



## The influence of osmo-priming on germination parameters of *Telfairia occidentalis* Hook f. (fluted pumpkin)

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**Abstract.** Fluted pumpkin (*Telfairia occidentalis* Hook F.) seed germination proceeds once adequate temperature and moisture content have been reached and dormancy is broken. Seed priming is a technique in which seeds are hydrated (control hydration) and dried to their original moisture content while preventing radicle emergence. The study aims to investigate the consequence of osmo-priming on the germination parameters of fluted pumpkin (*Telfairia occidentalis*). Laboratory studies were carried out using 36 seeds of fluted pumpkin which was osmoprimed with NaCl (0.05, 0.1 and 1 millimolar), MgCl<sub>2</sub> (0.05, 0.1 and 1 millimolar) and KCl (0.05, 0.1 and 1 millimolar). Data on germination percentage, growth parameters, and chlorophyll content showed a significant difference in germination percentages between osmoprimed seeds and control seeds. The time of germination in osmoprimed seeds was significantly reduced when compared with control. The germination rate index (64%) was different between controls and osmoprimed seeds with 0.05 millimolar KCl and 0.10 millimolar MgCl<sub>2</sub> (). The growth parameters of seedlings 15 days after sowing showed significant increase in the number of leaves, number of root branches and chlorophyll content. Seed osmopriming may be a sustainable method to increase crop production in *T. occidentalis*.

**Keywords:** Fluted pumpkin, seed priming, germination, seedling, vegetable.

## Introduction

Germination of fluted pumpkin (*Telfairia occidentalis* Hook F.) begins when the temperature and moisture content are appropriate and the dormancy is broken. For embryo growth, seeds must first absorb water to start their metabolism and boost their respiration (Fincer, 1989). Three phases can be differentiated during seed germination/sprouting, all of which are connected to water intake (Bewley *et al.*, 2013). Rapid water uptake (phase I) starts the metabolic process, which includes DNA and mitochondrial repair as well as protein synthesis using existing mRNA (Bewley *et al.*, 2013). Further water uptake is limited during phase II because the grain's water potential is nearly equal to that of its surroundings. The activation or lag phase is another name for this stage. Major metabolic changes, such as the manufacture of hydrolytic enzymes (such as-amylase, endoxylanase, and phytase) and other activities required for embryo growth, occur during this phase. A second rapid water uptake occurs in phase III (Bewley *et al.*, 2013; Nonogaki *et al.*, 2010). The appearance of radicle is known as germination (Nonogaki *et al.*, 2010) and is also known as sprouting (Lemmens *et al.*, 2019). Plant hormones such as abscisic acid (ABA), gibberellic acid (GA3), ethylene, auxins, cytokinins, and brassinosteroids influence germination and sprouting (Nonogaki *et al.*, 2010; Miransari and Smith, 2014). The expression of important genes regulates their synthesis and activity. ABA and GA3 are the most significant plant hormones for seed germination. They are created in the embryo and then transferred to the aleurone (Nonogaki *et al.*, 2010; Ma *et al.*, 2017). As response to GA3 aleurone cells produce and secrete hydrolytic enzymes in order to mobilize grain reserves and germination, whereas ABA inhibits these processes (Ma *et al.*, 2017).

As previously noted, phase II of the germination process is critical due to the significant metabolic changes that occur there (Di and Barbanti, 2012). Seed priming, a pre-sowing therapy, has an impact on this period. To begin metabolic activity, seeds are soaked in an osmotic solution and then dried to their original moisture level before sprouting/sowing (Di and Barbanti, 2012).

Hydro-priming and osmo-priming are two common priming methods (Ventura *et al.*, 2012). Grain imbibitions with water during a limited time period (714 h) is referred to as hydro-priming. It starts the above-mentioned phase II metabolism without putting the seeds under too much stress. The emergence of the radicle (phase III) is prevented during the process by drying the seeds to their original moisture level (Ventura *et al.*, 2012). Osmo-priming is a regulated procedure that restores 10 to 20% of complete hydration. Inducing abiotic stress conditions, in phase II physiological and biochemical activities are sustained. The applied negative water potential prevents the development of the radicle

(phase III) during the procedure (Bewley *et al.*, 2013; Di and Barbanti, 2012; Rehman *et al.*, 2010; Khan *et al.*, 2014). Higher water potentials (0.3 to 1.5 MPa) and short priming times (12 h to 2 days) are best for osmo-priming (Rehman *et al.*, 2010; Ghiyasi *et al.*, 2008; Salehzadeh *et al.*, 2009; Yari *et al.*, 2010). Oxidative processes occur when using more negative water potentials and/or longer priming times (Basra *et al.*, 2005). High molecular weight polyethylene glycol is the most commonly utilized solute for osmo-priming (PEG); it causes high osmotic pressure, which alters the availability of water in the germination medium (Hameed *et al.*, 2014). As a result, the amount of cellular damage caused by rapid water entry into a seed is minimized (Ventura *et al.*, 2012).

The seeds are soaked in aerated osmotic solutions containing potassium nitrate, potassium phosphate, potassium chloride salts, or polyethylene glycol (PEG) with varying water potentials and time lengths in osmopriming, the most popular priming method. The applied solutes are usually dissolved in water at a concentration that allows the seeds to drink a small amount of water to start the pre-germination metabolism. Before the major root or radicle emerges, the primed seeds are extracted from the osmoticum (Paparella *et al.*, 2005).

