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Evaluation of Biochemical Marker - Glutathione and DNA Fingerprinting of Biofield Energy Treated *Oryza sativa*

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Abstract: Food production needs to increase to satisfy the demand due to increasing human population worldwide. To minimize this food crisis, an increase in the rice production is necessary in many countries. The current study was undertaken to evaluate the impact of Mr. Trivedi's biofield energy treatment on rice (*Oryza sativa*) for its growth-germination of seedling, glutathione (GSH) content in seedling and mature plants, indole acetic acid (IAA) content in shoots and roots and DNA polymorphism by random amplified polymorphic-DNA (RAPD). The sample of *O. sativa* cv, 644 was divided into two groups. One group was remained as untreated and coded as control, while the other group was subjected to Mr. Trivedi for biofield energy treatment and denoted as treated sample. The growth-germination of *O. sativa* seedling data exhibited that the biofield treated seeds was germinated faster on day 3 as compared to control (on day 5). The shoot and root length of seedling was slightly increased in the treated seeds of 10 days old with respect to untreated seeds. Moreover, the plant antioxidant *i.e.* GSH content in seedling and in mature plants was significantly increased by 639.26% and 56.24%, respectively as compared to untreated sample. Additionally, the plant growth regulatory constituent *i.e.* IAA level in root and shoot was significantly ($p<0.05$) increased by 106.90% and 20.35%, respectively with respect to control. Besides, the DNA fingerprinting data using RAPD, revealed that the treated sample showed an average range of 5 to 46% of DNA polymorphism as compared to control. The overall results envisaged that the biofield energy treatment on rice seeds showed a significant improvement in germination, growth of roots and shoots, GSH and IAA content in the treated sample. In conclusion, the treatment of biofield energy on rice seeds could be used as an alternative way to increase the production of rice.

Keywords: Rice, Biofield Energy Treatment, *Oryza sativa,* Seedling, RAPD, Glutathione, Indole Acetic Acid

1. Introduction

In Asia, Latin America, and Africa, rice (*Oryza sativa*) is essential for the supplement of nutrition in much of the population as a staple food. Over half of the world's populations are consuming rice as their main food source. About half of the world's rice production is from China and India. The largest consumers are from China (about 30%) and about 25% consumers from India of the world's consumption. It is essential to increase the rice production in order to solve the crisis of rice as foodstuff [1]. The gross agricultural productivity depends on the most vital abiotic stress salinity *i.e.* dissolved salts in water. The metabolic impairment in the plant cell occurs due to the osmotic and toxic effects of salt concentration in water. Generation of reactive oxygen species (ROS) is the main output of such metabolic impairment during salinity stress [2-4]. The ROS such as superoxide radical (O^{2-}), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-) are produced through the reduction of molecular O_2 during aerobic metabolism in mitochondria. Apart from metabolic derived ROS, plant cell also produces singlet oxygen ($^1O_2$) in the chloroplast during photosynthesis [5, 6]. Among the various antioxidant pathways, the ascorbate–glutathione (ASC–GSH) cycle has been played an important role [7]. In plants, GSH is crucial for biotic and abiotic stress management. It is a pivotal component of the ASC–GSH cycle, a system that reduces poisonous hydrogen peroxide produced during photorespiration in peroxisomes. GSH and

GSH-dependent enzymes represent a regulated defense against oxidative stress not only against ROS but also against their toxic products. Plants communicate with a great variety of symbiotic partners, above and below ground. Continuous monitoring of signals of biotic and abiotic environmental influences allows the plants to generate appropriate response behavior [8]. Recent advances in molecular biology, development of polymerase chain reaction (PCR), and DNA sequencing have resulted in a powerful technique that can be used for the characterization of genetic diversity. Besides, the genetic diversity can also be assessed by the study of morpho-agronomic variability for plant breeders. This is the only approach still used by rice breeders. For characterization of genetic profile a powerful tool has been developed as the molecular marker, so called DNA fingerprinting [9]. Although more than 40,000 rice varieties were reported worldwide, while few species have been used in practical breeding. Therefore, better understanding regarding genetic makeup of used rice germplasm is a critical issue for rice breeding [10].

