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Research Article

Varieties of USDA Certified Organic *Raphanus Raphanistrum Subsp. Sativus* [L.] Response to Salinity Stress

Jonathan D Hulse[1]*, Scott Paxon[1], Rachel Meriwether[2], Laura Tinney[1], John Norvell[1], Joseph Mendrykowski[1], Ralph Streadwick[1], Isabella Leonard[1], Victor Calderas[1], Murrin Reed[1], Anthony Pully[1], Gerald Mahle[1], Katherine Spencer[1], Dominick Gregory[1], Desiree Cao[1], Juliana Rusnak[1], Paiton Jacko[3], BayleeAnn Pollett[4] and Carter West[1]

[1]*Department of Arts and Sciences, Blue Ridge Community College, United States of America*

[2]*Department of Horticulture, Blue Ridge Community College, United States of America*

[3]*Department of Health Sciences, Blue Ridge Community College, United States of America*

[4]*Department of Education, Blue Ridge Community College, United States of America*

***Corresponding Author:** Jonathan D Hulse, Department of Arts and Sciences, Blue Ridge Community College, United States of America.

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Abstract

R*aphanus Raphanistrum subsp. Sativus* [L.] or radish, is a major economic root crop species grown in the United States of America, and it is a distinct member of the *Brassicaceae* family. It is consumed worldwide for its crunchy texture and piquant flavor, which is often described as sharp or spicy. Globally, agricultural lands have become contaminated with salts in the soils, which can greatly affect productivity of those plots. Climate change is also elevating sea water levels, which has been shown to infiltrate aquifers, and can cause agricultural damage to croplands if used as irrigation water. This experiment tested various sodium chloride concentrations on different varieties of USDA Certified Organic *R. Raphanistrum subsp*. Sativus seeds. The results from this study showed significant statistical differences in germination and root length for different concentrations of sodium chloride. This study also showed significant differences between varieties of *R. raphanistrum subsp*. sativus at different sodium chloride concentrations.

Keywords: Agriculture; Agronomy; *Brassicaceae*; *Raphanus raphanistrum subsp. Sativus*; Salinity Stress

Abbreviations

C: Celsius; CMV: Cucumber Mosaic Virus; GMO: Genetically Modified Organism; M: Molar; MiRNA: Micro-RNA; PTES: Potentially Toxic Elements; NaCl: Sodium Chloride; USDA: United States Department of Agriculture; YMV: Youcai Mosaic Virus

Introduction

Brassicaceae is a large and diverse family of plants that includes dozens of economically important species [11]. This family includes the genus *Raphanus*, which is comprised of distinct species and recently identified subspecies [16,30,43,44]. Of the known species and subspecies in the genus *Raphanus*, some are edible crops, while others are noxious weeds that invade agricultural lands in Europe [43,44]. The cultivated radish belongs to the genus *Raphanus*, but there are discrepancies in species nomenclature [33,11,23,50]. Some research groups refer to cultivated radishes as *Raphanus Raphanistrum* subsp. *Sativus* [L.], while other research groups use *Raphanus sativus* [L.] [1-54]. We will refer to the cultivated radish as *Raphanus Raphanistrum* subsp. *Sativus* [L.] for the duration of this study.

Radishes are grown for their roots, leafy greens, seeds, sprouts, and is consumed worldwide for its texture as well as for its unique flavor profile [3,11]. It is understood that the pungent flavor profile of radishes comes from its diverse glucosinolate profile [30]. *R. Raphanistrum* subsp. *Sativus* is grown in Africa, Asia, Europe, North America, South America, and is cultivated in over 35 countries [3]. *R. Raphanistrum* subsp. *Sativus* prefers cool growing conditions and has a fast growth cycle, which makes it ideal for use as a cover crop, or in multiple rotations within agricultural fields [50]. Traditionally, *R. Raphanistrum* subsp. *Sativus* has been grown in soil, but recent research has experimented with alternative soilless growth systems [13,42].

Economically speaking, *R. Raphanistrum* subsp. *Sativus* is a major economic crop, with an annual production of over seven million metric tons, which represents over 2% of all vegetable crops produced worldwide [50]. *R. Raphanistrum* subsp. *Sativus* has many medicinal properties including anti-cancer, anti-microbial, cardio-protective, hepatoprotective, and organoleptic, to name a few [7,19,24,25,33,35,37,50]. *R. Raphanistrum* subsp. *Sativus* is recommended as a food source because of its high content of ascorbate, folate, riboflavin, vitamin A, vitamin B6, and vitamin C [50].

