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Invigoration of deteriorated seeds with cathodic water, ascorbic acid and other seed priming agents

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Abstract

A greenhouse pot experiment was conducted to compare the efficacy of cathodic water and ascorbic acid seed priming on the emergence and growth of deteriorated seeds of pea and pumpkin. Treatments that were investigated alongside cathodic water included cathodic water with pH adjusted to 7, calcium magnesium solution, calcium magnesium solution (with pH adjusted to 11), and distilled water. Electrolyte leakages from both the fresh and deteriorated seeds were also investigated. The study was conducted by subjecting seeds of the test species to controlled deterioration in an oven at 40°C for 32 days. The deteriorated seeds were thereafter invigorated with cathodic water, ascorbic acid, and other treatments. Fresh seeds of both species served as the control. The results indicated that electrolyte leakage from the deteriorated seeds was significantly higher than that of fresh seeds in both species. Invigorating the seeds with cathodic water led to significant ($p < 0.05$) emergence in both pea and pumpkin. Although all the treatments led to an improvement in both emergence and growth in both species, seeds treated with cathodic water generally performed best when compared with other treatments. More improvement in emergence and growth was also observed with pumpkin when compared with pea. While the successful invigoration of the deteriorated seeds, which led to the emergence and subsequent growth, may be linked to cell repairs and other biochemical changes in the invigorated seeds (not investigated in this study), the positive effects on the growth and biomass of the plant may be associated with improvements in chlorophyll content and chlorophyll fluorescence in pumpkin, and an increase in chlorophyll content in pea.

Keywords: Cathodic Water, Controlled Deterioration, Emergence, Growth, Invigoration.

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1. Introduction

Seeds are the genetic resource by which most higher plants are propagated. Based on their desiccation tolerance, seeds are classified as either recalcitrant or orthodox. While the recalcitrant seeds are desiccation-sensitive and cannot be stored for a long time, orthodox seeds are tolerant of desiccation and can be stored for a long time [1, 2]. Orthodox seeds' genetic resources can be conserved via conventional gene banking. However, during long-term storage, orthodox seeds deteriorate even under very good conditions; the best that can be done is to lower the rate of deterioration [1, 3]. The occurrence and severity of the observed effects of aging on germination and subsequent seedling growth increase with aging time and the level of applied stress [1, 3].

Deterioration of seeds during storage has been linked to the production of reactive oxygen species (ROS). Other factors that contribute to seed deterioration, also known as "seed aging," are relative humidity and temperature of the storage environment, genetics, mechanical damage, seed water content, presence of microbiome, and seed maturity [1, 4, 5]. Loss of seed vigor is associated with loss of membrane integrity [6-8]. In particular, membrane damage is ROS-mediated, which may lead to electrolyte leakage. Hence, electrolyte leakage has been recommended as an indicator of seed deterioration and physiological stress in some species [8, 9]. Other factors linked with aging in seeds are degradation and inactivation of enzymes due to changes in their macromolecular structures [1, 4, 10]. The reduction in enzymatic activities as a result of enzyme degradation has been reported to lower respiratory activities, which in turn lowers the energy (ATP) and assimilate supply of the germinating seeds, which consequently results in decreased germination and weaker seedling growth [4, 8, 10].

It has been proposed that seed priming (a seed invigoration technique) can be used to improve seed vigor, as well as the rate and uniformity of seedling emergence, which will ultimately enhance crop yields. Many techniques have been employed to prime seeds. The first step often involves hydrating seeds in solutions to allow sufficient imbibition to enable the early events in the germination process to occur, but not enough to permit radicle emergence [11, 12]. Solutions that are used for seed priming include water (hydropriming), osmotically active solutions such as polyethylene glycol (osmopriming), or salt solutions (halopriming). Other seed priming techniques include placing seeds between saturated jute mat layers (matripriming) and alternate soaking of seeds in tap water and drying before sowing (hardening) [13, 14]. Seed priming has been reported to have positive effects on the germination and growth of some vegetables, floriculture, and certain field crops [5, 12, 15].

