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Full Length Research Paper

Effect of electric treatment on germination, seedling growth and water uptake in chickpea seed

***Kovind Nisar[1], Kumar Sangma[1] and Singh Rani[2]**

[1]Department of Agrophysics, Faculty of Life Science, Rajiv Gandhi University, Arunachal Pradesh, India.
[2]Department of Agronomy, Faculty of Agriculture, Assam University, Assam, India.

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The chickpea (*Cicer arietinum*) seeds were exposed to the electric field at 270, 470, 670, 870 and 1070 V/cm for 15 min duration at a constant temperature (13°C). The effect of electric treatment on germination, seedling growth and water uptake was investigated. Electrically treated seeds have shown a delay in water absorption as compared to the control (unexposed); hence, there was an increase in mean germination time and transition time. Maximum delay was observed at 470 V/cm with 15 min exposure. Rate of water uptake was calculated experimentally. It increased at all field values. A new water uptake model was used to find water content absorbed by the seed at any instant as a function of the electric field at a constant temperature (13°C). Among the various treatments, 470 V/cm for 15 min yielded the peak performance (for root and shoot lengths).

Key words: Germination time, electric-treatment, water-uptake model, ferroelectric effect.

INTRODUCTION

Magnetic field treatment has been proved as a promising technique for agricultural improvements (Fischer et al., 2004; Florez et al., 2007; Rochalska et al., 2007; Podleoeny et al., 2005; Marks et al., 2010). There is a positive effect of magnetic field on seed germination whereas there is no clear view about the impact of the electric field on the plant growth. Moon et al. (2000) showed an inhibitory effect on germination of tomato seed when they were exposed with electric field (more than 12 kV/cm) for exposure time greater than 60 s; whereas Kiatgamjorn et al. (2002, 2003) found a positive effect of the electric field intensity 25 kV/m (longer height of stems and higher length of roots) on the growth of bean sprouting. Dymek et al. (2012) studied Pulsed Electric Fields (PEF) at varying voltages [0 (control), 110, 160, 240,

Mahajan and Pandey (2013) formulated new water uptake model without using w_{max}. They assumed that net

Corresponding author E-mail: kovind.nisar@yahoo.com

weight of water absorbed at any instant w - wdry is directly 320, 400 and 480 V] on barley seeds and showed that PEF exposure affects radicle emergence without significantly affecting the seeds gross metabolic activity. Chickpea (*Cicer arietinum*) which is also known as Gram seeds were treated with magnetic field and has been studied by many authors (Das et al., 2006; Mahajan et al., 2011, 2012; Vashisth et al., 2008) but up to date no author has studied the effects of low static electric field on Chickpea seeds.

The aim of the present study was to find the effect of low electric field (0 to 1200 V/cm) on germination, growth and water uptake of chickpea seeds at a constant temperature of 13°C. Another aspect of the present study is to explore a cause for change in germination and water uptake by the electrically treated seeds.

Water uptake model

Water absorption by the seed is an important tool to understand basic germinating processes. Water uptake curve can be divided into three phases (phases I, II and III). In phase I, there is rapid water absorption (independent of metabolic activity); in phase II, water absorption becomes small as the seed gets saturated in this phase (enzyme activation and synthesis increases in phase II. The activated enzymes break down storage materials like fats, proteins, carbohydrates and phosphorous containing compounds. In phase III, water uptake again increases because radical emergence and its elongation occurs. The rate of seed swelling (dw/dt) during hydration is often described by the equation (Meyer et al., 2007):

$$dw/dt = k(w_{max} - w) \quad (1)$$

This model assumed that rate of water uptake is linearly proportional to wmax - w; where wmax is weight of seed at full hydration, w is the weight of a seed at time t and wmax - w is water deficit in the seed. It was assumed that seed coat limits the imbibition. Solving aforementioned equation:

$$-kt = \ln [(w_{max} - w)/ w_{max}] \quad (2)$$

Equation 2 shows that the logarithm of water uptake versus time is a straight line. To find w at any instant t, value of two unknown parameters w_{max} and k are required. There is another model for water uptake, which assumes that water absorption by the seed follows diffusion kinetics. Difference in water potentials across the seed forces water to diffuse into the seed (Fick's first law of diffusion). Let D measures rate of swelling of seed (in the unit of the diffusion coefficient m2 s -1) and r denotes the radius of seed (seed is assumed to be spherical). Seed weight (w) starts increasing as imbibition proceeds satisfying diffusion equation (Meyer et al., 2007):

$$\frac{w}{w_{max}} = 1 - \frac{6}{\pi^2}\sum_{n=1}\frac{1}{n^2}\exp(-Dn^2\pi^2 t / r^2) \quad (3)$$

Diffusion equation shows that uptake statistics is a complicated sum of exponentials. There are two unknown parameters, D and w_{max}. To find w any instant t, w_{max} is required. To get the solution of w any instant t by using any of the two statistics, two unknown parameters are required. A prior guess about w_{max} is very difficult; therefore, to find w at any instant time t, a new model is proposed.

New water uptake model

Mahajan and Pandey (2013) formulated new water uptake model without using w_{max}. They assumed that net weight of water absorbed at any instant w - wdry is directly

proportional to weight of the seed (before soaking) and time interval between t and t + dt (that is dt) during which water is absorbed by the seed. It is inversely proportional to time t elapsed starting from when hydration was started. The equation of new water uptake model is:

$$w = kE \ln(t)\ k2 \quad (4)$$

Where w = [(w2 - w1)/ w1] × 100 [w1 is initial weight of seed (dry seed) and w2 is weight of seed after absorbing water at any time t).

MATERIALS AND METHODS

Static electric field in the present study is generated with a power supply and the test cell which is basically a condenser as shown in Figure 1. The test cell consisted of two circular aluminium plates of diameter 8 cm and adjustable inter-electrode gap. The test cell was connected to a fully adjustable DC high-voltage supply (0 to 12 kV). In the present research, Pusa - 362, a variety of desi Chickpea is used. Its area of adoption is Punjab, Haryana, Delhi, North Rajasthan and West U.P (India). This variety is bold seeded and is tolerant to wilt. Chickpea seeds of a single layer was loaded in between two electrodes. Two transparent circular high density polyethylene layers of the same diameter as that of electrode are kept above and below the seed layer inside the condenser to avoid direct contact of seeds with the electrodes. No heating effect was observed during the experiments.

