UNIVERSITY OF GHANA

COLLEGE OF BASIC AND APPLIED SCIENCES

EFFECT OF SEED PRIMING ON GERMINATION AND SEEDLING DEVELOPMENT OF PEPPER UNDER SALINITY STRESS

EMMANUEL AKRASI JUNIOR

WEST AFRICA CENTRE FOR CROP IMPROVEMENT

JULY, 2018

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BY

EMMANUEL AKRASI JUNIOR

(ID. No. 10602673)

THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF PHILOSOPHY IN SEED SCIENCE AND TECHNOLOGY.

WEST AFRICA CENTRE FOR CROP IMPROVEMENT

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DECLARATION

I hereby declare that except for references to works of other researchers, which have been duly cited, this work is my original research and that neither part nor whole has been presented elsewhere for the award of a degree.

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Emmanuel Akrasi Junior

(Student)

…………………………….

Dr. Naalamle Amissah

(Supervisor)

……………………………

Rev. Prof. Frank K. Kumaga

(Supervisor)

..…………………………..

Prof. E. Y. Danquah

(Supervisor)

ABSTRACT

Salinity stress affects crop production in various stages of plant growth with germination and seed development being the most sensitive to this stress. The need to raise uniform seedlings with better crop establishment is of utmost importance. Various seed priming methods have been employed in many crops to alleviate the effect of saline, drought and temperature stress. The objective of this study was to evaluate NaCl and PEG priming for enhanced germination and improved seedling establishment in pepper under salinity stress. A study to select a priming protocol using three concentrations (25, 50 and 100 mM) of each priming agent were applied on 'Legon 18' pepper seeds at three different durations of 12, 24 and 36 hours. Results indicated that 25 mM NaCl applied to 'Legon 18' seeds for 36 hours and 25 mM PEG for 24 hours, had the highest germination percentages, germination index, and rate of germination with lowered mean germination time after seeds were sown in seed trays. Further studies using best primed treatments of NaCl and PEG were used to assess their response to germination under salinity stress. Primed and unprimed seeds were sown in transparent plastic cups at different saline levels (0, 4.46 and 8.95 dS/m). The results revealed that NaCl and PEG priming improved germination percentage, germination index, seedling length, mean germination time and seedling vigour than control though there were no significant interaction between salinity and priming among the studied traits. Primed and unprimed seeds were transplanted after 30 days of being irrigated with tap water. Saline treatments (0, 4.46 and 8.95 dS/m) were imposed after transplanting for another 30 days. Priming treatments improved plant height, number of leaves, chlorophyll content, stem diameter and total leaf area although they were reduced by increase salinity. Also, priming treatments generally increased K^+/Na^+ and Ca^{+2}/Na^+ ratios in leaves, stems and roots of pepper seedling plants than in control at all saline levels. From the results, seed priming with NaCl and PEG can be employed to increase germination and seedling performance of Legon 18 pepper under salinity stress.

DEDICATION

This work is dedicated to God for the strength He supplied to accomplish this task.

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I express my sincere gratitude to God for His favour and abundant grace on my life. Without His divine protection, I will not have come this far. I express my profound gratitude to Dr. Naalamle Amissah, Rev. Prof. F. K. Kumagah and Prof. Eric Y. Danquah who served as my supervisors. I appreciate your continuous inspiration and needful criticisms on the thesis.

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Ca^{+2}	Calcium
$CaCl_2$	Calcium chloride
cm	centimetre
CV	Coefficient of velocity
DAP	Days after planting
DQI	Dickson Quality Index
dS/m	deciSiemens per metre
EC	Electrical Conductivity
FW	Fresh weight
g	gram
G.I	Germination index
K^+	Potassium
KCl	Potassium chloride
KNO_3	Potassium nitrate
Mg	Magnesium
mg	milligram
MGT	Mean germination time
mM	milli mole
MR	Rate of germination
N	Nitrogen
Na^+	Sodium

NaCl	Sodium Chloride
NaOCl	Sodium hypochlorite
PEG	Polyethylene glycol
SQ	Sturdiness quotient
ROS	Reactive oxygen species

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

1.0 INTRODUCTION

Pepper, commonly referred to as chilli is a Solanaceous crop in the same family with crops like tomato, eggplant, potato and tobacco (Dias *et al.*, 2013). *Capsicum annuum* is the most widely propagated species, though there are about 25 to 30 known species (Csilléry, 2006, Ravishankar *et al.*, 2003).

Pepper by adding flavour, pungency and certain mineral vitamins (e.g. Vitamins A and C) is considered an important component of many foods. Capsaicin, the main active ingredient in pepper seeds has medicinal purposes that are relevant for the treatment of various disorders such as indigestion, constipation, fevers, colds, and pains (Messiaen, 1992; Dagnoko *et al.*, 2013).

Pepper is an important cash crop for many small holder farmers in developing countries like Ghana, Nigeria, Pakistan and Ethiopia (Lin *et al.*, 2013). In 2017, Ghana produced approximately 119,804 metric tonnes of pepper and was ranked the 9^{th} highest producer of pepper in the world (FAOSTAT, 2017).

The yield of pepper is averagely estimated as 8.30 Mt/ha in Ghana and this is far below from the attainable yield of 32.30 metric tonnes per hectare projected by the Ministry of Food and Agriculture (MoFA, 2011). Lower yields in pepper production have been credited to a number of factors of which low soil fertility plays a major role (MiDA, 2010).

Salinity stress affects every facet of plant growth from germination of seeds through to vegetative growth and reproductive development. The effect of salt stress on plants are consequences of multifaceted interactions between the physiological, biochemical, and morphological processes involved in the germination of seed, seedling development and the uptake of nutrients and water (Akbarimoghaddam *et al.*, 2011).

Agriculture production is affected adversely worldwide by salinity which is the most important abiotic stress (Borsani *et al.*, 2003). Salinity harmfully affects almost every aspect of the physiology and biochemistry of plants and thereby decreases yield. This can be attributed to inappropriate management of irrigation and drainage, low precipitation, high evaporation and irrigation with saline waters (Munns and Tester, 2008). In Ghana, saline soils are predominant in the Coastal Savanna Agro-Ecological Zone that lies in the south eastern corner of the country. Soils within this zone are highly saline, not supportive of crop production and poor in nutrients (Asamoah *et al.*, 2013).

Raising seedlings is of much importance to crop production as delayed and reduced germination is a common occurrence which is of much concern to farmers. The reduction and delay in the germination of seeds holds up the ability of seedlings to achieve uniformity and have vigorous plant stand. In parts of the Coastal Savannah Agro Ecological zone of Ghana where pepper is grown, pepper farmers nurse their seeds either in the open field (seed beds) or in seed trays and later transplant to the field. Both categories of farmers face challenges in relation to salinity with the former often experiencing plant development problems after transplanting and the later encountering reduced germination. These outcomes are not surprising as salinity has been shown to delay or obstruct seed germination in various ways such as the reduction in water availability, deviation in the mobilization of stored reserves and affecting the structural organization of proteins thereby hindering plant growth and development (Ibrahim, 2016).

The harm caused by salinity to production of crops has been managed by various practices including the use of tolerant varieties, application of anti-saline chemicals, and use of halophytes and leaching and drainage (González-Núñez *et al.,* 2004, Qureshi *et al.,* 2007). The use of these practices has generally been uneconomical and difficult to operate. Seed priming techniques are simple ways of managing saline soils and help to reduce time to emergence, obtain uniformity in germination and better plant growth and development in many field and horticultural crops.Seed priming have been utilized in many horticultural crops to aid in early and uniform emergence and better plant stand (Farooq *et al.*, 2006; Basra *et al.*, 2005). Priming as a seed enhancement technique has effectively led to the increase in performance of seed under salinity and drought stress (Khan *et al.,* 2009; Bewley *et al.,* 2013). Seed priming has helped improve the tolerance of seeds under salinity stress in many crops including vegetables such as tomato (*Solanum lycopersicum* L.) (Pradhan *et al.*, 2015), hot pepper **(***Capsicum annuum* var. *acuminatum* L.) (Khan et al., 2009), lettuce (*Lactuca sativa* L.) (Nasri *et al.*, 2011), okra (*Abelmoschus esculentus* L.) (Dkhil *et al.*, 2014) and pepper **(***Capsicum annuum* L.) (Aloui *et al.*, 2014).

Food production is projected to increase by 38% by 2025 and by 57% by 2050 across the world (Wild, 2003). The demand of chilli in the world is on the rise and this continuous increase in demand means that there is still more room for development through increasing the productivity of land and raising yield potentials (Asravor *et al.*,2016). Final germination of seeds and uniformity of growth of many crops including pepper have been affected by salinity stress (Sivritepe *et al, 2003;* Sivritepe and Sivritepe, 2007). High salinity levels take 1.5 million hectares of land out of production each year (Pitman and Läuchli, 2002; Munns and Tester, 2008). Thus, 50% of cultivable lands will be lost by the middle of the 21st century (Wang *et al.*, 2003).

The objectives of this study are to:

1. determine priming protocol for Legon 18 pepper

2. evaluate the effects of different priming methods for enhanced germination and improved crop establishment in Legon 18 pepper under salinity stress
3. determine the relationships between priming and plant vigour in Legon 18 pepper cultivation

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Origin and distribution of pepper

Peppers (*Capsicum* spp. L), of the family Soalnaceae, along with crops like tomatoes, tobacco and potatoes such as Irish potato and white potato is an annual herb. They originated in South and Central America (Grubben and El Tahir, 2004) comprising of thirty species and are thought to have been introduced into West Africa by Portuguese traders during the 15th century (Purseglove, 1968). It is widely propagated in many countries in the world and is considered the most significant solanaceous crop after tomato (Yoon *et al.*, 1989).

China, Mexico, Turkey and Indonesia are known to be the leading pepper producers in the world (FAO, 2017). The annual production of pepper stands at 36,143,113MT that represents an annual yield of 9.97MT ha^{-1} (FAO, 2014).

2.2 Importance and uses of pepper

Pepper is an important commercial crop primarily produced for its fruits and seeds but used in several ways based on its hotness and colour. Consumer preference for the fruits are based on the shape, colour and degree of pungency (Berke *et al.*, 2005). Fruits can be cooked or consumed raw as vegetables in soups and stews, cut and processed in several forms as fresh, dried or grounded into fine powder for seasoning and flavouring.

According to Nadeem *et al.* (2011), chillies contains vitamin A and C which is very nutritious, it also contains antioxidants including; carotenoids, polyphenols, flavonoids and ascorbic acid. In the medicinal field, pepper is used in the treatment of cold and fevers and also to help in prevent constipation (Udoh *et al.*, 2005 and Patwardhan *et al.*, 2010). Due to these antioxidant compounds, chillies guard the human body from harm prompted by free radicals and reduce risk to cardiovascular diseases, asthma, headaches, diabetes and sore throat (Simonne *et al.*, 1997).

Pepper based extracts have also been employed in many pest management programmes in controlling plant pests (Oparaeke *et al.*, 2005) and in the security agencies they are used in the manufacture of tear gas to combat criminal activities.

2.3 Salinity and crop production

Salinity, an example of abiotic stress such as irradiation, drought, and heavy metal toxicity has detrimental effect on crop production. It has been revealed that, salt stress is most predominant in the arid, semiarid and coastal regions among the environmental stresses spreading easily in irrigated fields (Munns and Tester, 2008).

About 800Mha land area, which represents 6% of the world's total land area is said to be affected by salt (FAO, 2012). The proportion of arable land inflicted by saline conditions is of much concern. Each year, about 1.5 Mha of land are taken due to increase in salt levels (Pitman and Läuchhli, 2002; Munns and Tester, 2008). This will lead to about 50% of arable lands been lost to salinity by 2050 (Wang *et al.*, 2003).

High saline concentrations in soil or irrigation water affects germination of seeds and subsequent development of seedling thereby making several plants vulnerable to the effect of this stress (Hubbard *et al.*, 2012). Areas affected with saline soils hold sufficient amounts of soluble salts that reduce growth of most plants (Flowers and Flowers, 2005). Several crops like rice, maize, cowpea, soyabean, lettuce, cabbage, okro, to mention a few are known to be sensitive to a rise in salt levels

(Basu and Roychoudhury, 2014; Banerjee and Roychoudhury, 2016; Ibrahim, 2016). Tolerance to salinity of crops differs among species as well as the growth parameters taken.

2.4 Characteristics of Salt Affected Soils

Salinity stress is regarded as the most destructive of the abiotic stresses limiting crop production significantly (Allakhverdiev *et al.*, 2000). Salt affected areas are classified under two classes; saline and sodic. Saline soils are usually occupied with sodium ions, with sulphate and chloride being the prevailing anions. Values of pH and sodium adsorption ratios (SAR) of saline soils are lower than 8.5 and electrical conductivities higher than 4 dS/m.

Sodic soils have excessive sodium ion exchange sites and much accumulation of carbonate or bicarbonate anions. Sodium adsorption ratios (SAR) in sodic soils are high with also high pHs (>8.5)

2.5 Causes of salinity

Generally, causes of salt stress to plants are grouped into two. These are primary and secondary causes. Primary causes of salinity are consequences of the build-up of salts over long periods of time, accomplished by natural processes in the groundwater or soil. The weathering of parent materials containing dissolvable salts or the carrying of salt through wind and rain may be the cause of theses natural processes. These natural processes may be due to weathering of parent materials which contain soluble salts or carrying of salt through wind and rain. Saline levels of soils may be increased by climatic factors and water management systems. Most dry and semiarid terrains get under 500 mm of precipitation every year and this, combined with a yearly potential evapotranspiration of around 2000 mm increases salinization (Wanjogu *et al.*, 2001)

Secondary causes of salinity arise as a result of human factors including; inappropriate methods of irrigation, deforestation, and poor discharge of industrial waste water and large levels of salt deposition from intensive agriculture. Present day anthropogenic land utilization is expanding the region of areas affected with salt, which is a noteworthy environmental issue (Bridgman *et al.*, 2008). In many irrigated fields, there has been a notable rise in the water table due to over application of water coupled with inadequate drainage. Manchanda and Garg (2008) have indicated that a contributory factor to secondary salinity arises from most irrigation systems in the world.

2.6 Salinity effect on different stages of plant growth and development

2.6.1 Response of germination to salinity

The stages associated with germination and developments of seedlings are the two fundamental and vital phases for crop establishment and development (Hubbard *et al.*, 2012). The most sensitive stages of many crops to abiotic stress are germination and seedling development (Yadav *et al.*, 2011). Decrease in germination percentage and increase in time to germination has been observed among various crops when saline levels were increased (Rouhi *et al.*, 2011)

Germination of seeds is normally affected by the incidence of high levels of salt found within the seed planting region. Salts interfere with seed's ability to take up moisture. Seeds of crops are typically planted within the topmost (10cm layer) of the soil. The saline concentration within the top layer is higher compared to lower parts (Esechie, 1995). Germination of seeds become non uniform and seedling growth is deficient in salt affected soils (Ibrahim, 2016). Generally, low salt levels decrease rate of germination by inducing a state of dormancy (Shannon and Grieve, 1999) whereas high salt accumulation inhibits germination thereby decreasing total germination (Khan and Weber, 2008)

Salinity stress affects the time taken for seeds to germinate and also rate of germination (Mudgal, 2004). Läuchli and Grattan (2007) projected a summed up connection between germination percentage and the period taken for seeds to germinate after the addition of varying saline concentrations of water (Figure 1). Salt stress is known to affect several crops such as cabbage, okro and cowpea (Thiam *et al.*, 2013; Dkhil *et al.*, 2014; Sarker *et al.*, 2014; El-Shaieny, 2015). Yildirim & Güvenç (2006) reported that germination and rate of germination of eleven pepper cultivars decreased as salinity was increased. Similar results of decreased germination and longer time to germination of pepper under salinity stress has been reported (Yilmaz *et al.*, 2004).