The use of PEG during priming minimizes toxicity because it is not taken up by seeds due to its high molecular weight (Di and Barbanti 2012). High PEG concentrations, on the other hand, result in high viscosity, which inhibits oxygen transport and requires efficient aeration during priming (Paparella *et al.*, 2015). Several factors are thought to play a role in priming beneficial effects on sprouting. First, the increased water content is critical for activating enzymes involved in embryo growth and starchy endosperm mining (Mirza *et al.*, 2015). Second, priming activates biochemical cell repair processes, increases RNA content, and improves DNA replication (Di and Barbanti 2012; Khan *et al.*, 2014; Salehzadeh *et al.*, 2009; Mirza *et al.*, 2015). Third, increased activity of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione reductase seems to improve the defense system after priming (Di and Barbanti 2012; Zhang *et al.*, 2015). Primed crops absorb water and recover grain metabolism more quickly after sowing than non-primed grains (Hameed *et al.*, 2014). As a result, seed priming improves germination rates (Brocklehurst and Dearman, 1983, Hardegree 1994; Toklu *et al.*, 2015), emergence uniformity (Brocklehurst and Dearman, 1983; Toklu *et al.*, 2015), yield (Toklu *et al.*, 2015), and seedling resistance to unfavourable environmental conditions (Hardegree, 1994).

Following water intake (imbibition) in phase I, adequate water is required in metabolic processes to repair cellular components lost during the maturation drying period. Although various trials have been undertaken to explore the effects of pretreatment on seed germination performance of commercially important

crops. The present study was carried out to evaluate the effects of NaCl, MgCl<sub>2</sub> and KCl solutions as osmopriming reagents on seed germination and the seed parameters such as number of leaves, length of leaf, number of roots, branches per root, and length of nodes will be determined, Also the chlorophyll content will be determined. This study therefore seeks to address the extent osmopriming enhances germinability and germination parameters of the test plant.

Farmers of fluted pumpkins in tropical West Africa appear to be facing a huge difficulty in overcoming the problem of delayed germination. Osmopriming aids in dormancy breaking, improved germination and vigour, homogeneity of germination, and better development and early flowering (Mirmazloun *et al.*, 2020). It also makes plants more tolerant of abiotic stress like dehydration. To what extent can osmopriming enhance germinability and germination characteristics of the test plant? These are the questions that this research aims to solve.

## **Materials and methods**

### ***Plant materials***

Seeds of fluted pumpkin (*Telfairia occidentalis* Hook F.) were obtained from local farmers in Benin City, Edo State, Nigeria.

### ***Seed osmopriming treatment***

A total of 36 seeds of fluted pumpkin were treated. Three priming media at varied concentration levels were used as described below: NaCl (0.05, 0.1 and 1 mM, millimolar), MgCl<sub>2</sub> (0.05, 0.1 and 1 mM) and KCl (0.05, 0.1 and 1 mM). Osmopriming was done by soaking the seeds inside a plastic bottle containing the three media for 3 hours at room temperature, on the laboratory bench. The unprimed seeds were soaked in tap water for 3 hours (controls). The primed and unprimed seeds were planted right away in plastic bottles with the top half cut off and filled with sandy loamy soil. The plastics were then laid out in block design replicated three times. The plants were watered daily.

### ***Germination analysis***

Seed germination analysis was carried out for a period of two weeks. Data was collected on germination from day 1 to day 4 after sowing. A seed is considered germinated when the radicle emerges through the seed coat. Germination percentage was computed using the following formula:

$$\text{Germination percentage} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds in all replicates}} \times 100$$

### ***Determination of growth parameters***

Analyzed growth parameters were: number of leaves , length of leaf, number of roots, branches per root, and length of nodes. Data was collected for a period of 15 days after sowing. To determine plant moisture content (%), difference between plant fresh and dry weight was expressed as a percentage of the fresh weight. Plants were initially dried in an electric laboratory cabinet drying oven (Model - CAH-550, manufactured by Sepor, Wilmington) at 85°C for 4 hours. Chlorophyll content was also assessed. To do this, three leaves per plant were collected and placed in a chlorophyll meter to ascertain the chlorophyll content of the leaf. Chlorophyll content, measured as Chlorophyll Content Index (CCI) was determined by the hand-held Chlorophyll Concentration Meter (Model MC-100, by Apogee Instruments Inc., USA). Data was collected for a period of 15 days after sowing.

### ***Germination indices***

Germination indices calculated were done according the methods of Abdul-Baki and Andersen (1972); AOSA (1983); Scott *et al.* (1984); ISTA (1993); Al-Mudaris (1998); ISTA (1999); Sadeghi *et al.* (2001); Josep and Maria (2002).

#### **First day of germination (or Germinability) (FDG)**

It is the time when the first germination was recorded

#### **Last day of germination (LDG)**

This is the last day when the seed germination was reported

#### **Final germination percentage (FGP)**

This is the germination percentage attained by the plant even beyond the time period

#### **Peak period of germination (PPG) or Modal time of germination (MTG)**

It is the time in which highest frequency of germinated seeds are observed and need not be unique.

#### **Median germination time (MeGT), or Days required for 50% germination, T503,**

Days required for 50% germination, T50

T50 = Days required for 50% germination of the total number of seeds

#### **Time spread of germination, or Germination distribution (TSG)**

TSG = LDG - FDG

This is the time (in days) taken between the first and last germination events.

