MICROPROPAGATION OF SALT TOLERANT EXOTIC POTATO GENOTYPES AND THEIR *IN VITRO* BIOASSAY

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CERTIFICATE

This is to certify that the thesis entitled "MICROPROPAGATION OF SALT TOLERANT EXOTIC POTATO GENOTYPES AND THEIR IN VITRO BIOASSAY" 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 BIOTECHNOLOGY, embodies the result of a piece of bonafide research work carried out by MD. HABIBUR RAHMAN, Registration No. : 09 - 03621, under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma.

I further certify that any help or sources of information as has been availed of during the course of this work has been duly acknowledged & style of the thesis have been approved and recommended for submission.

Dated: June, 2016
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Dedicated to

My

Beloved Parents

ABBREVIATION AND ACRONYMS

BARI	..	Bangladesh Agricultural Research Institute
TCRC	..	Tuber Crops Research Center
DSI	..	Days to shoot initiation
DWDS	..	Days to well-developed shoot
DRI	..	Days to root initiation
DWDR	..	Days to well-developed root
TRN	..	Total root number
PRN	..	primary root number
SRN	..	secondary root number
FWS	..	Fresh weight of shoot
DWS	..	Dry weight of shoot
FWR	..	Fresh weight of root
DWR	..	Dry weight of root
IBA	..	Indole Butyric Acid
NaCl	..	Sodium chloride
mM	..	Mili mole
%	..	Percent
Cm	..	Centimeter
Mm	..	Milimeter
i.e.	..	id test (That is)
mg/L	..	Milligram per litre
MS	..	Murashige and Skoog
CV	..	Co-efficient of Variation
LSD	..	Least Significant Difference
CRD	..	Completely randomized design
DMRT	..	Duncan's Multiple Range Test
ANOVA	..	Analysis of variance
^{o}C	..	Degree Celsius
etc.	..	Etcetera
Conc.	..	Concentration
Ppm.	..	Parts per million
No.	..	Number
Viz.	..	Videlicet (namely)
GDP	..	Gross domestic product

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ABSTRACT

In vitro bioassay of nine exotic potato genotypes namely CIP102, CIP106, CIP111, CIP 117, CIP 120, CIP124, CIP127, CIP 136 and CIP 139 was conducted for salinity tolerance at Tissue Culture Lab, Tuber Crops Research Center (TCRC), Bangladesh Agricultural Research Institute (BARI), Gazipur-1701. Single node and root tip segments of these genotypes were cultured in MS media supplemented with 0.0 (control), 80, 100, 120, 140 and 160 mM NaCl. In case of *in vitro* shoot bioassay, CIP 139 was found as the most salt tolerant with the highest plant height (9.67 cm), number of nodes (9.50), number of leaves (13.60), number of roots (8.00), length of root (6.50 cm), fresh weight of shoot (509.0 mg) and fresh weight of root (205.60 mg) at 160 mM NaCl (14.61 dSm^{-1}). On the other hand, CIP 106 was found as the most salinity sensitive at 120 mM NaCl (10.96 dSm^{-1}) producing minimum plant height (7.17 cm), number of nodes (6.50), number of leaves (12.50), number of roots (9.70), length of roots (5.10 cm), fresh weight of shoot (572.3 mg) and fresh weight of root (244.4 mg) followed by CIP 136, CIP 117 and CIP 111 at same salinity level. However, CIP 127, CIP 102 and CIP 124 genotypes showed very good performance up to 140 mM NaCl (12.78 dSm^{-1}). *In vitro* root bioassay also revealed the highest salinity tolerance of CIP 139 up to 160 mM NaCl, CIP 127 and CIP 102 up to 140 mM NaCl as well as CIP 106 up to 120 mM salinity level. The root tips of experimented potato genotypes were not significantly affected up to 120 mM salinity level in comparison with control where CIP 120 was found as the lowest tolerant up to 120 mM salinity level in MS media. Among the 0.0 (control), 0.1, 0.5, 1.0 and 1.5 mg /L IBA concentrations supplemented with MS media 1.0 mg/ L was found best for rooting with early root initiation (6.57 days) and well development (10.48 days), highest root length (8.63 cm), 2nd highest root number (17.25) and maximum fresh weight of root (332.22 mg). Interestingly, there was no significant differences between 0.5 and 1.0 mg/L IBA for *in vitro* root induction and development in the experimented CIP potato genotypes.

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INTRODUCTION

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INTRODUCTION

Potato (*Solanum tuberosum* L.) belongs to the family Solanaceae introduced in Indian subcontinent in the 16th century through the merchant of Portugal and dispersed in whole region within 200 years cultivated about 180 countries around the world (Liljana et al. 2012). Nutritionally, it is an excellent and cheap source of carbohydrates, antioxidants, vitamins and minerals, macro and micro nutrients, polyphenols, carotenoids, free essential amino acids particularly lysine as well as more high quality protein than other root and tuberous crops (FAO 2014; Anonymous 2016, Khurana and Naik 2003). It is the most important non-cereal food crop and ranks fourth in terms of total global food production after maize, wheat and rice nourishing more than one billion people around the world (FAO 2016). Potato is commonly known as Alu in Bangladesh and has great demand throughout the year as important vegetable crops. Over the last two decades Bangladesh has emerged as one of the leading potato producing country in the world, ranking 7th position in the world but 3rd in the Asia (FAOSTAT 2016). Progressively high yielding varieties of potato have been cultivated in Bangladesh since 1960 and became popular day by day (Kundu et al. 2013). At present, potato is the 3rd most important cash crop after rice and wheat in Bangladesh (TCRC 2014-15). But in terms of total crop production, it ranks 2nd position after rice and 1st position (70.97 %) among edible vegetables (BBS 2015-16). In Bangladesh, its production is concentrated during the month of January to March covering an area of about 4,75,488 hectares (3.13 % of total cultivated area) having production of 94,74,098 metric tons (BBS 2015-16). Two types of potato cultivars viz. local or indigenous (14.51 %) and high yielding varieties (85.49 %) with an average yield potentially of 11.57 t/ha and 21.34 t/ha, respectively are cultivated in Bangladesh. But, national average yield of potato is still low (19.93 t/ha) compare to developed countries (BBS 2015-16). 21st century has been marked as climate change, environmental pollution and increased salinization of soil and water. Besides, increasing human population and reduction in land available for cultivation are two major threats for global agricultural sustainability (Shahbaz and Ashraf 2013). To face these challenges, significant increase in yields of major crop such as rice, wheat, potato and maize is required to fulfill the food requirements for the projected population by 2050 (Godfray et al. 2010). But global food production for more than 7.5 billion people have been facing constraints due to several factors. Among of them, salinity is one of the principle abiotic stresses for major reduction in cultivable land area, crop productivity and quality around the world especially in arid and semiarid regions (Munns 2005; Witzel et al. 2009). Over the time, it has become a recurrent problem in world agriculture (McWilliam 1996) where 20-25 % of the world's cultivated land (Khenifi et al. 2011; UNU-INWEH 2014) and 33% of irrigated agricultural lands (Shrivastava and Kumar 2015) are affected by different degrees of salinity causing

global economic losses of about US $ 27.3 billion per year (UNU-INWEH 2014). Since Bangladesh belongs to one of the seaside countries, the adverse impact of salinity is also significant here. The coastal areas of Bangladesh cover some 32% of the country in 19 districts (Mahmuduzzaman et al. 2014; World Bank 2014) having about 2.86 million hectares of coastal and off-shore lands (SRDI 2010). Bangladesh is an agriculture-based country having 30 % of her cultivable land located in coastal areas (Parveen et al. 2014). Gross and net-cropped areas in the coastal zone of Bangladesh are about 1,44,085 and 83,416 hectare, respectively (Islam et al. 2004). Out of 1.689 million hectares of coastal land 1.056 million hectares are affected by various degrees of soil salinity where 0.328, 0.274, 0.189, 0.161 and 0.101 million hectares of land are affected by very slight (S_1), slight (S_2), moderate (S_3), strong (S_4) and very strong salinity (S_5) respectively which reduces agricultural productivity remarkably (SRDI 2010). Tidal flooding during the wet season, direct inundation by storm surges and movement of saline ground and surface water during the dry season are the main reasons for salinity in that areas (Haque 2006; Dasgupta et al. 2014a). Saline affected land in our country had been increased 0.833 to 1.056 million hectares during 1973 to 2009 with an average increase of 26.7 % (SRDI 2010) causing economic loss of about 10.5 % in Barisal region and 7.5 % in Chittagong region for HYV rice production (Dasgupta et al. 2014b). Due to high soil and water salinity cropping intensity also reduced considerably (SRDI 2010) posing a potential threat to 15.6 % yield loss of HYV rice in coastal areas before 2050 (Dasgupta et al. 2014b). Besides, most of the vegetable crops are said to be very sensitive to saline condition and their yield decrease from 6-19% with each unit increase in salinity (SRDI 2010). Moreover, salinity and drought are interlinked factors which occur often simultaneously affecting almost all biochemical functions of plant (Solh and Ginkel 2014; Das et al. 2013). As a result, the potential impact of salinity has become a major concern for food security in Bangladesh to nourish her increasing vast population as well as a critical issue for adaptation to climate change (Dasgupta et al. 2015). However, growing salt-tolerant crops may be a solution as they can tolerate a certain amount of salinity without compromising production or quality. Potato has been reported as a moderately salinity sensitive crop, particularly in the early growth stages by Katerji et al. (2003), Shaterian et al. (2005) and Munira et al. (2015). Therefore, salt tolerant potato cultivars can be chosen for this purpose as we can get 7-8 times higher yield (t/h) and 5-6 times higher profit than the rice and wheat cultivation from the same amount of land in Bangladesh (Haque et al. 2007). Besides, easy production system, diversified use from dining table to junk restaurant and smart processing for industrial propose have further increased the importance of this starch rich vegetable crop in Bangladesh. In addition, export potential of potato is also very bright. According to Bangladesh Potato Export Association (BPEA 2016) 40,500 tons of potato has been exported to different countries in 2016 and it is expected that by 2030 potato will be one of the major export goods after garments, leather and frozen fish in Bangladesh. But for the saline susceptibility of HYVs and low yield potentiality, poor seed quality as well as disease susceptibility of most of the local cultivars available in our country are not suitable

economically for their cultivation in saline soil (Khatun et al. 2011; Rahman et al. 2013; Uddin et al. 2011). However, some of the high yielding exotic potato cultivars viz. CIP 102, CIP 106, CIP 111, CIP 117, CIP 120, CIP 127, CIP 136 and CIP 139 collected from International Potato Center (CIP), Peru have been reported as salt tolerant by Tuber Crops Research Centre (TCRC) of Bangladesh Agricultural Research Institute (BARI). Mass propagation of these salt tolerant CIP potato cultivars through tissue culture technique for commercial cultivation can play a vital role in seed tuber production as well as ensuring food security in Bangladesh. But before that they should be screened or assessed against salt stress. Assessment of salinity tolerance in the field is seasonally constrained, affected by climate, costly, labor intensive and may lack reliability due to combined salinity and water stress problems (Murshed et al. 2015). Besides, variable salt composition and uneven distribution in the field may also tend to confound field screening (Shannon 1984). Therefore, *in vitro* evaluations of salt stress effects on potato genotypes have been recently proposed as alternatives to field experiment (Rahman et al. 2008; Sajid and Aftab 2014). Moreover, understanding a correlation among salt stress responses of potato in field and *in vitro* condition is needed for potato production in south belt of Bangladesh. But unfortunately, there are very few reports available on salt tolerant potato varieties, their micropropagation and *in vitro* bioassay study in Bangladesh. Therefore, the present study was undertaken with the following aims-

1. To study the *in vitro* regeneration efficiency of CIP Potato genotypes under salt stress condition.
2. To identify the most effective potato genotype(s) for saline belt areas of Bangladesh.
3. To develop a suitable protocol for *in vitro* shoot and root bioassay of salt tolerant CIP Potato genotypes.
4. To develop a suitable protocol for the micropropagation of salt tolerant CIP Potato genotypes.

