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Influence of Pranic Agriculture on Morphological Traits, Chlorophyll Content and Genetic Polymorphism of Ridge Gourd (*Luffa acutangula* L. Roxb.) Assessed by RAPD Marker Analysis

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ABSTRACT

Pranic agriculture is a newly emerging concept of sustainable and eco-friendly agriculture. Pranic agriculture techniques are applied to plants before sowing and at the time of plant development to improve growth and yield. The present study aimed to understand the influence of pranic agriculture on growth, yield, and genetic polymorphism of ridge gourd. An increase in root length by 38%, stem girth by 8%, and the number of days taken for the premier harvest was advanced and found to be significant ($p<.05$) in pranic treated plots against the control. Chlorophyll content was 26% higher ($p<.05$) in pranic treated plants when compared to control. To find out the probable effects of pranic agriculture at molecular levels, RAPD marker analysis was carried out and average polymorphism up to 47% was observed between pranic and control treatments. Thus, pranic treatment was found to be very effective in increasing the overall growth and yield of ridge gourd. Further, in-depth studies are warranted about molecular mechanisms that are bringing changes in the plants after pranic treatment.

INTRODUCTION

Agriculture is a major production sector and plays a crucial role in ensuring food and nutritional security in India. In the present global climate change scenario, major challenges in agriculture production are the development of sustainable natural cropping systems by applying low-cost inputs like biofertilizers and biopesticides to reduce the burden on soil and soil microflora and to retain healthy natural ecosystem and biodiversity (Yadav et al., 2013). Among many attempts towards attaining a sustainable natural farming systems, pranic agriculture can be a complementary and supplementary method/science to the existing advanced crop production practices. Pranic agriculture is the application of prana to the plant through pranic healing techniques.

According to Master Choa Kok Sui, pranic healing is an ancient science and art of healing the bioplasmic body by using prana or life force (Sui, 2000). Prana or life force is a bioplasmic body or "Aura" which surrounds all the living organisms on earth like man, animal, plants, and microorganisms. The word bioplasmic refers to bio means life and plasma which is the fourth state of matter. Scientists with the help of Kirlian's photography have rediscovered the bioplasmic body that keeps the living organism healthy and alive (Kirlian, 1949). Plants absorb prana from sun, air, ground, and water (Sui, 2000). With limited agricultural land and an increasing human population, it is essential to enhance crop yield by improving photosynthetic activities. Photosynthesis is the foundation of life on earth providing the food, oxygen, and energy that sustains the biosphere and human civilization (Evans, 2013; Reynolds et al., 2011). Like any other animal, plants also experience abiotic and biotic stresses during its life cycle from seed germination to crop harvest. The effects of some stresses are self-repairable and some lead to heavy yield losses (Suzuki, Rivero, Shulaev,

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Blumwald, & Mittler, 2014; Wang, Vinocur, & Altman, 2003). Application of pranic healing on the soil before sowing, to the seed at the time of sowing and further treatment during different growth stages of the crop might lead to improved growth and yield and reverting the stress effects. Farmers can be trained to apply pranic treatment in their field and can be benefitted as it is a no-cost and no-loss method regarding the cost of cultivation is concerned.

