EFFECT OF INDUCED SALINITY STRESS ON GERMINATION AND ION ACCUMULATION OF COWPEA

SUMYEA BEHESTI

DEPARTMENT OF AGROFORESTRY AND ENVIRONMENTAL SCIENCE
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
DHAKA-1207

JUNE, 2021

Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207

PABX: +88029144270-9
Fax: +88029112649
Web site: www.sau.edu.bd

CERTIFICATE

This is to certify that the thesis entitles, **"EFFECT OF INDUCED SALINITY STRESS ON GERMINATION AND ION ACCUMULATION OF COWPEA"** *submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE IN AGROFORESTRY AND ENVIRONMENTAL SCIENCE,*** *embodies the result of a piece of bona fide research work carried out by* ***SUMYEA BEHESTI*** *Registration No.* ***14-05880*** *under my supervision and my guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated: June, 2021
Dhaka, Bangladesh

Dr. Nazmun Naher
Professor
Supervisor

Dedicated To

My Beloved Parents

And Husband

for their endless love, support and encouragement.

ACKNOWLEDGEMENTS

*All the praises and gratitude are due to the omniscient, omnipresent, and omnipotent "**Almighty Allah**", who has kindly enabled the author to complete her research work and complete this thesis successfully for increasing knowledge and wisdom.*

*The author sincerely desires to express her deepest sense of gratitude, respect, profound appreciation, and indebtedness to her research Supervisor, Professor **Dr. Nazmun Naher**, Department of Agroforestry and Environmental Science, Sher-e-Bangla Agricultural University, Dhaka for her kind and scholastic guidance, untiring effort, valuable suggestions, inspiration, co-operation and constructive criticisms throughout the entire period of the research work and the preparation of the manuscript of this thesis.*

*The author expresses heartfelt gratitude and indebtedness to her Co-supervisor, **Dr. A.K.M. Mahbubul Alam**, PSO, Bangladesh Agriculture Research Institute, Gazipur, for his cordial co-operation, valuable advice, and helpful suggestions for the successful completion of the research work.*

*The author is grateful to the honorable chairman, **Dr. Jubayer-Al-Mahmud**, Department of Agroforestry and Environmental Science, Sher-e-Bangla Agricultural University, Dhaka for her co-operation and advice and thanks to other teachers of the Agroforestry and Environmental Science department.*

*The author also expands her thanks to Assistant Prof. **Abdul Awal Chowdhury Masud** for his guidance in data analysis of the experiment and would also like to give thanks to all the staff who were engaged with the greenhouse of Bangladesh Agricultural Research Institute (BARI) for helping in data collection.*

*The author would also like to thank her **in-laws' family** and **well-wishers** for their cordial support, inspiration, and ceaseless patience, without which this study would not have been finished.*

*Finally, the author found no words to thank her beloved **parents and husband** for their unquantifiable love and continuous support, their sacrifice, never-ending affection, immense strength, and untiring efforts for bringing her dream to a proper shape. They were a constant source of inspiration, zeal, and enthusiasm in the critical moment of her studies.*

May Allah bless and protect them all.

Date: June, 2021 *The Author*

EFFECT OF INDUCED SALINITY STRESS ON GERMINATION AND ION ACCUMULATION OF COWPEA

ABSTRACT

Salinity is one of the most significant abiotic factors in arid and semi-arid areas of the world. This study was conducted to assess the potential for salt tolerance of cowpea genotypes during the germination and early vegetative growth. From the germination test, twenty best performed genotypes were grown at 0, 4, 8 and 12 dS/m at greenhouse of Bangladesh Agricultural Research Institute, Gazipur. The experiment was laid out in two factors Complete Randomized Design with two replications. Factor A was twenty cowpea genotypes and Factor B was four NaCl concentrations. From the result, it was found that TVU-2398 and TVU-1330 showed the highest SPAD value (53.1 and 53.62 SPAD unit). In the leaf, the highest Na^+ was found in TVU-1330 (0.206%) and K^+ in TVU-2398 (0.24%) and TVU-1330 (0.242%). In case of stem, the highest Na^+ was found in TVU-1330 (0.29%) and TVU-2398 (0.29%) and K^+ in TVU-1330 (0.206%). So, the genotypes TVU-1330, TVU-2398 and TVU-1059 were performed best under salinity condition than other genotypes.

ACRONYMS

% = Percent
^{0}C = Degree Celsius
Ca = Calcium
BARI = Bangladesh Agricultural Research Institute
BBS = Bangladesh Bureau of Statistics
ANOVA= Analysis of Variance
CEC = Cation exchange capacity
cm = Centimeter
CRD = Completely Randomized Design
CV = Co-efficient of Variation
DAT = Days After Transplantation
dS/m = Deci Siemens per meter
EC =Electrical conductivity
e.g. =For example
et al. =And others
g = Gram
GP = Germination Percentage
ha = Hector
K = Potassium
Kg = Kilogram
Km = Kilometer
LSD = Least Significant Difference
m = Meter
mg = Milligram
Mg = Magnesium
mm = Millimeter
mM = Mili mole
Na = Sodium
NaCl = Sodium Chloride
no. = Number
NSB = National Seed Board
ppm = Parts per million
PRC = Pulse Research Center
RARS = Regional Pulse Research Station
ROS = Reactive Oxygen Species
SAU = Sher-e-Bangla Agricultural University
SI = Serial
Sp. = Species
Var. = Variety
viz. = Namely

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER I

INTRODUCTION

Soil salinity is a serious threat to crop production, biodiversity, food, and nutritional security. The intrusion of saline water into cultivable lands because of sea-level rise as a consequence of worldwide warming exacerbates soil salinity in all the coastal areas of Bangladesh. Salinity affected area in Bangladesh has increased from 8,330 square km in 1973 to 10,560 square km in 2009 (SRDI, 2010). The coast of Bangladesh consists of 19 districts, covers 32% of the country and accommodates about 35 million people (Huq and Rabbani, 2011). The ecology of the coastal region especially within the southwest region is greatly concerned with salinity. Salinity may be a great constraint to growing crops, especially in rabi season (dry months) when soil salinity arises and reaches the height in March-April before monsoon starts (Haque *et al.*, 2008). Most crop plants are vulnerable to saline soil and cowpea (*Vigna unguiculata* L. Walp.) is taken into account highly at risk of salt stress (McKenzie, 1988). Cowpea is a crucial seed legume crop in Bangladesh. The cowpea (*Vigna unguiculate* L.) may be a legume crop grown for its green pods or dry seeds, which offer protein, vitamins, and minerals. It is also used for other purposes like fodder and as a manure crop (Halli and Angadi, 2019). Its dry seeds provide an equiponderant source of nutrients with numerous health benefits; they need high levels of protein (20.0–39.4%) and a low content of fat (3.1–30.4%) (Moustafa *et al.*, 2020). It's area of cultivation is approximately 14 million hectares, producing 4.5 million a lot of cowpeas annually in Africa, Asia, and America (Abdel *et al*., 2020). The cowpea has the aptitude of fixing atmospheric nitrogen (N) and grows well in infertile soils (Hashim *et al*., 2020). Hence, it could grow better in arid and semi-arid areas and under stress conditions than other

crops. The presence of salt at concentrations over 50 mM NaCl affects germination, seedlings' growth and total protein synthesis in cowpea cultivars (Dantas *et al*., 2005). Soil salinity may affect the germination of seeds either by creating a lower osmotic potential external to the seed preventing water uptake or through the toxic effects of Na^+ and Cl^- ions on the germinating seeds (Khajeh-Hosseini *et al*., 2003). Various strategies are adapted to address salinity stress like screening and selection, conventional breeding and use of transgenic supported morphological, physiological and biochemical traits. (Dasgan *et al.,* 2002) suggested that screening at the seedling stage is not only less laborious, less time-consuming and fewer expensive, but also has high reliability. While establishing appropriate salinity screening techniques, it is also important to grasp which of the physiological or biochemical processes is more sensitive to salt stress which will be used as an efficient selection criterion (Ashraf and Harris, 2004).

Salt stress can impair plant physiology, photosynthesis and absolutely important functions like cell extension and division (Maas and Hoffman, 1977). These aforementioned factors may lead to a big cowpea yield reduction (Dutta and Bera, 2014).

Phenotyping could be a substantial process in screening genotypes for a specific trait of interest. It is usually a labor-intensive, time-consuming and expensive task to undertake for plant breeders. Salt phenotyping are often disbursed in fields.

Hydroponics culture is becoming increasingly popular everywhere the planet. It is highly productive, conservative of water and land, and protective of the environment. Hydroponics has proved to be a wonderful alternative crop production system (Savvas, 2003). The cultivation of vegetable crops and also the achievement of high yields and

prime quality are possible with hydroponics even in saline or acidic soils, or non-arable soils with poor structure. An extra advantage of hydroponics is that the precise control of plant nutrition. Furthermore, the preparation of the soil is avoided in hydroponics, thereby increasing the potential length of cultivation time, which is a good means of skyrocketing the overall yield in greenhouses. The reason, imposing a reverse to hydroponics is increasingly related to environmental policies yet. A hydroponic system enables a substantial reduction of fertilizer application and a drastic restriction or perhaps a whole elimination of nutrient leaching from greenhouses to the environment (Avidan, 2000). Hydroponics offers a way of control over soil-borne diseases and pests, which is particularly desirable within the tropics, where infestations are a significant concern. Despite the considerable advantages of economic hydroponics, there are still some disadvantages. Nowadays, the principal disadvantages of hydroponics, are the high costs of capital and energy inputs, and therefore the high degree of management skills required for a successful production.

The hydroponic method is applying the nutrient solution to the plant roots. The nutrient solution is one among the foremost components for successful hydroponic crop production. The composition of nutrient solutions and therefore the optimization of nutrition in commercial hydroponics can reduce fertilizer costs. Moreover, to get high yield and good quality in commercial crops grown hydroponically, the nutrient solution supplied to the plants must be specific for the actual crop.

Although there is plenty of research being done on nutrient content levels of cowpea during a hydroponic system within the world there is little information on research done on the nutrient solution concentrations in our country.

Considering the above facts, the current research work was aimed to review with the subsequent objectives:

1. To assess the germination percentage of some cowpea genotypes through in vitro screening under salinity stress;

2. To examine the effects of salinity on chlorophyll content (SPAD value) and ion accumulation (Na^+, K^+) of cowpea genotypes at different salinity levels and

3. To find out the best performed salt tolerant cowpea genotypes during seedling growth stages at greenhouse

CHAPTER II

REVIEW OF LITERATURE

2.1 Soil salinity

Soil salinity is that the term used to designate a condition in which the soluble salt content of the soil reaches a level harmful to crops. Soil with an electrical conductivity of saturation extracts above 4 dS /m is termed saline soil. It contains a way over soluble salts, especially common salt. Soil salinity could be a major constraint of food production because it limits crop yield and restricts the utilization of uncultivated land (Flowers and Yeo, 1997).

Tanji (1990) said that soil salinity is that the concentration of dissolved mineral salts presents in water and soils on a unit basis or weight. According to Hemandez (2019), salt stress is taken into account one amongst the foremost widespread abiotic stresses and severely hampers crop production, especially in arid and semi-arid areas. Flowers (2005) said that salinity may be termed as abiotic stress and comprises all the issues because of salts primarily by an abundance of binary compound (NaCl) from irrigation or natural accumulation.

2.2 Soil salinity around the world and in Bangladesh

Soil salinity area is one in all the assorted effects of the changing environment and is rapidly increasing. Around 930 million ha of land is plagued by salinity worldwide. The severity of the matter is often gauged from the actual fact that salinity has increased by 6% over the last 45 years, with 77 million ha of land becoming saline. Within the next 50 years, another 15 million ha is in danger of becoming saline in Australia (Ghassemi *et al.*, 1995 and Munns, 2002). Approximately 33% of irrigated croplands

are plagued by salinity levels to varying degrees, and this might surpass 50% by 2050 (Gopalakrishnan, 2020).

According to FAO (2010), the overall worldwide area of land laid low with salinity is about 190 million per ha. Bradbury and Ahmad (1990) reported, one-third of the world's land surface is arid or semi-arid, out of which one-half is estimated to be littered with salinity. Waisal (1972) reported that over four-fifths of the surface of our earth is roofed with salt solution containing, among many other constituents approximately 0.5 m NaCl.

FAO (2017) reported that salt stress negatively effects 60 million hectares or around about 20% of the whole irrigated surface area within the world. Yasin *et al*. (1998) reported that out of 16.2 m ha of land under irrigation, quite 40,000 ha of land is lost to crop production annually in Pakistan. Bangladesh is extremely susceptible to sea-level rise (Brammer *et al.*, 1993). Naher *et al.* (2011) reported that coastal area in Bangladesh constitutes 20% of the country of which about 53% are full of different degree of salinity. The full coast runs parallel to the Bay of Bengal, forming a 710 km long coastline (CZP, 2005). The world lies at 0.9 to 2.1 meters above mean water level (Iftekhar and Islam, 2004). The Bangladesh Agriculture Census, from its 1996 to 2008 to preliminary 2019 results, has shown slight fluctuations within the percentage of completely landless households, from 10% to 13% to eight nationally and from 8% to 10% to 7% for the Khulna division, which has the three southwest coastal districts (BBS, 2019).

Soil Resource Development Institute (2000) showed that soil saline area within the country has increased to 1.02 million ha. Agricultural land employed in these areas is

incredibly poor, which is roughly 50% of the country's average (Petersen and Shireen, 2001). In Bangladesh, coastal areas of about 2.86 million ha are covered by 30% of the entire cropland of the country. Of this, nearly 1.056 million ha are suffering from varying degrees of salinity (Karim *et al*., 1990).

The problems of salinization are increasing, either because of bad irrigation drainage or agriculture practices. Despite its relatively small area, irrigated land is estimated to provide one-third of the world's food (Munns, 2002). Hasanuzzaman *et al*. (2013) reported that the arable land is continuously transforming into saline (1- 3% per year) either due to primary natural salinity or secondary irrigation-associated salinity and is anticipated to extend up to 50% land loss by 2050.

According to SRDI (2010), in Bangladesh out of coastal cultivable saline area, about thousand hectares of land are affected by non-saline to very slightly saline (2-4 dS/m), very slightly to slightly saline (4-8 dS/m), slightly to moderate saline (8-12 dS/m), moderate saline to strong saline (12-16 dS/m) and strong saline to very strong saline (>16 dS/m) respectively are scope to successfully crop production.

Table 1. Soil salinity class of Bangladesh

Land classification	**Salinity (dS/m)**
Non saline to very slightly saline	2-4
Very slightly saline to slightly saline	4-8
Slightly saline to moderate saline	8-12
Moderate saline to strong saline	12-16
Strong saline to very strong saline	>16

Source: SRDI, 2010

Salinity is predicted to own devastating global effects leading to up to 50% land loss by 2050 because the arable land is continuously transforming into saline (1-3% per year) either by natural salinity or induced by humans in Bangladesh (Mahajan and Tuteja, 2005 and Hasanuzzaman *et al.*, 2013). In general, soil salinity is believed to be mainly answerable for low land use additionally because the cropping intensity within the area (Rahman and Ahsan, 2001).

Significant seasonal patterns in surface water salinity have already been observed, with saltwater pushing inland into rivers and canals when droughts, dry season, and upstream dams reduce the amount of downstream flow of the Ganges and its distributaries (Salehin *et al*., 2018). The surface or shallow saltwater that has intruded inland can filter down vertically to salinize groundwater resources or seep into the encircling land to salinize the soil (Salehin *et al*., 2018). Acute increases in salinity have also been documented within the delta, as a results of flooding and storm surges from severe tropical cyclones within the North Ocean, like Cyclone Sidr in 2007 and Cyclone Aila in 2009 (Kabir *et al.*, 2002; Salehin *et al*., 2018). In step with the coastal zone policy (CZP, 2005) of the govt. of Bangladesh, 19 districts out of 64 are within the coastal zone covering a complete of 147 Upazilas of the country. The central coastal zone extends from the Feni River estuary to the eastern corner of the Sundarbans, covering Noakhali, Barisal, Bhola, and Patuakhali districts. The mouth of the Ganga-Brahmaputra-Meghna (GBM) Rivers occupies the western boundary of the central coast, and also the tide from the Bay of Bengal (BoB) inundates twice this coastline. Sediment and water discharge from the Meghna River (MR) of this estuary is that the peak within the country and therefore the third-highest among all the river confluences within the world (Syed *et al*., 2018).

Naher *et al*. (2011) found that the lands of the coastal area become saline because it comes involved with seawater by continuous inundation during high tides and ingress of seawater through cracks and sometimes cyclone induced storm surge. The severity of salinity is increasing within the coastal area during winter with the drying of the soil. Salinity causes an unfavorable environment and hydrological situation that restrict normal crop production throughout the year. It affects crops counting on the degree of salinity at the critical stages of growth, which reduces yield, and in severe cases, the overall yield is lost. Soil reaction values (pH) in coastal regions range from 6.0–8.4 (Haque, 2006 and Naher *et al.,* 2011). Observations within the recent past indicated that because of the increasing degree of salinity of some areas and expansion of salt-affected areas as a reason behind further intrusion of saline water, normal crop production becomes more restricted.

Generally, the exposure of plants to high levels of salt can prevent growth and retard developmental processes in many ways, like osmotic imbalance, cytotoxicity induced by excrescent Na^+ and Cl^-, and nutritional inconsistency (Soliman, 2020 and Abdel Latef, 2021). At a later stage of development, plants exposed to salinity experience increased oxidative stress due to the assembly of supernumerary amounts of reactive oxygen species (ROS). This leads to oxidative injuries to numerous cellular macromolecules, like lipids, proteins and nucleic acids, which eventually deactivate numerous important cellular processes in plants (Halli, 2019 and Fouad, 2021). All the key processes, like photosynthesis, and therefore the energy of plants also is littered with salinity stress. Salinity reduced the power of plants to soak up water, resulting in growth reduction, additionally on impaired metabolic processes almost like those caused by water stress (Ahmed, 2021 and Zhu, 2002) and warmth stress (Jahan, 2021).

The negative effect of salinity stress on growth, physiological aspects and productivity has been observed in several plant species, like the common bean *(Phaseolus vulgaris*) (Bargaz, 2016 and Shabana, 2020), wheat (*Triticum aestivum*) (Alnusairi, 2021), basil (*Ocimum basilicum*) (Nassar, 2016), and lupine (*Lupinus termis*) (Rady, 2020).

2.3. Fertility status of saline soils

Soil fertility is a very important factor for crop production. In general, the coastal regions of Bangladesh are quite low in soil fertility. Thus, additionally to salinity, plant nutrients in soils affect plant growth.

Soil reaction values (pH) range from 6.0-8.4 except Chittagong and Patuakhali, where the pH values range from 5.0-7.8. The soils are generally poor in organic matter content apart from Paikgachha Upazila of Khulna district, where the top soils contain high organic matter (7%). The organic matter content of the top soils ranges from but 1% to 1.5%. The low organic content in soils indicates the poor vigor of the coastal soils. The overall N contents of the soils are generally low, mostly around 0.1%. The low N content is also attributed to the low organic matter contents of most of the soils. Available P status of the soils ranges from 15-25 ppm. Some deficient P soils also are found in Chittagong, Barguna, Satkhira, and Patuakhali districts. Widespread Zn and Cu deficiencies are observed within the coastal regions (Karim et al., 1990 and Naher *et al.,* 2011).

Compared to other coasts, the central coast of Bangladesh is that the most dynamic because it is vastly irregular and broken (Rasheed, 2016). Geomorphologically, this area accreted an oversized tract of land after the 1950's Assam earthquake (Brammer

and H., 2012; Kawser *et al*., 2021) and is taken into account a vigorous zone of land erosion and accretion.

2.4 Centre of Origin, Domestication, and Distribution of Cowpea

Cowpea [*Vigna unguiculata* (L.) Walp.] is one of the important food and forage legumes within the semi-arid tropics that include parts of Asia, Africa, Southern Europe, Southern United States, and Central and South America (Singh, 2005; Timko *et al.*, 2007). The most cowpea producing countries of the planet are in geographical area, that is the Sudano-Sahelian vegetation region (Boukar *et al*., 2019). In terms of the metric weight unit production levels of cowpea grain, Nigeria is that the largest producer within the world (FAO, 2020).

Cowpea was likely introduced to the Indian sub-continent from Africa approximately 2000 to 3500 years ago (Allen, 1983). Spanish explorers are likely liable for introducing cowpea into the New World, bringing seed to the Indies within the 16th century. The plant presumably was introduced into Central and South America at about the identical time and made its due to the continental United States by 1700 (Purseglove, 1968). Presently, cowpea is grown throughout the tropic and subtropics areas around the world (Magloire, 2005).

According to Duke (1981), the origin and domestication of cowpea occurred in Africa mainly within the African Savannah. Probable centers of domestication are thought to be mainly in geographical area, African nation and South Africa (Vavilov, 1951). All of the present evidence suggests that cowpea originated in southern Africa. Probably, the Limpopo region was the middle of speciation of *Vigna unguiculata*, because of the

presence of the foremost primitive wild varieties (Ng and Marechal, 1985). It is one of the most ancient human food sources and has probably been cultivated since Neolithic times (Summerfield *et al*., 1974). It is center of origin and subsequent domestication being closely associated with pearl millet and sorghum in Africa. The precise origin of cultivated cowpea has been a matter of speculation and discussion for many years due to lack of archaeological evidence (Tindall, 1983). Previous speculation on the origin and domestication of cowpea has been based on botanical and cytological evidence, as well as information on its geographical distribution and cultural practices, and historical records (Ng and Marechal, 1985).

In Bangladesh, cowpea is commonly referred to as "Felon". Considering the emerging popularity, scopes, and opportunities, Bangladesh Agricultural Research Institute has invented two distinct types of cowpeas, BARI Cowpea-1 and BARI Cowpea-2. The germplasm of Cowpea-1 was collected from the Chittagong region through primary, secondary, and multi-spot trial basis selection. The most identifying characters of BARI Cowpea- 1 include light green erected leaves and stem, lifetime 125-135 days, ash-colored skin with blackish stripes, the load of 100 seed are 90-95 grams, yield 1.1- 1.4 tons per hectare, protein content 25-30% (Khrishi, 2016). On the opposite hand, BARI Cowpea-2 was originated from some variety lines of IITA invented pulse crops. The performances of the lines regarding yield capacity, resistance to pest and disease, lifetime, etc. were closely observed analyzed. The variability was developed and identified as a high-yielding variety through primary secondary and multi-spots trials.

In 1996, the National Seed Board (NSB) certified and released this variety as BARI Cowpea- 2 for commercial production. The identifying features of this variety include comparatively dark greenish leaves and stems, lifetime 120-130 days, skin is of ash

color, the weight of 100 seeds is100-120 grams; 75-80 grams without peel, yield 1.5 kg per hector (Khrishi, 2016). Since BARI Cowpea-1 was developed and released for commercial production in 1993, it absolutely was preferably introduced within the Chattogram region instead of BARI Cowpea-2 for its specific features suited better during this region.

In 2017, as a shot of the Department of Economics, Regional Agricultural Research Station (RARS), Hathazari, Chattogram, an adoption study was applied on BARI Cowpea-1 for assessing the status of adoption and socioeconomic impact of this technology (variety) on farmer's household income. It absolutely was imperative to grasp the status of the technology (BARI Cowpea-1) considering circumstances at the sector level and factors affecting adoption and non-adoption. the opposite associating organizations even have shown interest and cooperated in executing the study successfully. The DAE high officials had projected highly positive responses about this new study during the "Research extension Review workshop" at planner's session on 27 April 2017 at RARS, Hathazari, Chattogram. Their assistance was a really good means for disseminating the technologies at the sphere level.

Carbon dating of untamed cowpea remains from the Kimtampo rock shelter in central Ghana has shown that the oldest archaeological evidence of cowpea is found in Africa (Flight, 1976). Additionally, to the current evidence, Baudoin and Maréchal (1985) reported that, supported the six distributions of diverse wild cowpeas along the whole length of eastern Africa, from Ethiopia to Southern Africa, east and southern Africa to be the first region of diversity, and west and Central Africa to be the secondary center of diversity. These researchers also proposed Asia as a 3rd center of diversity. More modern studies strongly indicate that the best genetic diversity of primitive wild

varieties of cowpea are found within the region of the African continent currently encompassed by Namibia, Botswana, Zambia, Zimbabwe, Mozambique, Swaziland, and South Africa and also the most primitive species were observed within the Transvaal, Cape Town, and Swaziland (Padulosi, 1993). Supported this latter observation, Padulosi and Ng (1997) suggested that southern Africa is also the positioning of origin of cowpea with subsequent radiations of the primitive forms to other parts of southern and eastern Africa, and subsequently to geographic region and Asia. Human selection for larger seeds and better growth habits from natural variants in wild cowpeas likely led to diverse cultigroups and their domestication in Asia and in Africa (Ng, 1995; Padulosi and Ng, 1997; Ba *et al.*, 2004).