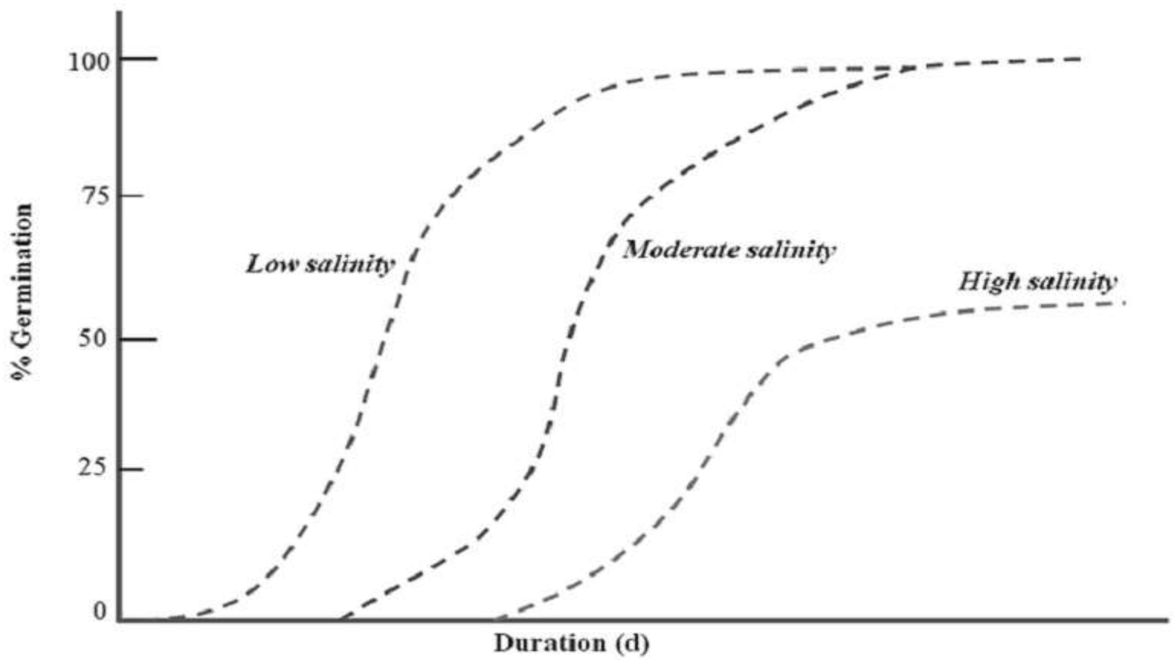

Figure 1: Germination and period taken for germination under different salinity stress (Source: Läuchli and Grattan, 2007).

2.6.2 Response of seedling growth to salinity

Seedling development, in most crops is more vulnerable to high concentrations of salt than in other developmental stages. One preliminary consequence of salinity to crop production is the decrease in rate of growth (Hasanuzzaman *et al.*, 2013). The mechanism by which salinity affects seedling development is influenced by the extent of time to which the seedling is exposed to salt. Plants control water transport in the wake of salt stress because adequate volume of water is needed to sustain growth and perform other cellular functions such as photosynthesis and other metabolic exercises (Horie *et al.*, 2012).

Plants lose water from cells when cultivated in saline environment within the first few minutes. After some hours, cells recover and increase to their normal size but the elongation rates are still affected and decreased. This brings down the growth rate of leaves and roots. Rate of division of cells over days are also influenced and contribute to bring down the rate of leaves and roots development (Munns, 2002).

Seedling length diminishes with increase in salinity (Akbarimoghaddam *et al.*, 2011). Seedling and initial vegetative stages are mostly susceptible to high salt concentrations in many plants (Hasanuzzaman *et al.*, 2013). It has been revealed by several authors that, salinity caused reduction in seedling length of cotton (Meloni *et al.*, 2001) sugar beet (Ghoulam *et al.*, 2002) and tomato (Romero-Aranda *et al.*, 2001).

2.6.3 Response of plant water relations to salinity

Plant growth reduction as a consequence of increased concentration of salt may be due to salinity effects on water relations. Romero-Aranda *et al.*, (2001), reported that increment in salt within the root region may result in a decline in leaf water potential and, consequently affect many plant developments. The osmotic impacts of salt on crops are the outcome of the lowering of the soil water potential in the root region as a result of increase in salt concentration. During low soil water potential, plants maintain turgor as their capacity to draw water from the soil is interfered (Sohan *et al.*, 1999) but plants accumulate solutes and maintain a potential rise for water entry at moderately higher soil water potential (Hasanuzzaman *et al.*, 2013). It has been reported by numerous authors that water and osmotic potential become more negative whereas tugour pressure rises when salinity is increased during crop production (Meloni *et al.*, 2001; Romero-Aranda *et al.*, 2001).

2.6.4 Response of plant height to salinity

A varied range of metabolic processes of is affected by salt stress resulting in stunted growth, reduced enzymatic activities and photosynthetic carbon metabolism. High concentrations of salt in soils or irrigation water may result in decreased plant height. Plant height studied in tomatoes and asparagus plants were affected by saline soils (Jumberi *et al.*, 2002). Similarly, plant height was reduced in rice crops grown under increasing saline concentrations in soil (Hasanuzzaman *et al.*, 2009). Height of pepper plants were observed to decrease with an increase in salinity (Hussein *et al.*, 2012; Sakr *et al.*,2015).

2.6.5 Response of root length and volume to salinity

Root structure (diameter, length, etc.) is an important factor as it determines how plants take up water in saline soil as well as nutrient absorption (Acosta-Motos*et al.*, 2017; Schleiff, 2008). The roots are the first point of contact when plants are exposed to saline soil. Roots are very sensitive to and reduce quickly with increase in salinity (Alshammary *et al.*, 2004).

Root length have been reported to reduce due to increase in salt water in many plants including maize (Khan *et al*, 2003), ryegrass (Nizam, 2011), and soyabean (Dolatabadian *et al.*, 2011).In pepper, root density decreased proportionally when saline irrigation was increased while non-salinized control had the highest average root density (De Pascale *et al.*, 2003).

2.6.6 Response of leaf area and number to salinity

Leaf area, according to Azevedo Neto and Tabosa (2000) is considered as an important parameter to assess the influence of salinity on plants. The reduction or decrease in leaf area of plants observed under salinity stress shows the arrest of leaf expansion, which eventually limits the area available for photosynthesis (Vadez *et al.*, 2005). Leaf area at both vegetative and flowering stages in mustard decreased with increasing sodium chloride salinity (Garg *et al.*, 2006). Romero-Aranda *et al.* (2001) in tomatoes, Najafian *et al.* (2008) in bitter almond and Jamil *et al.* (2007) in sugarcane observed reduction in leaf area due to increase in salinity.

Number of leaves may reduce significantly with increase in salinity. This results from the osmotic effect which may lead to death of leaves thereby reducing the photosynthetic ability of the plant which eventually affects yield (Hasanuzzaman *et al.*, 2013).

2.6.7 Response of dry matter production to salinity

Amirjani (2011) reported that salinity stress decreases plant dry matter and leaf area which eventually decreases crop yield. Shoot dry matter reduced when saline water of different concentrations were used in watering plants of mustard (Garg *et al.*, 2006). Saline treatments imposed reduced seed yield and shoot dry weight by 16.6% and 23.9% respectively. Irrigating water of EC 4.4 dS/m and 8.5 dS/m decreased weight of leaves and stem in pepper (De Pascal *et* *al.*, 2003). Similar results in shoot and root dry weight decreasing with increasing salinity in pepper production were observed by Yildirim and Güvenç (2006).

2.6.8 Physiological and Biochemical parameters response to salinity

2.6.8.1 Chlorophyll content

Photosynthesis and cell growth are two crucial processes noted to be influenced by salt stress (Munns *et al.*, 2006). Chlorophyll pigment responsible for photosynthesis comes under severe pressure under increased salt concentrations. Plants adjust to salt stress in a manner that depending on the extent and severity of the stress, plants build up osmotic compounds in order to sustain tissue metabolic activity. Plants also synthesize compatible solutes and adjust ion transport to restore cellular homeostatsis (Chaves *et al.*, 2009).

Several authors have noticed the adverse harm caused by salt stress on stomatal conductance, net photosynthesis and rate of transpiration as a result of variations in chlorophyll content and damage to chloroplast structure (Fidalgo *et al.*, 2004; Pinheiro *et al.*, 2008). Chlorophyll amount in maize was reported to decrease with sodium chloride application (Sepehr and Ghorbanli, 2006). Sahoo *et al.*, (2001) found a major decline in chlorophyll content in rice cultivars irrigated with 100 mM NaCl. Similar results were observed by Albassam (2001) in pearl millet.

2.6.8.2 Soluble protein

Soluble protein content was significantly decreased due to salt concentrations of 50, 100 and 150 mM NaCl in green bean and the effect increased with time from 42 to 72 hours (Yurekli *et al.*, 2004). Moussa (2004) observed a major decrease in protein content when plants were irrigated with 100 and 200 mM NaCl by 20.3% and 41.7% respectively. Similarly, decline in total soluble protein content have been shown in tomato, broad bean, rice, small flower bruguiera (*Bruguiera parviflora*) and Chinese spinach (*Basella alba*) plants under sodium chloride stress (Wang and Nil, 2000; Al-aghabary *et al.*, 2005; Parida and Das, 2005; Parvaiz and Satayavati, 2008).

2.6.8.3 Mineral nutrition

The roots being the first point of contact with high levels of sodium enhance the absorption of sodium. The effect of salt stress is noticed temporarily by prompting osmotic stress enabled by reduction in water availability and inducing toxicity in the long term due to nutrient contents being disproportional in the cytosol (Munns, 2005). The ion toxicity induced by salt stress is linked to excessive sodium and chlorine uptake leading to a deficiency in potassium and calcium and other nutrient imbalances (Marschner, 1995). The harmful influence of salt stress to plants are due to both water deficit from the incidence of high solute levels in the soil and changed K^+/ Na^+ ratios and high Na^+ concentrations that are unfavourable to crop plants. Zhang *et al.* (2001) submitted that, K^+ ions may be displaced from its carrier binding site by Na^+ ions and eventually impair K^+ uptake by plants and lower K^+ cytosolic concentrations.

Increased salinity decreased amount of nitrogen, phosphorus and potassium but increased sodium concentration in the shoot tissue in mustard (*Brassica napus*). There was an average decline in concentration of nitrogen, phosphorus and potassium of about 22% at the flowering stage when salinity level was $10dSm^{-1}$ whereas sodium concentration increased by 219% compared to control in mustard (Garg *et al.*, 2006).

Salinity stress adversely affects nitrogen uptake and use in many plant species (Burman *et al.*, 2002). Mineral deposition of nitrogen, phosphorus, potassium, magnesium, zinc, sulphur, copper and manganese were reduced by 30 - 60% in maize under salinity stress, while Ca deposition reduced by 90% (Beatriz and Bernstein, 2005).

2.7 Process of priming seeds

Seed priming is considered as a pre-sowing treatment where seeds are exposed to certain solutions for specified duration of time allowing seeds to hydrate nonetheless restrict emergence of the radicle (Ibrahim, 2016). Bewley *et al.* (2013) and Rosental *et al.* (2014), reported that the three phases of germination that occur when seeds are kept in water are i) imbibition, ii)the lag and iii) the protrusion of the radicle through the testa. Figure 2 shows a diagrammatic representation of these phases.

Water supplied to seeds during priming is controlled and keeps the moisture of seeds beneath that required for real germination to occur. At this state, seeds start many physiological processes that are linked to the initial segment of germination but are prevented from transitioning toward full germination (Paparella *et al.*, 2015). Seeds are held within the lag phase and are prevented from entering the phase where the radicle emerges, that is phase III (Figure 2)

Seeds are re-dried after been picked from the priming solution. The advantageous influence of the priming protocol is maintained after the re-drying and does not cause quick deterioration of seeds (Varier *et al.*, 2010; Ratikanta *et al.*, 2011). Seeds allowed entering phase III where radical emergence occur cause damages to seed by losing their vigour and viability (Rajjou *et al.*, 2012). Two factors that determine the longevity of primed seeds at this stage are the conditions that exist during the drying of seeds after priming and nature of storage. A hasty re-drying of seeds to their initial moisture level may alter concentrations of soluble carbohydrates and reduce tolerance of seeds to dehydration and longevity (Gurusinghe and Bradford, 2001).

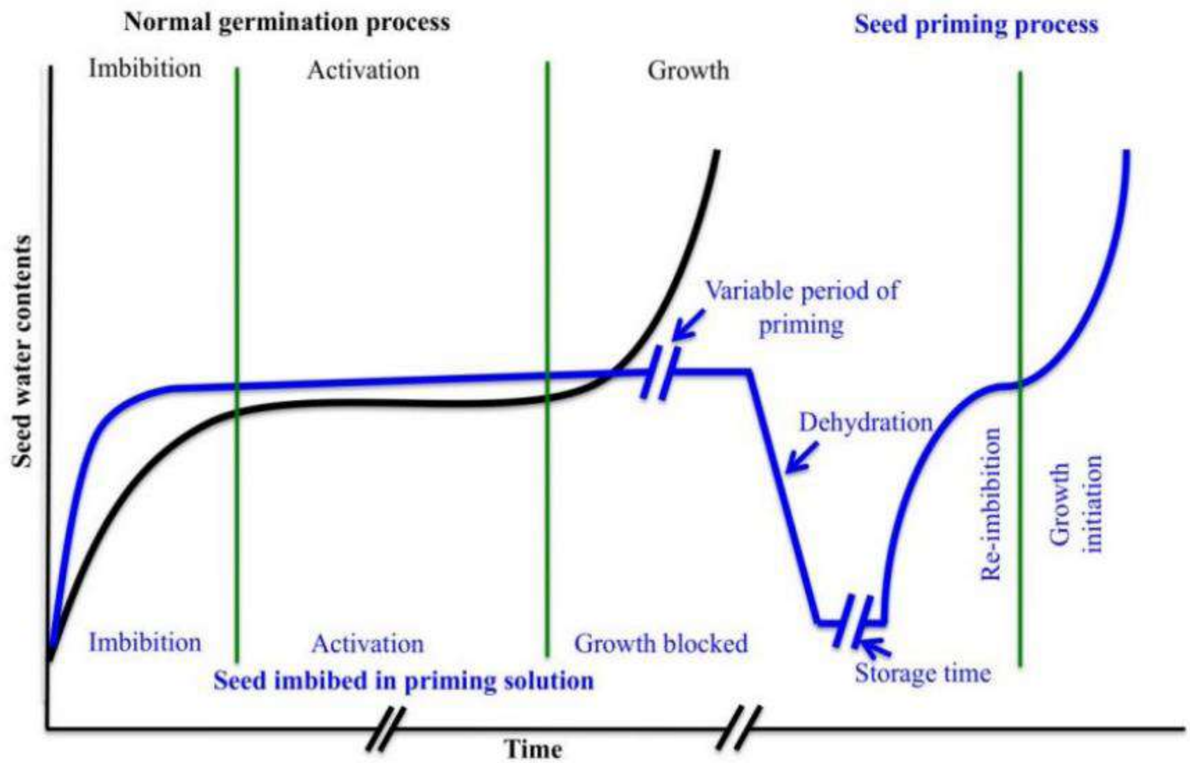

Figure 2: Seed priming process (Source: Rajjou *et al.*, 2012)

2.8 Priming Techniques

The classification of the several priming methods employed depends on the nature of the priming materials used. Priming methods such as osmopriming, hydropriming, hormonal priming, halopriming, solid matrix and hardening have been utilized to quicken germination, seedling development and increase crop yield under both normal and stress conditions. Eight priming techniques are used (Table 1) (Ibrahim, 2016). Hydropriming, halopriming, osmopriming and hormone priming are the most often used priming techniques out of the eight (Ibrahim, 2016). The duration of priming, water potential, vigour of seeds, plant variety and post-priming storage conditions determines the success of the priming used. The outcome of the technique therefore depends on the best strategy employed (Maiti and Pramanik, 2013)

Table 1: Seed priming techniques

Priming technique	Treatment composition
Hydropriming	Treated with water
Halopriming	Inorganic salt solution eg. NaCl, KCl
Osmopriming	Aqueous solution of osmolytes like polyamines, polyethelene glycol, etc.
Hormone priming	Plant hormones like gibberellic acid (GA) , salicylic acid (SA), abscisic acid (ABA), etc.
Hardening	Redrying of hydrated seeds
Solid matrix (Matriconditiong)	Mixing seeds with solid materials like vermiculate and water in known proportions
Physical	Irradiation, heat, etc.
Humidification and Stratification	Processing of seeds for instance with moist or cold treatments to enhance quicker germination

2.9 Physiological, molecular and biochemical changes prompted by priming

Seeds after priming have their imbibition and lag phases shortened (Khan *et al.*, 2009). Water absorption is facilitated by the swelling up of the embryo of primed seeds (Elouaer and Hannachi, 2012). Pre-germinative metabolic activities are induced when seeds are primed and this prepares seeds for the protrusion of their radicles (Farooq *et al.*, 2007). The resistance of endosperm during the imbibition phase is reduced by priming. Also, seed priming helps in repairing membranes and developing young embryos (Balestrazzi *et al.*, 2011; Bewley *et al.*, 2013). Seedling emergence obtained from various priming techniques are faster with higher vigour and develop better under stress conditions (Sadeghi *et al.*, 2011).