Effect of pranic Agriculture is studied recently in some agricultural and horticultural crops like tomato, cucumber, pole beans, brinjal, papaya and found an enhancement in seed germination and vigour, plant growth, leaf area, flower number, fruit growth, yield and nutritional quality of the crop. Drumstick (*Moringa olifera*) seeds were exposed to Pranic treatment and grown under greenhouse conditions and seedling growth was evaluated from 15-day-old seedlings. Pranic treatment improved germination percentage and seedling vigour index as compared to control (Prasad & Jois, 2019). Similarly, in Papaya (*Carica papaya*) pranic treatment has improved germination percentage, plant length, shoot length, number of leaves, leaf length, leaf diameter and seedling vigour index as compared to control (Prasad & Jois, 2020). In another study with Pole beans (*Phaseolus vulgaris*) treated with pranic agriculture showed a 3.6% higher germination percentage when compared to control. The time required for flowering and flower to fruit set was reduced by 2.2% and 3.2 % respectively in the pranic group when compared to control group (Yathindra, Jois, & D'Souza, 2017a). European cucumber pranic treated flowers started flowering earlier (5-6 days). The higher number of fruits per plant (14%) and plant yield (18%) was noticed in the pranic group when compared to control (Yathindra, Jois, Prasad, & D'Souza, 2017b). Like other vegetable crops in Tomato (*Solanum lycopersicum*) also there was an increase in plant height (18.5%), stem diameter (12%) and flowers per plant (31.7%) in pranic treatment as compared to control (Jois et al., 2015). Besides improvement in growth and yield, there was an increase in post-harvest characteristics/qualities like low fruit water loss, shrinkage, titrable acidity and total soluble solids contents during storage both at room and cold storage conditions in pranic treatment as compared to control (Jois et al., 2015). Brinjal fruit was stored at room temperature for 0, 6, 12 and 16 days and at the end of 16th day, pranic treated brinjal had significant improvement in firmness, non-decay, colour, gloss and non-shrivelling against control (Jois, Prasad, & Shalini, 2019). Antioxidant and polyphenol contents were found higher in pranic treated cucumber compared to control (Keerthika, Devaki, Suma, & Urooj, 2016).

Ridge gourd (*Luffa acutangula*) is a fruit eaten as vegetable popularly known as Kalitori, angled gourd and angled loofah, belongs to genus *Luffa* of *Cucurbitaceae* family. Ridge gourd is a famous vegetable consumed in Asian, African and Middle East countries. It is a common vegetable in the Indian diet. It is a nutritive vegetable and has a bitter taste if taken raw. Ridge gourd has been also used extensively in the Indian traditional system of medicines. Ridge gourd is reported to contain many phytochemicals such as flavonoids, saponins, luffangulin, sapogenin, oleanolic acid and cucurbitacin B (Rahman, Anisuzzaman, Ahmed, Rafiul Islam, & Naderuzzaman, 2008). Ridge gourd acts as an appetiser and it is healthy food and contains a good amount of fibre, vitamins and kind of minerals. Hence, with these above advantages, ridge gourd was chosen as one of the best *Cucurbitaceous* crops in the present study to find out the impact of pranic treatment on growth, yield, and chlorophyll content. More interesting and first of its kind, an attempt was initiated to understand the influence of pranic healing at the DNA level by carrying out RAPD marker analysis.

MATERIALS AND METHODS

Experimental site

Ridge gourd (*Luffa acutangular* variety Naga) field experiment was conducted at the College of Horticulture, Mysore during February, 2019. Each treatment was carried out in a 0.2 ha land area on red loamy soil. Timely required agronomic cultural practices like weeding, irrigation, and inter cultivations were carried out according to the package of practice recommendations. The general view of the experimental plot at the time of fruiting is presented in Fig. 1.

Treatment

Pranic energy was applied to the seeds for three weeks, land area for two weeks before sowing, and to the crop two weeks after germination. Each time the pranic energy was applied for a duration of 15 minutes at twice a week interval. Another group with conventional treatment was considered as control.

Fig. 1. Control (A) and Pranic treated (B) plot of Ridge gourd

Field Observations

To understand the influence of pranic treatment given to the seed, initial observations like percent germination and premature seedling death was recorded 20 days after sowing. Growth parameters like leaf area, plant height, stem girth, number of branches, root length and chlorophyll content were recorded at the vegetative stage, whereas days to the first male flower, days to the first female flower, days to 50% flower, number of male flowers, number of female flowers, percent fruit set, number of days taken for the first harvest, fruits

number per plant, fruit girth, fruit weight, fruit length, yield per plant and yield per acre were recorded at a reproductive stage of the crop.