Vigna unguiculata is thought by a range of names world-wide: within the English-speaking parts of Africa, it is referred to as cowpea whereas, within the Francophone regions of Africa, the name "niébé" is most frequently used. Local names for cowpea include "seub" in Senegal, "wake" in Nigeria, and "luba hilu" within the Sudan. In the USA, it is typically referred to as blackeye beans and blackeye or southern peas. On the Indian subcontinent it is called "lobia" and in Brazil it is "caupi." (Timko and Singh, 2008). In Ethiopia it is known by different names: "adengure" (Amaharic), "atera argobba" (Oromifa), "lakoma" (Wolaitegna), "gobo" (Kefigna) and "wee" (Kembategna) (Uppsala, 1989; Shackleton *et al.*, 2009).

2.5 Botanical Description of Cowpea

Cowpea is an annual herb reaching heights of up to 80 cm with a robust taproot and lots of spreading lateral roots within the dirt. Growth forms vary and lots of are erect, trailing, climbing, or bushy. Leaves are alternate and trifoliate. The first pair of leaves is simple and opposite. Leaves exhibit considerable variation in size and shape and they

are usually dark green. The stems are striate, smooth or slightly hairy and sometimes tinged with purple (Uppsala, 1989; Patel and Hall, 1990; Davis *et al*., 1991).

The flowers of cowpea are arranged in racemose or intermediate inflorescence at the distal ends of 5-60 cm long peduncles. Flowers are conspicuous, self-pollinating, borne on short pedicels and therefore the corollas is also white, dirty yellow, pink, pale blue, or purple. Early flowering cowpea genotypes can produce a crop of dry grain in 60 days, while longer season genotypes may require quite 150 days to mature, counting on the photoperiod. They are usually yellow when ripe, but may additionally be brown or purple (Fery, 2002).

There are usually 8-20 seeds per pod. Seeds vary considerably in size, shape and color. They are relatively large (2-12 mm long) and weigh 5-30 g/100 seeds. Seed shape is correlated thereupon of the pod. When individual seeds are separate from adjacent ones during development, but as crowding within the pod increases, the seeds become globular. The testa may be smooth or wrinkled, white, green, buff, red, brown, black, speckled, blotched, eyed or mottled in color neutral (Craufurd *et al.,* 1997). The cotyledons emerge above the ground indicating epigeal germination (Davis *et al.,* 1991).

2.6 Classification of Cowpea

Cowpea could be a dicotyledonous crop that belongs to the order Fabales, Leguminosae, subfamily Faboideae, tribe Phaseoleae, subtribe Phaseolineae and section Catiang (Verdcourt, 1970; Marechal *et al.,* 1978). The Fabaceae also contains the common bean (*Phaseolus vulgaris* L.*),* the mungbean (*Vigna radiata* L.) and

blackgram (*Vigna mugo*) among other legumes of economic importance (Ng and Marachel, 1985). It contains 22 chromosomes (2n = 2x = 22). The genus was initially divided into several subgenera based upon morphological characteristics, extent of genetic hybridization/reproductive isolation, and geographic distribution of species (Maréchal *et al.,* 1978). The main groupings consisted of the African subgenera *Vigna* and *Haydonia*, the Asian subgenus *Ceratotropis*, and also the American subgenera *Sigmoidotropis* and *Lasiopron*. Under the scheme proposed by Maréchal *et al*. (1978) cultivated cowpea was placed within the subgenus *Vigna*, whereas mungbean and blackgram were placed in the Asian subgenera. V. *unguiculata* subspecies 9 *unguiculata* includes four cultigroups: unguiculata, biflora (or cylindrica), sesquipedalis, and textilis (Ng and Maréchal, 1985). According to Mebeaselassie *et al.* (2011) the secondary gene pool of cowpea includes nine perennial subspecies.

2.7 The Importance of Cowpea

Cowpea plays a critical role within the lives of uncountable people within the developing world, providing them with a serious source of dietary protein that nutritionally complements low protein cereals and tuber crops. The nutritional profile of cowpea grain is comparable to it of other pulses with a comparatively low-fat content and a complete protein content that is two to four-fold above cereal and tuber crops. Cowpea contains a big variety of uses namely as a nutritious component within the human diet yet as nutritious livestock feed. Cowpea is used in any respect stages of growth as a vegetable crop. The tender green leaves are a very important food source in Africa and are prepared as a potherb, like spinach. Immature snapped pods are employed in the identical way as snap beans, often being mixed with other foods. Green cowpea seeds are boiled as a fresh vegetable, or even canned or frozen. Dry mature

seeds are suitable for cooking and canning (Magloire, 2005). Consistent with the study by Bittenbender (1990), cooked leaves contain two-thirds of the protein, seven times of the calcium, thrice of the iron, half the phosphorus, eight times of the riboflavin, five times of the niacin to the cooked seed. Similarly, Quin (1997) reported that on the average the nutrient content of mature cowpea seed is protein 23 - 32 %, fat = 1.9 %, fiber = 6.3 %, carbohydrate = 63.6 %, thiamine = 0.00074 %, riboflavin =0 .00042 %, and niacin = 0.00042 %. Cowpea seeds are a fashionable source of minerals and vitamins and among plants have one amongst the best 10 contents of B complex, a B-complex vitamin necessary during pregnancy to stop birth defects within the brain and spine (Hall *et al*., 2003).

In many areas of the planet, cowpea is that the only available high-quality legume hay for livestock feed. Cowpea fodder plays a very critical role in feeding animals during the time of year in many parts of Africa (Tarawali *et al*., 2002). Although protease inhibitors are found within the seed, the utilization of cowpea grain does not present any serious nutritional problems in animal nutrition and has been used as another to other costlier grain protein sources of animal feed (Singh, 2005).

Due to its unique ability to fix atmospheric nitrogen through its nodule's cowpea grows well in poor soils having more than 85 % sand, less than 0.2 % organic matter and low levels of phosphorus (Sanginga *et al.,* 2000). It is very good for quick growth and establishment and for increasing organic matter and improving soil structure. It has superb heat tolerance and good drought tolerance. Cowpea is additionally well recognized as a key component in crop rotation schemes because of its ability to help restore soil fertility for succeeding cereal crops (Tarawali *et al*., 2002). Cowpea is shade

tolerant and, therefore, compatible as an intercrop with maize, millet, sorghum, sugarcane and cotton as well as with several plantation crops (Shiringani, 2007). In addition, well-adapted, early maturing cowpea varieties capable of manufacturing seed in as few as 55 days after planting often provide farmers with the first source of food from the current harvest ahead of the other (Hall *et al.,* 2003). Hausa and Ebo tribes use cowpea medicinally; one or two seeds are ground and mixed with soil or oil to treat stubborn bowels (Magloire, 2005).

Trading of fresh produce and processed cowpea foods and snacks provide rural and concrete people with the chance for earning cash income in Ethiopia and other African countries (Bressani, 1985; Muluemebt, 2003). Together, these characteristics have made cowpea a crucial component of subsistence agriculture. (Carsky *et al.*, 2001).

2.8 Production Status of Cowpea

It is rather difficult to get reliable statistics on cowpea area and production because most countries do not maintain separate records on cowpea. Singh (2002) suggests that cowpea production and acreage are beyond FAO estimates, with worldwide production of 4.5 tones on 12 to 14 million ha, because the FAO estimates do not include the acreage and production figures in Brazil, India, and a few other countries. In line with FAOSTAT (2017), cowpea was grown on an estimated 11 million ha in Africa in 2017 with most of the assembly confined to geographical region (10.6 million ha), especially in Niger, Nigeria, land, Mali, and Senegal. Over 7.4 million heaps of cowpeas are produced worldwide, with Africa producing nearly 5.2 million tons. In step with FAOSTAT (2017), over 87% of cowpeas are produced in Africa. About 6.5 million metrics a lot of cowpeas are being produced annually on about 14.5 million hectares

worldwide (Boukar *et al*., 2018). Dry grain production is that the only commodity of cowpea that production estimates are generated on a worldwide basis. in step with FAO, approximately 4 million metric plenty of dry cowpea grain are produced annually on about 10 million ha worldwide.

In Bangladesh, cowpea is one of the foremost popular and most often cultivated pulse crops within the greater Chattogram, Vola and Feni regions of Bangladesh. Other areas are involved in cowpea production on comparatively small scales. In line with BBS (2012), the entire area and production of other pulses like Gari Kalai, Khesari, Maskhali, Mung, Motor, Masur, Arhar, Gram were estimated at 363,182.5 ha and 473497 metric tons respectively; of which cowpea contributed with notable number; which brings a complete area of 32,000 hectors under cowpea production; the recorded amount of production is 35,000 tons in total.

About 70% of cowpea production occurs within the drier Savanna and Sahelian zones of West and African nation, with about 8 million hectares, followed by about 2.4 million hectares in central and southern America, 1.3 million hectares in Asia, and about 0.8 million hectares in eastern and southern Africa) (FAO, 2005-2006). Nigeria is the largest producer and consumer of cowpea grain with approximately 5 million ha under cultivation with an annual yield estimate at 2.3 metric tons in 2004 (Singh, 2005). After Nigeria, Niger and Brazil are the subsequent largest producers with annual yields estimated at 1 million tons and 0.7 million tons respectively (Singh, 2002; Shiringani, 2007). Cowpea production within the United States is estimated at 80,000 million tons. (Fery, 2002; Timko *et al*., 2007).

2.9 Environmental Requirements

Cowpea is ideally suited to tropical lowlands, doing well in hot, dry and humid ecosystems. It is sensitive to frost. Cowpea may be grown under rain fed conditions further as by using irrigation or residual moisture along river or lake flood plains during the time of year, providing the range of minimum and maximum temperatures are between 28 and 30 ^{0}C during the season (Davis *et al.,* 1991). It performs well in agro-ecological zones where the rainfall range is between 500 and 1200 mm/year. It is more tolerant to high heat and extended drought periods and also well adapted to sandy and poor soils. However, best yields are obtained in well-drained sandy loam to clay loam soils with a pH between 5.5 and 6.5 (Davis *et al*., 1991; Dugje *et al*., 2009).

Temperature and photoperiod interaction with genotype and other aspects of the environment to work out the yield potential of seed legumes through their effects on the duration of the vegetative and reproductive growth stages (Shiringani, 2007). Heat adversely affects the productivity of the many crops. In turn, sensitivity to photoperiod may be moderated by temperature. Developing improved germplasm for warm environments requires an understanding of genetic variation for these responses (Patel and Hall, 1990). Generally, cowpea can give yield satisfactorily under greater diversity of climatic, soil, and cultural conditions than other leguminous crops but the factors chargeable for the broad adaptation are poorly understood (Patel and Hall, 1990).

2.10 Cowpea Production Constraints

2.10.1 Biotic stress

Worldwide, biotic stressors (roots and membrane pathogens) in large numbers result in low productivity and low-quality agricultural products. Destructive pests and pathogens

lead to food insecurity on every scale from the littlest to the most important thus resulting in massive monetary losses on a worldwide scale in terms of crop yield (Savary *et al*., 2019). The most production constraints concerning biotic stress factors limiting cowpea productivity are exemplified by a good range of organisms, including destructive pests; parasitic weeds, viral pathogens, bacterial pathogens, still as fungal pathogens (Boukar *et al.,* 2019).

Cowpea is liable to a good range of bacterial, fungal, and viral diseases and an oversized kind of insect pests that attack the crop in the slightest degree stages of growth (Singh, 2005; Timko *et al*., 2007). As an example, cowpea wilt caused by *Fusarium oscysporium*, cowpea root rust caused by a nematode *Meloidogyne* ssp and cowpea bacterial blight caused by *Xanthomonas vignicola*. Losses because of pest attacks or diseases will be as high as 90 % (IITA, 2003). A number of the most important insect enemies of cowpea are cowpea weevil (*Callosobruchus maculatus*), cowpea cuculus (*Chalcodermus sermus*), the southern cowpea weevil (*Mylabris quadrimaculatus*) and also the pod borer (*Maruca vitrata*) which may be a major Lepidopteran pest that inflicts severe damage to cowpea on farmer's fields. In its severe infestations yield losses of between 70 – 80 you will need been reported (Dugje *et al*., 2009). Several viruses also can attack cowpea. Plants infected during seedling stages could also be barren and fail to supply seeds. The simplest way to prevent large yield losses from virus diseases is to grow tolerant varieties (Emechebe, 1975).

2.10.2 Abiotic stress

To feed the increasing population from existing natural resources, significant advances are required within the field of agriculture production. Increasing agricultural

productivity from the prevailing arable land in an environmentally friendly manner is, however, an enormous challenge for the worldwide agricultural system (Robertson and Swinton, 2005). A possible way forward is to extend the efficiency and sustainability of current crop production practices together with incorporating modern agricultural biotechnology (McMichael, 2001). More specifically, increased efforts are needed to boost crop productivity from salt affected land and water by combining crop production and management practices and genetic improvement that are environmentally sustainable and socially acceptable (Galvani, 2007).

2.11 Soil Salinity

Salinization is the accumulation of water-soluble salts within the soil column or regolith to grade that incorporates a drastic impact on agricultural production, environmental health and economic welfare of the country (Rengasamy, 2006). Soil salinity may be a major environmental issue threatening agricultural productivity worldwide (Wang *et al.*, 2003). Estimates vary, but approximately 70% of the world's total acreage is tormented by salinity (Flowers *et al*., 1997). Most significantly, at present greater than 20 % of the worlds cultivated land and 14 approximately half of all irrigated land is affected by salinity (FAO, 2005-2006). Furthermore, there is also a dangerous trend of a 10% per annum increase within the saline area throughout the planet (Ponnamieruma, 1984). Additionally, salinity is an issue for agriculture because only some crop species and genotypes are adapted to saline conditions. It is being estimated that soil salinity, together with other abiotic stresses, is accountable for over 50% of crop production losses in major field crops (Mahajan and Tuteja, 2005; Alam *et al*. 2004). Therefore, ways must be found to attain this without resorting to unsustainable farming practices and without major increases with the amount of recent land under cultivation, which

might further threaten forests and biodiversity. Is is estimated that productivity will must increase by 20% in developed countries and by 60% in developing countries. Within the light of those demographic, agricultural and ecological issues, the threat and effects of salinity become even more alarming (Galvani, 2007; FAO, 2009a). Reducing the spread of salinization and increasing the salt tolerance of crops and improving species or genotypes to salt tolerance, particularly the high yielding ones are, therefore, problems with global importance (Wikipedia, 2010).

In the soil, the determination of the electrical conductivity (EC) serves to give a concept of the whole quantity of soluble salts and therefore the degree of salinity. The critical level of electrical conductivity of saturated soil paste extracts (ECe) for many crops is 4 dS/m. Saline soils are soils that contain soluble salts in quantities great enough to interfere with the expansion and productivity of most crop plants (Landon, 1991). They are characterized by the electrical conductivity of saturation extracts (ECe) at 25 ^{0}C of greater than 4 ds/m (equivalent to 40 mM), an exchangeable sodium percentage less than 15 and pH value less than 8.5 (Brady, 2002). Salinization of soils develops due to two sources; primary and secondary salinization. Primary salinization occurs because of natural processes including weathering of minerals and soils derived from saline parent rocks whereas secondary salinization results from improper agricultural management practices including poor water management, high evaporation, heavy irrigation and former exposure to seawater (Galvani, 2007). Of those two kinds of soil salinity, secondary salinization of arable land may be a source of major concern because it is adversely affected approximately one-third of the world's agriculturally productive land (FAO, 2008).

2.12 Effect of Salinity on Plant Growth

Salinity is one of the major abiotic stresses that has been significantly affecting plant growth and yield (Gharsallah *et al*., 2016). The continual increase in salinity in arable land because of poor cultivation practices and temperature change have devastating global effects, and it is estimated that about 50% of arable land are lost by the middle of the 21st century (Islam *et al*., 2019). To date, about 1,125 million hectares of agricultural lands have already been seriously suffering from salinity, thus it is considered a significant threat to agriculture (Islam *et al*., 2019; Sanower-Hossain, 2019). In China, a complete of 36.7 million hectares of land has been greatly stricken by salinity, of which 12.3 million hectares is agricultural land (Li-ping *et al*., 2015).

Salinity adversely affects important phonological, physiological and biochemical processes in plants ultimately resulting in a discount in plant growth and development (Ali, 2010; Asci, 2011). Salinity can inhibit plant growth in three major ways; water deficit arising from the more negative water potential (elevated osmotic pressure) of the soil solution; specific ion toxicity usually related to either excessive chloride or sodium uptake and nutrient ion imbalance when the surplus of Na^+ or Cl^- leads to a diminished uptake of K^+, Ca^{2+}, NO^{3-} or P, or impaired internal distribution of one or another of those ions (Khan *et al*., 2007; Abdelhamid *et al*., 2010). Plant response to salinity stress occurs in two phases: an initial and rapid response to the elevation in external force per unit area and a slower response because of the buildup of Na^+ inside the plant cells (Munns *et al*., 2006).

The osmotic effect, which develops due to increasing salt concentration within the root medium, may be a primary contributor to growth reduction within the initial stages of

plant growth (Munns and Tester, 2008; Akhtar and Hussain, 2009). This stage is characterized by a discount in seed germination, generation of latest leaves, leaf expansion, development of lateral buds resulting in fewer branches, or lateral shoots formation in plants (Munns and Tester, 2008). When salt concentrations within the soil medium increase, the osmotic potential of the medium decreases, restricting the flow of water and nutrients through the basis membrane leading to a large style of physiological and biochemical changes that inhibit plant growth, development and proteins synthesis (Taffouo *et al*., 2009a, 2010). Other effects of osmotic stress include inhibition of root and shoot growth, decrease in stomatal conductance resulting in a discount within the rate of photosynthesis, reduction in root and shoot dry biomass, reduction in leave numbers and injury on leaves, reduction in yield (Taffouo *et al*, 2009b; Kinfemichael Geressu, 2011). In saline soil, salt-induced water deficit is one of the main constraints for plant growth (Taffouo *et al*., 2010).

The ion toxicity occurs when certain ionic species from irrigation water make their way into the plant, altering K^+ /Na^+ ratios, and increasing Na^+ and Cl^+ on concentrations to those who are detrimental to plants due to their negative effects on important processes in plants, including enzymatic activity, protein metabolism, and balance of plant growth regulators (Munns *et al*., 2002). When salt concentration increases inside the plant, the salt starts to accumulate inside the older leaves and eventually they die (Munns, 2002). If these older leaves die at a rate greater than that at which new leaves generate, it reduces the capacity of plants to provide the carbohydrate requirements of younger leaves resulting in a discount in their rate of growth (Munns *et al*., 2006). This phase is also recognized by the looks of some specific symptoms of plant damage within the leaves like color change, tip burn, marginal necrosis and succulence (Munns and Tester,

2008). The shortening of the lifetime of individual leaves leads to growth and yield reductions in plants which ultimately lead to a reduction in overall crop productivity (Munns, 2002).

Several authors have reported the negative effect of NaCl on plant growth and development. As an example, a major growth reduction has been reported in cowpea (Taffouo *et al*, 2009b). Likewise, Jeannette *et al.* (2002) reported a big reduction within the plant height, shoot and root fresh and dry biomass of all Phaseolus species under elevated salt concentrations.

Concentrations of macronutrients and micronutrients were also decreased with salinity dose increment (Mohamed *et al.*, 2008). Due to nutrient deficiency and/or harmful effects of the saline toxic ions, the expansion parameters of cowpea plants were negatively affected. Many researchers reported that salinity caused an excellent reduction in yield and yield-related traits in cowpea. As an example, Ziska and Hall (1981) reported that vegetative growth was reduced by 9.0 you take care of each unit increase in electrical conductivity of the soil saturation extract beyond a threshold value of 1.6 dS/m and dry seed yield was reduced 12% for every unit increase beyond 4.9 dS/m of salinity. Another report by Summerfield *et al.* (1976) showed that the shoot yield of cowpea was reduced to 50% of the control, at soil salt levels like electrical conductivities determined on saturated extracts (ECe, 50 %) of 11.6 dS/m, by using soil salinized with a variety of osmotic levels of NaCl, $CaCl_2$ and Na_2SO_4.

2.13 Mechanism of Salinity Tolerance in Plants

Salt tolerance refers to the power of plants to survive and maintain their growth under saline conditions (Moller and Tester, 2007). According to Shannon and Noble (1990), plant salt tolerance is the inherent ability of the plant to face up to the consequences of high salts within the root zone or on the plant's surfaces without a big adverse effect. It is a complex, quantitative, genetic character controlled by many genes. There is never-ending spectrum of plant tolerance to saline conditions starting from glycophytes that are sensitive to salt, to halophytes that survive in very high concentrations of salt. Other indices of tolerance are proposed which are supported specific physiological characteristics, as an example, accumulation of a particular ion in shoots or leaves, or the assembly of a particular metabolite (Kinfemichael Geressu, 2011).

Plants use several mechanisms to tolerate salinity; salt avoidance (Salt exclusion), tissue tolerance or ion accumulation, osmotic adjustment (Volkamar *et al*., 1998). Salt avoidance is that the process whereby plants keep the ions removed from their sensitive parts through the passive exclusion of ions by a permeable membrane, the active expelling of ions by ion pumps, and by dilution of ions within the tissue of plants (Munns, 2002). These plants adjust their pressure level by producing compatible organic solutes like proline, glycine betaine and sugars (Munns and Tester, 2008). These compatible solutes have low relative molecular mass, are highly soluble in water, are electrically neutral, and do not interfere with plants' metabolic processes (Ashraf and McNeilly, 2004). They are believed to boost salt tolerance by contributing to osmotic balance and preserving enzyme activity within the presence of toxic ions. In glycophytes like beans, salt tolerance is related to Na^+ exclusion. However, in some salt-sensitive genotypes, salt tolerance is not always related to Na^+ exclusion. As an

example, while Na^+ exclusion was a general characteristic of several salt-tolerant wheat lines, a salt-sensitive line had a way lower shoot Na^+ concentration than the more tolerant lines (Schachtman and Munns, 1992). Thus, tolerance to salinity is not necessarily associated with the flexibility to exclude toxic ions.

Restriction of ions into roots or shoots is one of the foremost frequently reported differences between salt-tolerant and sensitive varieties. It is well-known that halophytes take up substantially high concentrations of ions as an adaptation to saline environments (Flowers and Flowers, 2005); however, some can sequester toxic ions not only in vacuoles but also in specialized organs like salt glands and bladders (Brady, 2002). As with salt restriction, salt accumulation within tissues is believed to be regulated and customarily sequestered faraway from cytosolic compartments containing the salt-sensitive metabolic machinery of the cell. In both glycophytes and halophytes, salt may accumulate preferentially in vacuoles, interstitial compartments, stems, or older leaves. The physical and genetic factors that effect ion compartmentation and distribution within plants are mostly unknown.

Tissue tolerance occurs when the ions have already been accumulated within the tissue of the plant, and that they are then compartmentalized into the plant's vacuole, thus controlling the salt concentration within the cytosol and maintaining a high cytosolic K^+/Na^+ ratio in their cells which protects the cytoplasm from ion toxicity and prevents the buildup of salts within the plasma membrane (Chinnusamy *et al*., 2005). This protects the cytoplasm from ion toxicity and prevents the buildup of salts within the semipermeable membrane (Apse and Blumwald, 2002).

2.14 Evaluation of salinity tolerance in controlled conditions

Screening large numbers of genotypes for salinity tolerance within the field is notoriously difficult because of the variability of salinity within fields (Daniells *et al.*, 2001) and also the enormous potential for interactions with other environmental factors, starting from soil chemical and physical properties to temperature, light compactness and seasonal fluctuations in rainfall. It would be of plant genotypes under controlled conditions are a widely used technique, which allows uniform and precise stress conditions to assess salinity tolerance (El-Hendawy, 2004). Specifically, screening plants in Petri plates and pots under laboratory and greenhouse conditions have remained efficient criteria.

Plants are screened for salinity tolerance concerning their agronomic and physiological traits. Under controlled environments, important agronomic traits include survival, germination percentage, phenotypic expression, and stability of traits over the environment (Jamil *et al*., 2005; Taffouo *et al*, 2009b; Kinfemichael Geressu, 2011). consistent with Menguzzo *et al*. (2000), important physiological traits include Na^+ and K^+ uptake ratio, Na^+ and Cl^- exclusion, K^+/Na^+ or Ca^{2+}/Na^+ discrimination, leaf water retention, and photosynthesis are important traits to pick out salt tolerance in genotypes. For several glycophytes, but not all, differences in salt tolerances between genotypes are closely related to reduced uptake and accumulation of Na^+ and/or Cl^- ions at the full plant, shoot and leaf level (El-Hendawy *et al*., 2007).

