Seed Osmopriming Improves Germination, Physiological, and Root Anatomical Attributes of Red Amaranth (*Amaranthus tricolor* L.) in Salinity Stress Condition

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ARTICLE INFO

Received: 2 Dec 2022
Received in revised: 9 Feb 2023
Accepted: 21 Feb 2023
Published online: 10 Apr 2023
DOI: 10.32526/ennrj/21/202200258

Keywords:
Osmopriming/ NaCl/ PEG/ Proline/ Red amaranth

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ABSTRACT

Salinity stress is a form of abiotic stress that threatens the sustainability of agriculture in almost all countries in the world. It has an impact in reducing plant productivity. Red amaranth (*Amaranthus tricolor* L.) is a vegetable crop that has high nutritional value, but extensive saline land area can cause red amaranth yields to decline. Osmopriming is a seed priming method in which seeds are immersed in a solution that has a high osmotic potential, such as PEG (polyethylene glycol) in order to increase germination under unfavorable conditions. This study determined the effect of osmopriming on germination, physiological, and root anatomical attributes of red amaranth roots under salinity stress conditions. The research design used a completely randomized design with two types of treatment, namely, osmopriming and salinity stress. Each treatment used three concentrations, seed osmopriming with 0%, 5%, and 10% of PEG and salinity stress of 0 mM, 50 mM, and 100 mM of NaCl. The measured parameters were germination, growth, physiological, and root anatomical characters. Osmopriming of seeds with 10% PEG increased germination as indicated by the germination percentage, time, and rate reaching 95.55%, 1.393 day, and 71.98%/day, respectively. Red amaranth plants that had been osmoprimed with 10% PEG grew faster when exposed to salinity stress. Application of PEG 5% and 10% increased total chlorophyll levels while decreasing proline levels and Ca-oxalate crystal density. Under salinity stress conditions, PEG application improved the root anatomical characters of red amaranth as shown by increased epidermis thickness, cortex thickness, and stele diameter. Priming application with 10% PEG has the potential to increase the tolerance of red amaranth to salinity stress.

1. INTRODUCTION

The need for vegetables continues to increase along with increasing public awareness of the importance of a well-balanced nutrition in building immunity. Red amaranth (*Amaranthus tricolor* L.) is a highly nutritious leafy vegetable crop. Amaranth leaves and stems are rich in protein including the essential amino acids lysine, arginine, histidine as well as vitamins (A, C, K, B2, B3, and B6), minerals such as magnesium, calcium, potassium, iron, phosphorus, zinc, and iron (da Silva et al., 2019) and a red color in the form of betacyanins, which acts as an antioxidant (Li et al., 2019). Environmental stresses such as salinity stress decrease water availability (drought) and nutrients, and limit efforts to increase plant productivity.

Salinity stress occurs when an excessive amount of dissolved salt in the soil causes a decrease in plant growth and productivity. Salinity causes osmotic stress which is followed by ion toxicity, nutrient imbalance, impaired ROS detoxification ability, decreased photosynthetic rate, and disturbances in the mechanism of stomata opening and closing (Menezes et al., 2017). The capacity of the root system to absorb water declines during the early stages of stress, whereas the transpiration rate of the leaves increases dramatically.

Citation: Amalia DR, Rachmawati D. Seed osmopriming improves germination, physiological, and root anatomical attributes of red amaranth (*Amaranthus tricolor* L.) in salinity stress condition. Environ. Nat. Resour. J. 2023;21(3):232-244. (https://doi.org/10.32526/ennrj/21/202200258)

The presence of ROS causes oxidative damage to various cellular components, such as proteins, lipids, and DNA (Boughalleb et al., 2017).

Seed germination is a critical stage of the plant life cycle. Priming is a seed treatment that allows seeds to carry out the process of imbibition and activation of early metabolic processes during seed germination while inhibiting growth and emergence of radicles (Arjmand et al., 2014). Seed priming as a pre-planting treatment technique is carried out to increase germination and growth under non optimal environmental conditions (Chen et al., 2021). Priming prepares the process of seed metabolism, making the seeds more ready to germinate. Priming serves several purposes, including the production of healthier and stronger seedlings, which allow plants to grow faster in the field, as well as seedlings are protected from disease or other stresses during the development process. Typically, seed priming results in seedlings having a more developed root system, which allows them to survive stressful conditions.

Osmopriming is one of the most common used priming methods by immersing seeds in a solution with a high osmotic potential which allows pre-germination metabolic activity to continue while preventing the emergence of radicles (Debbarma and Das, 2017). Polyethylene glycol (PEG) is an osmopriming agent that is often used because it is non-toxic and has a high molecular weight. The use of PEG can improve physiological and biochemical processes in seeds by controlled addition of water to imbibition media with low osmotic potential (Pallaoro et al., 2016). Osmopriming can boosts crop yields in addition to increasing the percentage of seed germination. Osmopriming with PEG has been shown to alleviate the effects of drought stress on *Ipomoea reptans* (Latifa and Rachmawati, 2020).

The purpose of this research is to discover the role of osmopriming in increasing the growth and resistance of red amaranth (*Amaranthus tricolor* L.) under salinity stress conditions in terms of germination, physiological, and anatomical characteristics of red amaranth roots. Through the study of germination, physiological and root anatomical characters, the role of osmopriming to alleviate the negative effect of salinity stress can be determined.

2. METHODOLOGY

2.1 Experimental site and design

This experiment was conducted in the greenhouse at the Karanggayam Research Station, Laboratory of Plant Physiology and Laboratory of Plant Structure and Development, Faculty of Biology, Universitas Gadjah Mada from February 2022 to July 2022.

Complete randomized design was used to arrange this research containing two variables: seed osmopriming and salinity stress. Seed osmopriming was performed with three levels of PEG concentration of 0%, 5%, and 10%, and salinity stress with three level of NaCl concentration of 0 mM, 50 mM, and 100 mM. Each treatment combination consisted of three replications. Osmopriming was carried out by soaking red amaranth seeds in PEG solution with three different concentrations (0%, 5%, and 10%) for 24 h. Subsequently, the seeds were dried at room temperature for 24 h and then the primed seeds were germinated in a petri dish. Three replications of 15 red amaranth seeds each were placed on petri dishes Ø 9 cm on one layer of filter paper. The seeds were supplied daily with distilled water during the germination trial which lasted four days (Amalia, 2022). Seed germination was characterized by the appearance of a radicle with a length of ±1 mm. Germination characters including germination percentage, mean germination time, mean germination rate and germination rate index were observed based on the method of Al-Mudaris (1998). Germination percentage was calculated according the formula as follows:

$$\text{Germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds for germination}} \times 100$$

The germination rate provides a measure of the time course of seed germination. Mean germination rate (MGR)=CV/100=1/T; where T is mean germination time and CV: coefficient of velocity. Mean germination time (MGT)=ΣFx/ΣF; where F is the number of seeds germinated on day x. Germination rate index is calculated as the ratio between the sum of the number of seeds germinated each day and the number of days elapsed between sowing and germination. Germination rate index (GRI)= G1/1+G2/2+...+Gi/i; where; G1 is the germination percentage on day 1, G2 is the germination percentage at day 2; and so on (Al-Mudaris, 1998).

Germinated seeds were grown in tray pots for 14 days. The 14-days old individual seedling of each cultivar was transferred into a pot consisting of 2 kg growing media (compost and soil with a ratio of 1:3, respectively) and acclimated for seven days before

being treated with salinity stress at NaCl concentration of 0 mM, 50 mM, and 100 mM. Application of salinity stress was carried out for 21 days every two days and the dose given is 150 mL per polybag. The red amaranth plants were harvested at 35 days after transplanted (DAP).