CHAPTER II
REVIEW OF LITERATURE

CHAPTER II
REVIEW OF LITERATURE

Agriculture plays an important role in the economy of Bangladesh contributing about 15 % of the country's GDP and around 43 % of total labour force are still involved in this sector somehow (BBS 2015-16). Potato is the second most important staple crops after rice in Bangladesh covering 54.24 % of total cultivated area under vegetable production (BBS 2015-16). Due to climate change about 53% of the coastal areas including 8 agro-ecological zones (AEZ) of 13 districts of Bangladesh are affected by different degrees of salinity pg. 19(Russel et al. 2013; Uddin et al. 2011) with ranges from 3.63 to 27.67 (dSm^{-1}) (Akhter et al. 2008) posing a critical environmental constraint to crop productivity (Mahmuduzzaman et al. 2014). Such as, as of now only about 8,992 hectares of land has been brought under potato cultivation in coastal areas of Barisal Division with production of 1,73,008 metric tons (BBS 2015-16). Cultivation of salt-tolerant crops is one of the most important strategies to solve the problem of salinity (Manchanda and Garg 2008). Plant tissue culture techniques in assistance with conventional breeding have become latest approaches for rapid propagation and evaluation of potato cultivars against salt stress (Byun et al. 2007) as well as making them tolerant against environmental stresses especially for salt stress (Rahman et al. 2008). The study of plant salt tolerance to identify crop sensitivity seems to be a fruitful and short time approach (Zhu 2007). Therefore, the present study was undertaken to provide efficient information as much as possible on the maximum salinity tolerance of different salt tolerant CIP potato cultivars against NaCl stress and their propagation efficiency using modified MS media composition under *in vitro* condition. But unfortunately, there are few research available on micropropagation of salt tolerant potato varieties in Bangladesh. However, the literatures which are most relevant to the present study are reviewed and cited here under the following headings-

2.1 Concept of plant tissue culture

Tissue culture technique is the back bone of plant biotechnology. Plant tissue culture is the collection of techniques used to regenerate or maintain plant cells, tissues or organs under aseptic and controlled environmental condition in an artificial and well defined liquid or semisolid nutrient medium. Plant tissue culture largely depends on the four fundamental abilities i.e. totipotency, dedifferentiation, competency and plasticity of plant. Among the different cultural media, MS media (Murashige and skoog 1962) supplemented with or without different concentration of hormones or other elements have been used extensively since 1962 in plant tissue culture. Gottlieb Haberlandt (1902), an Austrian botanist, is recognized as the father of plant tissue culture. On the other hand, this technique was first introduced in Bangladesh with jute in late 1970s in the department of Botany at Dhaka

University and with potato at Tuber Crops Research Centre (TCRC) of Bangladesh Agricultural Research Institute (BARI) since 1983.

2.2 Prospect of potato tissue culture in Bangladesh

Traditionally potato is propagated by seed tuber which is most expensive input covering at least 35-50% of total cost of potato production and every year about 7.5-9 lakh ton seed tubers are needed in Bangladesh where govt. and private sectors can contribute only about 10-15 % and the rest 85- 90 % are covered by the farmer (Begum et al. 2013). Moreover, these may be produced by the farmers or collected any sources having no definite standard with accumulation of pathogen, physiological decline or low multiplication rates (Haque et al. 2012). Under field condition overall 10-15 potato tuber can be produced from a single seed tuber of potato in a year. On the other hand, tissue culture techniques can provide about 1 lakh true to type, disease and virus free plantlets at the same time which can yield about 50 ton potato after cultivation (Alam 2008). Besides considerable amount of valuable potato stocks that is used as seed (2-2.5 t/h) can be saved for consumption as food (Haque et al. 2012). Therefore, plant tissue culture techniques being suitable for rapid and disease free plantlets production of potato can be used as an efficient and profitable alternative method for potato production in the country (Hossain et al. 2004; Kundu et al. 2012).

2.3 Explants for *in vitro* propagation of potato

Different parts of potato plant viz. stem segment, shoot apex, root tip segment, leaves, buds etc. can be used as explants for *in vitro* propagation of potato. Stem segments having 2-3 nodes from *in vitro* growing plant are routinely used to propagate potato (Wang and Hu 1985). Single-node cuttings are approximately 1-2 cm long with one leaf and one lateral (axillary) bud which are regularly used as explant for *in vitro* shoot bioassay for salinity tolerance (Zang et al. 1997, Khenifi et al. 2011). On the other hand, root tip segments of about 1 cm long of 1-2 weeks old are used as explant in root tip segment bioassay for salinity tolerance (Naik and Widholm 1993, Zhang et al. 1998). In commercial varieties like Diamant and Cardinal nodal segments have been reported best by Borna et al. (2010) and Hussain et al. (2005). While for agrobacterium mediated gene transfer leaf was reported best by khatun et al. (2012). Among the all explants, stem segments responded best in the present study which is in agreement with the Hussain et al. (2005), Khenifi et al. 2011, Naik and Widholm (1993) and Zhang et al. (1998).

2.4 Prospect of potato cultivation for food security and climate change adaptation in Bangladesh

Bangladesh is going to be one of the 'Global Leaders' in potato production despite her very low cultivable land achieving a spectacular 500 % growth which is the highest in the world (BPEA 2016). Bangladesh has been eying a bright prospect of potato for food security and climate change adaptation. Draw-down of water table, global warming and other socio-economic limitations has made farmers looking for climate smart, less water consuming and profitable farming option. Potato might be the best option. Since, cultivation, harvest, and processing of potato are easier compared to other field crops and its cultivation is less water consuming and low labor intensive. Potato might be a safeguard against production failure of cereal crops as more than 70% yield can be harvested if it is harvested after or at 60 days. Potato is less water consuming crop requiring 1 to 3 irrigation compared to boro rice requiring 25-35 times irrigation. Moreover, potato is a soil friendly crop compared to rice, maize and wheat. With no exclusion of existing crop, cultivation area can be expanded more 4 lakh ha land following T-aman-Potato-Boro cropping pattern to increase cropping intensity. Besides, inter cropping of potato with maize and other vegetables (pumpkin, water melon) is becoming popular to the farmers day by day. Export potential of potato is also very bright. Bangladesh started potato export in 1999 with a 126 tons consignment to Malaysia but now, its export jumped to 40,500 tons to different countries (BPEA 2016). If things go right, potato is expected to be one of the major export goods after garments, leather and frozen fish by 2030 (BPEA 2016). High quality starch, alcohol, glucose are produced from potato. If industrial use of potato is promoted by government by setting up industries, especially in two major potato hub viz. Munsiganj and North Bengal, there is a huge opportunity to earn foreign currency. Considering yield and comparative profitability potato surpasses rice, jute and wheat. One hectare potato field give 20-30 tons of potato while rice and wheat give 4.0 tons and 3.0 tons respectively. One hectare rice farming offers TK15-20 thousands profit against Tk.1.0-1.5 lakh gained from potato farming in same area. Research and extension of potato technologies is quite satisfactory in Bangladesh. Tuber Crops Research Centre (TCRC) of Bangladesh Agricultural Research Institute (BARI) has been successful to develop 77 potato varieties so far 2017. In fine it may be concluded that, with respect to production technology, harvesting, processing, marketing, net profit and consumer acceptance potato can be considered as the most promising crop in Bangladesh and will be the best alternative for food security and climate change adaptation in years to come by branding her as a 'country of potato'.

2.5 Coastal Bangladesh and Salinity

Bangladesh is a deltaic plain having 710 km long coastline running parallel to the Bay of Bengal (SRDI 2010). The coastal area of Bangladesh covers 19 districts facing or near the Bay of Bengal, encompassing 148 sub districts and the Exclusive Economic Zone, accounting for 32 % of the land area and 25.7 % of the population of Bangladesh (BBS

2011, World Bank 2014). The 19 districts are Jessore, Narail, Gopalganj, Shariatpur, Chandpur, Satkhira, Khulna, Bagerhat, Pirozpur, Jhalakati, Barguna, Barisal, Patuakhali, Bhola, Lakshmipur, Noakhali, Feni, Chittagong, and Cox's Bazar (World Bank 2014). Due to climate change 12 of the coastal districts, comprising 51 sub districts (covering 50 % of the land area of the coastal zone), already face a combination of cyclone risk, salinity, and tidal water movement above critical levels (World Bank 2014). Today 1.2 million hectares of land (15 % of the country's total arable land) under agriculture is within the coastal embankment system sustaining the livelihoods of more than 37 million people (World Bank 2014). Farmers mostly cultivate low yielding, traditional rice varieties during wet season but most of the land remains fallow in the dry season (January- May) because of soil salinity (SRDI 2010). Salinity generally increases almost linearly from August (post-monsoon) to late May (pre-monsoon) from around 1 ppt (parts of salt per thousand gram of soil or water) in August to 8 ppt or more in April. At the end of May, the salinity level drops sharply because of rainfall and increased upstream flow of freshwater and reaches at minimum level in the wet season, usually during September or early October (World Bank 2014). In a word, crop yields, cropping intensity, production levels and people's quality of livelihood are much lower than that in other parts of the country, which have enjoyed the fruits of modern agriculture technologies (SRDI 2010; Hussain 2008).

2.6 Soil salinization

Presence of excessive soluble salts such as KCl, K_2SO_4, $MgCl_2$, $MgSO_4$, NaCl, and Na_4SO_4 in the soil causes soil salinization (Lewis 1984). It is induced when surface soil accumulates toxic ions through evaporation of surface irrigation water or by the migration of toxic ions upward in the soil from shallow groundwater resulting secondary salinization (Blaylock et al. 1994). In this present study, salinity stress is mainly equated with NaCl stress as it is one of the dominant salt components (70 %) of the salinity affected land areas (Lewis 1984). Soil salinity level is described as EC (dSm^{-1}), while *in vitro* level is expressed as mM, %, mg l^{-1} or ppm, which are inter-convertible but not readily converted to dSm^{-1}. An EC of 1 dSm^{-1} is approximately 11 mM NaCl or 640 mg NaCl /L (Lewis 1984).

2.7 Plant responses to soil salinity

Generally, plants are stressed in three ways in saline soils a) low water potential of the root medium leads water deficit b) the toxic effects of the Na^+ and Cl^- and C) nutrient imbalance by depression in uptake and/or shoot transport (Marschner 1995). On the other hand, Munns et al. (1995) cited a two-phase response i.e. osmotic and specific ion effects on plants due to salinity stresses where osmotic stress occurs first and induces an equal growth reduction in both salt sensitive and tolerant plants. Then specific ion stress leads to the build-up of toxic ion levels in salt-sensitive but not tolerant plants because salt

sensitive plants are less capable of excluding or compartmentalizing salts from plant cells. Halophytes can survive on saline soils by a) excluding the salts from their roots and/or secreting them through specialized salt glands on the leaf surfaces; b) compartmentalizing the salts they take up into cell vacuoles (Ashraf 1994). Under saline conditions these plant usually develop succulent leaves with thick cuticles. Serrano and Gazella (1994) reported that under low soil osmotic potential cells dehydration and loss of turgor occur in glycophyte like potato which promote the plant to take up Na^+ and Cl^- from the soil or to produce different osmolytes such as glycerol, sucrose, pralines in the cytoplasm of cells to resist the external osmotic pressure, reduce enzyme activities and inhibit other ion uptake e.g. K^+ and Ca^{+2} necessary for normal plant growth.