The genome of *R. Raphanistrum* subsp. *Sativus* was sequenced in 2015 by Mitsui., *et al*. and subsequent 'omic' studies have reveled tremendous details into the growth of this economically important organism [27,34,49,52,53]. *R. Raphanistrum* subsp. *Sativus* has been shown to exhibit a 2n = 2x = 18 chromosome profile and has been shown to express over 40 different MiRNA's within their root tissues [51]. Modern 'omic' studies are trying to understand *R. Raphanistrum* subsp. *Sativus*'s response to various stresses, including biotic and abiotic factors [27,34,49,52,53].

Just like any crop species, *R. Raphanistrum* subsp. *Sativus* is susceptible and resistant to a variety of different diseases, which can cause stress to the plant [10,54]. Yoon and colleagues showed that some strains were susceptible to Cucumber Mosaic Virus (CMV), while others were resistant to CMV [54]. In 2017, Choi and colleagues released a first report of Youcai Mosaic Virus (YMV) infecting *R. Raphanistrum* subsp. *Sativus* [10]. Other viral infections of *R. Raphanistrum* subsp. *Sativus* include but are not limited to Papaya Leaf Curl Virus and Pedilanthus Leaf Curl Virus [18,21]. Lee and colleagues report that *R. Raphanistrum* subsp. *Sativus* is susceptible to the fungal pathogen *Fusarium oxysporum* [Schlecht], while Garibaldi., *et al*. showed susceptibility of radish to *Fusarium equiseti* [Wollenw.] [15,23]. Arefin's research group states that *R. raphanistrum* subsp. *sativus* is susceptible to the fungal pathogen *Alternaria brassicae* [Berk] and *Alternaria brassicicola* [Schwein] [2]. In 2014, Cai and colleagues provided a first report of the fungi *Leptosphaeria biglobosa* [Shoemaker and Brun] on *R. Raphanistrum* subsp. *Sativus* [9]. Petrie (1986) reported *Albugo candida* [Pers.], the causal agent of white rust and stag head, on *R. raphanistrum* subsp. *sativus* [40]. Lakra showed that *R. Raphanistrum* subsp. *Sativus* is susceptible to the downy mildew pathogen *Peronospora parasitica* [Pers.] [22]. Bacterial pathogens of *R. Raphanistrum* subsp. *Sativus* include *Pectobacterium* [Hauben] spp., *Pseudomonas solanacearum* [Smith], *Pseudomonas cannabina pv. Alisalensis* [Gardan], to name a few [26,28].

R. Raphanistrum subsp. *Sativus'*s ability to grow quickly makes it an ideal model organism for agronomic and botanical research. *R. Raphanistrum* subsp. *Sativus* has served as a model organism for a variety of agricultural experiments, including abiotic stress, anti-microbial, biochemical, cover crops, heavy metal accumulation, genetics, and post-harvest spoilage [14,17,29,31,45,47,52]. *R. Raphanistrum* subsp. *Sativus* has been a used as a model crop for developing new techniques for vegetable preservation, which reduces post-harvest waste in food systems [17]. *R. Raphanistrum* subsp. *Sativus* has been a model organism to show associations with diverse insect pollinator species, which is an increasingly important topic in the 21st century [11]. *R. Raphanistrum* subsp. *Sativus* has also been used to shown concentration of Potentially Toxic Elements (PTES) and heavy metals in plant tissues, as a result from irrigation from contaminated waters [4,31].

Utilizing *R. Raphanistrum* subsp. *Sativus* to understand the effects of abiotic stress is increasingly important to researchers as the environment on Earth changes, and food resources become harder to cultivate. Climate change is one of the greatest factors effecting worldwide agricultural production. As the climate changes, abiotic stresses become more prominent, and threaten humans' ability to cultivate food. Extreme weather events and rising ocean waters are a result of climate change. Abiotic stress is often defined as any non-living entity that provides trauma to a host plant, which is not limited to moisture, pH, pollution, salinity, and temperature [6,8,12,47-49,51,52].