Four replications of Chickpea seeds (brown in color) comprising of 40 seeds in each set were exposed for 15 min at electric field of 270, 470, 670, 870 and 1070 V/cm. All electrically exposed chickpea seeds of each set were kept in between thin cotton cloth layers. Cotton layer containing seeds were transferred on a bed of sponge sheet of 1.8 cm thickness kept inside transparent plastic boxes of dimension 20 × 13 × 4 cm3 with lid. Sponge sheets in each box were damped with an equal amount of tap water. All the seeds were soaked on the same day and at the same time, ensuring that all the external conditions are same for each set of seeds during experiment. In case of necessity in subsequent days, equal amount of water was added to the sponge of all set of samples. Using Equation w = [(w2 - w1)/ w1] × 100 (Nizam, 2011), water uptake was measured by weighing the seeds before and after soaking at a fixed interval of time. In the present study, constant temperature 13°C is used for germination.

Sprouting experiment was performed in India at Patiala (Pb) (Khalsa College, Physics Laboratory) in between 13th January to 17th January 2013. Number of germinated seeds was counted after a certain time interval and the shoot length of every germinated seed was also measured using thread. This was done to minimize the error in measurement of shoot length. Shoot length of individual seed was added to get total shoot length. A seed was considered to be germinated when radical came out with more than 2 mm length. Sprouting was finished on 17th January then the seeds of each sample were transferred in different earthen pots. It was ensured that all the pots had the same type of soil (same by weight also) and the same amount of moisture content. Pots were kept in open natural environment.

After 28 days from the start of experiment, chickpea plants were taken out from pots and their root and shoot lengths were measured. Average temperature and day length variation in 28 days during chickpea plant growth was 10 to 16°C and 10 h 19 min 51s to 11 h 03 min, respectively. Mean germination time (Quintanilla et al., 2000; Ranal et al., 2006; Salehzade et al., 2009) is calculated as:

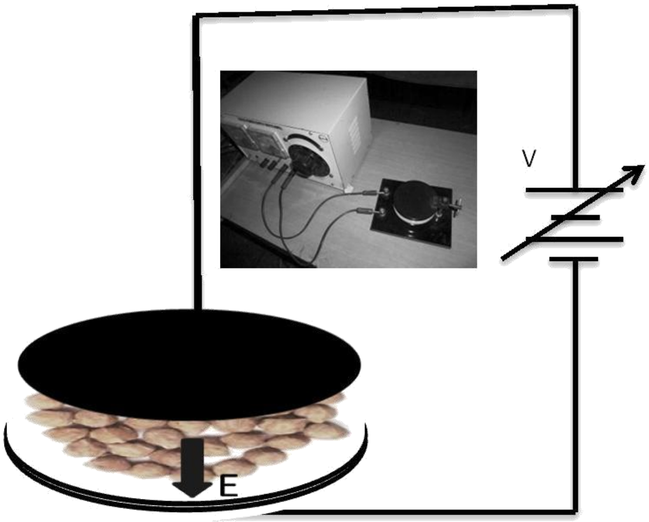

Figure 1. Experimental set up used in the present investigation.

$$\bar{t} = \frac{\sum_{i=1}^{k} n_i \times t_i^k}{\sum_{i=1}^{k} n_i} \;..................(1) \qquad (5)$$

In Equation 5, t_i signifies the time from the start of the experiment to the ith observation (in a day or hour), n_i signifies number of seeds germinated for ith observation and t_k signifies the last time of germination for kth observation. To find the number of un-germinated seeds *N* at any instant of time *t* in each set of samples a statistical equation is used:

$$N = (N_k - N_i) \exp[-(t - t_0)] \qquad (6)$$

N_k is the total number of seeds in the sample. N_i is the number of seeds germinated initially at time t_0. Substituting $(t - t_0) = 1/\lambda_E$ in Equation 6, *we* get $N = 0.368 (N_k - N_i)$. The constant λ_E is reciprocal of time $(t - t_0)$ at which, the number of seeds left in the un-germinated state reduces to 0.368 time the number of seeds in the sample at time t_0. Transition time between un-germinated and germinated state can be defined from Equation 6, it is:

$$\dot{t} = (1/\lambda_E) + t_0 \qquad (7)$$

Nested iterative procedures and least squares regressions are assimilated to estimate the germination electric constant .

RESULTS

Figure 2 shows the percentage of water uptake that increases with imbibition time. Water uptake curves are the function of the electric field strength (by which the chickpea are exposed). All curves satisfy proposed water uptake model (Equation 4). At any time *t = 10 h,* say(Figure 2), water absorption (%) for control, 270, 470, 670, 870 and 1070 (V/cm) are 76.29, 30.36, 26.78, 62.95, 58.32 and 73.86, respectively. Water absorbed at 470 V/cm is approximately 1/3rd as compared to the control. Figure 2 shows that there is more delay in water absorption at low field values as compared to high field values. Maximum delay is shown at 470 V/cm. Figure 3 shows that the seed water content (%) (g water/g dry weight) increases linearly with the logarithm of time. These lines again justify water uptake model (Equation 4). All straight lines have different slopes (k_E). The value of slope (k_E) signifies the rate of water uptake and is called the coefficient of water uptake. The value of k_E for control, 270, 470, 670, 870 and 1070 V/cm are 18.2, 31.95, 33.01, 20.55, 24.96 and 21.33 (h^{-1}), respectively. Rate of water uptake is increased for all field values. It is highest in between 270 and 470 V/cm (Figure 4). As compared to control an increase in root length (Figure 5) for 270, 470, 670, 870 and 1070 V/cm are 16.74, 42.42, 3.59, 7.24, 24.91 (%) and increase in shoot length are 14.07, 30.68, 6.56, 13.66, 29.85 (%), respectively. Among the various values of the electric field, 470 V/cm for 15 min exposures gave best results.

