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HIGH VOLTAGE ELECTRIC FIELD EFFECTS ON THE GERMINATION RATE OF TOMATO SEEDS

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Abstract. This paper presents a discussion about the application of electricity that stimulates the growth of the plant to a great extent. This paper also demonstrates that exposure to the action of a high voltage electric field can be an effective tool for the enhancement of germination. The experiments were carried out on tomato seeds. A pilot treatment unit was developed and used for the study. Under normal conditions (no treatment), only about 76% of a reference sample of such seeds were germinated. Other seed samples were subjected to 50 Hz electric fields ranging from 10 to 30 kV cm^{-1} with exposure time ranging from 10 to 30 sec. In the optimum laboratory test, 100% of the seeds were germinated. The weight of the resulting tomato seedlings was greater than that of untreated ones. The germination tests proved the efficiency of this method. Ozone generation by partial discharges between seeds seems to be the main effective parameter that enhances the growth.

Keywords: electrostatic stimulation, ozone generation, germination dynamics

INTRODUCTION

Recently the world population has been accelerating its growth rapidly and will be greater than nine thousand million in 2050 (United Nations 2008). However, it is difficult to obtain farmlands due to urbanisation. Thus, food shortage may become a serious issue in near future. Hence, there are requirements of bioresearch to improve the harvest efficiency of food plants. Various studies are being conducted in that area. The stimulation of plant growth to a great extent is possible by applying electricity in the form of magnetism, monochrome light and sound (Stone 1999). This little-known technology, called Electro-culture, can accelerate growth rates, increase yields and improve crop quality (Bailey 1999). The various approaches to electro-culture include: antennas, static electricity, direct and alternating current, magnetism, radio frequencies, monochrome and intermittent lighting and sound. The energies are applied to seed, plants, soil or water and nutrients. Electro-culture can protect plants from diseases, insects and frost. These methods also reduce the requirements for fertiliser or pesticides. Farmers can grow bigger and better crops in less time, with less effort and at a lower cost.

Plant growth as well as the biological processes of seeds can be accelerated or inhibited by high intensity electric fields. The mechanism of these effects is still insufficiently known. Electrostatic treatment is assumed to enhance seed vigour by influencing the biochemical processes which involve free radicals, and by stimulating the activity of proteins and enzymes (Morar *et al.* 1999). Corona discharges also seem to affect the biological activity of seeds (Lynikiene 2006).

Destruction of microorganisms in liquids by using high intensity electric fields has been thoroughly investigated by many scientists (Moon 2000, Kuzmanov 2010, Lynikiene 2003, Gui 2003). A review of the efforts on the inactivation of microorganisms by pulsed electric fields can be found in (Songnum 2011). The electric fields effects were mainly attributed to the field-induced intensification of the biological processes in seeds.

To understand seed enhancement technique, a study was carried out in Mahatma Phule Agricultural University. The authors aimed to analyse high voltage electrostatic field application, using tomato seeds as an experimental system. The data presented in this paper demonstrate that exposure to a high intensity field can be an effective tool for germination improvement.

EXPERIMENTAL PROCEDURE

Tomato is the important cash crop in the world. There are several varieties. The experiments were carried out on tomato seeds of the Rajashri variety developed by Mahatma Phule Agricultural University. Batches of fresh seeds was selected for the tests.

An experimental set up was designed as shown in Figure 1.

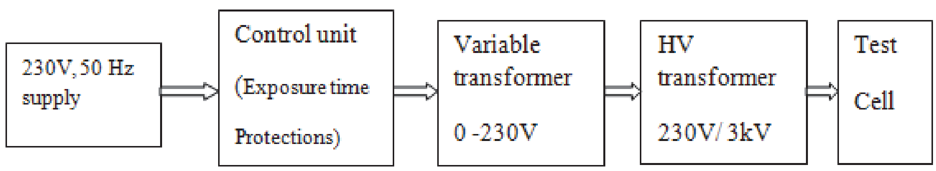

Fig. 1. Block diagram for HV test set up as a pilot unit

The single phase AC supply was used as a main source of electricity. This supply was given to a controller which controls exposure time as well as protection against short circuit. A variable transformer was used to vary incoming voltage as per requirement. This voltage was fed to a HV transformer of 230 V/3 kV rating. Thus a fully adjustable AC high voltage supply was available. This supply was fed to electrodes in the test cell. The test cell consisted of two electrodes of aluminium plate with 50 mm length and width, and 4 mm thickness. Inter-electrode gap was 1 mm. The electrode plates were covered with thin high density polypropylene film (HDPF) of 0.1 mm thickness to avoid contacts between the seeds and the electrodes. Hence the electrostatic field was developed. The seeds were loaded one layer over the electrode covered by HDPF films. No heating effect was noticed during the experiments, even when the maximum voltage was applied to the electrode system.