It is reported that nearly 50% of agricultural crop lands are affected by salinity due to contaminated irrigation waters [47,48]. When soils become contaminated with salt, plants lose their ability to procure water, and excess salt can negatively affect their metabolism [47,48]. It is reported that *R. Raphanistrum* subsp. *Sativus* has a low to moderate sensitivity to salinity stress [32]. Recent research by Sun., *et al*. [47,48] and Wang., *et al.* [51,52] have investigated the molecular mechanisms behind salinity stress in *R. Raphanistrum* subsp. *Sativus.* Paromita and colleagues studied the effect of salinity on germination rates of *R. Raphanistrum* subsp. *Sativus* cv. Tasakishan Mula-1, Drui, Red Bombay, at 0.66 - 12 ds m^{-1} of NaCl [39]. Marcelis and Hooijdonk [32] studied the effect of salt stress on *R. Raphanistrum* subsp. *Sativus* cv. Saxa Nova, and they investigated the mass of the root after exposure to varying salt concentrations. Norren and Ashraf [38] conducted a study on germination rate of *R. Raphanistrum* subsp. *Sativus* cv. Red Neck, Lal Pari, Mino Japani, 40 Days, Mannu Early, and Desi, in different concentrations of sodium chloride (0mM, 60mM, 120mM, 180mM, 240mM). Their study showed a decrease in germination rate as sodium chloride concentrations increased. Overall, there is substantial literature studying the effects of salinity stress on *R. Raphanistrum* subsp. *Sativus*, as compared with other major crop species. Unfortunately, little research has investigated USDA Organic varieties of *R. Raphanistrum* subsp. *Sativus*, and their ability to tolerate saline conditions.

This study was intended to test the effects of different salinity concentrations on germination rate and root length of two different varieties of USDA Certified Organic *R. Raphanistrum* subsp. *Sativus.* Varying concentrations of sodium chloride (NaCl) were applied to *R. Raphanistrum* subsp. *Sativus* cv. Cherry Belle and *R. Raphanistrum* subsp. *Sativus* cv German Giant seeds in-situ, to test the effects of contaminated irrigation water on the productivity of this economically important species. To date, USDA Certified Organic *R. raphanistrum* subsp. *sativus* cv. Cherry Belle and *R. Raphanistrum* subsp. *Sativus* cv German Giant varieties have not been tested for salinity tolerance in this manner.

Materials and Methods

Seed stock

Seeds of *R. Raphanistrum* subsp. *Sativus* cv. Cherry Bell and German Giant were purchased from Back to the Roots®. The seeds were marked as USDA Organic, which assures that they are non-GMO, and were certified by ECOCERT SA in Oakland California, USA. *R. Raphanistrum* subsp. *Sativus* cv. Cherry Belle seeds came from Lot C, while *R. Raphanistrum* subsp. *Sativus* cv. German Giant seeds came from Lot B. The seed packages are labeled as 'Heirloom' varieties, with a sell by date of 12/2022.

Germination rate

Germination rate was measured in-situ by the following technique. Sterile 100 x 15 mm Falcon® petri plates were lined with sterile 100 mm medium density Thermo Fisher® filter paper disks. Thermo Fisher® Transfer pipettes were used to apply three milliliters of Sodium Chloride (NaCl) to the filter paper disks. Concentrations of 0.0 M, 0.1 M, 0.2 M, 0.3 M, 0.4 M, and 0.5 M were used for this experiment. Then, ten seeds of *R. raphanistrum* subsp. *sativus* were evenly placed on the filter paper, and the petri plates were sealed with Parafilm M®. The petri plates were placed in natural light under a 14 light/10 dark regiment for one week at 22°C. Replicates of ten petri plates were created for each concentration and each variety of seeds, for a total of 120 petri plates or 1200 seeds. Germination was measured as the percentage of seeds that germinated from 100 seeds. Germination was assessed by the root penetrating the seed coat and emerging onto the filter paper disk.