Although plants have been reported to have internal mechanisms to counteract the damaging effects of ROS bursts, such systems include antioxidative systems, which are composed of metabolites such as ascorbate, glutathione, tocopherol, and enzymatic scavengers such as superoxide dismutase (SOD), peroxidases, and catalases [16, 17]. ROS would normally be quenched by the endogenous antioxidant system. However, at high levels of stress, the ability of protective mechanisms is insufficient to neutralize the damage caused by ROS production within the seed. An approach to solving this problem is to supply exogenous antioxidants such as ascorbic acid; however, success has been variable, and some antioxidants are cytotoxic at high concentrations [10, 16, 18]. One such antioxidant that has been recently reported to be very effective at invigorating deteriorated seed is cathodic water [11, 15, 19].

Cathodic water is the cathodic fraction of an electrolyzed, dilute ionic solution of calcium and magnesium chloride [20, 21]. Although the pH of the CaMg solution is 7, the pH of cathodic water is 11.2 [20, 21]. It must be noted that cathodic water has strong reducing power, and its use in seed priming obviates the need for exogenously supplied potentially toxic chemical antioxidants. The intuition leading to the discovery of cathodic water has been substantiated with remarkable successes. For example, cathodic protection was used as a medium during explant excision, as the solvent for cryoprotectant solutions, and as the medium for post-cryo thawing and rehydration in the cryopreservation of Strychnos gerrardii [16]. It has also been successfully used in the invigoration of some orthodox seeds [11, 15, 19]. On the other hand, ascorbic acid is a popular antioxidant that has long been used in seed priming. The use of ascorbic acid has been reported to improve germination and seed vigor of tomato [22] and rice [23].

In this study, seeds of the test species (pea and pumpkin) were subjected to controlled deterioration at 40°C and 100% relative humidity to allow for gradual seed deterioration and to avoid accelerated death of seed embryos. Although the aim of this study was to compare the efficacy of cathodic water with that of ascorbic acid, this study also examined the influence of some other treatments, which include cathodic water with pH adjusted to 7, calcium magnesium solution, calcium magnesium solution with pH adjusted to 11, and distilled water. The comparisons were made in terms of seedling emergence, plant mortality, and plant growth.

2. Methodology

2.1. Study Area

Seed germination was done in a growth room set at 16 h dark / 8 h light (52 µmol m^{-2} s^{-1}) photoperiod. The pot experiment was done in a greenhouse, with an average temperature of 23.5°C and average relative humidity of 67%, at GPS *coordinates*: S 29°48'59.8" E 30°56'37.4". Seeds used were purchased a year before the trial from Grovida seeds, a local seed company based in Durban. The seeds were stored at -5°C.

2.2. Controlled Deterioration of Seeds

The seeds of each species were examined for size; only seeds of similar sizes were used for this study. The initial water contents of the test species were determined gravimetrically. The water contents for both species were subsequently raised to 14% in a vapor chamber. The seeds were then sealed in airtight glass jars and kept in an oven at 40°C. Different glass jars were used for each of the species. Samples (25 seeds x 4 replicates) were taken at 4-day intervals and sterilized for 10 minutes

in 1% aqueous sodium hypochlorite. The seeds were germinated on water agar. Sampling was done until almost complete loss of germination vigor in both species (32 days).

2.3. Seed Electrolyte Leakage

Electrolyte leakage (S m^{-1} g^{-1}) was measured for both the deteriorated seeds and the control (fresh seeds). Three seeds (1 g) were immersed in 50 ml of distilled water in glass tubes placed in a water bath for 24 hours at 25°C. Thereafter, the leachate was measured for electrical conductivity using a CM 100-2 multi-cell conductivity meter (Reid and Associates, South Africa) [24].

2.4. Preparation of Calcium Magnesium (CaMg) Solution and Cathodic Water

A solution containing 1 µM $CaCl_2$ and 1 µM $MgCl_2$ known as calcium magnesium (CaMg) solution was prepared, autoclaved, and stored at -5 °C until needed. The pH of CaMg solution was ~ 7.

Cathodic water was prepared by electrolysing the CaMg solution [25]. Two 200 ml glass beakers were filled with water containing CaMg solution and platinum electrodes were immersed in the solution, the anode in one beaker and the cathode in the other. To form a complete circuit, an agar-based salt bridge was inserted to connect the two beakers. The CaMg solution was electrolysed by the provision of a 60 V potential difference using a Bio-Rad Powerpac (BioRad, Hercules, California, USA) at 400 mA for 1 h at room temperature. The electrolysis yielded anodic (oxidizing) water with a pH of *c*. 2.4, and cathodic (reducing) water with a pH of *c*. 11.2. The anodic water was discarded while the cathodic water was used within one hour of preparation.