Several laboratory experiments were performed to determine whether high intensity electric field exposure on tomato seeds causes any change in germination and growth of the seedlings. Each test involved several exposures of a particular sample to AC fields, at setting of the applied voltage (10 kV, 20 kV, 30 kV) per cm. The exposure time varied from one test to another within the range of 10-30 sec. The intervals between the samples ranged from 10 to 60 sec. Three replicates of 50 seeds/lot were treated for different combinations.

LABORATORY TESTS

Germination test

Germination tests were carried out immediately after one day of electrostatic field exposure. Seeds were sown on top of moistened blotter paper in covered transparent polyethylene boxes. The boxes were kept in a germinator where temperature and humidity were controlled as per ISTA standards, at 25^{o}C, 80% respectively. The first count was taken on the fourth day while the final count was measured on the tenth day as given in Table 1. The percentage of seedling emergence was presented as the percentage of normal seedling. For detailed study, seedling growth parameters were measured at the tenth day. Data based on observations of seedling root shoot length, fresh weight, dry matter weight were collected.

Enzyme activity

The assay of enzyme activity and lipid peroxidation was carried out within seven days after the seeds were exposed to electrostatic field. Three replicates of 50 seeds/treatment were allowed to imbibe for 10 hr., then they were hand homogenised in an iced mortar with pestle in 4 ml of 0.1 M potassium phosphate buffer (pH 7.0), followed by centrifuging at 10,000 g for 20 min; the supernatant obtained was used for determining enzyme activity and total soluble protein content (Thmmaiah 2009).

Lipid peroxidation was measured by the thiobarbituric acid (TBA) test that determines malondialdehyde (MDA) as an end product of lipid peroxidation. Three replicates of ten seeds per treatment were allowed to imbibe for 10 h, then hand homogenised in a mortar with pestle in 4 ml of 5% (v/v) trichloroacetic acid (TCA), followed by centrifuging at 10,000 g for 20 min; the supernatants obtained were used for MDA determination. To 1 ml of supernatant, 1ml of 20% TCA containing 0.5% (w/v) TBA was added. The mixtures were heated at 95°C for 30 min, and then quickly cooled in an ice water bath to room temperature. The mixtures were then centrifuged at 10,000 g for 10 min, and the absorbance of the supernatants was read at 532 nm. The value of non-specific absorption at 600 nm was subtracted. The amount of the MDA-TBA complex was calculated from the extinction coefficient 155 m M^{-1} cm^{-1} (Huang 1999).

Table 1. Germination percentage with three replicates

Treatment		R_1	R_2	R_3
V_1T_1	10 kV, 10 Sec	98	100	98
V_1T_2	10 kV, 20 Sec	98	98	100
V_1T_3	10 kV, 30 Sec	98	98	98
V_2T_1	20 kV, 10 Sec	100	98	98
V_2T_2	20 kV, 20 Sec	100	100	100
V_2T_3	20 kV, 30 Sec	100	98	98
V_3T_1	30 kV, 10 Sec	98	98	98
V_3T_2	30 kV, 20 Sec	98	100	98
V_3T_3	30 kV, 30 Sec	100	100	100
Control		92	92	92

Statistical analysis

A completely randomised design was employed in all laboratory experiments and a factorial randomised block design was used in the seedling growth test. The objective of these designs was to permit treatment comparisons to be made with the greatest possible precision. Correspondingly, in the statistical analysis of the results, the variation under control, such as between blocks in a randomised block design, was segregated from the residual error variation. The analysis of variance technique was capable of fruitful application to a diversity of practical problems. Basically, it was used to classifying and cross-classifying statistical results and testing whether the means of specified classification differ significantly. In this way it was determined whether the given classification was important in affecting the results. Data analysis was performed using Excel 2007. Two way ANOVA was calculated for each parameter. Curvilinear regression analysis was also adopted for representing germination as a dependent variable in equation form using the voltage applied.