2.2 Growth and physiological analysis

Growth parameters including plant height was measured from the base to the shoot tip every three days from the 14 DAP to 35 DAP, and root length was measured from the base to the tip of the longest root at 35 DAP. Dry weight of shoots and roots were recorded at 35 DAP.

Physiological parameters observed include total chlorophyll content, proline content, oxalic acid content, and Ca-oxalate crystal density. The total chlorophyll content was analyzed from 0.1 g leaf samples. Samples were homogenized in 80% cold acetone and the absorbance of supernatants was measured using GENESYS 10 UV Scanning, Thermo Scientific at the multi-wavelength of 645 nm and 664 nm according to the method of Yoshida (1976). The chlorophyll content was expressed in mg/g FW (fresh weight).

The proline level of samples was measured according to the Bates method (Bates et al., 1973). Leaf samples about 0.25 g were homogenized in 5 mL of 3% sulfosalicylic acid, and 1 mL of filtered supernatant was reacted with 1 mL of CH_3COOH, 1 mL of ninhydrin acid then incubated at 94°C for 1 h and chilled in an icebox. Two mL of toluene were added to separate the proline from the organic phase. The absorbances were measured at 520 nm and the proline content was determined by comparing the results with a standard curve of proline.

The oxalic acid content was determined with the principle of permanganometry titration according to Fitriani et al. (2016). Two grams of red amaranth leaves was crushed to a fine powder and then added to 100 mL aquadest. The extract was heated for 20 min and then filtered. The filtrate was diluted with aquadest up to 250 mL. Then, 50 mL diluted filtrate was added with 1 mL of H_2SO_4 and titrated with 0.01 N of $KMnO_4$ until the equivalent point was reached. Oxalic acid content is calculated using the following formula:

$$\text{Oxalic acid normality} \quad V_K \times N_K = V_o \times N_o$$

$$V_o \times N_o = \frac{\text{Oxalic acid mass (mg)}}{\text{EW}}$$

$$\text{Oxalic acid content (mg/g)} = \frac{\text{Oxalic acid mass (mg)}}{\text{Sample weight (g)}}$$

Where; V_K=Volume of potassium permanganate (mL); N_K=Normality of potassium permanganate (N); V_O=Volume of sample (mL); N_O=Normality of oxalic acid (N); EW=Equivalent weight of oxalic acid=63.

Ca-oxalate Crystal Density was determined according to the method described by Harijati (Harijati et al., 2011). The stem was free-hand sectioned, and the sections were placed in 70% alcohol before being examined under a microscope and documented with an optilab. Formula for calculating Ca-oxalate Crystal Density as follow:

$$\text{Ca-Oxalate Crystal Density (Crystal/mm}^2\text{)} = \frac{\text{Total observed crystals}}{\text{View area (mm}^2\text{)}}$$

2.3 Root anatomical analysis

A cross section of the root 5 cm from the base was prepared using the embedding method (Ruzin, 1999). The root anatomical structure including epidermis thickness, cortex thickness and stele diameter were observed using a binocular light microscope (BOECO BM-180) with an optilab. Measurements of epidermis thickness, cortex thickness and stele diameter were performed using Image Raster software.

2.4 Data analysis

The significant differences of the data between all treatments in each parameter were tested and obtained from the ANOVA test, were then followed by the Duncan multiple range test with a significance level of 5% using SPSS Software (IBM-SPSS Ver 25.00.US).

3. RESULTS AND DISCUSSION

3.1 Germination attributes

Osmopriming with PEG had a positive and significant effect ($p<0.05$) (Table 1) on germination parameters by increasing germination percentage, mean germination rate, and germination rate index, and decreased mean germination time of red amaranth seeds compared to control seeds without osmopriming treatment. Table 1 showed that the highest percentage of germination, mean germination rate, and germination rate index were found in the 10% PEG priming treatment with values of 95.55%, 71.98%, and 80.37%, respectively. These results were not significantly different ($p\geq0.05$) with 5% of PEG but significantly different ($p<0.05$) with control without

PEG treatment. The control seeds showed the lowest results compared to the application of 5% and 10% of PEG with the germination percentage of 82.22%, mean germination time of 2.132 days and mean germination rate of 46.94%/day.

Table 1. Seed germination attributes *A. tricolor* after osmopriming treatment

Osmopriming (% PEG)	Germination percentage (%)	Mean germination time (day)	Mean germination rate (%/day)	Germination rate index
0	82.22±3.85[a]	2.132±0.085[a]	46.94±1.83[a]	45.92±0.64[a]
5	91.11±3.84[b]	1.386±0.054[b]	71.67±2.88[b]	74.81±5.13[b]
10	95.55±3.85[b]	1.393±0.095[b]	71.98±5.08[b]	80.37±2.31[b]

The number followed by the same letter in the same column has no significant difference based on DMRT α=0.05, n=3.

These results are in line with the results of Kim et al. (2022) which showed an increase in germination properties of *Aruncus dioicus*, such as germination percentage, rate, and time to achieve 50% germination (T_{50}) compared to unprimed seeds. Improvement in germination properties caused by PEG could be facilitating water absorption and protein synthesis. Besides that, priming treatments with PEG prolongs phase II (activation) in the germination process where membrane and DNA repair occurs, accumulation of β-tubulin, and mobilization of food reserves (Ghiyasi and Tajbakhsh, 2013). Prolongs phase II (activation) in the germination process where membrane and DNA repair occurs, accumulation of β-tubulin, and mobilization of food reserves (Ghiyasi and Tajbakhsh, 2013). Starch degradation and greater sugar accumulation in primed seeds contributed positively to the attributes of better germination and stress tolerance (Lei et al., 2021). During the osmopriming treatment, the enzymes ATPase, ACC synthetase and isocitrate lyase which play a role in membrane repair also increased, thus increasing membrane integrity, and reducing metabolic leakage (Rachma et al., 2016; Raj and Raj, 2019).

Osmopriming also contributes to increase the expression of aquaporin proteins that facilitate the transport of water across cell membranes (Alleva et al., 2012). The potential for seed germination is increased by increased aquaporin expression because it enhances the water supply to the growing tissue (Raj and Raj, 2019). Additionally, osmopriming increases the accumulation of LEA (Late Embryogenesis Abundant) protein which is important for maintaining macromolecules and cell structure. This can increase seed tolerance when grown under abiotic stress conditions, such as salinity stress (Battaglia and Covarrubias, 2014).

3.2 Growth and physiological attributes

Salinity stress significantly decreased the height of red amaranth plants (Figure 1), particularly in those that had not received osmopriming treatment. Meanwhile, osmopriming-treated plants exhibited better growth than control plants despite being subjected to salinity stress, especially when treated with 10% PEG.

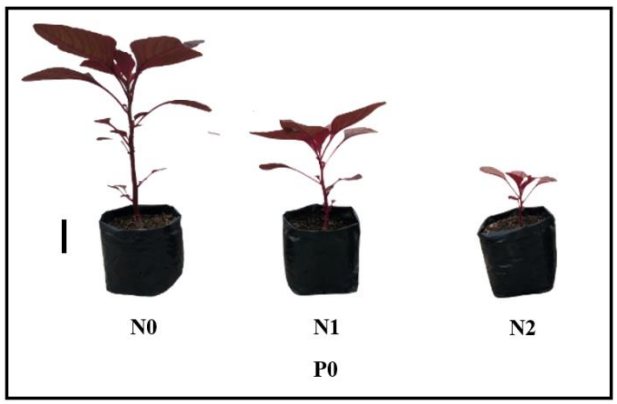


Figure 1. Plant height of red amaranth (*A. tricolor*) plants aged 5 weeks after osmopriming application with PEG (P0: 0%; P1: 5%; P2: 10%) and NaCl stress (N0: 0 mM; N1: 50 mM; N2: 100 mM). Bar=5 cm.