Changes that occur during priming include division of cells and elongation, stimulation of stress responsive proteins and plasm membrane fluidity which improves the performance of seeds and provide quicker and uniform germination (Sivritepe *et al.*, 2003; Kubala *et al.*, 2015). Coupled with these, priming may produce a moderate environmental stress during seedling development (Yacoubi *et al.*, 2011). In 2013, Pastor *et al.* stated in literature that, stress tolerance achieved by primed seeds is an indication that “primed plants do not forget” and that priming helps imprint stress memory on seeds. Seeds that are primed are first given pre-germination abilities such as heightened energy metabolisms, endosperm weakening and quick reserve mobilization (Pandita *et al.*, 2007; Chen and Arora, 2011). Priming enables alteration of inactive dormant seeds into a germinating position thereby improving its germination potential (Ibrahim, 2016). Also, priming enacts an abiotic stress that suppresses radicle emergence of seeds but excites stress responses, inducing cross-tolerance. A “priming memory” in seeds is formed by these two schemes which seeds later rely on during exposure to later stress by making primed seeds more tolerant to stress (Bruce *et al.*, 2007; Chen and Arora, 2013; Pastor *et al.*, 2013).

Priming increased the activities of many enzymes such as proteases associated with the metabolism of proteins, carbohydrates and lipids mobilization (Gamboa-deBuen *et al.*, 2006; Di Girolamo and Barbanti, 2012). Varier *et al.*, (2010) reported that such enzymes are necessary for breaking down macromolecules resulting in growth and expansion of the embryo. Prompt DNA replication and repair, reduction in leakage of metabolites and increase in RNA are facilitated by priming (Netondo *et al.*, 2004; Afzal *et al.*, 2008; Paparella *et al.*, 2015)

During the priming of seeds, numerous germination associated qualities are enhanced (Sharma *et al.*, 2015). Developments of seedlings are quickened as biophysical procedures essential for germination are heightened (Posmyk *et al.*, 2009). Seed priming reconstructs the gene expression for production of antioxidants and protects cells against oxidative harm (Wahid *et al.*, 2008; Kubala *et al.*, 2015)

2.10 Seed priming and salinity stress

Osmotic adjustment, the process by which plants overpower osmotic harm induced by salinity is achieved as a result of the buildup of inorganic solutes or the production of organic solutes like sugars which help plants to respond to reduced water potential from outside (Munns, 2005; Zhu *et al.*, 2011). The presence of organic solutes inside seeds and the activity performed by protective enzymes is an indication that plants are adjusting to salinity stress by promoting the reduction of cell osmotic potential (Matias *et al.*, 2015). Seed priming may intensify these factors, which can diminish the influence of salinity to plants (Kubala *et al.*, 2015)

The harmful effects of salt stress on emergence in seedling development is lessened by seed priming as seedlings stimulate potassium and calcium buildup, reducing sodium and chlorine uptake (Ashraf *et al.*, 2003; Bakht *et al.*, 2011). Ashraf (2004) stated that, reduction in osmotic potential is achieved thereby increasing water uptake of plants. Cherel (2004) stated that, "a key role fulfilled by Potassium is to ensure a balance in membrane potential and tugour, activate enzymes and regulate osmotic pressure within the cells of plants". Calcium is also a contributory factor for the structure of the cell wall, elongation and division of cells (Yadav *et al.*, 2009). A vital role played by calcium is to regulate nutrients and water uptake across cell membranes (Summart *et al.*, 2010). According Gobinathan *et al.* (2009), calcium alleviates the harmful influence of sodium on development of plants.

The process of salt stress leads to the alteration of normal cellular metabolism due to the presence of reactive oxygen species (ROS) which degrades protein and nucleic acids, peroxides lipids and produce malondialdehyde (MDA) (Mittler, 2002; Mei and Song, 2010).Cells hold many antioxidants (enzymatic and non-enzymatic) which protect seeds and scavenge ROS (Gupta, 2010; Das *et al.*, 2015). Seed priming increases the activities of antioxidant enzymes (Sharma *et al.*, 2014) and germination is enhanced through the defense mechanisms caused by these changes (Sedghi *et al.*, 2014).

$KN0_3$ used as a priming agent under salt stress increased soluble sugars and proteins during germination in pigeon pea (Verma and Srivastava, 1998) and increased the activities of phytase and acid phosphatase in shoots and roots of lettuce (Nasri *et al.*, 2011). $CaCl_2$, KCl and NaCl priming had a significant increase in proline buildup in pepper (Aloui *et al.*, 2014). Also, PEG and hydro-priming of sunflower seeds resulted in an increase in proline content when cultivated under saline conditions (Moghanibashi *et al.*, 2013). Leaves of faba bean (*Vicia faba)* seeds primed increased total phenolic content, potassium, calcium, Ca^{+2}/Na^{+} and K^{+}/Na^{+} under salt stress when primed with melatonin (Dawood and El-Awadi, 2015). Similarly, other authors have reported on the advantageous effect of seed priming to alleviate salt stress like sand priming (Nawaz *et al.*, 2012), β-amino butyric acid (Jisha and Puthur, 2015), Choline priming (Salama *et al.*, 2011) and glyciebetaine (Korkmaz and Şirikçi, 2011).

The review of literature clearly demonstrates the benefits of priming as it has been used to alleviate the effect of salinity in crop production by increasing the activities of enzymes and antioxidants, maintaining cell structure, increasing water uptake and maintaining a balance in mineral accumulation in order to reduce ion toxicity in plants.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Priming of seeds in the laboratory

3.1.1 Pepper variety

Pepper variety 'Legon 18' was used as the seed material. The seeds were obtained from the Department of Crop Science of the University of Ghana.

3.1.2 Priming Agents

Priming agents used were Sodium Chloride (NaCl) of molecular weight 58.44 and Polyethylene glycol (PEG) of molecular weight 8000. Seeds were soaked in either priming agents at three different concentrations (25 mM, 50 mM, and 100 mM)

3.1.3 Methodology

Seeds were soaked in the priming agents after they were surfaced sterilized in 1% NaOCl for three minutes and subsequently seeds were thoroughly washed in distilled water four times. The seeds were allowed to stay in the priming agents for three different time durations, namely; 12, 24 and 36 hours at room temperature. The priming regime was staggered so that seeds were removed from the solutions at the same time. Seeds of pepper soaked in distilled water for the same time durations served as controls. The priming of pepper seeds was done at the Biotechnology Laboratory of the University of Ghana. Nine treatment combinations each of NaCl and PEG priming protocol were used (Table 2).

Table 2: Nine NaCl and PEG treatment combinations, concentrations and time durations

Treatment Code	Concentration	Time duration
T1	25 mM	36 hours
T2	50 mM	36 hours
T3	100 mM	36 hours
T4	25 mM	24hours
T5	50 mM	24hours
T6	25 mM	12 hours
T7	50 mM	12 hours
T8	100 mM	12 hours
T9	100 mM	24hours

After seeds were primed in the respective priming agents for the specified time, they were taken out and washed in distilled water three times. 'Legon 18' pepper seeds were later dried in and kept in a refrigerator for a day after the priming regime until further use.

3.2 Experiment 1

3.2.1 Determination of optimum priming protocol for 'Legon 18' pepper

In order to estimate the effect of priming on germination traits of 'Legon 18' pepper, a Completely Randomized Design factorial experiment with 3 replications was used. Experimental factors included 3 priming materials (NaCl, PEG and Control), 3 concentrations (25, 50 and 100 mM) and 3 time durations (12, 24 and 36 hours).

Pepper seeds stored in the refrigerator at a temperature of 4^0C were taken out and sown in plastic seed trays under shade in the Sinna Garden of the Department of Crop Science of the University of Ghana. The trays were filled with 1:1 part of top soil and cocoa peat (v/v). The trays were watered and seeds sown thereafter. One seed per hole was sown in the seed trays for each treatment. Each treatment had 25 seeds and the experiment was replicated three times. In all, 22 seed trays were used for the exercise. Watering was done once a day and germination of seeds were monitored and recorded daily from day one to fifteen. The following data were taken:

Germination percentage: The formula for estimating the germination percentage was:

$$\text{Germination \%} = \frac{\text{Number of germinated seedlings}}{\text{Total number of seeds planted}} \times 100\%$$

Germination Index: Germination index was calculated using the formula:

$$\text{Germination Index} = \sum\left(\frac{\text{Gt}}{\text{Tt}}\right)$$

where Gt represents the number of seeds that germinated on day d and Tt is the number of days.

Coefficient of velocity: Coefficient of velocity (CV) was calculated following the formula proposed by Scott *et al.*, (1984).

$$\text{CV} = 100\left[\frac{\sum \text{Ni}}{\sum \text{NiTi}}\right]$$

Where N represents the number of seeds germinating on day i,and Ti represents the number of counted days.

Mean Germination Time: Mean germination time (MGT) was calculated by the formula proposed by Dezfuli *et al.*, (2008).

$$\text{MGT} = \Sigma\text{Dn} / \Sigma\ \text{n}$$

Where n represents the number of seeds that germinated on day D, and D being the number of days counted from the beginning of germination of seeds.

Mean Germination Rate: Rate of germination was calculated using the formula

$$\mathrm{MGR} = \left[\frac{1}{\mathrm{MGT}}\right]$$

Where MGT is the mean germination time

3.3 Experiment 2

3.3.1 Assessing germination of primed 'Legon 18' pepper under salinity stress

3.3.1.2 Determination of saline treatments

In order to estimate the effect of priming on seedling germination of 'Legon 18' pepper under salinity, a Completely Randomized Design factorial experiment with 3 replications was used. Experimental factors included 3 priming materials (25 mM NaCl, 25 mM PEG and Control) and 3 saline levels (0, 4.46 and 8.95 dS/m).

Soil samples were obtained from three pepper growing areas (Ada, Sege and Nakankope) to determine seed priming effect of seeds to salinity stress. The soil samples were analysed for their salinity contents at the Department of Soil Science of the University of Ghana. The electrical conductivities (EC), pH and Na content of the soil samples were undertaken (Table 3).

After soil samples were analyzed, two saline levels; 4.46 dS/m and 8.95 dS/m were selected to be used together with the control (tap water) as the saline treatments for the experiment. The saline treatments were attained by adding 2.5g NaCl to a litre of distilled water for the 4.46 dS/m and 5g/l NaCl for the 8.95 dS/m.

Table 3: pH, EC and Na content of soil samples collected from six different pepper farms

Sample number	Area	pH	EC (dS/m)	Na (Cmol/kg)
1	Ada	5.82	0.66	13.49
2	Ada	6.98	0.24	13.61
3	Sege	4.92	0.04	13.48
4	Sege	6.22	8.38	17.55
5	Nakamkope	6.95	6.95	22.76
6	Nakamkope	7.50	7.50	19.82

3.3.2 Planting of primed seeds

The best primed treatments from Experiment 1 for both NaCl and PEG were selected and used to assess the effect of priming of pepper under salinity stress. Priming protocol in 3.1.3 was followed. Plastic cups were filled with 1:1 part of top soil and cocoa peat (v/v). Transparent plastic cups were inserted in wire mesh and the set up placed under natural shade in the Sinna Gardens of the Department of Crop Science of the University of Ghana for 15 days. A seed per cup was sown. Each treatment consisted of 10 plastic transparent cups and the set up was replicated three times. Each treatment was irrigated with 150 ml of the required saline water treatment. The seeds were watered once daily. Germination was monitored and recorded daily. The saline treatments of 4.46 dS/m and 8.95 dS/m were applied every other day in order to avoid the build-up of salt.

Germination Index, Mean germination time, Germination percentage, Coefficient of velocity and Rate of germination were obtained as in Experiment 1. Other parameters recorded after the 15 days were:

Shoot length: This was determined by selecting 5 seedlings at random per replicate and taking their lengths from the soil level to the tip of the shoot with a metre rule and expressing the mean values in centimetres (cm).

Root length: The length of root was determined by measuring the length of the roots of 5 randomly selected seedlings per replicate from the base of the plant to the tip of the longest root with a meter rule. The values obtained were expressed in centimetres (cm).

Seedling fresh weight: Total fresh weights of seedlings were obtained by carefully washing off the soil attached to the roots of 5 randomly sampled seedlings with water and taking their weights in milligrams (mg) with an electric balance.

Seedling Dry weight: Total dry weight of the five sampled seedlings were recorded after been kept in an oven for 48 hours at 40°C. Dry weight was recorded and expressed in milligrams (mg)

Seedling vigour index: The formula for calculating vigour index of seedlings was proposed by Abdul-Baki and Anderson (1973) and the value obtained was expressed in percentages.

$$\text{Vigour Index} = (\text{Shoot length} + \text{Root length}) \text{ x Germination percentage}$$

Stress tolerance index: The equation proposed by Dhopte and Livera's (1989) was followed in calculating stress tolerance index.

$$\text{Stress Tolerance Index} = \frac{\text{Vigour index of treated seedling}}{\text{Vigour index of control seedling}} \times 100$$

3.4 Experiment 3

3.4.1 Assessing growth of transplanted seedling of primed 'Legon 18' pepper under salinity stress.

This experiment was to determine the response of seed priming of 'Legon 18'pepper variety to salinity stress after seedlings had been raised for a month. The best primed treatments of each priming agent (NaCl and Polyethylene glycol) obtained from Experiment 1 were used as priming treatments. Priming protocol in 3.1.3 was followed. Seed trays were filled with 1:1 mixture of top soil and cocoa peat (v/v). Control seeds were not primed. Pepper seeds were sown in seed trays and monitored for a month. The same design used for Experiment 2 was used for this experiment. After four weeks when the seedlings had attained 4 to 5 leaves, they were transplanted from the seed trays into larger plastic buckets of height 15 cm and diameter of 12 cm. Each bucket was filled with top soil of quantity of 2 kg. They were placed in a greenhouse at the University of Ghana Farms.

The saline treatments were then imposed on the plants as in Experiment 2. Each treatment had 6 buckets and was replicated 3 times. In all, 162 buckets were used for this study. The saline treatments consisted 4.46 dS/m and 8.95 dS/m. Tap water of EC of 150 µS/cm was used as the control treatment. The seedlings were watered daily with equal amounts of water for each treatment.