2.8 Effect of salinity on plant

Salinity limits the yield of crops by affecting the metabolism of plants and causes important modification in different biochemical and molecular processes (Allakhverdiev et al. 2000; Zhu 2007). Rate of photosynthesis and respiration in crop plants is severely interfered causing reduced plant growth and low productivity at high salinity level (Silva et al. 2001; Zhang et al. 2005; Fidalgo 2004). Higher level of salinity disrupts plant roots making water deficiency, nutrients imbalance by altering uptake and transport, ionic stress by higher Na^+ and Cl^- accumulation, cell membrane ineffectiveness and interfering cellular processes like cell division and genotoxicity resulting in reduced plant growth, development and yield (Munns 2005). Romero-Aranda and Syvertsen (1996) found that accumulation of Na^+ and Cl^- in the leaves caused stomatal closure and reduction of total chlorophyll content in leaves which ultimately limit the photosynthesis of plant.

2.9 Bioassay of potato for salinity tolerance

According to Sparague (1973) bioassay refers to a test in which the quantity or strength of material is determined by the reaction of a living organism to it. A plant bioassay for salinity (NaCl) tolerance quantifies the impact of NaCl on the growth process of the tested plant(s) and ranks them either *in vivo* or *in vitro*. As of now, only a small number of potato genotypes has been evaluated for the salinity tolerance under outdoor, greenhouse or *in vitro* conditions. Field trials (Ahmad & Abdullah 1979; Levy 1992; Paliwal and Yadav 1980) and outdoor pot trials (Levy et al. 1988) were primarily focused on NaCl or mixed salt effects on tuber yields. Greenhouse pot trials were used to examine salinity tolerance of genotypes under NaCl or mixed salt irrigation solutions and were based on tuber yield (Heuer and Nadler 1995), relative reduction of foliage dry weight (Bilski et al. 1988) or haulm fresh weight (Naik and Widholm 1993) but not for yield. *In vitro* evaluations of NaC1 or mixed salt stress effects on potato genotypes were recently proposed as alternatives to the costly, labor intensive and

sometime problematic field based evaluation (Rahman et al. 2008). For this single node cuttings (Naik and Widholm 1993; Zhang at al. 1993), five node cuttings (Morpurgo 1991), root tip segments or suspension cultures (Naik and Widholm 1993) were used and measured on one or more growth parameters at one or more salinity levels *in vitro*. Morpurgo (1991) found a correlation between root fresh weights of *in vitro* plantlets derived from 5 node cuttings and tuber fresh weights from saline irrigation in field. Naik and Widholm (1993) found another correlation between *in vitro* root tip segments in saline medium and haulm fresh weight in green house pot trials.

2.10 *In vitro* response of potato on shoot length against NaCl stress

Kenelif (2011) found that growth parameters of six cultivars of potato viz. Bartina, Spunta, Cardinal, Desirée, Timate and Fabula were inversely proportional with the NaCl concentration. Plantlets growing in the presence of increasing NaCl concentrations were found in decreased shoot and root length for all potato cultivars in the experiment of Pour et al. (2010). Zaman et al. (2015) found no growth in Asterix, Cardinal, Challenger, Desiree, Hermis, Kroda, Sh-5 and Sante at 80 mM and 100 mM NaCl where maximum plant height (6.5 cm) was found in Kroda at 60 mM NaCl. Sudhersan et al. (2012) reported reduced shoot growth in potato at *in vitro* study due to salt stress by increasing salt concentration in MS media from 750 - 4000 ppm. Aghaei et al. (2009) also reported the reduced shoot length in potato up to 90 mM and 120 mM NaCl in his two separate studies. Zhang and Donnelly (1997) found low growth and development in potato at 75 mM NaCl fortified in MS media. Rahman et al. (2008) found that plantlet growth was not affected by 25 mM NaCl in MS media and generally it was almost similar to control levels whereas 75 and 100 mM NaCl media significantly reduced plantlet growth compared with the control for three potato cultivar viz. Atlanta, Shepody and Shilbilaty.

2.11 *In vitro* response of potato on number of roots against NaCl stress

Number of roots per plant was also badly affected by NaCl stress supplemented in MS media. Zaman et al. (2015) found maximum number of roots (8.3) per plant at control and minimum number of roots (1.9) per plant at 60 mM NaCl. Sudhersan et al. (2012) reported reduced roots plant^{-1} in potato varieties by increasing salt in MS media from 750 - 4000 ppm. Farhatullah et al. (2002) reported that the studied potato varieties failed to develop roots even at 1% NaCl added in MS media. Naik and Widholm (1993) also reported poor root development and inhibited plant growth in potato at and above 100 mM NaCl.

2.12 *In vitro* response of potato on length of roots against NaCl stress

Zaman et al. (2015) found maximum root length (3.6 cm) at control and minimum (0.9 cm) was studied at 60 mM NaCl. Naik and Widholm (1993) described severely reduced root length in all tested potato cultivars by increasing salt treatments up to 75 mM NaCl. The investigation of Rahman et al. (2008) showed reduced plant root length at 75 and 100 mM NaCl. Sudhersen et al. (2012) also observed reduced rooting in potato due to NaCl addition in MS media from 750 - 4000 ppm. Farhatullah et al. (2002) reported that salt stress had the diverse effect on the rooting and root growth in potato. Pour et al. (2010) reported decreased root length of potato growing in the increasing NaCl concentrations.

2.13 *In vitro* effect of NaCl stress on leaf and node number of potato

Zaman et al. (2015) found maximum number of nodes plant^{-1} (8.8) up to 60 mM NaCl in Kroda where Cardinal and Desiree produced 5.6 and 5.2 nodes plant^{-1} respectively at same salinity level. Aghaei et al. (2009) reported white Desiree potato as moderately tolerant to salt stress and all tested potato varieties showed overall stunted growth due to salt stress. The inter-nodes plant^{-1} in potato was reduced at 75 mM NaCl in an *in vitro* study performed by Mahmoud et al. (2009). Mahmoud et al. (2009) found lower potato vigor at salinity level greater than 50 mM NaCl. Zhang et al. (2005), Sanchez et al. (2003) and Silva et al. (2001) found late tuberization, mal development of leaves, slow rate of tuber filling and small sized potatoes at and above 80 mM NaCl. Soil affected by 50 mM NaCl can reduce 50% growth hand yield of potato plants (Sayari et al. 2005).

2.14 *In vitro* response of potato on fresh weight of shoot against NaCl stress

Zaman et al. (2015) found maximum shoot weight plant^{-1} (0.166 g) in Kroda up to 60 mM NaCl. Rahman et al. (2008) reported reduced shoot mass of potato varieties Shepody, Atlanta and Shilbilaty at 75 mM and 100 mM NaCl in MS media. A reduced fresh shoot weight plant^{-1} and dry shoot weight plant^{-1} was revealed in different potato varieties at 90 - 120 mM NaCl by Aghaei et al. (2009). The findings of Pour et al. (2010) also found fresh shoot weight reduction in the tested potato cultivars by increasing NaCl level from 25 to 100 mM. An adverse effect on fresh shoot weight of *in vitro* potato was envisaged by Farhatullah et al. (2002). Similar findings were furnished by Askari et al. (2012) who measured reduced fresh shoot weight in potato cultivar Agria by increasing NaCl in MS media from 50 to 150 mM.

2.15 *In vitro* effect of NaCl stress on fresh weight of root of potato

With the increase level of NaCl conc. in culture media, the fresh weight of root of tested cultivars was found significantly decreased in the study of Levy et al. (1988), Zhang et al. (1993). The study of Naik and Widholm (1993) revealed limited rate of root weight plant^{-1} due to increased level of NaCl in MS media. Significantly reduced root mass was reported in potato at 75 mM and 100 mM NaCl in MS media by Rahman et al. (2008). Askari et al. (2012) also reported severe loss of root weight in potato cv. Agria by increasing NaCl stress in MS media from 50 to 150 mM.

2.16 Effect of different concentrations of IBA on root growth and development of potato

Parveen et al. (2014) found 0.5 mg /L IBA best for rooting supplemented with half strength PROP medium for three environmental stress tolerant potato varieties viz. Shadaghuti, Challisha and Zaubilati While Sarker and mostafa (2002) reported 0.1 mg /L IAA best for rooting. Hoque (2010) found combination of 2.0 mg/L kinetin and IAA best for multiple shoot and root regeneration in MS media for five potato verities viz. Diamond, Cardinal, Granulla, Ultra, Dheera and Provinto. Molla et al. (2011) found IBA 0.5 mg / L best for the microprapagation of potato cv. Asterix.

CHAPTER III
MATERIALS AND METHODS

CHAPTER III
MATERIALS AND METHODS

3.1 Time and location of the experiment

Experiments were conducted at the Tissue Culture Lab, Tuber Crops Research Centre (TCRC), Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur-1701 during the period of April 2015 to January 2016. Three experiments were conducted during the entire period of time to fulfill the objectives of the present study. The experiments were-

1. *In vitro* shoot bioassay of different CIP potato genotypes for salinity tolerance.
2. *In vitro* root bioassay of different CIP potato genotypes for salinity tolerance.
3. Effect of different concentrations of IBA on root development of CIP potato genotypes.

The materials and methods used in these investigations are described below under the following heads and sub-heads of this chapter.

3.2 Experimental materials

3.2.1 Plant materials

Nine high yielding salt tolerant exotic potato genotypes viz. CIP102, CIP106, CIP111, CIP 117, CIP 120, CIP124, CIP127, CIP-136 and CIP-139 were collected from the Tuber Crops Research Centre (TCRC), Bangladesh Agricultural Research Institute (BARI), Gazipur-1701 and used as plant materials in the experiments.

3.2.2 Explants

In vitro regenerated potato plantlets of these above mentioned genotypes were used as source material for explants. Stem segments having 2-3 nodes from *in vitro* growing plantlets were used as explants for micropropagation (subculture) and determining IBA effect on root development. On the other hand, stem segments having single node with one leaf and one lateral (axillary) bud and root tip segments of about 1 cm long of 1-2 week old were cut from the micropropagated plantlets and used as explants in shoot and root tip segment bioassay respectively for salinity tolerance.

Plate 1.a. *In vitro* raised potato plantlets

Plate 1.b. Shoot/stem of studied plantlets

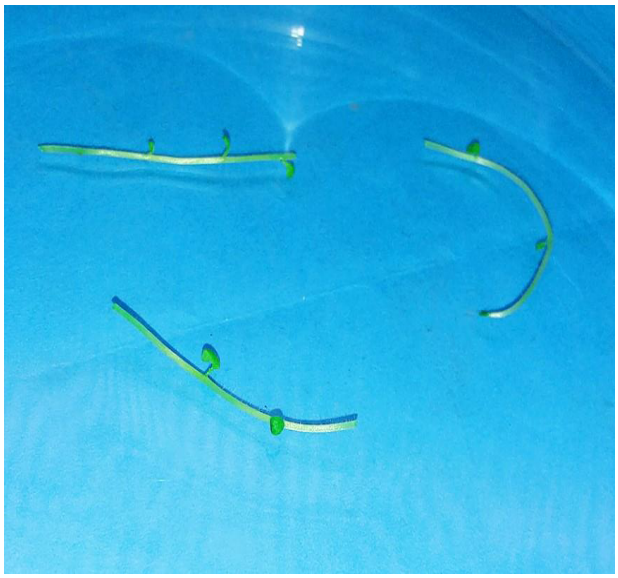


Plate 1.c. Stem segments for micropropagation

Plate 1.d. Root tip of about 1 week old

Plate no. 01 (1.a – 1.d): Plant materials used as explants in different experiments of this study

3.2.3 Instruments and glassware

Metal instruments like forceps, scissors, scalpels, spatulas, aluminum foils etc. were sterilized in an autoclave at a temperature of 121^0 C for 45 minutes at 1.06kg/cm^2 (15 PSI) pressure. Different glassware were viz. pipette, test tube, beaker, conical flask, flat bottom

flask, petri dishes, measuring cylinders (25 ml, 50 ml, 100 ml, 500 ml, 1000 ml) etc. were used for media preparation and other purposes in this experiment.