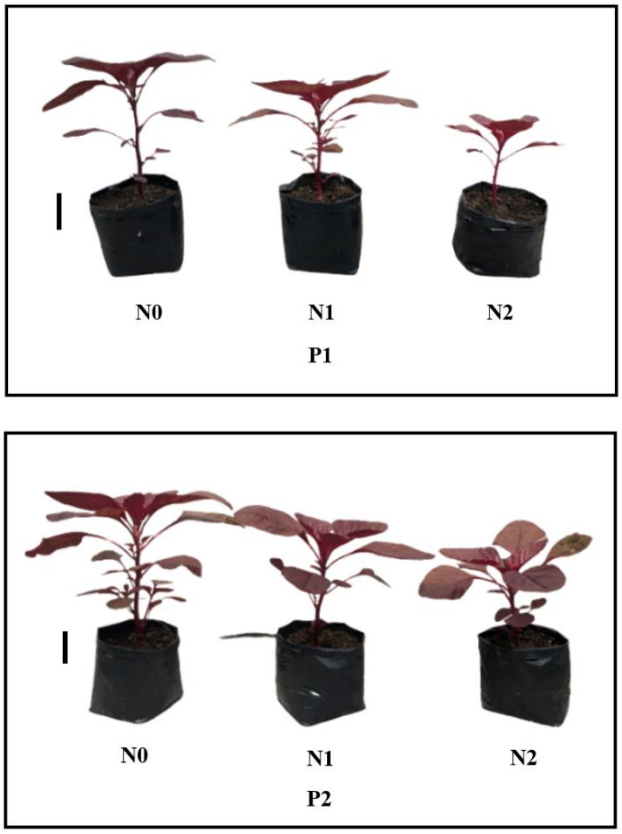


Figure 1. Plant height of red amaranth (*A. tricolor*) plants aged 5 weeks after osmopriming application with PEG (P0: 0%; P1: 5%; P2: 10%) and NaCl stress (N0: 0 mM; N1: 50 mM; N2: 100 mM). Bar=5 cm (cont.).

Plant height decreased as the concentration of NaCl increased. This is caused by increased osmotic pressure which inhibits the absorption and transport of water. Another negative effect of excess NaCl is a decrease in water potential due to stomatal guard cells losing turgor pressure causing disruption of the regulation of opening and closing of stomata. This situation hampers the gas exchange of CO_2 and O_2 and ultimately reduces the rate of photosynthesis. Another impact is the distribution of assimilates, such as carbohydrates and the distribution of various growth hormones (Kotagiri and Viswanatha, 2017). In addition, the excess concentration of NaCl causes the production of ROS (Reactive Oxygen Species) in plants to increase. Excess ROS production causes a series of oxidative damage to chlorophyll, carbohydrates, lipids, DNA and proteins that significantly affect cellular components and cell membranes (Alzahrani et al., 2019). Meanwhile, plants that were given osmopriming treatment showed good tolerance in salinity stress conditions as indicated by the increased height in red amaranth plants. Osmopriming stimulates gene expression to produce various antioxidant compounds that can protect plant cells from oxidative damage, therefore the process of division and expansion of plant cells becomes more optimal even when plants are under stress (Kubala et al., 2015). The presence of antioxidant compounds can reduce oxidative damage by reducing lipid peroxidation and increasing the stability of plant cell membranes. Osmopriming also increases the effectiveness of mobilizing several compounds, such as proteins, amino acids, and dissolved sugars from sources organ to various plant sink tissues during plant exposure to stress. Additionally, osmopriming stimulates plant growth through the mechanism of modification of sucrose metabolizing enzymes. Osmopriming increases the activity of the enzyme sucrose synthase (SS) which causes an increase in plant growth under various stress conditions. Since sucrose is the primary assimilate form transported in plants, sucrose metabolism is thought to be the primary factor in determining the sink strength. Sink strength describes the ability of sink organs to distribute assimilate to support plant growth, development, and maintenance (Kaur et al., 2005).

The increase in plant height under salinity stress in the osmopriming treatment was related to the root system (Figure 2). The plant height and root length significantly decreased as the NaCl concentration increased. According to Menezes et al. (2017), plants

experienced osmotic stress due to salinity stress which will decrease their ability to absorb water and nutrients and gradually reduce the rate of photosynthesis and result in decreased of photosynthate. This affects the translocation of photosynthate from source to sink and carbohydrate metabolism in leaves, causing a reduction in overall plant growth (Morales et al., 2012). On the other hand, osmopriming stimulates plant growth through modification of sucrose metabolic enzymes by increasing the activity of sucrose synthase, which acts as a sucrose cleavage enzyme. Sucrose acts as the main carbon link between sources and sinks and is the main form of assimilate transported in plants (Kaur et al., 2005).

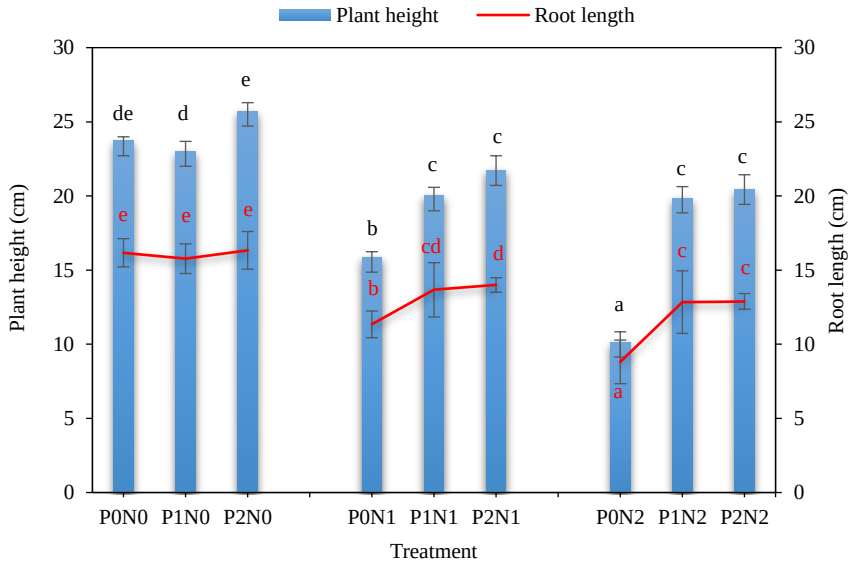


Figure 2. Plant height and root length of red amaranth (*A. tricolor*) aged 5 weeks after osmopriming application with PEG (P0: 0%; P1: 5%; P2: 10%) and NaCl stress (N0: 0 mM; N1: 50 mM; N2: 100 mM). Mean with the same letter in each character has no significant difference based on DMRT α=0.05, n=3.

The increase in root length due to osmopriming was caused by increased metabolic activity at the beginning of the germination period which overall had a valuable effect on the plant growth and development. Osmopriming has a positive effect on several biochemical activities including the breakdown of hydrolytic enzymes, protein and RNA synthesis, and DNA replication. Decomposition of food reserves and synthesis of metabolites for earlier germination of seed priming led to faster sugar availability for embryonic development. Faster sugar availability has a positive impact on plant growth and development, one of which is forming a stronger root system (Hussian et al., 2014). In contrast to the root system is related to the capacity of plants to obtain water and nutrients from the deepest layers of the soil, the growth of plant height shows the capacity of plants to obtain and use resources obtained from the soil (Price et al., 2014). As a result, roots determine growth and development of above-ground plant (Fort et al., 2013).