Before transplanting of the seedlings into the buckets, plant height, chlorophyll content, stem diameter and number of leaves were recorded. These parameters were taken twice after every two weeks (15 and 30 days after transplanting). The following data were also taken; Germination Index, Coefficient of velocity, Germination percentage, Mean germination time, Rate of germination and Stress tolerance index were obtained as in Experiment 1 and Experiment 2.

Plant height: This was determined by measuring the perpendicular height in centimetres (cm) of three records per replicate plants using a metre rule. The seedlings were measured from the soil level to the tip of the shoot.

Number of leaves: Number of leaves was counted at 1 month when seedlings were transplanted from trays to plastic buckets. Leaves were also counted at 2 and 4 weeks after transplanting.

Chlorophyll content: The chlorophyll content was determined by using Chlorophyll content meter Model CCM-200.

Stem diameter: This was determined by using a Vernier calliper to measure the diameter of plants and expressed in millimetres (mm) every two weeks after seedlings were transplanted into plastic bowls.

Total leaf area (TLA): Total leaf area was calculated following the formula proposed Kumar *et al.* (2002)

$$TLA\ (cm^2) = L \times l \times 0.80 \times N \times 0.662$$

Where L = length of leaf; l = width of leaf and N = total number of leaves

Shoot fresh weight: Shoot fresh weight was recorded by reading the value of the weights of three shoot samples per replicate from an electronic balance and recorded in grams.

Root fresh weight: The total fresh weight of root was determined by carefully rinsing off soil from the roots with tap water of three sampled plants and their weight in grams taken with an electronic balance

Shoot and Root dry weight: Shoot and root dry weights were determined after fresh weights of shoot and root samples were kept in an oven at 40°C for 48 hours.

Dickson Quality Index: The equation for calculating this quality index was proposed by Dickson *et al.* (1960).

$$\text{Quality Index} = \frac{\text{Total Seedling Dry wt(g)}}{\frac{\text{Height (cm)}}{\text{Diameter (mm)}} + \frac{\text{Shoot weight(g)}}{\text{Root weight(g)}}}$$

Sturdiness Quotient (SQ): Sturdiness quotient was determined by the formula

$$\text{SQ} = \frac{\text{H}}{\text{D}}$$

Where H, is height in cm of plant and D is stem diameter in mm

Nutrient content determination: Determination of Potassium, Sodium, Calcium and Magnesium. Subsamples of dried ground leaves, stems and roots were dry ashed at 550 °C for 4 h and thoroughly mixed with 250 mL of deionized water. The filtrate was analyzed with an atomic absorption spectrophotometer.

Protein content: 2 g of oven dried sample was mashed into powder and digested in a Kjeldahl digestion flask. A 20 ml solution made of H_2SO_4 and a Kjeldahl catalyst was used to boil the mashed sample until a clear mixture was obtained. The resulting mixture was filtered into a 250 ml flask. Distilled water was added to fill up the 250 ml mark of the flask. The filtrate was then connected for distillation for steam distillation of Ammonia. 150 ml of the distilled Ammonia, to which has been added 50 ml of 45% sodium hydroxide solution was poured into a conical flask holding 100 ml 0.1 HCl and methyl red indicator. The excess acid in the flask after the reaction between the ammonia and the acid was back titrated against 2.0M NaOH with a colour change from red to yellow. Nitrogen content was estimated before protein levels in solution were determined by multiplying estimated Nitrogen to a conversion factor of 6.25.

Total Soluble Sugars (TSS): Soluble sugar estimation was done by the method proposed by Dey (1990). Fresh leaf sample (0.5g) was reserved for one hour in 10ml of alcohol at 60^0C in an incubator. A 25 ml flask was used in decanting the extract and the residue was re-extracted. The final volume was filled up by the addition of alcohol to the 25 ml mark. 1ml aliquot was moved to a thick walled test tube and 1.0 ml of 5% phenol was added and thoroughly mixed. 5 ml of analytical grade sulphuric acid was then added to it and mixed thoroughly by vertical agitation with a glass rod. The test tube was cooled in the air for exothermic reaction to take place. The absorbance was recorded at 485 nm on Jenway 6305 spectrophotometer. The resultant concentration was determined against a standard curve obtained by using glucose solution. The amount of sugar was expressed as mg/g FW.

Estimation of total phenolic content (TPC): Concentration of total phenolic in pepper leaves were obtained following the Folin-Ciocalteau method. Determination of TPC was adopted from the method proposed by Singleton and Rossi (1965). Distilled water of volume 3.9 ml and 0.5 ml of Folin's reagent was added to 0.1ml extract. Incubation of the tube was done for 3 minutes at room temperature. Two millilitres of sodium carbonate was added and placed in a boiling water bath for 60 seconds. Absorbance was read at 650 nm after the blue colour was formed. The standard curve was constructed by using gallic acid as the standard.

Estimation of total flavonoid content (TFC) using aluminium chloride method: Total flavonoid content was determined after the method proposed by Zhishen *et al.,* (1999) with slight modifications. Distilled water was added to the 0.1 ml extract to make a volume of 5 ml. Five minutes later, 0.3 ml of $NaNO_2$ and 3 ml of 10% $AlCl_3$ was added to the 5 ml solution. Two millilitres of 1M NaOH was added to after 6 minutes. The absorbance was measured at 510 nm. Rutin was used as a standard for constructing the calibration curve.

Standard curves were used to determine the concentrations of soluble sugars, flavonoids and phenolic in the pepper leaves (Table 4).

Table 4: Compound and their standard curves

Flavonoid compound	Wavelength (nm)	Standard curves
Gallic acid	650	Y = 0.267x - 0.60
Rutin	510	Y = 0.594x + 0.03
Glucose	485	Y= 0.003x + 0.04

Statistical Analysis of Data

All data collected on Experiments 1, 2 and 3 were subjected to Analysis of Variance using Genstat statistical software (Edition 12) and differences in means were separated at 5% level of significance using Duncan Multiple Range Test (DMRT).

CHAPTER 4

4.0 RESULTS

4.1 Experiment 1

4.1.1 Germination percentage of primed 'Legon 18' pepper seeds

The highest germination percentages of 93.3% were observed for priming with 25 mM NaCl for 36 hours and 25 mM PEG for 24 hours. There was no significant interaction between concentration and time in respect of germination with NaCl primed treatments, but PEG primed treatments showed significant interactions. Priming protocols that had the lowest germination percentages than control were observed in 50 mM NaCl for 12 hours and 50 mM PEG for 36 hours (Table 5 and 6)

4.1.2 Germination Index of primed 'Legon 18' pepper seeds

Significant interactions of concentration and time were not observed for NaCl primed treatments, however, interactions were observed with PEG primed treatments. The maximum germination index for seeds primed with NaCl treatments was 3.4 and was observed in priming seeds with 25 mM NaCl for 36 hours (Table 5). The highest germination index of 3.1 for PEG treatments was noticed from seeds primed with 25 mM PEG for 24 hours (Table 6).

4.1.3 Coefficient of velocity of primed 'Legon 18' pepper seeds

Significant differences were not observed as a result of the interaction between concentration and time of priming with NaCl and PEG (Table 5 and 6).The highest CVs however were recorded for 100 mM Nacl at 36 hours (21.9) and 25 mM PEG at 12 hours (21.8).

4.1.4 Mean germination time of primed 'Legon 18'

Mean germination time values for 'Legon 18' pepper seeds primed with NaCl and PEG are presented in Tables 5 and 6 respectively. There were significant interactions between concentration and time with regards to PEG primed treatments but NaCl primed treatments showed no interaction effect. The best mean germination time for NaCl treatments was 7.19 when seeds were primed with 25 mM NaCl for 36 hours (Table 5) whereas seeds primed in PEG with 25 mM concentration for 24 hours had the lowest mean germination of 7.48 for PEG treatments (Table 6).

4.1.5 Rate of germination of primed 'Legon 18'

Highest mean rate of germination were observed for priming seeds with PEG at a concentration of 25 mM for 24hours (Table 6). NaCl priming with 25 mM for 36 hours obtained the highest rate of germination of 0.14 (Table 5). There was no interaction between concentration and time for both NaCl and PEG primed treatments.

The best priming protocols that gave overall good response to studied seed germination traits were 25 mM for 36 hours for NaCl primed treatments and 25 mM for 24 hour for PEG primed treatments.

Table 5: NaCl priming on the germination of 'Legon 18' pepper

Concentration	Time	Germination Index	Germination Percentage	Coefficient of velocity	Mean germination time	Rate of germination
Control	12h	2.5ab	80.0ab	21.4a	9.3d	0.11a
	24h	2.7ab	78.7ab	19.5a	7.6abc	0.13bcd
	36h	2.3a	80.0ab	18.3a	8.2abcd	0.12abcd
25 mM	12h	3.0bc	90.7ab	18.9a	8.0bc	0.12abcd
	24h	2.9abc	89.3ab	15.2a	7.4a	0.14d
	36h	3.4c	93.3b	15.9a	7.2a	0.14d
50 mM	12h	2.3a	76.0a	20.8a	8.8cd	0.11ab
	24h	3.2bc	92.0b	17.1a	7.5a	0.13cd
	36h	3.2bc	93.3b	20.2a	7.6ab	0.13bcd
100 mM	12h	2.6ab	84.0ab	16.9a	8.3abcd	0.12abcd
	24h	3.0bc	92.0b	19.2a	8.0abc	0.13abcd
	36h	2.5a	82.7ab	21.9a	8.7bcd	0.12abc
Concentration (C)		NS	NS	NS	NS	NS
Time (T)		*	NS	NS	*	*
C x T		NS	NS	NS	NS	NS

Table 6: PEG priming on the germination of 'Legon 18' pepper

Concentration	Time	Germination Index	Germination Percentage	Coefficient of velocity	Mean germination time	Rate of germination
Control	12h	2.4b	80.0b	21.4a	9.3c	0.11a
	24h	2.9b	78.7b	19.5a	7.6a	0.13b
	36h	2.7b	80.0b	18.3a	8.1ab	0.12ab
25 mM	12h	2.5b	80.0b	21.8a	8.2ab	0.12ab
	24h	3.1b	93.3b	16.8a	7.5a	0.13b
	36h	2.6b	85.3b	17.0a	8.8bc	0.11a
50 mM	12h	2.8b	90.7b	18.0a	8.2ab	0.12ab
	24h	2.5b	82.7b	15.4a	8.3abc	0.12ab
	36h	1.7a	58.7a	17.3a	8.9bc	0.11a
100 mM	12h	2.5b	82.7b	14.4a	8.5abc	0.12ab
	24h	2.6b	84.0b	19.4a	8.3abc	0.12ab
	36h	2.6b	85.3b	16.6a	8.3abc	0.12ab
Concentration (C)		NS	NS	NS	NS	NS
Time (T)		NS	NS	NS	*	*
C x T		*	*	NS	*	NS

Means expressed in columns followed by same letter are not different (P=0.05), according to Duncan's multiple range test.
NS not significant. **, * at 1 and 5 % significant levels respectively

4.2 Experiment 2

4.2.1 Germination percentage and germination index of primed 'Legon 18' seeds under different salinity stress

The two best primed treatments for NaCl and PEG from Experiment 1 were selected and used as the priming treatments together with a control (non-primed) for Experiments 2 and 3. The saline treatments were 0 dS/m, 4.46 dS/m and 8.95 dS/m.

Interaction between salinity and priming did not show any significant differences among treatments. However, priming treatments had higher germination percentages than control at all saline levels. NaCl priming obtained 80, 66.7 and 56.7% as the highest for 0, 4.6 and 8.5dS/m respectively (Table 7).

Germination index reduced as salinity increased among priming treatments. Increased sodium chloride salinity significantly reduced index in primed and control seeds. However, no significant interaction between salinity and priming were observed in respect of germination index. Control seeds had the least germination index at all saline levels (Table 7).

4.2.2 Coefficient of velocity, mean germination time and rate of germination of primed seeds under different salinity stress.

Significant differences were not seen in the interaction effect of salinity and priming on coefficient of velocity. The highest CV of 22.5 was recorded for control seedlings irrigated under 4.46 dS/m with the lowest (13.6) obtained from control plants irrigated with tap water (Table 7)

Mean germination time increased among priming treatments as salinity increased. Mean germination time were lower in primed treatments as salinity increased than control. At high stress level of 8.95 dS/m, NaCl priming had the lowest mean germination 8.0 (Table 7). It was observed that salinity increases had a negative impact on time taken for seeds to germinate.

Rate of germination also decreased as salinity was increased. However, priming treatment showed higher rates than control. Significant differences were not observed between the interaction of salinity and priming on the rate of germination.

Table 7: Germination percentage (G %), germination index (G.I), coefficient of velocity (CV), Mean germination time (MGT) and rate of germination (MR) of 'Legon 18' pepper under salinity stress.

Salinity	Primer	G.I	G%	CV	MGT	MR
0 dS/m	NaCl	1.3c	80.0f	20.8a	7.5a	0.13bc
	PEG	1.1c	76.7ef	20.8a	7.5a	0.14c
	Control	0.7ab	63.3cd	13.6 a	8.9b	0.11abc
4.46 dS/m	NaCl	0.8b	66.7de	21.5a	7.8a	0.13bc
	PEG	0.7ab	63.3cd	17.4a	8.5ab	0.12abc
	Control	0.6ab	53.3bcd	22.5a	8.9ab	0.11ab
8.95 dS/m	NaCl	0.6ab	56.7bcd	19.5a	8.0a	0.13abc
	PEG	0.6ab	53.3abc	18.5a	8.8ab	0.11bc
	Control	0.5a	43.3a	22.1a	9.5b	0.105a
Salinity (S)		**	**	NS	NS	NS
Priming (P)		*	*	NS	*	*
S x P		NS	NS	NS	NS	NS

Values are means of 3 replicates. Means within column followed by different letters are significantly different using Duncan Multiple range test.
NS not significant. **, * at 1 and 5 % significant levels respectively

4.2.3 Seedling Vigour of primed 'Legon 18' under salinity stress

The effect of salt stress on vigour index of primed seeds was evaluated at different salinity levels (0, 4.46 and 8.95 dS/m) (Figure 3). Seedling vigour index was not significantly different between the interaction of salinity and priming. Primed treatments however showed higher vigour than control at all levels of salinity imposition. NaCl primed seeds showed highest vigour index at all levels of salinity having 589.9, 443.1 and 349.2 at 0 dS/m, 4.46 dS/m and 8dS/m respectively.

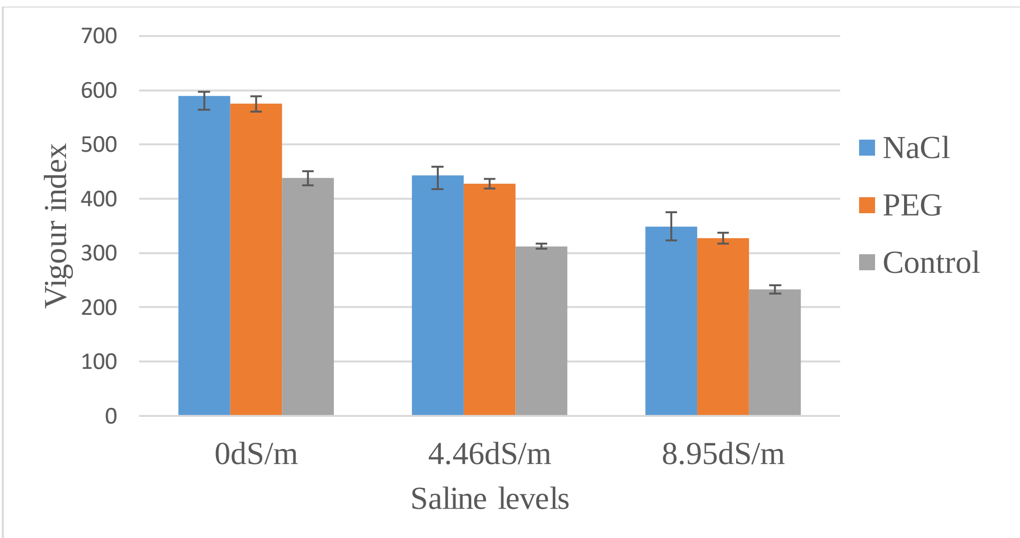

Figure 3: Vigour index of primed and unprimed seeds at different salinity levels

4.2.4 Stress Tolerance Index of primed 'Legon 18' under different salinity stress

The results revealed that increase in salinity decreased values of stress tolerance index (STI). However, seeds obtained from NaCl and PEG priming had higher stress tolerance values than control seeds (Figure 4). Stress tolerance indices of 59.2 and 56.9 % were maintained by primed seeds of NaCl and PEG respectively at highest salinity stress of 8.95 dS/m with control having 53.2%.