2.15 Approaches for alleviating the impact of salinity

To raise crop productivity under saline conditions numerous physical and chemical approaches exist for improving agricultural productivity in saline environments. These

include drainage and leaching of excess salt from the foundation zone, chemical amelioration of soils, and crop-based management practices (Rains and Goyal, 2003). However, except for being extremely costly and time-consuming, these techniques are non-applicable in many instances because of the unavailability of improved irrigation and drainage systems. Alternatively, researchers are working towards developing salt-tolerant crop varieties using selective breeding techniques over the past century; however, none of these efforts has proven successful (Ashraf, 2010; Yamaguchi and Blumwald, 2005). During the last decade, developing salt tolerant plants through modern biotechnology has been accorded very high research priority in plant biotechnology research and development. Recently, plant biotechnology has been perceived as an efficient and economic means of tailoring plants for salinity tolerance and has been pursued vigorously to boost the quantitative and qualitative traits of crop plants including tolerance to biotic and abiotic stresses in numerous crops (Wang *et al*., 2003).

2.16 Impact of salinity on nutrient solution

Salinity can inhibit plant growth by a variety of mechanisms, including low external water potential, ion toxicity and interference with the uptake of nutrients, particularly K^+ (Tester and Davenport, 2003). In saline soil, salt induced water deficit is one in all the most important constraints for plant growth (Taffouo *et al*., 2010a). A study had shown that the increasing NaCl concentration in nutrient solution adversely affected cowpea shoot and roots, K^+ concentration, and K/Na ratio (Al-Karaki, 2000). The important basic nutrient solutions consider in their composition only nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur and that they are supplemented with micronutrients.

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted during the period from December 2019 to April 2020 to study the effect of salt tolerance capability in cowpea (*Vigna unguiculata*) under salt stress. The materials and methods describe a short description of the experimental site, the climatic condition of the culture room, experimental materials, treatments and design, methods of the study, data collection procedure and data analysis. The study was conducted by two experiments. At first, the germination was tested at the laboratory and the second experiment was in the greenhouse. The detailed materials and methods that were used to conduct the study are presented below-

3.1 First Experiment: Assessment the germination percentage of cowpea genotypes

- **Experimental design**: Completely randomized design (CRD)
- **Number of replications:** Two
- **Data collection:** Germination percentage (%)
- **Factors of the experiment-**

  Factor A: Cowpea genotypes - Thirty genotypes (G_1, G_2 ,…….G_{19}, G_{30})

  Factor B: Different salinity levels – T_1 = 0 dS/m NaCl (Control), T_2 = 4 dS/m NaCl, T_3 = 8 dS/m NaCl and T_4 = 12 dS/m NaCl

In the first experiment, germination percentage was tested at Pulse Research Centre (PRC) Lab, Bangladesh Agricultural Research Institute (BARI), Gazipur at room temperature during December 2019. This experiment comprised of thirty genotypes of

cowpea were collected from PRC, Gazipur. The salt solution was prepared artificially by dissolving the calculated amount of commercially available NaCl with tap water to make 40, 80 and 120 Mm NaCl solution. The electrical conductivity (EC) of the respective salt solutions was equivalent to 4, 8 and 12 dS/m (1 dS/M=10 mM NaCl) respectively and 0.30 dS/m for tap water (control). Before the experiment, Petridishes (Pyrex) (87 mm diameter, 15mm height) were thoroughly washed with distilled water, rinsed with de-ionized water and dried in an oven. Three layers of Whatman No.2 filter paper were placed in each Petridis. Ten seeds of each cowpea genotypes per treatment were placed in a petridis on filter paper at almost equal distances from each other. An equal volume of salt solution was added to the dishes to maintain the concentration of salt treatment constant. Ten ml of the appropriate solution were applied on alternate days to each petridis. Germination percentage was determined. Seed germination was evaluated after every 12 hours. After 24 hours, seeds had started to germinate. The germinating seeds were counted at regular intervals until the sixth day from the start of the experiment. A seed was considered germinated when both plumule and radical had emerged >2mm.

Plate 1. Cowpea seed germination at petridis in the PRC lab

Control 4 dS/m

8 dS/m 12 dS/m

Plate 2. Germination differences due to NaCl stress in Petri dish experiment

3.1.1 Data Collection:

Germination percentage (GP)

Germination percentage was calculated using the following formula (Kinfemichael and Melkamu, 2008):

$$\text{Germination percentage (\%)} = \frac{\text{Total number of germinated seeds}}{\text{Total number of planted seedss}} \times 100\%$$

3.2 Second Experiment: Effect of different salinity level on ion accumulation in leaf and stem of selected cowpea genotypes

- **Experimental design**: Completely randomized design (CRD)
- **Number of replications:** Two
- **Factors of the experiment**

  Factor A: Cowpea genotypes - Twenty genotypes (G_1, G_2 ,…….G_{19}, G_{20})

  Factor B: Different salinity levels – T_1 = 0 dS/m NaCl (Control), T_2 = 4 dS/m NaCl, T_3 = 8 dS/m NaCl and T_4 = 12 dS/m NaCl

Plate 3. Seedlings of cowpea in hydroponic

3.2.1 Preparation of Solutions

The second experiment was performed in a greenhouse through a hydroponic system. The growing of plants in nutrient solutions without an inert medium (such as soil) to provide mechanical support. So, some solutions were used in this experiment which are described in detail below-

Nutrient solution

Hoagland and Arnon's (1940) solution were used as the nutrient solution in this research. It is a hydroponic nutrient solution. The nutrient compositions of this solution were the mixture of –

$5MnSO_4.H_2O+CuSO_4.5H_2O+ZnSO_4.5H_2O+\{(NH_4)_6.Mo7O24.4H_2O\}$ and H_3BO_3 and 5 mL of each solution was taken for 50 L of water.

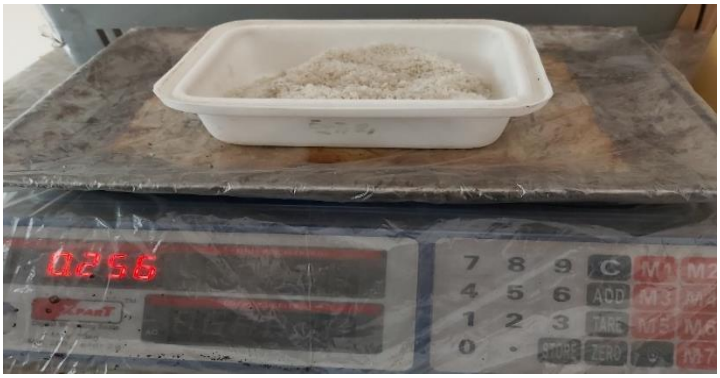
Stock solution

H2SO4+MgSO4, K2H2SO4 and Fe (EDTA) were used as macro and micro nutrient stock solution and 250 mL of each solution was taken for 50 mL of water.

NaCl solution

NaCl solution with different salinity levels of 4, 8 and 12 dS/m were obtained by dissolving 8.4, 16.8 and 25.2 gm of pure NaCl in 3 liters of water per pot for the experiment until the treatment level reached the desired EC. Tap water was used for this experiment. The EC of the tap water was measured by EC meter.

All the treatments were started after one week of seedlings transplantation into the pots. The pH was 5.8 and EC was 2.8 dS/m, respectively were maintained in the nutrient solutions. After seven days of cowpea seedlings transplantation, 1/2 strength of nutrient solution was used. 240 plants of twenty genotypes for four different treatments and 40 pots were used in this experiment.



Salt measurement

Filling with tap water

Adding stalk solution through pepette

Mixing the salt and solution with water

Measuring pH through pH meter

Plate 4. Preparation of salt solution

3.2.2 Growing media preparation for seedling rising

The newly collected sands were placed at seven plastic trays. As the sands were fresh, no sterilization was needed for sands. Every plastic tray was filled with sand at 1/3rd of the tray's height. Then water was sprinkled over the sands.

3.2.3 Seed sowing

Cowpea seeds were sterilized before sowing for 60 sec using 75% ethanol and then washed with distilled water. The seeds were placed in plastic trays containing sands to allow them for germination. Each tray was used for sowing three genotypes and each genotype of a tray was separated by hard plastic sheets. Genotype names were marked on trays with markers beside every genotype set. 15-20 seeds of each genotype following two replications were planted in each genotype chamber. After sowing the seeds, water was sprinkled in plastic trays and then the trays were covered with polythene sheets to remove evaporation loss.

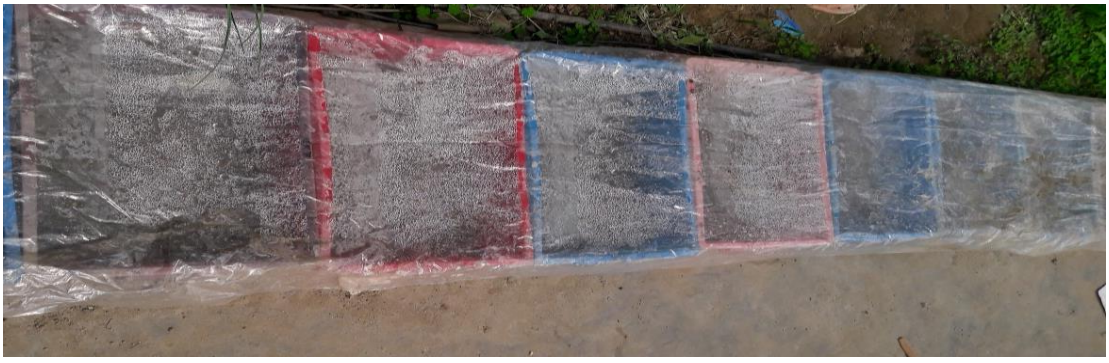


Plate 5. Covering the plastic trays with polythene sheet after sowing seeds

Plate 6. Germination of cowpea seedlings

3.2.4 Transplanting of cowpea seedlings

Seeds were germinated within seven days of sowing and after seven days the best-grown seedlings of each genotype were transplanted to the plastic pots. For transplanting the seedlings, to avoid root damage, the trays were flooded with water to make the roots lose. Then the previously cleaned pots were filled with tap water and each pot was contained 3 L of water. The roots of the plants were submerged in the nutrient solution.

Plate 7. Submerged the seedlings before transplanting

Plate 8. Transplanting the seedlings

3.2.5 Growing Environment

Pots were kept in the greenhouse. Seedlings were set in the pot through foam slices if the seedlings cannot fall into the water as they are so tender and thin. The water was changed after being in the pots for seven days and the pots were refilled with new freshwater and this time the nutrient, stock and NaCl solutions were added to the water (two expanded leaves stage). In this study, three different concentrations of NaCl (4,8 and 12 dS/m) were applied to the plants, and control plants were grown in Hoagland's solution without NaCl. The solution was changed every seven days. The plants were culture in the nutrient solution. An air pump was placed on the nutrient solution pots for proper aeration. One air pump connected with forty small pumps was set in forty pots. The greenhouse was kept clean and tidy during the time of the experiment to avoid any kind of pathogenic growth.

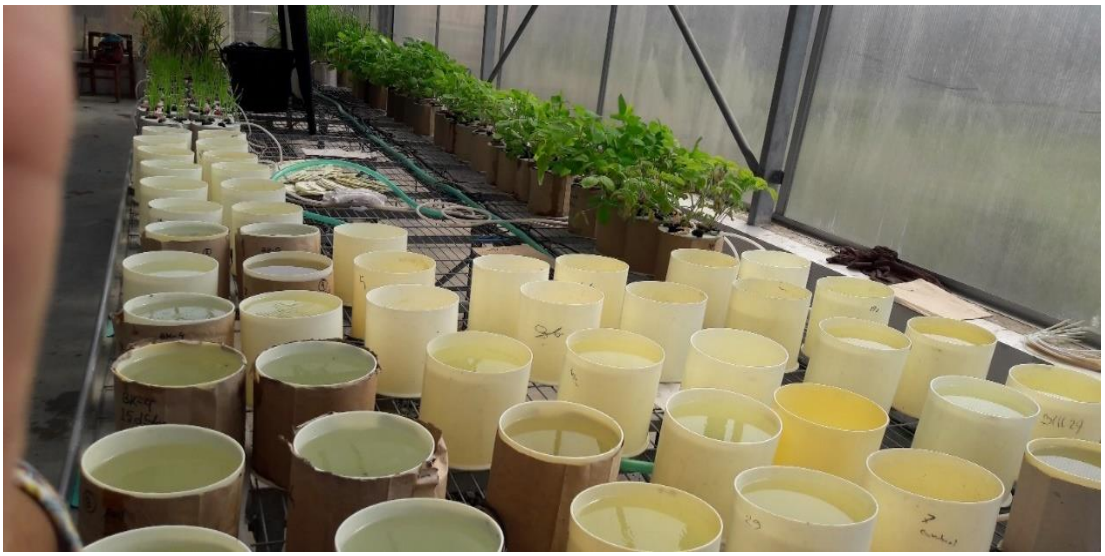


Plate 9. Organizing and preparing pots for seedling transplantation

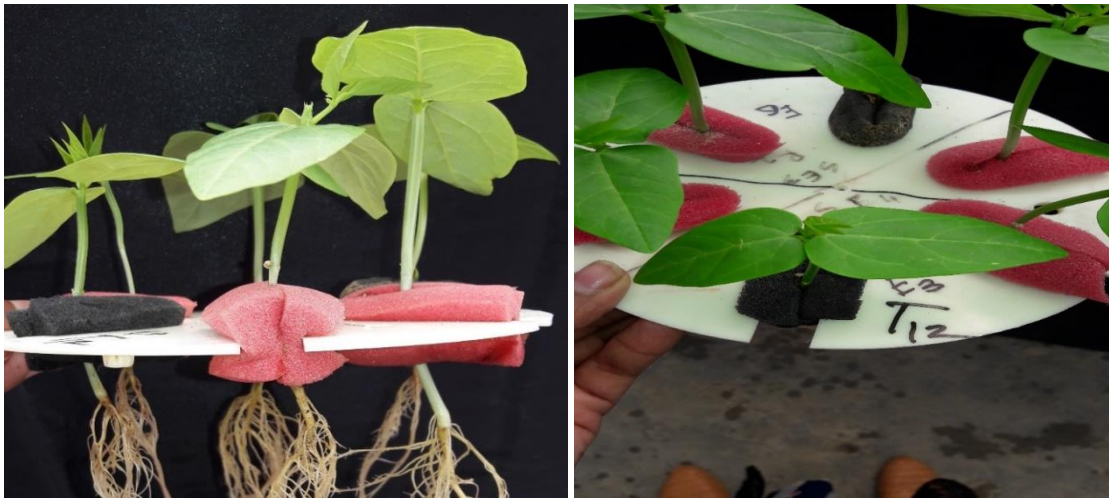


Plate 10. Settings the seedlings in pots through foam

Plate 11. Filling the pots with nutrient solution at 7 DAT

Plate 12. Polluted water

Plate 13. Changing the solution and adding new solution at 14 DAT

3.2.6 Measurements of physiological traits and growth parameters

Chlorophyll content (SPAD value) measurement

The leaf greenness and the photosynthetic rate (Pr) of the uppermost fully expanded trifoliate leaves of cowpea were chosen and their average was determined by using the SPAD chlorophyll meter (SPAD-502; Minolta Co., Ltd, Japan). For the measurement of SPAD value, three random leaves were selected from each treatment of each genotype.

Plate 14. Measurement of chlorophyll content by using SPAD 502 chlorophyll meter

Na^+ and K^+ content determination of leaf and stem

Na^+ and K^+ content determination was done at the laboratory of the Pulse Breeding Center (PRC) of Bangladesh Agricultural Research Institute (BARI). The uppermost fully expanded leaves were used for the analysis of Na^+ and K^+ content in the consecutive NaCl experiment. The leaves were ground with a pestle and a mortar and 50 mg of the leaves was used for subsequent measurements.

The Na^+ and K^+ ions were extracted according to Mitsuya *et al.* (2002). In brief, the leaves were homogenized in 10 ml of 1 M HCl solution at 90℃ for 2 h and then incubated using a rotor (EYELA MMS−3010, Tokyo Rikakikai Co., Ltd, Japan) for 24 h at room temperature. The solutions were diluted and filtered and an atomic absorption spectrophotometer (Shimadzu, Atomic Absorption/Lame Spectrophotometer; model-AA, 610s) following Hitachi, Ltd., (1986) was used to measure the concentration of Na^+ and K^+ of leaf and stem.

3.2.7 Data Collection

Data were recorded from the plants during the experiment. Data were collected from each plant. Each pot was regarded as an experimental unit. Following data were collected from the pot experiment-

1) Chlorophyll content (SPAD value) measurement

2) Na^+ accumulation in leaf

3) Na^+ accumulation in stem

4) K^+ accumulation in leaf

5) K^+ accumulation in stem

3.2.8 Statistical Analysis

The data obtained from greenhouse experiment was analyzed statistically for analysis of variance (ANOVA) using the Statistix 10 statistical software. Multiple comparisons of several means were set up using the ANOVA method following by all pairwise analysis using the Least Significant Difference (LSD) test at 5 % significance level.

CHAPTER IV

RESULTS AND DISCUSSION

In this experiment, efforts were made to find out the effect of salt stress on germination, SPAD value and ion accumulation contributing parameters of twenty cowpea genotypes. Data on different parameters were analyzed statistically and the results have been presented in this chapter.

4.1 Mean Effects of Salinity on Germination Percentage

Figure (1) shows that the high germination percentage was recorded in distilled water at a control treatment, whereas gradual decreases in the percentages of germination were noticed with the increase of salt concentrations at all treatments. From figure (1), it was observed that at the control condition maximum varieties showed a higher germination percentage but TVU-84 had a germination percentage of zero.

A decreasing trend was observed in all the genotypes with the increase of salinity. From figure (4), it was observed that at 12 dS/m germination percentage were decreased significantly in all genotypes and some genotypes like TVU-84, TVU-374, TVU-393, TVU-523 and TVU-1034 germination percentage were zero. That means at 12 dS/m, these five genotypes did not survive whereas TVU-415, TVU-456, TVU-1280, TVU-1330, TVU-1477, TVU-1556, TVU-1609 and TVU-2398 had a germination percentage of 100% even in 12 dS/m salinity.

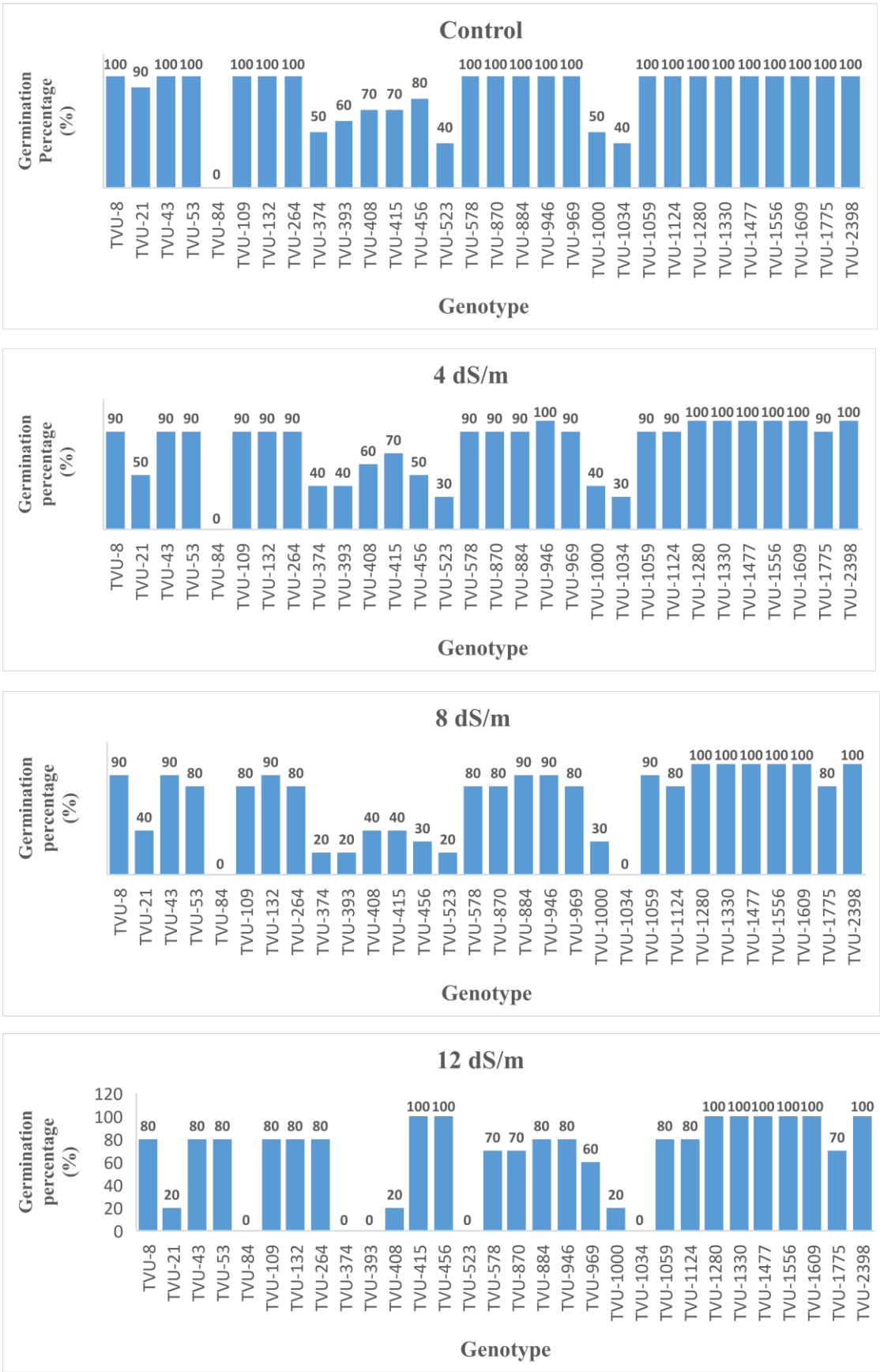


Figure 1. Effect of different level of salinity on germination percentage (%) of thirty cowpea genotypes

Seed germination is delayed with enhanced salinity. This coincides with the findings of Amador and Dieguez (2007), Mahmood *et al*. (2009), Muhammad and Hussain (2010), and Ghaloo *et al*. (2011), who reported that a rise in NaCl concentrations increases the times to germination. This might be the plumule and radial growth decreases at high levels of salinity.

Germination reduction under salinity stress could be the reason of dormancy increases in crop seeds also under salinity stress (Khajeh-Hoosseini *et al*., 2003). Seed germination could also be full of salinity through either creating external osmotic potential or toxic effect of Na^+ and Cl^- ions and in germination under saline conditions, the high pressure of saline water is made leading to capillary rise resulting in more salts density at seed depth than at lower profile, which reduces time and percentage of germination as reported by Murillo-Amador *et al*. (2000).

4.2 Selection of genotypes on the basis of germination percentage

Cowpea genotypes were selected on the basis of germination percentage obtained from first experiment. Among the thirty genotypes, TVU-2398 (100%), TVU-1609 (100%), TVU-1556 (100%), TVU-1477 (100%), TVU-1330 (100%), TVU-1280(100%), TVU-456 (100%), TVU-415 (100%), TVU-1124 (80%), TVU-1059 (80%), TVU-946 (80%), TVU-884 (80%), TVU-264 (80%), TVU-132 (80%), TVU-109 (80%), TVU-53 (80%), TVU-43 (80%), TVU-8 (80%), TVU-1775 (70%), TVU-770 (70%) and TVU-578 (70%) had higher germination percentage. Other cowpea genotypes showed the lower percentage and the percentage were less than 70%. So those genotypes were not selected for the further experiment.

4.3 Effect of salinity on selected genotypes on different traits

4.3.1 Parametric significance on chlorophyll content (SPAD value)

Table 2 shows data on ANOVA results of chlorophyll content (SPAD value) at 14 DAT. From this table, genotype (0.000) followed by treatment (0.000) impacts chlorophyll content (SPAD value) significantly since P values of them are lower than 0.05. Their interaction has a P-value (0.0001) lower than 0.05, which indicates its significance on

the chlorophyll content (SPAD value). A low CV value of 1.90% provides a precise analysis of the parameters for the chlorophyll content (SPAD value) at 14 DAT.