Salinity stress causes a decrease in several growth parameters. Salinity stress significantly reduced root and shoot dry weight, also root to shoot ratio, especially at 100 mM NaCl concentration (Table 2). These results were in line with the finding of Omami et al. (2006) where shoot growth was reduced and at 50 and 100 mM NaCl the reduction was greater in *A. tricolor* and Accession '83 than in *A. hypochondriacus* and *A. cruentus*. Additionally, Hoang et al. (2019) stated that applying a NaCl concentration of 100 mM to *Amaranthus tricolor* L. caused a 34% decrease in plant height, 40% in leaf number, and 58% in leaf area index. Because of osmotic stress, high NaCl concentrations can induce a decrease in plant water content. This condition correlates with a decrease in the rate of photosynthesis which leads to a reduction in CO_2 fixation, which in turn reduces assimilate production and has a negative impact on the accumulation of plant biomass (Benincasa et al., 2013). A decrease in the dry weight of a plant, both roots and shoots, also indicates an increase in metabolic energy demands and a decrease in carbon availability as a form of plant response to salinity stress (Puvanitha and Mahendran, 2017).

Table 2. Root and shoot dry weight of red amaranth (*A. tricolor*) aged 5 weeks after PEG osmopriming and NaCl stress

Parameters	Osmopriming (% PEG)	NaCl concentration (mM)			Average
		0	50	100	
Root dry weight (g)	0	1.39±0.36^{de}	0.67±0.57^{b}	0.41±0.35^{a}	0.82±0.43^{p}
	5	1.42±0.42^{de}	1.03±0.19^{c}	0.72±0.05^{b}	1.06±0.22^{q}
	10	1.52±0.35^{e}	1.30±0.17^{d}	0.73±0.57^{b}	1.18±0.36^{r}
	Average	1.44±0.38^{z}	1.33±0.31^{y}	0.62±0.32^{x}	
Shoot dry weight (g)	0	7.50±0.34^{bc}	5.70±0.15^{b}	2.61±0.21^{a}	5.27±0.70^{p}
	5	7.87±0.30^{c}	6.82±1.01^{bc}	3.84±2.60^{a}	6.18±1.30^{p}
	10	8.28±0.52^{c}	7.05±0.29^{bc}	6.53±0.24^{bc}	7.29±0.35^{q}
	Average	7.88±0.39^{z}	6.52±0.48^{y}	4.32±1.01^{x}	
Root to shoot ratio (g)	0	0.10±0.02^{ab}	0.09±0.02^{ab}	0.07±0.01^{a}	0.08±0.01^{p}
	5	0.11±0.01^{b}	0.10±0.02^{ab}	0.08±0.02^{ab}	0.09±0.01^{p}
	10	0.11±0.01^{b}	0.15±0.01^{c}	0.08±0.01^{ab}	0.11±0.01^{q}
	Average	0.10±0.01^{y}	0.11±0.01^{y}	0.07±0.01^{x}	

The number followed by the same letter in row and column of each parameter has no significant difference based on DMRT α=0.05, n=5.

Osmopriming increased root dry weight, shoots dry weight, and root to shoot ratio under various conditions (Table 2). The dry weight of roots and shoots, as well as root to shoot ratio, increased as the PEG concentration increased. Under conditions of salinity stress, the application of PEG 10% was able to significantly increase the dry weight of roots and shoots ($p<0.05$) compared to PEG 5% and PEG 0%. The increase in root dry weight, shoots dry weight, and root to shoot ratio in plants due to osmopriming correlated with the increase in root length. These results are in line with those of Latifa and Rachmawati (2020) which found a positive correlation between root length and dry weight of osmopriming *Ipomoea reptans* plants. A well-developed root system increases the reach of the roots in absorbing wider water so that more water and nutrients are available. These conditions increase photosynthetic activity and contribute positively to the accumulation of plant dry weight (Khan et al., 2015). Additionally, osmopriming improves the ability of plants to make osmotic adjustments, enabling them to retain their stomata, photosynthetic activity, and cellular turgor potential (Abid et al., 2017). Osmopriming also increases the rate of metabolic processes involved in germination processes, such as the synthesis of RNA, protein, and DNA during hydration of the embryo. The increase in the amount of nucleic acid caused by RNA synthesis occurs at the time of priming and after priming. In addition, the respiration process and ATP production were higher in seeds that were given primed seeds compared to those that were not given priming treatment, thereby supporting the accumulation of plant dry weight (Parera and Cantliffe, 1994).

Salinity stress results the lower chlorophyll content (Table 3). The decrease in total chlorophyll of salt-stressed plants is a result of either slow synthesis or fast breakdown, indicating that there was a photoprotection mechanism through reducing light absorbance by decreasing chlorophyll contents (Kibria and Hoque, 2019). From the research of Mane et al. (2010) has stated that at concentrations of NaCl 50 mM, the level of chlorophyll a and b was significantly reduced, followed by a decrease in the net photosynthetic rate. Chlorophyll plays an important role in carbon assimilation and maintains photo-synthetic capacity. The decrease in chlorophyll levels occurred due to an increase in chlorophyllase activity and chlorophyll degradation. In order to reduce the amount of ROS produced by chloroplasts, plants limit the amount of chlorophyll in their systems. This is one of their adaptive methods to prevent oxidative stress (Pospíšil, 2016).

The decrease in chlorophyll content at high NaCl concentrations occurs because plants experience a deficiency of magnesium ions. Magnesium functions as a key atom for chlorophyll synthesis which plays a role in the activity of the pigment-protein complex to collect photons in photosystem I (PSI) and photosystem II (PSII) (Chaudhry et al., 2021). Reduced chlorophyll pigment can also be associated with the formation of ROS, such as H_2O_2 which causes lipid peroxidation and chlorophyll damage (Nxele et al., 2017). Decreasing chlorophyll levels due to

salinity stress can reduce turgor pressure on leaves, causing water deficits, closing of stomata, and decreased stomatal conductance which ultimately reduces the rate of photosynthesis and inhibits photoassimilate transport (Wulandari et al., 2021). The PEG osmopriming effect increased total chlorophyll levels when compared to controls (Table 3). This is related to the osmopriming effect which can increase the concentration of important ions, one of which is the Mg^{2+} ion which acts as the main mineral constituent in the formation of chlorophyll (Lei et al., 2021). Increasing chlorophyll levels can increase the performance of photosystem II in the photosynthetic process resulting in an increase in the net CO_2 photosynthetic rate which overall has a positive impact on plant yields (Abdelhamid et al., 2019).

Table 3. Total chlorophyll and proline levels of red amaranth (*A. tricolor*) aged 5 weeks after PEG osmopriming and NaCl stress

Parameters	Osmopriming (% PEG)	NaCl concentration (mM)			Average
		0	50	100	
Total chlorophyll (mg/g)	0	3.490±0.10^{c}	3.162±0.05^{b}	2.523±0.02^{a}	3.058±0.06^{p}
	5	3.749±0.07^{cd}	3.474±0.14^{c}	3.569±0.36^{cd}	3.597±0.19^{q}
	10	3.878±0.07^{d}	3.632±0.04^{c}	3.552±0.07^{c}	3.687±0.06^{q}
	Average	3.705±0.08^{c}	3.422±0.08^{b}	3.215±0.15^{a}	
Proline (µmol/g)	0	0.653±0.25^{ab}	0.811±0.09^{bc}	0.957±0.03^{c}	0.807±0.12^{q}
	5	0.626±0.03^{ab}	0.513±0.14^{a}	0.740±0.12^{abc}	0.626±0.10^{p}
	10	0.616±0.10^{ab}	0.739±0.15^{abc}	0.628±0.08^{ab}	0.661±0.11^{p}
	Average	0.632±0.13^{x}	0.688±0.13^{xy}	0.775±0.08^{z}	

The number followed by the same letter in row and column of each parameter has no significant difference based on DMRT α=0.05, n=3.

