

Germination of eggplant, okra and rice seeds soaked in alkaline water

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Germination of Eggplant (*Solanum melongena* Linn.

), Okra (*Abelmoschus esculentus* (L.) Moench), and Rice (*Oryza sativa* Linn.)

Seeds Soaked in Alkaline Water In Partial Fulfillment of the Requirements In

Bi 160 and Bi 160. 1 First Semester, AY 2012-2013 A Research Paper

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Manila University Submitted by: Medina, Kryzka August 2012 I. Introduction

The life cycle of plants is characterized by the change in ploidy of

generations; with diploid generations giving rise to haploid generations and vice versa.

The diploid generation, commonly called the sporophyte, produces spores via meiosis. Through mitosis these haploid cells develop into a multicellular gametophyte that can be a separate plant in itself or a part of a plant. The gametophyte gives rise to male and female gametes that in turn produce sperm and egg cells, respectively. In gymnosperms and angiosperms, the haploid generations bring forth seeds through fertilization and subsequent growth and development (Solomon et al. 2008). A seed is associated with the independence of the sporophyte generation.

It is comprised of a young plant embryo, an endosperm, a perisperm, and a testa or seed coat making it physiologically and structurally equipped to propagate a new plant. The main function of seeds is to give rise to new plants; thus allowing the continuity of the generation. The emergence of a plant starts with seed germination. Dormant seeds have low moisture content and minimal metabolic activity; such seeds are called quiescent

wherein none of the germination processes is occurring (Bewley and Black 1994, Bewley 1997).

Germination is instigated by the imbibition of water by a quiescent seed and concluded by the initiation of the elongation of the embryonic axis, usually the radicle.

(Bewley and Black 1994, Bewley 1997). Seed germination consists of three phases: (1) water imbibition, (2) mitochondrial activity and protein synthesis, and (3) visible germination. The first phase, characterized by influx of water into the cells of the dry seed, results to the leakage of solutes and metabolites from the cells through the temporary perturbations in the structure of the cell membranes.

The imbibition of water also leads to the onset of metabolic activity. The second phase, characterized by an increase in metabolic activity, is evidently accompanied by high mitochondrial activity and protein synthesis.

Mitochondrial activity includes cellular respiration and ATP synthesis. Protein synthesis results gives rise to various proteins required for germination and development of the seed. Finally, the third phase is characterized by the emergence of the radicle from the structures that encloses the embryo (Bewley 1997). Seed germination will only occur if environmental conditions are favorable.

These include optimum temperature, moisture, and light among others; still, specific requirements vary among different species (Monaco et al.

2002). Factors that affect germination are not limited to the aforementioned environmental conditions; salinity, acidity and alkalinity can also influence germination. Increasing germination rate is possible through the manipulation of environmental factors such as alkalinity. Perveen et al. (2008) compared the germination rate of barley seeds soaked in alkaline solutions with varying concentrations and pH levels.

Germination rate after 48 hours was 35% for the control, distilled water; while germination rate of seeds soaked in solutions of $\text{Ca}(\text{OH})_2$, KOH , and $\text{Mg}(\text{OH})_2$ were 60, 66 and 62%, respectively. Likewise, a study by Huo and Simpson (1994) promoted germination of barley seed through the utilization of alkaline solutions. They found out that the efficacy of alkaline treatments is highly dependent on maturity, presence of hulls and moisture content of freshly harvested barley grains. They utilized KOH and NaOH solutions of varying concentrations; immersion time of seed in the solutions also varied. KOH solutions promoted seed germination by 100%.

Immersion time of the seed is inversely proportional to the germination rate.

The results of the two studies however are expected, as barley is highly tolerant to alkaline soils (Perveen et al. 2008, Huo and Simpson 1994). Koger et al. (2004) studied the factors that can affect seed germination, seedling emergence and survival of texasweed. Among the factors that they considered was pH where they utilized buffer solution with pH 4 to 10.

Germination of texasweed seeds followed a quadratic response to pH; germination rate was greater than 42% at pH 5-9 while pH 4 and 10 resulted to germination rate of less than 32% (Koger et al.