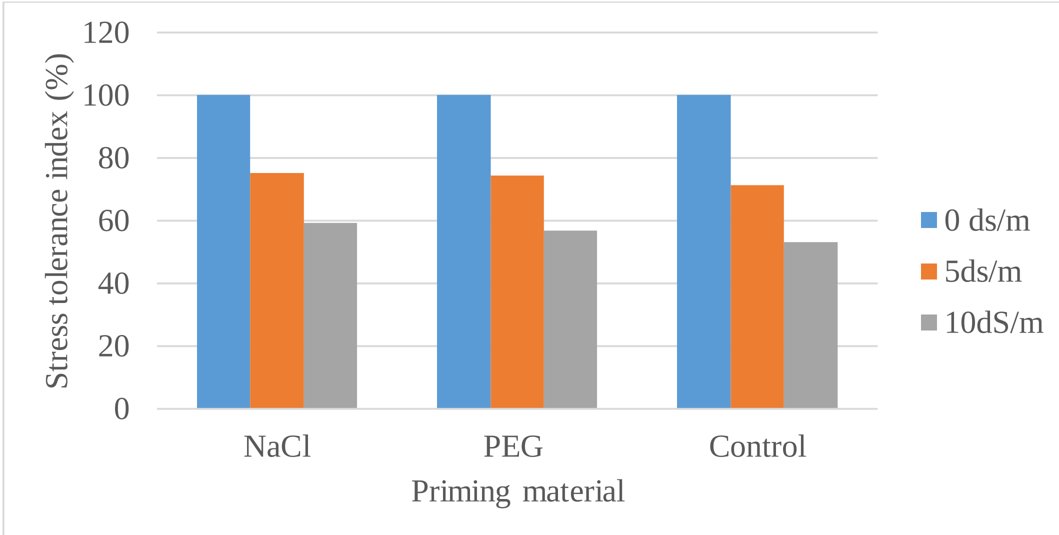

Figure 4: Stress tolerance index of primed and unprimed seeds at different salinity levels

4.2.5 Seedling, shoot and root length of primed 'Legon 18' under different salinity stress

The mean length of seedling, shoot and root taken at 15 DAP (Table 8). Salinity and priming interactions showed no significant differences among treatments. However, heights of seedling obtained from primed treatments were higher than in control plants at salinity levels. High shoot lengths were recorded in PEG primed seeds at 4.46 and 8.95 dS/m salinity levels (Table 8).

4.2.6 Fresh and dry weight of primed 'Legon 18' under different salinity stress

Salinity had a marked effect on fresh and dry weights of 'Legon 18' pepper as they decreased with increasing saline levels (Table 8). Significant differences were observed between the interaction of salinity and priming on fresh and dry weights (P=0.05). NaCl and PEG priming had higher fresh and dry than control at all saline levels.

Table 8: Shoot height, root height, seedling fresh and dry weight, and seedling vigour of primed and unprimed 'Legon 18' pepper at different salinity levels

Salinity	Priming	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Fresh weight (mg)	Dry weight (mg)
Control	NaCl	3.6b	4.5de	7.4d	490.0f	37.7c
	PEG	3.3ab	4.7c	7.5d	503.3f	37.7c
	Control	3.0ab	4.3cde	6.9c	386.7e	28.0b
4.46 dS/m	NaCl	3.0ab	4.0bcd	6.6c	366.7de	25.7b
	PEG	3.1ab	4.0bc	6.8c	353.3cde	25.3b
	Control	2.8ab	4.1bcd	6.3b	343.3cd	26.3b
8.95 dS/m	NaCl	2.5a	3.4b	6.2b	316.7bc	26.0b
	PEG	2.9ab	3.6b	6.1b	303.3ab	23.0ab
	Control	2.8ab	3.1a	5.4a	270.0a	18.3a
Salinity (S)		*	**	**	**	**
Priming (P)		*	NS	**	**	*
Interaction (S x P)		NS	NS	NS	`*	*

Means expressed in columns followed by same letter are not different (P=0.05), according to Duncan's multiple range test.
NS not significant, **, * at 1 and 5 % significant levels respectively

4.3 Experiment 3

Table 9: Chemical composition of top soil used in pot experiment

% N	% C	% S	P	Ca	Mg	K	Na
0.0888	0.7721	0.0038	2.531	1.49	0.608	0.893	0.105

4.3.1 Number of leaves, stem diameter, plant height and chlorophyll content of primed and unprimed 'Legon 18' pepper seedlings at 30, 45 and 60 DAP under different salinity levels.

Number of leaves counted on 45 and 65 DAP increased among primed and control treatments when saline treatments were applied after seedlings were transplanted, i.e. Day 30 (Figure 5). Though there was an increase in all treatments with regards to number of leaves, the rate of increase was reduced as salinity increased. The highest mean number of leaves recorded under tap watering (0 dS/m) at day 60 was 36, recorded in seeds primed with PEG. At moderate salinity of 4.46 dS/m, highest number of leaves recorded on the same day was 22 recorded from NaCl primed seeds. At salinity level of 8.95 dS/m, the highest number of leaves recorded was 24 from PEG primed seeds. From the trend in number of leaves obtained at 30, 45 and 60 DAP, it was observed that as salinity increased, number of leaves reduced in all treatments. However, primed treatments had more leaves than control at all levels of salinity.

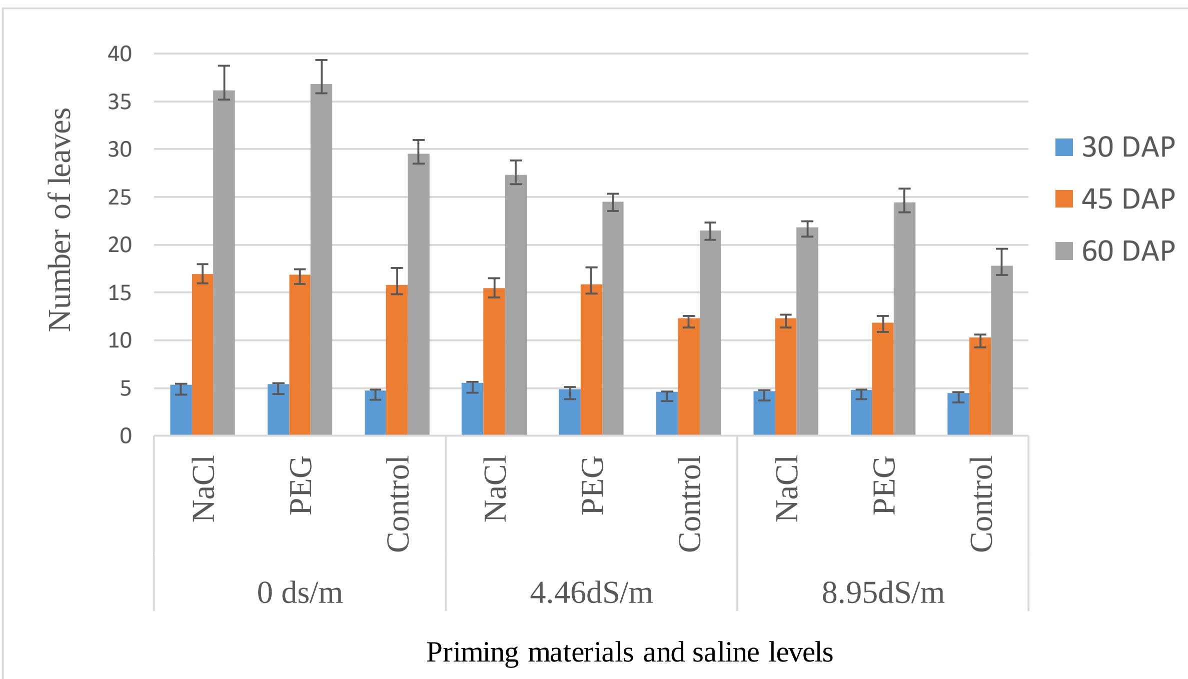

Figure 5: Salinity and seed priming on number of leaves at 30, 45 and 60 DAP

Chlorophyll contents in plants recorded on 45 DAP increased from their levels at 30 DAP but decreased at 60 DAP among all treatments. The trend in chlorophyll content at 30, 45 and 60 days is shown in Figure 6. Similar to the number of leaves, chlorophyll contents read were higher in primed treatments than control at all levels of salt stress. The lowest recorded mean value of chlorophyll content at 60 DAP (30 days of salt water treatment) was 10.46 from control plants with NaCl primed plants having the highest chlorophyll content of 11.59 under 8.95 dS/m watering.

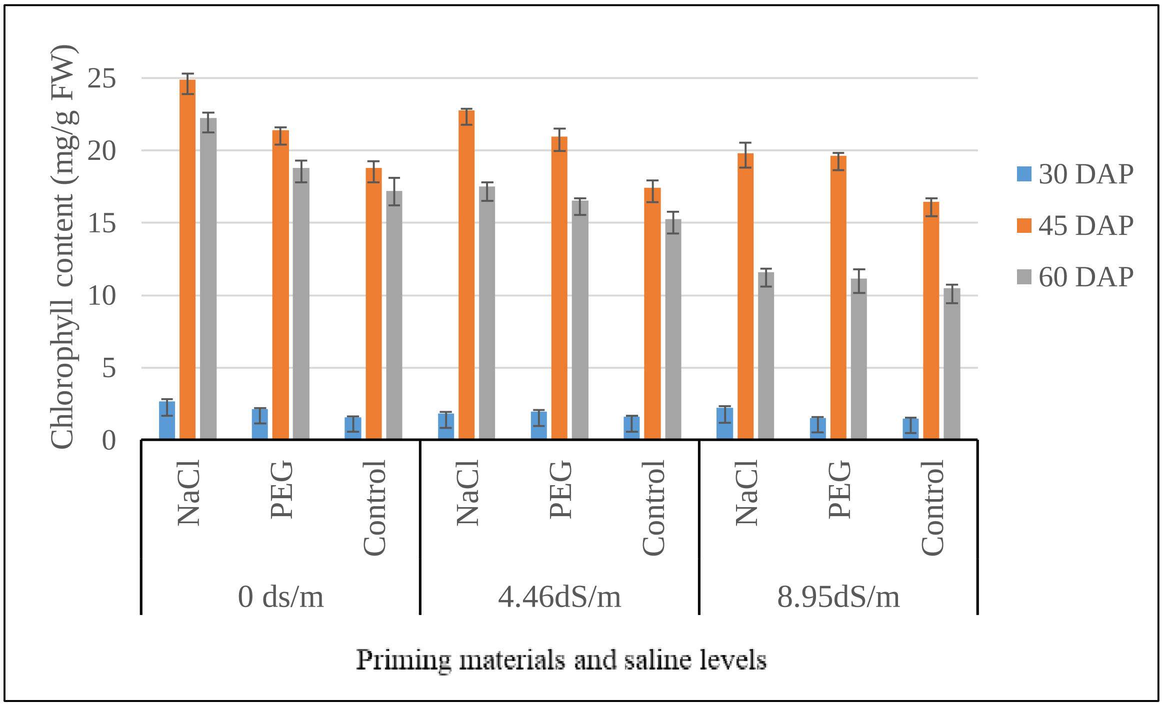

Figure 6: Salinity and seed priming on chlorophyll content at 30, 45 and 60 DAP

Salinity and seed priming had an effect on plant height studied at 30, 45 and 60 DAP. Plant height increased as number of days increased among treatments. A clear trend observed was that mean height of plants was affected by salinity, so treatments under 4.46 dS/m and 8.95 dS/m had shorter plants than their counterparts watered with tap water (0 dS/m) (Figure 7). Plants increased at a slower rate when salinity increased. At highest salinity level (8.95 dS/m), mean plant height recorded on day 60 were 7.06, 7.17 and 5.99cm for seeds primed in NaCl, PEG and control respectively. Plants irrigated with tap water on the same day had 16.6, 13.5 and 12.6cm plant height for NaCl, PEG and control respectively. Salinity reduced plant height at 60 DAP almost by half in all treatments at highest saline level.

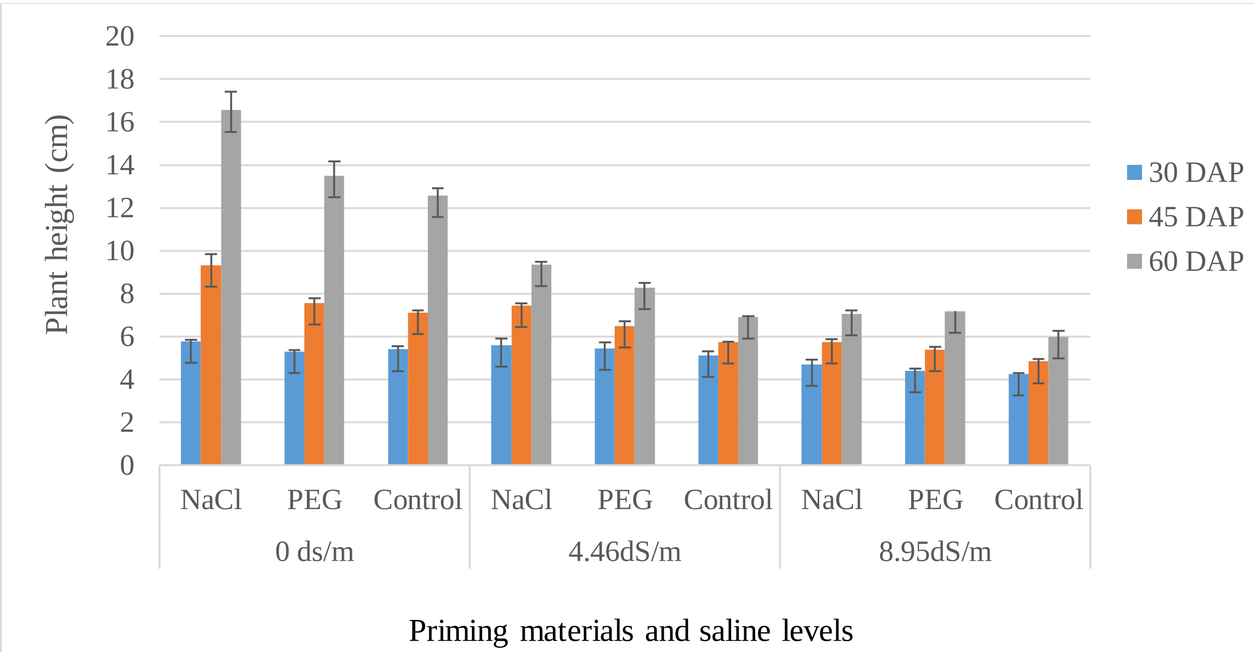

Figure 7: Salinity and seed priming on plant height at 30, 45 and 60 DAP

Stem diameter increased from 30, 45 and 60 DAP studied treatments. Primed treatments had the highest increase in stem diameter from time when saline treatments were imposed. 4 weeks after saline treatment were imposed (60th day), stem diameter increased at a slow rate with salinity increases among treatments. Primed treatments were longer in diameter of stem at all levels of salinity than control treatments (Figure 8).