2.5. Seed Priming with Cathodic Water and Other Treatments

Hydration of the controlled deteriorated (CD) seeds was done between 20 layers of single-ply paper towels with 50 ml of the priming solutions for 24 hours. Domestic aluminum foil was folded around the paper towels and sealed with cellophane tape to allow hydration of the seeds to take place. After hydration, the seeds were dried back to the original mass in the open air (on tables in the seed laboratory) for 7 days. The seeds were subsequently kept in airtight glass jars and stored in a refrigerator at 4°C until use.

Controlled deteriorated seeds were hydrated in six priming solution treatments: cathodic water (CW), cathodic water with pH adjusted to 7 (CW pH=7), calcium magnesium solution (CaMg), calcium magnesium solution with pH adjusted to 11 (CaMg pH=11), ascorbic acid (As), and distilled water (DW). Unprimed fresh seeds (FSC) and aged seeds (ASC) served as the controls.

2.6. Plant Growth

The growth study was conducted as a greenhouse pot experiment. Eight hundred grams of Grovida potting mix were weighed into each pot of 2-liter size. In each pot, five seeds from each treatment were sown. Nutrients were supplied to the plants using multi-feed fertilizer (at 1 g multi-feed fertilizer per 100 ml H_2O^{-1}). Watering was done based on observation. Plants were thinned to 2 and 1 plant/pot at 4 and 6 weeks after planting, respectively. The plants were grown for 14 weeks. Pots were arranged in the greenhouse in a completely randomized design (CRD).

2.7. Data Collection

Seedling emergence was observed daily and recorded. Time to the first seedling emergence in each pot was also observed. Dead plants were counted and recorded as mortalities.

The chlorophyll ($C_{55}H_{72}O_5N_4Mg$) content was measured with a SPAD chlorophyll content meter on three fully expanded, non-senescing leaves of similar physiological maturity (the third, fourth and fifth leaf from the terminal bud). Three measurements were taken per leaf, and values were expressed as chlorophyll content index (CCI). Chlorophyll content index is a measure of the transmission (T) ratio of light at wavelengths of 653 nm to 931 nm light in the transmission spectrum of a green leaf (CCI = %T931nm/%T653nm).

Chlorophyll fluorescence (Fv/Fm), the ratio of the variable (Fv) to maximum fluorescence (Fm), which is a measure of potential photochemical efficiency of photosystem II (PSII), was done at eight weeks after planting. The measurement was done using a Li-Cor 6400XT portable photosynthesis measuring system (Li-Cor, Lincoln, NE). Three measurements were taken on the third leaf from the terminal bud across all treatments and replicates (n=12 per treatment). All leaves were fully expanded and non-senescing. Measurements were taken after the plants were dark-adapted for 40 min [26]. The stem girth was measured with vernier calipers, a day preceding harvesting, 5 cm above soil level.

At harvesting, the plants were carefully pulled out of the potting mix to avoid damage to the roots, washed, and separated into root, stem, and leaves. The root and stem lengths were measured. The plant parts were dried at 70°C until constant mass (4 d) and their mass determined. All the leaves were counted for all treatments and replicates, and the control. Individual leaf area (cm^2) was measured using a leaf area meter (Licor, CI-202 Area Meter, Lincoln, Nebraska, USA). Measurement was done across all treatments and the control [27].

2.8. Data Analyses

The data collected were subjected to an analysis of variance (ANOVA) procedure using GenStat Release 18.1 (PC/Windows Vista) [28]. The means of the treatments were separated for the least significant difference at 5% ($LSD_{0.05}$). The data for seed ageing were further subjected to correlation and regression analyses. Thus, mathematical functions expressing correlations and regression relationships between the number of days seeds were in the oven and the germination

percentage of seeds were obtained using the curve fitting program of TableCurve 2D v5.01.01 (Systat Software Inc., San Jose, CA, USA, 2002).