3.2.4 Culture media

Murashige and Skoog (MS) (1962) medium has been greatly used as a culture medium since 1962 with or without the supplementation of different plant growth regulators. Hormones were added separately to different media according to their requirement. Stock solutions of hormones were prepared before of media preparation and stored at 4 $\pm 1^0$C temperature. The following treatments were applied in this study.

3.2.4.a Treatments used in the *in vitro* shoot and root bioassay of CIP Potato genotypes for salinity tolerance

Treatments	Composition
1. T_0 (Control)	Simple MS Media without NaCl
2. T_1	80 mM NaCl in MS Media (7.31 dsm^{-1})
3. T_2	100 mM NaCl in MS Media (9.13 dsm^{-1})
4. T_3	120 mM NaCl in MS Media (10.96 dsm^{-1})
5. T_4	140 mM NaCl in MS Media (12.78 dsm^{-1})
6. T_5	160 mM NaCl in MS Media (14.61 dsm^{-1})

3.2.4.b Treatments used for the effect of IBA on root development of CIP Potato genotypes

Treatments	Composition
1. T_0 (Control)	Simple MS Media without IBA
2. T_1	0.1 mg IBA / L in MS Media
3. T_2	0.5 mg IBA / L in MS Media
4. T_3	1.0 mg IBA / L in MS Media
5. T_4	1.5 mg IBA / L in MS Media

3.3 Experimental Methods

3.3. 1 Preparation of Murashige and Skoog (MS) stock solution

Chemical composition of MS (Murashige and Skoog, 1962) medium is tabulated in the Appendix I. The preparation of stock solutions of macronutrients, micronutrients, FE-EDTA, vitamins, amino acids and growth regulators is the first step in the preparation of MS media from stock solution. That's why these stock solutions were prepared at first and stored appropriately for use.

Stock solution of growth regulators were prepared separately by dissolving the desired quantity of ingredients in appropriate solvent and the required final volume was made with distilled water for ready use to expedite the preparation of medium wherever needed.

3.3.1. a Macronutrients (MS stock solution, 10X)

The required quantities of major salts (Appendix I) were weighed and dissolved thoroughly in 750 ml of distilled water in a 1000 ml beaker and final volume was made to 1000 ml by further adding water. The strength of the solution was 10 timed of that used in culture medium. The solution was poured into a reagent bottle (Durand, Scotland) and stored in a refrigerator at 4±1° C.

3.3.1. b Micronutrients (MS II stock solution, 100X)

The required quantity of minor salts (Appendix I) were weighed and successively dissolved in 750 ml of distilled water in a 1000 ml beaker. Final volume was made to 1000 ml through adding distilled water. The strength of the solution was 100 times of that used in culture medium. The stock solution was also poured into a clean reagent bottle (Durand, Scotland) and store in a refrigerator at 4±1°C.

3.3.1. c Iron EDTA (MS II stock solution, 100X)

The required quantities (Appendix I) of Na_2 EDTA (Ethylene di-amine tetra acetic acid, di-sodium salt) and $FeSO_4.7H_2O$ were weighed and dissolved separately in a 500 ml beaker. Then mixed together in a 1000 ml beaker and was heated until yellowish color appears. Final volume was made to 1000 ml through adding distilled water. The strength of the solution was 100 times of that used in culture medium. The stock solution was also poured into an amber color bottle and storage at 4±1°C.

3.3.1. d Organics (MS IV stock solution, 100X)

The required quantities of organic constituents and vitamins except myoinositol (Appendix I) were weighed and dissolved in 750 ml of distilled water in a 1000 ml beaker and made up to 1000 ml by adding distilled water. The strength of the solution was 100 times of that used in culture medium. The stock solution was also poured into a clean reagent bottle (Durand Scotland) and stored in a refrigerator at 4±1°C.

3.3.1. e Myoinositol (MS IV stock solution, 100X)

The solution was made 100 times the final strength of the medium. Required amount of myoinsitol (Appendix I) was dissolved in 250 ml distilled water in a clean beaker until the salt dissolved completely. The final volume was made up to the mark by adding the distilled water. The solution was also filtered and stored in refrigerator at 4±1°C.

3.3.2 Preparation of growth regulators (Hormonal stock solutions)

To prepare the stock solution of IBA, 1 mg powder was placed on a clean weighing boat and dissolved with 0.1 N NaOH solvent. The mixture was then washed off with distilled water and collected in a 100 ml measuring cylinder and was made up to the volume with distilled water. The solution was then poured into a reagent bottle (Durand, Scotland) and stored at 4±1ºC.

The requirement amount of this solution for each treatment was calculated using dilution factor as $V_1S_1=V_2S_2$ where,

V_1= Volume of the stock solution

S_1= Strength of the stock solution

V_2= Volume of the resultant solution

S_2=Strength of the resultant solution

3.3.3 Preparation of other stock solutions

3.3.3. a Preparation of 1N NaOH

To prepare of 1 N NaOH stock solution, 40 g of NaOH pellets were dissolved in 1 L of double distilled water. Prepared solution was stored in a glass bottle and kept in cool and dry place. This solution was used to adjust pH in culture media preparation by increasing the pH meter reading.

3.3.3. b Preparation of 1N HCl

To prepare of 1 N HCl stock solution, 36.5 g of HCl substances were dissolved in 1L double distilled water. Prepared solution was stored in a glass bottle and kept in cool and dry place. This solution was used to adjust pH in culture media preparation by decreasing the pH meter reading.

3.3.3. c Preparation of 70 % ethanol

70 ml 99.9 % ethanol was dissolved poured in a100 ml measuring cylinder. Then 30 ml double distilled water was added to make final volume (100ml) for 70% ethanol and stored in a glass bottle. This solution was made fresh each time before use. It was used for sterilization purpose.

3.4 Preparation of culture media from MS stock solution

To prepare 1 L of culture media the following steps were followed:

Step -1. 500 ml of sterile double distilled water was poured into in a 1000 mL beaker.

Step -2. 100 mL from prepared MS Stock I (10 x), 10 mL from prepared MS Stock II (100X), 10 mL from prepared MS Stock III (100X) and 10 mL from prepared MS Stock IV (100x) were added in the beaker.

Step -3. 30g of sucrose was added and gently stirred to dissolve these ingredients completely with the help of a hot plate magnetic stirrer.

Step -4. Required amount of NaCl, prepared IBA stock or hormonal supplements of different concentration for different media were measured and added to the solution as required and mixed well.

Step - 5. Finally the volume was made up to 1000 mL with addition of sterile double distilled water.

Step - 6. The pH was adjusted at 5.8

Step - 7. 8g agar was added to the mixture and heated for 10 minutes in an electric oven for melting of agar.

Step -8. The media were distributed in test tubes or other culture vessels.

Step -9. Then sterilization of media was done by autoclaving at 1.06kg/cm^2 (15 PSI) pressure with 121^0 C for 20 minutes.

Step -10. Finally the media were stored in culture room for future use.

3.5. Sterilization technique

Aseptic condition is the pre requisite for *in vitro* techniques and for that all instruments, glassware and culture media were sterilized.

3.5.1. Sterilization of culture media

The culture tubes having prepared media were autoclaved at 1.06kg/cm^2 (15 PSI) pressure with 121^0 C for 20 minutes. In case of root bioassay, at first culture media were taken different conical flask and autoclaved at 1.06kg/cm^2 (15 PSI) pressure with 121^0 C for 20 minutes. Then autoclaved media were poured into sterile petri dishes under a Laminar Air Flow Cabinet and were allowed to cool before use. All the test tubes and petri dishes were parafilmed and marked with permanent marker or sticker to indicate specific treatment. After sterilization the media were stored at 21±2^{0}C for several hours to make them ready (semi-solid) for inoculation with explants.

3.5.2. Sterilization of glassware and instruments

All types of glassware and instruments viz. culture vessel, beaker, petri dishes, pipette, plastic caps, test tubes, conical flasks, forceps, scalpels, needles, spatulas, etc. were first rinsed with liquid detergent (Trix) and washed thoroughly with tap water until the detergent

was removed completely. Then they were rinsed with double distilled water. Finally all the glassware, instruments, H_2O, aluminum foil etc. were sterilized for two times in an autoclave at a temperature of 121^0 C for 45 minutes at 1.06kg/cm^2 (15 PSI) pressure.

3.5.3. Sterilization of culture room and transfer area

The culture room was cleaned by washing the floor and walls with a detergent or Lysol (germicide) followed by wiping with 70 % ethyl alcohol. This process of sterilization of culture room is repeated at regular intervals. The transfer area was also sterilized twice a month by 70 % ethyl alcohol. Laminar Air flow Cabinet was usually sterilized by switching on the cabinet and UV light was used for 20 minutes and after sterilization by UV light transfer work was delayed for at least 5 minutes to ensure safe environment. Then the working surface of Laminar Air flow Cabinet was sterilized by wiping with cotton soaked with 70% ethyl alcohol prior to start the transfer work.

3.6. Precaution to ensure aseptic condition

All inoculation and aseptic manipulation were carried out in laminar air flow cabinet. The cabinet was usually switched on half an hour before working with UV light for 20 minutes to kill the germs before use. The instruments like scalpels, forceps, needles etc. were pre sterilized by autoclaving and subsequent sterilization was done by dipping in 70% ethyl alcohol followed by flaming and cooling method inside the laminar air flow cabinet. Hands were also sterilized by wiping with cotton soaked with 70% ethyl alcohol. All glassware and instruments except media were kept inside laminar air flow cabinet to reduce the chances of contamination. Glass plate, distilled water, petri dishes etc. were sterilized in autoclave by following the same method of media sterilization. The neck of the culture vessels were flamed before closing it with the cap. Aseptic conditions were followed in each and every operation to avoid the contamination of cultures.

3.7. Culture method

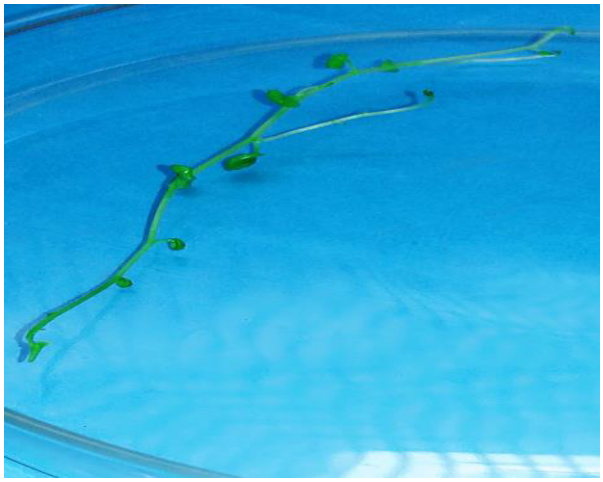
The following culture methods were employed in the present investigation

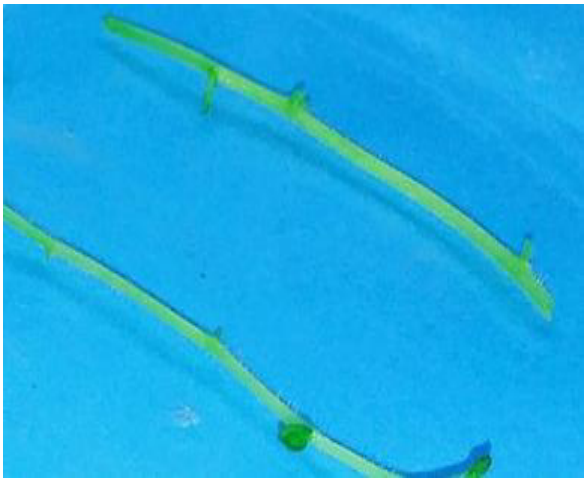
1. Explant culture **2.** Subculture

3.7.1. Explant culture

3.7.1. a. Preparation of explants for subculture

In vitro regenerated potato plantlets (microplants) of the above mentioned genotypes were used as source materials. 3-4 weeks old microplants (8-9 cm in height) were taken out from the test tubes and placed on sterilized petri dishes under laminar air flow cabinet. Leaves and stem segments containing two or three nodes were excised by a sterilized scalpel and forceps.