Table 2. ANOVA results of chlorophyll content (SPAD value) at 14 DAT

Source	DF	SS	MS	F	P
Treatment	3	1323.90	441.301	653.48	0.0000
Genotype	19	4405.83	231.886	343.38	0.0000
Treatment*Genotype	57	93.75	1.645	2.44	0.0001
Error	80	54.02	0.675		
Total	159	5877.50			
CV	1.90%				

Table 3 contains data on ANOVA results of chlorophyll content (SPAD value) at 28 DAT. From this table, genotype (0.000) and treatment (0.000) have significant impacts on chlorophyll content (SPAD value) as P values of them are lower than 0.05. Their interaction has a P-value (0.9999) higher than 0.05, which indicates it is non-significant on the chlorophyll content (SPAD value) at 28 DAT. A low CV value (1.39%) provides a precise analysis of the parameters for the chlorophyll content (SPAD value) at 28 DAT.

Table 3. ANOVA results of chlorophyll content (SPAD value) at 28 DAT

Source	DF	SS	MS	F	P
Treatment	3	836.24	278.748	499.44	0.0000
Genotype	19	4422.08	232.741	417.01	0.0000
Treatment*Genotype	57	11.91	0.209	0.37	0.9999
Error	80	44.65	0.558		
Total	159	5314.88			
CV	1.39%				

Table 4 appears data on ANOVA results of chlorophyll content (SPAD value) at 42 DAT. From this table, genotype (0.000) followed by treatment (0.000) impacts shows significantly since P values of them are lower than 0.05. Their interaction has a P-value (0.9398) higher than 0.05, which indicates it is non-significant on the chlorophyll content (SPAD value) at 42 DAT. CV value of 1.95% is low which provides a precise analysis of the parameters for the chlorophyll content (SPAD value) at 42 DAT.

Table 4. ANOVA results of chlorophyll content (SPAD value) at 42 DAT

Source	DF	SS	MS	F	P
Treatment	3	732.04	244.014	236.66	0.0000
Genotype	19	4213.10	221.742	215.06	0.0000
Treatment*Genotype	57	39.76	0.698	0.68	0.9398
Error	80	82.48	1.031		
Total	159	5067.39			
CV	1.95%				

4.3.2 Effect of salinity on chlorophyll content (SPAD value)

Figure (2) shows that the total chlorophyll contents were negatively affected by 4 dS/m, 8 dS/m and 12 ds/m salinity concentrations during the 14 DAT of growth. However, plants exposed to lower salinity (4 dS/m NaCl) had the highest chlorophyll content (SPAD value), SPAD unit 48.11, which was significantly higher than the other treatments, including the control. Then it decreases gradually followed by 8 dS/m (42.48 SPAD unit), almost equal to control and 12 ds/m (40.36 SPAD unit) which is the lowest SPAD value at 14 DAT.

During the 28 DAT, salinity concentrations positively affected the chlorophyll values with increasing plant age and were significantly different from one another at $p \leq 0.05$. The highest SPAD-56.79 values were obtained at 12 dS/m NaCl concentration followed by 8 dS/m, 4 dS/m and the control. At this week control had the lowest SPAD value (50.61 SPAD unit).

During the 42 DAT, chlorophyll values have negatively affected all treatments by salinity except the control. The control had the highest SPAD-55.61 value, which was significantly higher than all salt treatments. Other treatments had lower SPAD values followed by 12 dS/m, 8 dS/m and 12 dS/m when compared to 28 DAT.

It is concluded that all the treatments (0, 4, 8 and 12 dS/m) affected the SPAD value of all the genotypes at 14, 28, and 42 DAT. But 12 dS/m affected more on SPAD value than other treatments.

T_1= 0 dS/m, T_2= 4 dS/m, T_3= 8 dS/m, T_4= 12 dS/m

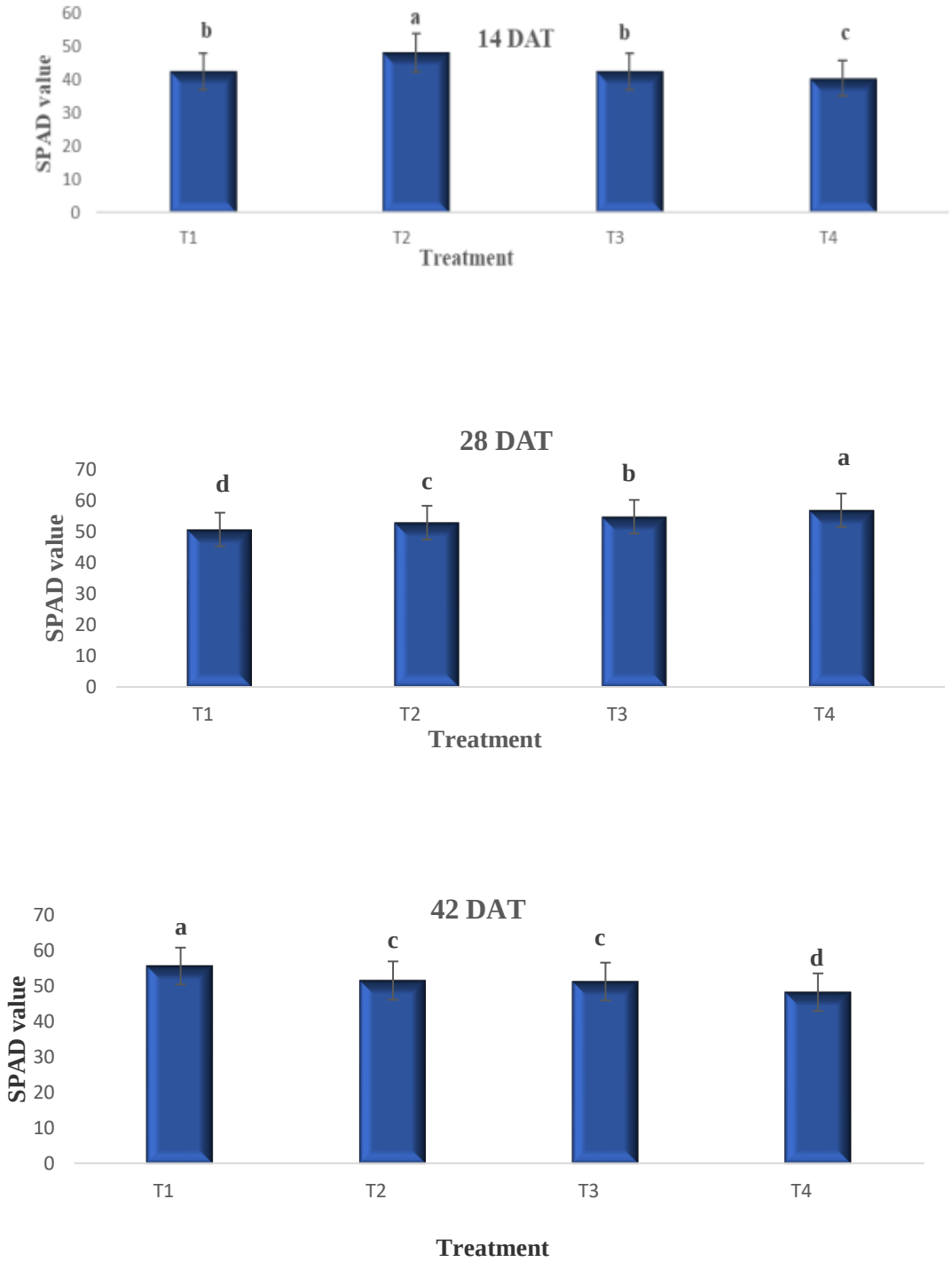


Figure 2. Effect of different salinity level on chlorophyll content (SPAD value) of cowpea genotypes at different DAT

Photosynthetic activity is considered one of the major factors which control plant growth (Chandrasekeran *et al*., 2019). The salinity indirectly slows down photosynthesis in plants and photosynthesis is directly associated with stomatal conductance, transpiration, and water potential. Leaf photosynthesis are often can be lowered by the reduction of stomatal conductance as a result of water imbalance under salt stress. The rate of photosynthesis in salt-tolerant species generally has been least affected than that in salt-sensitive species (Iqbal *et al*., 2019).

4.3.3 Effect of genotypes on chlorophyll content (SPAD value)

From figure 3, at 14 DAT, TVU-2398 and TVU-1330 had showed the highest SPAD value (SPAD unit 53.6) and (SPAD unit 53.1) whereas TVU-43 showed the lowest SPAD value (SPAD unit 35.7).

Similarly, at 28 DAT, TVU-2398 (SPAD unit 61.88) and TVU-1330 (SPAD unit 61.57) showed almost the same and the highest SPAD value, whereas TVU-870 (SPAD unit 45.72) and TVU-43 (SPAD unit 45.69) also showed almost the same and the lowest SPAD value.

At 42 DAT, TVU-2398 (SPAD unit 60.09) and TVU-1330 (SPAD unit 59.95) expressed the highest SPAD value. On the other and TVU-43 (SPAD unit 44.47) showed the lowest SPAD value than other genotypes.

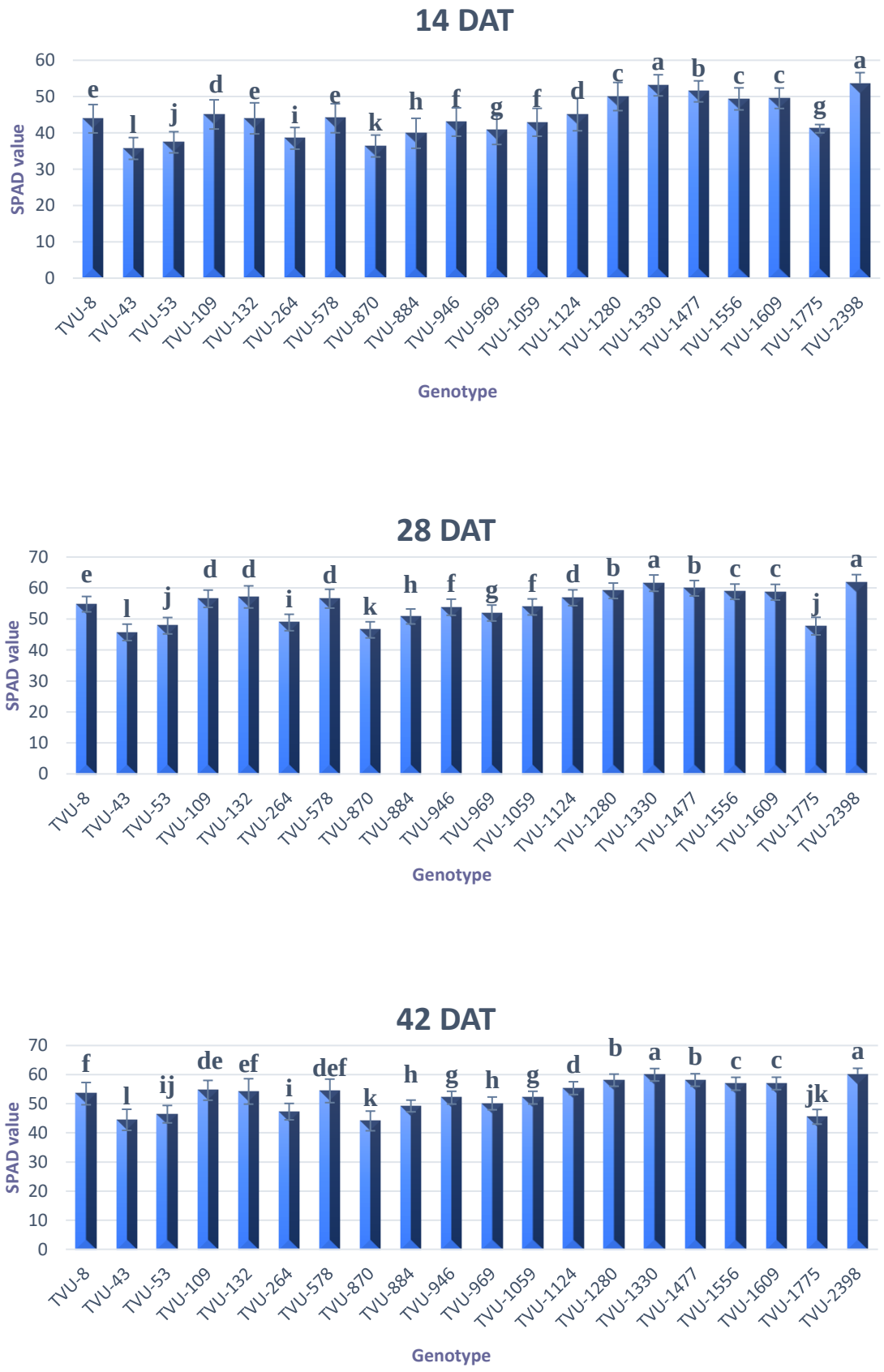


Figure 3. Effect of cowpea genotypes on chlorophyll content (SPAD value) at different DAT

Chlorophyll contents were reduced markedly at high salinity concentration treatments, especially with aged plants. It might be due to the reason that the total chlorophyll and the proportion of its components depended on the biological process and development stages of the plant and also on the type and concentration of the salt. Ahmed *et al*., (1978) and Hajer *et al*., (1993) also obtained similar findings in the case of the tomato plant.

4.3.4 Interaction effect of salinity and genotype on chlorophyll content (SPAD value) after transplantation

Interaction effect of salinity and genotype on SPAD value at 14 DAT

Table 5 shows the interaction effect on SPAD value at 14 DAT between salinity and genotypes of cowpea was found significant**.** From the table it was observed that the highest SPAD value 57.80 was obtained from the interaction, $T_2 \times G_{20}$ and the lowest SPAD value 32.60 was found from the interaction $T_4 \times G_2$ which was statistically close to $T_4 \times G_8$.

Interaction effect of salinity and genotype on SPAD value at 28 DAT

Table 6 shows the interaction effect on SPAD value at 28 DAT between salinity and genotypes of cowpea was found non-significant. From the table it was observed that the highest SPAD value 64.75 was obtained from the interaction, $T_4 \times G_{15}$ and the lowest SPAD value 42.60 was found from the interaction $T_1 \times G_2$.

Interaction effect of salinity and genotype on SPAD value at 42 DAT

Table 7 shows the interaction effect on SPAD value at 42 DAT between salinity and genotypes of cowpea was found non-significant. From the table it was observed that the highest SPAD value 63.3 was obtained from the interaction, T1 × G15 and the lowest SPAD value 41.15 was found from the interaction $T_4 \times G_2$.

Table 5. Interaction effect of salinity and genotype on SPAD value at 14 DAT

Treatment combination	Mean	Treatment combination	Mean
T1 × G1	42.750 klmn	T3 × G1	42.600 lmn
T1 × G2	34.850 wx	T3 × G2	35.700 vw
T1 × G3	36.600 uv	T3 × G3	36.700 uv
T1 × G4	44.000 kl	T3 × G4	44.250 jk
T1 × G5	42.700 klmn	T3 × G5	42.500 lmn
T1 × G6	38.200 rstu	T3 × G6	37.600 tu
T1 × G7	42.700 klmn	T3 × G7	43.000 klmn
T1 × G8	35.550 vw	T3 × G8	35.750 vw
T1 × G9	38.600 rst	T3 × G9	38.450 rst
T1 × G10	42.000 mno	T3 × G10	41.700 no
T1 × G11	39.800 pqr	T3 × G11	39.550 qrs
T1 × G12	41.900 no	T3 × G12	41.700 no
T1 × G13	43.600 klm	T3 × G13	43.600 klm
T1 × G14	48.900 h	T3 × G14	48.900 h
T1 × G15	34.650 wx	T3 × G15	34.500 wx
T1 × G16	50.800 ef	T3 × G16	50.600 efg
T1 × G17	48.500 h	T3 × G17	48.700 h
T1 × G18	49.050 gh	T3 × G18	48.800 h
T1 × G19	41.850 no	T3 × G19	41.700 no
T1 × G20	53.150 cd	T3 × G20	52.850 cd
T2 × G1	49.600 fgh	T4 × G1	40.650 opq
T2 × G2	39.700 qrs	T4 × G2	32.600 y
T2 × G3	41.600 no	T4 × G3	34.750 wx
T2 × G4	50.700 ef	T4 × G4	41.400 nop
T2 × G5	50.000 fgh	T4 × G5	40.800 opq
T2 × G6	42.650 klmn	T4 × G6	35.600 vw
T2 × G7	49.800 fgh	T4 × G7	40.650 opq
T2 × G8	40.650 opq	T4 × G8	32.600 y
T2 × G9	45.800 ij	T4 × G9	36.700 uv
T2 × G10	48.700 h	T4 × G10	39.600 qrs
T2 × G11	45.700 ij	T4 × G11	38.150 stu
T2 × G12	48.500 h	T4 × G12	39.600 qrs
T2 × G13	51.700 de	T4 × G13	41.500 no
T2 × G14	55.600 b	T4 × G14	46.650 i
T2 × G15	39.450 qrs	T4 × G15	51.650 e
T2 × G16	55.600 b	T4 × G16	48.750 h
T2 × G17	53.650 c	T4 × G17	46.600 i
T2 × G18	53.500 c	T4 × G18	46.800 i
T2 × G19	41.550 no	T4 × G19	39.550 qrs
T2 × G20	57.800 a	T4 × G20	50.700 ef
CV		**1.90%**	
Level of significance at 5%			

Table 6. Interaction effect of salinity and genotype on SPAD value at 28 DAT

Treatment combination	Mean	Treatment combination	Mean
T1 × G1	52.000	T3 × G1	55.750
T1 × G2	42.600	T3 × G2	46.650
T1 × G3	44.700	T3 × G3	48.800
T1 × G4	53.100	T3 × G4	57.550
T1 × G5	52.300	T3 × G5	58.700
T1 × G6	45.800	T3 × G6	49.800
T1 × G7	52.800	T3 × G7	57.800
T1 × G8	42.800	T3 × G8	46.700
T1 × G9	48.000	T3 × G9	51.650
T1 × G10	50.800	T3 × G10	54.700
T1 × G11	48.900	T3 × G11	52.800
T1 × G12	50.950	T3 × G12	54.800
T1 × G13	53.900	T3 × G13	57.900
T1 × G14	58.700	T3 × G14	62.500
T1 × G15	42.800	T3 × G15	46.800
T1 × G16	57.050	T3 × G16	60.750
T1 × G17	56.000	T3 × G17	59.700
T1 × G18	55.700	T3 × G18	59.550
T1 × G19	44.200	T3 × G19	48.750
T1 × G20	59.100	T3 × G20	62.750
T2 × G1	53.700	T4 × G1	57.700
T2 × G2	44.800	T4 × G2	48.700
T2 × G3	46.900	T4 × G3	50.900
T2 × G4	55.800	T4 × G4	59.700
T2 × G5	56.900	T4 × G5	60.600
T2 × G6	48.000	T4 × G6	51.850
T2 × G7	55.700	T4 × G7	59.900
T2 × G8	44.600	T4 × G8	48.800
T2 × G9	49.800	T4 × G9	53.800
T2 × G10	52.900	T4 × G10	56.800
T2 × G11	51.000	T4 × G11	55.000
T2 × G12	52.800	T4 × G12	57.000
T2 × G13	55.800	T4 × G13	59.800
T2 × G14	60.600	T4 × G14	63.500
T2 × G15	44.700	T4 × G15	64.500
T2 × G16	59.000	T4 × G16	62.900
T2 × G17	57.800	T4 × G17	61.750
T2 × G18	57.700	T4 × G18	61.600
T2 × G19	47.000	T4 × G19	50.850
T2 × G20	60.900	T4 × G20	64.750
CV		**1.39%**	
Level of significance		**ns**	

Table 7. Interaction effect of salinity and genotype on SPAD value at 42 DAT

Treatment combination	Mean	Treatment combination	Mean
T1 × G1	57.850	T3 × G1	52.200
T1 × G2	48.500	T3 × G2	42.750
T1 × G3	50.000	T3 × G3	44.900
T1 × G4	58.950	T3 × G4	53.200
T1 × G5	60.050	T3 × G5	52.400
T1 × G6	51.000	T3 × G6	46.100
T1 × G7	59.500	T3 × G7	53.000
T1 × G8	48.400	T3 × G8	43.000
T1 × G9	52.050	T3 × G9	48.000
T1 × G10	55.200	T3 × G10	50.700
T1 × G11	53.300	T3 × G11	48.700
T1 × G12	55.300	T3 × G12	50.800
T1 × G13	58.250	T3 × G13	53.850
T1 × G14	61.000	T3 × G14	58.800
T1 × G15	63.300	T3 × G15	42.900
T1 × G16	61.300	T3 × G16	56.800
T1 × G17	60.200	T3 × G17	55.800
T1 × G18	60.100	T3 × G18	55.700
T1 × G19	49.200	T3 × G19	44.000
T1 × G20	62.950	T3 × G20	58.900
T2 × G1	51.250	T4 × G1	52.600
T2 × G2	44.150	T4 × G2	41.500
T2 × G3	44.300	T4 × G3	46.500
T2 × G4	52.600	T4 × G4	53.700
T2 × G5	51.650	T4 × G5	52.850
T2 × G6	45.400	T4 × G6	46.650
T2 × G7	52.400	T4 × G7	52.800
T2 × G8	44.500	T4 × G8	42.500
T2 × G9	47.700	T4 × G9	49.200
T2 × G10	50.500	T4 × G10	51.950
T2 × G11	48.650	T4 × G11	49.800
T2 × G12	50.600	T4 × G12	51.700
T2 × G13	53.600	T4 × G13	55.750
T2 × G14	58.400	T4 × G14	59.600
T2 × G15	42.500	T4 × G15	47.700
T2 × G16	56.700	T4 × G16	57.800
T2 × G17	55.700	T4 × G17	55.750
T2 × G18	55.500	T4 × G18	56.600
T2 × G19	43.800	T4 × G19	45.100
T2 × G20	58.700	T4 × G20	59.800
CV		**1.95%**	
Level of significance		**ns**	

Salinity stress caused swelling of membranes in chloroplasts of sensitive plants which affected their chlorophyll content, or it occurred due to excess ions (Na^+ and Cl^-) in leaves which induced loss of chlorophylls (Wahid *et al*., 2004 and Arulbalachandran *et al*., 2009). Accumulation of toxic ions under salinity stress reduced the water and osmotic potential that further caused disturbances in photosynthetic processes (Khan *et al*., 2010). Loss of chlorophyll content caused chlorosis of leaves that later turned into necrosis. These adverse effects finally caused senescence and plant death. The results are in agreement with the earlier findings on mungbean (Sehrawat *et al*., 2013b; 2013c). Reduction in chlorophyll content is probably due to the inhibitory effect of the accumulated ions of various salts on the biosynthesis of the different chlorophyll fractions.

4.4 Effect of salinity level on ion accumulation in plant parts

4.4.1 Effect of salinity level on Na^+ accumulation

Salinity can directly affect nutrient uptake. Salt stress had a significant impact on Na^+ uptake by cowpea plants (Figure 4). Concentrations of Na^+ significantly increased in parallel to the quantity of NaCl ($p<0.05$). Compared to control treatment Na^+ concentration increased with the increasing salinity in leaf and stem of all the varieties. Under saline conditions plant absorbs different mineral ions for osmoregulation. When the absorption of Na^+ within the shoot is unusually high, then the physiological processes are suffered from Na^+ toxicity. Moreover, Na^+ competes with Ca^{2+} from the identical binding site in the plasmalemma thus creating physiological chaos in the cell function.

4.4.2 Parametric significance on Na^+ accumulation in leaf

Table 8 shows data on ANOVA results of Na^+ accumulation in leaf. From this table, genotype (0.000) followed by treatment (0.000) impacts Na^+ accumulation in leaf significantly since P values of them are lower than 0.05. Their interaction has P value (0.0000) lower than 0.05, which indicates its significance on Na^+ accumulation in leaf. A low CV value of 2.00% provides a precise analysis of the parameters for Na^+ accumulation in leaf.

Table 8. ANOVA results of Na^+ accumulation in leaf

Source	DF	SS	MS	F	P
Treatment	3	1.77959	0.59320	64565.72	0.0000
Genotype	19	0.03910	0.00206	223.98	0.0000
Treatment*Genotype	57	0.01840	0.00032	35.14	0.0000
Error	80	0.00073	0.00001		
Total	159	1.83783			
CV	2.00 %				

4.4.3 Effect of Na^+ accumulation in leaf by different salinity level

Figure (4) shows the effect of different treatments on Na^+ accumulation in leaf in nutrient solution. It shows that the total sodium concentration increased significantly in leaves of cowpea plants in the presence of NaCl in the nutrient solution.

