon Phytopure DNA Extraction Kit

Genomic DNA of control and treated cashew leaves were isolated by standard method using nucleon phytopure DNA extraction kit (Amersham Bioscience: Cat# RPN 8510). In this process, cell wall of the plant was first lysed by reagents contained potassium SDS that formed the complex with protein and polysaccharides. After this, chloroform was added with Nucleon PhytoPure proprietary resin (contains free boric acid), which removed the polysaccharides and removed it from plant tissue sample. The DNA recovery was facilitated after semi-solid fraction formed with chloroform. The isolated DNA from all the samples were further studied for DNA fingerprinting analysis [20].

2.8.2. DNA Fingerprinting by RAPD Analysis

To test whether the alterations after biofield treatment could reach the genetic level, the DNA from the different samples were compared against each other for polymorphism *i.e.* to check whether there were changes before and after treatment. Comparisons were made (using 5 RAPD primers) between DNA isolated from control leaves against treated for each of the two treated varieties, V4 and V7, while also among the treated samples of both varieties. RAPD analysis was carried out between control and treated sample. The control sample was divided in two sets (C1 and C2), while two sets of both varieties of cashew *i.e.* V4 (A1 and G1) and V7 (A2 and G2) were selected for DNA fingerprinting. DNA of plant sample was analysed in two sets for fingerprinting study between controls and treated as C1, A1 and G1; and C2, A2 and G2, while polymorphism among two treated varieties of cashews as A1 and G1; and A2 and G2 were also studied. Two sets of RAPD primers were used for both the experiments as RPL 4A, RPL 6A, RPL 13A, RPL 18A, and RPL 20A; and RPL 4A, RPL 13A, RPL 18A, RPL 19A, and RPL 20A for genetic variation analysis among control-treated and in treated groups respectively.

Amplifications were performed with denaturation at 94ºC for 4 min, followed by 10 cycles annealing at 94ºC for 1 min, annealing at 35ºC for 1 min and extension at 72ºC for 2 min. Further, second step include 35 cycles of annealing at 94ºC for 1 min, annealing at 38˚C for 1 min and extension at 72ºC for 1.5 min. The final extension cycle was carried out at 72ºC for 7 min. The above detailed PCR conditions were followed for all the combinations of samples for RAPD analysis. The PCR reaction mixture (12 µL) of control and treated groups of DNA template were visualized on 1.5% agarose gel and size of each fragment was estimated using 100 bp Ladder (Genei^{TM}, Cat # RMBD19S). For clear visualization of bands ethidium bromide dye (1.5 µL of 0.5 µg/mL final concentration) was added and gel images were processed in gel documentation system [21].

The percentage of polymorphism was calculated using following equation-

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

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

2.9. Statistical Analysis

Data from chlorophyll content analysis of cashew leaves in all the groups were expressed as mean ± S.E.M. and analyzed using one way ANOVA test to ascertain statistical differences between control and treated groups at the end of the experiment. Further, Post-hoc analysis was performed using Tukey Test. A probability level of $p<0{\cdot}05$ was considered as statistically significant as compared to the control.

3. Results and Discussion

3.1. Growth, Yield, and Yield Attributes of Cashew Plants

The biofield treated fields were completely organic as no chemicals of any kind, not even fertilizers, were used on the treated plots, although regular practices followed on control farms included several installments of fertilizers, pesticides and fungicides due to the high incidence of disease and the requirement of nutritional supplements in the region. The luxuriant green and healthy growth of trees in the treated

plots were observed when compared with the comparatively far poorer growth, incidence of diseases found in trees in the control plots (Fig. 1). There appeared to be a bluish aura in the air, and a visible luster in the leaves, which was also found in the well-over three times higher number of larger sized nuts obtained from treated trees. The treated plot plants were at least 13-14 feet in height, like that of a 6 to 7 years old cashew tree of the same variety and in the same region. The canopy observed was large and wide, and the plants were disease free and highly vigorous (Fig. 1). The branches grew lower to the base of the tree compared to cashew trees of the same varieties (V4 and V7) in the untreated farm. The branches were thicker and stronger with more secondary and tertiary branches than trees of the same variety. Inherent strength of the trees to resist the diseases and insects were observed. The mortality of the control plots in the farms was around 35-40% in the first year as compared with a loss of only 0.5% within the first 3½ years in the treated farms.

The flowering patterns of the treated cashew tree were observed as that every branch was flowered heavily. The panicles were thick, strong and showed a large number of female flowers. Instead of the expected 6-8 nuts per bunch on average, there were 12-18 nuts per bunch in biofield treated plot plants (Fig. 1).