In the present work, the level of GSH in plant cell was taken as a biochemical marker and also characterized the genetic profile of *O. sativa* by RAPD. Nowadays, biofield energy treatment has been known as lucrative surrogate approach that may be useful in that concern. The National Center for Complementary and Integrative Health (NCCIH), allows the use of Complementary and Alternative Medicine (CAM) therapies like biofield energy as an alternative in the healthcare field. About 36% of US citizens regularly use some form of CAM [11], in their daily activities. CAM embraces numerous energy-healing therapies; biofield therapy is one of the energy medicine used worldwide to improve the overall human health. Mr. Trivedi's unique biofield treatment (The Trivedi effect®) has been extensively contributes in scientific communities in the field of agricultural science [12-15] and chemical science [16]. Due to the necessity of rice as the prime food resource and to improve overall productivity of rice plants an effective control measure need to be established. Under these circumstances, the present work was undertaken to evaluate the effect of biofield energy treatment on rice in relation to germination growth in seedlings, level of GSH and IAA and the molecular analysis using DNA fingerprinting.

2. Materials and Methods

The seeds of O*ryza sativa* (*O. sativa* cv, 6444) were divided into two parts. One part was considered as control, no treatment was given. The other part was coded as treated and subjected to Mr. Trivedi's biofield treatment. The random amplified polymorphic DNA (RAPD) analysis was performed using Ultrapure Genomic DNA Prep Kit; Cat KT 83 (Bangalore Genei, India).

2.1. Biofield Treatment Strategy

The treated sample of rice seeds was subjected to Mr. Trivedi's biofield treatment under laboratory conditions. Mr. Trivedi provided the treatment through his unique energy transmission process to the treated group without touch. The treated sample was assessed for growth germination of seedlings, glutathione (GSH) level and indole acetic acid (IAA) content in roots and shoots of rice plant.

2.2. Growth Germination of Rice Seedlings

Control and treated rice seeds (*Oryza sativa*) were soaked for 6 hours in distilled water. The water soaked seeds were wrapped with moist tissue paper and kept in dark condition for germination. The percent of germinated seeds and length of root and shoot were recorded.

2.3. Measurement of Glutathione in Rice Leaves

For the extraction of GSH approximate 5 gm of rice leaves were crushed and mixed with 5 mL of 80% chilled methanol (as a solvent). Then the extract was sonicated for about 10 minutes. Then 1 mL of 5% tricholoroacetic acid (TCA) was added to the extract. This sample was used for the analysis of GSH content. The GSH levels were estimated as per Moron *et al*. TCA was taken as blank [17].

2.4. Measurement of Indole Acetic Acid (IAA) Content in Shoots and Roots of Rice Seedlings

For the extraction of IAA approximate 200 mg plant tissue was grinded with 5 mL of 80% chilled methanol. The extract was filtered through Whatmann filter paper (No. 1). After filtration the final volume of extract was made upto 10 mL using 80% chilled methanol. Then optical density was measured after 30 minutes at 530 nm using ultra-violet visible spectrophotometer. IAA was analyzed using Tang and Bonner's method. Freshly prepared Salkowski's reagent was used for the detection of IAA content in shoots and roots of rice seedlings [18].

2.5. 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 [19]. Approximate 200 mg of plant tissue (seeds) were grind to a fine paste in approximately 500 µL of CTAB buffer. The mixture (CTAB/plant extract) was transferred to a microfuge 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 transfer to a clean microfuge tube. After mixing with chloroform and iso-amyl alcohol followed by centrifugation the aqueous layers were isolated which contains DNA. Then, ammonium acetate followed by chilled absolute ethanol was added to precipitate the DNA and stored at -20oC. The RNase treatment was provided to remove any RNA materials followed by washing with DNA free sterile solution. The quantity of genomic DNA was then measured at 260 nm with the help of a spectrophotometer [20].

2.6. Random Amplified Polymorphic DNA (RAPD) Analysis

DNA concentration was considered about 25 ng/µL using

distilled deionized water for polymerase chain reaction (PCR) experiment. RAPD analysis was performed on the treated sample of rice seeds using five RAPD primers, which were labelled as RPL 6A, RPL 13A, RPL 16A, RPL 18A, and RPL 19A were adopted from earlier studies. 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 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. Final extension cycle 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 gel was subsequently stained with ethidium bromide and viewed under UV-light [21]. 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 control plant.