RESULTS AND DISCUSSION

Germination test results

With reference to several filler trials, the parameters for the voltage applied and duration of time of exposure were selected. Based on statistical requirements the experiment was planned for factorial randomised block design. There were nine treatment combinations with three replications and one control. All samples were treated as per the process selected. Next day each lot of seeds was put to the germination trials using petri dishes. The samples were kept in a germinator. On the fourth day, germination count was taken. On the tenth day the final count was made, and fresh weights and root shoot lengths were measured (Figs. 2, 3). After that, as per International Seed Testing Association (ISTA) recommendation, the samples were put in an oven for 17 hrs, at 60^{o}C, for measurement of dry matter content. Then the weights of all samples were noted. Using these results, seed vigour1 and seed vigour2 were calculated as given in Table 4.

Seed vigour1 = Germination % × Root shoot length
Seed vigour2 = Germination % × Seedling dry weight

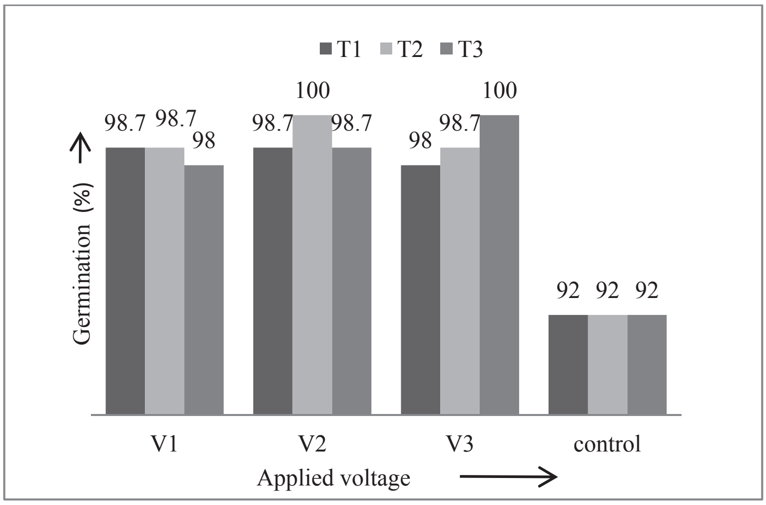

Fig. 2. Germination as a function of applied voltage

Table 4. Seed vigour calculation

Treatment	Replicates	Fresh weight	Dry matter	Seed vigour1	Seed vigour2
Control		0.11	0.016	7856.8	1.472
V1T1 10 kV, 10 Sec	R1	0.21	0.018	8241.8	1.764
	R2	0.21	0.017	9300	1.7
	R3	0.24	0.02	9437.4	1.96
V1T2 10 kV, 20 Sec	R1	0.2	0.017	8457.4	1.666
	R2	0.2	0.016	8202.6	1.568
	R3	0.21	0.015	8820	1.5
V1T3 10 kV, 30 Sec	R1	0.16	0.019	9339.4	1.862
	R2	0.17	0.02	8486.8	1.96
	R3	0.17	0.019	8300.6	1.862
V2T1 20 kV, 10 Sec	R1	0.14	0.018	8540	1.8
	R2	0.14	0.016	8437.8	1.568
	R3	0.14	0.015	8839.6	1.47
V2T2 20 kV, 20 Sec	R1	0.12	0.018	9660	1.8
	R2	0.13	0.019	9620	1.9
	R3	12	0.017	10220	1.7
V2T3 20 kV, 30 Sec	R1	0.24	0.018	9020	1.8
	R2	0.25	0.02	8104.6	1.96
	R3	0.22	0.017	8428	1.666
V3T1 30 kV, 10 Sec	R1	0.17	0.016	7898.8	1.568
	R2	0.17	0.019	7536.2	1.862
	R3	0.2	0.019	8790.6	1.862
V3T2 30 kV, 20 Sec	R1	0.13	0.016	8202.6	1.568
	R2	0.15	0.018	9050	1.8
	R3	0.15	0.019	8526	1.862
V3T3 30 kV, 30 Sec	R1	0.13	0.013	8120	1.3
	R2	0.13	0.018	8910	1.8
	R3	0.13	0.015	8080	1.5