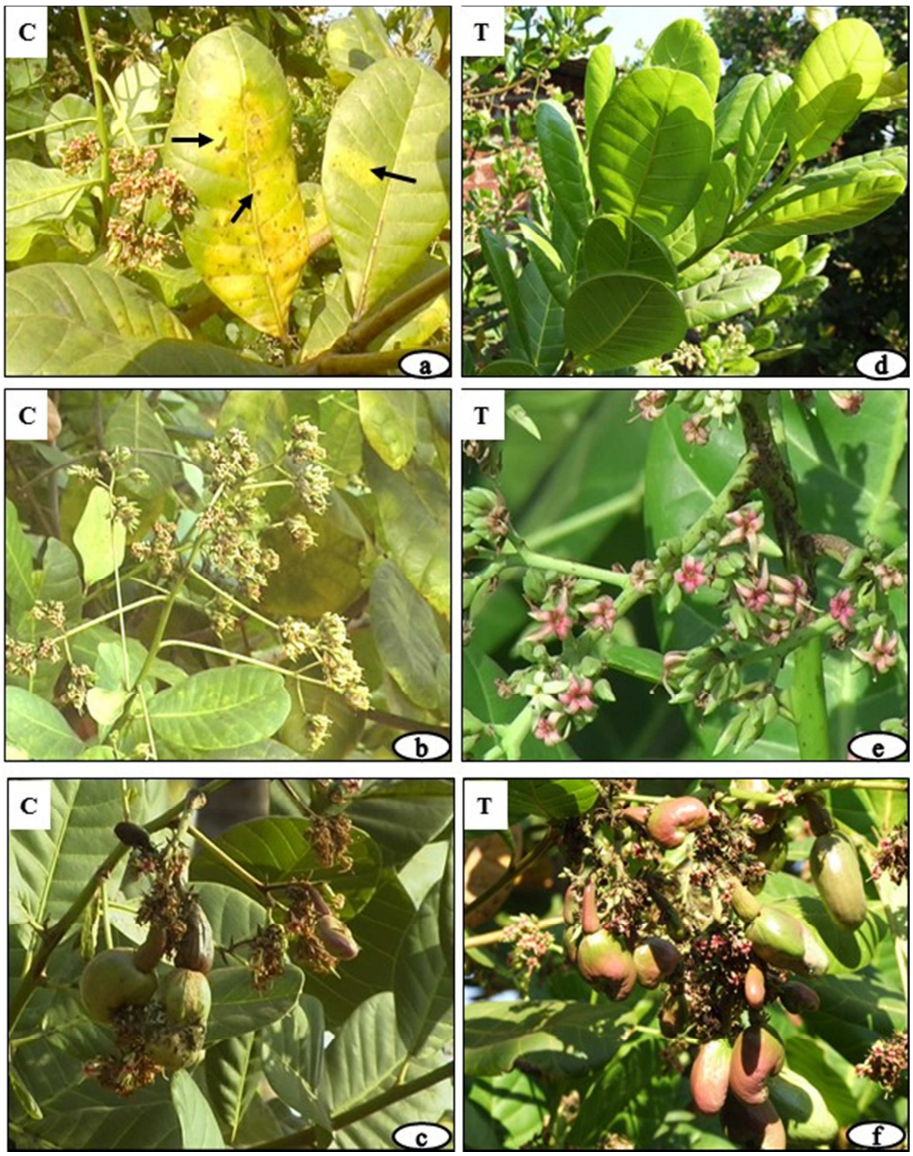

Figure 1. *Effect of biofield treatment on cashew, (a) Control leaves with infections, (b) control plants with irregular flowering pattern, (c) cashew fruits with improper growth and infections, (d) biofield treated farm plants with healthy leaves, containing more chlorophyll, (e), better flowering, that improves the productivity, and (f) disease resistant and healthy cashew of biofield treated farms.*

The leaves showed brighter, greener and shinier color, which indicated an increase in amount of chlorophyll in the leaves as compared with the control plants. The thickness of the leaves were more with larger surface area than all other trees of the same varieties growing in the same region of untreated farm, indicated that higher photosynthesis and energy harnessed by the planta which were more resistant to weather and pest damage. The veins of the leaves were also very thick and this indicated a strong flow of nutrients around the leaves. Biofield treatment showed improved overall

growth contributing parameters of cashew plants, which directly correlated to the cashew productivity. Although, amount of female flower, healthy plant, no infection are related to high yield [22], biofield treated farm produced more female flowers, which might leads to the high productivity of cashew.

3.2. Analysis of Chlorophyll Content After Biofield Treatment

The results of chlorophyll content were analyzed and observed before and after biofield treatment, it showed that the chlorophyll content has reported to be increased in the treated plants. The chlorophyll a content Results showed that chlorophyll a content was increased by 31% in farm 1, while 29.6% in farm 2 as compared with the control. The chlorophyll a content in control was reported as 0.742 mg/g, while in treated group it was increased as 0.966 and 0.962 mg/g in farm 1 and farm 2, respectively. However, the chlorophyll b content was increased from 0.244 mg/g (control) to 0.480 mg/g (farm 1) and 0.472 mg/g (farm 2) in treated farms. Overall, the percentage increase in chlorophyll b in farm 1 and 2 was 96.7% and 93.4%, respectively. Further, the total chlorophyll content in control was reported as 0.986 mg/g, while biofield treated farm 1 and 2 showed values as 1.441 and 1.435 mg/g concentration. Therefore, the total chlorophyll content after biofield treatment was increased by approximately 45.5% in both the biofield treated farms.