Na^+ accumulation in leaf was positively affected by the treatments (T_1, T_2, T_3 and T_4). However, plants exposed to higher salinity (12 dS/m NaCl) had the highest Na_+ content (0.306%), which was significantly higher than the other treatments, including the control. Then it decreases gradually followed by 8 dS/m (0.234%), 4 ds/m (0.089%) and at control (0.01%) Na^+ content was lowest in cowpea leaf.

It is concluded that all the treatments (0, 4, 8 and 12 dS/m) affected Na^+ content in cowpea leaf of all the genotypes in nutrient solution. But 12 dS/m affected more positively on Na^+ content in cowpea leaf than other treatments.

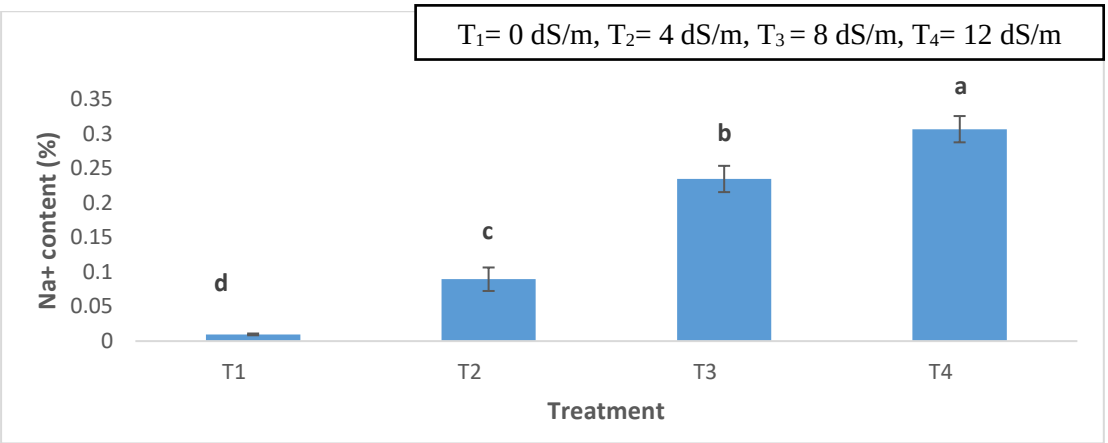


Figure 4. Effect of Na^+ accumulation in leaf by different salinity level

4.4.4 Effect of Na^+ accumulation in leaf by different cowpea genotypes

Figure (5) shows the effect of genotype on Na+ accumulation in leaf. From the figure it is observed that the sodium concentration was highest in TVU-1330 (0.206%) and lowest in TVU- 8 (0.131%) and TVU-1124 (0.134%). Salinity raised Na^+ concentration in all the leaves but after TVU-1330, some others genotype like TVU-870 (0.198%) and TVU-1556 (0.193%) were also comparatively higher than other genotypes. Similarly, along with TVU-8 and TVU- 1124, salinity decreased Na^+ concentration in leaves of some other genotypes like TVU-264 (0.138%) and TVU-53 (0.148%) were also comparatively lower than other genotypes.

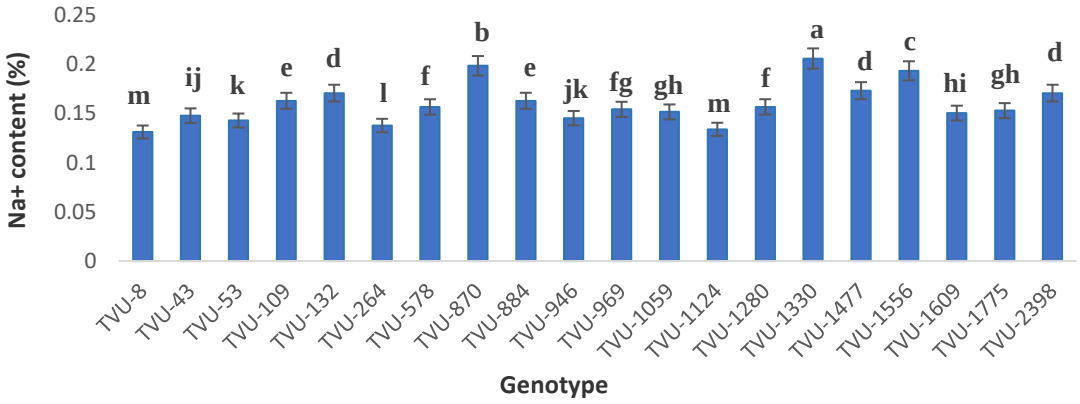


Figure 5. Effect of Na^+ accumulation in leaf by different cowpea genotypes

The damage of chloroplasts ultrastructure caused by salinity has been reported in rice (Mitsuya *et al.*, 2003). This study revealed that chloroplasts were reduced. Salt injury is due to Na^+ accumulating in transpiring leaves to excessive levels, exceeding the ability of the cells to compartmentalize these ions in the vacuole. Ions then build up rapidly in the cytoplasm and inhibit enzyme activity, or they build up in the cell walls and dehydrate the cell (Flowers, 1986).

4.4.5 Interaction effect of salinity and genotype on Na^+ accumulation in leaf

The interaction effect on Na^+ accumulation in leaf between treatment and genotypes of cowpea was found significant **(**Table 9). From the table it was observed that the highest Na^+ content in leaf (0.367%) was obtained from the interaction, $T_4 \times G_{15}$ and the lowest Na^+ accumulation (0.027%) was found from the interaction $T_1 \times G_1$.

The damage of the root and leaf of the plants were exposed to salinity was caused by either osmotic effects or ionic toxicity due to Na^+ accumulation in the plant tissues (Munns, 2002). High salt concentration in soils lowers soil water potential. As a result, plants can no longer take up water. Ion toxicity due to excessive sodium ion causing

the decrease of ion acquisition, displaces calcium ion from the plasma membrane of root hairs, leading to a membrane leakage and inhibits many important enzymes (Munns, 2002) and causes a nutrient imbalance in the tissues Munns, 1986).

Table 9. Interaction effect of salinity level and genotype on Na^+ accumulation in leaf

Treatment combination	Mean	Treatment combination	Mean
T1 × G1	0.0275 z	T3 × G1	0.1875 r
T1 × G2	0.0493 x	T3 × G2	0.2075 p
T1 × G3	0.0491 x	T3 × G3	0.2075 p
T1 × G4	0.0495 x	T3 × G4	0.2375 m
T1 × G5	0.0505 wx	T3 × G5	0.2475 l
T1 × G6	0.0385 y	T3 × G6	0.1975 q
T1 × G7	0.0492 x	T3 × G7	0.2475 l
T1 × G8	0.0520 wx	T3 × G8	0.2875 h
T1 × G9	0.0495 z	T3 × G9	0.2575 k
T1 × G10	0.0490 z	T3 × G10	0.2275 n
T1 × G11	0.0498 x	T3 × G11	0.2175 o
T1 × G12	0.0493 x	T3 × G12	0.2375 m
T1 × G13	0.0385 y	T3 × G13	0.1975 q
T1 × G14	0.0495 x	T3 × G14	0.2275 n
T1 × G15	0.0515 wx	T3 × G15	0.2975 g
T1 × G16	0.0505 wx	T3 × G16	0.2575 k
T1 × G17	0.0515 y	T3 × G17	0.2775 i
T1 × G18	0.0495 x	T3 × G18	0.2175 o
T1 × G19	0.0495 x	T3 × G19	0.2075 p
T1 × G20	0.0505 wx	T3 × G20	0.2475 l
T2 × G1	0.0725 w	T4 × G1	0.2675 j
T2 × G2	0.0875 v	T4 × G2	0.2875 h
T2 × G3	0.0775 w	T4 × G3	0.2875 h
T2 × G4	0.0875 v	T4 × G4	0.3175 e
T2 × G5	0.0975 v	T4 × G5	0.3275 d
T2 × G6	0.0775 w	T4 × G6	0.2775 i
T2 × G7	0.0725 w	T4 × G7	0.2975 g
T2 × G8	0.1375 t	T4 × G8	0.3575 b
T2 × G9	0.0775 w	T4 × G9	0.3075 f
T2 × G10	0.0775 w	T4 × G10	0.2775 i
T2 × G11	0.0975 v	T4 × G11	0.2975 g
T2 × G12	0.0725 w	T4 × G12	0.2875 h
T2 × G13	0.0725 w	T4 × G13	0.2675 j
T2 × G14	0.0825 v	T4 × G14	0.3075 f
T2 × G15	0.1475 s	T4 × G15	0.3675 a
T2 × G16	0.0975 v	T4 × G16	0.3275 d
T2 × G17	0.1375 t	T4 × G17	0.3475 c
T2 × G18	0.0775 w	T4 × G18	0.2975 g
T2 × G19	0.0875 v	T4 × G19	0.3075 f
T2 × G20	0.1075 u	T4 × G20	0.3175 e
CV		**2.0%**	
Level of significance at 5%			

4.4.6 Parametric significance on Na^+ accumulation in stem

Table 10 shows data on ANOVA results of Na^+ accumulation in stem. From this table, genotype (0.000) followed by treatment (0.000) impacts Na^+ accumulation in stem significantly since P values of them are lower than 0.05. Their interaction has P value (0.0000) lower than 0.05, which indicates its significance on Na^+ accumulation in stem. A low CV value of 2.00% provides a precise analysis of the parameters for Na^+ accumulation in stem.

Table 10: ANOVA results of Na^+ accumulation in stem

Source	DF	SS	MS	F	P
Treatment	3	2.18502	0.72834	76743.07	0.0000
Genotype	19	0.06447	0.00339	357.52	0.0000
Treatment*Genotype	57	0.02652	0.00047	49.02	0.0000
Error	80	0.00076	0.00001		
Total	159	2.27676			
CV	1.93 %				

4.4.7 Effect of Na^+ accumulation in stem by different salinity level

Figure (6) shows the effect of different treatments on Na^+ accumulation of stem in nutrient solution. It shows that the total sodium concentration increased significantly in stems of cowpea plants in the presence of NaCl in the nutrient solution.

Na^+ accumulation in stem was positively affected by the treatments (T_1, T_2, T_3 and T_4). However, plants exposed to higher salinity (12 dS/m NaCl) had the highest Na_+ content (0.306%), which was significantly higher than the other treatments, including the control. Then it decreases gradually followed by 8 dS/m (0.234%), 4 ds/m (0.089%) and at control (0.01%) Na^+ content was lowest in cowpea stem.

It is concluded that all the treatments (0, 4, 8 and 12 dS/m) affected Na^+ content in cowpea stem of all the genotypes in nutrient solution. But T_4 affected more positively on Na^+ content in cowpea stem than other treatments.

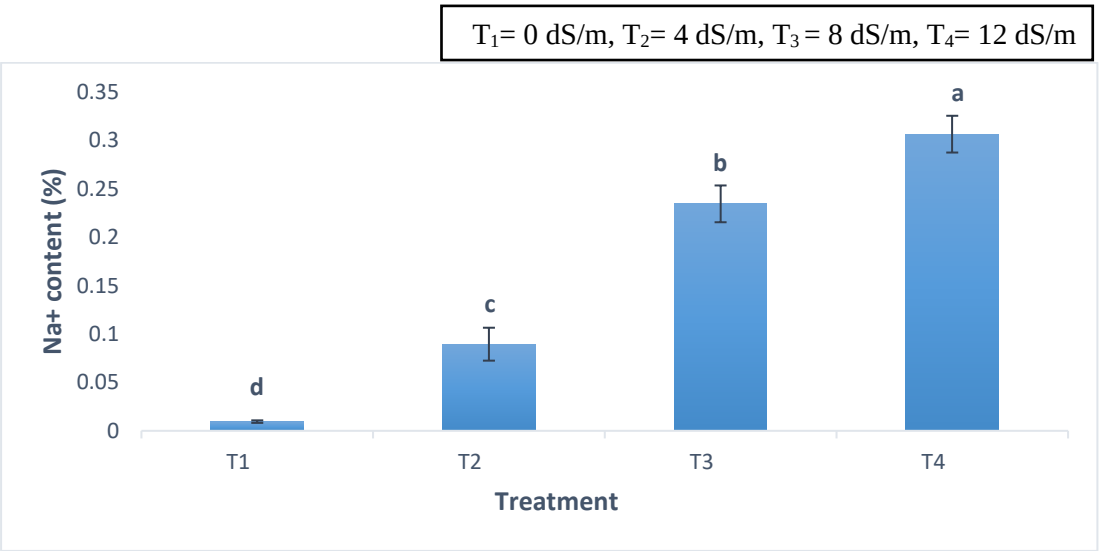


Figure 6. Effect of Na^+ accumulation in stem by different salinity level

When the salt stress is strengthened, the Na^+ content in stems and leaves will be also increased, which is the same as the conclusion that some researchers think that the Na^+ content in stems will be increased with the increasing NaCl concentration. (Munns, 2006).

4.4.8 Effect of Na^+ accumulation in stem by different genotypes of cowpea

Figure (7) shows the effect of genotype on Na+ accumulation in stem. From the figure it is observed that the sodium concentration was highest in TVU-1330 (0.206%) and lowest in TVU- 8 (0.131%) and TVU-1124 (0.134%). Salinity raised Na^+ concentration in all the stems but after TVU-1330, some others genotype like TVU-870 (0.198%) and TVU-1556 (0.193%) were also comparatively higher than other genotypes. Similarly,

along with TVU-8 and TVU- 1124, salinity decreased Na^+ concentration in stems of some other genotypes like TVU-264 (0.138%) and TVU-53 (0.148%) were also comparatively lower than other genotypes.

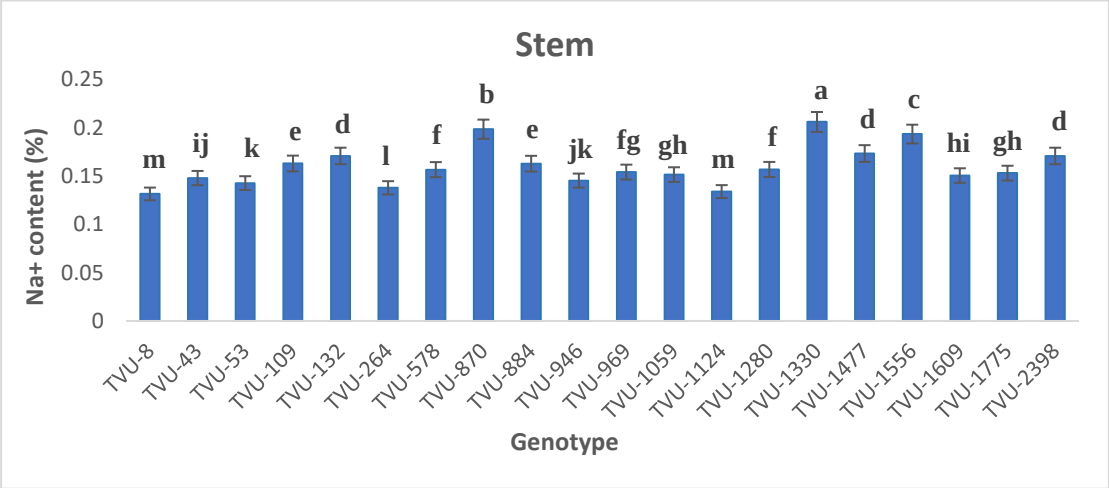


Figure 7. Effect of Na^+ accumulation in stem by different cowpea genotypes

Different genotypes act differently under saline conditions. Sweet sorghum can accumulate Na^+ in roots and limit the transportation of Na^+ up to shoots under salt stress, which is termed salt exclusion (Dai *et al.*, 2014), an important salt-tolerance-related process in monocotyledonous crops, including rice, maize and sorghum. In line with Tester and Danenport (2003), the key mechanism of salt tolerance is that the ability of plants to manage Na^+ uptake from soil. T. *tetragonoides* has shown the highest Na^+ content all told versions of salt stress, including the control group. Increased Na^+ content together with increasing salt concentrations was reported for lettuce (Ünlükara *et al.* 2008), New Zealand spinach Yousif *et al.* (2010).

4.4.9 Interaction effect of salinity and genotype on Na^+ accumulation in stem

The interaction effect on Na^+ accumulation in stem between salinity and genotypes of cowpea was found significant **(**Table 11). From the table it was observed that the highest Na^+ content (0.367%) was obtained from the interaction, $T_4 \times G_{15}$ and the lowest Na^+ content (0.033%) was found from the interaction $T_1 \times G_1$.

Table 11. Interaction effect of salinity and genotype on Na^+ accumulation in stem

Treatment combination	Mean	Treatment combination	Mean
T1 × G1	0.0330 z	T3 × G1	0.1875 r
T1 × G2	0.0400 z	T3 × G2	0.2075 p
T1 × G3	0.0385 z	T3 × G3	0.2075 p
T1 × G4	0.0405 z	T3 × G4	0.2375 m
T1 × G5	0.0405 z	T3 × G5	0.2475 l
T1 × G6	0.0385 z	T3 × G6	0.1975 q
T1 × G7	0.0400 z	T3 × G7	0.2475 l
T1 × G8	0.0515 y	T3 × G8	0.2875 h
T1 × G9	0.0385 z	T3 × G9	0.2575 k
T1 × G10	0.0388 z	T3 × G10	0.2275 n
T1 × G11	0.0400 z	T3 × G11	0.2175 o
T1 × G12	0.0385 z	T3 × G12	0.2375 m
T1 × G13	0.0395 z	T3 × G13	0.1975 q
T1 × G14	0.0405 z	T3 × G14	0.2275 n
T1 × G15	0.0515 y	T3 × G15	0.2975 g
T1 × G16	0.0505 y	T3 × G16	0.2575 k
T1 × G17	0.0515 y	T3 × G17	0.2775 i
T1 × G18	0.0405 z	T3 × G18	0.2175 o
T1 × G19	0.0405 z	T3 × G19	0.2075 p
T1 × G20	0.0505 y	T3 × G20	0.2475 l
T2 × G1	0.0625 x	T4 × G1	0.2675 j
T2 × G2	0.0875 v	T4 × G2	0.2875 h
T2 × G3	0.0675 x	T4 × G3	0.2875 h
T2 × G4	0.0875 v	T4 × G4	0.3175 e
T2 × G5	0.0925 v	T4 × G5	0.3275 d
T2 × G6	0.0675 x	T4 × G6	0.2775 i
T2 × G7	0.0725 w	T4 × G7	0.2975 g
T2 × G8	0.1375 t	T4 × G8	0.3575 b
T2 × G9	0.0775 w	T4 × G9	0.3075 f
T2 × G10	0.0675 x	T4 × G10	0.2775 i
T2 × G11	0.0925 v	T4 × G11	0.2975 g
T2 × G12	0.0725 w	T4 × G12	0.2875 h
T2 × G13	0.0625 x	T4 × G13	0.2675 j
T2 × G14	0.0825 v	T4 × G14	0.3075 f
T2 × G15	0.1475 s	T4 × G15	0.3675 a
T2 × G16	0.0925 v	T4 × G16	0.3275 d
T2 × G17	0.1375 t	T4 × G17	0.3475 c
T2 × G18	0.0775 w	T4 × G18	0.2975 g
T2 × G19	0.0875 v	T4 × G19	0.3075 f
T2 × G20	0.1075 u	T4 × G20	0.3175 e
CV		**1.93%**	
Level of significance at 5%			

Gu *et al*. (2016) reported that in cabbage seedlings a rise in Na^+ and Cl^- concentration in roots, stems and leaves of cabbage seedlings was the principal contributor to declining ratios. Crop salt tolerance is therefore considerably variable between species, moreover between genotypes and cultivars of the identical species, because of reliance on different salt tolerance components (Janardhan *et al.,* 1986; Maas, 1993; La Bella *et al.*, 2019).

4.5 Effect of salinity level on K^+ accumulation in plant parts

Potassium concentration in cowpea is higher than the concentration of other nutrients. Potassium helps in the vigorous growth of cowpea and stimulates in early flowering and setting of fruits, thereby increasing the amount and production of cowpea per plant. Maintenance of adequate levels of K^+ is important for plant survival in saline habitats. Potassium is the most distinguished inorganic plant solute, and as such makes an important contribution to the low osmotic potential within the stele of the roots that is a prerequisite for turgor-pressure-driven solute transport in the xylem and the water balance of plants (Marschner, 1995). Moreover, it is a vital co-factor for several enzymes like pyruvate kinase (Mahajan and Tuteja, 2005). Because of K^+'s importance, salt tolerant plants must maintain a top level of potassium in their cells (Volkmar *et al.,* 1998).

4.5.1 Parametric significance on k^+ accumulation in leaf

Table 12 shows data on ANOVA results of Na^+ accumulation in leaf. From this table, genotype (0.000) followed by treatment (0.000) impacts Na^+ accumulation in leaf significantly since P values of them are lower than 0.05. Their interaction has P value (0.0000) lower than 0.05, which indicates its significance on Na^+ accumulation in leaf.

A low CV value of 2.00% provides a precise analysis of the parameters for Na^+ accumulation in leaf.

Table 12. ANOVA results of k^+ accumulation in leaf

Source	DF	SS	MS	F	P
Treatment	3	0.30918	0.10306	1268.43	0.0000
Genotype	19	0.11806	0.00621	76.48	0.0000
Treatment*Genotype	57	0.01892	0.00033	4.09	0.0000
Error	80	0.00650	0.00008		
Total	159	0.45266			
CV	5.02 %				

4.5.2 Effect of K^+ accumulation in leaf by different level of salinity

Figure (8) shows the effect of different treatments on K^+ accumulation in leaf in nutrient solution. Leaf K^+ concentration was decreased significantly by increasing salinity levels and this was compensated by the accumulation of sodium.

K^+ accumulation in leaf was negatively affected by the treatments (T_1, T_2, T_3 and T_4). However, plants exposed to higher salinity (12 dS/m NaCl) had the lowest K^+ content (0.124%), which was significantly lower than the other treatments, including the control. Then it increases gradually followed by 8 dS/m (0.151%), 4 ds/m (0.207%) and at control (0.235%) K^+ content was highest in cowpea leaf.

It is concluded that all the treatments (0, 4, 8 and 12 dS/m) affected K^+ content in cowpea leaf of all the genotypes in nutrient solution. But control affected more positively and 12 dS/m affected more negatively on K^+ content in cowpea leaf than other treatments.

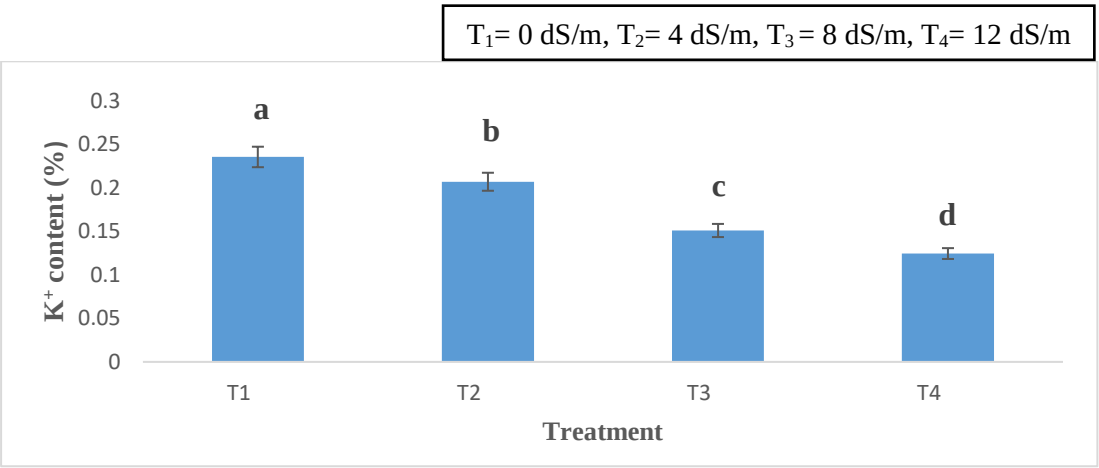


Figure 8. Effect of K^+ accumulation in leaf by different level of salinity

High Na^+ content inhibits the uptake of K^+ ions which is an important element for growth and development (James *et al*. 2011). The Na^+ toxicity problem mainly results from this lack of discrimination. Because there is competition between Na^+ and K^+ for uptake by Na^+, K^+ co transporters, Na^+ blocks K^+ acquisition. Moreover, Na^+ may block K^+ uptake through K^+ specific transporters in root cells. High K^+ concentrations in the stroma are necessary for the uptake of optimum photosynthetic capacity under stress conditions (Chow *et al.*, 1990).