Vashisth et al. (2008) exposed seeds of chickpea in batches to static magnetic fields of strength from 0 to 250 mT in steps of 50 mT for 1 to 4 h and showed that among the various combinations of field strength and duration, 50 and 150 mT for 2 h exposures gave best results. Figure 5 shows increase in chickpea plant growth in 28 days. Data shows that at all the field values, germination (after germination effects) is enhanced. From various exposed field values, maximum increased germination occurs first at 470 V/cm. It implies that after germination, effects are linked with rate of water uptake (k_E) (Figure 4). For any time $t - to = 18\ h$ (say) (Figure 6) percentage of seeds left in the sample for control, 270, 470, 670, 870 and 1070 V/cm are 10, 24, 42, 25, 25 and 22%, respectively. It shows that all seed samples shows retardation in germination capacity as compared to control when seeds are pre-treated with electric field. The retarding effects in germination capacity is linked with water absorption curves of Figure 2. The reason for this retardation is a delay in water absorption by the seed. Maximum retardation happens at 470 V/cm. Figure 7 shows variation of electric transition constant varies with the electric field. It is having least value at 470 V/cm and maximum at control. Experimental data of germination capacity at any instant (for different values of intensity of the electric field control, 270, 470, 670, 870 and 1070 V/cm) for 15 min exposure fitted well in re-formulated Malthus-Verhulst equation:

$$N_g(t) = N_k N_i / [N_i + (N_k - N_i) \exp\{- á_E N_k (t - t_0)\}]$$

Where, $á_E$ is germination rate coefficient which is a function of the electric field if other parameters are kept constant (Pietruszewski, 2001, 2002; Pietruszewski et

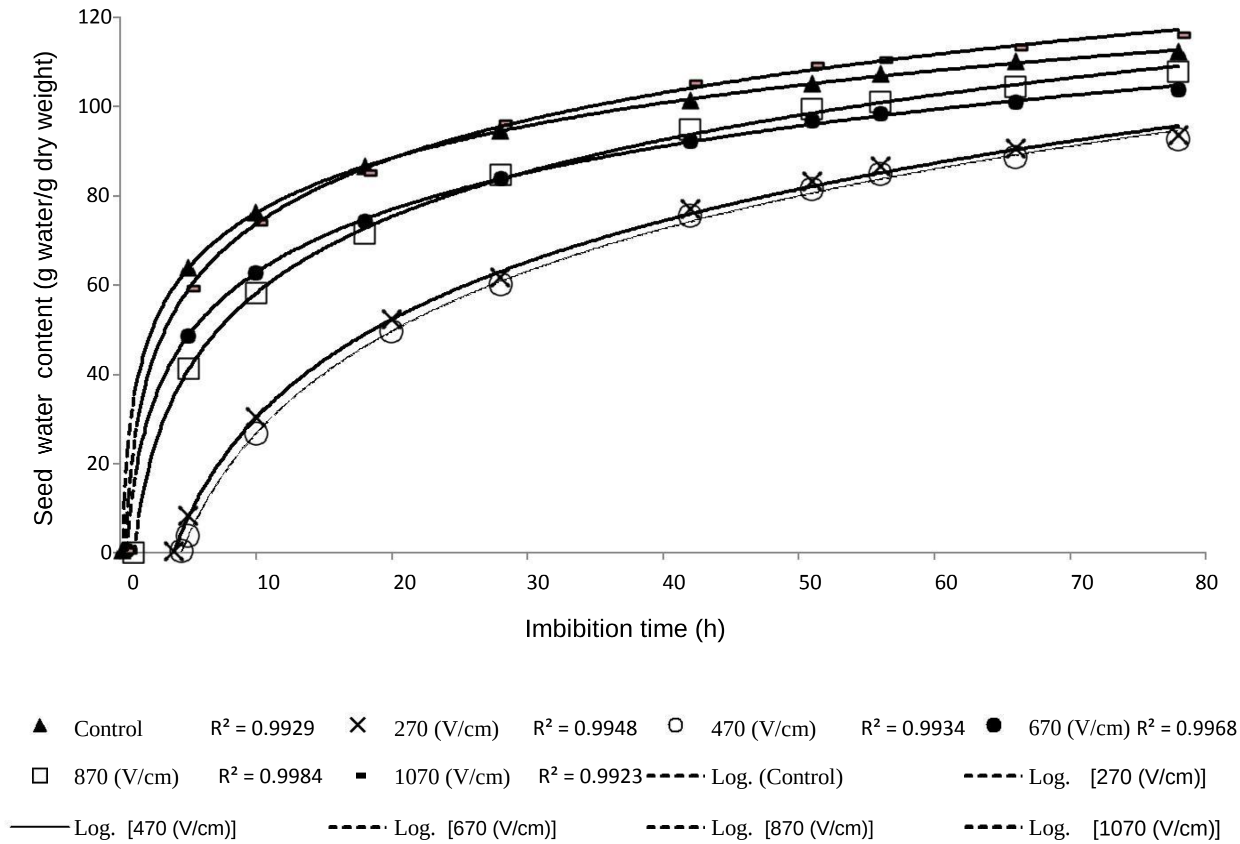

Figure 2. Variation of seed water content with imbibition time showing delay in water absorption for electrically treated chickpea seeds as compared to control (unexposed) for all values of electric field.