Table 1. *Effect of biofield treatment on chlorophyll content of cashew leaves.*

Groups	Chl a (mg/g)	Chl b (mg/g)	Total Chlorophyll (mg/g)
Control Farm	0.742 ± 0.026	0.244 ± 0.027	0.986 ± 0.052
Farm 1	0.966 ± 0.093	0.480 ± 0.070**	1.441 ± 0.162
Farm 2	0.962 ±0.073	0.472 ± 0.037**	1.435 ± 0.106

All the values are expressed as mean ±S.E.M. Chl b show significant value as ** $P = 0.008$, n=5

Chlorophyll content can act as an index for age/nutrient status of leaves, which might be related to overall growth and productivity, as it will direct the photosynthesis. Young cashew plants can maintain relatively high photosynthetic activity, under different environmental conditions [23], which can be correlated that plants of biofield treated farm may have the adaptation in the stress conditions.

These aspects have to be studied at transcription and translation level of each enzyme. Then only it could be detected the phenomenon that has been developed in cells for the development of specific enzyme. As the observations suggest that the chlorophyll b content is exactly double in quantity in the leaves of the treated plant, and that was well known that chlorophyll a and b that are the main sites of solar energy absorption. It is also known that the proportion of chlorophyll a to b is 1: 0.33 in normal cashew plants.

However, the plants treated with The Trivedi Effect® showed high proportion of chlorophyll content with respect to the control, and chlorophyll b ratio after treatment was reported as 1:2. The results showed that the chlorophyll a and b in the photo system has been increased in multifold levels after biofield treatment. It could lead to increased energy level in the chloroplast, which could be helped for the development of more amount of photosynthate, and indirectly for the product of more amount of assimilates in the leaf for the production of end products. Hence, it can be said that leaves were showed showing shininess and free from pests and diseases. However, the exact mechanism by which plant were producing such types of enzymes that are useful for making that plant to become immune for different calamities is still the area of research. Therefore, the enzymes produced by these plants can be studied, especially different types of kinase enzymes and protein synthesis in detail as well as nitrogen metabolism in these plant.

3.3. Pathological Examination of Cashew

Samples from both the varieties (V4 and V7) were collected from ten different locations for the pathological examination, and were compared with the control for the presence of diseases such as fungi or bacterial infections. The use of fertilizers and pesticides were restricted in the biofield treated farms, while in control farm chemicals were supplied as per recommended level. The results of the pathological analysis showed the presence of fungi namely *Colletotrichum gloeosporioides* and *Botryodiplodia theobromae* even after the regular application of fertilizers in the control farms. The reports suggested the absence of any fungal phytopathogens in both the farms treated with biofield energy, in spite of the absence of fertilizers. The infection of phytopathogenic fungus such as *Colletotrichum gloeosporioides* and *Botryodiplodia theobromae* were highly reported causing gummosis, a threatening disease for cashew plants [24], study results showed that the treated farm plants were free from fungus infection.

These results suggested that both the treated farms were free from the diseases such as anthracnose, die back, pink disease and blossom blight. But on the contrary, the control farm plants tested positive for the presence of diseases, which were approximately ten feet away from each other. The air column above the control, and biofield treated cashew farms were expected to be similar containing same amount of spore load of disease causing fungi. This suggests that the air-borne fungi were not able to infect the biofield treated cashew plants.

Aerobiology principles suggest that it would be easy for the air-borne pathogens to travel through the air currents from control farm to treated [25], but results suggested that still no infection has been detected, which means that the plants have developed an inbuilt resistance against these diseases. Therefore, any type of fungi that may be responsible for the diseases were not observed in the treated trees.

There are possibilities that either something is preventing the spread of spores or the trees have the ability to resist spore infection in the biofield treated plants as compared

with the control. For the next one year, both the farms were observed and found free from disease without applications of any pesticides, fungicides and fertilizers. Biofield treated farms showed that cashew trees were survived for more than three years without any disease, hence resulted in completely organic trees. Cashew trees observed in biofield treated farms were with lustrous green leaves, healthy nuts, and free from infections.