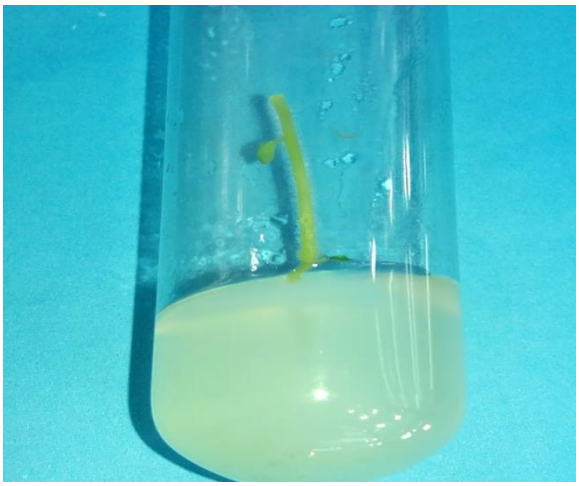


2. a. *In vitro* plant **2. b. Stem segments**

Plate no. 02 (2.a-2.b): Preparation of explants for subculture

3.7.1. b. Inoculation of explants

Stem segments were arranged horizontally on each culture tube having Murashige and Skoog (MS, 1962) medium supplemented with 0.5 mg/L IBA. Inoculation of explants was done in Laminar Air Flow Cabinet. Each culture tube contained 30 ml sterile medium. Explants were placed vertically on medium and mouth of the bottle was quickly flamed and capped tightly. After proper labeling mentioning treatment code, inoculation date etc. culture jars were transferred incubation room.



3.a CIP 127 for subculture **3.b CIP 139 for subculture**

Plate no. 03 (3.a - 3.b): Inoculation of explants for subculture

3.7.1. c. Incubation of culture

The culture jars containing explants were incubated in a growth room with 21 ± 1^{O}C under 2000 lux of fluorescent light with 16/8 hours photoperiod for regeneration. The culture jars were checked daily to note the growth response and contamination.

3.7.2. Sub-culture

Generally when height of the regenerated plantlets reached the top of culture tubes at about 20 - 25 days were sub cultured on respective media.

3.7.2. a. Sub culture of single-node cuttings for *in vitro* shoot bioassay

Single nodes were cut from 18- 22 days old micropropagated plantlets. Nodes were selected from the middle of each plantlet. The shoot apex and base were discarded to achieve the most consistent response to salinity. Each single-node cutting was 1-2 cm long with 1 leaf and 1 axillary bud and individually cultured in a 25 x 150 mm Pyrex glass test tube. Each tube contained 10 ml of MS media supplemented with 0, 80, 100, 120, 140, and 160 mM NaCl.

4.a Stem segments with single node **4.b CIP 139 at 160 mM NaCl**

Plate no. 04 (4.a – 4.b): Sub culture of single-node cuttings for *in vitro* shoot bioassay

Five single-node cuttings of each genotype were used at each salinity level (treatment) and repeated for five times. The cultures were incubated in growth room under cold fluorescent light for 16/8 photoperiod at a temperature of 21 ± 1^0C. The culture jars were checked daily to note the development and contamination. The experiment lasted for 4 weeks and data were collected on different shoot and root parameters such as days to shoot and root initiation, number of leaves per plant, shoot and root length, number of root per plant etc.

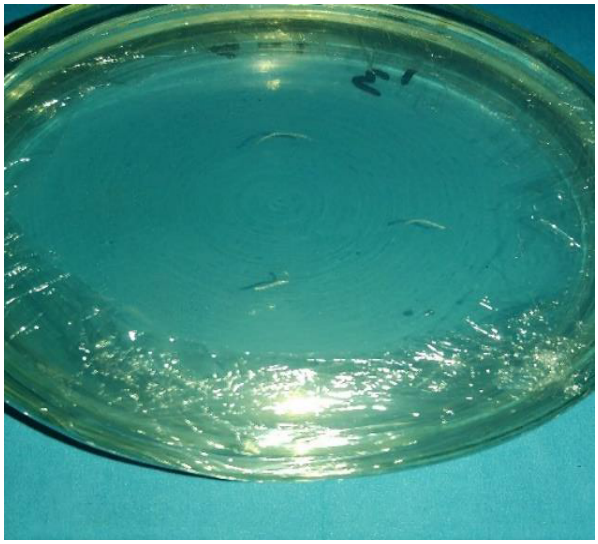
3.7.2.b. Sub culture of root tip segments for *in vitro* root bioassay

One cm long root tip segments were cut directly from 1-2 weeks old growing plants cultured in MS media supplemented with 0.5 mg IBA/L. Then the root tip segments (1 cm long) of five potato genotypes viz. CIP 102, CIP 106, CIP120, CIP127, CIP 139 were placed into 9 cm diameter petri dishes containing 20 ml semi-solid MS medium supplemented with 0, 80,100, 120,140 and160 mM NaCl. Three to four root tip segments were kept in each petri dish for each genotype at each salinity level. Incubation was done in the dark at 21 ± 2^0C. Each experiment lasted 4 weeks and was performed 3 times. The data were collected on root extension (final length minus initial length) and root growth relative to growth in control medium.

5. a One week old root

5. b Root tip segments

5. c Transfer of media in petri dishes

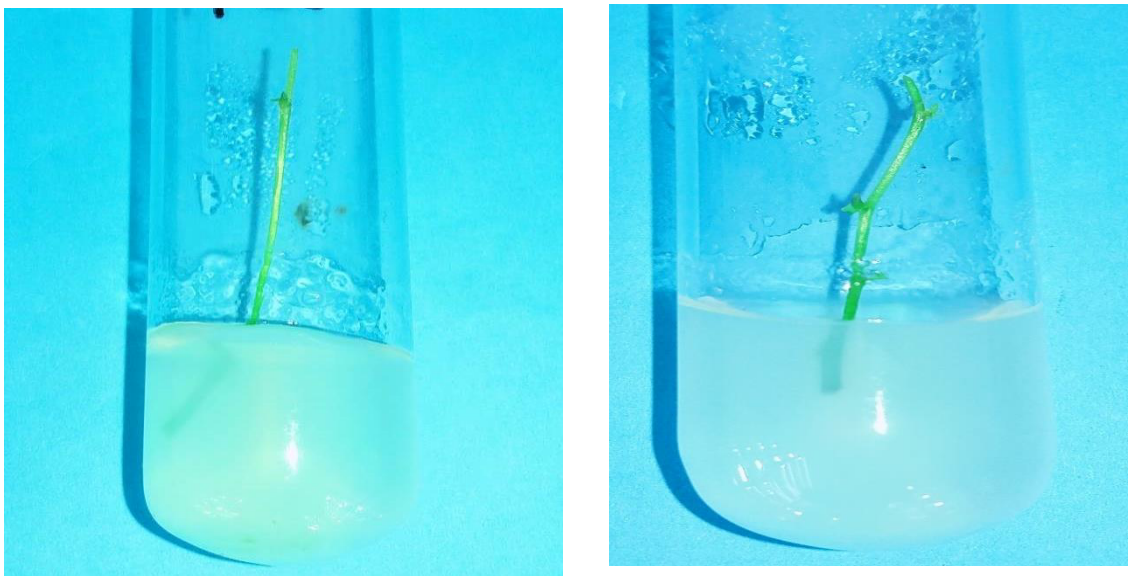


5. d Inoculation of root tip

Plate no. 05 (5.a- 5.d): Sub culture of root tip segments for *in vitro* root bioassay

3.7.2.c. Sub culture for determining IBA effect on root development of CIP Potato genotypes

Stem segments having 2-3 nodes from *in vitro* regenerated potato plantlets were used for determining IBA effect on root development. Leaves were removed by a sterilized scalpel and forceps. Then stem segments were inoculated in media supplemented with different concentration of IBA viz. $\mathbf{T_0}$ = Control; $\mathbf{T_1}$ = 0.1 mg IBA / L; $\mathbf{T_2}$ = 0.5 mg IBA / L; $\mathbf{T_3}$ = 1.0 mg IBA / L; $\mathbf{T_4}$ = 1.5 mg IBA / L



6.a. CIP 117 at 0.1 mg IBA /L **6.b. CIP 127 at 1.0 mg IBA /L**

Plate no. 06 (6.a-6.b): Sub culture for determining IBA effect on root development

3.8. Calculation of Data

3.8. a. Data recorded: Development of plantlets was observed and the following data were recorded for over all experiment:

01. Days required for shoot initiation and well-developed shoot
02. Length of the shoot / Plant height (cm)
03. Number of leaves per explant
04. Number of nodes per explant
05. Fresh and dry weight (mg) of the shoot
06. Days to root initiation and well-developed root
07. Primary and secondary root length (cm)
08. Number of primary, secondary and total roots per plant
09. Fresh and dry weight (mg) of the root
10. Actual (mm) and relative (%) extension / length of root tip segments

3.8. b. Methods of data recording are given below:

01. Days required for shoot and root initiation

The cultures were observed at alternate days starting from 3rd day of inoculation and continued up to 28th day for shoot and root initiation. Any change or development in culture when observed was recorded as days to shoot initiation and any development or outcome of root was recorded as days to root initiation.

02. Days to well-developed shoot and root

Days required for vigorous growth of shoot bearing buds and leaves were considered as days to well-developed shoot. Days required for vigorous growth of root having 1 or 2 secondary roots were considered as days to well-developed root.

03. Length of the shoot / plant height (cm)

The length or height of the plant was measured against a ruler in cm at 28th days after culture. The length from the base of plantlet to the tip of the plantlet was considered as height of the plant. In case of multiple shoot, the length of the tallest plant was considered as plant height and measured in cm.

04. Length of the root (cm)

Root length of the plantlet was measured against a ruler in cm at 28th day after culture. The length from the base of plantlet to the tip of the root was considered as length of the plant. In case of multiple root, the length of the longest root was considered as root length and measured in cm.

05. Number of leaves per explant

The number of leaves of plant was counted at 28th day after culture. In case of single stem plantlet, al the leaves were counted from base to tip of the plantlet as 1 to 15 and in case of multiple stem plantlet, all the leaves counted in plantlet were divided by the total number of stems of the plantlet.

06. Number of nodes per explant

It was counted at 28th day after culture from base to tip of the plantlet as 1 to 15.

07. Number of roots per explant

It was counted at 28th day after culture. All the primary, secondary and tertiary (if available) roots were counted in number as 1 to 8 and sum total of each was divided by the sum total of plantlet for average total root number per plant.