Total Chlorophyll Content

Total chlorophyll content of leaves was estimated using Dimethyl sulphoxide (DMSO) in young leaves at 55 days after sowing as given by Shoaf & Lium (1976). Fresh leaf tissue of 100 mg was cut into small pieces and incubated for 30 minutes in 7.0 ml of DMSO at 65^0C. At the end of the incubation period, leaf tissue was discarded and also supernatant was decanted. Volume was made until 10 ml with DMSO and the absorbance of extract was read at 652 nm using DMSO as a blank. The total chlorophyll content was calculated by using the following formula and expressed as mg/g of fresh weight.

$$\text{Total chlorophyll} = 27.8\ (A_{652}) \times \frac{V}{AxWx1000} \quad \text{.....1)}$$

Where: A = Absorbance at specific wavelength (652 nm), V = Final volume of the chlorophyll extract (ml) and W= Weight of leaf sample (g).

RAPD Marker Analysis

RAPD marker analysis was aimed to study the effect of pranic treatment on plant DNA characteristics using a standard methodology (Babu et al., 2014). DNA was isolated separately from the young fresh leaf of ridge gourd from control (untreated) and pranic treated groups according to the method of Rezadoost, Kordrostami, & Kumleh (2016) with slight modifications. The DNA quality was observed on 0.8% agarose gel and purity was checked by the A_{260}/A_{280} absorbance ratio using a nanodrop. The plant genomic DNA was subjected to PCR using arbitrary universal oligonucleotide primers (Table 1) for tracing genetic polymorphism. The reaction primed in a final volume of 25 µl contained the following components; DNA template: 5 µl (10 ng), primer: 2 µl (20 pmol), dNTPs: 5 µl (0.2 mM), *Taq* DNA polymerase: 0.5 U, PCR buffer: 2.5 µl, $MgCl_2$: 1.5 mM and H_2O: 8.5 µl. The PCR program was executed with one cycle of denaturation at 95°C/3 minutes, followed by 35 cycles of 95°C/45 seconds, 36-40°C/1 minute, 72°C/1 minute, and a final extension of 72°C/5 minutes. Amplified PCR products along with a 10 kb DNA ladder were separated by 1.8% (w/v) agarose gel and was stained with ethidium bromide and documented using a gel doc system. The percentage of polymorphism was calculated as.

$$\text{Percent polymorphism} = \frac{A}{B} \times 100 \quad \text{.....2)}$$

Where: A = number of polymorphic bands, B = total number of bands.

Statistical Analysis

The paired *t-test* was carried out for the morphological and yield data using Microsoft Excel and SPSS software, and the level of significance was expressed at p=.05.

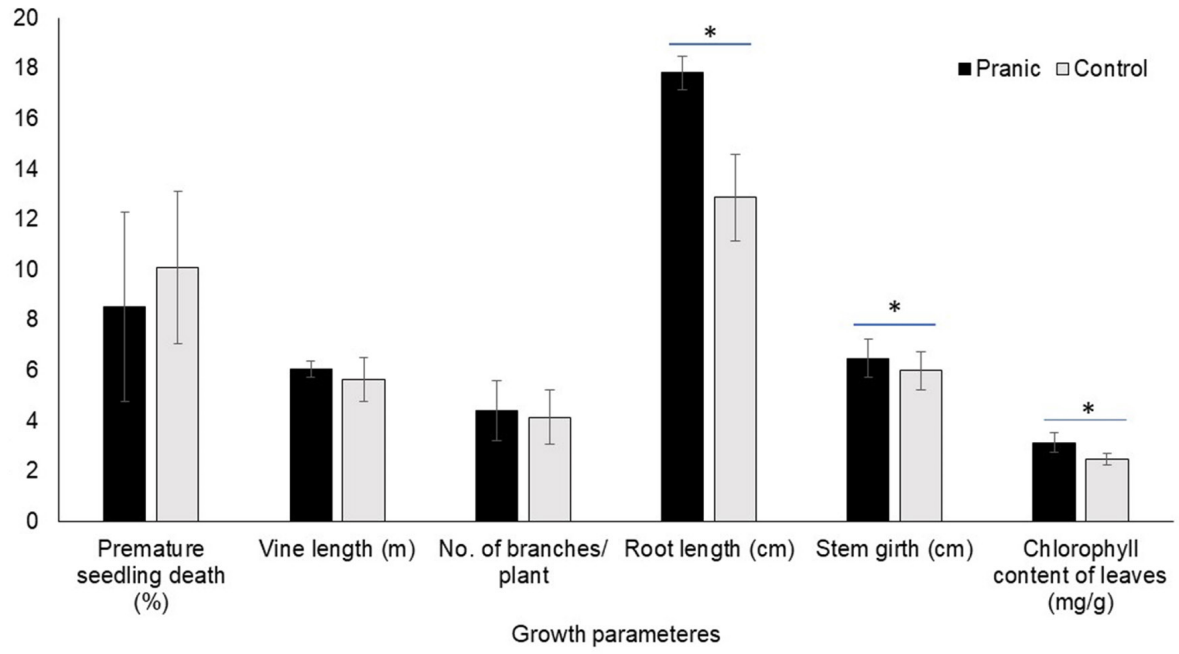
Table 1. Universal oligonucleotide primers used for RAPD-PCR