Na^+ contains a strong inhibitory effect on K^+ uptake by cells, probably by inhibiting K^+ transporters. Additionally, membrane depolarization caused by large cytosolic Na^+ influx leads to increased K^+ efflux through depolarization-activated outward-rectifying K+ channels (Adams, 2014 and Sun *et al*. 2009). According to Hakim *et al*. (2014), K^+ ions decreased more in the roots than in the stems when salinity stress increased. Salt-tolerant plants usually accumulate low Na^+ and high K^+ as critical salt-sensitive plants, through selective uptake mechanisms (Ismail, 2007 and Platten, 2013).

4.5.3 Effect of K^+ accumulation in leaf by different cowpea genotypes

Figure (9) shows the effect of genotype on K^+ accumulation in stem at nutrient solution. From the figure it is observed that the potassium concentration was highest in TVU-1330 (0.24) and TVU-2389 (0.242%) and lowest in TVU-1556 (0.137%). Salinity reduced K^+ concentration in all the leaves but after TVU-1556, some others genotype like TVU-870 (0.15%) and TVU-8 (0.193%) were also comparatively lowered than other genotypes. Similarly, along with TVU-2389 and TVU-1330, K^+ concentration was increased in leaves of some other genotypes like TVU-1124 (0.227%) were also comparatively higher than other genotypes.

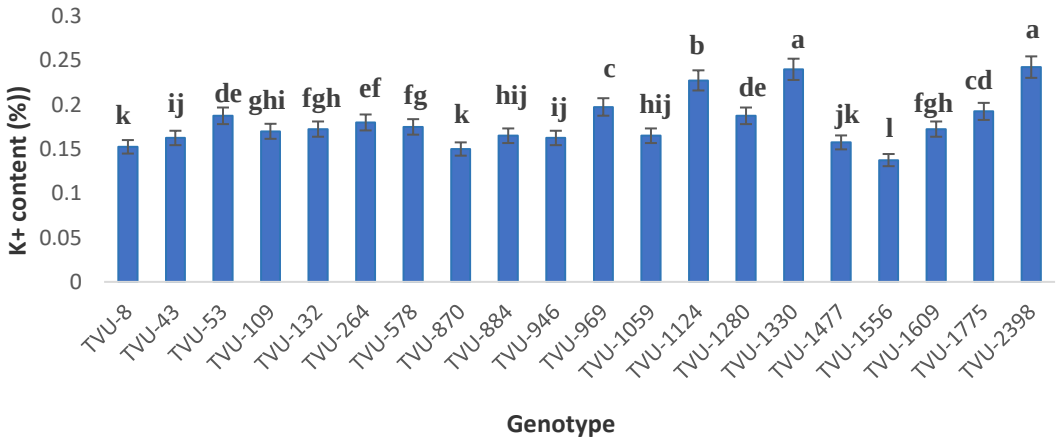


Figure 9. Effect of K^+ accumulation in leaf by different cowpea genotypes

4.5.4 Interaction effect of treatment and genotype on k^+ accumulation in leaf

The interaction effect on k^+ accumulation in leaf between treatment and genotypes of cowpea was found significant **(**Table 12). From the table it was observed that the highest K^+ content in leaf (0.297%) was obtained from the interaction, $T_1 \times G_{15}$ which was statistically close to $T_1 \times G_{20}$. The lowest K^+ content (0.097%) was found from the interaction $T_4 \times G_8$ which was statistically close to $T_4 \times G_{20}$.

Table 13. Interaction effect of salinity and genotype on K^+ accumulation in leaf

Treatment combination	Mean	Treatment combination	Mean
T1 × G1	0.1975 hi	T3 × G1	0.1275 op
T1 × G2	0.2175 fg	T3 × G2	0.1275 op
T1 × G3	0.2475 cd	T3 × G3	0.1575 lm
T1 × G4	0.2275 ef	T3 × G4	0.1275 op
T1 × G5	0.2275 ef	T3 × G5	0.1375 no
T1 × G6	0.2375 de	T3 × G6	0.1475 mn
T1 × G7	0.2375 de	T3 × G7	0.1375 no
T1 × G8	0.2075 gh	T3 × G8	0.1175 pq
T1 × G9	0.2275 ef	T3 × G9	0.1175 pq
T1 × G10	0.2375 de	T3 × G10	0.1275 op
T1 × G11	0.2475 cd	T3 × G11	0.1575 lm
T1 × G12	0.2875 b	T3 × G12	0.1275 op
T1 × G13	0.2175 fg	T3 × G13	0.2175 fg
T1 × G14	0.2375 de	T3 × G14	0.1875 ij
T1 × G15	0.2975 a	T3 × G15	0.1275 op
T1 × G16	0.2075 gh	T3 × G16	0.1275 op
T1 × G17	0.1775 jk	T3 × G17	0.1175 pq
T1 × G18	0.2175 fg	T3 × G18	0.1375 no
T1 × G19	0.2475 cd	T3 × G19	0.1575 lm
T1 × G20	0.2975 a	T3 × G20	0.2375 de
T2 × G1	0.1675 kl	T4 × G1	0.1175 pq
T2 × G2	0.1875 ij	T4 × G2	0.1175 pq
T2 × G3	0.2075 gh	T4 × G3	0.1375 no
T2 × G4	0.1975 hi	T4 × G4	0.1075 qr
T2 × G5	0.2075 gh	T4 × G5	0.1175 pq
T2 × G6	0.1975 hi	T4 × G6	0.1275 op
T2 × G7	0.2075 gh	T4 × G7	0.1175 pq
T2 × G8	0.1775 jk	T4 × G8	0.0975 r
T2 × G9	0.2075 gh	T4 × G9	0.1075 qr
T2 × G10	0.1775 jk	T4 × G10	0.1075 qr
T2 × G11	0.2375 de	T4 × G11	0.1475 mn
T2 × G12	0.1975 hi	T4 × G12	0.1175 pq
T2 × G13	0.2475 cd	T4 × G13	0.1575 lm
T2 × G14	0.2075 gh	T4 × G14	0.1175 pq
T2 × G15	0.2575 bc	T4 × G15	0.1475 mn
T2 × G16	0.1775 jk	T4 × G16	0.1175 pq
T2 × G17	0.1575 lm	T4 × G17	0.0975 r
T2 × G18	0.2075 gh	T4 × G18	0.1275 op
T2 × G19	0.2275 ef	T4 × G19	0.1375 no
T2 × G20	0.2675 b	T4 × G20	0.1675 kl
CV		**5.02%**	
Level of significance at 5%			

4.5.5 Parametric significance on K^+ accumulation in stem

Table 14 shows data on ANOVA results of K^+ accumulation in stem. From this table, genotype (0.000) followed by treatment (0.000) impacts K^+ accumulation in stem significantly since P values of them are lower than 0.05. Their interaction has P value (0.0000) lower than 0.05, which indicates its significance on K^+ accumulation in stem. A low CV value of 2.05% provides a precise analysis of the parameters for K^+ accumulation in stem.

Table 14. ANOVA results of K^+ accumulation in stem

Source	DF	SS	MS	F	P
Treatment	3	0.22445	0.07482	2850.10	0.0000
Genotype	19	0.17807	0.00937	357.02	0.0000
Treatment*Genotype	57	0.01438	0.00025	9.61	0.0000
Error	80	0.00210	0.00003		
Total	159	0.41899			
CV	2.05 %				

4.5.6 Effect of K^+ accumulation in stem by different level of salinity

Figure (10) shows the effect of different treatments on K^+ accumulation in stem in nutrient solution. Stem K concentration was decreased significantly by increasing salinity levels and this was compensated by the accumulation of sodium.

K^+ accumulation in stem was negatively affected by the treatments (T_1, T_2, T_3 and T_4). However, plants exposed to higher salinity (12 dS/m NaCl) had the lowest K^+ content (0.198%), which was significantly lower than the other treatments, including the control. Then it increases gradually followed by 8 dS/m (0.234%), 4 ds/m (0.269%) and at control (0.297%) K^+ content was highest in cowpea stem.

It is concluded that all the treatments (0, 4, 8 and 12 dS/m) affected K^+ content in cowpea stem of all the genotypes in nutrient solution. But control affected more positively and 12 dS/m affected more negatively on K^+ content in cowpea stem than other treatments.

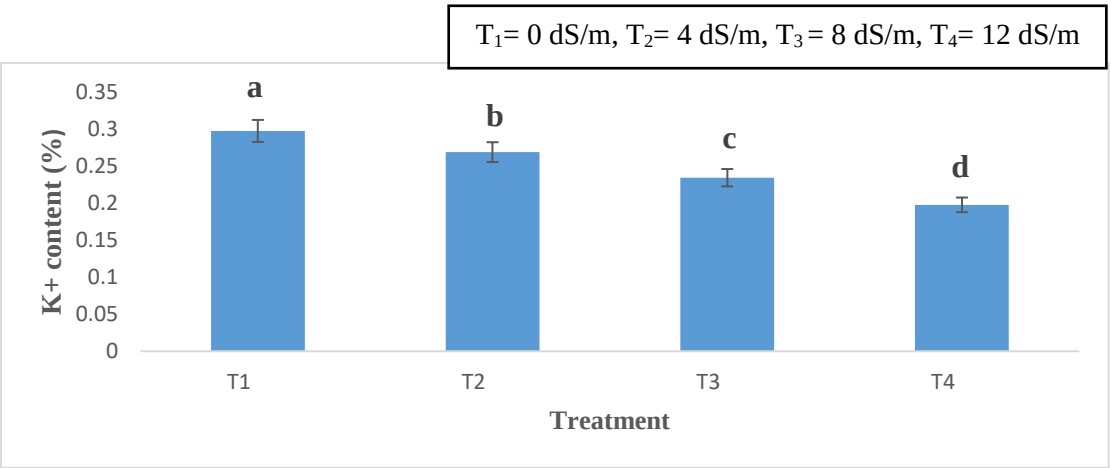


Figure 10. Effect of K^+ accumulation in stem by different salinity level

Increased salinity of up to 8000 ppm decreased concentrations of K. This may be due to an ionic imbalance resulting from a disorder in the integrity of the plasma membranes of cells (Guo *et al*., 2019) in addition to a converse relationship between Na and other elements (Wakeel *et al.*, 2019). High Na+ interferes with K+ nutrition and disturbs efficient stomatal regulation, which results in a depression of photosynthesis and growth (Tavakkoli *et al*. 2010). Bartha *et al*. (2015) reported that selected cultivars of lettuce have shown evident differences in potassium content between 50 and 100 mmol/L NaCl concentrations.

4.5.7 Effect of K^+ accumulation in stem by different cowpea genotypes

Figure (11) shows the effect of genotype on K^+ accumulation in stem at nutrient solution. From the figure it is observed that the potassium concentration was highest

and same in TVU-1330 (0.29%) and TVU-2398 (0.29%). The lowest K^+ was in TVU-8 (0.18%) and TVU-870 (0.18%). Salinity reduced K^+ concentration in all the stems but after TVU-8 and TVU-870, TVU-132 (0.192%) were also comparatively lowered than other genotypes. Similarly, along with TVU-1330 and TVU-2398, K^+ concentration was increased in stems of some other genotypes like TVU-1059 (0.28%) and TVU-1477 (0.28%) were also comparatively higher than other genotypes.

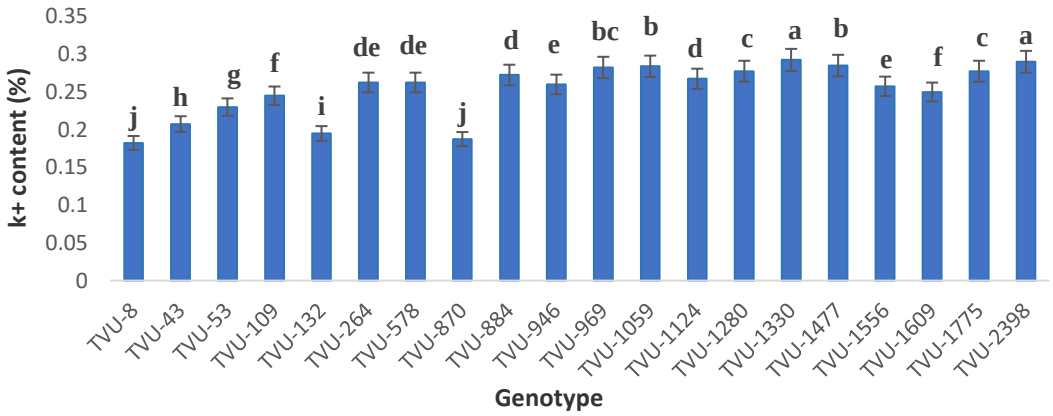


Figure 11. Effect of K^+ accumulation in stem by different cowpea genotypes

4.5.8 Interaction effect of salinity and genotype on k^+ accumulation in stem

The interaction effect on k^+ accumulation in stem between treatment and genotypes of cowpea was found significant **(**Table 15). From the table it was observed that the highest K^+ content in stem 0.337% was obtained from the interaction, $T_1 \times G_{12}$ which was statistically close to $T_1 \times G_{15}$ and the lowest K^+ content (0.147%) was found from the interaction $T_4 \times G_8$ which was statistically close to $T_4 \times G_1$.

Higher salt concentrations remarkably decreased K^+ ion in leaves, shoots, and roots of all for kiwifruit genotypes as compared to control. The same decreasing behavior of K^+ ion under NaCl was observed in kiwifruit (Tian *et al.,* 2011). The decline in K^+ ion under higher salt stress conditions was possibly due to a higher accumulation of Na^+ ion contents in various plant tissue.

Table 15. Interaction effect of salinity and genotype on K^+ accumulation in stem

Treatment combination	Mean	Treatment combination	Mean
T1 × G1	0.2175 lm	T3 × G1	0.1675 q
T1 × G2	0.2475 ij	T3 × G2	0.1875 op
T1 × G3	0.2875 ef	T3 × G3	0.1975 no
T1 × G4	0.2975 de	T3 × G4	0.2175 lm
T1 × G5	0.2275 kl	T3 × G5	0.1775 pq
T1 × G6	0.3075 cd	T3 × G6	0.2375 jk
T1 × G7	0.3075cd	T3 × G7	0.2475 ij
T1 × G8	0.2175 lm	T3 × G8	0.1775 pq
T1 × G9	0.3175 bc	T3 × G9	0.2575 hi
T1 × G10	0.3125 c	T3 × G10	0.2375 jk
T1 × G11	0.3275 ab	T3 × G11	0.2675 gh
T1 × G12	0.3375 a	T3 × G12	0.2775 fg
T1 × G13	0.3175 bc	T3 × G13	0.2575 hi
T1 × G14	0.3275 ab	T3 × G14	0.2575 hi
T1 × G15	0.3375 a	T3 × G15	0.2875 ef
T1 × G16	0.3075 c	T3 × G16	0.2775 fg
T1 × G17	0.3075 cd	T3 × G17	0.2275 kl
T1 × G18	0.2975 de	T3 × G18	0.2375 jk
T1 × G19	0.3225 ab	T3 × G19	0.2575 hi
T1 × G20	0.3275 b	T3 × G20	0.2375 jk
T2 × G1	0.1875 op	T4 × G1	0.1475 r
T2 × G2	0.2075 mn	T4 × G2	0.1775 pq
T2 × G3	0.2375 jk	T4 × G3	0.1875 op
T2 × G4	0.2575 hi	T4 × G4	0.1975 no
T2 × G5	0.1975 no	T4 × G5	0.1675 q
T2 × G6	0.2875 ef	T4 × G6	0.2075 mn
T2 × G7	0.2875 ef	T4 × G7	0.1975 no
T2 × G8	0.1975 no	T4 × G8	0.1475 r
T2 × G9	0.2775 fg	T4 × G9	0.2075 mn
T2 × G10	0.2875 ef	T4 × G10	0.1975 no
T2 × G11	0.3075 cd	T4 × G11	0.2175 lm
T2 × G12	0.3175 bc	T4 × G12	0.2275 kl
T2 × G13	0.2875 ef	T4 × G13	0.1975 no
T2 × G14	0.3075 cd	T4 × G14	0.2075 mn
T2 × G15	0.3175 bc	T4 × G15	0.2175 lm
T2 × G16	0.2975 de	T4 × G16	0.2275 kl
T2 × G17	0.2775 fg	T4 × G17	0.2075 mn
T2 × G18	0.2575 hi	T4 × G18	0.1975 no
T2 × G19	0.2975 de	T4 × G19	0.2175 lm
T2 × G20	0.2875 ef	T4 × G20	0.2025 n
CV		**2.05%**	
Level of significance at 5%			

CHAPTER V

SUMMARY, CONCLUSION AND RECOMMENDATION

SUMMARY

Salinity is one of the major abiotic factors affecting agricultural productivity worldwide. This research was carried out to evaluate the salt tolerance at germination and seedling stages of cowpea genotypes in vitro and greenhouse experiments. To achieve the objectives, 30 genotypes were used in the experiment germination test was done at Pulse Research Centre Lab, Bangladesh Agricultural Research Institute (BARI), Gazipur at room temperature. The experiments were arranged in a completely randomized design in a factorial combination with two replications. For both experiments, four different degrees of salinity: control, 4, 8, and 12 dS/m of NaCl were designated as T_1, T_2, T_3 and T_4 respectively, were used to evaluate the relative tolerance of cowpea genotypes for salinity tolerance for germination percentage and various morpho-physiological traits. From the result it was showed that germination was gradually decreased with increasing salinity and germination was delayed at higher salt stress.

From the thirty genotypes, twenty genotypes which performed best (70-100%) were chosen in terms of germination percentage at 12 dS/m for the next experiment.

A pot experiment was conducted at greenhouse of Bangladesh Agricultural Research Institute (BARI), Gazipur during the period from December 2019 to April 2020. Significant variations and adaptability among stressed and non-stressed plants were observed in all genotypes. The data recorded from different characters were statistically

analyzed to find out the significance of difference of different levels of salinity on SPAD value and ion accumulation by leaves and stems of cowpea.

Every salt treatment delayed the emergence of plumule and radicle compared to the control and the delay was more pronounced with higher salt concentrations. TVU-415, TVU-456, TVU-1280, TVU-1330, TVU-1477, TVU-1556, TVU-1609 and TVU-2398 had a germination percentage of 100% even in 12 dS/m salinity. On the other hand, TVU-84, TVU-374, TVU-393, TVU-523 and TVU-1034 had a germination percentage of zero.

At 14 DAT, the highest chlorophyll content (SPAD value) was recorded at 4 dS/m (48.11 SPAD unit) and the lowest was at 12 dS/m (40.36 SPAD unit). At 28 DAT, the highest SPAD value was recorded at 12 dS/m (56.79 SPAD unit) and the lowest was at 4 dS/m (50.61 SPAD unit). At 42 DAT, the highest SPAD value was recorded at control (55.61 SPAD unit) and the lowest was at 4 dS/m (50.23 SPAD unit).

TVU-2398 (SPAD unit 53.62) and TVU-1330 (SPAD unit 53.1) showed the highest chlorophyll content (SPAD unit) whereas TVU-43 showed the lowest SPAD value (SPAD unit 35.7) at 14 DAT. At 28 DAT, TVU-2398 (SPAD unit 61.88) and TVU-1330 (SPAD unit 61.57) showed almost the same and the highest SPAD value, whereas TVU-43 (SPAD unit 45.69) and TVU-870 (SPAD unit 45.72) showed almost the same and the lowest SPAD value. At 42 DAT, TVU-2398 (SPAD unit 60.09) and TVU-1330 (SPAD unit 59.95) expressed the highest SPAD value. On the other hand, TVU-43 (SPAD unit 44.47) showed the lowest SPAD value than other genotypes.

Na^+ content in leaf was highest (0.306%) at 12 dS/m and it decreases gradually and was lowest at control (0.01%) in cowpea leaf. K^+ content in leaf plants was highest (0.235%) at control and it decreases gradually and was lowest at 12 dS/m (0.01%) in cowpea leaf.

Na^+ concentration in leaf was highest in TVU-1330 (0.206%) and lowest in TVU- 8 (0.131%) and TVU-1124 (0.134%) than other genotypes. K^+ concentration in leaf was highest in TVU-2398 (0.242%) and TVU-1330 (0.24%) amd lowest in TVU-1556 (0.137%).

K^+ concentration in stem was highest in TVU-1330 (0.29%) and TVU-2398 (0.29%) and lowest content was in TVU-8 (0.137%) along with TVU-870 (0.18%) than other genotypes. On the other hand, the highest Na^+ content in stem was in TVU-1330 (0.206%) and lowest were in TVU- 8 (0.131%) and TVU-1124 (0.134%).

The interaction effect of salinity and variety was statistically significant in all the maximum parameter. The result of pot experiment showed that genotypes TVU-1330 (G15), TVU-2398 (G20) and TVU-1059 (G12) genotypes were performed best under salinity condition than other genotypes and had the highest salt tolerant ability whereas the smallest value was recorded in genotype TVU-8 (G1) and TVU-870 (G8). Germination percentage was also 100% from the day one to last date of these genotypes.

CONCLUSION

In vitro and greenhouse screening method proves to be a perfect method to screen an oversized set of genotypes with fewer efforts and accuracy. Based on the study results, it may be concluded that -

1. TVU-415, TVU-456, TVU-1280, TVU-1330, TVU-1477, TVU-1556, TVU-1609 and TVU-2398 genotypes had a germination percentage of 100% at 12 dS/m salinity.
2. TVU-2398 (SPAD unit 53.62) and TVU-1330 (SPAD unit 53.1) showed the highest chlorophyll content (SPAD value) at 42 DAT.
3. The highest Na^+ content in leaf was in TVU-1330 (0.206%) and lowest in TVU- 8 (0.131%) and TVU-1124 (0.134%).
4. The highest Na^+ content in stem was in TVU-1330 (0.206%) and lowest in TVU- 8 (0.131%) and TVU-1124 (0.134%).
5. K^+ concentration in leaf was highest in TVU-2398 (0.242%) and TVU-1330 (0.24%) and lowest content was in TVU-8 (0.137%) along with TVU-870 (0.18%) than other genotypes.
6. K^+ concentration in stem was highest in TVU-1330 (0.29%) and TVU-2398 (0.29%) and lowest was in TVU-8 (0.137%) and TVU-870 (0.18%) than other genotypes.

Considering the salinity tolerance and stability performance, genotypes TVU-1330 (G_{15}), TVU-2398 (G_{20}) and TVU-1059 (G_{12}) of cowpea genotypes were performed best under salinity condition than other genotypes and had the highest salt tolerant ability.

RECOMMENDATION:

Based on the result of this study, the following recommendations were suggested for a further selection of genotypes for salinity tolerance -

- The current study considered only twenty genotypes of the massive cowpea genotypes in Bangladesh; therefore, it is difficult to form an overall conclusion for all of the genotypes in Bangladesh for its salinity tolerance. The findings of the study must be considered as preliminary and need further confirmation on field conditions.

- It should be mentioned that the concentrations used for inducing salinity conditions during a limited time of exposure may improve the germination percentage as a seed priming treatment. So appropriate concentrations and exposure time is required to simulate saline conditions.

- Since the root of the plant is in direct contact with water and nutrient solution, it absorbs nutrients and water more directly than soil. It is, therefore, necessary to consider the Na^+ and K^+ accumulation to evaluate the salt tolerance of plants.

- The finding was only based on some basic traits. Therefore, when all necessary facilities are available, identification of the salt-tolerant genes by using different molecular tools is highly recommended.

CHAPTER VI

REFERENCES

Abdel-Azeem, A. Nada, A.A., O'Donovan, A., Kumar Thakur, V., Elkelish, A. (2019). Mycogenic Silver Nanoparticles from Endophytic Trichoderma Atroviride with Antimicrobial Activity. *J. Renew. Mater*. **7**: 171–185.

Abdelhamid, M. T., M. B. Shokr and M.A. Bekheta. (2010). Effects of induced salinity on four *vicia faba* cultivars differing in their broomrape tolerance. Fourteenth International Water Technology Conference, IWTC, Cairo, Egypt.

Abdel Latef, A.A.H., Tahjib-Ul-Arif, M., Rahman. (2021). M.S. Exogenous auxin-mediated salt stress alleviation in faba bean (*Vicia faba* L.). *Agronomy*. **11:** 547.

Adams, E. and Shin, R. (2014). Transport, signaling, and homeostasis of potassium and sodium in plants. *J. Integr. Plant Biol.* **56:** 231–249.

Ahmed A. M., Heikal M. M. and Shaddad M. A. (1978). Photosynthetic activity, pigment content and growth of *Helianthus annus* and *Linum usitatissimum* plants as effectd by salinization treatments. *Bull. Fac. Sci. Assiut Univ*. **7**: 49-56.