004). Lin et al. (2012) studied the salinity-alkalinity tolerance of wheat in seed germination and early seedling growth. They mixed two neutral salts (NaCl and Na₂SO₄) and mixed two alkaline salts (NaHCO₃ and Na₂CO₃) to generate two separate stress groups, salt and alkali. It was found that germination percentage significantly decreased through the increased alkalinity of the solution. The decreased germination of the seeds under alkali stress is attributed to the influence of high pH, which results to the inhibition of ion uptake and disruption of ionic balance of plant cells (Lin et al.

012). This study was undertaken to determine the effect of commercial alkaline water to the germination of eggplant, okra, and rice seeds. Drinking alkaline water has become a trend in society for the past years for the alleged benefits; proponents of alkaline water claim that it stimulates seed germination. More expensive than distilled or purified water, alkaline water has a short shelf life. The utilization of expired alkaline water that purportedly stimulates germination will provide an alternative use for the undrinkable water. II.

Materials and Methodology Assay Location

The experiment proper was carried out inside a room in the Medina Residence, which is located at 5 Capt. Miyong St. , San Roque, Marikina City, Metro Manila, Philippines. The room experienced limited sunlight with temperatures that vary from 18°C to 28°C. The set-up was placed on top of a table to eliminate the possibility of animal foraging. Species Selection The experiment entailed the utilization of three species of plants.

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The first species, *Oryza sativa* Linn. , was preselected while the last two species were left to the discretion of the proponent of the study.

Prudent seed selection was done to ensure the preservation of the diversity of the plant species that will be utilized for the experiment. *O. sativa*, commonly called rice, is a monocot plant. It belongs to the family of grasses, Poaceae, and is the main source of carbohydrates in Asian countries.

Solanum melongena Linn. , commonly called eggplant, in contrast is a dicot plant. The shrub belongs to the nightshade family, Solanaceae, and is used as a source of vitamins and minerals in tropical and temperate countries.

Abelmoschus esculentus (L.) Moench, commonly called okra, is a herbaceous dicot plant.

It belongs to the hibiscus family, Malvaceae, and is also used as a source of vitamins and minerals in tropical and subtropical countries (Asis et al.

1980, Merrill 1912). Table 1 shows the complete plant portrait of each

species. Table 1. Plant portraits of rice, eggplant, and okra seeds. Plant

Portrait| Scientific Name| *Oryza sativa* Linn. | *Abelmoschus esculentus* (L.

) Moench| *Solanum melongena* Linn. | Common Name| Rice, palay (Tag.)|

Gumbo, okra (Tag.)| Eggplant, talong (Tag.)| Family Name (scientific)|

Poaceae| Malvaceae| Solanaceae| Family Name (common)| Grass| Hibiscus|

Nightshade| Growth habit| Grass| Herb| Shrub|

Part(s) of plant used| Fruit| Fruit| Fruit| Description of economic uses| Source

of starch| Source of vitamins and minerals| Source of vitamins and minerals|

Cultivation Techniques| Transplanting, Direct seeding| Sowing,

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Transplanting| Slope terracing (monsoon), Pit system (summer)| Product Preparation| Milling, Boiling| Boiling, Frying, Preserving in brine| Frying, Grilling| Miscellaneous| None| None| None| Region of presumed origin| China| India| Uncertain| Current area of cultivation| Tropical and temperate countries| Tropical and subtropical countries| Tropical and temperate countries| References| (Merrill 1912, Asis et al. 980)| (Merrill 1912, Asis et al. 1980)| (Merrill 1912, Asis et al. 1980)| Material Acquisition Initial material acquisition transpired during class hours, Dr. Padolina provided 100 rice seeds (NSIC Rc 240; Tubigan 22) and six sheets of kitchen paper. While plastic bags were provided, the proponent of the study opted to utilize six 900-mL plastic containers bought from SM Marikina Department Store. Seed acquisition of the last two species occurred outside class hours. Seeds were obtained from commercial packets bought from Handyman, specifically the smooth green okra and long purple eggplant varieties of Ramgo International Corporation.