Increasing the concentration of NaCl enhanced proline levels, while osmopriming with PEG significantly reduced proline levels ($p<0.05$) (Table 3). Proline levels in the osmopriming treatment with 5% and 10% PEG concentrations were lower than 0% PEG. The proline level increased as the increase in NaCl concentration the highest proline level was shown in the 100 mM NaCl concentration treatment. Proline accumulation is related to plant response to external osmotic changes. The increase in proline levels under salinity stress was caused by the breakdown of proline rich proteins (PRPs) (Abdelaziz et al., 2018). Proline acts as an osmoprotectant which is produced to protect cells from the adverse effects of salinity stress (Ahmad et al., 2013). Proline acts as a protective enzyme combats the effects of free radicals, maintains protein structure, as well as nitrogen reserves. (Abid et al., 2020). The decrease in proline levels due to osmopriming treatment showed that the application of osmopriming with PEG could reduce the level of salinity stress in plants. This is related to the accumulation and synthesis of antioxidant enzymes, such as peroxide enzymes (POD) and superoxide dismutase (SOD). Peroxidase enzymes play a role in catalyzing the reduction of H_2O_2, peroxynitrite, and various other organic hydroperoxides that can trigger oxidative stress in plants (Uddin et al., 2021), while SOD is able to catalyze the elimination of ROS by reducing them to O_2 and H_2O_2 so that the levels of free radicals in cells are reduced within the safe range (Das and Roychoudhury, 2014).

Increases in dissolved oxalate levels under salinity stress were accompanied by increases in the density of calcium oxalate crystals (Figure 3). Control plants (P0) showed higher levels of dissolved oxalate and calcium oxalate crystal density compared to plants that treated with 5% (P1) and 10% (P2) of PEG osmopriming treatments. The lowest levels of dissolved oxalate and calcium oxalate crystal density were found in red amaranth which was primed with 10% PEG.

In general, the application of osmopriming reduces the dissolved oxalic acid levels and the density of calcium oxalate crystals (Figure 3). The decrease in oxalate due to osmopriming may be due to an increase in the activity of the enzyme oxalate oxidase, an enzyme involved in the breakdown of oxalate to CO_2 and H_2O_2 (Maksimov et al., 2004). The CO_2 compounds formed can be used as a carbon source in photosynthesis, while H_2O_2 compounds will be degraded into H_2O and O_2 by ROS scavenger compounds (Tooulakou et al., 2016). The reduction in oxalate levels can also happen through the decarboxylase mechanism in addition to the oxidation process. Oxalate decarboxylase catalyzes the decarboxylation of oxalate to produce formic acid and CO_2 (Cai et al., 2018).

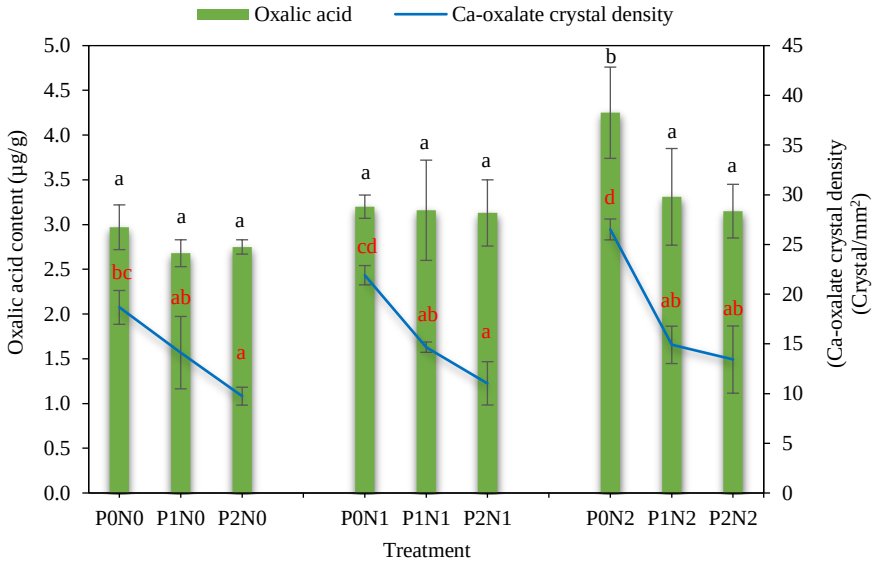


Figure 3. Ca-oxalate crystal density and oxalic acid content of red amaranth (*A. tricolor*) aged 5 weeks after osmopriming application with PEG (P0: 0%; P1: 5%; P2: 10%) and NaCl stress (N0: 0 mM; N1: 50 mM; N2: 100 mM). Mean with the same letter in each character has no significant difference based on DMRT α=0.05, n=3.

Based on research by Wang et al. (2011), plants accumulate various organic acids, such as citric, formic, lactic, acetic, succinic, malic, and oxalic acids under salinity stress. Organic acids are important intermediaries in carbon metabolism in plant cells and play a role in controlling cell physiology, including signaling messengers and absorbing nutrients from the soil. Overall, it can increase plant resistance to a certain extent in less than optimum environmental conditions (Fang et al., 2021). Oxalic acid crystals, particularly calcium oxalate crystals, are formed for the regulation of calcium, metal detoxification, and herbivore defense (Nakata, 2021). Oxalic acid can bind minerals, such as calcium which can trigger the formation of crystals (Xu et al., 2006).

3.3 Root anatomical attributes

Roots are organs that are directly affected by the salinity stress of the growing media. Salinity stress has a negative impact on plant structure and root systems. From Table 4, increasing the concentration of NaCl decreased the thickness of the epidermis, cortex thickness, and diameter of the stele. A NaCl concentration of 100 mM caused the most significant decrease in decreasing epidermal thickness, cortex thickness, and stele diameter. These results are in line with the research of Yildiz et al. (2020) which reported that excess concentration of NaCl in the root area causes the roots to be unable to absorb water and nutrients optimally due to external conditions of low water potential. This condition inhibits the process of cell expansion and growth in the plant root area. It was also reported by Boughalleb et al. (2009) that NaCl stress caused a decrease in root cortex thickness in *Nitraria retusa* L. plants and a reduction in stele and xylem areas in *Medicargo arborea* L. given a NaCl stress of ≥200 mM. The decrease in the cortex thickness is caused by cells that experience a decrease in size under stress conditions. The structure of the epidermis as a protective tissue can also be affected by high concentrations of NaCl. In addition, the toxicity of Na^+ causes inhibition of photosynthate transport to the root area, water dan solute transport, causing a significant decrease in root volume and diameter (Hasanuzzaman et al., 2022). The decrease in the value of each parameter is also due to the negative effect of salinity stress which causes changes in the shape of the cells to become flatter when compared to the control. Excessive NaCl accumulation causes changes in the uptake process of various important elements, such as Al, Ca, Fe, and Mg (Hasan and Miyake, 2017).

Meanwhile, osmopriming with PEG tended to significantly increase epidermal thickness, cortex thickness, and stele diameter ($p<0.05$) under salinity stress conditions, especially osmopriming with 10% PEG concentration. The osmoprimed plants showed better root anatomical structure, both under normal and stressed conditions. This can be related to the early growth and development of seeds. Rehman et al. (2021) reported that osmoprimed seeds had a larger embryonic surface area thereby increasing the availability of nutrients for the developing radicle and

increasing the embryo axis during germination compared to control seeds.