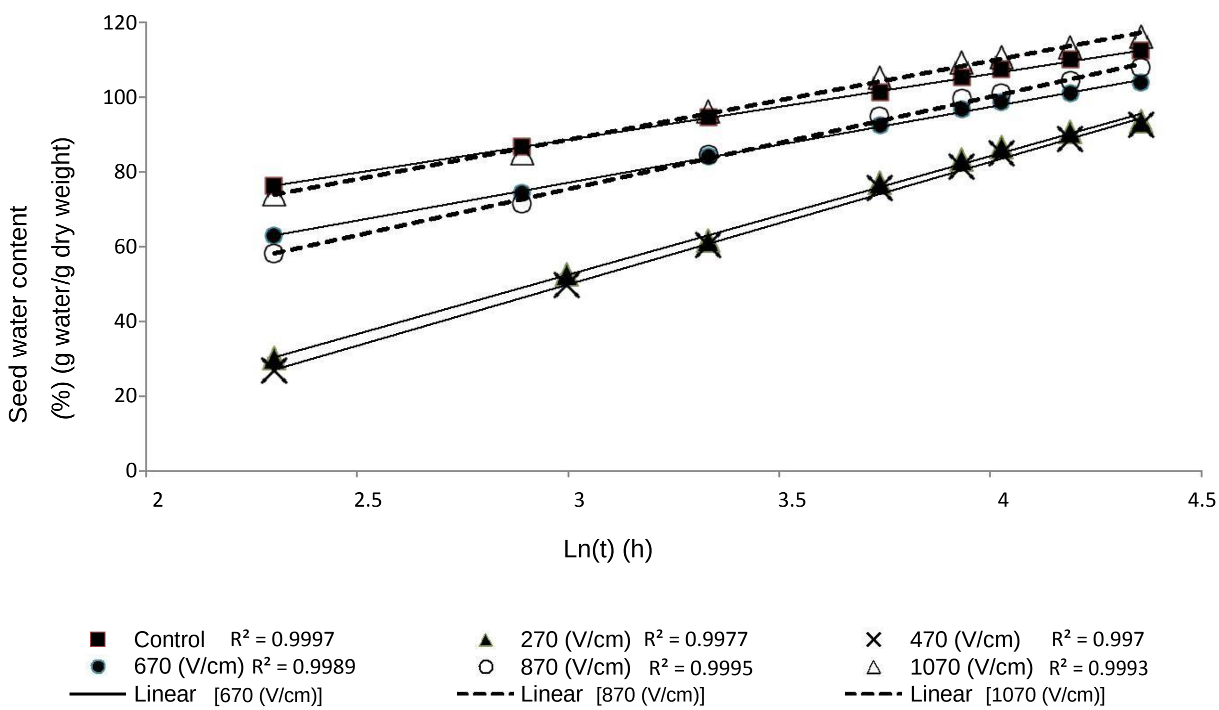

Figure 3. Graph showing the (%) water uptake with linear dependency to logarithm of time (h).

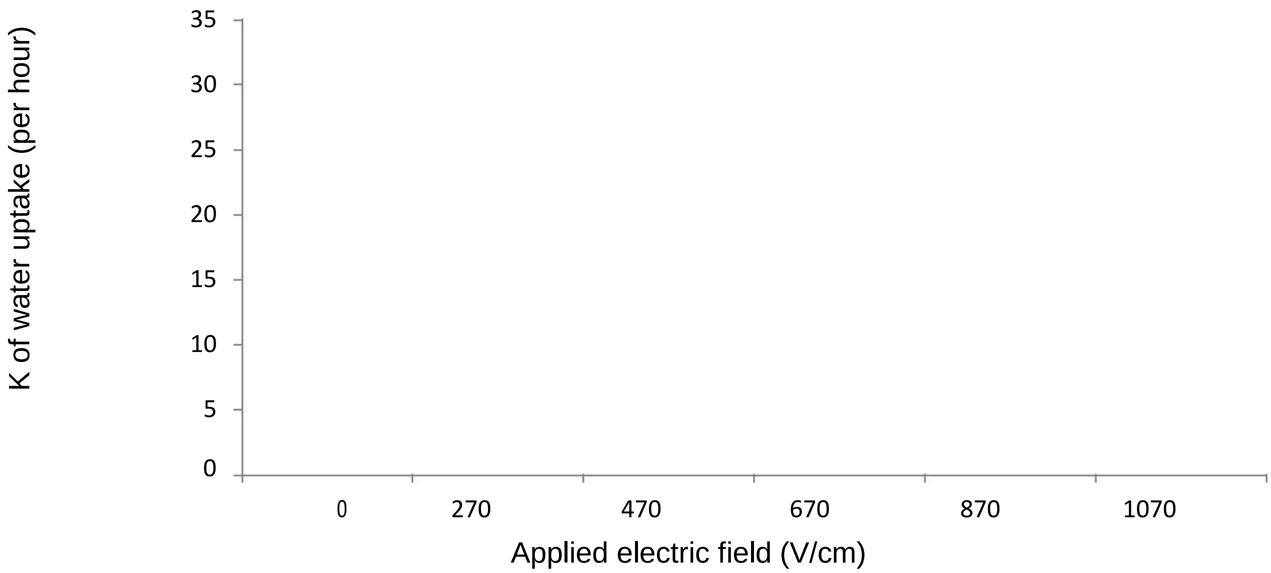

Figure 4. Variation of coefficient of water uptake *k* is a function of exposed electric field for chickpea seeds.