3. Results and Discussions

Controlled deterioration at 40°C and 100% relative humidity was done to allow for gradual seed deterioration and to avoid accelerated death of seed embryos. At 4 d of ageing both species maintained 100% germination which was similar to the initial germination obtained at the start of the experiment (Figure 1). As the ageing time increased, a gradual but continuous decline in germination continued in both species. Controlled deterioration was terminated at 32 d as deterioration beyond 32 d would have killed all the seeds. The percentage loss in germination at 32 d of controlled deterioration was 92% for pea and 98% for pumpkin (Figure 1). The loss in seed germination as the age of controlled deterioration increased is represented by the equations $y = 98.87+1.34x^{1.5}-0.76x^{2}+0.08x^{2.5}$ (pumpkin) and y = LgstcDoseRsp (-4.65,104.5621.085.78) (pea).

In this study, a strong correlation between seed germination and the duration of controlled deterioration of pea and pumpkin occurred (Figure 1). The decline in germination because of controlled deterioration of the seed may be due to the production of reactive oxygen species. The ROS may have resulted in seed damage and consequently, a reduction in germination due to ROS attack [3, 29, 30]. Reduction in germination may also be occasioned by the death of some seed embryo during controlled deterioration of the seed [30]. It has been reported that many systems within seed tissues become deteriorated in the process of ageing [17, 31, 32]. Of significance is damage to the cell membrane. Membrane damage may have led to electrolyte leakage and consequently loss in seed vigor [6-8]. Electrolyte leakage measured was significantly higher than those of fresh seeds in both pea and pumpkin (Figure 2). Strong inverse correlation has been reported between loss of seed germination and electrolyte leakage in *Diplotaxis tenuifolia* and *D. erucoides* [33] and wheat [34]. Hence, electrolyte leakage has been recommended as a measure of seed vigor for pea and pumpkin [35].

In both species, the fresh seed control had 100% emergence, which is significantly ($P>0.05$) higher than any of the aged seed treatments (CW, CW (pH=7), CaMg, CaMg (pH=11), DW, and As) (Table 1). Seedling emergence was significantly ($p<0.05$) delayed in all the aged seeds treatments, even though the seeds were primed with cathodic water, ascorbic acid and other priming solutions (Table 1). While the first emergence in the fresh seed treatment occurred in both species at 5 d after planting, the first seedling emergence occurred at 7 d (pea) and 14 d (pumpkin) after planting for the CD seed treatments (Table 1). There was no emergence in the aged seeds control treatment; hence, the treatment was discarded in further study and analysis. The seeds that were aged for 32 days recorded germinations of 8.34% (pea), 1.67 % (pumpkin), but 0% emergence when planted. However, in both species, after priming, 30.0% (pea) and 32.5% (pumpkin) emergence were achieved after CW treatment. Other treatments also achieved some level of emergence (Table 1). It is clear from the results obtained in this study that CW and CW (pH=7) enhanced earlier emergence when compared with CD seeds treated with other priming solutions. While the delay in emergence may be due to the loss of seed vigor caused by seed deterioration, priming may have lead to varying recovery of vigor as a result of seed priming.

All plants from the fresh seed control survived to maturity (Table 1). Apart from fresh seed treatment and CW primed treatments, seed deterioration caused significant post-emergence mortalities in both species (Table 1). Of the seedlings derived from the controlled deteriorated seeds treatments, mortality occurred in all the treatments except the CW treatments. The occurrence of post-emergence mortality in this study may be due to loss of seed vigor caused by seed deterioration [26]. It seems likely that the absence of mortality in seeds treated with CW was due to the strong reducing power of cathodic water on ROS. Physiological activities leading to seed repairs occur, particularly in phase 2 of seed priming [4] and include DNA repairs, an increase in enzymatic activities, cell membrane repairs, and protein refolding [4]. Generally, the most significant improvements in emergence, both in total and early seedling emergence, and the most significant reductions in mortality occurred in seeds primed with cathodic water treatment and CW (pH=7).