Ahmed, H.I.S., Badr, A., El-Shazly, H.H.,Watson, L., Fouad, A.S.; Ellmouni, F.Y. (2021). Molecular Phylogeny of *Trifolium* L. Section Trifolium with Reference to Chromosome Number and Subsections Delimitation. *Plants*. **10:** 1985.

Akhtar, P. and F. Hussain. (2009). Growth performance of *Vicia sativa L*.under saline conditions. *Pak. J. Bot*. **41**(6): 3075-3080.

Alam, M. Z., T. Stuchbury, R.E.L. Naylor and M.A. Rashid. (2004). Effect of salinity on growth of some moderm rice cultivar. *J. Agron*. **3**: 1-10.

Allen, D. J. (1983). The pathology of tropical food legumes. *Crop Science*. **38**: 1261-1271.

Ali, A. (2010). Exploring the possibility of transforming food crops for salinity tolerance using the TMT gene encoding thiol *methyltransferase* enzyme. A M.Sc. Thesis Presented to the University of Waterloo in Environment and Resource Studies Waterloo, Ontario, Canada.

Al-Karaki G. N. (2000). Growth, water use efficiency, and sodium and potassium acquisition by tomato cultivars grown under salt stress, *J. Plant Nutr*., **23**, 1-8.

Allison, M.A., Khan, S.R., Goodbred, J.S.L. and Kuehl, S.A. (2003). Stratigraphic evolution of the late Holocene Ganges– Brahmaputra lower delta plain, Sedimentary Geology. **155**: 317–342.

Alnusairi, G.S.H., Mazrou, Y.S.A.; Qari, S.H., Elkelish, A.A., Soliman, M.H., Eweis, M., Abdelaal, K., El-Samad, G.A., Ibrahim, M.F.M. and El Nahhas, N. (2021). Exogenous nitric oxide reinforces photosynthetic efficiency, osmolyte, mineral uptake, antioxidant, expression of stress-responsive genes and ameliorates the effects of salinity stress in wheat. *Plants*. **10:** 1693.

Amador, B.M., Troyo-Dieguez, E., Garcia-Hernandez, J.L., Lopez-Aguilar, R., Avila-Serrano, N.Y., Zamora-Salgado, S., Rueda-Puente, E.O., and Kaya, C. (2006). Effect of Nacl salinity in the genotypic variation of cowpea (*Vigna unguiculata)* during early vegetative growth. *Sci. Hort.* **108**(4): 423-431.

Amador, B.M. and Dieguez, E.T. (2007). Effects of salinity on the germination and seedling characteristics of cowpea (*Vigna unguilata* (L.) Walp). *Australian J. of Exp. Agric.* **40** (3): 433-438.

Apse, M. P. and E. Blumwald. (2002). Engineering salt tolerance in plants. Current Opinion in Biotechnology. **2**: 146-150.

Arulbalachandran, D., Sankar, G.K. and Subramani, A. (2009). Changes in metabolites and antioxidant enzyme activity of three Vigna species induced by NaCl stress. *American Eurasian J. Agron.* **2**:109-116.

Asci, O.O. (2011). Salt tolerance in red clover (*Trifolium pratense L*.) seedlings. *African J. of Biotech*. **10**(44): 8774-8781.

Arshad, M. and A. Rashid. (2001). Nitrogen uptake and dry matter production by tomato plants under salt stress. *Pak. J. Biol. Sci*. **4**: 397-399.

Ashraf, M and A. Waheed. (1992). Screening chickpea (*Cicer arietinum L.)* for salt tolerance. *J. Agri. in the Tropic and S.tropics*. **93**:45-55.

Ashraf, M. (2002). Breeding for salinity tolerance in plants. *Plant Sci.* **13**:17-42.

Ashraf, M., and T. McNeilly. (2004). Salinity tolerance in Brassica oilseeds. *Critic. Rev. in Plant Sci*. **23**: 157-174.

Ashraf, M. (2010). Inducing drought tolerance in plants. Biotechnology Advances. 169-183.

Ashraf, M. and Harris, J.C. (2004). Potential biochemical indicators of salinity tolerance in plants. *Plant Sci*. **166**: 3–16.

Avidan, A. (2000). The use of substrates in Israel. World congress on Soilless Culture on 'Agriculture in the coming millennium. 'Maale Hachamisha, Israel. p.17.

Ba, FS, R. E. Pasquet and P. Gepts. (2004). Genetic diversity in cowpea [*Vigna unguiculata* (L.) *Walp.*] as revealed by RAPD markers. *Genet Res. Crop Evol.* **51**:539–550.

Bargaz, A., Nassar, R.M.A., Rady, M.M., Gaballah, M.S., Thompson, S.M., Brestic, M., Schmidhalter, U., Abdelhamid, M.T. (2016). Improved salinity tolerance by phosphorus fertilizer in two *Phaseolus vulgaris* recombinant inbred lines contrasting in their P-efficiency. *J. Agron. Crop Sci.* **202:** 497–507.

BARI and ICBA. (2007). Development of integrated salinity management techniques for the coastal ecosystem of Bangladesh. Annual report of A Collaborative Research Project between BARI and ICBA. 6-14.

BBS (Bangladesh Bureau of Statistics) (2010). Statistical year book of Bangladesh. Statistics Division. Ministry of Planning, Government of the Peoples Republic of Bangladesh, Dhaka.

BBS. Year book of agricultural statistics. (2011). Bangladesh Bureau of Statistics, Statistics Division, Ministry of Planning, Government of the Peoples Republic of Bangladesh, Dhaka.

BBS (Bangladesh Bureau of Statistics) (2012). Statistical year book of Bangladesh. Statistics Division. Ministry of Planning, Government of the Peoples Republic of Bangladesh, Dhaka. p. 472.

Bangladesh Bureau of Statistics. (2019). Preliminary report on Agriculture census 2019. Bangladesh Bureau of Statistics.

Bartha C., Fodorpataki L., Martinez-Ballesta M. del C., Popescu O. and Carvajal M. (2015). Sodium accumulation contributes to salt stress tolerance in lettuce cultivars. *J. of App. Botan. and Food Quality*. **88:** 42–48.

Baudoin, JP and R. Maréchal. (1985). Genetic diversity in *Vigna*. **In**: Singh SR, Rachie KO (eds) Cowpea Research, Production and Utilization. John Wiley and Sons, Ltd., Chichester, NY. pp. 3–9.

Bayuelo-Jimenez, J.S., D.G. Debouck and J.P Lynch. (2002). Salinity tolerance in Phaseolus species during early vegetative growth. *Crop Sci*. **42**: 2148–2192.

Bittenbender, H. C. (1990). Handling and storage of cowpea (*Vigna unguiculata (L.) Walp.)* as a leaf vegetable. *Trop. Agric*. **69**:197-200.

Brady, N.C. (2002). The Nature and Properties of Soils. New Jersey, USA, Prentice Hall.

Brammer, H., Asaduzzaman, M. and Sultana, P. (1993). Effects of climate and sea-level changes on the natural resources of Bangladesh. briefing document No. 3, Bangladesh Unnayan Parishad (BUP), Dhaka. Pp.5-7.

Brammer, H. (2012). The physical Geography of Bangladesh. The University Press Limited, Dhaka.

Bressani, R. (1985). Nutritive value of cowpea. **In:** Cowpea Research, Production and Utilization. Singh SR, Rachie KO (eds) John Wiley and Sons, Ltd., Chichester, NY. Pp. 353–359.

Bybordi, A, J. Tabatabael. (2009). Effect of salinity stress on germination and seedling properties in canola cultivars (*Brassica napus L*.). *Hort. Agrobot. Cluj*. **37**(1): 71-76.

Bray, E.A., Bailey-Serres, J. and Weretilnyk, E. (2000). Responses to abiotic stresses. **In:** Buchanan, B.B., Gruissem, W. and Jones, R.L. (eds). Biochemistry and Molecular Biology of Plants. pp. 1158-1203. American Society of Plant Physiologists, Rockville.

Bradbury, M. and Ahmad, R. (1990). The effect of silicon on the growth of *Prosopis juliflora* growing in saline soil. *Plant and Soil*. **125**: 71- 74.

Bremner J. M. and Mulvaney C. S. (1982). Total nitrogen. **In:** "Methods of Soil Analysis (Part 2)", 2nd ed., ed. by A. L. Page, R. H. Miller and D. R. Keeny, American Society of Agronomy, Inc. and Soil Science Society of America, Inc., Madison, Wisconsin. Pp. 595–622.

Boukar O., Belko N., Chamarthi S., Togola A., Batieno J. and Owusu E. (2019). Cowpea (*Vigna unguiculata*): genetics, genomics and breeding. *Plant Breed.* **138:** 415–424.

Canci, H., M. Cagirgan and I.Toker. (2004). Genotypic variation for root and shoot growth at seedling stage in chickpea mutants. *Int. Chickpea and Pigeonpea Newsletters*. **11**:11-13.

Carsky, R. J., B. B. Singh and B. Oyewole. (2001). Contribution of early season to late season maize in the savanna zone of West Africa. *Biol. Agric. and Hort*. **18**: 303-316.

Chandrasekaran M, Chanratana M, Kim K, Seshadri S and Sa T. (2019). Impact of arbuscular mycorrhizal fungi on photosynthesis, water status, and gas exchange of plants under salt stress-a meta-analysis. *Front Plant Sci.* **10**:457.

Chinnusamy, V., A. Jagendorf and J. Zhu. (2005). Understanding and improving salt tolerance in plants. *Crop Sci*. **45**:437-448.

Chow W. S., Marylin C. B., Anderson J. M. (1990). Growth and photosynthetic responses of spinach to salinity: Implications of K+ nutrition for salt tolerance. *Australian J. of Plant Physiol.* **17:** 563–578

Coulibaly, S., RS. Pasquet, R. Papa, P. Gepts. (2002). AFLP analysis of the phenetic organization and genetic diversity of cowpea [*Vigna unguiculata (L.) Walp.*] reveals extensive gene flow between wild and domesticated types. *Theor Appl Genet*. **104**:258–266.

Craufurd, PQ, RJ.Summerfield, RH. Ell, EH. Roberts. (1997). Photoperiod, temperature and the culinaris Medic.) to salt stress. *J. Agron. and Crop Sci.* **170:**103-112.

Cuartero, J. and R. Fernandez-Munoz. (1999). Tomato and salinity. *Sci. Hort*. **78**: 83–125.

CZP (2005). Coastal Zone Policy, Ministry of Water Resources, Government of the People's Republic of Bangladesh, Dhaka.

Dai, L. Y., Zhang, L. J., Jiang, S. J. and Yin, K. D. (2014). Saline and alkaline stress genotypic tolerance in sweet sorghum is linked to sodium distribution. *Acta Agric Scandinavica.* **64:** 471–481.

Daniells, I. G., J. F., Holland, R. R.Young, C. L.Alston and A. L.Bernardi. (2001). Relationship between yield of grain sorghum (Sorghum bicolour) and soil salinity under field conditions. *Aust J. Exp. Agric*. **41**:211-217.

Dantas, B.F., de Sa Ribeiro, L. and Aragao, C.A. (2005). Physiological response of cowpea seeds to salinity stress. *Revis. Brasileira Sement*. **27**: 144–148.

Dasgan, H.Y., Aktas, H., Abak, K. and Cakmar, I. (2002). Determination of screening techniques to salinity tolerance in tomatoes and investigation and investigation of genotypes response. *Plant Science*. **163**: 695–703.

Davis, D.W., E.A. Oelke, E.S. Oplinger, J.D. Doll, C.V. Hanson and D.H. Putnam. (1991).

Demir M. and I. Arif. (2003). Effects of different soil salinity levels on germination and seedling growth of safflower *(Carthamus tinctoriusl*), *Turkish J. Agric.* **27**: 221- 227.

DPIRD (Department of Primary Industries and Regional Development) (2019). Govt. of Western Australia.

Dudeck, A. E, S. Singh, C. E. Giordano, T. A. Neel and D. B. McConnell. (1983). Effects of sodium chloride on cynodon turfgrasses. *J. Agron*. **75** (6): 927-930.

Dugje, I. Y., L. O. Omoigui, F. Ekeleme, A. Y. Kamara and H. Ajeigbe. (2009). Farmers' Guide to Cowpea Production in West Africa. IITA, Ibadan, Nigeria.

Duke, J. A. (1981). Handbook of Legumes of World Economic Importance. Plenum Press, New York. Pp. 345.

Dutta, P. and Bera, A. K. (2014). Effect of NaCl salinity on seed germination and seedling growth of mungbean cultivars. Agricultural research communication center. *Leg. Res.* **37**(2): 161-164.

EARO (Ethiopian Agricultural Research Organization). (2004). Directory of Released Crop Varieties and Their Recommended Cultural Practices. Addis Ababa, Ethiopia.

EIAR (Ethiopian Institute of Agricultural Research). (2007). The Effect of Liming and Phosphate Fertilizer Application on the Acid Properties of Soils under Major Crops Grown in Ethiopia. Proposal on acid soil management in Ethiopia.

Emechebe, A. M. (1975). Some aspects of crop diseases in Uganda. Kampala, Uganda, Makerere University.

Epstin E., Norlyn J. D., Rush D. W., Kingsbury. R. R., Kelley D. B., Cunning Gham G. A. and Wrona A. F. (1980). Saline culture of crops a genetic approach. *Sci*. **210**: 399-404.

El-Hendawy, S. E., Y. Hu, and U. Schmidhalter. (2007). Assessing the suitability of various physiological traits to screen wheat genotypes for salt tolerance. *J. Integr. Plant Biol*. **49**: 1–9.

El-Hendawy, S.E. (2004). Salinity Tolerance in Egyptian Spring Wheat Genotypes. A PhD dissertation presented to Center of Life Science of Technical University of Munich. Pp.10.

Estan M. T., Martinez-Rodriguez M. M., Perez-Alfocea F., Flowers T. J. and Bolarin M. C. (2005). Grafting raises the salt tolerance of tomato through limiting the transport of sodium and chloride to the shoot. *J. of Exp. Bot.* **6**: 703-712.

FAO (Food and Agriculture Organization). (2003). FAO Production Year Book, Rome Italy.

FAO (Food and Agriculture Organization). (2005-2006). The state of food and agriculture. Document prepared for the International Conference on Worlds" State of Food. FAO, Rome, Italy.

FAO (Food and Agriculture Organization). (2008). FAO Land and Plant Nutrition Management service. Online: (http://www.fao.org/ag/agl/agll/spush).

FAO (Food and Agriculture Organization). (2009). How to Feed the World in 2050: Technology Challenge? Food and Agriculture Organization (FAO) High-Level Expert Forum, Rome, Italy.

FAO (Food and Agriculture Organization of the United Nations) (2010). Land and Plant Nutrition Management Service. Retrieved from http:// www. fao. org/ag/a /agll/spush.

FAO (Food and Agriculture Organization) (2013). FAO Yearbook Production. p. 53.

FAO (2020). World Food and Agriculture - Statistical Yearbook 2020. Rome.

FAOSTAT (2017). Food and Agriculture Operation of the United Nations.

Fateme, K., Rayhaneh, A., Akbar, M. and Giti, E. (2010). Effect of hydro and osmopriming in two commercial chickpeas (*Cicer arietinum L*.) cultivars on germination, growth parameters and nodules number in salt stress condition. *Iran J. of Plant Biol*. **2**: 71–86.

Fatokun. (2009). Online: http://review.org/2009/03/christian-a-fatokun-the-guardian-of biosafety. December 2/2010.

Fery, RL. (2002). New opportunities in *Vigna*. **In**: Trends in New Crops and New Uses. Janick J, Whipkey A (eds). ASHS, Alexandria, VA. pp. 424–428.

Flight, C. (1976). The Kintampo culture and its place in the economic prehistory of West Africa. In: Origins of African Plant Domestication. Harlan, J.R., J.M.J. de Wed and A.B.L Stemler (eds). Mouton. The Hague, Netherlands. pp. 212-217.

Flowers, T. J., and S. A. Flowers. (2005). Why does salinity pose such a difficult problem for plant breeders? *Agri. Water Manag*. **78**:15-24.

Flowers, T. J., A. Garcia, M. Koyama and A. R.Yeo. (1997). Breeding for salt tolerance in crop plants-the role of molecular biology. *Acta, Physiol, Plant*. **19**:427-433.

Fouad, A., Hegazy, A.E., Azab, E., Khojah, E. and Kapiel, T. (2021). Boosting of Antioxidants and Alkaloids in Catharanthus roseus Suspension Cultures Using Silver Nanoparticles with Expression of CrMPK3 and STR Genes. *Plants*. **10:** 2202.

Gaber, A., Refat, M.S., Belal, A.A.M., El-Deen, I.M., Hassan, N., Zakaria, R., Alhomrani, M., Alamri, A.S., Alsanie, W.F. and Saied, E.M. (2021). New Mononuclear and Binuclear Cu (II), Co (II), Ni (II), and Zn (II) Thiosemicarbazone Complexes with Potential Biological Activity: Antimicrobial and Molecular Docking Study. *Molecules*. **26:** 2288.

Galvani, A. (2007). The challenge of the food sufficiency through salt tolerant crops. *Rev. in Environ. Sci. and Biot*. **6**: 3-16.

Ghaloo, S.H., Soomro, Z.A., Khan, N.U., Bibi, Z. Khan, I.U., Kakar, M.S., Taran, S.A. and Rajper, A.A. (2011). Response of wheat genotypes to salinity at early growth stages. *Pakistan J. Bot*. **43**(1): 617-623.

Gharsallah, C., Fakhfakh, H., Grubb, D., and Gorsane, F. (2016). Effect of salt stress on ion concentration, proline content, antioxidant enzyme activities and gene expression in tomato cultivars. *AoB Plants.* **8**: plw055.

Ghassemi F., Jakeman A.J. and Nix H.A. (1995). Salinization of Land and Water Resources: Human Causes, Extent, Management and Case Studies. UNSW Press, Sydney, Australia, and CAB International, Wallingford, United Kingdom. *J. of Plant Physiol*. **22**:875–884.

Gopalakrishnan, T. and Kumar, L. (2020). Modeling and mapping of soil salinity and its impact on Paddy Lands in Jaffna Peninsula, Sri Lanka. *Sustainability*. **12**: 8317.

Gu M.F., Li N., Shao T.Y., Long X.H., Brestič M., Shao H.B., Li J.B. and Mbarki S. (2016). Accumulation capacity of ions in cabbage (*Brassica oleracea* L.) supplied with sea water. *Plant, Soil and Environ*. **62:** 314–320.

Guo, Q., Liu, L. and Barkla, B. J. (2019). Membrane lipid remodeling in response to salinity. *Int. J. Mol. Sci.* **20:** 4264.

Gupta R. K., Singh R. R., and Abrol I. P. (1989). Effect of simultaneous changes in sodicity and pH on the hydraulic conductivity of an alkali soil under rice culture. *Soil Sci*. **147** (1): 28-33.

Hasanuzzaman, M., Nahar, K., Fujita, M., Ah-mad, P., Azooz, M.M. and Prasad, M.N.V. (2013). Ecophysiology and responses of plants under salt stress. Springer, New York. pp. 25-87.

Hasanuzzaman, M., Hossain, M.A., Silva, J.A.T. and Fujita, M. (2012). Plant responses and tolerance to abiotic oxidative stress: antioxidant defenses are a key factor. In Crop stress and its management: perspectives and strategies. Springer, Berlin, New York. pp. 261-316.

Hajer A. S., Malibari A. A., Al-Zahrani H. S. and Almaghrabi O. A. (2006). Responses of three tomato cultivars to sea water salinity. Effect of salinity on the seedling growth. *African J. of Biotech*. **5** (10): 855-861.

Hakim, M.A., Juraimi, A.S., Hanafi, M.M., Ismail, M.R., Rafii, M.Y., Islam, M.M. and Selamat, A. (2014). The effect of salinity on growth, ion accumulation and yield of rice varieties. *J. Anim. Plant Sci*. **24:** 874–885.

Hall, AE., N. Cisse, S.Thiaw, HOA. Elawad and JD. Ehlers. (2003). Development of cowpea cultivars and germplasm by the Bean/Cowpea CRSP. *Field Crops Res.* **82**:103–134.

Halli, H.M. and Angadi, S.S. (2019). Effect of land configuration on rain water use efficiency, yield and economics of cowpea (*Vigna unguiculata* L.) in maize-cowpea sequence cropping under rainfed condition of Northern Transitional Zone. *Legume Res. Int. J.* **4**: 211–215.

Haque, S.A. (2006). Salinity problems and crop production in coastal regions of Bangladesh. *Pakistan J. Bot*. **38**: 1359-1365.

Haque, M.A., Jharna, D.E., Hoque, M.F., Uddin, M.N. and Saleque, M.A. (2008). Soil solution electrical conductivity and basic cations composition in the rhizosphere of lowland rice in coastal soils. *Bangladesh J. of Agri. Res*. **33**: 243–250.

Hashim, A.M., Alharbi, B.M., Abdulmajeed, A.M., Elkelish, A., Hozzein, W.N. and Hassan, H.M. (2020). Oxidative Stress Responses of Some Endemic Plants to High Altitudes by Intensifying Antioxidants and Secondary Metabolites Content. *Plants*. **9**: 869.

Hernández, J.A. (2019). Salinity tolerance in plants: Trends and perspectives. *Int. J. Mol. Sci.* **20**: 2408.

Hoagland, D. R. and Arnon, D. I. (1950). California Agricultural Experiment Station. *Circular*. **347:** 32.

Huq, S. and Rabbani, G. (2011). Climate change and Bangladesh: Policy and institutional development to reduce vulnerability. *J. of Bangladesh Stud.* **13**: 1–10.

Iftekhar, M.S. and Islam, M.R. (2004). Managing mangroves in Bangladesh: A strategy analysis. *J. of Coast. Cons.* **10**: 139-146.

IITA (International Institute of Tropical Agriculture). (2003). Crops and Farming Systems. Available at: http://www.iita.org/crop/cowpea.htm. Accessed on 26/03/2011.

Iqbal S, Basra SM, Afzal I, Wahid A, Saddiq MS, Hafeez MB, Jacobsen SE. (2019). Yield potential and salt tolerance of quinoa on salt-degraded soils of Pakistan. *J. Agron. Crop. Sci.*, **205 (**1):13-21.

Islam, F., Wang, J., Farooq, M. A., Yang, C., Jan, M. and Mwamba, T. M. (2019). "Rice responses and tolerance to salt stress," in Advances in Rice Research for Abiotic Stress Tolerance. eds. M. Hasanuzzaman, M. Fujita, K. Nahar, and J. Biswas. Woodhead Publishing. Pp. 791–819.

Ismail, A.M., Heuer, S., Thomson, M.J. and Wissuwa, M. (2007). Genetic and genomic approaches to develop rice germplasm for problem soils. *Plant Mol. Biol*. **65:** 547–570.

Jahan, M.S., Guo, S., Sun, J., Shu, S., Wang, Y., Abou El-Yazied, A., Alabdallah, N.M., Hikal, M., Mohamed, M.H.M. and Ibrahim, M.F.M. (2021). Melatonin-mediated photosynthetic performance of tomato seedlings under high-temperature stress. *Plant Physiol. Biochem.* **167**: 309–320.

James R.A., Blake C., Byrt C.S. and Munns R. (2011). Major genes for Na^+ exclusion, Nax1 and Nax2 (wheat HKT1;4 and HKT1;5), decrease Na^+ accumulation in bread wheat leaves under saline and waterlogged conditions. *J. of Exp. Bot*. **62:** 2939–2947.

Jamil, M., E.S. Rha. (2004). The effect of salinity (NaCl) on the germination and seedling of sugar beet (*Beta vulgaris L*.) and cabbage (*Brassica oleracea capitata L*.). *Korean J. Plant Res*.**7**: 226-232.

Jamil, M., C.C. Lee, S.U. Rehman, D.B. Lee, M. Ashraf and E.S. Rha. (2005). Salinity (NaCl) Tolerance of Brassica Species at Germination and Early Seedling Growth. *EJEAF Chem*. **4**(4): 970-976.

Janardhan, K. V., Patil, B. N., and Raiker, D. S. (1986). Relative tolerance of Safflower (*Carthamus tinctorius* L.) varieties to saline water irrigation. *Indian J. Plant Physiol*. **29**: 118–124.

Jeannette, S., R. Craig and J.P. Lynch. (2002). Salinity tolerance of phaseolus species during germination and early seedling growth. *Crop Sci*. **42**: 1584-1594.

Kaveh, H., N. Hossein, F.Mohammad and V.J. Safieh. (2011). How Salinity Affect Germination and Emergence of Tomato Lines. *J. biol. environ. sci.* **5**(15): 159-163.