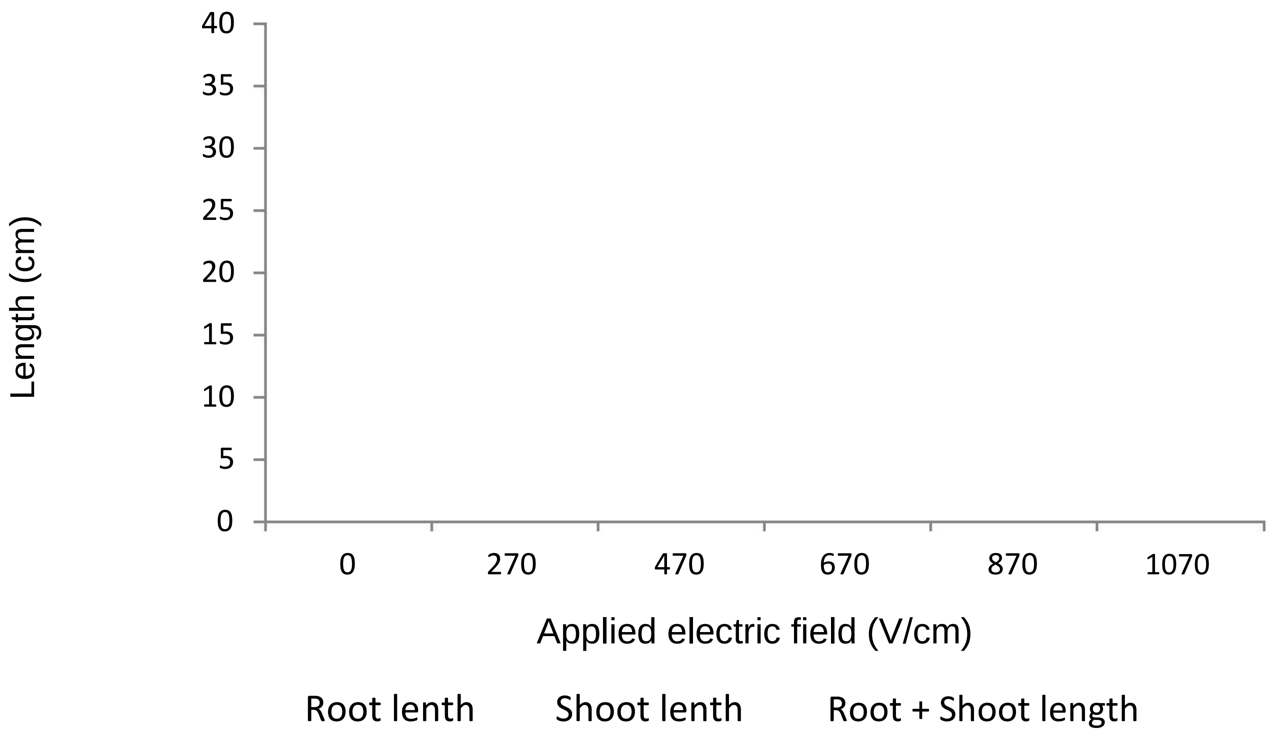

Figure 5. The bar graph showing the variation of average root length, shoot length and root + shoot length with applied electric field for 28 days old plant [$p_{(Root\ length)} < 0.001$ and $p_{(shoot\ length)} < 0.001$].

al., 2010; Mahajan and Pandey, 2011).

The trend obtained for these curves is shown in Figure 8. Mean germination time, transition time and time taken for the first seed to germinate are all physical parameters which describes the earlier germination effects and are calculated by using different formulas (Equations 5 and 7) (Figure 9). There is an increase in all these times (mean germination time, transition time and time taken for the first seed to germinate) at all electric field values and is highest at 470 V/cm. Seedling length *y* of a group of 40

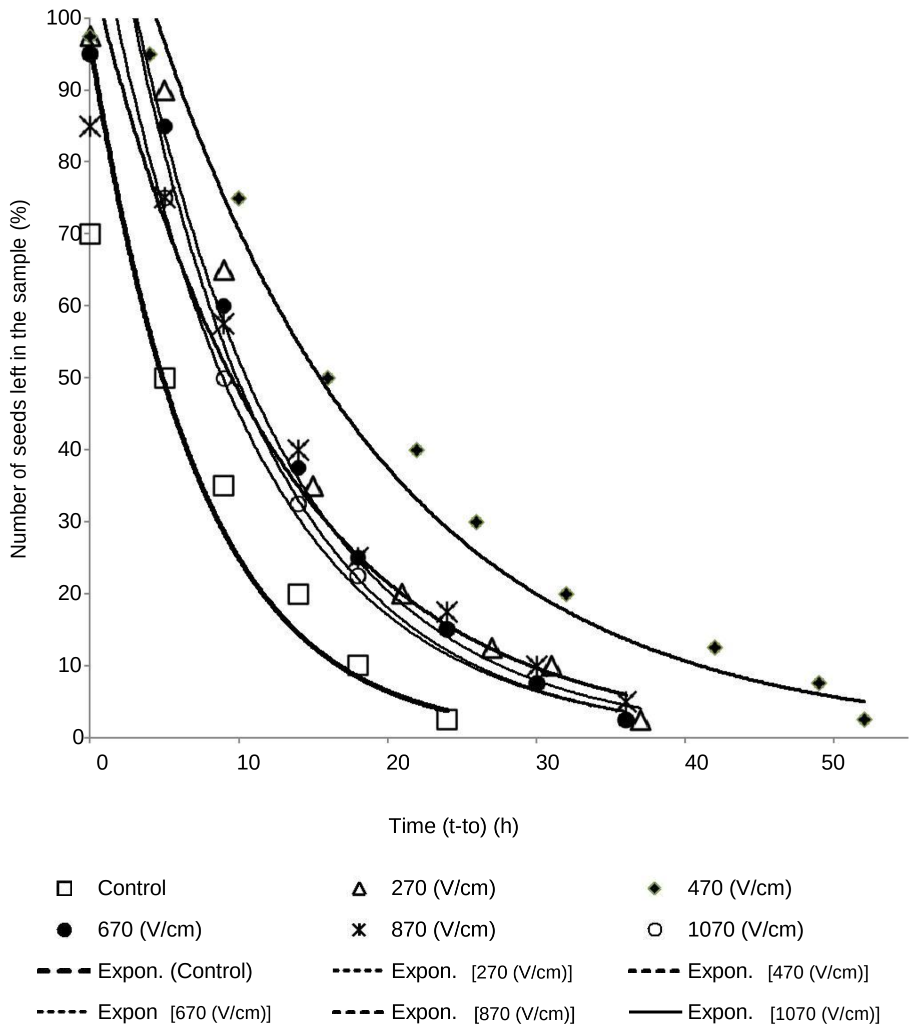

Figure 6. Variation of germination data with applied electric field on chickpea seeds along with decay function.

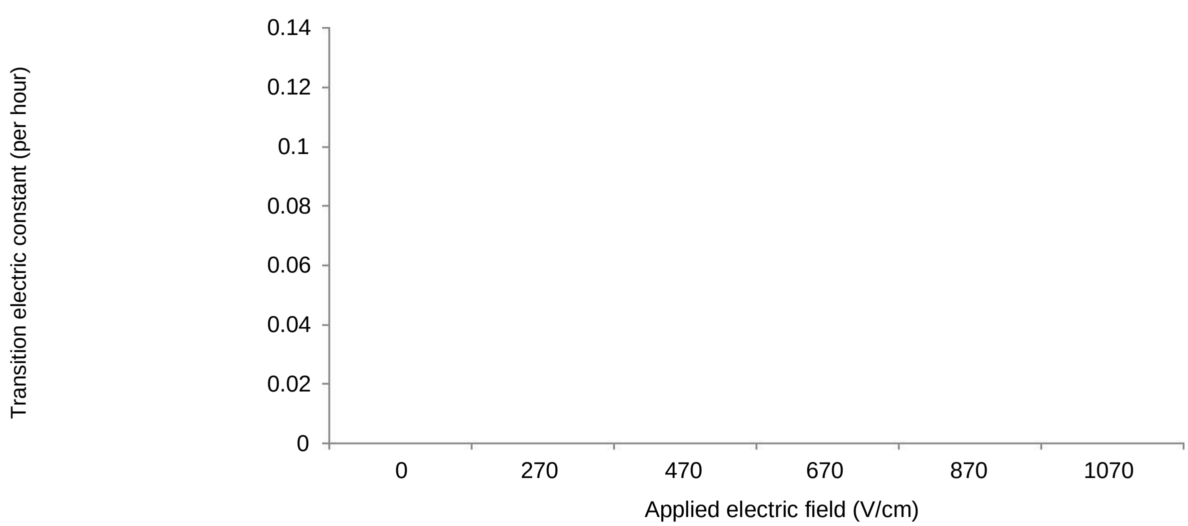

Figure 7. Bar graph showing variation in transition electric constant with increasing electric field on the chickpea seeds.