3.4. Biophoton Emission Analysis

Biophoton emission (BE) or autoluminescence imaging is an alternate monitoring system that can be observed or correlate with the stress status of plants similar to delayed fluorescence. In this experiment, extremely weak light emissions were used to monitor the physical state of a plant [18]. Biophoton emission reports of cashew nuts samples showed emitted signals of different strengths and shapes. The strength in b type cashew obtained from the biofield treated farm plants were reported to be high in measurements with same amount of nuts. This experiment was designed to highlight the difference between two shapes. The signal decayed quite differently in two types. In order to compare the shapes, the detected photon numbers were divided by NB1, counts were measured in the first bin of 50 min, so that all decay curves starts from the same value. The decay curves of four measurements with b type are same and so are the decay curves with other four measurements with n type, i.e. obtained from control land, as marketed cashew. The decay curves obtained from b and n types are significantly different. The difference lies as a higher value in b type after 10 sec that persisted for 100 sec, while lower value of t_0 in b type, *i.e.* t_0 is equals to 0.17 in b and 0.37 in n. The lower value of B_2 in b type, showed that value of B_2 equals to 0.76 in b and 3.4 in n. The results calculated from MATLAB are summarized in Table 2. The graph obtained after biophoton emission analysis was shown in Fig. 2.

Table 2. *Biophoton Emission Analysis of biofield treated cashew using MATLAB software.*

Type	B_0	B_1	B_2	t_0	F_{min}
b1s1N	9.31E-05	7.14E-10	7.66E-01	0.171	3.46E-03
b1s2N	5.08E-06	5.79E-11	7.63E-01	0.170	3.49E-03
n1s1N	5.26E-05	2.09E-11	3.51E+00	0.380	2.53E-02
n1s2N	9.32E-06	2.15E-11	3.38E+00	0.373	2.42E-02
b2s1N	6.68E-05	2.91E-10	6.58E-01	0.156	4.44E-03
b2s2N	6.66E-05	2.86E-11	6.58E-01	0.157	4.26E-03
n2s1N	8.11E-05	1.22E-11	3.05E+00	0.354	2.16E-02
n2s2N	6.34E-05	1.50E-12	3.09E+00	0.356	2.22E-02

Notation b1s1N b (or n) type, *i.e.* 1 is for day 1, s denotes stimulation, after first measurement, N was calculated after dividing F_{min}, which gives the least square fit from 2000 points. The value of B_1 was very small and not well determined. This is a general situation with seeds which have dormant life structures. The values of B_0 were also small and not well determined. The values of F_{min} further points out that b type of cashews give much better fit as compared with the other. The factors that indicated the higher vitality are, large value of B_0, small will be the value of t_0. The large value of B_1 results in small value of B_2. All the three factors indicate higher vitality of b type cashews after biofield treatment compared with untreated cashew plants.

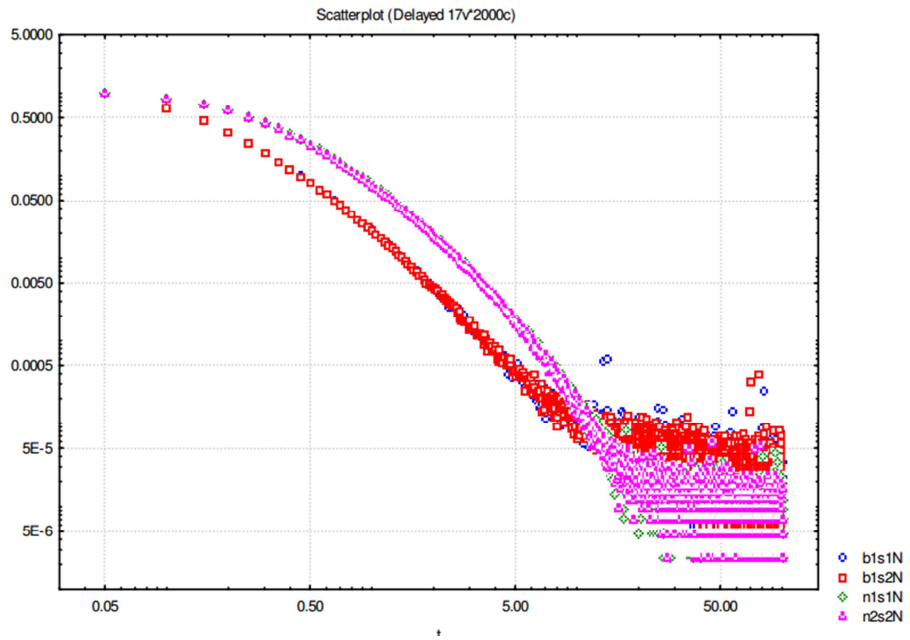

Figure 2. *Scattered plot of biophoton emission of cashew.*

The fruit and nuts of treated trees had a very unique shininess and were 60% bigger in size. The nuts showed 300% more vigor, strength and vitality than the control cashew nuts as reported in a biophoton emission test. This showed that these large nuts were very crispy and had a sweet taste. They had high density and their shelf life was significantly more when compared to the control cashew nuts. So, it could be concluded that biofield treated farm

plants have characteristic kinetics and intensity, with less stress as compared with the control farm plants.