Table 2. Variety and source of rice, eggplant and okra seeds. Common name| (1) Rice seed, (2) Eggplant seed, (3) Okra seed| Variety/ Cultivar| (1) PhilRice 31132, (2) Long purple, (3) Smooth green| Source of Seeds| (1) IRRI, (2) Commercial seed packet – Ramgo International Corporation, (3) Commercial seed packet – Ramgo International Corporation| A liter of distilled water (Absolute), with pH 7, was acquired for the control group while three liters of alkaline water, with pH 7. 7, from Grandmaster Water Station was acquired for the experimental group. Experimental proper only started after the alkaline water has expired.

The values of pH of the distilled and alkaline water were obtained through the use of a pH meter. **Experimental Design** The experiment was performed by using the seeds of three species of plants; a total of three set-ups with an experimental and control group were accomplished, each corresponding to different species. Only one replicate per treatment was utilized; each treatment contained fifty seeds. Three of the six plastic containers were labeled with " Control", while the other half were labeled with " Experimental". The paper towels were placed inside the six containers; afterwards the seeds were positioned on top of the towels, five in a single row and ten in a single column for easier observation.

Two separate spray bottles were then used to water the control and experimental groups with distilled water and alkaline water, respectively. To prevent the rapid escape of moisture, the containers were covered with cling wraps. Sixteen holes were then stabbed into the plastic cover to allow passage of air. **Observation Period** The experiment proper went on for 9-15 days until no germination has been observed in each treatment. Seed germination is tantamount to the emergence of the radicle.

Each night, the treatments were observed; seeds that have sprouted radicles were counted and recorded. The germinated seeds were then disposed of to prevent overlapping of percent germination per time interval. Seeds that procure molds after a few days of observation were thrown out to prevent contamination of the seed group. Moistening of the paper towels was done after data collection or as often as needed. **Data Collection** As mentioned, the seeds were observed every night for fifteen days. The number of

germinated seeds and the number of ungerminated seeds were entered into a Microsoft Office Excel file.

Percent germination per day and cumulative percent germination were derived from the data using equations in Microsoft Office Excel 2011. Statistical Analysis The statistical analysis of the results was performed by the use of the Chi-Square Contingency Table Test. The test was used to determine whether or not the difference between the germination rates of the control and experimental group were significant for each species. P-values less than 0.05 were considered significant.

The data were presented using marked line graphs through the use of Microsoft Excel 2011.

Percent germination per day and cumulative percent germination of the treatments were plotted for each species. To visualize the variation of the percent germination of species within each treatment four line graphs were constructed; two depicting percent germination per day and two depicting cumulative percent germination. For better illustration of the deviation between treatments the graphs only depicted percent germination over 10 days. III. Results Figure 1 illustrates the cumulative percent germination of the control and the experimental groups of rice seeds.

The control group had a cumulative percent germination of 94% at the end of the observation period, compared to the 92% of the experimental group. Though the control group had a larger cumulative percent germination the experimental group germinated faster, as the apex cumulative percent germination of the control group (Day 4) was achieved a day after the

experimental group (Day 3). According to the chi-square contingency table test, the difference between the cumulative percent germination of the two treatments is not significant ($1.0 < p < 0.05$, see Appendix).

It should be noted that the ungerminated seeds for both treatments were contaminated with molds 10-12 days after the initiation of the experiment.

Fig 1. Cumulative percent germination of control and experimental *Oryza sativa* Linn. seeds Fig 1. Cumulative percent germination of control and experimental *Oryza sativa* Linn. seeds Unlike the rice seeds, okra seeds in the alkaline water treatment had a higher cumulative percent germination (96%) than the okra seeds in the distilled water treatment (94%). Figure 2 depicts the cumulative percent germination of the okra seeds under alkaline water and distilled water treatments.

Similarly, the treatment that germinated faster was the treatment that had a lower cumulative percent germination. Statistical analysis showed that the difference between the cumulative percent germination of the two treatments is not significant ($1.0 < p < 0.05$, see Appendix). The presence of molds was observed in both treatments, corresponding to the ungerminated seeds. Fig 2.

Cumulative percent germination per day of control and experimental *Abelmoschus esculentus* (L.) Moench seeds Fig 2. Cumulative percent germination per day of control and experimental *Abelmoschus esculentus* (L.) Moench seeds Lastly, eggplant seeds immersed in distilled water or alkaline water both had a cumulative percent germination of 92% (Refer to Figure 3).