08. Fresh weight of the shoot and root (mg)

The fresh weight of root and shoot were measured in mm by a digital balance at 28th day after culture using following formula:

$$\textbf{Fresh weight of shoot / root (mg)} = \frac{\text{Total weight of shoot / root}}{\text{Total number of shoot / root measured}}$$

09. Dry weight of the shoot and root (mg)

Shoot and root were collected at 28th day after culture and dried in an oven at 30^0 C for 48 hours. Then dry weight of the shoot and root was measured in mg by a digital balance using following formula:

$$\textbf{Dry weight of shoot / root (mg)} = \frac{\text{Total weight of shoot / root}}{\text{Total number of shoot / root measured}}$$

10. Actual extension (length) (cm) and relative growth (%) of root tip segments

Actual extended length of root tip segments was measured against a ruler in cm at 28th day after culture by subtracting initial root length from final root length. Relative root length indicates the extension of root tip segments comparing to control. It is expressed in percentage and calculated by following formula:

$$\textbf{Relative growth of root tip segments (\%)} = \frac{\text{Actual root length (cm)}}{\text{Root length in control (cm)}} \times 100$$

3.9 Experimental design and statistical analysis

All the experiments were conducted under laboratory conditions where all the factors were homozygous. For this reason, experiments were arranged in two factorial (Genotype and Treatment) Completely Randomized Design (CRD) with 5 replications for each treatment. Data for the characters were statistically analyzed by MSTATE-C (1990) software for *in vitro* shoot and root bioassay and Statistix10 software for determining IBA effect on root development. The analysis of variance (ANOVA) for different characters was performed and means were compared by the Duncan's Multiple Range Test (DMRT) for *in vitro* shoot and root bioassay and LSD for determining IBA effect on root development both at 5% probability level.

CHAPTER IV
RESULTS AND DISCUSSIONS

CHAPTER IV
RESULTS AND DISCUSSIONS

Three experiments were conducted under the laboratory condition to study the CIP Potato genotypes against different salinity level and IBA concentrations. In this study, basal MS (Murashige and Skoog, 1962) media supplemented with different concentrations of NaCl viz. **T_0:** Control; **T_1:** 80 mM; **T_2:**100 mM; **T_3:** 120 mM; **T_4:** 140 mM; **T_5:** 160 mM were used for *in vitro* shoot and root bioassay. On the other hand, MS media supplemented with different concentrations of IBA viz. **T_0** = Control; **T_1** = 0.1 mg / L; **T_2** = 0.5 mg / L; **T_3** = 1.0 mg/ L; **T_4** = 1.5 mg/ L were used for determining IBA effect on root growth and development. The results and possible interpretations have been described under the following headings:

Experiment no. 01: *In vitro* shoot bioassay of different CIP Potato genotypes for salinity tolerance

4.1.1 Effect on days required for shoot initiation and well-developed shoot

4.1.1.a Days required for shoot initiation (DSI) and well-developed shoot (DWDS) as influenced by CIP Potato genotypes at *in vitro*

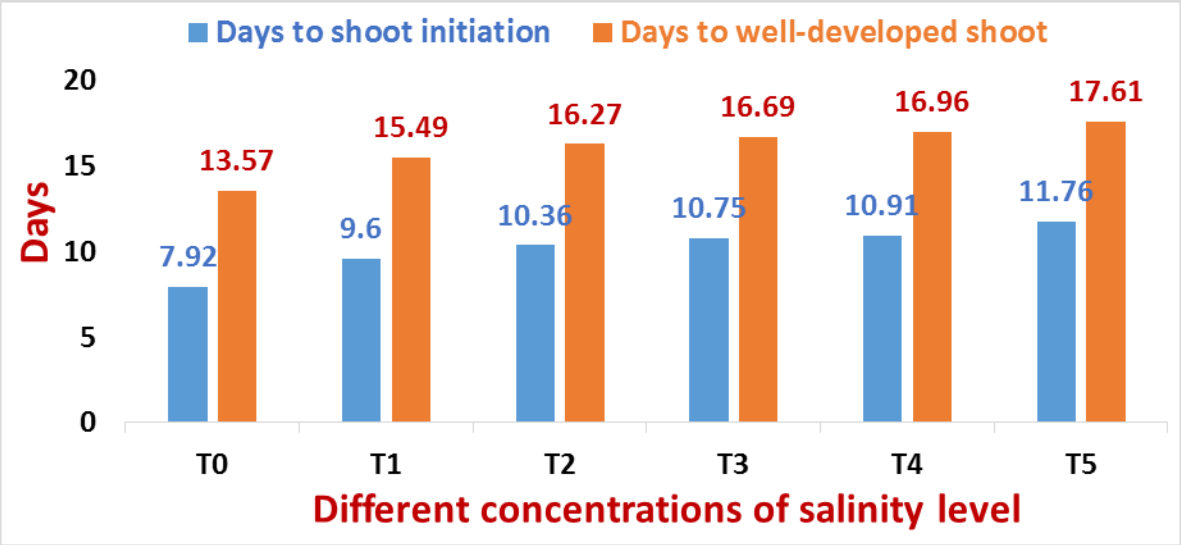
Response on days required for shoot initiation and well-developed shoot of eight potato genotypes varied significantly under salt stress condition **(Table 01)**. Among the eight potato genotypes, CIP 127 needed the lowest time for both of shoot initiation and well-developed shoot with 8.63 and 14.89 days respectively followed by CIP 102, CIP 111, and CIP 117. On the other hand, CIP 124 responded lately for both of shoot initiation and well-developed shoot with 11.35 and 17.30 days respectively followed by CIP 136, CIP 139 and106.

Table no. 01: Days required for shoot initiation and well-developed shoot As influenced by CIP Potato genotypes at *in vitro*

Genotypes	Days to shoot initiation	Days to well-developed shoot
CIP 102	9.57 f	15.24 e
CIP 106	10.54 d	15.93 c
CIP 111	9.91 e	15.62 d
CIP 117	9.91 e	15.81 c
CIP 124	**11.35 a**	**17.30 a**
CIP 127	**8.63 g**	**14.89 f**
CIP 136	11.06 b	17.17 a
CIP 139	10.76 c	16.82 b
CV (%)	2.62	1.60
$LSD_{(0.05)}$	0.176	0.171

4.1.1.b Days required for shoot initiation (DSI) and well-developed shoot (DWDS) as influenced by different concentrations of salinity level

With the increase level of salinity on media both DSI and DWDS were affected by the salinity levels for all genotypes which ranged from 7.92 to 11.76 days for DSI and 13.75 to 17.61 days for DWDS **(Figure 1).** This is strongly in agreement with **Sudhersan et al. (2012)** who reported higher concentrations of salt in the culture media greatly affected the shoot initiation and well development.



Where, T_0 : Control; **T_1 :** 80 mM NaCl; **T_2 :**100 mM NaCl; **T_3 :** 120 mM NaCl; **T_4 :** 140 mM NaCl; **T_5 :** 160 mM NaCl

Figure no. 01: Days required for shoot initiation and well-develope shoot as influenced by different concentrations of salinity level

4.1.1.c Combined effect of genotypes and salinity level on days required for shoot initiation (DSI) and well-developed shoot (DWDS)

There was significant variation in the combined effect of genotypes and different salinity levels on shoot initiation and well-developed shoot where with the increase level of salinity plant response for DSI and DWDS needed more days **(Table 02)**. CIP 127 was found best with earlier response for both DSI and DWDS up to 140 mM salinity level followed by CIP 117 and CIP 111 up to 100 mM salinity level. Among the all interactions, CIP 127 responded earlier (6.37days) at simple MS media and CIP 124 responded lately (12.40 days) at 160 mM salinity level for DSI. Likewise, for well-developed shoot CIP 127 needed the lowest time (11.83 days) at control and CIP 124 needed the highest time (18.47 days) at 160 mM salinity level which was statistically similar with CIP 136 (18.43 days) at same salinity level (**Table 02).**

Table no. 02: Combined effect of genotypes and salinity level on days required for shoot initiation and days required for well-developed shoot

Genotypes	Treatment	Days to shoot initiation		Days to well-developed shoot	
CIP 102	**T_0**	7.47	qr	12.90	o
	T_1	8.17	o	13.60	n
	T_2	10.20	hi	15.60	i
	T_3	10.37	g-i	15.67	i
	T_4	11.20	f	16.83	ef
	T_5	12.03	a-d	16.83	ef
CIP 106	**T_0**	9.63	j-l	14.83	jk
	T_1	9.97	i-k	14.93	jk
	T_2	10.27	g-i	15.57	i
	T_3	10.60	gh	15.93	hi
	T_4	11.37	ef	16.77	ef
	T_5	11.43	ef	16.80	ef
CIP 111	**T_0**	6.53	s	12.97	o
	T_1	9.27	lm	15.63	i
	T_2	9.96	i-k	16.23	gh
	T_3	10.20	hi	16.43	fg
	T_4	10.27	g-i	16.57	e-g
	T_5	11.17	f	17.03	e
CIP 117	**T_0**	7.10	r	12.13	p
	T_1	8.73	n	14.60	kl
	T_2	9.56	kl	15.90	hi
	T_3	11.30	ef	17.50	d
	T_4	11.40	ef	17.70	cd
	T_5	11.40	ef	17.73	cd
CIP 124	**T_0**	9.50	kl	15.90	hi
	T_1	10.50	gh	16.90	ef
	T_2	10.70	g	16.90	ef
	T_3	12.10	a-c	17.87	b-d
	T_4	12.10	a-c	18.07	a-c
	T_5	**12.40**	**a**	**18.47**	**a**
CIP 127	**T_0**	**6.37**	**s**	**11.83**	**p**
	T_1	7.53	p-r	13.57	n
	T_2	8.00	op	14.23	lm
	T_3	9.03	mn	15.13	j
	T_4	9.30	lm	15.60	i
	T_5	11.7	d-f	17.87	b-d
CIP 136	**T_0**	7.87	o-q	13.90	mn
	T_1	10.10	h-j	16.20	gh
	T_2	10.20	hi	16.43	fg
	T_3	11.73	c-e	17.70	cd
	T_4	12.23	a-c	18.27	ab
	T_5	12.33	a	18.43	a
CIP 139	**T_0**	8.00	op	14.07	m
	T_1	10.23	g-i	16.30	gh
	T_2	11.77	b-e	17.93	b-d
	T_3	12.00	a-d	18.10	a-c
	T_4	12.03	a-d	18.13	a-c
	T_5	12.30	ab	18.17	a-c
CV (%)		**2.62**		**1.60**	
LSD $_{(0.05)}$		**0.419**		**0.4195**	

Where, T_0 : Control; **T_1 :** 80 mM NaCl; **T_2 :** 100 mM NaCl; **T_3 :** 120 mM NaCl;**T_4 :** 140 mM NaCl; **T_5 :** 160 mM NaCl

4.1.2 Effect on days required for root initiation and well-developed root

4.1.2.a Days required for root initiation and well-developed root as influenced by CIP Potato genotypes at *in vitro*