An increase in α-amylase and β-amylase activity during the early stages of germination is associated to improved root anatomical structure, which then enhances the availability of energy from starch metabolism and stimulates root growth (Farooq et al., 2019). Priming treatment can also promote root viability and reduce damage to root cells and tissues by maintaining the integrity of root cell membranes.

Table 4. Epidermis thickness, cortex thickness, and stele diameter of red amaranth (*A. tricolor*) aged 5 weeks after PEG osmopriming and NaCl stress

Parameters	Osmopriming (% PEG)	NaCl concentration (mM)			Average
		0	50	100	
Epidermis thickness (µm)	0	33.34±4.18^{abc}	31.27±2.76^{ab}	26.53±2.01^{a}	30.38±2.98^{p}
	5	37.02±0.92^{bcd}	42.51±6.61^{d}	39.64±6.44^{cd}	39.72±13.97^{q}
	10	41.57±5.38^{d}	35.28±3.23^{bcd}	30.60±3.77^{ab}	35.81±4.13^{q}
	Average	37.31±3.50^{z}	36.35±4.20^{xy}	32.25±4.07^{x}	
Cortex thickness (µm)	0	204.67±35.96^{bc}	158.63±11.60^{ab}	117.26±20.97^{a}	160.18±22.84^{p}
	5	216.83±15.02^{c}	205.68±42.56^{bc}	194.82±16.51^{bc}	205.77±24.70^{q}
	10	220.87±18.24^{c}	217.39±15.56^{c}	201.55±43.27^{bc}	213.27±25.70^{q}
	Average	214.12±23.07^{y}	192.57±23.24^{xy}	171.21±26.91^{x}	
Stele diameter (µm)	0	1,376.83±88.32^{cd}	984.35±68.53^{a}	808.59±174.65^{a}	1,056.59±110.50^{p}
	5	1,508.15±101.28^{de}	1,387.17±54.39^{cd}	1,011.47±100.72^{ab}	1,302.26±85.46^{q}
	10	1,649.29±192.67^{e}	1,438.59±200.95^{cde}	1,230.39±55.07^{bc}	1,439.42±115.17^{r}
	Average	1,511.42±127.42^{z}	1,270.03±107.95^{y}	1,016.81±110.14^{x}	

The number followed by the same letter in row and column of each parameter has no significant difference based on DMRT α=0.05, n=5.

Increasing the concentration of NaCl causes changes in the shape of the epidermal cells (Figure 4). Under normal conditions, the epidermis is round and neatly arranged around the cortex, whereas when the plant is under salinity stress, the epidermis will be compressed, thereby the shape is slightly flattened with an irregular arrangement. This correlates with a decrease in the area of epidermal cells (Hasan and Miyake, 2017). The decrease in cortex thickness due to salinity stress is related to a strategy of plant adaptation to shorten the distance of water transport into the stele and xylem so that roots are more effective in transporting water and nutrients. Rosawanti et al. (2015) has reported that the decrease in cortex thickness occurs due to decreased food supply and cortical cell turgidity. The decrease in root conductivity to water and minerals that occurs in response to high NaCl concentrations results in a reduction in the ability of the roots to absorb nutrients. Stele diameter decreases because the stele parenchyma shrinks during salt stress (Hasan and Miyake, 2017). A decrease in xylem and phloem area can also occur, causing obstacles to the transportation of water and important substances which have an impact on structure of the roots (Atabayeva et al., 2013).

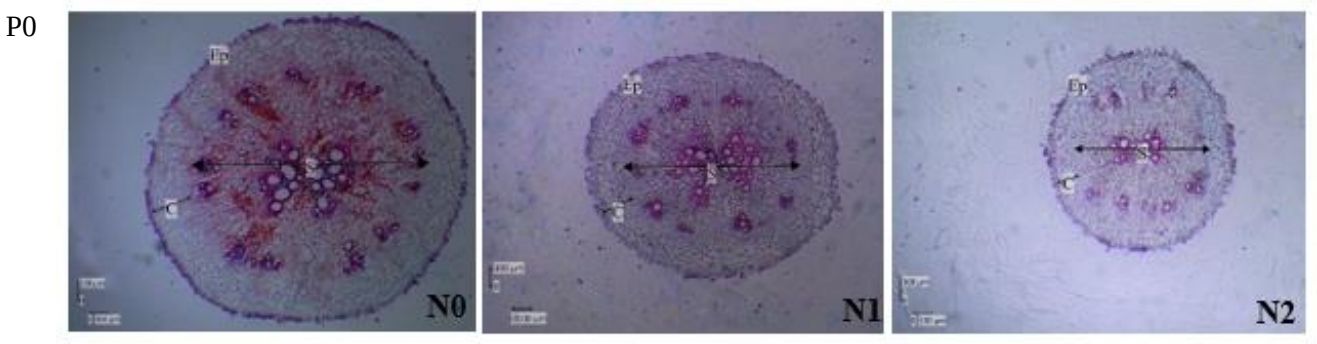
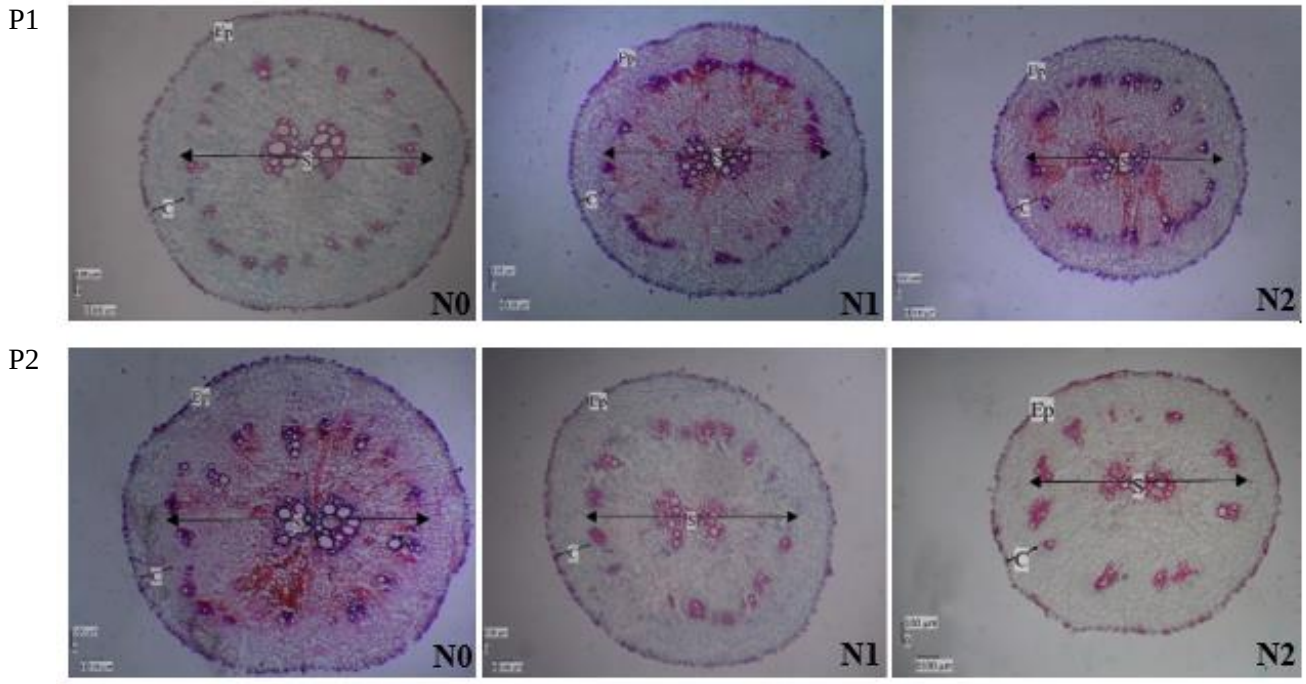


Figure 4. Cross section of red amaranth root (*A. tricolor*) plants aged 5 weeks after osmopriming application with PEG (P0: 0%; P1: 5%; P2: 10%) and NaCl stress (N0: 0 mM; N1: 50 mM; N2: 100 mM). Bar=100 µm. (Note: Ep=Epidermis, C=Cortex, S=Stele)



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4. CONCLUSION

According to the study's results, priming with PEG 6000 improved germination percentage, mean germination rate, germination rate index, plant height, leaf number, root length, chlorophyll content, and characteristics of red amaranth roots (epidermis thickness, cortex thickness, and stele diameter) under salinity stress conditions, but decreased proline, soluble oxalate, and Ca-oxalate crystal density levels. It indicates that priming with PEG 6000 reduces salinity stress in red amaranth.