Sl. No.	Primer	Sequence	Tm (°C)	Reference
1.	GE2	5'-GTTTCGCTCC-3'	38	GE healthcare (Arif *et al* 2010)
2.	GE3	5'-GTAGACCCGT-3'	37	GE healthcare (Arif*et al* 2010)
3.	OPL-12	5'- GGGCGGTACT-3'	41	Operon technologies
4.	RPL 19A	5'-CACACTCCAG-3'	37	Genei (Bangalore)

RESULTS AND DISCUSSION

Seed Germination and Premature Seedling Death

Pranic treated ridge gourd showed a higher germination percentage (89%) as compared to the control (88%). Premature seedling death was higher in the control plot (10%) as compared to the pranic plot (8.5%) (Fig. 2). There was no significant difference effect among the treatments, but higher germination and seedling survival was observed in pranic treatment than control. Treating the seeds with pranic energy before sowing might have played a role as seed priming. Seed priming is believed to bring about some biochemical changes in the metabolism within the seed, which further favors germination and growth of seedlings (Jisha & Puthur, 2014). Spiritual practices of *Sriyantra*, pyramid, and *MahaMrtyunjayaMantra* were studied on green gram and fenugreek seed germination. Green gram seeds were kept for germination in front of paper sri yantra and two models of pyramids (plywood and plastic) and the control sample was kept under the normal white paper. The Plywood pyramid has shown maximum percent emergence and radical length. Plastic pyramid has shown a maximum percentage of fresh weight. Paper *Sriyantra* has shown maximum percentage change in the dry weight of germinated seeds. In another experiment, fenugreek seeds are treated with *MahaMrtyunjayaMantra* chanting for 108 times and without chanting is considered as control. Mantra chanting has given exponential significance in seed emergence, radical length, fresh weight, and dry weight as compared to control (Jungyun, Jeeye, & Kumar, 2016).



Remarks: * Significance at 0.05 level

Fig. 2. Effect of pranic treatment on growth parameters of ridge gourd

Vine and Root Growth

Ridge gourd vine length and number of branches per plant were found numerically higher in the pranic plot (6.05 m and 4.4) as compared to the control plot (5.63 m and 4.15) respectively. However, interestingly root length and stem girth were significantly higher in the pranic plot (17.82 cm and 6.48 cm) as compared to the control plot (12.87 cm and 6 cm) (Fig. 2). The root is one of the most important parts of the plant from where the plant absorbs necessary water and minerals and supplies to the above-ground plant parts. There is always an interdependent relationship that exists between the root and shoots called a source to sink relationships. Active root supplies a sufficient amount of water, nutrients, and phytohormones to shoots, and in turn, healthy leaf synthesizes photosynthates and supplies to root for its growth and uptake (Qi, Hu, Song, & Zhang, 2019). An increase in the root growth might have influenced the growth of above-ground parts like vine, leaf, and stem in pranic treatment. Another probable reason can be attributed to the increase in the synthesis of the IAA hormone in the root and shoot tips, and it helps in cell elongation and apical dominance (Paque & Weijers, 2016). This might have further lead to the increase of root length and stem girth followed by vine length and leaf area.