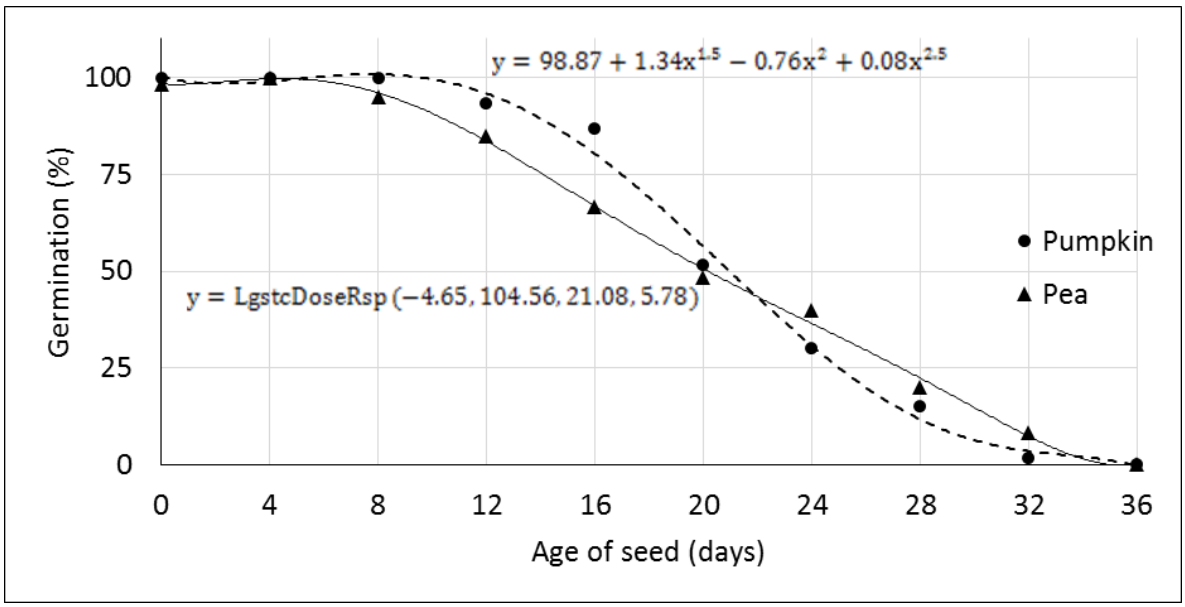

Figure 1.
Ageing curve for pea and pumpkin at 40 °C and 100% relative humidity with sampling done at 4 d intervals for a period of 36 d.