### Mean germination time, MGT

$$T = \frac{\sum_{i=1}^k N_i T_i}{\sum_{i=1}^k N_i}$$

Where S1, S2, S3, Sn are number of seeds that germinated per lot (or petri dish) at day 1, day 2, day 3 ... day n. The lower the MGT, the faster a seed population has germinated. It is also called Length of Germination Time (LGT) or Germination Resistance (GR) or Sprouting Index (SI). It is the average length of time required for maximum germination of a seed lot.

### Mean germination rate, MGR

$$\text{MGR} = 1 / \text{MGT}$$

### Germination rate index (GRI)

$$\text{GRI} = [ \text{GP1}/1 + \text{GP2}/2 + \text{GP3}/3 + \dots + \text{GPn}/n ]$$

Where GP1 is germination percentage at 1st day, GP2 is germ percent at 2 days, GPn is germ percent at n days. GRI reflects the percentage of germination on each day of the germination period.

### Speed of accumulated germination, SAG

$$\text{SAG} = [ (\text{GP1}/1 + (\text{GP1}+\text{GP2})/2 + (\text{GP1}+\text{GP2}+\text{GP3})/3 + \dots + (\text{GP1}+\text{GP2}+\text{GP3}+\dots+\text{GPn})/n ]$$

Where GP1 is germination percentage at 1st day, GP2 is germ percent at 2 days, GPn is germ percent at n days. GRI reflects the percentage of germination on each day of the germination period.

### Corrected germination rate index (GRI corrected)

$$S_{corrected} = \text{GRI} / \text{FGP}$$

### Timson's Index, (TI) or Germination Energy Index

$$n = \sum_{i=1}^t G_i$$

$$\text{GEI} = ( \text{GP1} + \text{GP2} + \text{GP3} + \dots + \text{GPn} )$$

Where GP1, GP2, ..., GPn are the germination percentages at day 1, 2, ... and n respectively.

**Modified Timson's Index, (TI<sub>mod</sub>)**

TI<sub>mod</sub> = Timson's index (TI) divided by the number of intervals (t).

$$T_{mod} = \frac{T}{t}$$

**Germination index, GI**

$$GI = 10 \times (S1+S2+S3+ \dots +Sn) / (1*S1 + 2*S2 + 3*S3 + \dots + n * Sn)$$

Where S1, S2, S3, Sn are number of seeds that germinated per lot (or petri dish) at day 1, day 2, day 3 ... day n

**Mean daily germination, MDG**

MDG = FGP / d, where d is the number of days it took to first arrive at the FGP

**Daily germination speed, DGS**

DGS = 1 / MDG. This is the reciprocal of MDG

**Germination Value (Czabator)**

$$GV = PV \times MDG$$

Where, PV is the peak value and MDG is the mean daily germination percentage from the onset of germination.

**Coefficient of velocity of germination, CVG**

$$CVG = [(G1+G2+G3+ \dots +Gn) / (1*G1 + 2*G2 + 3*G3 + \dots + n * Gn)] * 100$$

Where G1, G2, G3, Gn are germination percent per lot (or petri dish) at day 1, day 2, day 3 ... day n. CVG gives an indication of the rapidity of germination.

**Germination capacity, GC**

$$GC = FGP / N$$

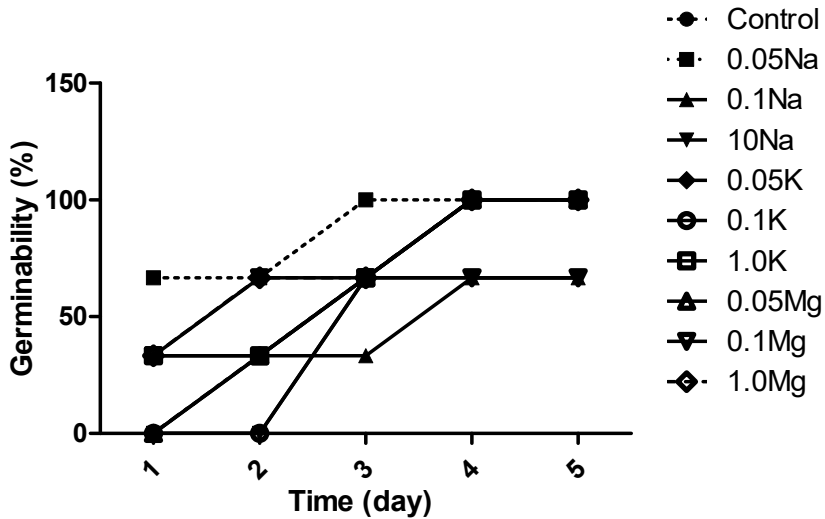
Where N is number of seeds used in the bioassay

***Statistical analysis***

The data collected on seed germination analysis, number of leaves, length of leaf, number of roots, branches per root length, length of nodes and chlorophyll content were subjected to two-way analysis of Variance (ANOVA).

## Results

The effect of osmopriming of *Telfairia occidentalis* seeds on germinability and germinating percentage is shown in Fig. 1. Germination began after four days of sowing when compared to the control, germination increased to 100%.



**Figure 1.** Osmopriming effects on the germinability of *Telfairia occidentalis*

**Table 1.** ANOVA summary Table to show source of variation due to germination and experimental treatment (osmopriming concentrations)

Source of Variation	Sum-of-squares	Mean square	F	% of total variation	P value
Treatments	7756	861.7	3.277	13.58	<b>0.0052**</b>
Days	39890	9973	37.93	69.85	<b>&lt; 0.0001***</b>
Error	9466	262.9			

It was important to show if variations observed in the study were either due to time of germination or to the osmotic treatments applied. Results showed that treatment application accounted for 13.58% of total variations observed in the study (Table 1). Germination time accounted for 69.85% variation.