ACKNOWLEDGEMENTS

This research was supported by the Research Directory of Universitas Gadjah Mada through Final Project Recognition Programs (RTA). Letter Task No. 3550/UN1.P.III/Dit-Lit/PT.01.05/2022. The authors extend our gratitude to the Faculty of Biology Universitas Gadjah Mada for supporting this research.

REFERENCES

Abdelaziz MN, Xuan TD, Mekawy AMM, Wang H, Khanh TD. Relationship of salinity tolerance to Na^+ exclusion, proline accumulation, and antioxidant enzyme activity in rice seedlings. Agriculture 2018;8(166):1-12.

Abdelhamid MT, El-Masry RR, Saleh DD, Mazhar MF, Ragab R, Oba S, et al. Mechanisms of seed priming involved in salt stress amelioration. In: Hasanuzzaman M, Fotopoulos V, editors. Priming and Pretreatment of Seeds and Seedlings Implication in Plant Stress Tolerance and Enhancing Productivity in Crop Plants. Singapore: Springer; 2019. p. 219-51.

Abid M, Hakeem A, Shao Y, Liu Y, Zahoor R, Fan Y, et al. Seed osmopriming invokes stress memory against post-germinative drought stress in wheat (*Triticum aestivum* L.). Environmental and Experimental Botany 2017;145:12-20.

Abid M, Zhang YJ, Li Z, Bai DF, Zhong YP, Fang JB. Effect of Salt stress on growth, physiological and biochemical characters of four kiwi fruit genotypes. Scientia Horticulturae 2020;271:1-12.

Ahmad P, Azooz MM, Prasad MNV. Ecophysiology and Responses of Plants under Salt Stress. New York: Springer; 2013. p. 16.

Al-Mudaris MA. Notes on various parameters recording the speed of seed germination. Der Tropenlandwirt 1998;99:147-54.

Alleva K, Chara O, Amodeo G. Aquaporins: Another piece in the osmotic puzzle. FEBS Letters 2012;586:2991-9.

Alzahrani SM, Alaraidh IA, Migdadi H, Alghamdi S, Altaf KM, Ahmad P. Physiological, biochemical, and antioxidant properties of two genotypes of *Vicia faba* grown under salinity stress. Pakistan Journal of Botany 2019;51:786-98.

Amalia DR. The Effect of Seed Osmopriming with PEG 6000 (Polyethylene Glycol) on Growth and Morphophysiological of Red Amaranth (*Amaranthus tricolor* L.) in NaCl Stress [dissertation]. Faculty of Biology, Universitas Gadjah Mada; 2022.

Arjmand HS, Sharafi S, Jouyban Z, Akhlaghi S. Effects of osmopriming on seed germination, emergence and field performance. Journal of Applied Science and Agriculture 2014;9(4):1569-73.

Atabayeva S, Nurmahanova A, Minocha S, Ahmetova A, Kenzhebayeva S, Aidosova S, et al. The effect of salinity on growth and anatomical attributes of Barley seedling (*Hordeum*

vulgare L.). African Journal of Biotechnology 2013; 12(18):2366-77.

Bates LS, Waldran RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant Soil 1973;39:Article No. 205.

Battaglia M, Covarrubias AA. Late embryogenesis abundant (LEA) proteins in legumes. Frontiers in Plant Science 2014;4(190):1-11.

Benincasa P, Pace R, Quinet M, Lutts S. Effect of salinity and priming on seedling growth in Rapeseed (*Brassica napus* var oleifera Del.). Acta Scientiarum Agronomy 2013;35(4):479-86.

Boughalleb F, Denden M, Tiba B. Anatomical changes induced by increasing NaCl salinity in three fodder shrubs, *Nitraria retusa*, *Atriplex halimus*, and *Medicago arborea*. Acta Physiologiae Plantarum 2009;31:947-60.

Boughalleb F, Abdellaoui R, Nbiba N, Mahmoudi M, Neffati M. Effect of NaCl stress on physiological, antioxidant enzymes and anatomical responses of *Astragalus gombiformis*. Biologia 2017;72(12):1454-66.

Cai X, Ge C, Xu C, Wang X, Wang S, Wang Q. Expression analysis of oxalate metabolic pathway genes reveals oxalate regulation patterns in Spinach. Molecules 2018;23(6):Article No. 1286.

Chaudhry AH, Nayab S, Hussain SB, Ali M, Pan Z. Current understandings on magnesium deficiency and future outlooks for sustainable agriculture. International Journal of Molecular Sciences 2021;22(4):1-18.

Chen X, Zhang R, Xing Y, Jiang B, Li B, Xu X. The efficacy of different seed osmopriming agents for promoting sorghum germination under salt stress. PLoS ONE 2021;16(1):1-14.

da Silva JG, Bianchini A, Costa PMC, de Almeida Lobo F, de Ameida JPM, de Moraes MF. Amaranth response to water stress. Journal of Experimental Agricultural International 2019;40(1):1-9.

Das K, Roychoudhury A. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Frontiers in Environmental Science 2014;2(53):1-13.

Debbarma M, Das SP. Priming of seed: Enhancing growth and development. International Journal of Current Microbiology and Applied Sciences 2017;6(12):2390-6.

Fang S, Hou X, Liang X. Response mechanisms of plants under saline-alkali stress. Frontiers in Plant Science 2021; 12(667458):1-20.

Farooq M, Usman M, Nadeem F. Seed priming in field crops: Potential benefits, adoption and challenges. Crop and Pasture Science 2019;70:731-71.

Fitriani H, Nurlailah D, Rakhimna. Oxalic acid content in spinach. Medical Laboratory Technology Journal 2016;2(2):51-5. (in Indonesian).

Fort F, Jouany C, Cruz P. Root and leaf functional trait relations in Poaceae species: Implications of differing resource-acquisition strategies. Journal of Plant Ecology 2013; 6(3):211-9.

Ghiyasi M, Tajbakhsh M. Osmopriming alleviates drought stress in Soybean (*Glycine max* L.) seeds during germination and early growth stages. Journal of Applied Biological Sciences 2013;7(1):27-32.

Harijati N, Arumingtyas EL, Handayani R. The effect of calcium on the size and density of crystal of calcium oxalate in Porang (*Amorphophallus muelleri* Blume). Jurnal Pembangunan dan Alam Lestari 2011;1:72-139. (in Indonesian)

Hasan R, Miyake H. Salinity stress alters nutrient uptake and causes the damage of root and leaf anatomy in maize. KnE Life Sciences 2017;3(4):219-25.