3. Statistical Analysis

Data from growth germination of seedling and indole acetic acid (IAA) were expressed as Mean ± S.E.M. and analyzed through a Student's t-test to ascertain statistical differences between control and treated rice seeds at the end of the experiment. A probability level of $p<0{\cdot}05$ was considered as statistically significant as compared to the control.

4. Results and Discussion

4.1. Growth Germination of Rice Seedlings

Oryza sativa is one of the few plant species that has the ability to tolerate prolonged soil flooding or complete submergence conditions. Elongation rate of submerged shoot organs is faster than normal rate to develop aerenchyma. However, the rice seeds are able to germinate in anaerobic environment by means of coleoptile elongation [22]. The growth germination of rice seedling data of control and treated samples are shown in Table 1.

Table 1. *Growth-germination of rice (Oryza sativa) seedlings on 10 days old plant.*

Group	Germination (Day)	Germination (%)	Length (cm) (Mean ± S.E.M.)	
			Shoot	Root
Control	5th	100	5.7 ± 0.013	8.8 ± 0.05
Treated	3rd	100	5.8 ± 0.021	8.9 ± 0.05

n = 100; S.E.M.: Standard error of mean

Based on the obtained results, the control seeds of *O. sativa* were absolutely germinated on day 5, while the biofield treated seeds were germinated on day 3 with 100% germination. After germination, the tenth days old rice plants shoot and roots were measured. The shoot length in control sample was 5.7 cm and in treated sample it was 5.8 cm (n =100). The shoot length in the treated sample was slightly increased as compared to the control. Moreover, the length of root in control sample was 8.8 cm and in the treated sample it was 8.9 cm (n = 100). The root length in the treated sample was also slightly increased with respect to the control. The seeds of majority plant species have failed to germinate due to deprive of oxygen and causes metabolic abnormality *i.e.* anoxia. Based on responses to availability of oxygen, seeds can be categorized. The starchy seeds (e.g. *O. sativa*) have the capability to maintain high energy metabolism under oxygen deprive condition as compared with the fatty seeds (*e.g. Ricinus communis*) [23, 24]. Based on the findings, it is assumed that the early germination in biofield energy treated sample may be due to increase in the ability of oxygen mediated metabolism that ultimately shortens the germination time as compared with the untreated seeds. However, several researchers have reported the, decline in oxygen concentration affect germination of oat and barley. In the same situation *i.e.* deprive in oxygen, rice can behaves different phenomenon. The growth of root was suppressed, while the growth of shoot was increased [25].

4.2. Measurement of Glutathione in Rice Leaves

Sulphur is an essential component of all living organisms for protein synthesis. It is the integral constituent of various amino acids and cellular endogenous components like GSH. GSH (γ-L-glutamyl-L-cysteinylglycine) is a sulphur containing thiol tripeptide, found in most of the organisms including plants. Deficiency of sulphur retard the growth of shoot, while did not affect the growth of the roots [8, 26]. The level of endogenous GSH of control and treated samples in both seedling and mature plants are illustrated in Table 2.

Table 2. *The glutathione (GSH) level on seedlings and mature plants in rice (Oryza sativa).*

Leaves type	Group	Endogenous glutathione (mM)	Increased (%)
Seedling	Control	0.163	639.26
	Treated	1.205	
Mature	Control	0.521	56.24
	Treated	0.814	