The effects of the treatments on germination time have been presented in Table 2. In the control group, germination occurred after 47.93 hours. However, when the seeds were osmoprimed with 0.05mM NaCl, the time it took for the seeds to germinate was significantly reduced to 23.96 hours. When



seeds were primed with 0.1 millimolar NaCl, germination time was also 23.96 hours. Seeds primed with 1.00 mM KCl, 0.10 mM MgCl<sub>2</sub>, and 1.00 mM MgCl<sub>2</sub> all took the same amount of time to germinate (23.96 hours). There were no significant differences when seeds were primed with KCl irrespective of the concentration applied. However, with the application of 0.10 millimolar and 1.00 millimolar NaCl as osmoprimer the time taken for the last germination period was significantly reduced to 60.58 hours. The peak period of germination was less (47.93 hours) when seeds were primed with 0.10 millimolar MgCl<sub>2</sub> compared to 95.85 hours in the control.

**Table 2.** Effects of treatments on germination time

Day	Control	0.05 Na	0.1 Na	10 Na	0.05 K	0.1 K	1.0 K	0.05 Mg	0.1 Mg	1.0 Mg	LSD (0.05)	p-value
First Day of Germination	47.93	23.96*	23.96*	71.89*	47.93	71.89*	23.96*	47.93	23.96*	23.96*	19.3	0.006
Last Day of Germination	95.85	71.89*	95.85	71.89*	95.85	95.85	95.85	95.85	47.93*	95.85	22.31	0.031
Final Germination Percent	90.87	90.87	60.58*	60.58*	90.87	90.87	90.87	90.87	60.58*	90.87*	28.62	0.022
Peak period of Germination	95.85	71.89*	95.85	71.89*	95.85	95.85	95.85	95.85	47.93*	95.85	22.31	0.031
Median Germination Time	67.9	19.97*	23.96*	23.96*	61.91	67.9	67.9	64.9	23.96*	39.94*	26.73	0.013
Time Spread of Germination	47.93	47.93	71.89*	46.66	47.93	23.96*	71.89*	47.93	23.96*	71.89*	19.5	<0.001
Mean Germination Time	3.33	0.21	0.28	3	3.33	3.58	3	3.33	1.67	2.87	NA	NA
Mean Germination Rate	0.29	4.75	3.56	0.3	0.29	0.28	0.33	0.29	0.59	0.35	NA	NA

Table 3 shows the impact of seed priming on germination indexes. The germination rate index was minimally differed between the control and those of seeds osmoprimered with 0.05 millimolar KCl and 0.10 millimolar MgCl<sub>2</sub> (63.65). Modified Timson’s index was the least (22.22) in seeds primed with 1.00 millimolar NaCl and the highest in both the control (77.77) and seeds primed with 0.05 millimolar NaCl. Coefficient of velocity of germination was 29 in the control, 30 in seeds primed with 1.00 millimolar NaCl and 476 when seeds were primed with 0.05 millimolar NaCl.

The growth parameters of the seedlings at 15 days after sowing (Table 4) showed significant increases in the number of leaves for those seeds that were primed with 0.10 millimolar NaCl (16 leaves) and those primed with 1.00

millimolar MgCl<sub>2</sub> (9 leaves), compared to the control (4 leaves). Although there were minimal differences in chlorophyll content index in seeds osmoprimed with Na, K and Mg chloride respectively as compared to the control (21.50), were significantly increased in chlorophyll content index in seeds osmoprimed with 0.05 millimolar KCl, 1.00 millimolar KCl and 1.00 millimolar MgCl<sub>2</sub> (30.20 – 36.40) (P < 0.05). The number of roots branches increased significantly from 31 in seeds primed with 1.00 millimolar NaCl to 4 in seeds primed with 0.05 millimolar NaCl.

**Table 3.** Impact of seed priming on germination Indices

Day	Control	0.05Na	0.1Na	10Na	0.05K	0.1K	1.0K	0.05Mg	0.1Mg	1.0Mg
Germination Rate Index	63.65	133.33	77.67	22.22	63.67	47	96.98	97.22	66.67	113.66
Speed of accumulated Germination Corrected	99.96	211.12	141.65	22.22	83.3	64.1	169.36	100	83.33	160.5
Germination Rate Index	0.64	1.33	1.16	0.33	0.64	0.47	0.97	0.97	1	1.13
Timson's Index	233.3	233.34	166.66	66.67	200	166.67	233.33	200	100	266.67
Modified Timson's Index	77.77	77.78	41.65	22.22	50	41.67	58.33	50	50	66.67
Germination Index	2.99	46.67	35.7	3.33	2.99	2.78	3.3	2.99	5.99	3.48
Mean Daily Germination	25	33.33	16.67	22.22	25	25	25	25	33.33	25
Daily Germination Speed	0.04	0.03	0.06	0.05	0.04	0.04	0.04	0.04	0.03	0.04
Germination Value	2500	3333	1111	1481	2500	2500	2500	2500	2222	2500
Coefficient of velocity of germination	29	476	357	30	29	28	33	29	59	35
Germination capacity	33.33	33.33	22.22	22.22	33.33	33.33	33.33	33.33	22.22	33.33

Figure 2 shows the treated and control seedlings with roots exposed at 13 days after sowing (Figure 2a), while the treated and controlled seedlings without root exposure have been presented in Figure 2b. By the 16 days, results showed improved growth capacity for the osmoprimed seedlings at 16 days after planting (Figure 2c).