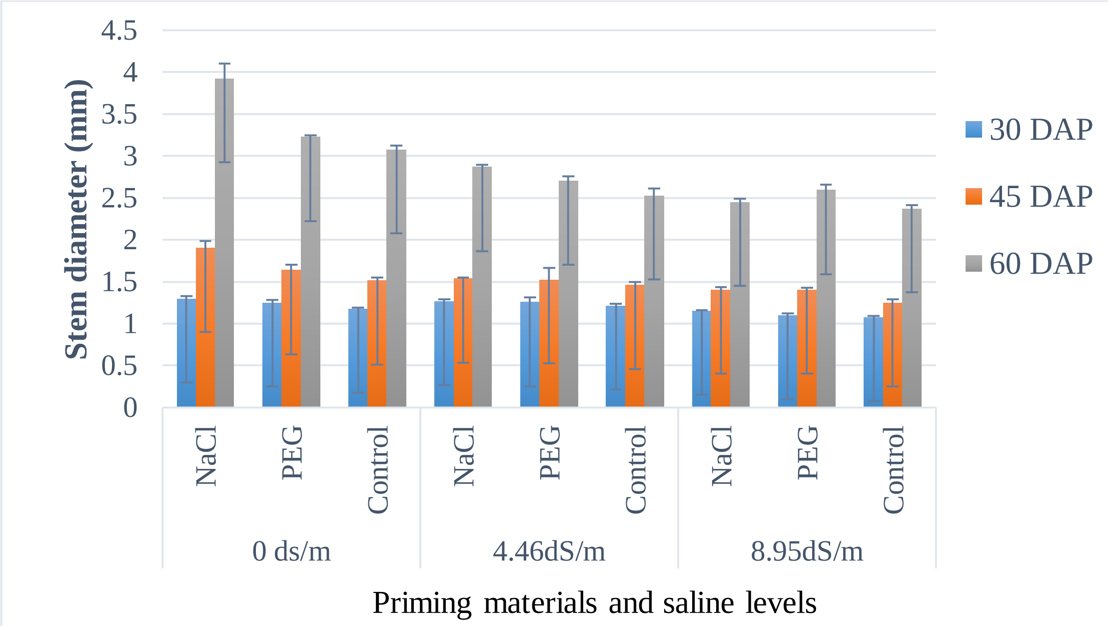

Figure 8: Salinity and seed priming on stem diameter at 30, 45 and 60 DAP

4.3.2 Salinity and priming on plant height at 60 DAP

No significant effect was observed in the interaction of salinity and priming on plant height. Salinity had a marked influence on plant height as it reduced the height of plants when salinity levels were increased. Irrigating plants with 8.95 dS/m reduced plant height more than 50% in NaCl primed and control seedlings and almost by half in PEG primed seedlings than those irrigated with tap water at 60 DAP (Table 10).

4.3.3 Seed priming and salinity on number of leaves at 60 DAP

The interaction effect of salinity and priming on number of leaves was not significant on the number of leaves. However, increase in salinity reduced the number of leaves produced by plants. The highest reduction observed among treatments irrigated with 8.95 dS/m. Table shows the mean effect of salinity and priming on number of leaves (Table 10).

4.3.4 Salinity and priming on chlorophyll content at 60 DAP

Salinity and priming had no significant effect on their interaction on chlorophyll content (Table 10). The chlorophyll content reduced among priming treatments as salinity increased. Chlorophyll content was lower in control seedlings at 60 DAP at all saline levels than in seedlings obtained from priming treatments.

4.3.5 Salinity and priming on total leaf area at 60 DAP

The total leaf area reduced as salinity levels increased in all treatments (Table 10). There were significant differences observed among the interaction effect of salinity and priming at P=0.01. Priming treatments had higher leaf area than control treatments at 0, 4.46 and 8.65dS/m. NaCl primed treatments had the highest total leaf area of 29.9, 20.58 and 13.61cm^2 for 0, 4.46 and 8.65 dS/m respectively.

4.3.6 Salinity and priming on stem diameter at 60 DAP

There were significant differences observed in the interaction effects of salinity and priming on stem diameter at P= 0.01 (Table 10). Salinity reduced diameter in stems of plants yet, seedlings primed in NaCl and PEG had bigger diameters at all levels of salinity than in control seedlings.

Table 10: Salinity and priming on plant height, number of leaves and total leaf area of 'Legon 18'pepper at 60 days after planting

Salinity	Priming	Plant Height (cm)	Stem Diameter (mm)	Chlorophyll content	No. of leaves/plant	Total Leaf Area (cm^2)
0 dS/m	NaCl	16.6d	3.9e	22.2d	36.0c	29.9f
	PEG	13.5c	3.2d	18.8c	37.0c	19.4de
	Control	12.6c	3.1cd	17.2bc	30.0bc	18.7cde
4.46 dS/m	NaCl	9.4b	2.9bcd	17.5bc	27.0b	20.6e
	PEG	8.3ab	2.7abc	16.6bc	25.0ab	15.2bc
	Control	6.9a	2.5ab	15.3b	22.0ab	16.1bcd
8.95 dS/m	NaCl	7.1ab	2.5ab	11.6a	22.0ab	13.6b
	PEG	7.2ab	2.6ab	11.2a	24.0ab	9.4a
	Control	6.0a	2.4a	10.5a	18.0a	6.0a
Salinity (S)		**	**	**	**	**
Priming (P)		*	*	*	*	**
Interaction (S x P)		NS	*	`NS	NS	*

Means expressed in columns followed by same letter are not different (P=0.05), according to Duncan's multiple range test.
NS not significant, **, * at 1 and 5 % significant levels respectively

4.3.7 Salinity and priming on Dickson Quality Index at 60 DAP

Highest values for Dickson's Quality Index of 1.71, 0.86, and 0.42 corresponding to treatments irrigated under salinity levels of 0, 4.46 and 8.95 dS/m were recorded under NaCl primed treatments (Table 11). The least values of Dickson Quality Index were observed in control treatments at all levels of salinity. ANOVA table showed significant differences among the interaction between salinity and priming (P=0.05)

Table 11: Salinity and priming on Sturdiness Quotient (SQ) and Dickson Quality Index (DQI) of 'Legon 18' pepper at 60 days after planting

Salinity	Priming	SQ	DQI
0 dS/m	NaCl	4.22c	1.71f
	PEG	4.18c	0.94e
	Control	4.08c	0.87e
4.46 dS/m	NaCl	3.26b	0.87e
	PEG	3.07ab	0.73d
	Control	2.76ab	0.50c
8.95 dS/m	NaCl	2.89ab	0.42c
	PEG	2.76ab	0.28b
	Control	2.51a	0.15a
Salinity (S)		**	**
Priming (P)		*	**
Interaction (S x P)		NS	**

Means expressed in columns followed by same letter are not different (P=0.05), according to Duncan's multiple range test.
NS not significant. **, * at 1 and 5 % significant levels respectively

4.3.8 Salinity and priming on Sturdiness Quotient at 60 DAP

Treatments primed in NaCl and PEG were observed to have higher sturdiness quotient than control treatments at all saline levels (Table 11). Combined ANOVA showed significant differences (P=0.05) in salinity and priming (P=0.01). No significant difference was observed in the interactions of salinity and priming (Table 11).

4.3.9 Salinity and priming on nutrient composition in stems, roots and leaves of 'Legon 18' pepper

There were significant differences observed between the interactions of salinity and priming on the composition of sodium and potassium in leaves. No significant differences were observed in the interaction of salinity and priming on calcium and magnesium compositions (Table 12).

Significant differences were obtained in the interaction of K/Na ratio and Ca/Na ratio in leaves (Table 12).

Interaction effects of salinity and priming were significantly different among treatments in relation to their accumulation of sodium and potassium in their stems. Similarly in leaves, no significant difference was recorded in the interaction of salinity and priming on calcium and magnesium content in stems. There were significant differences in K/Na ratio and Ca/Na ratios in stems based on the interaction effect of salinity and priming (Table 13)

Table 12: Nutrient composition in leaves obtained from primed and unprimed 'Legon 18' pepper at different salinity levels at vegetative stage (60 DAP).

Salinity	Priming	Na	K	Ca	Mg	K/Na	Ca/Na
0 dS/m	NaCl	14.2a	22.3a	36.2a	14.4a	1.6g	2.6c
	PEG	72.0b	234.0c	36.5a	14.4a	3.3h	0.5b
	Control	980.0i	211.0b	36.5a	14.4a	0.2a	0.1a
4.46 dS/m	NaCl	406.0d	408.0f	36.6a	14.6a	1.0e	0.1a
	PEG	357.0c	443.0h	36.2a	14.4a	1.2f	0.1a
	Control	419.0e	373.0d	36.0a	14.5a	0.9d	0.1a
8.95 dS/m	NaCl	427.0f	429.0g	37.1a	14.8a	1.0e	0.1a
	PEG	706.0h	449.0i	37.1a	14.8a	0.6b	0.1a
	Control	591.0g	403.0e	37.3a	14.9a	0.7c	0.1a
Salinity (S)		**	**	NS	NS	**	**
Priming (P)		**	**	NS	NS	**	**
Interaction S x P		**	**	NS	NS	**	**

Means expressed in columns followed by same letter are not different (P=0.05), according to Duncan's multiple range test.
NS not significant. **, * at 1 and 5 % significant levels respectively

Table 13: Nutrient composition in stems obtained from primed and unprimed 'Legon 18' pepper at different salinity levels at vegetative stage (60 DAP)

Salinity	Priming	Na	K	Ca	Mg	K/Na	Ca/Na
Control	NaCl	32.1a	24.0a	38.1d	15.2a	0.8c	1.19g
	PEG	50.5b	262.0c	34.3a	13.7a	5.2g	0.68f
	Control	954.3i	185.3b	33.9a	13.6a	0.2a	0.04a
4.46 dS/m	NaCl	405.0e	407.6f	36.3bc	14.5a	1.0e	0.09d
	PEG	337.0c	423.0h	34.6ab	13.8a	1.3f	0.10e
	Control	396.0d	350.0d	34.9ab	13.7a	0.9d	0.09d
8.95 dS/m	NaCl	426.0f	428.0i	37.2d	14.8a	1.0e	0.09d
	PEG	666.0h	419.4g	34.2a	13.4a	0.6b	0.05b
	Control	571.0g	383.0e	33.6a	14.2a	0.7b	0.06c
Salinity (S)		**	**	NS	NS	**	**
Priming (P)		**	**	**	*	**	**
Interaction S x P		**	**	*	NS	**	**

Means expressed in columns followed by same letter are not different (P=0.05), according to Duncan's multiple range test.
NS not significant. **, * at 1 and 5 % significant levels respectively

Significant differences were observed in roots accumulation of sodium and phosphorus with no significant differences in calcium and magnesium levels with respect to the interaction of salinity and priming. Also, K/Na ratio and Ca/Na ratios in roots were significantly different based on the interaction of salinity and priming (Table 14).

Table 14: Nutrient composition in roots obtained from primed and unprimed 'Legon 18'pepper at different salinity levels at vegetative stage (60 DAP)

Salinity	Priming	Na	K	Ca	Mg	K/Na	Ca/Na
0 dS/m	NaCl	24a	19a	33.7a	13.5a	0.79c	1.41f
	PEG	62.2b	274c	35.9b	14.3a	4.40f	0.58e
	Control	953i	185b	33.4a	13.3a	0.19a	0.04a
4.46 dS/m	NaCl	375e	377f	33.1a	13.5a	1.01e	0.09c
	PEG	350c	449i	36.8b	14.7a	1.28f	0.11d
	Control	366d	320d	33.6a	13.4a	0.87d	0.09c
8.95 dS/m	NaCl	387f	389g	33.9a	13.5a	1.00e	0.09c
	PEG	696.5h	438h	36.5b	14.5a	0.63b	0.05e
	Control	532g	344e	32.4a	13.0a	0.65b	0.06b
Salinity (S)		**	**	NS	NS	**	**
Priming (P)		**	**	**	*	**	**
Interaction S x P		**	**	**	NS	**	**

Means expressed in columns followed by same letter are not different (P=0.05), according to Duncan's multiple range test.
NS not significant. ** , * at 1 and 5 % significant levels respectively

4.3.9 Total phenolic in leaves of primed and unprimed 'Legon 18' pepper at different salinity levels

Significant differences were observed from the interaction between salinity and priming on total phenolic in leaves of pepper. At 0 dS/m and 4.46 dS/m the highest concentration of total phenolic were observed in control plants. At saline level of 8.95 dS/m, PEG seedlings had the highest concentration of total phenolic in their leaves (Figure 9).

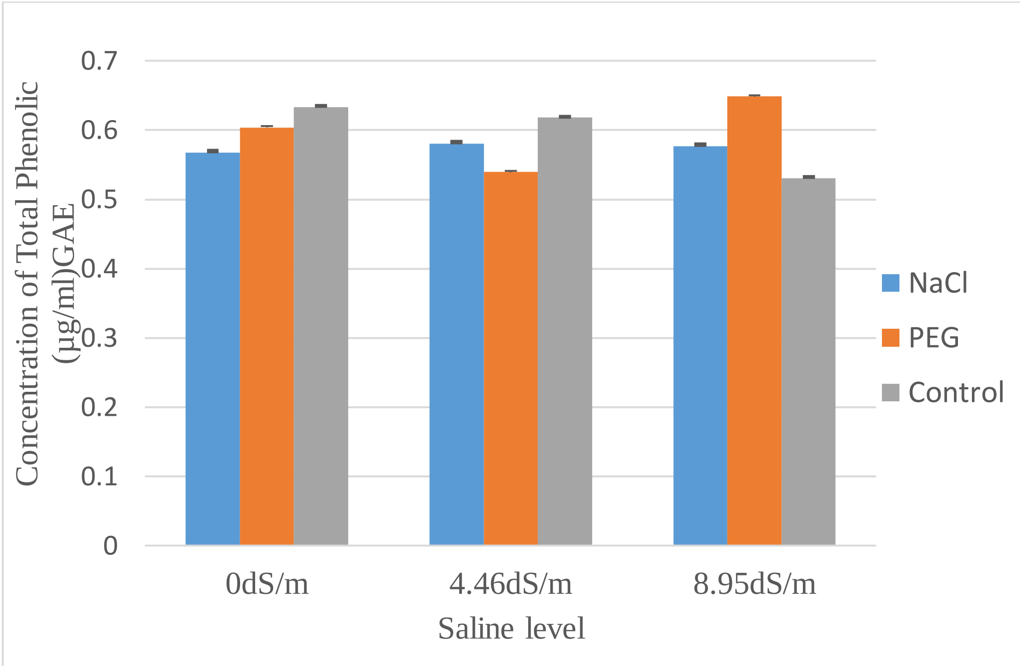

Figure 9: Salinity and priming on total phenolic in leaves of pepper

4.3.10 Flavonoid content in leaves of primed and 'Legon 18' pepper at different salinity levels

Salinity and priming interaction effect showed significant differences among treatments in concentrations of flavonoid in leaves harvested at 60 DAP. The highest recorded flavonoid concentration for 0, 4.46 and 8.95 dS/m were found in control, PEG and NaCl respectively (Figure 10)