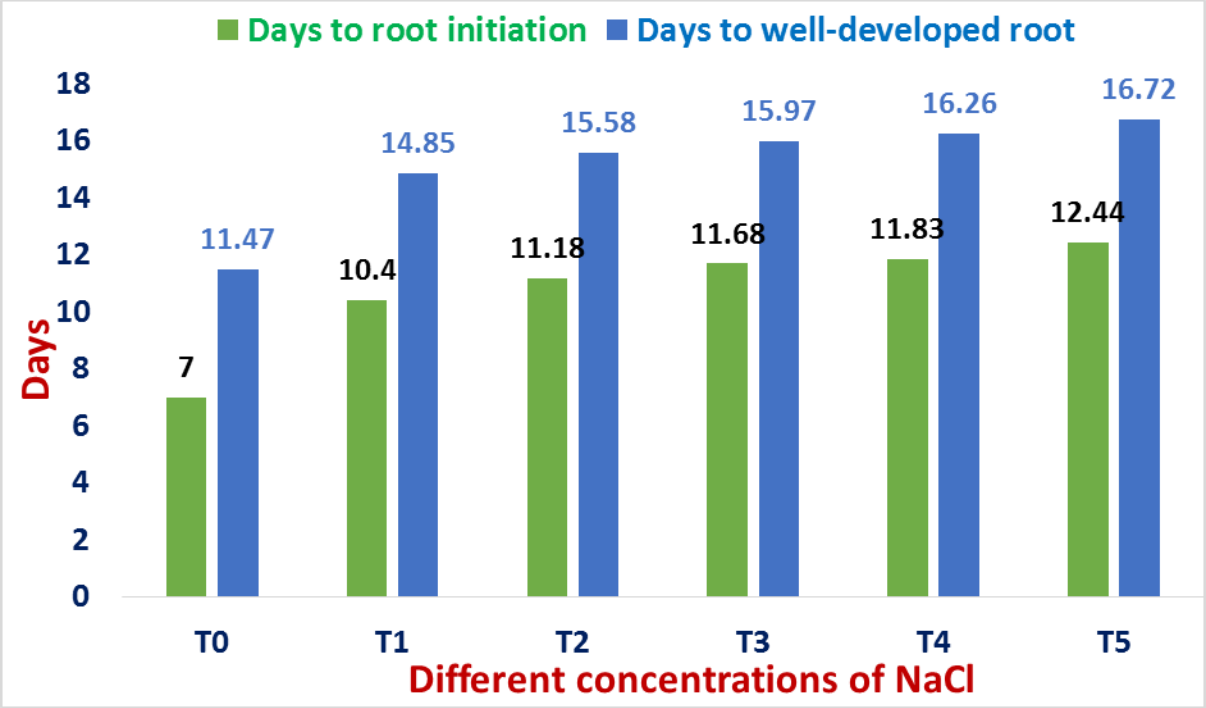
Among the eight potato genotypes, CIP 127 took minimum time (9.55 days) on average genotypic response for root initiation followed by CIP 106, CIP 111, CIP 117 and CIP 124 while maximum time (11.73 days) was needed for CIP 136 followed by CIP 102 and CIP 139 **(Table 03**). Likewise, CIP 127 needed lowest time (14.16 days) and CIP 136 needed highest time (16.43 days) for the well development of their roots.

Table no. 03: Days required for root initiation and well-developed root as influenced by CIP Potato genotypes at *in vitro*

Genotypes	Days to root initiation	Days to well-developed root
CIP 102	11.56 ab	16.20 b
CIP 106	10.06 e	14.26 f
CIP 111	10.22 de	14.57 e1
CIP 117	10.42 d	14.77 d
CIP 124	11.20 c	15.42 c
CIP 127	**9.55 f**	**14.16 f**
CIP 136	**11.73 a**	**16.43 a**
CIP 139	11.31 bc	15.43 c
CV (%)	3.70	3.09
LSD $_{(0.05)}$	0.264	0.098

4.1.2.b Days required for root initiation (DRI) and well-developed root (DWDR) as influenced by different concentrations of salinity level

The results revealed that due to the increased level of salt stress in media both DRI and DWDR were increased gradually from 7.00 to 12.44 days and 11.47 to 16.72 days respectively **(Figure 02)** where all the treatments were statistically significant from one another. This is in agreement with **Khenifi et al. (2011)** who reported that the addition of NaCl to the culture media adversely affected the growth of potato cultivars.



Where, T_0 : Control; **T_1 :** 80 mM NaCl; **T_2 :**100 mM NaCl; **T_3 :** 120 mM NaCl; **T_4 :** 140 mM NaCl; **T_5 :** 160 mM NaCl

Figure no. 02: Days required for root initiation and well-developed root as influenced by different concentrations of salinity level

4.1.2.c Combined effect of genotypes and different salinity level on days required for root initiation (DRI) and well-developed root (DWDR)

Plantlets of studied genotypes showed different response to the root initiation against different degrees of salinity **(Table 04).** Interaction effect of genotypes against different salinity levels revealed that CIP 127 responded better up to 160 mM salinity level for DRI and DWDR followed by CIP 117 up to 120 mM salinity level while CIP 136 responded lately followed by CIP 102, CIP 139 and CIP 124 from 80 mM salinity level to upward.

Among the all interactions, CIP 127 responded earlier for both root initiation (5.47 days) and well-developed root (9.97 days) at salt free MS media and CIP 136 responded lately for both root initiation (14.27 days) and well-developed root (18.03 days) at MS media supplemented with 160 mM salinity level **(Table 04).** However, **Morpurgo (1991)** reported that some cultivar such as cv. Serrana can produced roots in MS liquid medium containing 150 mM NaCl under *in vitro* condition.

Table no. 04: Combined effect of genotypes and salinity level on days required for root initiation and well-developed root

Genotypes	Treatment	Days to root initiation		Days to well-developed root	
CIP 102	T_0	6.60	n	11.20	z
	T_1	10.00	ij	14.53	st
	T_2	12.27	d	16.97	e
	T_3	13.00	bc	17.33	d
	T_4	13.47	b	17.90	a
	T_5	13.60	b	17.60	bc
CIP 106	T_0	7.00	mn	11.67	xy
	T_1	10.00	ij	14.63	rs
	T_2	11.00	gh	15.70	l
	T_3	11.00	gh	15.77	kl
	T_4	11.30	g	16.20	ij
	T_5	11.60	e-g	16.33	hi
CIP 111	T_0	7.60	m	11.80	wx
	T_1	10.00	ij	14.37	t
	T_2	10.57	hi	14.73	q-s
	T_3	10.60	hi	15.23	no
	T_4	11.00	gh	15.57	lm
	T_5	11.57	fg	15.73	l
CIP 117	T_0	6.53	n	10.90	ij
	T_1	9.43	jk	13.83	u
	T_2	9.80	ij	13.87	u
	T_3	11.57	fg	14.83	p-r
	T_4	12.00	d-f	15.59	lm
	T_5	13.00	bc	16.59	jk
CIP 124	T_0	8.60	l	12.67	v
	T_1	10.50	hi	14.73	q-s
	T_2	11.60	e-g	15.50	lm
	T_3	12.00	d-f	16.23	h-j
	T_4	12.23	de	16.47	gh
	T_5	12.27	d	16.90	ef
CIP 127	T_0	**5.46**	**o**	**9.97**	**ij**
	T_1	9.20	kl	13.67	u
	T_2	9.53	jk	14.33	t
	T_3	10.47	hi	14.93	pq
	T_4	10.50	hi	15.03	op
	T_5	10.60	hi	15.33	mn
CIP 136	T_0	7.60	m	12.00	w
	T_1	11.00	gh	15.53	lm
	T_2	11.43	fg	15.77	kl
	T_3	12.37	cd	16.27	hi
	T_4	12.60	cd	16.67	fg
	T_5	**14.27**	**a**	**18.03**	**a**
CIP 139	T_0	6.60	n	11.53	y
	T_1	12.00	d-f	17.07	e
	T_2	12.53	cd	17.33	d
	T_3	12.60	cd	17.40	cd
	T_4	12.60	cd	17.43	cd
	T_5	13.00	bc	17.83	ab
CV (%)		**3.70**		**3.09**	
LSD $_{(0.05)}$		**0.646**		**0.239**	

Where, T_0 : Control; **T_1**: 80 mM NaCl; **T_2**:100 mM NaCl; **T_3**: 120 mM NaCl; **T_4**: 140 mM NaCl; **T_5:** 160 mM NaCl

4.1.3 Effect on plant height (cm) at *in vitro*

4.1.3.a Response of genotypes on plant height (cm) at *in vitro*

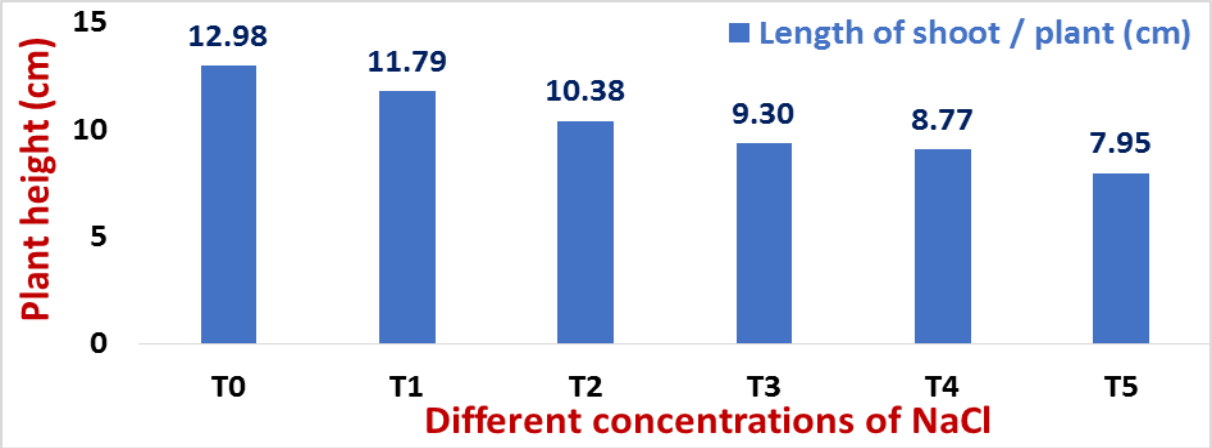
Different genotypes showed significant variations as to the plant height (cm) **(Table 05)** where CIP 102 produced longest shoot length (12.68 cm) followed by CIP 127 (11.23), CIP 139 (10.82) and CIP 124 (10.67). On the other hand, CIP 111 had smallest plant height (8.66 cm) which was statistically similar with CIP 117 (8.68 cm) and CIP 136 (8.99 cm).

Table no. 05: Response of genotypes on plant height at *in vitro*

Genotypes	Plant height (cm)
CIP 102	**12.68 a**
CIP 106	8.99 d
CIP 111	**8.66 e**
CIP 117	8.68 e
CIP 124	10.67 c
CIP 127	11.23 b
CIP 136	8.99 e
CIP 139	10.82 c
CV (%)	5.15
$LSD_{(0.05)}$	0.349

4.1.3.b Effect of salinity level on plant height (cm) at *in vitro*

Plant height of all studied genotypes was gradually reduced with the increase of NaCl concentration in culture media **(figure 03).** Plantlets of tested potato genotypes were found tolerant up to 100 mM NaCl salinity level. Then plant height was reduced to 9.30 cm at 120 mM, 8.77 cm at 140 mM and further to 7.95 cm at 160 mM salinity level. The findings of **Rahman et al. (2008)** also reported reduced plant shoot length at 75 and 100 mM of NaCl in MS media.



Where, T_0: Control; **T_1**: 80 mM NaCl; **T_2**:100 mM NaCl; **T_3**: 120 mM NaCl; **T_4**: 140 mM NaCl; **T_5**: 160 mM NaCl

Figure no. 03: Effect of salinity level on plant height (cm) at *in vitro*

4.1.3.c Combined effect of genotypes and salinity level on plant height (cm) at *in vitro*

Highly significant differences were recorded among the eight potato genotypes for plant height where CIP 139 showed the greatest tolerance up to 160 mM salinity level with 9.67 cm plant height **(Table 06 and Plate 07)** followed by CIP 102 and CIP 124 with 9.45 and 9.43 cm plant height respectively at same salinity level. On the other hand, CIP 106 emerged as most sensitive genotype to salt stress with 8.50 cm tall plantlets up to 100 mM salinity level followed by CIP 136 with 9.50 cm plant height at same salinity level. However, CIP 127, CIP 111 and CIP 117 were found vigorous up to 140 mM salinity level with 9.0 cm, 8.51 cm and 8.10 cm plant height respectively. Among the all interactions, CIP 127 produced maximum plant height (16.83 cm) at simple MS media and CIP 111 produced minimum (4.50 cm) at MS media having 160 mM salinity level **(Table 06). Aghaei et al. (2009)** also reported the reduced plant height in potato up to 90 mM and 120 mM NaCl in his two separate studies.