Hasanuzzaman M, Parvin K, Anee TI, Masud AAC, Nowroz F. Salt stress responses and tolerance in soybean. In: Hasanuzzaman M, Nahar K, editors. Plant Stress Physiology-Perspectives in Agriculture. IntechOpen; 2022. p. 1-37.

Hoang HL, Guzman C, Cadiz N, Dang HT. Physiological and phytochemical responses of red amaranth (*Amaranthus tricolor* L.) and green amaranth (*Amaranthus dubius* L.) to different salinity levels. Agricultural Research Communication Centre 2019;2(9):1-7.

Hussian I, Ahmad R, Farooq M, Rehman A, Amin M, Bakar MA. Seed priming: A tool to invigorate the seeds. Scientia Agriculturae 2014;7(3):122-8.

Kaur S, Gupta AK, Kaur N. Seed priming increase crop yield possibly by modulating enzymes of sucrose metabolism in chickpea. Journal of Agronomy and Crop Science 2005;191:81-7.

Khan AH, Singh AK, Maurya KN, Yadava RK. Effect of different seed priming treatments on germination, growth, biochemical changes, and yield of wheat varieties under sodic soil. International Journal of Science and Research 2015; 4(7):Article No. 14061502 .

Kibria MG, Hoque Md A. A Review on plant responses to soil salinity and amelioration strategies. Open Journal of Soil Science 2019;9:219-31.

Kim SH, Su YL, Jae YH. The effect of seed priming on the germination properties of *Aruncus dioicus*. Seed Science and Technology 2022;50(2):221-6.

Kotagiri D, Kolluru VC. Effect of salinity stress on the morphology and physiology of five different coleus species. Biomedical and Pharmacology Journal 2017;10(4):1639-49.

Kubala S, Garnczarska M, Wojtyla Ł, Clippe A, Kosmala A, Żmieńko A, Quinet M. Deciphering priming-induced improvement of rapeseed (*Brassica napus* L.) germination through an integrated transcriptomic and proteomic approach. Plant Science 2015;231:94-113.

Latifa A, Rachmawati D. The effect of seed osmopriming on growth and morphophysiology of Kangkoong (*Ipomoea reptans* Poir) in drought stress. Jurnal Agronomi Indonesia 2020;48(2):165-72. (in Indonesian).

Lei C, Bagavathiannan M, Wang H, Sharpe S, Meng W, Yu J. Osmopriming with polyethylene glycol (PEG) for abiotic stress tolerance in germinating crop seeds: A Review. Agronomy 2021;11(11):Article No. 2194.

Li G, Meng X, Zhu M, Li Z. Research progress of betalain in response to adverse stresses and evolutionary relationship compared with anthocyanin. Molecules 2019;24(17):Article No. 3078.

Maksimov IV, Surina OB, Sakhabutdinova AR, Troshina NB, Shakirova FM. Changes in the phytohormone levels in wheat calli as affected by salicylic acid and infection with *Tilletia caries*, a bunt pathogenic agent. Plant Physiology 2004; 51:228-33.

Mane AV, Karadge BA, Samant JS. Salinity induced changes in photosynthetic pigments and polyphenols of *Cymbopogon Nardus* (L.) Rendle. Journal of Chemical and Pharmaeutical Research 2010;2:338-47.

Menezes RV, Azevedo Neto ADD, Ribeiro MDO, Cova AMW. Growth and contents of organic and inorganic solutes in amaranth under salt stress. Pesquisa Agropecuária Tropical 2017;47:22-30.

Morales SG, Trejo-Tellez LI, Merino FCG, Caldana C, Espinosa-Victoria D, Cabrera BEH. Growth, photosynthetic activity, and potassium and sodium concentration in rice plants under salt stress. Acta Scientiarum-Agronomy 2012;34(3):317-24.

Nakata P. Plant calcium oxalate crystal formation, function, and its impact on human health. Frontiers in Biology 2012; 7:254-66.

Nxele X, Klein A, Ndimba BK. Drought and salinity stress alters ROS accumulation, water retention, and osmolyte content in sorghum plants. South African Journal of Botany 2017; 108:261-6.

Omami EN, Hammes PS, Robbertse PJ. Differences in salinity tolerance for growth and water-use efficiency in some amaranth (*Amaranthus* spp.) genotypes. New Zealand Journal of Crop and Horticultural Science 2006;34(1):11-22.

Pallaoro DS, Camili EC, Guimarães S, de Figueiredo e Albuquerque MC. Methods for priming maize seeds. Journal of Seed Science 2016;38(2):148-54.

Parera CA, Cantliffe DJ. Pre-sowing seed priming. Horticultural Reviews 1994;16:109-41.

Pospíšil P. Production of reactive oxygen species by photosystem II as a response to light and temperature stress. Frontiers in Plant Science 2016;7:1-11.

Price CA, Wright IJ, Ackerly DD, Niinemets U, Reich PB, Veneklaas EJ. Are leaf functional traits 'invariant' with plant size and what is 'invariance' anyway? Functional Ecology 2014;28:1330-43.

Puvanitha S, Mahendran S. Effect of salinity on plant height, shoot and root dry weight of selected rice cultivars. Scholars Journal of Agriculture and Veterinary Sciences 2017;4(4):126-31.

Rachma TNS, Damanhuri, Saptadi D. Viability and vigor of Cacao (*Theobroma cacao* L.) in some different invigoration media. Plantropica Journal of Agricultural Science 2016;1(2):72-80. (in Indonesian).

Raj AB, Raj SK. Seed priming: An approach towards agricultural sustainability. Journal of Applied and Natural Science 2019;11:227-34.

Rehman A, Nadeem F, Farooq M. Role of seed priming in root development and crop production. In: Rengel Z, Djalovic I, editors. The Root Systems in Sustainable Agricultural Intensification. United States: John Wiley and Sons Ltd.; 2021. p. 221-5.

Rosawanti P, Ghulamahdi M, Khumaida N. Anatomical and physiological responses of soybean root to drought stress. Jurnal Agronomi Indonesia 2015;43(3):186-92. (in Indonesian).

Ruzin SE. Plant Microtechnique and Microscopy. New York: Oxford University Press; 1999.

Tooulakou G, Giannopoulos A, Nikolopoulos D, Bresta P, Dotsika E, Orkoula MG, et al. Alarm photosynthesis: Calcium oxalate crystals as an internal CO_2 source in plants. Plant Physiology 2016;171:2577-85.

Uddin S, Nafees M. Effect of seed priming on growth and performance of *Vigna radiata* L. under induced drought stress. Journal of Agriculture and Food Research 2021;4:1-8.

Wang X, Geng S, Ri YJ, Cao D, Liu J, Shi D, et al. Physiological responses and adaptive strategies of tomato plants to salt and alkali stresses. Scientia Horticulturae 2011;130:248-55.

Wulandari Y, Triadiati T, Sulistyaningsih Y, Suprayogi A, Rahminiwati M. Salinity stress affects growth and physiology of mulberry (*Morus* sp.). IOP Conference Series: Earth and Environmental Science 2021;948:1-11.

Xu HW, Ji XM, He ZH, Shi WP, Zhu GH, Niu JK, et al. Oxalate accumulation and regulation is independent of glycolate oxidase in rice leaves. Journal of Experimental Botany 2006;57(9):1899-908.

Yildiz M, Poyraz I, Caudar A, Ozgen Y, Beyaz R. Plant response to salt stress. In: Abdurakhmonov IY, editor. Plant Breeding: Current and Future Views. IntechOpen; 2020. p. 1-19.

Yoshida S, Forno DA, Cock JH, Gomez KA. Laboratory Manual for Physiological Studies of Rice. 3rd ed. Philippines: The International Rice Research Institute. 1976.