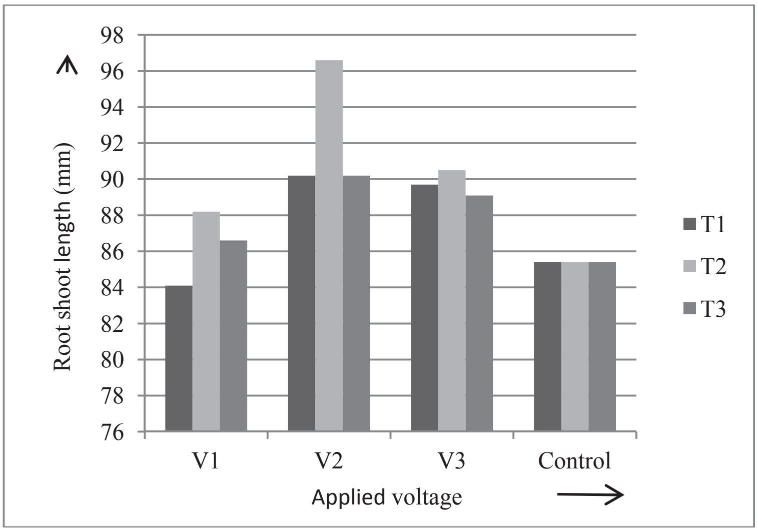

Fig. 3. Root shoot length as a function of applied voltage

Enzyme activity results

These values were related to the basic mechanism which explains stimulating effects of electric field exposure. Ozone generation by partial discharges between seeds and the activation of OH radicals under the action of the high-intensity electric field were assumed to be responsible for the intensification of the biological processes. The process had been reported to be time dependent. The above described seed exposure was employed at three different high voltage levels. Laboratory tests showed that the germination energy of the treated seed samples increased as compared to untreated ones. The development of root shoot for electrically treated tomato seeds was faster as can be seen in Figure 3.

The electric field exposure of seed may result in breaking of hydrogen bonding in ultra structural elements of cell. This structural alteration may increase enzyme activity depending on the strength of electric field and the time of exposure. From the biochemical analysis, it was observed that the reaction of lipid peroxidation was reduced for the treatment V_2T_2. Hence seed balance was improved, which resulted in faster growth as well as increased germination count.

Statistical analysis results

From the observations, it was seen that germination percentage was significantly increased due to treatment given to seeds as shown in Table 1. All the data were averages of three replicates. Treatments V_2T_2, V_3T_3 were more efficient, as given in Table 2. But, considering the combined impact of growth parameters such as root shoot length (Tab. 3), seed vigour1, seed vigour2, the V_2T_2 method showed the best results. As a general observation, all the methods were superior to that of the control. Hence, in ANOVA, control Vs treatment got CD at 5% as well as 1% highly significant (Tab. 2). To achieve significance between voltage and duration intervals, their ranges should be revised. At this stage, it was observed that electrostatic field had a significant impact on tomato seed germination.

The difference of germination between treated and untreated seeds was statically very significant. The mass increase of the roots with the applied voltage up to 20 kV cm^{-1} was highly significant. These results can be interpreted in relation to the data regarding the action of the electric field. The curvilinear regression analysis was carried out. Mathematically, the local optimum point was found at voltage gradient of 1.69 kV mm^{-1}. This voltage justified the best performance of the V_2T_2 method.