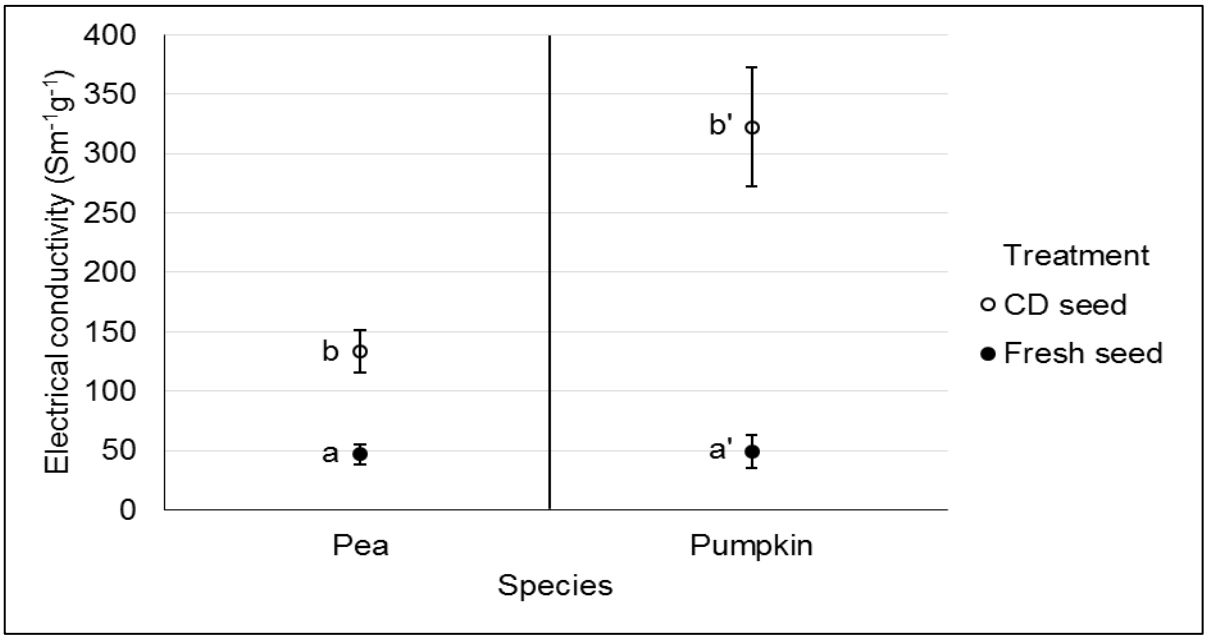

Figure 2.
Electrolyte leakages of the fresh (•) and controlled deteriorated (o) seeds of pea and pumpkin measured after 32 d of controlled deterioration at 40°C and 100% relative humidity. Bars are mean ± 2se with letters that are not similar, indicating that the electrolyte leakage of the fresh and controlled deteriorated seeds are significantly ($p<0.05$) different.

Table 1.
Emergence and mortality of control deteriorated seeds of pea and pumpkin treated with cathodic water and other treatments after 18 days of seeding.

	*FD Emergence		Emergence (%)		Mortality (%)	
Treatments	**Pumpkin**	**Pea**	**Pumpkin**	**Pea**	**Pumpkin**	**Pea**
Fresh seed control	5.0^{d}	5.0^{cd}	100.0^{e}	100.0^{e}	0.0^{a}	0.0^{a}
Cathodic water	14.0^{c}	7.0^{abc}	32.5^{d}	30.0^{d}	0.0^{a}	0.0^{a}
Cathodic water (pH=7)	15.0^{bc}	7.0^{abc}	30.0^{cd}	27.5^{cd}	6.3^{ab}	8.3^{a}
CaMg solution	18.0^{ab}	7.0^{abc}	20.0^{b}	22.5^{cd}	33.3^{c}	45.8^{c}
Camg (pH=11)	18.0^{ab}	8.0^{ab}	22.5^{bc}	20.0^{bc}	41.6^{c}	37.5^{bc}
Deionized water	17.0^{abc}	5.0^{cd}	22.5^{bc}	22.5^{cd}	29.1^{c}	19.2^{ab}
Ascorbic acid	19.0^{a}	8.0^{ab}	17.5^{b}	12.5^{b}	25.0^{bc}	37.5^{bc}
Mean	15.1	6.6	30.6	29.4	19.3	21.2
Lsd $_{0.5}$	-	-	8.94	7.88	13.56	13.09

Note: *FD emergence (First Day of Emergence) is the number of days before the emergence of the first seedling in each treatment. Figures along the same column with different letters are significantly ($p<0.05$) different.

Table 2.
Effect of cathodic water treatment on the root length, stem girth shoot length and shoot/root ratio of pea and pumpkin at 14 weeks after planting.

	Pumpkin			Pea		
Treatments	**Root length (cm)**	**Stem girth (mm)**	**Shoot length (cm)**	**Root length (cm)**	**Stem girth (mm)**	**Shoot length (cm)**
Fresh seed control	28.0^{cd}	9.5^{ab}	22.5^{c}	31.7^{b}	2.4^{c}	35.7^{c}
Cathodic water	25.3^{bcd}	12.4^{b}	15.3^{abc}	26.5^{ab}	2.2^{bc}	35.0^{bc}
Cathodic water (pH=7)	30.0^{d}	9.7^{ab}	17.5^{bc}	24.7^{ab}	2.1^{bc}	33.3^{abc}
CaMg solution	17.0^{a}	7.9^{a}	7.0^{a}	24.3^{ab}	2.1^{bc}	34.3^{bc}
CaMg (pH=11)	20.0^{ab}	6.5^{a}	11.0^{ab}	22.0^{ab}	2.4^{c}	27.0^{ab}
Deionized water	21.3^{abc}	8.5^{a}	17.3^{bc}	19.7^{ab}	1.9^{b}	27.5^{abc}
Ascorbic acid	17.0^{a}	7.1^{a}	10^{ab}	18.0^{a}	1.37^{a}	25.5^{a}
Mean	22.7	8.8	14.38	23.8	2.1	31.2
$Lsd_{0.05}$	4.369	2.342	5.761	7.92	0.267	5.455

Note: Values along the same column with different letters are significantly ($p<0.05$) different. Cathodic water (pH=7) is cathodic water with its pH adjusted to 7, CaMg (pH=11) is CaMg solution with its pH adjusted to 11.