Table 5 shows the two-way analysis of variance set out to determine the sources of variation in number of leaves, chlorophyll content, number of root branches, moisture content as well as dry weight measurement. For each of

these parameters, the primary treatments were compared against the measured parameters and results as presented in Table 5 show a mean square of 1.31 for number of leaves, 0.08 for chlorophyll content and 1.74 for dry weight measurement.

**Table 4.** Growth parameters of seedling 15 days after sowing

Groups	#No of leaves	Chlorophyll content (CCI)	Internode (cm)	#No primary root branches	#Av. Number of secondary root branches	Plant moisture content (%)	Plant dry wt. (g)
Control	4	21.5	0.4	5	10	2.8	7
0.05Na	7	29.3	1.1	4	9	18.9*	2.4*
0.1Na	3	27.2	0.3	5	14*	9.7	6.5
10Na	1	21	0.5	31*	3*	15.6	2.7*
0.05K	5	30.2*	2.3	5	15*	18.2*	4.5
0.1K	16*	27.1	1.1	18*	7	27.5*	4
1.0K	2	35.3*	2.8*	2	9	15.4	5.5
0.05Mg	8	23.1	1.2	6	11	2.3	8.5
0.1Mg	3	29.3	0.5	15*	8	14.0	4.3
1.0Mg	9*	36.4*	1.1	28*	5*	17.1*	2.9*
Mean	6	28.1	1.1	11.9	9.1	14.1	4.7
Variance	19	27.4	0.6	112	14	56.3	4.1
<b>LSD(0.05)</b>	5	8.7	1.6	8	4	13.2	3.7
<b>p-value</b>	0.043	0.007	0.293	0.001	0.062	<0.001	0.173

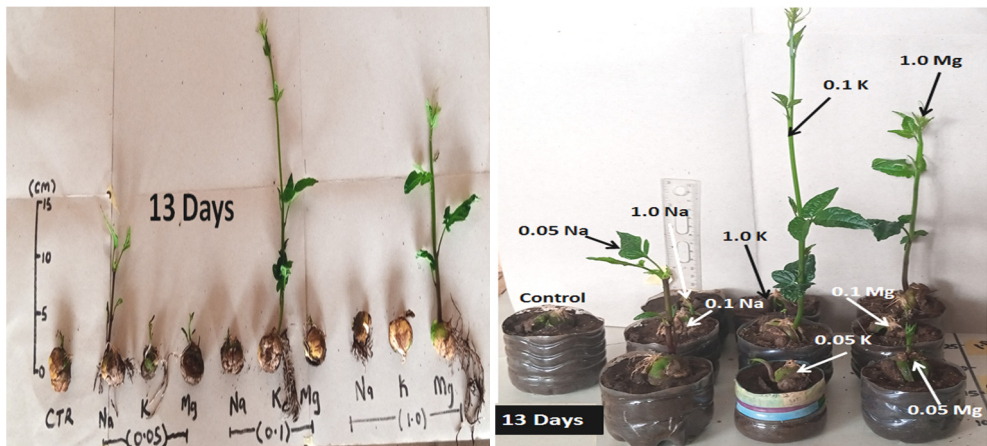
#Means are presented to the nearest integer

\* Means differ significantly from the control ( $p < 0.05$ )

## Discussion

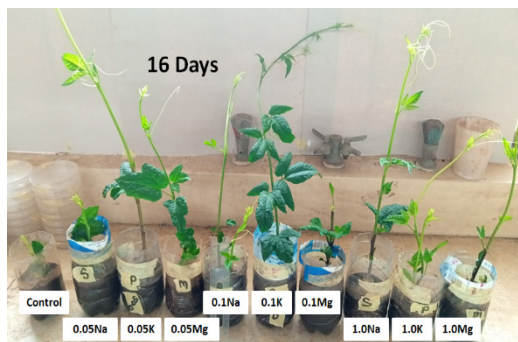
The study on the effects of osmo-priming on the germination parameters of fluted pumpkin (*Telfairia occidentalis*) was carried out. This study revealed that osmopriming of seed of *Telfairia occidentalis* caused significant increases in the germination percentage compared to the control. The significant increase in germination percentage on osmoprimed seeds could be related to the activation of physiological germination processes in primed seeds, resulting in better germination through seed coat activation and softening. This finding was similar to that of Gurusinghe *et al.* (1999), who found that during priming, radical tip cells of some tomato seed lots proceeded through the cell cycle.

Several osmotica have been proven to have beneficial effects on germination (Lemrasky and Husseini, 2012). The effect of osmopriming on enhanced germination percentage could be explained by an increase in the activity of essential enzymes such as amylase and proteases (Dell-Aquila and Tritto, 1990), which play a critical part in the seed embryo's growth and development. On the appropriate application of osmopriming treatment, Dell-Aquila *et al.* (1984) found a link between the pattern of water absorption, the reactivation of mitotic activity, and the initiation and synchronization of germination.



(a)

(b)



(c)

**Figure 2.** Treated and control plant seedlings at different times after exposure to osmopriming regimes: (a) Treated and control seedlings (roots exposed) 13 days after sowing; (b) Treated and control seedlings 13 days after sowing; (c) Treated and control seedlings 16 days after sowing.