3.5. Shelf-Life Study of Cashew

Raw cashew kernels start decaying once they have been shelled and peeled, and do not last more than 9 months unless refrigerated or vacuum packed. Even, the shelf life is highly affected by the relative humidity, thickness of the polythene bags, and the duration of storage [26]. Cashew kernel which had been stored unsealed and un-refrigerated for a period of one year in the warm Indian climate was tested along with fresh cashew kernel harvested in the next year. They were compared against UN standards for international trade in cashew kernels and were found to be acceptable as high quality edible cashew. The reports and comparisons are displayed in Table 3. Cashew nuts have high oil content and a hard outer covering. When left in the shell, they can be warehoused up to 12 months without degradation. The cashew kernel was extracted and removed from the outer coverings, and their degradation depends on their quality, method of packaging as well as the temperature, humidity, infections and other environmental hazards. Good quality cashew may decay in 3 to 9 months. The shelf life test analyze for edible quality and chemicals produced through the decay of oil in the cashew. According to UN specifications for International Trade in cashew kernels, the cashew should be free from any rancid tastes, foreign smells, and pests, retaining the appropriate color without blemishes, have high density, crispness and a sweetish taste, maximum tolerated values of free fatty acid (as oleic acid) 1%, peroxide 5 meq/kg and moisture content not exceeding 5%. In addition, *p*-anisidine is also used to determine the extent of decay, as it is produced in the decay of oil as iso-peroxide.

A magnesium level of 292 mg per 100 g contributes 79% to the nutrition value. High magnesium content in these cashews directly related to their benefit for a healthy heart. In raw form, these cashews contain 82.5 milligrams of magnesium per ounce, or 21 percent of the daily recommended value. Magnesium also called the heart healthy mineral, works against conditions like high blood pressure, muscle spasms, migraine headaches, tension, soreness, fatigue and also works in conjunction with calcium to support healthy muscles and bones in the human body. The experimental results suggest the altered level of magnesium in biofield treated group as compared with the control, which might be helpful as a nutritive value of cashew.

Cashews have a lower fat content than most other nuts and approximately 75% of their fat is unsaturated fatty acids, of which about 75% is oleic acid, as the more free acid content might be responsible for rancidity of the cashew fruits, and results suggest the level was decreased in both the treated groups as compared with their respective control (Table 3).

Table 3. *Variation in characteristics of control and treated cashew samples after biofield treatment.*

Parameter	Control C1	Treated T1	Control C2	Treated T2	UN Value	Typical value
Description	Brownish green with shells	Off white whole cashews	Off-white	Off-white	–	–
Taste, smell	–	Sweetish, no rancid smells	Sweetish, no rancid smells	Sweetish, no rancid smells		–
Moisture content %	5.35	4.44	3.27	3.55	Less than 5%	3-5%
Magnesium mg/100g	281.04	244.98	272.24	275.31		273 (Rich Source)
Free fatty acid (as oleic acid)%	9.69	0.21	0.27	0.14	Less than 1%	
Peroxide value	Nil	Nil	Nil	Nil	Less than 5	
Iodine value	17.78	38.61	16.12	24.25		Comparable with typical
p-anisidine value	2.49	0	Nil	0.66		Lower is better, typical upto 6mL/g

C1 and T1: Control and treated in first year; C2 and T2: Control and treated in second year; UN values: UN Quality recommendation for international trade

The iodine value (or "iodine adsorption value" or "iodine number" or "iodine index") is the mass of iodine in grams that is consumed by 100 grams of a substance. The amount of iodine solution thus required to keep the solution yellow/brown is a measure of the amount of iodine sensitive reactive groups. One application of the iodine number is the determination of the amount of un-saturation in the fatty acids. This un-saturation is in the form of double bonds which react with iodine compounds. The higher the iodine number suggest high unsaturated fatty acid in the fat. The experimental results showed a high iodine value in cashews from biofield treated farm, as compared to control in both the groups.

The peroxide value of an oil or fat is a technique to measure the extent of rancidification occurred during storage. The best test for auto oxidation (oxidative rancidity) was to determine the peroxide value. Peroxides are the intermediates in the auto oxidation reaction. Although, our experimental results does not found the level of peroxide in control or treated groups.

The oxidative process of oils and fats is one of the main causes of the deterioration of the principal organoleptic and

nutritional characteristics of foodstuffs. The complex oxidation process can be summarized into two phases: in the first one fat acid react with oxygen and determine odorless compounds as peroxides; during the second phase the peroxides degrade into many substances as volatile aldehydes, responsible of the rancid odor and flavor, and in a non-volatile portion.

The primary oxidation products are normally measured with Peroxide Value test (PV) and the secondary products with *p*-anisidine test. Anisidine value (AnV) represents the level of non-volatile aldehydes, primarily 2-alchene present in the fat. The value of p-anisidine in treated group cashews was decreased to zero, in first group as compared with the control value.