Chlorophyll Content

Pranic treated leaves had a high significantly of chlorophyll content (3.14) as compared to control (2.48) (Fig. 2). Chlorophyll is a green pigment and a vital component of photosynthesis. The increase in chlorophyll might have helped for the increase in photosynthesis and ultimately higher growth and yield (Kura-hotta, Satoh, & Katoh, 1987). Similarly, in ridge gourd variability and character association study regarding yield and yield attributing characters like chlorophyll was carried out by Koppad, Chavan, Hallur, Rathod, & Shantappa (2015). Chlorophyll content in the study was high in genotype Arabhavi Local 2.47 and 2.39 mg/g fresh weight at 45 and 90 DAS, respectively. Pranic energy might have improved some energy and ion equilibrium process of photosynthesis which takes place in Photosystem I and II by transfer of light energy to chemical energy and synthesis of carbohydrates and ATP. In another recent study, the combined use of energy art and energy art treated water had a significant impact in improving the growth of lettuce and bok choy plants. The impact was found in physiological development, especially in terms of carotenoid and chlorophyll content (Lee & Wu, 2019).

Flower Parameters

Flower initiation, days to 50% flowering, and number of female flowers are the most important traits for the fruit yield except for the number of female flowers per plant, all other flowering parameters are on par with each other in pranic and control groups. Whereas, the most important trait number of female flowers bearing capacity was higher in pranic treated plants (43.9) than control plants (40.1) (Fig. 3). The male/female flower ratio was less in pranic treated plants (2.35) as compared to control plants (2.45). The ratio indicates that pranic agriculture has reduced the number of male flowers and increased female flowers. A similar variation in flower ratio was found in Cucumber (Yamasaki, Fujii, & Takahashi, 2003) and Bitter gourd (Ghani et al., 2013). An increase in female flower is an important trait determining the final fruit yield per plant by increasing the number of pistillate flowers and fruit set. An early appearance of male and female flowers on the vine indicates crop earliness, reported by Tyagi, Sharma, Siddiqui, & Khandelwal (2010) in ridge gourd.

Yield Traits

Percent fruit set was high in pranic treatment (2.46%) when compared to control (2.35%) (Table 2). The number of days taken for the first harvest was significantly lower in pranic treatment (55.25 days) as compared to control (61.5 days). In pranic treatment first fruit harvest was advanced by almost 5-6 days indicates that an increase in the number of fruit pickings and ultimately higher yield. Pranic treatment probably has advanced fruit initiation time by altering some hormones involved in flower initiation. It also might have played a role in healing the plants by damage from external oxidative stresses and lead to early recovery. Similarly, the number of fruits per plant (7.7), fruit weight (468.6), yield per plant (3.62), and yield per acre (13.4) are higher in pranic treatment as compared to control (Table 2). The yield of a crop is decided by many of the morphological parameters, improvement in root and shoot girth and chlorophyll content might have accounted majorly for improvement in yield. The application of agri-wave technology on tomatoes remarkably stimulated seedling growth. The fresh weight of the branch, stems, and leaves of the treated

tomatoes are significantly higher (59.53%) than that of the control group. The fresh weight and yield of riped tomatoes is 30.73% and 13.89% higher than untreated tomatoes (Hou & Mooneyham, 1999). In some studies, the application of biofield energy treatment has documented an increase in growth and yield compared to untreated in lettuce and tomato (Shinde, Sances, Patil, & Spence, 2012). Tyagi, Sharma, Siddiqui, & Khandelwal (2010) also reported that the number of fruits per vine had a higher positive relationship to the total yield. Long roots help in better absorption of water and nutrients from the root zone leading to the vigorous growth of the vine. By applying pranic energy there is an improvement in the capacity of two important sources of plants *viz.* root and leaf and ultimately leads to the increase of source to sink capacity and has increased the yield. Application of pranic energy might have promoted the metabolic activities and photosynthesis in plants in-turn enhancing vegetative growth leading to higher shoot and root growth and finally effective on overall grain yield.