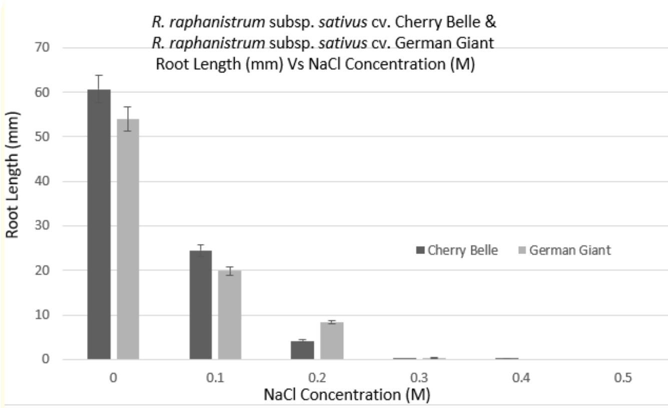
Measuring root length

Root length was measured after one week of incubation in conditions as mentioned above. Briefly, germinated seeds were removed from the petri plates with sterile forceps and placed on a sterile lab bench. The roots were stretched linearly, and precisely measured in millimeters with a metric ruler. The measurements were recorded in a Microsoft Excel® workbook and saved for future analysis.

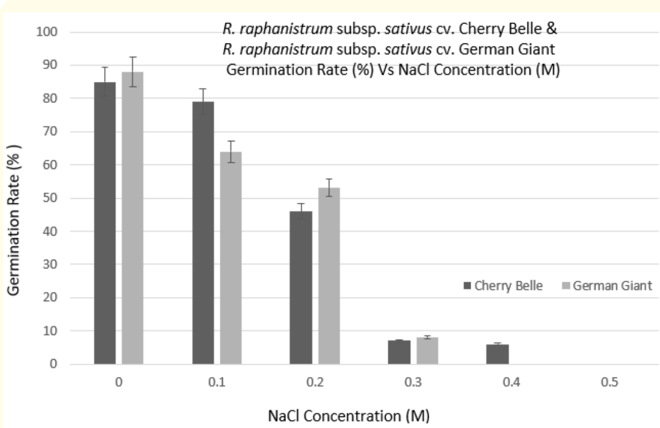
Statistical analysis

Statistical analysis was completed using Microsoft Excel® - Data Analysis options. The Analysis ToolPak® and Solver Add-In® was installed previous to the statistical analysis. Before ANOVAs, Means, and Two-Tailed T-Tests Assuming Equal Variance were performed in Microsoft Excel®, F-Tests were completed to look for equal variances. Comparisons were made between the control group (0.0M NaCl) to the treatment groups (0.1M, 0.2M, 0.3M, 0.4M, and 0.5M NaCl) for each variety. Average germination rate and average root length was calculated for each treatment and each variety. ANOVAs and T-Tests were used to make comparisons between different concentrations of NaCl and different varieties. Data was recorded into a Microsoft Excel® workbook.

Results and Discussion



Graph 1: Root length of USDA Certified Organic *R. Raphanistrum subsp. Sativus* cv. Cherry Belle and *R. Raphanistrum subsp. Sativus* cv. German Giant under varying concentrations of Sodium Chloride. Error Bars represent an α value of 0.05.



Graph 2: Germination rate of USDA Certified Organic *R. Raphanistrum subsp. Sativus* cv. Cherry Belle and *R. Raphanistrum subsp. Sativus* cv. German Giant under varying concentrations of Sodium Chloride (NaCl). Error Bars represent an α value of 0.05

Average germination rate was calculated for each treatment (0.0M, 0.1M, 0.2M, 0.3M, 0.4M, and 0.5M) NaCl and each variety (Cherry Belle/German Giant) used for this study (Graph 1, graph 2, table 1, table 2). Two-tailed T-Tests (assuming equal variances) were calculated (Table 3) after the data set passed F-Tests. ANVOA's (Single Factor) were calculated to see if there was a difference between the treatment groups used in this study (Supplemental Data). T-Tests were calculated to see if there was a significant difference between germination rate and NaCl concentration, as well

Variety	Concentration (M)	Germination rate (%)	Root Length (mm)
German Giant	0	88	53.9495
German Giant	0.1	64	19.84444444
German Giant	0.2	53	8.35
German Giant	0.3	7	0.365
German Giant	0.4	0	0
German Giant	0.5	0	0

Table 1: Average Germination rate and Root length of *R. Raphanistrum* subsp. *Sativus* cv. German Giant.