The oxidative status of a fat should be evaluated considering both its primary and secondary oxidation. In fact it can happen that a fat that has initially a high peroxide value, kept in stock for a long time in absence of oxygen, endures a secondary oxidative process that determines the decrease of peroxide value but the increase of anisidine value. Additionally, *p*-anisidine test on oil is an indicator of excessive oil deterioration in deep frying process. So, it can be assumed that cashews obtained from biofield treated farms could be stored for long time, without any deterioration as compared with the control cashews.

3.6. RAPD Analysis of Cashew

DNA fingerprinting of control and treated cashew plants were performed with different combinations as among control and treated to determine the epidemiological relatedness and genetic characteristics. RAPD analysis was performed to study the correlation based on genetic similarity or mutations between the cashew plants grown in the biofield treated and the control farm. RAPD study required short nucleotide primers, which were unrelated to the known DNA sequences of the target genome. DNA polymorphism can be efficiently detected using PCR primers and identify inter-strain variations among plant species in the treated samples. The degree of relatedness and genetic mapping can be correlated between similar or different treated sample [27].

Random amplified polymorphic-DNA fragment patterns of cashew plants from control and biofield treated farms samples were generated using five RAPD primers, and 100 base pair DNA ladder. RAPD analysis between control and treated samples are presented in Fig. 3 and 4, as between C1, A1 and G1; and C2, A2 and G2 respectively. DNA polymorphism among two treated varieties *i.e.* A1 and G1; and A2 and G2 are presented in Fig. 5 and 6. The polymorphic bands observed using eight different primers in control and treated samples were marked by arrows. The RAPD patterns of treated samples showed some unique and polymorphic bands using five primers. DNA polymorphism analyzed by RAPD analysis, the total number of bands, common, and unique bands are summarized in Table 4, 5, 7, and 8. The level of polymorphism in terms of percentage values between tested samples were varied and summarized in Table 6 and 9. However, this technique has the potential to detect polymorphism throughout the entire genome of two variety of cashew before and after treatment.

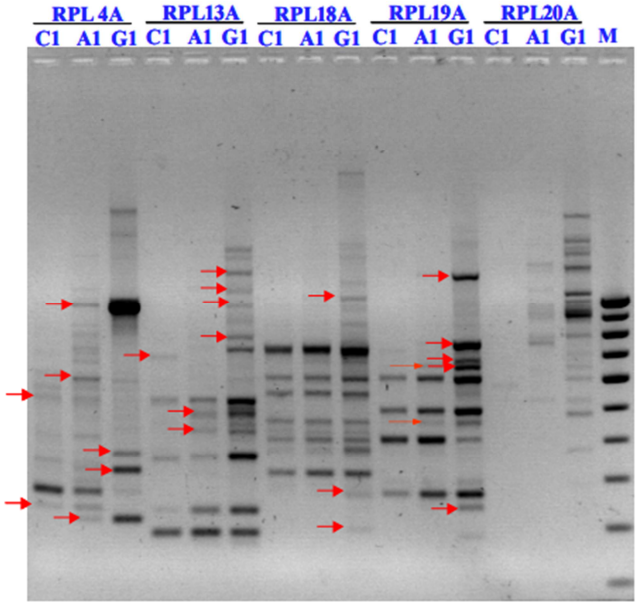

Figure 3. *Random amplified polymorphic-DNA (RAPD) profile of cashew (V4) generated using Genei five RAPD primers. C1: Control; A1: Treated A1; G1: Treated G2; M: 100 bp DNA Ladder.*

Table 4. *DNA polymorphism of control and biofield treated cashew V4 variety (C1, A1, and G1) using random amplified polymorphic DNA (RAPD) analysis.*

S. No.	Primer	Band Score	Common bands in control and treated	Unique band		
				Control	TSA1	TSG1
1	RPL 4A	12	2	2	1	2
2	RPL 13A	13	4	1	2	4
3	RPL 18A	12	8	-	-	3
4	RPL 19A	10	5	-	2	5
5	RPL 20A	-	-	-	-	-

TSA: treated sample A1; TSG1: treated sample G1.

The DNA polymorphism of control and biofield treated cashew V4 variety (C1, A1, and G1) using five primers in RAPD analysis showed maximum band scores with primer RPL13A, along with unique bands in both the treated samples of cashew V4.