The concentration of endogenous GSH in control seedling was 0.163 mM and in treated sample was 1.205 mM. The result indicated that the GSH level in seedling was increased 639.26% in the biofield energy treated sample as compared to the naive seeds. Furthermore, the concentration of GSH in mature plants leaves of treated sample was 0.814 mM as compared with the untreated seeds *i.e.* 0.521 mM. The result revealed 56.24% increased GSH content in the leaves of mature plants in treated group (Table 2). The antioxidant network in plants is complex in nature. Due to hazardous

environmental conditions ROS can be generated in plants. To fight and maintain a steady-state level against ROS, plants itself can evolve several antioxidant enzymes including GSH. It helped to scavenge the excessive ROS through redox-homeostatic mechanism [27-29]. More generation of ROS impart intrinsic metabolism in plant cell negatively [30]. In this experiment, it is assumed that the increased in GSH content in the biofield treated sample might accelerate the rate of intrinsic metabolism. Hence, this data were well supported with early germination of rice seedling in the biofield treated group (Table 1). Nowadays, some unequivocal evidence has emphasized that apart from redox homeostatic and cell signaling, GSH plays an important role as defense reaction. Based on mutant and mapping resources information from *Arabidopsis* mutants database, a small molecule of thiol compound plays as a heart of regulator of plants growth, development and defense responses against environmental hazardous [31].

4.3. Measurement of Indole Acetic Acid (IAA) Content in Shoots and Roots of Rice Seedlings

Auxins are the first class of plant hormones responsible for growth and development of plants. IAA is one of the principal auxin *i.e.* plant growth substance produced by several plant-associated commensal bacteria. Among plant microbiota some bacteria are pathogenic to plants. Auxin production is the key factor for determination of plant pathogenicity. More level of IAA is less chances of plant infection [32, 33]. The IAA content in rice shoots and roots of both control and treated samples are shown in Table 3.

Table 3. *The indole acetic acid (IAA) content in shoots and roots of rice (Oryza sativa).*

IAA in rice root					
Group	**IAA (µg/g)**			**Mean IAA (µg/g) (Mean ± S.E.M.)**	**% Increase**
	Sample 1	**Sample 2**	**Sample 3**		
Control	0.7	0.8	1.1	0.87 ± 0.12	106.90
Treated	1.4	1.8	2.2	1.80* ± 0.23	
IAA in rice shoot					
Control	4.0	4.3	3.8	4.03 ± 0.15	20.35
Treated	5.1	5.3	4.15	4.85 ± 0.35	

IAA: Indole acetic acid; S.E.M.: Standard error of mean; n = 3; *$P< 0.05$

In this experiment, the IAA content of control sample was 0.87 µg/g, while in treated sample it was increased significantly ($p<0.023$) to 1.80 µg/g in rice roots. There was 106.90% increased of IAA content in rice roots after biofield energy treatment. Furthermore, the level of IAA in rice shoots was also increased in the treated group as 4.85 µg/g as compared to the control *i.e.* 4.03 µg/g. The data showed 20.35% increase in the IAA constituent in the shoots of biofield treated group. Based on the findings, it is assumed that the increased IAA content in roots and shoots after biofield energy treatment might be helpful for their growth and overall development of plants. Besides, high level of auxins *i.e.* IAA in plants cells indirectly indicates the less numbers of pathogenic bacteria, because only the plant-associated beneficial bacteria are able to produce high abundance of auxins. There are several methods existed for the determination of IAA in growing roots and shoots. However, all the methods are time-consuming. In this experiment we have used Salkowski test based on colorimetric principle for estimation of IAA, as this test is very simple, rapid, and cheap [34-36]. This technique was also proven for its usefulness for the screening of mutant bacteria affected auxins synthesis [37].

4.4. Random Amplified Polymorphic DNA (RAPD) Analysis

Based on several reports, it was established that the polymorphic DNA is responsible to give information about an ideal genetic markers. This was happened due to its selectively neutral nucleotide sequence and distinct genomes pattern [38, 39]. Here, RAPD was used as a DNA fingerprinting technique for evaluation of rice seeds. The control and treated samples were evaluated based on their various RAPD patterns. It is very simple to detect because there is no need of DNA sequence information or synthesis of specific primers. It is a preferred tool being used nowadays to correlate the genetic similarity or mutations between species. The simplicity and wide field acceptability of RAPD technique due to short nucleotide primers, which were unrelated to known DNA sequences of the target organism [40]. The DNA fingerprinting by RAPD method was performed using five primers in the control and treated samples. The data are shown in Fig. 1, and the polymorphic bands are marked by arrows. The RAPD patterns of treated sample showed some unique and dissimilar patterns.