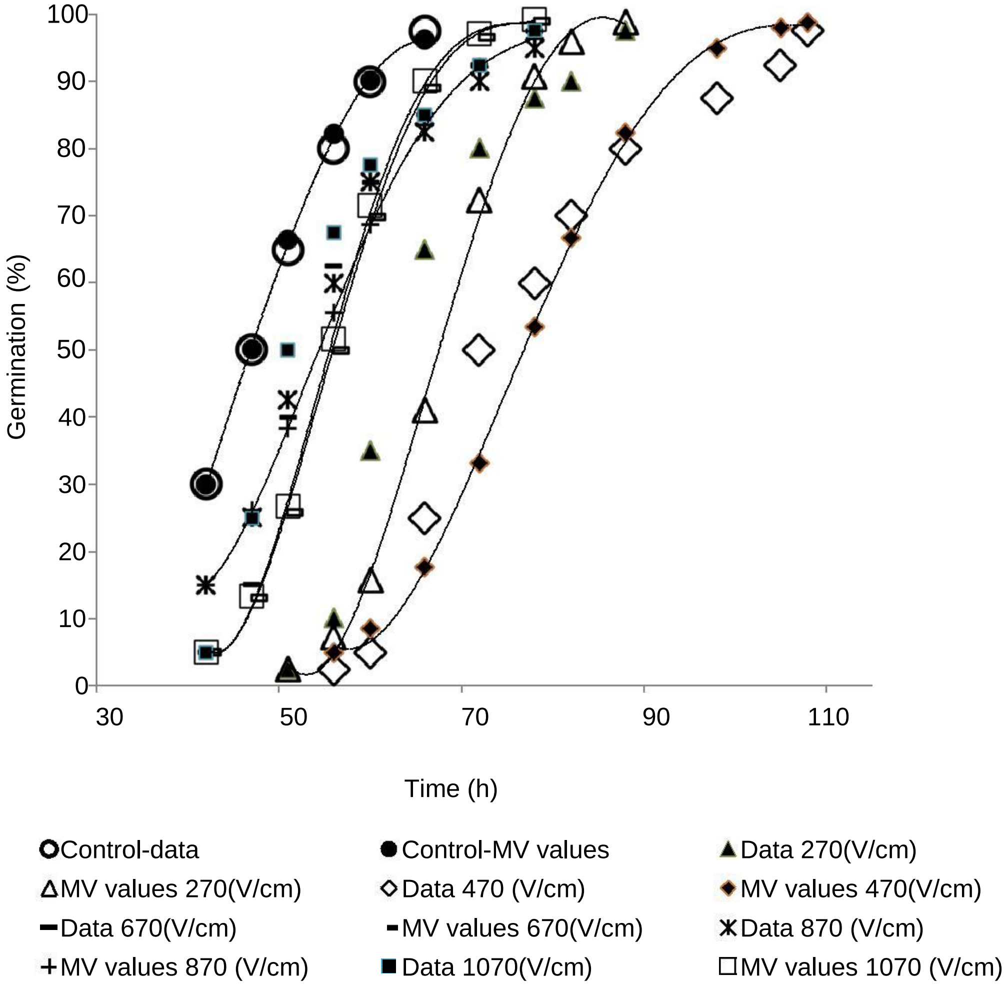

Figure 8. Variation of germination data with electric field on chickpea seeds along with MV function.

seeds (Figure 10) grows with logarithmic of time at any instant *t* and can be calculated by using equation for control $y_{control}$ = 86.902ln(t) - 314.63) as well as for treated seeds $y_{270V/cm}$ = 63.656ln(t) - 249.27, $y_{470/cm\ V/cm}$ = 51.944ln(t) - 209.62, $y_{670V/cm}$ = 91.903ln(t) - 344.95, $y_{870V/cm}$ = 88.533ln(t) - 331.67 and $y_{1070\ V/cm`}$ = 88.705ln(7) - 329.04 with the electric field. Experimental data fitted well (R^2 very close to 1) in all these equations. highest at 470 V/cm. Seedling length *y* of a group of 40 seeds (Figure 10) grows with logarithmic of time at any instant *t* and can be calculated by using equation for control $y_{control}$ = 86.902ln(t) - 314.63) as well as for treated seeds $y_{270V/cm}$ = 63.656ln(t) - 249.27, $y_{470/cm\ V/cm}$ = 51.944ln(t) - 209.62, $y_{670V/cm}$ = 91.903ln(t) - 344.95, $y_{870V/cm}$ = 88.533ln(t) - 331.67 and $y_{1070\ V/cm`}$ = 88.705ln(7) - 329.04 with the electric field. Experimental data fitted well (R^2 very close to 1) in all these equations.

DISCUSSION

Meyer (2007) discussed water uptake by the seed as $-kt = \ln[(w_{max} - w)/w_{max}]$ that is the logarithm of water uptake versus time is a straight line (Equation 2). In this model, there are two unknown parameters w_{max} and k which are required for the calculation of weight of the seed or water uptake at any instant *t*. W_{max} is the weight of seed at full hydration. It is not feasible to get prior information about w_{max}. Therefore, the use of this water uptake model and diffusion model (Equation 3) becomes limited. On the other hand (Mahajan and Pandey, 2013) formulated a new model in which water uptake grows linearly with the logarithm of time (Equation 4). This model has been verified experimentally as shown in Figure 3. The plus point of this model is that it is free from w_{max}. Electrical nature of seeds can be described by their dielectric properties (Nelson, 2010). The influence of electric fields on the seed provides a means for sensing those processes which are responsible for seed germination. Let E_0 be the electric field produced by an external voltage applied to the plates of a parallel plate capacitor (plate area *A* and plate separation *d*). Seeds layer of thickness *t* (*t*<d) is then introduced between the plates.