Seed priming approaches improved germination percentage, emergence, and seedling stand, according to Basra *et al.* (2003). In fact, priming causes a variety of biochemical changes in the seed that are essential to start the germination process, such as dormancy breaking, inhibitor hydrolysis or metabolism, imbibitions, and enzyme activation (Ajouri *et al.*, 2004). Some earlier research has suggested that priming triggers some or all of the processes that occur prior to germination and that these processes continue after the seed is re-desiccated (Asgedom and Becker, 2001). As a result, primed seed can quickly ingest and revive the seed metabolism after sowing, resulting in a higher germination percentage and less physiological variability in germination (Rowse, 1995).

**Table 5.** Two way ANOVA to determine sources of variation

Source of Variation	% of total variation	Sum-of-squares	Mean square	F	P value
<b>Number of leaves</b>	0.52	2.621	1.31	17.05	< 0.0001
Priming treatments	99.2	495.5	55.06	716.2	< 0.0001
Residual		1.384	0.07687		
<b>Chlorophyll content</b>	61.25	284.5	8.09	< 0.0001	30.63
Priming treatments	694.1	716.2	91.66	< 0.0001	77.12
Residual	1.938				0.1077
<b>No primary root branches</b>	11.03	12.63	0.39	0.0004	5.516
Priming treatments	2815	716.2	99.33	< 0.0001	312.8
Residual	7.86				0.4367
<b>Plant moisture</b>	15.46	35.44	1.08	< 0.0001	7.731
Priming treatments	1411	718.7	98.64	< 0.0001	156.8
Residual	3.927				0.2182
<b>Dry weight measurement</b>	1.817	57.27	1.74	< 0.0001	0.9087
Priming treatments	102.3	716.2	97.99	< 0.0001	11.36
Residual	0.2856				0.01587

In the control group, the first day of germination took 47.93 hours. When the seed was osmoprimed with NaCl at 0.05 milligrams per kilogram, the time it took for the seed to germinate on the first day was dramatically reduced to 23.96 hours. Primed seeds with 0.1 millimolar NaCl, first germination took 23.96 hours. Seeds primed with 1.00 millimolar KCl, 0.10 millimolar MgCl<sub>2</sub>, and 1.00 millimolar MgCl<sub>2</sub> all took the same amount of time to germinate on the first day (23.96 hours). When seeds were primed with KCl, regardless of the concentration

used, there was no significant difference. The time taken for the last germination period was significantly reduced to 60.58 hours when 0.10 millimolar and 1.00 millimolar NaCl were used as osmoprimers. Seeds primed with 0.10 millimolar MgCl<sub>2</sub> had a shorter peak period of germination (47.93 hours) compared to 95.85 hours in the control. Seed priming resulted in an increase in anti-oxidants such as glutathione and ascorbate in seed, according to Huns and Sung (1997). By reducing lipid peroxidation activity, these enzymes increase germination speed. Priming has been shown to increase the speed of germination in sorghum, sunflower, and melon (Foti *et al.*, 2002; Sivritepe *et al.*, 2003; Demir Kaya *et al.*, 2006).

When comparing the growth parameters of the seedlings 15 days after sowing, those primed with 0.10 millimolar NaCl (16 leaves) and those primed with 1.00 millimolar MgCl<sub>2</sub> (9 leaves) showed significant increases in the number of leaves compared to the control (4 leaves). Although there were minimal differences in chlorophyll content in seeds osmoprimed with Na, K, and Mg chloride as compared to the control (21.50), chlorophyll content index in seeds osmoprimed with 0.05 millimolar KCl, 1.00 millimolar KCl, and 1.00 millimolar MgCl<sub>2</sub> (30.20 – 36.40) ( $P < 0.05$ ) was significantly increased. The number of root branches increased significantly from 31 with 1.00 millimolar NaCl-primed seeds to 4 in 0.05 millimolar NaCl-primed seeds. The primed seed's early emergence could be attributable to the completion of pregermination metabolic activities, preparing the seed for radicle protrusion, and the primed seed germinated quickly after planting when compared to untreated dry seed (Arif, 2005). Chemically primed seeds have been shown to have a better germination pattern and vigor level than nonprimed seeds (Ruan *et al.*, 2002). Nascimento and West (1998) found that seed coat adhesion was reduced during the emerging of muskmelon seeds. The improvement in germination and vigor of normal/low-vigor seed could be related to food reserve mobilization, activation and re-synthesis of certain enzymes, and the commencement of DNA and RNA synthesis during osmotic priming (Basra *et al.*, 2003). When the barrier to germination was eliminated, the embryos grew quickly (Basra *et al.*, 2003).

Priming can help to improve seed quality (Arif *et al.*, 2008). Sung *et al.* (1993) found that priming reduced seed secretion and, as a result, decreased EC, which was consistent with Xiang *et al.* (1995) findings. Priming has been proved to be an effective approach in numerous experiments, particularly for seeds with low vigor (Varier *et al.* 2010, Flors *et al.* 2007, Soeda *et al.* 2005). The effects of priming applied to seeds of many domesticated crop species have helped agriculture. Good examples include barley (Ajouri *et al.*, 2004), soybean (Arif, 2005), canola (Basra *et al.*, 2003), carrot (Brocklehurst and Dearman, 1983), and sorghum (Foti *et al.*, 2002).

## Conclusions

The results of this study revealed that germination in osmoprimed seedlings increased to 100%. The number of leaves, root branches, and chlorophyll content were all higher than in control seeds. This knowledge can be applied to fluted pumpkin farming on a larger scale. Osmopriming causes the vegetative growth of the plant to increase; this thus improves food security by increasing production.