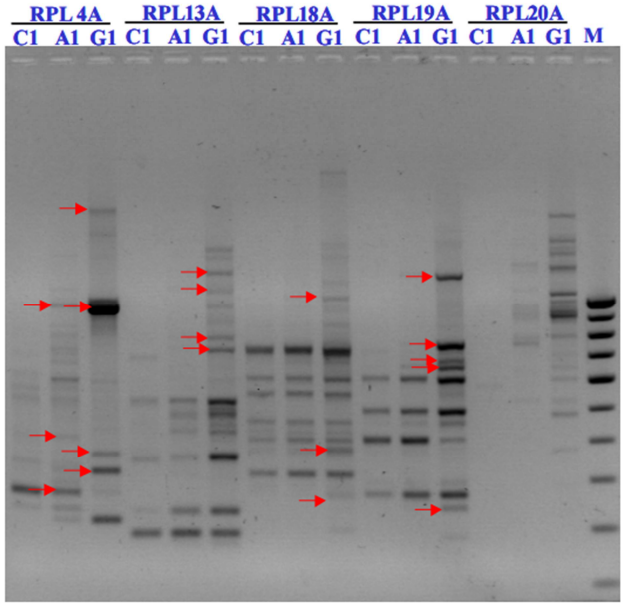

Figure 4. *Random amplified polymorphic-DNA (RAPD) profile of cashew (V4) generated using Genei five RAPD primers. A1: Treated A1; G1: Treated G2; M: 100 bp DNA Ladder.*

Table 5. *DNA polymorphism of two variety of V4 biofield treated cashew (A1 and G1) using random amplified polymorphic DNA (RAPD) analysis.*

S. No.	Primer	Band Score	Common bands among A1 and G1	Unique band	
				TSA1	TSG1
1	RPL 4A	12	2	3	4
2	RPL 13A	13	6	-	4
3	RPL 18A	14	7	-	3
4	RPL 19A	12	5	-	4
5	RPL 20A	-	-	-	-

TSA: treated sample A1; TSG1: treated sample G1.

The DNA polymorphism among treated cashew V4 variety (A1 and G1) using five primers in RAPD analysis showed maximum band scores with primer RPL19A, and most of the unique bands were found in TSG1 sample against all the tested primers expect RPL20. The true polymorphism *i.e.* 100% was detected with primers RPL 13A and RPL 19 A in V4 variety of cashew after biofield energy treatment.

Table 6. *Level of polymorphism of V4 variety of cashew using five RAPD primers.*

S. No.	Primer	C1 and TSA1	C1 and TSG1	TSA1 and TSG1
1	RPL 4A	42%	42%	70%
2	RPL 13A	60%	100%	66%
3	RPL 18A	0%	37%	42%
4	RPL 19A	0%	100%	80%
5	RPL 20A	-	-	-
6	Average polymorphism	20%	55%	51%

C1: control; TSA1: treated sample A1; TSG2: treated sample G2

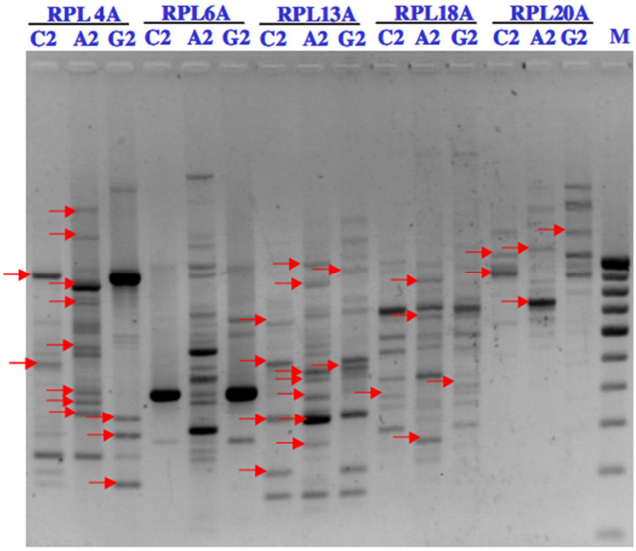

Figure 5. *Random amplified polymorphic-DNA (RAPD) profile of cashew (V7) generated using Genei five RAPD primers. C2: Control; A2: Treated A2; G2: Treated G2; M: 100 bp DNA Ladder.*

Table 7. *DNA polymorphism of control and biofield treated cashew V7 variety (C2, A2, and G2) using random amplified polymorphic DNA (RAPD) analysis.*

S. No.	Primer	Band Score	Common bands in control and treated	Unique band		
				C2	TSA2	TSG2
1	RPL 4A	21	4	2	8	3
2	RPL 6A	-	-	-	-	-
3	RPL 13A	18	2	4	7	2
4	RPL 18A	15	4	1	3	1
5	RPL 20A	13	2	2	2	1

C2: Control; TSA2: treated sample A2; TSG2: treated sample G2.