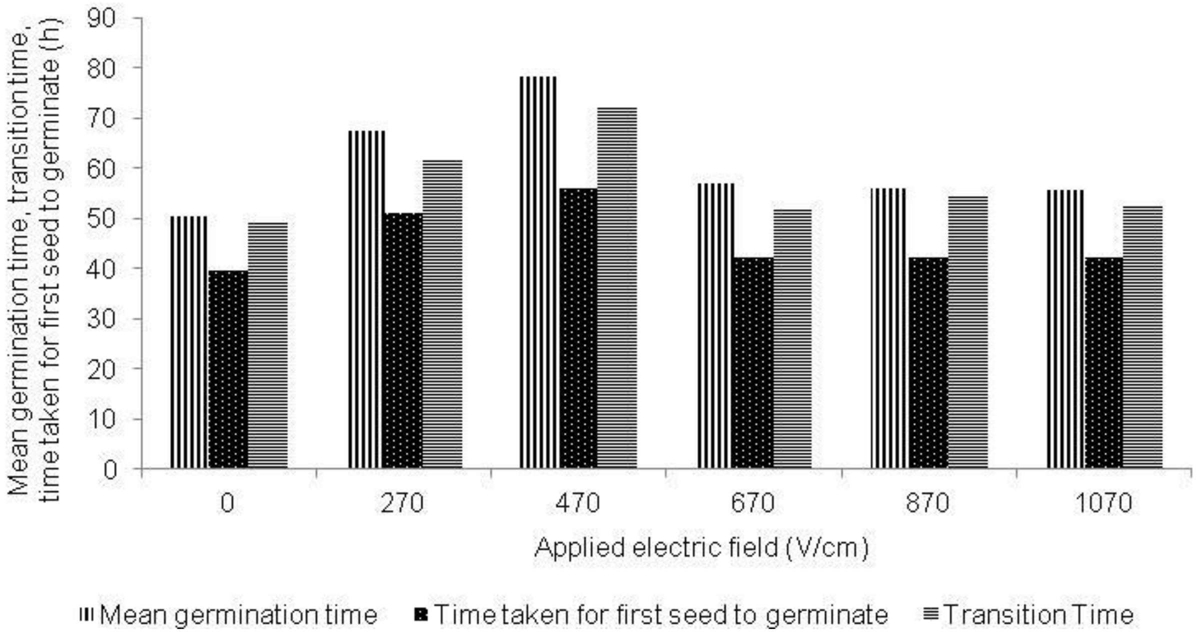

Figure 9. Variation in transition germination time, mean germination time and time taken for first seed to germinate with increasing intensity of the electric field.

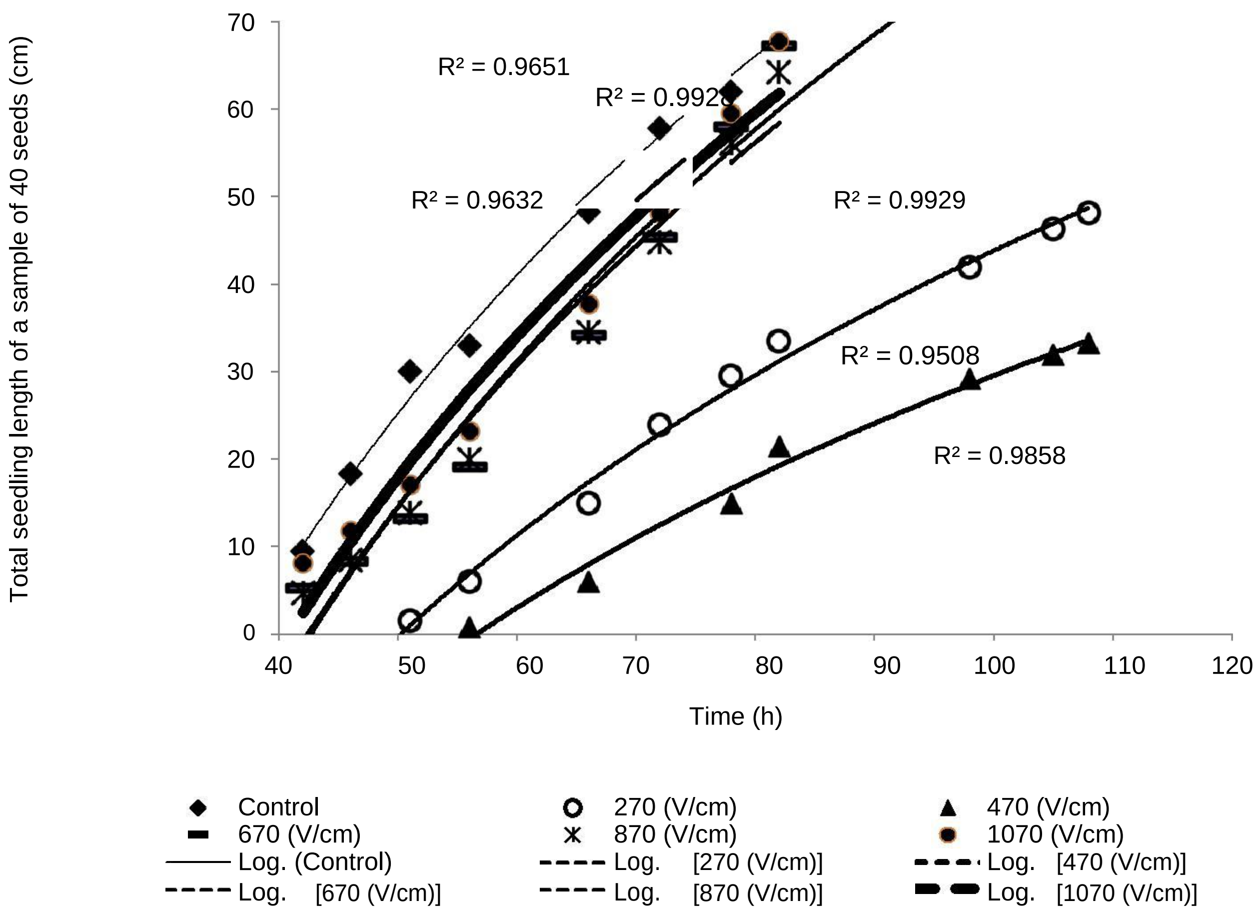

Figure 10. Variation of total seedling length of 40 seeds with electric field.