Table 2. Two way ANOVA for germination percentage

Two way interaction table

	10 sec	20 sec	30 sec	Mean	
10 kV cm^{-1}	98.67	98.67	98.00	**98.44**	
20 kV cm^{-1}	98.67	100.00	98.67	**99.11**	
30 kV cm^{-1}	98.00	98.67	100.00	**98.89**	
Mean	**98.44**	**99.11**	**98.89**	**98.81**	Treat. Mean
Control mean		**92.00**		**98.13**	Grand mean

ANOVA

Source	DF	SS	MSS	F	SE	CD 5%	CD 1%
Replications	2	0.26	0.13	0.18	0.26	NS	NS
Treatments	9	138.1	15.34	21.14	0.49	1.46	5.94
Con Vs Treat	1	125.3	125.39	172.73	0.61	1.84	7.49
Voltage	2	2.07	1.03	1.42	0.28	NS	NS
Time	2	2.07	1.03	1.42	0.28	NS	NS
V xT	4	8.59	2.14	2.95	0.49	NS	NS
Error	18	13.06	0.72				
Total	29	151.46				CV (%)	0.868

DF = Degrees of freedom; SS = Sum of squares; MSS = Mean sum of squares; SE = Standard Error; CD = Critical Difference; CV = Coefficient of variation, NS – not significant.

Table 3. Root shoot length data

Treatment		Root shoot length (mm)			
		R1	R2	R3	Av
V_1T_1	10 kV, 10 Sec	84.1	93	96.3	91.13
V_1T_2	10 kV, 20 Sec	86.3	83.7	88.2	86.06
V_1T_3	10 kV, 30 Sec	95.3	86.6	84.7	88.86
V_2T_1	20 kV, 10 Sec	85.4	86.1	90.2	87.23
V_2T_2	20 kV, 20 Sec	96.6	96.2	102.2	98.33
V_2T_3	20 kV, 30 Sec	90.2	82.7	86	86.3
V_3T_1	30 kV, 10 Sec	80.6	76.9	89.7	82.4
V_3T_2	30 kV, 20 Sec	83.7	90.5	87	87.06
V_3T_3	30 kV, 30 Sec	81.2	89.1	80.8	83.7
Control		76	95	84	85.4

PILOT INSTALLATION FOR TOMATO SEED TREATMENT

A pilot installation set was set up at Mahatma Phule Agricultural University, Rahuri. This set was only for initial treatment application. By using this, voltage level was confirmed to apply electrostatic field for tomato seeds for enhancement of germination. The fresh seed lot was used for trials. So the germination for the control lot was also relatively better. Due to aging, seed deterioration took place. Hence to observe the impact of seed age on germination, more trials were planned. For further trials, older seed lots will be used to check the viability of seed due to the aging effect.

CONCLUSIONS

1. Exposure to the action of a high-intensity AC electric field can be an effective tool for enhancement of germination
2. The modification in germination and subsequent seed vigour are statistically significant when electrically treated samples are compared with untreated ones
3. The application of voltage gradient as 20 kV cm^{-1} for 20 second duration is found the most suitable for the best germination
4. This method was found very simple for adoption; hence commercialisation of the method will be advantageous

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WPŁYW WYSOKO NAPIĘCIOWEGO POLA ELEKTRYCZNEGO NA SZYBKOŚĆ KIEŁKOWANIA NASION POMIDORA

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S t r e s z c z e n i e. Artykuł przedstawia wyniki badań zastosowania pola elektrycznego do stymulacji wzrostu roślin. W pracy wykazano, że poddanie nasion działaniu pola elektrycznego wysokiego napięcia może być skutecznym narzędziem do stymulacji ich kiełkowania. Doświadczenia prowadzono na nasionach pomidora. Opracowano i zastosowano pilotażowe urządzenie do stymulacji nasion polem elektrycznym. W warunkach normalnych (bez działania pola elektrycznego), wykiełkowało około 76% nasion z próbki referencyjnej (kontrolnej). Inne próbki nasion poddano działaniu pola elektrycznego o częstotliwości 50 Hz w zakresie od 1 do 3 $kV \cdot mm^{-1}$, przy czasach ekspozycji od 10 do 30 s. W optymalnych warunkach testu laboratoryjnego wykiełkowało 100% nasion. Masa wyrosłych z tych nasion siewek pomidora była większa niż siewek wyrosłych z nasion nie poddawanych działaniu pola elektrycznego. Testy kiełkowania dowiodły skuteczności tej metody. Wytwarzanie ozonu poprzez częściowe wyładowania pomiędzy nasionami wydaje się być głównym powodem stymulującym wzrost.

S ł o w a k l u c z o w e: stymulacja elektrostatyczna, wytwarzanie ozonu, dynamika kiełkowania