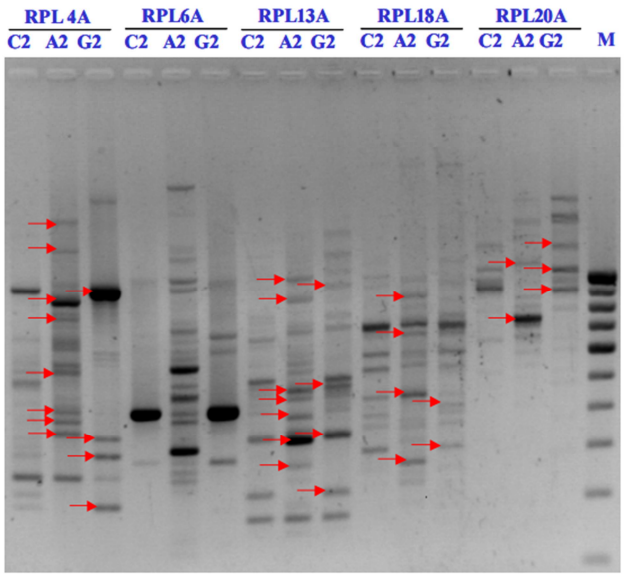

Figure 6. *Random amplified polymorphic-DNA (RAPD) profile of cashew (V7) generated using Genei five RAPD primers. A2: Treated A2; G2: Treated G2; M: 100 bp DNA Ladder.*

Table 8. *DNA polymorphism of two variety of V7 biofield treated cashew (A2 and G2) using random amplified polymorphic DNA (RAPD) analysis.*

S. No.	Primer	Band Score	Common bands among A2 and G2	Unique band	
				TSA2	TSG2
1	RPL 4A	17	3	8	3
2	RPL 6A	-	-	-	-
3	RPL 13A	18	4	7	4
4	RPL 18A	15	4	4	4
5	RPL 20A	12	1	2	1

TSA2: treated sample A2; TSG2: treated sample G2.

Table 9. *Level of polymorphism of V7 variety of cashew using five RAPD primers.*

S. No.	Primer	C2 and TSA2	C2 and TSG2	TSA2 and TSG2
1	RPL 4A	25%	62%	85%
2	RPL 6A	-	-	-
3	RPL 13A	77%	90%	91%
4	RPL 18A	44%	44%	60%
5	RPL 20A	60%	60%	55%
6	Average polymorphism	41%	51%	58%

C2: control; TSA2: treated sample A2; TSG2: treated sample G2

The DNA polymorphism among treated cashew V7 variety using five primers in RAPD analysis showed true polymorphism *i.e.* ranged from 25% to 91% after biofield energy treatment.

The improved yield and consistency of results across multiple kinds of cashew samples suggested the efficacy of biofield energy treatment on cashew plants. The results suggested that biofield energy may interact sufficiently with plants genetic materials, which stands the plant in disease free environment, with improved cashew fruits in plant, and are able to produce healthier plants with higher yield as compared with the control. High level of genetic diversity has been reported in cashew using various RAPD primers [28]. However, biofield treatment has been reported with improved overall plant health of *Withania somnifera* and *Amaranthus dubius*. Leaf, stem, flower, seed setting, and immunity parameters were reported to be improved after biofield treatment. Chlorophyll a, chlorophyll b and total chlorophyll concentration were consistently higher in treated plants along with genetic variability using RAPD DNA fingerprinting [14]. The impact of biofield treatment on yield of ginseng, blueberry [9], and growth and yield of lettuce and tomato were reported [13]. Similarly, biofield energy treatment on plots grown with cashew plants were high immunity, better growth of plant, fruits, more life span, and also genetic variability, this all suggested that biofield treatment could be new and alternative approach to increase the yield of cashew and other agricultural crops.

4. Conclusions

In the present study, two varieties of cashew was examined for the impact of biofield treatment on selected plots, and were reported to be very beneficial in terms of overall growth related parameters and productivity. All the tress grown in biofield treated lands were very healthy in terms of flowering pattern, veins of the leaves, canopy of the plants, free from pest attack, and related diseases, even without any application of chemical fertilizers in the treated farms. The chlorophyll a and b content in biofield treated lands plants were increased by approximately 30% and 93% respectively, while total chlorophyll content was increased by 45% in both the farms as compared with the control. The pathological examination showed the presence of fungi namely *Colletotrichum gloeosporioides* and *Botryodiplodia theobromae* in control farm, even after the application of fertilizers, while absent in biofield treated farm plants. Biophoton analysis results suggested that cashew fruits of biofield treated farms plants had a very unique shininess and were 60% bigger in size, with high density. The nuts showed 300% more vigor, strength and vitality as compared with the control cashew nuts. The shelf life study of control and biofield treated farm cashew showed sweeter taste, less moist, changes magnesium level, and free fatty acids as compared with the control. The iodine content was significantly high in the treated group, while *p*-anisidine was also decreased as compared with control values. However, RAPD analysis showed a high level of polymorphism among control and treated samples, while the level of true polymorphism among the V4 variety of cashew was ranged from 0 to 100%, and in V7 variety, it ranged from 25 to 91% using the different set of RAPD primers. So, it can be concluded that the cashew fruits and plants from biofield energy treated farms are completely organic, more life span, high nutritive value, and free from fungal infection with

better shelf life as compared to the cashews of control farms.

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