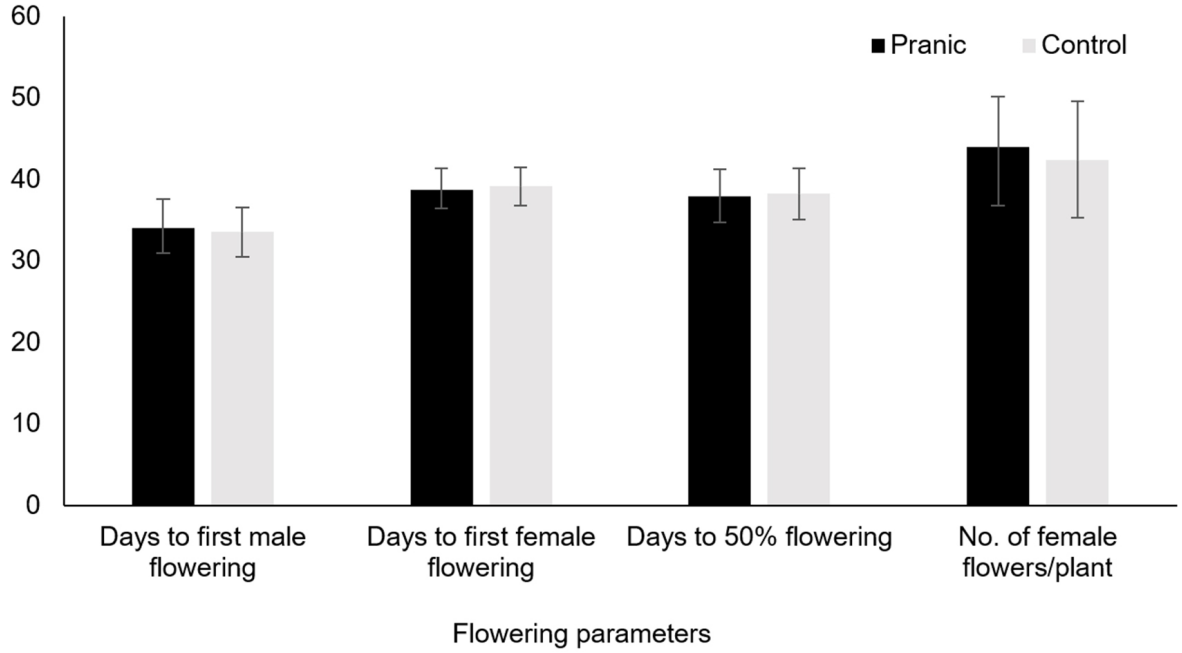


Fig. 3. Effect of pranic treatment on flower parameters of ridge gourd

Table 2. Effect of pranic treatment on yield parameters of ridge gourd

Yield parameters	Pranic		Control		t-test Statistics	
	Mean	S.D	Mean	S.D	t-stat	Critical value
Percent fruit set (%)	2.46	0.50	2.35	0.48	-0.90	2.0
No. of days taken for first harvest	55.25	3.86	61.5	3.00	-2.55	2.44
Fruits number/plant	7.70	1.82	7.50	1.98	0.40	2.00
Fruit length (cm)	45.23	2.63	45.68	4.36	-0.27	2.10
Fruit girth (cm)	4.70	0.36	4.95	0.56	-1.16	2.10
Fruit weight (g)	468.62	69.56	459.74	121.87	-0.20	2.10
Yield per plant (kg)	3.62	1.35	3.42	1.04	0.62	2.0
Yield per acre (t)	13.4	1.63	12.66	1.63	0.64	2.44