Electric field inside the seed now gets modified (reduced) and becomes E:

$E = E_0 - P/\epsilon$

Where, P is total dipole density and ϵ is electric permittivity of the seed.

Polarisation P is directly proportional to the reduced value of the electric field (Kittel, 2011). Ferroelectric crystals (like KDP type, KH_2PO_4) exhibit some residual electric dipole moment upon removal of external electric field (Kittel, 2011). Present experimental data of seed germination and water uptake shows small magnitude of electric dipole moment that is left inside the seed even upon removal of the electric field (ferroelectricity). Water is a polar molecule. A dipole-dipole interaction (interaction between water dipoles and electrically stressed dipoles inside the seed) leads to make either a delay in water absorption or increased in water absorption. Vashisth et al. (2010) in their study have shown that at certain combinations of magnetic field and exposure time, the germination rate is enhanced more. Their observation indicates that the internal energy of the seed responds positively when there is an appropriate combination of magnetic field and exposure time. In electrically treated seeds from 0 to 1200 V/cm at 13°C, there is an electric field 470 V/cm (15 min exposure) which stimulates chickpea seeds more as shown in Figure 5. The reason of this is the dipole dipole interactions (water dipole and micro dipoles inside the electrically treated seed). Some water dipoles are replled by the seed dipoles (when water dipoles approach with similar charge as the charge present on the dipoles inside the seed), the result is a delay in water absorption.

Some water dipoles are accelerated towards the seed (when water dipoles approach with a charge which is of opposite in nature to the charge present on electrical dipoles inside the seed), where an increase in the rate of water uptake occurs. Das and Bhattacharya (2006) found that electro magnetic field (EMF) strength of 0.88 T for 80 min has maximum stimulating effect on germination of gram seeds (*Cicar arietium*) and it may be assumed that under this condition the three-cell water potential forces act in the same direction on germinated seeds. Many authors (Arauz et al., 2010; Dias, 2001; Miller et al., 2003) have used some statistical functions for cumulative germination like Weibull function $y = M(1-\exp(-k(t-z))^c))$, Morgan-Mercer-Flodin function $y = (bk + M(t^c))/(k + (t^c))$, Richards function $y = M(1-\exp(-k(t-z)))^{(1/(1-c))}$, Mitscherlich function $y = M(1-\exp(-k(t-z)))$, Gompertz function $y = M(\exp(-\exp(-kt + b)))$ and logistic functions $y = M/(1 + \exp(-kt + b))$. In these functions, y is cumulative germination at time t and M, k, z, b and c are empirically derived constants. *M* is the asymptote, *k* is the rate of increase, *z* is the lag in germination, *b* is related to the lag, and *c* is a shape parameter. Many of these existing functions have no biological meaning moreover, all these functions are growth function. On the other hand, we used a decay function $[N = (N_k - N_i) \exp\{ - (t- t_0)\}]$ to find the seed (%) left during germination with a minimum number of unknown parameters. Moreover, this function is also having biological meaning (its constant can be related with electric field or any other factor which affects the germination) and gives best fitting with experimental data (Figure 6) (as fitting coefficient R^2 : R^2 (control) = 0.94, R^2 (270 V/cm) = 0.95, R^2 (470 V/cm) = 0.94, R^2 (670 V/cm) = 0.97, R^2 (870 V/cm) = 0.98, R^2 (1070 V/cm) = 0.97).

With the help of this function, number of un-germinated seeds *N* left in the sample can be calculated at any instant *t.* Let N_k be the total seeds in a sample. At time $t = t_0$, the seeds germinated are N_i then $N = (N_k - N_i) \exp[-(t- t_0)]$. Upon substituting $(t- t_0) = 1/$ then $N = 0.368 (N_k - N_i)$. Germinating electric constant is defined as reciprocal of time $(t- t_0)$ at which, the number of seeds left in the un-germinated state reduces to 0.368 time the number of seeds in the sample at time t_0. Transition time can be defined with the help of germinating electric constant as t = (1/) + t_0. This equation shows that lesser the value of more in the value of transition time will be (from un-germinated to germinated state). Values of at different electric field is calculated and found that it is less at 470 V/cm (Figure 7); therefore conversion of state from ungerminate to germinate becomes difficult at this electric field. Transition time values calculated using Equation *7* for different value of exposed electric field to seeds is very close to mean germination values calculated using Equation 5 (Figure 9). Both times are peaked at 470 V/cm. Time taken for the first seed to germinate also exhibits the same trend as that of transition time and mean germination time. The reason for poor response in early germination parameters lies in the fact that there was a delay in water absorption at the start of germination for electrically treated seeds (Figures 2 and 3).

Seedling length (y) grows with logarithmic of time for control as well as for treated group of seeds (with the electric field). Seedling growth formula is $y = á_1 \ln (t) + á_2$ (where $á_1$ and $á_2$ are some constants and are the function of the electric field (if other parameters are kept constant which effects the germination). Karadavut (2010) com-pared some statistical growth models for describing seedling growth in the early period and found quadratic model ($y = a + bx + cx^2$) that explained better than other models for seedling growth, but our findings proves that seedling growth is logarithm (logarithm of time) wise (Figure 10) (as fitting coefficient R^2 comes nearly 1). It is not quadratic growth.

Conclusions

1) The pre-sowing electric field treatment on chickpea seed introduces delay in water uptake.
2) ~470 V/cm 15 min electric field exposure at 13°C to

chickpea seed is critical as at this value, there is the maximum delay in water absorption. This delay increases mean germination time, transition time and time taken for first seed to germinate.

3) The rate of water uptake is maximum at 470 V/cm (15 min exposure) electric field to chickpea seed. It improves the post germination factors like root length and shoot length of 28 days old plant.

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