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## References

- Abdul-Baki, A., & Anderson, J.D. (1973). Vigor determination in Soybean seed by multiple criteria. *Crop Sci.* 13: 630-633.
- Ajouri, A., Haben, A., & Becker, M. (2004). Seed priming enhances germination and seedling growth of barley under conditions of P and Zn deficiency. *J Plant Nutr Soil Sci* 167: 630 – 636.
- Al-Mudaris, M.A. (1998). Notes on various parameters recording the speed of seed germination. *Der Tropenlandwlr*t, Beltrage zur tropischen Landwirtschaft und Veterinarmedizin, 99. Jahrgang, Oktober 98, S. 141 - 154.
- Arif, M. (2005). Seed priming improves emergence, yield and storability of soybean. PhD thesis in Agronomy. NWFP Agricultural University Peshawar, Pakistan.
- Asgedom, H., & Becker, M. (2001). Effects of seed priming with nutrient solutions on germination, seedling growth and weed competitiveness of cereals in Eritrea. In: Proceedings of. Deutscher Tropentag, University of Bonn and ATSAF, Magrraf Publishers Press, Weickersheim. 282p.
- Association of Official Seed Analysis (AOSA). (1983). Seed Vigor Testing Handbook. Contribution No. 32 to the handbook on Seed Testing.
- Basra, S.M.A., Ullah, E., Warriach, E.A., Cheema, M.A., & Afzal, I. (2003). Effect of storage on growth and yield of primed canola seeds (*Brassicana apus*). *Int J Agric Biol* 5: 117 – 120.
- Bewley, J.D., Bradford, K.J., Hilhorst, H.W.M., Nonogaki, H. (2013) Seeds: Physiology of Development, Germination and Dormancy. *Springer Publisher*, New York, USA. 399p.
- Brocklehurst, P.A., & Dearman, J. (1983). Interactions between seed priming treatments and nine seed lots of carrot, celery and onion. *Ann Appl Biol* 102: 577 – 584.
- Dell’Aquila, A., Pignone, D., & Carella, G. (1984). Polyethylene glycol 6000 priming effects on germination of aged wheat seed lots. *Biol Plant* 26: 166 – 173.
- Dell’Aquila, A., & Tritto, V. (1990). Ageing and osmotic priming in wheat seeds: effects upon certain components of seed quality. *Ann Bot* 65: 21 – 26.

- Demir Kaya, M., Okçu, G., Atak, M., Çikili, Y., & Kolsarici, Ö. (2006). Seed treatment to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.). *Euro J Agro* 24: 291 – 295.
- Di Girolamo, G., & Barbanti, L. (2012). Treatment conditions and biochemical processes influencing seed priming effectiveness. *Italy J Agro* 7(25): 178 – 188.
- Fincher, G.B. (1989). Molecular and cellular biology associated with endosperm mobilization in germinating cereal grains. *An Rev Plant Phy & Mol Bio* 40: 305 – 346.
- Flors, V., Paradís, M., García-Andrade, J., Cerezo, M., Bosch, G.C., & Pilar, G.A.P. (2007). A tolerant behavior in salt-sensitive tomato plants can be mimicked by chemical stimuli. *Plant Signaling Behav* 2: 50 – 57.
- Foti, S., Cosentino, S.L., Patane, C., & D'Agosta, G.M. (2002). Effect of osmoconditioning upon seed germination of Sorghom (*Sorghom Bicolor* (L). Moench) under low temperatures. *Seed Sci Tech* 30: 521 – 533.
- Ghiyasi, M., Siyahjani, A. A., Tajbakhsh, M., Amirnia, R., & Salehzadeh, H. (2008). Effect of osmopriming with polyethylene glycol (8000) on germination and seedling growth of wheat (*Triticum aestivum* L.) seeds under salt stress. *Res J Biol Sci* 3: 1249 – 1251.
- Gurusinghe, S.H., Cheng, Z., & Bradford, K.J. (1999). Cell cycle activity during seed priming is not essential for germination advancement in tomato. *J Exp Bot* 50(330): 101 – 106.
- Hameed, A., Sheikh, M.A., Farooq, T., Basra, S.M.A., & Jamil, A. (2014). Chitosan seed priming improves seed germination and seedling growth in wheat (*Triticum aestivum* L.) under osmotic stress induced by polyethylene glycol. *Philippian Agr Sci* 97: 294 – 299.
- Hardegree, S.P. (1994). Drying and storage effects on germination of primed grass seeds. *J Range Manage* 47: 196 – 199.
- ISTA (1993). Handbook for seedling evaluation. International Seed Testing Association, Zurich, Switzerland.
- Josep, A.R., & Maria, M. (2002). Seed germination and reproductive features of *Lysimachia minoricensis* (Primulaceae), a wild extinct plant. *Ann Bot* 89: 559–562.
- Khan, M.B., Gurchani, M.A., Freed, S., Jabran, K., & Hussain, M. (2014). Osmopriming improves the emergence, growth, nutrient uptake and soluble sugar contents of wheat seedlings. *Soil Environ* 33: 142 – 148.
- Lemmens, E., Moroni, A.V., Pagand, J., Heirbaut, P., Ritala, A., Karlen, Y., Lê, K., Van den Broeck, H.C., Brouns, F.J.P.H., De Brier, N., & Delcour, J.A. (2019). Impact of cereal seed sprouting on its nutritional and technological properties: a critical review. *Comprehensive Rev Food Sci Food Safety* 18: 305 – 328.
- Lemrasky, M.G., & Hosseini, S.Z. (2012). Effect of seed priming on the germination behavior of wheat. *Int J Agr Crop Sci* 4: 564 – 567.
- Ma, Z., Bykova, N.V., & Igamberdiev, A.U. (2017). Cell signaling mechanisms and metabolic regulation of germination and dormancy in barley seeds. *Crop J* 5: 459 – 477.
- Miransari, M., & Smith, D.L. (2014). Plant hormones and seed germination. *Environ Exp Bot* 99: 110 – 121.