RAPD Marker Analysis

RAPD analysis gel picture and results between pranic treated and control plant samples using four arbitrary primers are represented in Fig. 4 and Table 3. The DNA polymorphism bands obtained between control and pranic are represented in arrows on the left side of the lane. Totally 43 scorable bands, 20 polymorphic bands and 24 monomorphic bands were obtained between pranic and control groups. Percent polymorphism obtained by arbitrary primers GE 2, GE 3, OPL-12, and RPL-19A was 41.7%, 36.4%, 53.8%, and 57.1% respectively. The percent polymorphism ranged from 36.4% to 53.8%, and the highest was observed in RPL 19A primer. The size of the amplified product varied from 400bp to 3000bp. Average percent polymorphism of 47.3% was obtained between the groups indicates that there is wide variability between the two groups. The pranic treatment has imparted considerable variability not only on plant morphology and chlorophyll content but also on plant DNA. Results of RAPD analysis give basic information and confirmation that the pranic treatment is bringing remarkable change on plants at the DNA level. The results of the present study can be a baseline for further understanding of the actual mechanism and site of action of pranic treatment on the plant at the cellular level. The additional DNA bands which are found in the pranic treated plant might be associated with the increase in the root length and stem girth of the plant. Similarly, days are taken for fruit maturity and an increase in chlorophyll content might also have some relationship with the additional polymorphic bands in pranic treated plants.