Table 3.
Effect of cathodic water seed treatment on the root mass, stem mass, leaves mass, and total biomass of pea and pumpkin at 14 weeks after planting.

Treatments	**Root**	**Stem**	**Leaf**	**Shoot**	**Total biomass (g)**	**S/R ratio**
	mass (g)					
	Pumpkin					
Fresh seed control	0.14^{a}	0.35^{ab}	1.27^{ab}	1.62^{ab}	1.76^{a}	12.06^{b}
Cathodic water	0.43^{b}	0.81^{c}	2.65^{d}	3.46^{c}	3.89^{c}	8.58^{ab}
Cathodic water (pH=7)	0.48^{b}	0.76^{c}	2.50^{cd}	3.26^{c}	3.74^{bc}	7.07^{a}
CaMg solution	0.29^{ab}	0.43^{ab}	1.76^{bcd}	2.20^{bc}	2.48^{abc}	7.65^{a}
CaMg (pH=11)	0.21^{a}	0.54^{c}	1.57^{abc}	2.11^{abc}	2.32^{ab}	10.05^{ab}
Deionized water	0.20^{a}	0.35^{ab}	0.94^{ab}	1.29^{ab}	1.48^{a}	6.98^{a}
Ascorbic acid	0.13^{a}	0.18^{a}	0.61^{a}	0.79^{a}	0.92^{a}	6.22^{a}
Mean	0.27	0.49	1.62	2.11	2.37	8.37
$Lsd_{0.05}$	0.19	0.31	0.99	1.26	1.44	2.50
	Pea					
Control	1.42^{c}	1.47^{c}	0.46^{b}	2.89^{c}	3.35^{c}	7.05^{abc}
Cathodic water	1.21^{c}	1.48^{c}	0.29^{ab}	2.68^{c}	2.98^{c}	9.67^{bc}
Cathodic water(pH=7)	1.35^{c}	1.46^{c}	0.28^{a}	2.82^{c}	3.10^{c}	9.96^{c}
CaMg solution	0.57^{ab}	0.73^{ab}	0.33^{ab}	1.29^{ab}	1.62^{b}	4.33^{a}
CaMg (pH=11)	0.84^{b}	0.91^{b}	0.30^{a}	1.75^{b}	2.05^{ab}	5.94^{abc}
Deionized water	0.55^{ab}	0.49^{a}	0.15^{a}	1.04^{ab}	1.19^{a}	7.29^{abc}
Ascorbic acid	0.43^{a}	0.52^{a}	0.21^{a}	0.95^{a}	1.16^{a}	4.49^{ab}
Mean	0.91	1.01	0.29	1.92	2.21	6.96
$Lsd_{0.05}$	0.35	0.37	0.16	0.69	0.79	3.38

Note: Figures along the same column (each species) with different letters are significantly ($p<0.05$) different.

Table 4.
Effect of cathodic water seed treatment on the number of leaves, leaves areas, chlorophyll content and chlorophyll fluorescence of pea and pumpkin at 14 weeks after planting

Treatments	**Number of leaves**	**Leaves Area (cm^2)**	**Chlorophyll content (CCI)**	**Chlorophyll fluorescence (Fv/Fm)**
	Pumpkin			
Fresh seed control	8.0^{ab}	1025.0^{c}	50.30^{b}	0.738^{bc}
Cathodic water	11.7^{d}	1295.0^{d}	50.63^{b}	0.749^{c}
Cathodic water (pH= 7)	10.0^{bcd}	1225.0^{d}	50.77^{b}	0.681^{abc}
CaMg solution	11.0^{cd}	336.0^{a}	44.10^{a}	0.740^{bc}
CaMg (pH= 11)	9.0^{bc}	292.0^{a}	44.36^{a}	0.612^{a}
Deionized water	6.7^{a}	710.0^{b}	41.13^{a}	0.647^{ab}
Ascorbic acid	8.0^{ab}	227.0^{a}	42.80^{a}	0.670^{abc}
Mean	9.2	730.0	46.30	0.691
Lsd (5%)	1.9	141.6	3.92	0.091
	Pea			
Fresh seed control	120.0^{b}	628.0^{b}	68.80^{c}	0.768^{d}
Cathodic water	105.3^{b}	573.0^{b}	68.80^{c}	0.702^{c}