Variety	Concentration (M)	Germination rate (%)	Root Length (mm)
Cherry Belle	0	85	52.77
Cherry Belle	0.1	79	24.44
Cherry Belle	0.2	46	4.2
Cherry Belle	0.3	7	0.044
Cherry Belle	0.4	6	0.305
Cherry Belle	0.5	0	0

Table 2: Average Germination rate and Root length of USDA Certified Organic *R. Raphanistrum* subsp. *Sativus* cv. Cherry Belle.

as root length and NaCl concentration, for both varieties used in this study (Table 3).

Results of the T-Tests showed significant differences between most of the treatment groups (Table 3). *R. Raphanistrum* subsp. *Sativus* cv. Cherry Belle showed significant differences between all treatments except 0.0M vs 0.1M and 0.3M vs 0.4M for germination rate. *R. Raphanistrum* subsp. *Sativus* cv. Cherry Belle showed significant differences between all treatments except 0.3M vs 0.4M, 0.3M vs 0.5M, and 0.4M vs 0.5M for root length. *R. Raphanistrum* subsp. *Sativus* cv. German Giant showed significant differences between all treatments except 0.4M vs 0.5M for germination rate. *R. Raphanistrum* subsp. *Sativus* cv. German Giant showed significant differences between all treatments except 0.3M vs 0.4M, 0.3M vs 0.5M, and 0.4M vs 0.5M for root length.

The data shows that there are significant differences between germination rate and the concentration of NaCl, for both varieties tested during this study. The data also shows that there are significant differences between root length and the concentration of NaCl, for both varieties tested during this study. ANOVA's show

T-Test	CB - GR (%) vs Concentration	CB - RL (mm) vs Concentration	GG - GR (%) vs Concentration	GG - RL (mm) vs Concentration
0 vs 0.1	0.709001756		0.00700526	2.6717E-05
0 vs 0.2	0.006424549	5.18613E-08	2.01093E-05	3.62727E-08
0 vs 0.3	4.178E-10	1.13729E-08	1.53526E-13	1.91419E-09
0 vs 0.4	1.20893E-09	1.25685E-08	5.3254E-16	1.69611E-09
0 vs 0.5	4.53676E-11	1.12485E-08	5.3254E-16	1.69611E-09
0.1 vs 0.2	0.001431412	4.0675E-05	0.008712111	0.008712111
0.1 vs 0.3	1.43288E-11	2.94933E-06	7.76196E-07	5.53675E-05
0.1 vs 0.4	5.59809E-12	9.54016E-06	5.08515E-08	4.41564E-05
0.1 vs 0.5	1.60589E-13	2.87958E-06	5.08515E-08	4.41564E-05
0.2 vs 0.3	0.000157615	0.00087703	3.30229E-07	4.40233E-06
0.2 vs 0.4	0.000103753	0.002085088	6.17274E-09	2.0604E-06
0.2 vs 0.5	1.33723E-05	0.000793039	6.17274E-09	2.0604E-06
0.3 vs 0.4	0.773046143	0.166874593	0.00488525	0.086640135
0.3 vs 0.5	0.015004964	0.040708551	0.00488525	0.086640135
0.4 vs 0.5	0.014234209	0.115248874	#DIV/0!	#DIV/0!

Table 3: Results of the Two-Tailed T-Test (Assuming Equal Variance) for different varietites of *R. Raphanistrum* subsp. *Sativus.*

CB = Cherry Belle, GG = German Giant, GR= Germination Rate, RL = Root Length, Yellow indicates a p-value below the α value of 0.025.

there were significant differences between varieties for root length at 0.2M NaCl. No other differences were observed after ANOVA's and T-Tests were performed.

Overall, *R. Raphanistrum* subsp. *Sativus* cv. German Giant had the highest germination rate (88%) for the control (0.0M NaCl), while *R. Raphanistrum* subsp. *Sativus* cv. Cherry Belle had a slightly lower germination rate (85%) for the control (0.0M NaCl). At 0.1M NaCl, *R. Raphanistrum* subsp. *Sativus* cv. Cherry Belle had a higher germination rate (79%), while *R. Raphanistrum* subsp. *Sativus* cv. German Giant had a lower rate of germination (64%). While there is large difference between the germination rate of these two varieties at 0.1M, ANOVA showed a P-vale of 0.08, which is larger than our alpha value of 0.05. With a larger sample size, there might be significant differences between the two varieties at this concentration. At 0.2M NaCl, *R. Raphanistrum* subsp. *Sativus* cv. German Giant had the highest germination rate (53%), while *R. Raphanistrum* subsp. *Sativus* cv. Cherry Belle had a lower germination rate (46%). At 0.3M NaCl, *R. Raphanistrum* subsp. *Sativus* cv. German Giant had a slightly higher germination rate (8%), while *R. Raphanistrum* subsp. *Sativus* cv. Cherry Belle had a slightly lower germination rate (7%). At 0.4M NaCl, *R. Raphanistrum* subsp. *Sativus* cv. Cherry Belle had a higher germination rate (6%), while none of the *R. Raphanistrum* subsp. *Sativus* cv. German Giant seeds germinated.