- Mirmazloun, I., Kiss, A., Erdélyi, É., Ladányi, M., Németh, É. Z., & Radácsi, P. (2020). The Effect of Osmopriming on Seed Germination and Early Seedling Characteristics of *Carum carvi* L. *Agriculture*. 10(4):94.
- Mirza, S.R., Ilyas, N., & Batool, N. (2015). Seed priming enhanced seed germination traits of wheat under water, salt and heat stress. *Pure Appl Bio* 4: 650 – 658.
- Nascimento, W.M., & West, S.H. (1998). Priming and Seed Orientation affect emergence and seed coat adherence and seedling development of muskmelon transplants. *Horti Sci* 33: 847 – 848.
- Nonogaki, H., Bassel, G.W., & Bewley, J.D. (2010). Germination - still a mystery. *Plant Sci* 179: 574 – 581.
- Paparella, S., Araújo, S.S., Rossi, G., Wijayasinghe, M., Carbonera, D., & Balestrazzi, A. (2015). Seed priming: state of the art and new perspectives. *Plant Cell Report* 34: 1281 – 1293.
- Rehman, S.U., Khalil, S.K., Khan, A.Z., Subhan, F., Younis, M., Muhammad, Y., & Jan, N. (2010). Poly ethylene glycol (PEG) osmopriming affects phenology, plant height and biomass yield of rainfed wheat. *Saudi J Anaesth* 26: 337 – 348.
- Rowse, H.R. (1995). Drum Priming- A non-osmotic method of priming seeds. *Seed Sci Tech* 24: 281 – 294.
- Ruan, S., Xue, Q., & Tylkowska, K. (2002). The influence of priming on germination of rice *Oryza sativa* L. seeds and seedling emergence and performance in flooded soil. *Seed Sci Tech* 30: 61 – 67.
- Sadeghi, H., Fardin, K., Iela, Y., & Saman, S. (2011). Effect of seed osmopriming on seed germination behavior and vigor of soybean (*Glycine max* L.). *ARPN J Agric Bio Sci*, 6(1): 39-43.
- Salehzadeh, H., Shishvan, M.I., Ghiyasi, M., Forouzin, F., & Siyahjani, A.A. (2009). Effect of seed priming on germination and seedling growth of wheat (*Triticum aestivum* L.). *Research J Biol Sci* 4: 629 – 631.
- Scott, S.J., Jones, R.A., & Williams, W.A. (1984). Review of data analysis methods for seed germination. *Crop Science*, 24:1192-1199.
- Sivritepe, N., Sivritepe, H.O., & Eris, A. (2003). The effects of NaCl priming on salt tolerance in melon seedling grown under saline conditions. *Scientia Horti* 97: 229 – 237.
- Soeda, Y., Konings, M.C.J.M., Vorst, O., van Houwelingen, A.M.M.L., Stoopen, G.M., & Maliepaard, C.A. (2005). Gene expression programs during Brassica oleracea seed maturation, osmopriming, and germination are indicators of progression of the germination process and the stress tolerance level. *Plant Phys* 137: 354 – 368.
- Sung, J. M., & Chang, Y. H. (1993). Biochemical activities associated with priming of sweet corn seeds to improve vigor. *Seed Sci Tech* 21: 97 – 105.
- Toklu, F., Baloch, F.S., Karaköy, T., & Özkan, H. (2015). Effects of different priming applications on seed germination and some agromorphological characteristics of bread wheat (*Triticum aestivum* L.). *Turkey J Agri Forest* 39: 1005 – 1013.
- Varier, A., Vari, A.K., & Dadlani, M. (2010). The subcellular basis of seed priming. *Curr Sci* 99: 450 – 456.

- Ventura, L., Donà, M., Macovei, A., Carbonera, D., Buttafava, A., Mondoni, A., Rossi, G., & Balestrazzi, A. (2012). Understanding the molecular pathways associated with seed vigor. *Plant Phy Biochem* 60: 196 – 206.
- Xiang, X., Fu, J.R., Xiang, X., & Fu, J.R. (1995). The ways to increase vigor of wampee (*Clausena lansium*) seeds. *J Trop Subtrop Bot* 5: 39 – 44.
- Yari, L., Aghaalikani, M., & Khazaei, F. (2010). Effect of seed priming duration and temperature on seed germination behavior of bread wheat (*Triticum aestivum* L.). *ARPJ Agric Bio Sci* 5: 1 – 6.
- Zhang, F., Yu, J., Johnston, C.R., Wang, Y., Zhu, K., Lu, F., Zhang, Z., & Zou, J. (2015). Seed priming with polyethylene glycol induces physiological changes in sorghum (*Sorghum bicolor* L. Moench) seedlings under suboptimal soil moisture environments. *PLoS* 10: 1 – 15.