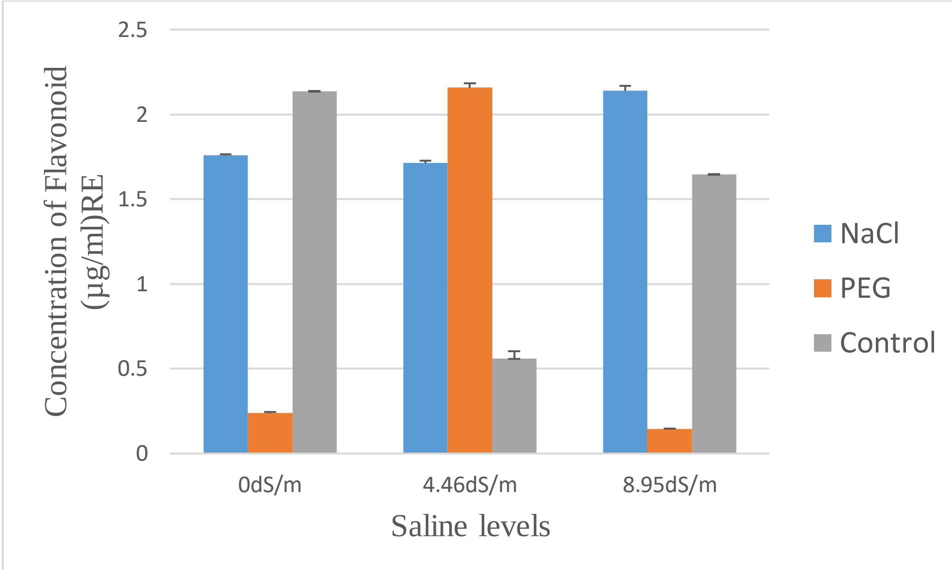

Figure 10: Salinity and priming on flavonoid in leaves of pepper

4.3.11 Protein content in leaves of primed and unprimed 'Legon 18' pepper at different salinity levels

For all priming treatments studied, the concentration of protein decreased at saline level of 4.46 dS/m. There was an increase observed in concentration of protein for NaCl primed leaves and control at 8.95 dS/m. Significant differences were obtained from the interaction of salinity and priming (P=0.01). At increased salinity of 8.95 dS/m, NaCl primed seedlings had the highest concentration of protein in their leaves (Figure 11)

4.3.12 Soluble sugars in leaves of 'Legon 18' pepper at different salinity levels

Significant differences were observed among treatments based on the interaction of salinity and priming. PEG priming obtained the highest accumulation of soluble sugars in leaves at 4.46 and 8.95 dS/m while control had the highest when tap water (0 dS/m) was used to irrigate (Figure 12).

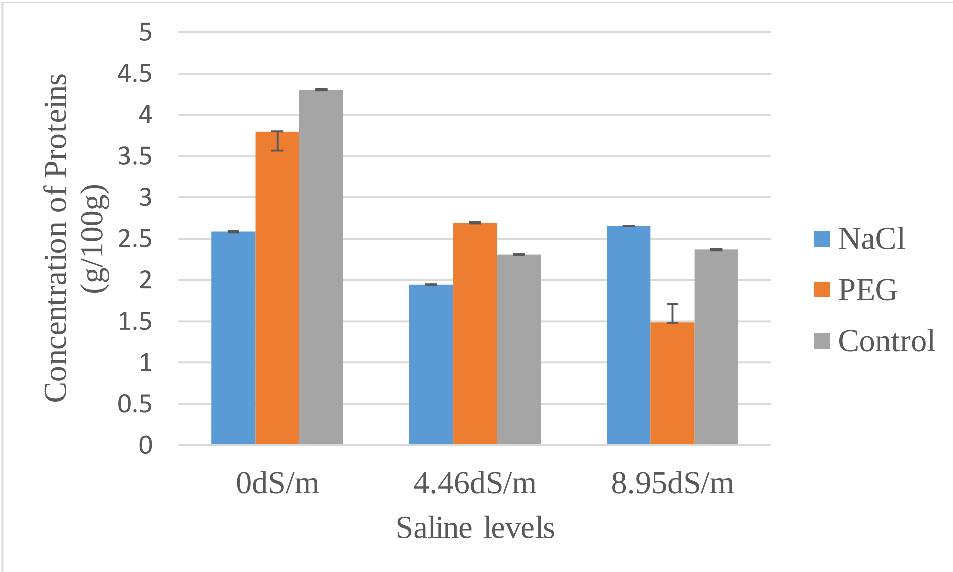

Figure 11: Salinity and priming on proteins in leaves of pepper

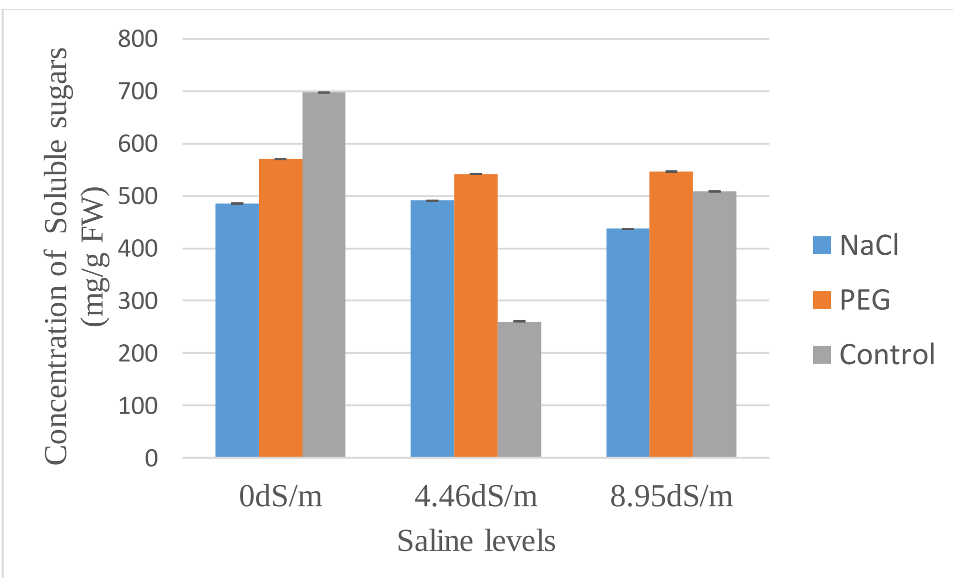

Figure 12: Salinity and priming on soluble sugars in leaves of pepper

CHAPTER 5

5.0 DISCUSSION

5.1.2 Seed Germination parameters

Variations in seed germination parameters were observed after seeds were sown in seed trays. The highest germination percentage of 93.3% was obtained in 25 mM NaCl primed seeds for 36 hours and 25 mM PEG primed seeds for 24 hours. Also, lowest germination was recorded in pepper seeds primed with 50 mM NaCl for 12 hours (76%) and 50 mM PEG for 36 hours (58.7%). In general, priming with NaCl and PEG had better germination than control. This could be attributed to pre-germination metabolic processes associated with priming which prepare seeds for radicle protrusion (Farooq *et al.*, 2007). The differences observed in the germination percentage when different concentrations of the same priming agent was used at different time durations can be attributed to the varying response of seeds to different concentrations of same priming agent. Aloui *et al.* (2014) observed variations in the response of three pepper cultivars to germination percentage when seeds were primed in different concentrations of priming solutions (KCl, $CaCl_2$ and NaCl) at different durations for 12, 24 and 36 hours respectively. Ameri *et al.* (2011), observed an increase in germination percentage with primed seeds after priming pepper 'Carlifornia Wonder' with NaCl and other priming agents. Osmo-priming of hot pepper seeds in PEG 6000 for 7 days improved seed germination than in control seeds at different temperatures (15, 20 and 25^0C) (Pandita *et al.*, 2007).

Treatments with the highest germination indices (3.6 and 3.1) were 25 mM NaCl for 36 hours and 25 mM PEG for 24 hours respectively. The high germination index of primed treatments compared to the control is an indication of better vigour of primed seeds. Sadeghi *et al.* (2011) reported of high germination index in soybean after osmopriming seeds in -1.2 MPa PEG 6000 for 12 hours. Variations in the germination index could have resulted from different concentrations (25, 50 and 100 mM) of NaCl and PEG and time used in priming of seeds.

Mean germination times for pepper seeds were significantly influenced by treatments. The lowest mean germination time of 7.2 and 7.5 were recorded for 25 mM NaCl for 36 hours and 25 mM PEG for 24 hours respectively. Reduced mean germination time in primed seeds may be due to improved imbibition of water which decreases resistance of endosperm and causes seeds to germinate quicker than control. Khan *et al.* (2009) observed no significant differences in mean germination time among primed and control seeds when pepper seeds were primed in 1.0 mM NaCl for 48 hours. Mean germination time was however reduced in primed pepper seeds 'Anaheim chilli' when seeds were primed in 50 mM NaCl for 24 hours (Aloui *et al*, 2014). Pandita *et al.* (2007) also observed a reduction in mean time to germination after pepper seeds were primed in -1.0MPa PEG.

The highest rate of germination was obtained for 25 mM NaCl for 36 hours (0.139) and 25 mM PEG for 24 hours (0.134). Primed seeds emerge faster and uniformly as a result of changes that take place during priming such as increase water absorption and pre-germinative activities which lead to cell elongation and division (Sivritepe *et al.*, 2003). Nagarajan *et al.* (2003), observed a 38% speed in germination when carrot seeds were primed with PEG-6000 of osmotic potentials of -0.5 and -1.0 MPa.

There were no significant differences among priming treatments in relation to coefficient of velocity (CV). However highest CVs were recorded for priming with NaCl (21.9) and PEG (21.8). Aloui *et al.* (2014) however recorded significant increases in CV as a result of priming 'Anaheim

Chilli', 'Beldi' and 'Baklouti' pepper with NaCl (50 mM for 24 hours), KCl (10 mM for 36 hours) and $CaCl_2$ (10 mM for 36 hours) respectively. The significant differences obtained by Aloui *et al.* (2014) may be due to the variations within the cultivars used.

5.2 Salinity and priming on seed germination parameters

Salinity stress affected germination percentage and germination index of seeds as it decreased with increase in saline levels. Aloui *et al.* (2017) reported that, beyond EC levels of 3dS/m, pepper growth and production are affected. Priming treatment of NaCl (25 mM for 36 hours) and PEG (25 mM for 24 hours) had higher germination percentages and germination indices than control at all studied saline levels. Amjad *et al.* (2007) and Yadav *et al.* (2011) concluded that priming increased pepper seed germination under salinity stress. PEG-6000 priming increased germination percentages of Eucalyptus seeds under salinity stress after seeds were primed in PEG solutions of osmotic potentials of -1.0 and -1.5 MPa. Seed germination and speed of germination decreased as salinity increased (José *et al.*, 2016). The reduction in germination percentage and germination index as saline levels increased may be attributed to the alteration in water uptake due to the high concentrations of salt. Aloui *et al.*, (2017) observed a reduction in germination index with increase in salinity with primed seeds obtaining higher germination indices than the control.

Mean germination time and rate of germination were not significantly affected by the interactions of salinity and priming in this study. However, priming had significant effect on these parameters. Primed treatments of NaCl (25 mM for 36 hours) and PEG (25 mM for 24 hours) had lower mean germination time and higher mean rate of germination than control at all saline levels. Fuller *et al.* (2012), in wheat observed reduced mean germination time with priming with PEG 6000 at osmptic potential of -1.0 MPa while salinity increases lengthened the days to emergence.

Results obtained showed a decline in vigour of seedlings as salinity increased. Though no significant differences were shown with the interaction of salinity and priming, primed treatments had higher seed vigour than non-primed or control seeds. Khan *et al.* (2009) observed improvement in seedling vigour after priming seeds of pepper with 1mM NaCl. The higher vigour of primed seeds may be attributed to the metabolic processes that take place during priming including repairing of membrane and developing immature embryos (Bewley *et al.*, 2013).

5.3 Salinity and priming on length of shoot and roots of 'Legon 18' pepper

Salinity reduced shoot and root length of 'Legon 18' pepper as it increased. This reduction in length in shoot and root may have arisen due to imbalance in nutrient uptake and reduced water uptake due to high osmotic potential (Musyimi *et al.*, 2007; Yildirim & Güvenç, 2006). Activities of various plant growth regulators like cytokinins and auxins in young seedlings tissue may be affected with increase in salinity thereby affecting growth (Tehseen *et al.*, 2016). In this study, shoot height of seedlings obtained from PEG priming had longer shoots than control at all saline levels. NaCl priming had shorter shoot length than control at 8.95 dS/m. Seedlings obtained from primed treatments had longer root than control at all saline levels with the exception of 4.46 dS/m where root length of control was longer than primed treatments. Khan *et al.* (2009) and Abdollahi and Jafari (2012), reported of increase in shoot and root length of pepper and canola primed with NaCl of concentrations 1.0 mM and 1% respectively. Similarly, Farooq *et al.* (2005), found increase in root length of tomato after priming with -1.1 MPa PEG 8000 solution. Amjad *et al.* (2007) also reported of increase in shoot length in pepper when primed with 1% NaCl solution with no significant increase in root length. Significant improvement in length of shoot and roots of seedlings from primed treatments over non-primed treatments may be attributed to earlier germination induced by priming (Farooq *et al.*, 2005)

5.4 Salinity and priming on fresh and dry weight of 'Legon 18' pepper

Fresh and dry weights of pepper seedlings were significantly reduced with increase in salt concentrations. Yildirim & Güvenç (2006), observed decreasing weight in shoot and root fresh and dry weights among 11 cultivars of pepper irrigated with saline solutions of 0, 85, 170 and 215 mM NaCl solutions for 14 days. Salt stress can affect seed germination by decreasing the uptake of water and nutrient thereby delaying and or making seedlings germinate at a reduced rate. The interaction of salinity and priming showed significant differences among treatments. Priming with 25 mM NaCl for 36 hours and 25 mM PEG for 24 hours resulted in higher fresh and dry weights than control at all saline levels. Amjad *et al.* (2007) observed increase in fresh and dry weight of pepper seedling primed with agents including 1% NaCl and -1.25 MPa PEG 8000.

5.5 Salinity and priming on number of leaves, stem diameter, total leaf area and chlorophyll content

The response of seed priming treatments to salinity stress showed a decrease in number of leaves with increase in salinity. The reduction in number of leaves was more pronounced in control seeds than in primed treatments. Under salinity stress, plant nutrient uptake is impaired which may result in crops not developing well. Bajehbaj (2010) reported that -1.0 MPa KNO_3^- priming resulted in higher number of leaves in sunflower. Under salinity stress, Pradhan *et al.* (2015), observed increase in number of leaves of primed tomato plants obtained from -0.5, -1.0, -1.5 and -2.0 MPa PEG 6000 priming than in control.

Stem diameter was reduced in seedlings of control treatments than in primed treatments when salinity increased. This reduction in size of diameter of stems may be due to the reduced water uptake and imbalance in mineral accumulation by seedlings which affected cell division and elongation (Munns, 2002). Growth of stem is usually affected by high salt concentrations (Acosta-

Motos *et al.*, 2017). Nakaune *et al.* (2012) observed bigger diameters when seeds were primed with 300 mM NaCl than control in tomato.

Total leaf area at 60 DAP was significantly affected by salinity and priming. Highest total leaf areas were 29.91, 20.58 and 13.61cm^2 for 0, 4.6 and 8dS/m respectively. These values were all recorded for NaCl primed seedlings with control seedlings obtaining the lowest leaf area at all saline levels. Increase in leaf area of primed seedlings may have resulted from the ability of primed seedlings to maintain cell wall structure and elongation and regulate uptake of nutrients for growth. Cell wall properties are transformed under saline conditions, with a decrease in photosynthetic rate which eventually leads to decrease in total leaf area (Franco *et al.*, 1997; Rodriguez *et al.*, 2005).