Kabir M. E. (2002). Role of nutrients (N, P and K) on salinity tolerance of mungbean (*Vigna radiata L*. Wilezek). An M. S. Thesis. Dept. of Agronomy, BSMRAU, Salna, Gazipur.

Karim, Z., Ibrahim, A.M., Iqbal, A. and Ahmed, M. (1990). Drought in Bangladesh Agriculture and Irrigation Schedules for Major Crops, Bangladesh Agricultural Research Council (BARC), Soils Public. pp 11.

Kawser U., Hoque A. and Nath B. (2021). Observing the impacts of 1950s great Assam earthquake in the tectono-geomorphological deformations at the Young Meghna Estuarine Floodplain of Bangladesh: evidence from Noakhali Coastal Region. *Arabian J. Geosci.* **14**: 306.

Khan, M.A., A. Qayyum, and E. Noor. (2007). Assessment of wheat genotypes for salinity tolerance. Proceedings of 8th African Crop Science Conference, Octo.27-31, El-Minia, Egypt. pp: 75-78.

Khan, N.A., Syeed, S., Masood, A., Nazar, R. and Iqbal, N. (2010). Application of salicylic acid increases contents of nutrients and antioxidative metabolism in mungbean and alleviates adverse effects of salinity stress. *Int. J. Plant Biol*. **1**(1): 11.

Khajeh-Hosseini, M., Powell, A.A. and Bimgham, I.J. (2003). The interaction between salinity stress and seed vigor during germination of soybean seeds. *Seed Sci. and Tech.* **31**: 715–725.

Kinfemichael G. and Melkamu G. (2008). Response of some lowland growing sorghum (*Sorghum bicolor L. Moench*) accessions to salt stress during germination and seedling growth. *Afrcan. J. of Agri. Res*. **3** (1): 044-048.

Kinfemichael Geressu. (2011). The Response of Some Haricot Bean (*Phaseolus vulgaris)* Varieties for Salt Stress during Germination and Seedling Stage. *Cur. Res. J. Biol. Sci*. **3**(4): 282-288.

Khrishi, P.H. (2016). A hand book of agro-technology. Joydebpur, Gazipur: Published by Bangladesh Agricultural Research Institute.

Taïbi K., Taïbi F., Abderrahim L.A., Ennajah A., Belkhodja M. and Mulet J.M. (2016). Effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidant defence systems in Phaseolus vulgaris L. *South African J. Bot.* **105**: 306-312.

La Bella, S., Tuttolomondo, T., Lazzeri, L., Matteo, R., Leto, C. and Licata, M. (2019). An agronomic evaluation of new Safflower (*Carthamus tinctorius* L.) germplasm for seed and oil yields under Mediterranean climate conditions. *Agron*. **9:** 1–16.

Landon, J. R. (1991). Booker Tropical Soil Manual. A Handbook of Soil Survey and Agricultural Land Evaluation in the Tropics and Sub-Tropics. 1st Edn., Longman, London. pp: 185.

Li-ping, L., Xiao-hua, L., Hong-bo, S., Zhao-Pu, L., Ya, T. and Quan-suo, Z. (2015). Ameliorants improve saline-alkaline soils on a large scale in northern Jiangsu Province, China. *Ecol. Eng.* **81:** 328–334.

Maathuis F. J. M. and Amtmann A. (1999). K^+ nutrition and Na^+ toxicity: the basis of cellular K^+ /Na^+ ratios. *Ann. Bot*. **84**: 123-133.

Magloire, N. (2005). The Genetic, Morphological and Physiological Evaluation of African Cowpea Genotypes. An M.Sc. Thesis presented to the University of the Free State, Faculty of Natural and Agricultural Sciences, Bloemfontein.

Maggio A., Pascale S., Angelino G., Ruggiero C. and Barbieri G. (2004). Physiological response of tomato to saline irrigation in long-term salinized soils. *European J. of Agron.* **2** (2): 149-159.

Mahajan, S. and N. Tuteja (2005). Cold, salinity and drought stresses: An overview. *Arch. Biochem. Biophysiol*. **444**: 139-158.

Mahmood, A. T., Latif, T. and Arif, M. (2009). Effect of salinity on growth, yield and yield components in basmati rice germplasm. *Pakistani J. Bot*. **41**(6): 3035-3045.

Marechal, R., J. M. Mascherpa and F. Stainier. (1978). Etude taxonomique d'un groupe d'especes des genres *Phaseolus* et Vigna (Papilonaceae) sur la base des donnees morphologiques et polliques, traitees pour l'analyse informatique. *Boissiera*. **28**: 1-273.

Marschner H. (1995). Mineral Nutrition of Higher Plants (Academic Press, London), Ed 2. 45-56.

Maas, E.V. and J. A. Poss. (1989). Effects of salinity on germination, growth and yield of cowpea. *Sci*. **7**: 29-40.

Maas, E.V. and G. J. Hoffman. (1977). Crop salt tolerance-current assessment. *J. of the Irrig. and drain. Div.* **103**: 115-134.

Mass E.V. (1986). Salt tolerance of plants. *App. Agri. Res.* **1**: 12-26.

McKenzie. (1988). Tolerance of plants to soil salinity. In: Proceedings of Dryland Salinity Control Workshop. Calgary, AB. pp. 246–251.

McMichael, A. J. (2001). Impact of climate and other environmental changes on food production and population health in the coming decades. *Proceed. of the Nutri. Soci.* **60**: 195-201.

Mebeaselassie, Andargie, R. S. Pasquet, B. S. Gowda, G. M. Muluvi and M. P. Timko. (2011). Construction of an SSR-based genetic map and identification of QTL for domestication traits using recombinant inbred lines from a cross between wild and cultivated cowpea (*V. unguiculata (L.) Walp.). Mol. Breed*. **28**: 413-420.

Meneguzzo,S, F. Navari-Izzo, and R. Izzo. (2000). NaCl effects on water relations and accumulation of mineral nutrients in shoots roots and cell sap of wheat seedling. *J. of Plant Physiol*. **156**: 711-716.

Mitsuy S., M. Kawasaki, M. Taniguchi and H. Miyake. (2003). Light dependency of salinity-induced chloroplast degradation. *Plant Prod. Sci*. **6:** 219-223.

Mohamed, M., M. Hussein, M. Shaaban and AK. M. El-Saady. (2008). Response of Cowpea Plants Grown Under Salinity Stress to PK-Foliar Applications. *American J. of Plant Physiol*. **3**: 81-88.

Moller, I. S. and M. Tester. (2007). Salinity tolerance of Arabidopsis: a good model for cereals? *Trends in Plant Sci.* **12**: 5 34-540.

Moud, A. M. and K. Maghsoudi. (2008). Salt Stress Effects on Respiration and Growth of Germinated Seeds of Different Wheat (*Triticum aestivum* L.) Cultivars. *World J. of Agri. Sci.* **4** (3): 351-358.

Moustafa-Farag, M., Elkelish, A., Dafea, M., Khan, M., Arnao, M.B., Abdelhamid, M.T., El-Ezz, A.A., Almoneafy, A., Mahmoud, A. and Awad, M. (2020). Role of Melatonin in Plant Tolerance to Soil Stressors: Salinity, PH and Heavy Metals. *Molecules*. **25:** 5359.

Muhammad, Z. and Hussain, F. (2010). Effect of NaCl salinity on the germination and seedling growth of some medicinal plants. *Pakistani J. Bot.* **42**(2): 889-897.

Muluemebt Dinku. (2003). Survey of cowpea storage methods extent of loss due to pulse beetle (*Callosobruchus maculates fabricus)* and its management in gambella. An M. Sc. Thesis Presented to the School of Graduate Studies of Alemaya University, College of Agric., Alemaya.

Munns R. (2002). Comparative physiology of salt and water stress. *Plant, Cell and Environ*. **25:** 239-250.

Munns, R., S. Husain, A. R. Rivelli, R. A. James, A. G. Condon, M. P. Lindsay, E. S. Lagudah, D. P. Schachtmann, and R. A. Hare. (2002). Avenues for increasing salt tolerance of crops, and the role of physiologically based selection traits. *Plant and Soil.* **247**: 93-105.

Munns, R., R. A. James, and A. Lauchli. (2006). Approaches to increase the salt tolerance of wheat and other cereals. *J. of Exp. Botany.* **57**: 1025-1043.

Munns, R., and M. Tester. (2008). Mechanism of salinity tolerance. *Ann. Rev. of Plant Biotec*. **59**: 651-681.

Murillo-Amador, B. and E. Troyo-Die'guez. (2000). Effects of salinity on the germination and seedling characteristics of cowpea [*Vigna unguiculata* (L.) Walp.]. *Australian J. of Exp. Agric*. 40(3): 433-438.

Naher, N., Uddin, M.K. and Alam, A.K.M.M. (2011). Impacts of salinity on soil properties of coastal areas in Bangladesh. *Agrivita*. **33(**2): 3–10.

Nassar, M.A.; Azoz, D.N.; Wessam, S.; Serag El-Din, M. (2019). Improved growth and productivity of basil plants grown under salinity stress by foliar application with ascorbic acid. *Middle East J. Agric.* **8:** 211–225.

Ng, N. Q. and R. Marechal. (1985). Cowpea taxonomy, origin and germplasm. **In:** Cowpea Research, Production and Utilization. Singh, S.R and K.O. Rachie (eds). John Wiley and Sons, Chichester. pp. 11-12.

Padulosi, S. (1993). Genetic diversity, taxonomy and eco geographic survey of the wild relatives of cowpea (V. *unguicullata*). Ph.D. Thesis submitted to the University of Catholique Lovain-la-Neuve, Belgique.

Padulosi, S., G. Laghetti, B. Pienaar, N.Q. Ng and P. Perrino. (1991). Survey of wild Vigna in southern Africa. FAO Plant Genetic Resources News 83/84. Pp.4–8.

Padulosi, S. and N.Q. Ng. (1997). Origin, taxonomy and morphology of *Vigna uiculungata (L.) Walp*. **In:** Advances in Cowpea Research. Singh, B.B., D.R. Mohan Raj, K.E. Dashiell and L.E.N. Jackai (eds). IITA, Ibadan, Nigeria. pp. 1-11.

Patel, P.N. and A.E. Hall. (1990). Genotypic variation and classification of cowpea for reproductive responses to high temperature under long photoperiods. *Crop Sci.* **30**: 614621.

Petersen, L. and S. Shireen. (2001). Soil and water salinity in the coastal area of Bangladesh. SRDI. Pp. 15-18.

Pitman, M. G. and A. Lauchli. (2002). Global impact of salinity and agricultural ecosystems. In A. Lauchli and U. Luttge (Eds.). Salinity: Environment - Plants - Molecules (pp. 3-20). Kluer, Netherland.

Platten, J.D., Egdane, J.A. and Ismail, A.M. (2013). Salinity tolerance, Na+ exclusion and allele mining of HKT1; 5 in Oryza sativa and O. glaberrima: Many sources, many genes, one mechanism? *BMC Plant Biol*. **13:** 32.

Ponnamieruma, P. N. (1984). Role of cultivars tolerance in increasing rice production on saline land. **In:** Staples, R. C., Toenniessen, G. H., (Eds.), Salinity tolerance in plants strategies for crop improvement, John Wiley and Sons, New York. Pp 255-271.

Purseglove, JW. (1968). Tropical Crops - Dicotyledons. Longman, London, UK.

Quin, F. M. (1997). Advances in cowpea research. Co-publication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). Ibadan, Nigeria.

Rady, M.M., Taha, R.S. and Mahdi, A.H.A. (2016). Proline enhances growth, productivity and anatomy of two varieties of *Lupinus termis* L. grown under salt stress. *S. African. J. Bot.* **102:** 221–227.

Rahman, M.M. and Ahsan, M. (2001). Salinity constraints and agricultural productivity in coastal saline area of Bangladesh, Soil Resources in Bangladesh: Assessment and Utilization.

Rahman M. M. (2002). Efficiency of Indigenous Mulches on the growth and Development of Quality Protein Maize under Zero Tillage condition; An M.S. thesis in Crop Botany, submitted to Bangladesh Agricultural University, Mymensingh, Bangladesh.

Rahman M. A. (1999). Morpho-Physiological Adaption of Quality Protein Maize (QPM) as effected by Indigenous Mulches; An M.S. thesis in Crop Botany, submitted to Bangladesh Agricultural University, Mymensingh, Bangladesh.

Rains, D. W., and S. S. Goyal. (2003). Strategies for managing crop production in saline environments: An overview. *J. of Crop Prod*. **7**:1-10.

Rasheed. (2016). K.B.S. Rasheed Bangladesh Resource and Environmental Profile. A H Development Publishing House, Dhaka.

Rengasamy, P. (2006). World salinization with emphasis on Australia. *J. Exp. Bot*. **57**(5): 1017-1023.

Robertson, G. P. and S. M. Swinton. (2005). Reconciling agricultural productivity and environmental integrity: a grand challenge for agricultural. *Front. in Ecol. and Environ.* **3**: 38-46.

Sairam, R. K., K. V. Rao and G. C. Srivastava. (2002). Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolytes concentrations. *Plant Sci*. **163**: 1037-1046.

Salehin, M., Chowdhury, M. M., Clarke, D., Mondal, S., Nowreen, S., Jahiruddin, M., and Haque, A. (2018). Mechanisms and drivers of soil salinity in coastal Bangladesh. In: R. J. Nicholls, C. W. Hutton, W. N. Adger, S. E. Hanson, M. M. Rahman, and M. Salehin (Eds.), Ecosystem Services for Well-Being in

deltas: Integrated assessment for policy analysis. *Springer International Publishing.* https://doi.org/10.1007/978-3-319-71093-8.

Sanginga, N., O. Lyasse and B. B. Singh. (2000). Phosphorus use efficiency and nitrogen balance of cowpea breeding lines in low P soil of derived savanna zone in West Africa. *Plant and Soil*. **220**: 119-128.

Sanower-Hossain, M. (2019). Present scenario of global salt affected soils, its management and importance of salinity research. *Int. Res. J. Biol. Sci.* **1:** 1–3.

Savary S., Willocquet L., Pethybridge S. J., Esker P., Mcroberts N., Nelson A. (2019). The global burden of pathogens and pests on major food crops. *Nat. Ecol.* **3**:430.

Savvas, D. (2003). Hydroponics: A modern technology supporting the application of integrated in greenhouse. *J. Food Agric. Environ.* **1**: 80-90.

Sehrawat, N., Bhat, K.V., Sairam, R.K. and Jaiwal, P.K. (2013b). Screening of mungbean (*Vigna radiata* L. Wilczek) genotypes for salt tolerance. *Int. J. Plant An. Env. Sci*. **4**:36-43.

Syed H., Choi G. and Byeon S. (2018). A numerical approach to predict water levels in ungauged regions—Case study of the Meghna River estuary Bangladesh. *Water*. **10:** 110.

Schachtman, D. P. and R. Munns. (1992). Sodium accumulation in leaves of *Triticum* species that differ in salt tolerance. *Func. Plant Biol.* **19**: 331–340.

Shabana, A.I.,Mostafa, D.M. and El-Hady, M.A.M. (2020). Effect of biological, chemical and physical agents on common bean plant under saline conditions. *J. Plant Prod.* **11:** 609–616.

Shackleton, C. M., W. Pasquini and A. W. Drescher. (2009). African Indigenous Vegetables in Urban Agriculture. 1st edition published Earthscan, UK and USA.

Shannon, M. C., and C. L. Noble. (1990). Genetic approaches for the developing economic salt-tolerant crops. In "Agricultural Salinity Assessment and Management" (K. K. Tanji, Ed.), ASCE Manuals and Reports on Engineering Practice. ACSE, New York. pp. 161-185.

Shannon, M. C. (1997). Adaptation of plants to salinity. *Adv. Agron*. **60**: 75–120.

Shiringani, R. P. (2007). Effects of planting date and location on phenology, yield and yield components among selected Cowpea varieties. An M.Sc. Mini-Dissertation Submitted to the School of Agricultural and Environmental Sciences, Faculty of Science and Agriculture University of Limpopo, South Africa.

Siddiqui H., Hayat S. and Bajguz A. (2018). Regulation of photosynthesis by brassinosteroids in plants. *Acta Physiol Plant.* **40**(3):59.

Singh, B. B., Y. Mai-Kodomi and T. Terao. (1997). A simple screening method for drought tolerance in cowpea. *Indian J. of Gen*. **59** (2): 211-220.

Singh, B. B. (2002). Recent genetic studies in cowpea. In: Fatokun CA, Tarawali SA, Singh BB, Kormawa PM, Tamo M(eds) Challenges and Opportunities for Enhancing Sustainable Cowpea Production. Ibadan, Nigeria. *Intl. Inst. Trop. Agric*. **3:**13.

Singh, B. B. (2003). Improving the production and utilization of cowpea as food and fodder. *Field Crops Res*. **84**: 169–150.

Singh, B. B. (2005). Cowpea [*Vigna unguiculata* (L.) *Walp*. **In:** Singh RJ, Jauhar PP (eds) Genetic Resources, Chromosome Engineering and Crop Improvement. Volume 1, CRC Press, Boca Raton, FL, USA. pp. 117–162.

Soliman, M.H., Abdulmajeed, A.M., Alhaithloul, H., Alharbi, B.M., El-Esawi, M.A., Hasanuzzaman, M. and Elkelish, A. (2020). Saponin Biopriming Positively Stimulates Antioxidants Defense, Osmolytes Metabolism and Ionic Status to Confer Salt Stress Tolerance in Soybean. *Acta Physiol. Plant*. **42:** 114.

SRDI (2000). Soil salinity in Bangladesh. Soil Resources Development Institute. Ministry of Agriculture, Dhaka-1215. pp. 40-42.

SRDI (2010). Saline soils of Bangladesh. SRMAF project, Ministry of Agriculture, Govt. Peoples Republic of Bangladesh. pp. 35.

Summerfield, R. J., P. A.Huxley, P. J. Dart and A. P. Hughes. (1976). Some effects of environmental stress on seed yield of cowpea (*Vigna unguiculata* (L.) *walp.*). *Prima Plant and Soil*. **44** (3): 527-546.

Summerfield, R. J., P. A. Huxley and W. Steel. (1974). Cowpea (*Vigna unguiculata* (L.) *Walp*.). *Field Crop Abst*. **27**: 301-312.

Sun, J., Dai, S., Wang, R., Chen, S., Li, N., Zhou, X., Lu, C.,Shen, X., Zheng, X. and Hu, Z. (2009). Calcium mediates root K+/Na+ homeostasis in poplar species differing in salt tolerance. *Tree Physiol.* **29:** 1175–1186.

Taffouo, V. D., J. K. Kouamou, M. T. Ngalangue, J. R.Ndongo D. D., Siegfried, A. N.,Ndjeudji and A. Akoa. (2009a). Growth, ionic distribution and yield response of some cowpea cultivars grown under salt stress. African Crop Science Conference Proceedings, Uganda. **9:** 151 – 155.

Taffouo, V. D., J. K. Kouamou, M. T. Ngalangue, A. N. Ndjeudji and A. Akoa. (2009b). Effects of Salinity Stress on Growth, Ions Partitioning and Yield of Some Cowpea (*Vigna unguiculata* L. *Walp*.) Cultivars. *Int. J. of Bot*. **5**: 135-143.

Taffouo, V. D., L. Meguekam, A. Amougou, A. Ourry. (2010). Salt stress effect on germination, plant growth and accumulation of metabolites in five leguminous plants. *J. Agric. Sci. Technol.* **4**(2): 27-33.

Tanji, K.K. (1990). Nature and extent of agricultural salinity. **In:** Agricultural Salinity Assessment and Management, Manuals and Reports on Engineering Practices No.71. K.K. Tanji (Ed.). American Society of Civil Engineers, New York. pp. 1-17.

Tarawali, SA, B. B. Singh, SC. Gupta, R. Tabo, F. Harris. (2002). Cowpea as a key factor for a new approach to integrated crop–livestock systems research in the dry savannas of West Africa. **In:** Fatokun CA, Tarawali SA, Singh BB, Kormawa PM, M Tamo (eds) Challenges and Opportunities for Enhancing Sustainable Cowpea Production. Intl Inst Tropical Agric, Ibadan, Nigeria. pp. 233–251.

Tavakkoli E., Rengasamy P. and McDonald G.K. (2010). High concen-trations of Na+ and Cl– ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress. *J. of Exp. Botan.* **61:** 4449–4459.

Tester M. and Davenport R. (2003). Na^+ tolerance and Na^+ transport in higher plants. *Annals Botany*. **91** (5). 503-527.

Tian N., Wang J. and Xu Z. (2011). Overexpression of Na^+/H^+ antiporter gene AtNHX1 from *Arabidopsis thaliana* improves the salt tolerance of kiwifruit (*Actinidia deliciosa*). *South African J. Bot*. 77: 160-169

.

Timko, M. P., and B. B. Singh. (2008). Cowpea, a Multifunctional Legume. Pp. 227-258. **In:** P.H. Moore, R. Ming (eds.). Genomics of Tropical Crop Plants. Academic Press, Springer Verlag, Berlin.

Tindall, H.D. (1983). Vegetables in the Tropics. Macmillan Press, London.

Turan, M.A., Turkmen, N. and Taban, N. (2007). Effect of NaCl on stomatal resistance and proline, chlorophyll, Na, Cl and K concentrations of Lentil Plants. *J. Agron.* **6**: 378-381.

UN (United Nations). (2008). World population prospects: The 2008 Revision. United Nations Population Division. UK. pp. 313–325. Available online at: http://esa.un.org/UNPP/.

Ünlükara, A., Cemek, B., Karaman, S. and Ersahin, S. (2008): Response of lettuce (*Lactuca sativa var. crispa*) to salinity of irrigation water. *New Zealand J. of Crop and Hort. Sci.* **36:** 263–271.

Uppsala. (1989). Flora of Ethiopia, *Pittosporaceace* to *Araliaceace*. A.A and Asmara. *Ethiopia.* **3:** 174.

Vaillancourt, R.E. and Weeden, N.F. (1992). Chloroplast DNA polymorphism suggests a Nigerian center of domestication for the cowpea, *Vigna unguiculata* (Leguminosae). *Am. J. Bot.* **79**: 1194–1199.

Vavilov. (1951). The origin, variation, immunity and breeding cultivated plants. *Chronica Botanica*. **13**:1-364.

Verdcourt, B. (1970). Studies in Leguminosae-Papilionoideae for the flora of tropical East Africa. IV. *Kew Bull*. **24**: 507-569.

Volkamar, K. M., Hu, Y. and Steppuhn, H. (1998). Physiological responses of plants to salinity: a review. *Canadian J. of Plant Sci.* **78**:19-27.

Wahid, A., Hameed, M., and Rasul, E. (2004). Salt injury symptom, changes in nutrient and pigment composition and yield characteristics of mung bean. *Int. J. Agric. Biol.* **6**:1143-1152.

Waisal, Y. (1972). Biology of Halophytes. Aca. Press, New York. Pp. l-14.

Wakeel, A., Farooq, M., Qadir, M. and Schubert, S. (2011). Potassium substitution by sodium in plants. *Crit. Rev. Plant Sci*. **30:** 401–413.

Wang, W., Vinocur, B. and Altman, A. (2003). Plant responses to drought, salinity and extreme temperature: towards genetic engineering for stress tolerance. *Planta*. **218**: 1-14.

Wikipedia. (2010). Soil Salinity. Available: http://en.wikipedia.org/wiki/Soil_salinity/ Dec.

Yamaguchi, T., and Blumwald, E. (2005). Developing salt-tolerant crop plants: challenges and opportunities. *Trends in Plant Sci*. **10**: 615-620.

Yasin, M., Zada R. and Niazi, B.H. (1998). Effectiveness of chemical and biotic methods for reclamation of saline-sodic soil. *Pakistani J. Soil Sci.* **15**: 179-182.

Yousif B.S., Nguyen N.T., Fukuda Y., Hakata H., Okamoto Y., Masa-oka Y. and Saneoka H. (2010): Effect of salinity on growth, mineral composition, photosynthesis and water relations of two vegetable crops; New Zealand spinach (*Tetragonia tetragonioides*) and water spinach (*Ipomoea aquatica*). I*nt. J. of Agric. and Biol.* **12:** 211–216.

Zayed, M.A. and Zeid, I.M. (1997). Effect of water and salt stresses on growth, chlorophyll, mineral ions and organic solute content, and enzyme activity in mung bean seedlings. *Biol. Plant*. **40**: 351–6.

Ziska, L. H., and A. E. Hall. (1981). Effects of salinity on germination, growth and yield of cowpea. *Irrig. Sci.* **3**: 247-257.