Cathodic water (pH= 7)	105.3[b]	573.0[b]	68.80[c]	0.702[c]
CaMg solution	80.5[a]	422.0[a]	48.60[a]	0.638[a]
CaMg (pH= 11)	69.0[a]	412.0[a]	51.90[ab]	0.686[bc]
Deionized water	59.0[a]	321.0[a]	55.17[b]	0.658[ab]
Ascorbic acid	66.0[a]	359.0[a]	54.17[b]	0.638[a]
Mean	86.3	477.00	57.45	0.685
Lsd (5%)	21.95	111.90	4.73	0.032

Cathodic water-treated seeds had the best growth parameters, such as total biomass, root length, stem girth and shoot lengths, among the CD seed treatments (Tables 2 and 3). In pumpkin, cathodic water treatment performed better than fresh seed control treatment in terms of shoot mass, root mass, stem girth, and leaf area (Table 3). Invigoration with CW increased leaf size, number, and chlorophyll content, with some of the values being significant ($p<0.05$) (Table 4). In pea, the chlorophyll fluorescence for all treatments except the control was lower than $0.75 \leq Fv/Fm \leq 0.86$, which is the established range for stress-free plants (Table 4). Similarly, in pumpkin, all treatments, including the control, were below the range. CW with 0.749 may be considered as being the only treatment within the range (Table 4).

After growth for 14 w the total biomass, root and shoot length, and stem girth of CW invigorated CD of pumpkin and pea were not significantly different from the control (Table 2). While other treatments permitted some successful germination/emergence, their growth was usually significantly poorer than that of the controls. Surprisingly, deteriorated seeds of pumpkin invigorated with cathodic water produced seedlings that grew better than fresh seeds, possibly because of a poor initial vigor of the seeds used in this study. Although the seeds were stored at 5°C, the seeds may have lost some vigor due to natural ageing [29]. Some measure of ageing in the fresh seeds was suggested by the photochemical efficiency of photosystem II (Fv/Fm) of the plants derived from the fresh seeds, which was 0.75 rather than values over 8 that occur in healthy plant (Table 4) [36]. Cathodic water treatment may have also favoured the production of chlorophyll, in both test species, the chlorophyll content of the plants derived from CW and CW (pH=7) were significantly higher than those of plants derived from other CD treatments. The improvement in chlorophyll content may have resulted in higher photosynthetic efficiency of the plants, which is crucial to growth and dry matter accumulation in plants. Chlorophyll plays a role in light energy harvest by plants [11, 37]. Also, larger leaf areas as observed in CW treatments may have provided greater surface area for photosynthetic activities, and subsequent partitioning of photoassimilates in favour of vegetative growth [38-40]. Cathodic water may have reduced the oxidative stress damage in the leaves of plants derived from CW treatments [41, 42] and probably reduction in the controlled deterioration induced hangover effects on the plants derived from CW and CW (pH=7) treatments [26].

Generally, the performance of the 6 CD treatments is in the order CW ~ CW (pH=7) > CaMg ~ CaMg (pH=11) > DW > As. While the outstanding performance of CW and CW (pH=7) treatments may be due to the reducing power of CW, that of CaMg may be linked to its nutritional role in plant growth. Calcium and magnesium are readily soluble and absorbable plant macronutrients; hence, they may have served as an additional source of plant nutrients, especially at the early stages of seedling growth. Seed priming with plant nutrients, otherwise known as nutrients priming, such as zinc, manganese, boron, and phosphate, has been reported to have a significant influence on the growth of maize (Muhammad et al., 2015). The successful use of ascorbic acid may be attributed to its antioxidant properties. However, from the result of this study, the antioxidant properties of ascorbic acid may not be as strong as that of cathodic water.

5. Conclusion and Recommendations

In general, seeds invigorated with CW performed better than those primed using existing techniques such as water (hydro priming), ascorbic acid (osmo priming), and calcium magnesium solution (nutrient priming). Adjusting the pH of CW and the CaMg solution did not result in striking differences in plant emergence and growth when compared with the original solutions. Hence, it may be unnecessary to consider such pH adjustments as separate treatments in future studies. The mechanism of action of the ameliorative effects of cathodic water should be further investigated, and biochemical germination enzymes, such as amylase, as well as deoxyribonucleic acid (DNA) concentration and purity, should be explored in future germination studies.

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