Interaction between salinity and priming was not significantly different for chlorophyll content. However, at all saline levels, chlorophyll content at 60 DAP was higher in primed treatments (NaCl and PEG) than in the control. Decline in chlorophyll content in plants under saline stress may result from ion toxicity resulting from the uptake of Na^+ which may inhibit leaf expansion. The findings obtained is similar to those reported by Sepehr and Ghorbanli (2006), who reported of lower chlorophyll content in maize due to salinity. Furthermore, reduction in chlorophyll content due to salinity may be a resulting effect of decreased leaf area which have been reported to decreases photosynthetic processes in leaves (Bajehbaj *et al.*, 2009). The results of improved chlorophyll content in primed treatments than control under salinity stress agree with results reported in earlier studies (Aloui *et al.*, 2014; Pradhan *et al.*, 2015)

5.6 Salinity and priming on plant height

Plant heights taken at 60 DAP showed a reduction in all primed treatments and the control as salinity was increased. At the seedling stage, salt stress has been reported to affect plant growth by osmotic and ionic stresses which consequently cause oxidative stress that is responsible for the reduction of growth in plants (Hasanuzzaman *et al.*, 2013). Sakr *et al.* (2015), found that pepper plants decreased in height as a result of increase salinity. Though the interaction between salinity and priming in the present study was not significant, pepper seedlings obtained from 25mM NaCl for 36 hours and 25mM PEG for 24 hours priming had taller plants than control at all saline levels. Yadav *et al.* (2011) reported that plant height recorded after 60 days of transplanting in cold and salt condition of 'California Wonder' pepper seedlings obtained after priming in 16.7 mM PEG-6000 solution had taller plants than unprimed seeds. Priming of seeds with NaCl under salinity stress resulted in taller plants in sunflower (Bajehbaj, 2010) and cotton (Bakht *et al.*, 2011).

5.7 Mineral composition in roots, leaves and stem of primed pepper seedlings at different salinity levels.

The K/Na ratios obtained at all saline levels in stems, leaves and roots at 0 and 4dS/m were higher in primed treatments than in control. At saline level of 8.95 dS/m, however, control treatment had higher K/Na ratios than PEG in leaves, stems and roots. Transport and uptake of minerals have been studied in plants under salinity stress. Na competes with the uptake of K under saline conditions basically as a result of greater concentrations of Na (Khan *et al.*, 2009). Plants ability to limit the uptake of toxic minerals and maintain normal ion contents show greater tolerance to salinity stress. Priming treatments having higher K/Na ratios in roots stems and leaves in the present study than the control at all saline levels could be as a result of the priming materials ability to increase the activities of antioxidant enzymes (Sharma *et al.*, 2014) and enhance germination through the defense mechanisms in reducing sodium and chlorine uptake (Ashraf *et al.*, 2003; Sedghi *et al.*, 2014).

Ca/Na ratios in leaves were higher in primed treatments than control at all saline levels. In stems and roots of pepper seedlings, at saline level of 8.95, Ca/Na ratio was higher in the control than

PEG primed treatment. The higher K/Na and Ca/Na ratios generally obtained from primed treatments in this study may be the reason why primed treatments had better plant stand in terms plant height, number of leaves, chlorophyll content and stem diameter than the control under the different saline levels. Theerakulpisut *et al.* (2016), reported of enhanced growth in rice as a result of lowered Na^+/K^+ (higher K^+/Na^+), greater membrane stability and chlorophyll content after priming rice with KNO_3, mannitol and wood vinegar

CHAPTER 6

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Priming of 'Legon 18' pepper seeds with 25 mM concentration of NaCl for 36 hours and 25 mM concentration of PEG for 24 hours resulted in better germination percentages and germination index compared to all the other concentration (25 ,50 and 100 mM) and times (12, 24 and 36 hours) combinations used for priming. They also had lower mean germination time and faster rate of germination than all other concentration and time durations.

Germination percentage and germination index of seedlings obtained from PEG and NaCl priming performed better when germinated under irrigation water with electrical conductivities of 0, 4.46 and 8.95 dS/m. Though there were no significant interactions between salinity and priming on all seed germination parameters studied (germination percentage, germination index, coefficient of velocity, mean germination time, seedling length, seed vigour and rate of germination), NaCl primed seedlings performed better at all saline levels in terms of germination percentage, germination index, mean germination time, mean rate of germination, and seedling vigour.

There were significant differences obtained in total leaf area, Dickson quality index, fresh weight, dry weight, K/Na and Ca/Na ratios in pepper seedlings raised under different salinity stress. NaCl primed seedlings followed by PEG primed seedlings had higher leaf area and greater Dickson quality index. Plant height, chlorophyll content, stem diameter and number of leaves were all affected by salinity, however, primed seedlings had higher and better plant stand at all studied parameters than the control. Seed priming can be used to alleviate the effect of salinity stress in pepper seedling development.

6.2 Recommendation

- Further studies using other varieties of pepper should be conducted to assess the effect of priming under salinity stress.
- Different priming agents should be employed to assess their effect under salinity stress of 'Legon 18' pepper seedling development.

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APPENDIX

EXPERIMENT 1

NaCl PRIMING

Appendix 1: Analysis of Variance (ANOVA) for Coefficient of Velocity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	11	87.75	7.98	0.67	0.752
Residual	24	285.71	11.90		
Total	35	373.47			

Appendix 2: ANOVA for Germination percentage

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	11	1146.2	104.2	1.01	0.463
Residual	24	2464.0	102.7		
Total	35	3610.2			

Appendix 3: ANOVA for Germination Index

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	11	3.6537	0.3322	2.08	0.064
Residual	24	3.8245	0.1594		
Total	35	7.4782			

Appendix 4: ANOVA for Mean Rate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	11	0.00218790	0.00019890	3.23	0.008
Residual	24	0.00147999	0.00006167		
Total	35	0.00366789			

Appendix 5: ANOVA for Mean Germination Time

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	11	12.9238	1.1749	3.30	0.007
Residual	24	8.5487	0.3562		
Total	35	21.4725			

PEG PRIMING

Appendix 6: ANOVA for Coeffiecient of Velocity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	11	164.18	14.93	0.66	0.759
Residual	24	540.92	22.54		
Total	35	705.10			

Appendix 7: ANOVA for Germination percentage

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	11	2392.89	217.54	3.50	0.005
Residual	24	1493.33	62.22		
Total	35	3886.22			

Appendix 8: ANOVA for Germination Index

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	11	3.6987	0.3362	2.45	0.032
Residual	24	3.2952	0.1373		
Total	35	6.9939			

Appendix 9: ANOVA for Mean Rate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	11	0.00174752	0.00015887	2.29	0.043
Residual	24	0.00166172	0.00006924		
Total	35	0.00340923			

Appendix 10: ANOVA for Mean Germination Time

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	11	8.1010	0.7365	2.39	0.036
Residual	24	7.3933	0.3081		
Total	35	15.4943			

EXPERIMENT 2

Appendix 11: ANOVA for Germination percentage

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salinity	2	2251.85	1125.93	30.40	<.001
Primer	2	1207.41	603.70	16.30	<.001
Salinity.Primer	4	14.81	3.70	0.10	0.981
Residual	18	666.67	37.04		
Total	26	4140.74			

Appendix 12: ANOVA for Germination Index

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salinity	2	1.01350	0.50675	27.66	<.001
Primer	2	0.35605	0.17802	9.72	0.001
Salinity.Primer	4	0.16922	0.04230	2.31	0.097
Residual	18	0.32980	0.01832		
Total	26	1.86856			

Appendix 13: ANOVA for Coefficient of Velocity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salinity	2	32.49	16.25	0.78	0.472
Primer	2	20.52	10.26	0.49	0.618
Salinity.Primer	4	132.64	33.16	1.60	0.218
Residual	18	374.00	20.78		
Total	26	559.65			

Appendix 13: ANOVA for Mean Germination Time

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salinity	2	2.8894	1.4447	2.43	0.116
Primer	2	8.2005	4.1003	6.90	0.006
Salinity.Primer	4	0.9235	0.2309	0.39	0.814
Residual	18	10.7015	0.5945		
Total	26	22.7149			

Appendix 14: ANOVA for Mean Rate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salinity	2	0.0007350	0.0003675	2.90	0.081
Primer	2	0.0015462	0.0007731	6.09	0.010
Salinity.Primer	4	0.0002066	0.0000516	0.41	0.801
Residual	18	0.0022841	0.0001269		
Total	26	0.0047718			

Appendix 15: ANOVA for Seedling Vigour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salinity	2	244097.	122049.	76.92	<.001
Primer	2	94068.	47034.	29.64	<.001
Salinity.Primer	4	1614.	404.	0.25	0.903
Residual	18	28560.	1587.		
Total	26	368341.			

Appendix 16: ANOVA for Dry Weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salinity	2	690.667	345.333	43.77	<.001
Primer	2	155.556	77.778	9.86	0.001
Salinity.Primer	4	122.444	30.611	3.88	0.019
Residual	18	142.000	7.889		
Total	26	1110.667			

Appendix 17: ANOVA for Fresh Weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salinity	2	123474.1	61737.0	128.22	<.001
Primer	2	18607.4	9303.7	19.32	<.001
Salinity.Primer	4	10148.1	2537.0	5.27	0.005
Residual	18	8666.7	481.5		
Total	26	160896.3			

Appendix 18: ANOVA for Shoot Length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salinity	2	0.49583	0.24791	6.97	0.006
Primer	2	0.68645	0.34323	9.65	0.001
Salinity.Primer	4	0.09241	0.02310	0.65	0.634
Residual	18	0.64007	0.03556		
Total	26	1.91476			

Appendix 19: ANOVA for Root Length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salinity	2	5.15976	2.57988	37.08	<.001
Primer	2	0.37609	0.18804	2.70	0.094
Salinity.Primer	4	0.26489	0.06622	0.95	0.457
Residual	18	1.25247	0.06958		
Total	26	7.05320			

Appendix 20: ANOVA for Seedling Length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salinity	2	8.42312	4.21156	82.19	<.001
Primer	2	2.03036	1.01518	19.81	<.001
Salinity.Primer	4	0.11641	0.02910	0.57	0.689
Residual	18	0.92240	0.05124		
Total	26	11.49230			

EXPERIMENT 3

ROOTS

Appendix 21: ANOVA for Ca/Na ratio

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salinity	2	2.10166255	1.05083127	26076.86	<.001
Primer	2	0.98637467	0.49318733	12238.67	<.001
Salinity.Primer	4	1.87504559	0.46876140	11632.53	<.001

Residual	18	0.00072535	0.00004030		
Total	26	4.96380816			

Appendix 22: ANOVA for K/Na

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salinity	2	5.1403939	2.5701969	7518.36	<.001
Primer	2	11.5740685	5.7870343	16928.29	<.001
Salinity.Primer	4	20.1151344	5.0287836	14710.24	<.001
Residual	18	0.0061534	0.0003419		
Total	26	36.8357502			

Appendix 23: ANOVA for Ca

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salinity	2	0.212	0.106	0.11	0.900
Primer	2	55.555	27.777	27.78	<.001
Salinity.Primer	4	4.631	1.158	1.16	0.362
Residual	18	18.000	1.000		
Total	26	78.398			

Appendix 24: ANOVA for K

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salinity	2	309032.667	154516.333	1.545E+05	<.001
Primer	2	80938.667	40469.333	40469.33	<.001
Salinity.Primer	4	57899.333	14474.833	14474.83	<.001
Residual	18	18.000	1.000		
Total	26	447888.667			

Appendix 25: ANOVA for Na

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salinity	2	2.033E+05	1.017E+05	1.017E+05	<.001
Primer	2	5.965E+05	2.982E+05	2.982E+05	<.001
Salinity.Primer	4	1.206E+06	3.016E+05	3.016E+05	<.001
Residual	18	1.800E+01	1.000E+00		
Total	26	2.006E+06			

Appendix 26: ANOVA for Mg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salinity	2	0.204	0.102	0.10	0.904
Primer	2	8.414	4.207	4.21	0.032
Salinity.Primer	4	0.376	0.094	0.09	0.983
Residual	18	18.000	1.000		
Total	26	26.995			

STEM

Appendix 27: ANOVA for Na

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salinity	2	2.259E+05	1.130E+05	1.130E+05	<.001
Primer	2	6.364E+05	3.182E+05	3.182E+05	<.001
Salinity.Primer	4	1.127E+06	2.818E+05	2.818E+05	<.001
Residual	18	1.800E+01	1.000E+00		
Total	26	1.989E+06			

Appendix 28: ANOVA for K

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salinity	2	360606.447	180303.223	1.803E+05	<.001
Primer	2	32668.647	16334.323	16334.32	<.001
Salinity.Primer	4	68183.773	17045.943	17045.94	<.001
Residual	18	18.000	1.000		
Total	26	461476.867			

Appendix 29: ANOVA for Ca

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salinity	2	0.711	0.355	0.36	0.706
Primer	2	53.893	26.947	26.95	<.001
Salinity.Primer	4	5.890	1.472	1.47	0.252
Residual	18	18.000	1.000		
Total	26	78.493			

Appendix 30: ANOVA for Mg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salinity	2	0.108	0.054	0.05	0.948

Primer	2	7.226	3.613	3.61	0.048
Salinity.Primer	4	1.433	0.358	0.36	0.835
Residual	18	18.000	1.000		
Total	26	26.767			

Appendix 31: ANOVA for K/Na ratio

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salinity	2	8.0849837	4.0424919	5236.29	<.001
Primer	2	16.0010491	8.0005246	10363.17	<.001
Salinity.Primer	4	29.4529985	7.3632496	9537.70	<.001
Residual	18	0.0138963	0.0007720		
Total	26	53.5529277			

Appendix 32: ANOVA for Ca/Na

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salinity	2	1.84184095	0.92092047	81377.07	<.001
Primer	2	0.69935194	0.34967597	30899.09	<.001
Salinity.Primer	4	1.29580348	0.32395087	28625.89	<.001
Residual	18	0.00020370	0.00001132		
Total	26	3.83720007			

LEAVES

Appendix 33: ANOVA for Na

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salinity	2	2.466E+05	1.233E+05	1.233E+05	<.001
Primer	2	7.066E+05	3.533E+05	3.533E+05	<.001
Salinity.Primer	4	1.178E+06	2.946E+05	2.946E+05	<.001
Residual	18	1.800E+01	1.000E+00		
Total	26	2.132E+06			

Appendix 34: ANOVA for K

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salinity	2	412650.527	206325.263	2.063E+05	<.001
Primer	2	35585.727	17792.863	17792.86	<.001
Salinity.Primer	4	55909.853	13977.463	13977.46	<.001

Residual	18	18.000	1.000		
Total	26	504164.107			

Appendix 35: ANOVA for Ca

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salinity	2	4.347	2.174	2.17	0.143
Primer	2	0.012	0.006	0.01	0.994
Salinity.Primer	4	0.809	0.202	0.20	0.934
Residual	18	18.000	1.000		
Total	26	23.169			

Appendix 36: ANOVA for Mg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salinity	2	0.895	0.448	0.45	0.646
Primer	2	0.010	0.005	0.00	0.995
Salinity.Primer	4	0.025	0.006	0.01	1.000
Residual	18	18.000	1.000		
Total	26	18.930			

Appendix 37: ANOVA for Ca/Na

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Primer	2	3.654045	1.827023	1363.08	<.001
Salinity	2	5.435369	2.717685	2027.57	<.001
Primer.Salinity	4	7.068322	1.767080	1318.36	<.001
Residual	18	0.024127	0.001340		
Total	26	16.181863			

Appendix 38: ANOVA for K/Na

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Primer	2	5.5873197	2.7936598	9628.81	<.001
Salinity	2	3.8840164	1.9420082	6693.45	<.001
Primer.Salinity	4	8.7149443	2.1787361	7509.37	<.001
Residual	18	0.0052224	0.0002901		
Total	26	18.1915029			