The control concentration (0.0M NaCl) showed that *R. Raphanistrum* subsp. *Sativus* cv. Cherry Belle had the largest average root length of 60.66mm, while *R. Raphanistrum* subsp. *Sativus* cv. German Giant had an average root length of 53.94mm. At 0.1M NaCl, *R. Raphanistrum* subsp. *Sativus* cv. Cherry Belle had the largest average root lenth of 24.44mm, while *R. Raphanistrum* subsp. *Sativus* cv. German Giant had an average root length of 19.84mm. Interestingly, at 0.2M NaCl, *R. Raphanistrum* subsp. *Sativus* cv. German Giant had the largest average root length of 8.35mm, while *R. Raphanistrum* subsp. *Sativus* cv. Cherry Belle had an average root length of 4.2mm. At 0.3M NaCl, the root lengths were slightly higher for *R. Raphanistrum* subsp. *Sativus* cv. German Giant (0.365mm), while *R. Raphanistrum* subsp. *Sativus* cv. Cherry Belle had an average average of 0.044mm. At 0.4M NaCl, the root lengths were slightly higher for *R. Raphanistrum* subsp. *Sativus* cv. Cherry Belle 0.305mm, where the root length for *R. Raphanistrum* subsp. *Sativus* cv. German Giant was 0.0mm. There was no root length data collected for 0.5M, since none of the seeds germinated at that concentration.

This study adds to the knowledge of salinity tolerance in *R. Raphanistrum* subsp. *Sativus*, and will hopefully benefit agriculturalists in the future. Previous studies have examined salinity tolerance in *R. Raphanistrum* subsp. *Sativus*, but they tested different varieties than this study. Kanjevac and colleagues examined various mechanisms that effected germination rate of *R. Raphanistrum* subsp. *Sativus* [20]. Su., *et al*. examined the effect of different salt concentrations on germination rate and root length of *R. Raphanistrum* subsp. *Sativus,* but looked at cv. Jiujin, Changfeng, Shunyuan, Nanpan, May, and Red. [46]. Munir [36] conducted a study to examine the effect of different salt concentrations on root biomass and chlorophyl concentrations of *R. Raphanistrum* subsp. *Sativus* cv. Deci White and Lal Pari, but did not examine Cherry Belle or German Giant. Ayyub., *et al*. [5] investigated the effects of salt stress on the morphological, physiological, and biochemical characters of *R. Raphanistrum* subsp. *Sativus* cv. Desi Long, Local Japanese, Laal Pari, 40 Days, Meno, and Green Neck.

Many other studies have examined salinity tolerance, but this expands knowledge to USDA Certified Organic *R. Raphanistrum* subsp. *Sativus* cv. Cherry Belle and *R. Raphanistrum* subsp. *Sativus* cv German Giant. This study will be useful to agriculturalists and farmers who are trying to grow USDA Certified Organic radishes in salt contaminated soils.

Conclusion

Raphanus Raphanistrum subsp. *Sativus* is a major economic crop, and is cultivated world-wide for its roots, leafy greens, seeds, as well as sprouts. Throughout the world, agricultural lands are becoming contaminated with salty ocean water from irrigation with polluted aquifers. *R. Raphanistrum* subsp. *Sativus* was shown to exhibit varying levels of salinity tolerance. The USDA Certified Organic varieties Cherry Belle and German Giant showed different germination rates and different root lengths when exposed to varying concentrations of Sodium Chloride. Differences occurred between varieties at 0.2M NaCl, which is half the salt concentration of the world's oceans. This information will help agriculturalists and farmers find the most adapted variety of radishes for varying levels of salt contamination in agricultural lands.

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