Though it was observed that the control eggplant seeds, similar to okra

seeds, germinated faster than the treated eggplant seeds. The control group reached maximum percent germination two days (Day 6) before the experimental group (Day 8).

The chi-square contingency table test cannot be utilized as the same number of control and treated eggplant seeds germinated. Molds, which characterize ungerminated seeds, were seen in both treatments. Fig 3.

Cumulative percent germination of control and experimental *Solanum melongena* Linn. seeds Fig 3.

Cumulative percent germination of control and experimental *Solanum melongena* Linn. seeds IV. Discussion Prevalence of high cumulative percent germination was observed in all of the three plant species; with 92% as the lowest cumulative percent germination observed in the control and experimental groups of the eggplant seeds. While more untreated rice seeds germinated than those that germinated in alkaline water, the opposite was true for the okra seeds. As mentioned, no difference was observed in the germination of eggplant seeds.

Since the difference between the percent germination of the control and experimental groups were not significant, these observations are not sufficient to conclude that alkaline water promotes, inhibits or have no effect in the germination of okra, rice, and eggplant seeds. The inconclusive results may be attributed to the small difference between the pH of the distilled water (pH 7) and that of the alkaline water (pH 7.7). Though pH is measured in a logarithmic scale, the actual difference (0.01) between the

concentration of OH⁻ ions found in alkaline water and distilled water was insufficient to affect seed germination.

Likewise, the prevalence of high percent germination for all three plant species may be attributed to the small difference between the concentration of OH⁻ ions in distilled and alkaline water.

While Monaco and his colleagues specified that optimum conditions of seed germination vary among different species (2002) the minimally basic alkaline water may have not affected germination of the three species. Moreover, studies show that seed germination follows a quadratic response to pH; where optimum percent germination is observed in pH levels 7-9 (Koger et al. 2004, Mehrafarin et al. 2011).

The two treatments fall within the optimum pH range for seed germination. Studies on the influence of alkaline conditions in seed germination commonly used varying concentrations of KOH solutions.

Significant difference between the percent germination of the control and experimental groups showed that alkaline conditions promoted seed germination. Conclusive results were obtained due to the extreme difference in the concentration of OH⁻ ions (Hou and Simpson 1994, Perveen et al. 2008). Though the influence of alkaline water on seed germination was not elucidated it can still be utilized to germinate seeds.

As mentioned, expired alkaline water is unfit for human consumption.

The seemingly analogous results of the control and experimental groups will allow substitution with expired alkaline water in seed germination. V.

Conclusion This study was not able to elucidate the effect of alkaline water in seed germination. High percent germination was observed in all treatments. While no difference was observed in the germination of eggplant seeds immersed in alkaline and distilled water, the results of the experimental and control groups of rice and okra seeds proved to be insignificant.

The minimal disparity seen in the germination of seeds using distilled and alkaline water may be attributed to the small difference between pH levels. Though inconclusive, the study promotes the use of expired alkaline water for seed germination for practical purposes. Future studies that determine the effect of alkaline conditions to seed germination can be furthered by the use of varying concentrations of KOH solutions wherein one can control the pH level for each treatment. Significant results may be used to compare percent germination of each species. More replicates for each treatment is also suggested.

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8th ed. Thomson Brooks/ Cole; 2008. Appendix Table 2. Chi-square contingency table for *Oryza sativa* L. | Germinated Seeds| Not germinated seeds| Total| Control| 47| 3| 50| Experimental| 46| 4| 50| Total| 93| 7| | | df| 1| | ? | 0.

05| | | χ^2 | 0. 153609831| | | p-value| 1. 0 < p < 0. 05| Table 3. Chi-square contingency table for *Abelmoschus esculentus* (L.) Moench | Germinated Seeds| Not germinated seeds| Total| Control| 47| 3| 50| Experimental| 48| 2| 50| Total| 95| 5| | | df| 1| | ? | 0.

05| | | χ^2 | 0. 210526316| | | p-value| 1. 0 < p < 0. 05|

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