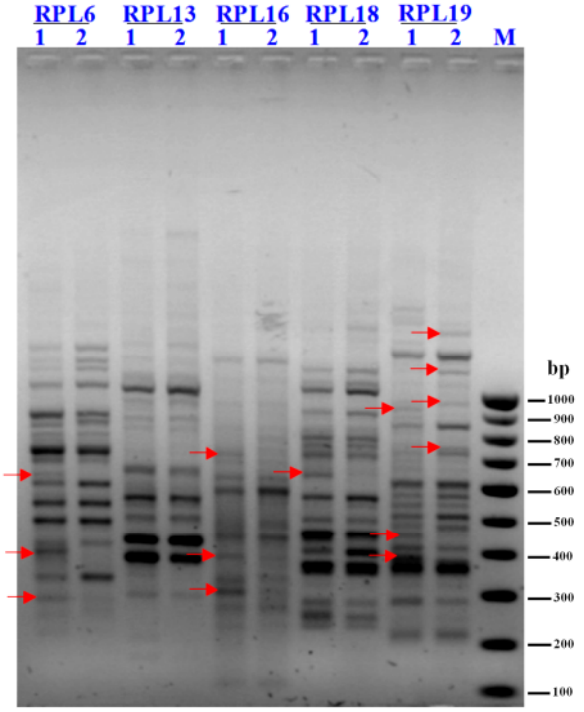

Figure 1. *Random amplified polymorphic-DNA (RAPD) profile of rice seeds (Oryza sativa) generated using Genei five RAPD primers, RPL 6A, RPL 13A, RPL 16A, RPL 18A and RPL 19A. 1: Control; 2: Treated; M: 100 bp DNA Ladder.*

DNA polymorphism analyzed by the RAPD analysis was presented in Table 4. The level of polymorphism was found in an average range of 5 to 46% in the treated sample as compared to the control in rice seeds after the biofield energy treatment. The highest change in DNA sequence (46%) was observed in the treated group with RPL 19A primer as compared to the control. A very less change was found in the treated group with RPL 16A primer as compared to the control. However, RPL 13A primer did not show any response against the treated sample. Biofield energy treatment could be responsible to improve the GSH and IAA content in rice shoots and roots. Based on the findings of growth germination pattern of seedling, GSH level, and IAA content followed by RAPD analysis there was positive impact of Mr. Trivedi's biofield energy treatment on the seeds of *O. sativa*. Based on these results, it is expected that biofield energy treatment has the scope to be an alternative approach related to improve the plant growth, development and simultaneously could be reduce the pathogenicity.

Table 4. *DNA polymorphism analyzed by random amplified polymorphic-DNA (RAPD) analysis of rice seeds (Oryza sativa).*

S. No.	Primer	Band Scored	Common Band in Control and Treated	Unique Band	
				Control	Treated
	RPL 6A	17	13	3	−
	RPL 13A	13	13	−	−
	RPL 16A	12	8	3	−
	RPL 18A	16	15	1	−
	RPL 19A	20	11	3	4

−, No band

5. Conclusions

Based on study outcome, the biofield energy treated *O. sativa* showed faster with 100% germination as compared to the control. Moreover, the GSH content in treated sample was increased significantly by 639.26% of *O. sativa* seedling and 56.24% in mature plants as compared with their respective control. Apart from this, the plants growth regulating constituent IAA was also increased significantly by 106.90% in rice roots, while 20.35% was increased in rice shoots as compared to the control. RAPD analysis data of the treated sample showed an average range of 5 to 46% of polymorphism among the primers as compared to the control. In conclusion, the present investigation demonstrates that Mr. Trivedi's unique biofield treatment could be utilized as an alternate therapeutic approach concurrent with other existing therapy to improve the productivity of rice in the field of agriculture in the near future.

Abbreviations

ROS: Reactive oxygen species; ASC–GSH: Ascorbate–glutathione; PCR: Polymerase chain reaction; NCCIH: National Center for Complementary and Integrative Health; CAM: Complementary and Alternative Medicine; RAPD: Random amplified polymorphic; DNA: Deoxy ribonucleic acid; TCA: Tricholoroacetic acid; CTAB: Cetyl-trimethyl-ammonium bromide

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