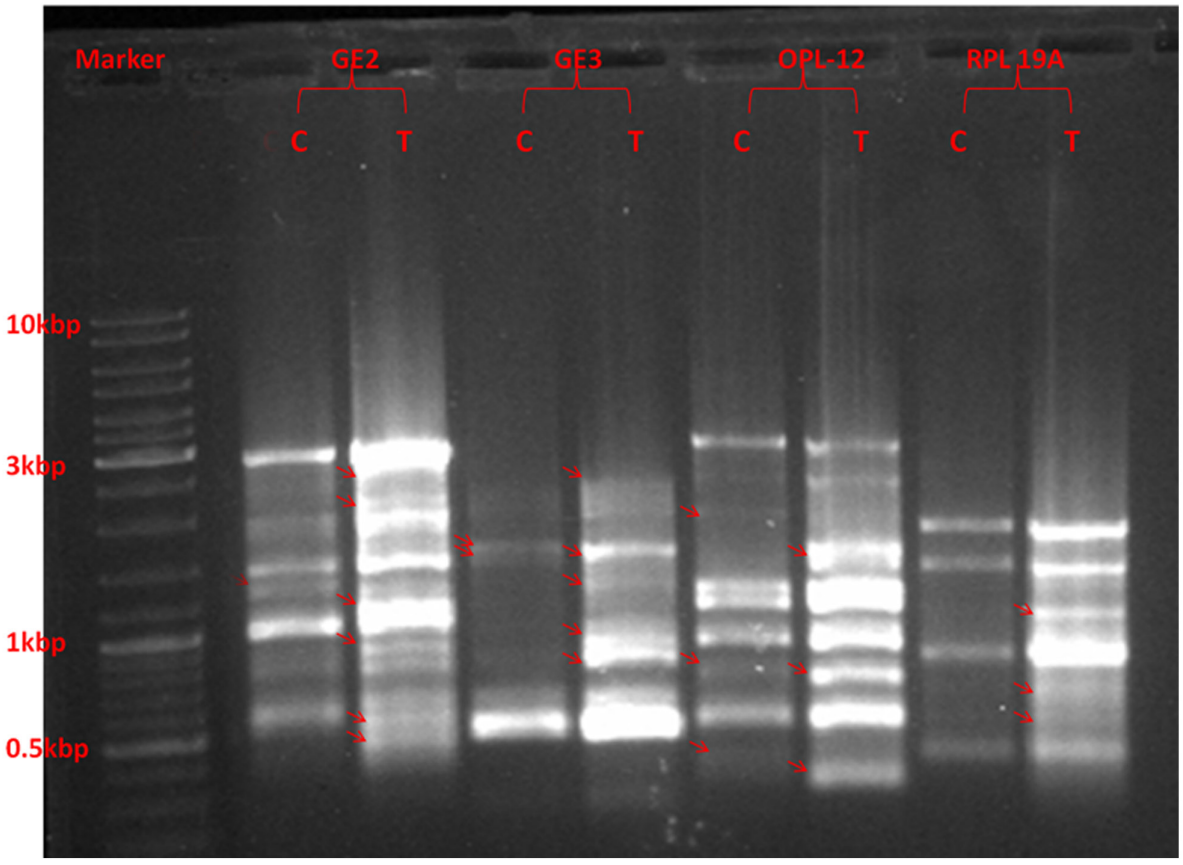


Fig. 4. RAPD banding patterns of ridge gourd using primers GE2, GE3, OPL-12, RPL 19A, Lane M: 10kb ladder; C: Control, T: Treated. Red arrow indicates the presence of unique bands

Table 3. DNA polymorphism detected by RAPD analysis for ridge gourd

Polymorphism	Primer				Total	Average
	GE2	GE3	OPL-12	RPL19A		
No. of scorable bands	12	11	13	7	43	10.8
No. of polymorphic bands	5	4	7	4	20	5
No. of monomorphic bands	7	7	6	4	24	6
Percent Polymorphism (%)	41.7	36.4	53.8	57.1	189	47.3

Pranic energy applied might have changed the expression of genes and protein synthesis related to the morphological and yield variations. External and internal factors bring changes in plant gene expression and regulation. External factors bring about upregulation or downregulation of the genes or receptors responsible for specific functions (Hasegawa, Bressan, Zhu, & Bohnert, 2000; Mizoguchi, Ichimura, Yoshida, & Shinozaki, 2000). In some studies, external factors like light and temperature influence gene expression related to change in photosynthetic pigment composition (Esteban et al., 2015) and flavonol accumulation (Neugart, Krumbein, & Zrenner, 2016) in different plants. All living cells can receive and process signals that originate outside their cell membranes. Similarly, pranic energy applied externally near the plant micro space might have acted as a stimulus to plants and have brought variation in gene expression related to morphology and yield. Genetic diversity studies were carried out using RAPD markers in different *Cucurbitaceae* family vegetables like Ridge gourd (Hoque & Rabbani, 2009), Pointed gourd (Khan, Rabbani, Islam, Rashid, & Alam, 2009), Indian ash gourd (*Benincasahispida)* (Pandey et al., 2008), Ash gourd [*Benincasahispida* (Thunb.) Cogn.] (Resmi & Sreelathakumary, 2011), Bitter gourd (Rathod, Narasegowda, Papanna, & Simon, 2008) showed 81.5%, 79.5%, 73%, 90% and 48.3% polymorphism, respectively. In another study, 93 accessions of Okra, comprise 50 West African genotypes and 43 Asian genotypes were assessed for genetic distinctiveness and relationships using the RAPD marker and they exhibited 89% similarity with nine and eight clusters in each group (Aladele, Ariyo, & de Lapena, 2008). Similarly, RAPD analysis was adopted to find out salt tolerance of segregating F_2 progenies crossed between domestic salt-sensitive cultivar and natural salt-tolerant wild type Tomato parents (Ezin, Dasenka, Agbobatinkpo, Ahanchede, & Handa, 2018).

Wheat and pea seeds germinated rapidly and produced longer roots within 20 minutes by projecting 'Qi Energy'. RAPD analysis was carried out using eleven selected primers in wheat, and pea, seven primers amplified polymorphism in wheat, and five primers in pea seeds. The supply of more Qi energy during rapid cell division, growth and differentiation influenced on accelerated germination. Qi Energy might have changed the DNA at the promoter or regulatory region and brought out the changes in gene expression (Bai et al., 2000).

CONCLUSION

Pranic treated ridge gourd showed better and higher on growth, early maturity, chlorophyll content, and yield when compared to control plants. Polymorphism observed in pranic treatment over control is evident that pranic energy application bringing changes at a molecular level also. Further studies are needed to find out the underlying biochemical and molecular mechanisms behind this variation. This technique could be very promising for farmers to achieve global agriculture sustainability.

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