Table no. 06: Combined effect of genotypes and salinity level on plant height (cm) at *in vitro*

Genotypes	Plant height (cm)					
	T_0	T_1	T_2	T_3	T_4	T_5
CIP 102	16.73 a	13.20 d	12.73 e	12.50 e	11.50 g	**9.45 kl**
CIP 106	15.00 b	11.10 gh	**8.50 m**	7.17 o	6.57 p	5.57 q
CIP 111	11.43 g	10.03 ij	8.90 lm	8.60 lm	**8.51 lm**	4.50 r
CIP 117	11.00 h	9.40 kl	8.50 m	8.17 mn	**8.10 mn**	7.90 n
CIP 124	12.50 e	12.00 f	10.43 ij	10.17 ij	9.50 k	**9.43 kl**
CIP 127	**16.83 a**	13.50 c	10.00 j	9.60 jk	**9.00 l**	8.43 mn
CIP 136	12.60 e	11.00 h	**9.50 k**	7.67 o-n	6.77 p	6.43 p
CIP 139	12.17 ef	11.60 g	10.50 i	10.80 ih	10.20 ij	**9.67 jk**
CV (%)	**5.15**					
LSD (0.05)	**0.856**					

Where, T_0 : Control; **T_1**: 80 mM NaCl; **T_2**:100 mM NaCl; **T_3**: 120 mM NaCl; **T_4**: 140 mM NaCl; **T_5:** 160 mM NaCl

7.a. CIP 139 at 160 mM NaCl

7.b. CIP 102 at 140 mM NaCl

7.c. CIP 124 at 160 mM NaCl

7.d. CIP 127 at 140 mM NaCl

7.e. CIP 111 at 140 mM NaCl

7.f. CIP 117 at 140 mM NaCl

7.g. CIP 136 at 120 mM NaCl

7.h. CIP 106 at 100 mM NaCl

7.i. CIP 127 at control

Plate no. 07 (7.a-7.i): Combined effect of genotypes and salinity level on plant height (cm) at *in vitro*

4.1.4. Effect on number of leaves at *in vitro*

4.1.4. a Response of genotypes on number of leaves per plant at *in vitro*

In respect of leaves number, CIP 139 produced highest leaves number (16.13) on average genotypic response followed by CIP 117, CIP 124 and CIP 127. On the other hand, the lowest number of leaves (11.98) was recorded in CIP 136 followed by CIP 102, CIP 111 and CIP 106 **(Figure 04).**

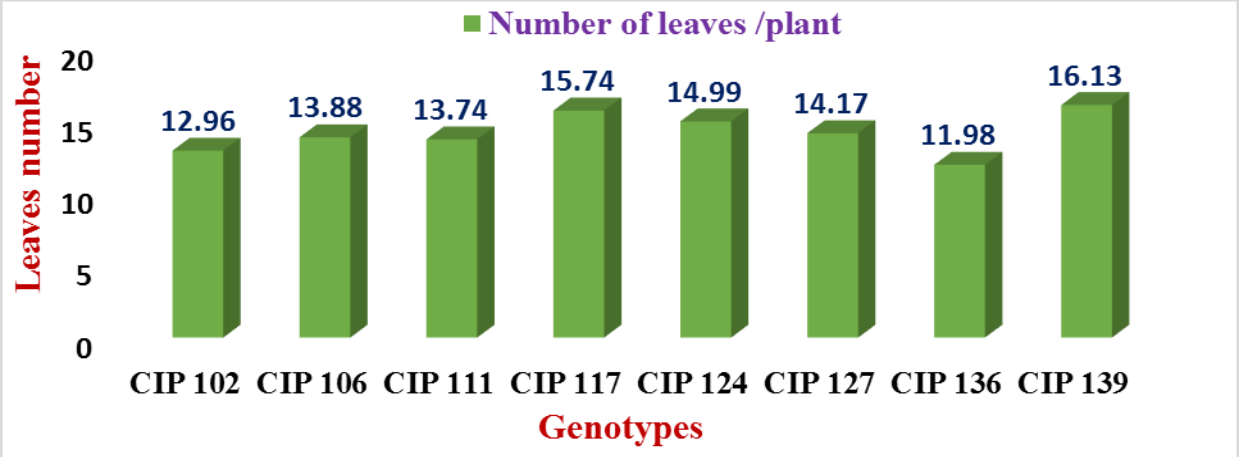


Figure no. 04: Response of genotypes on number of leaves per plant at *in vitro*

4.1.4.b Effect of salinity level on number of leaves per plant at *in vitro*

The study revealed that leaves development was not severely affected by different levels of NaCl in MS media. All the studied genotypes were able to produce enough leaves up to 160 mM salinity level where maximum leaves (15.13) was recorded at MS media free from NaCl stress which was statistically similar with MS media having 80 mM NaCl (14.95). On the other hand, minimum number of leaves (11.78) was found at 160 mM NaCl stress which was statistically significant from rest of the treatments **(Table 07).**

Table no. 07: Effect of salinity level on number of leaves per plant at *in vitro*

Treatment	Number of leaves /plant	
T_0	**15.13**	**a**
T_1	14.95	ab
T_2	14.76	b
T_3	14.36	c
T_4	14.20	c
T_5	**11.78**	**d**
CV (%)	3.59	
LSD $_{(0.05)}$	0.2916	

Where, T_0: Control; **T_1:** 80 mM NaCl; **T_2:** 100 mM NaCl; **T_3:** 120 mM NaCl; **T_4:** 140 mM NaCl; **T_5:** 160 mM NaCl

4.1.4.c Combined effect of genotypes and salinity level on number of leaves per plant at *in vitro*

Significant difference was found among the genotypes in respect of number of leaves per plant against different salinity level **(Table 08).** All the tested genotypes were able to produce sufficient leaves up to 160 mM salinity level where CIP 139 produced maximum leaves (13.60) against highest salt stress (160 mM NaCl) followed by CIP 127 (12.50), CIP 117 (10.50) which was statistically similar with CIP 111 (10.50) at same salinity level. On the other hand, minimum leaves were produced by CIP 136 (9.47) at 160 mM salinity level which was statistically similar with CIP 124 (9.50) followed by CIP 106 (9.73) and CIP 102 (9.73) at same salinity level. Among the all interactions, CIP 106 produced maximum leaves (18.57) at salt free MS media **(Plate 08). Murshed et al. (2005)** found significant differences from 100 to 200 mM salinity level where Diamant and Loane produced 7.67 and 4.67 leaves at 160 mM even Sylvana and Amarin showed tolerant at 200 mM salinity level with 3.67 leaves for each.

Table no. 08: Combined effect of genotypes and salinity level on number of leaves per plant at *in vitro*

Genotypes		Number of leaves per plant					
		T_0	T_1	T_2	T_3	T_4	T_5
01	**CIP 102**	14.83 fg	14.50 g	13.50 h	13.40 h	11.77 jk	9.73 lm
02	**CIP 106**	**18.57 a**	16.50 e	13.50 h	12.50 ij	12.47 ij	9.73 lm
03	**CIP 111**	15.43 f	15.37 f	13.50 h	13.17 hi	10.50 l	10.50 l
04	**CIP 117**	16.50 c	15.37 f	15.17 fg	14.43 g	13.50 h	10.50 l
05	**CIP 124**	17.50 c	15.50 f	15.43 f	13.50 h	12.50 ij	9.50 m
06	**CIP 127**	16.40 e	15.50 f	14.60 g	14.50 g	11.50 k	12.50 ij
07	**CIP 136**	13.50 h	13.47 h	12.50 ij	12.50 ij	10.43 l	**9.47 m**
08	**CIP 139**	17.33 cd	16.57 de	16.50 e	16.43 e	16.33 e	**13.60 h**
CV (%)		**3.59**					
LSD (0.05)		**0.825**					

Where, T_0: Control; **T_1:** 80 mM NaCl; **T_2:** 100 mM NaCl; **T_3:** 120 mM NaCl; **T_4:** 140 mM NaCl; **T_5:** 160 mM NaCl

8.a CIP 139 at 160 mM salinity level

8.b CIP 117 at 140 mM salinity level

8.c CIP 111 at 140 mM salinity level

8.d CIP 136 at 120 mM salinity level

8.e CIP 139 at 100 mM salinity level

8.f CIP 106 at control

Plate no. 08 (8.a-8.f): Combined effect of genotypes and salinity level on number of leaves per plant at *in vitro*

4.1.5. Effect on number of nodes per plant at *in vitro*

4.1.5.a Response of genotypes on number of nodes per plant at *in vitro*

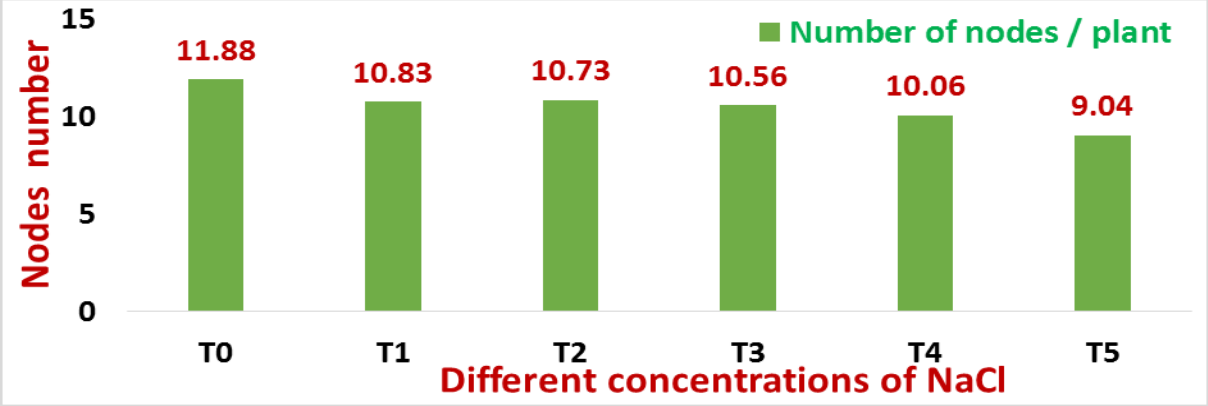
The tested potato genotypes responded differently to the number of nodes and showed significant differences to this parameter **(Table 09).** CIP 124 produced maximum nodes (11.37) which was statistically similar with CIP 139, CIP 127 and CIP 102. On the other hand, minimum number of nodes (8.817) were found in CIP 106 which was statistically similar with CIP 136 followed by CIP 111 and CIP 117.

Table no. 09: Response of genotypes on number of nodes / plant at *in vitro*

Genotypes	Number of nodes / plant
CIP 102	11.16 ab
CIP 106	8.82 d
CIP 111	10.22 c
CIP 117	10.80 b
CIP 124	11.37 a
CIP 127	11.30 a
CIP 136	9.16 d
CIP 139	11.31 a
CV (%)	6.26
$LSD_{(0.05)}$	0.436

4.1.5.b Effect of salinity level on number of nodes per plant at *in vitro*

Different treatments showed pronounced variation at different levels of NaCl in respect of nodes number per plant. The highest number of nodes (11.88) was developed at simple MS. On the contrary, the lowest number of nodes (9.04) was developed at 160 mM NaCl salinity level. Interestingly, statistically similar number of nodes was developed at 80, 100 and 120 mM NaCl salt stress level (**figure 05).**



Where, T_0 : Control; **T_1**: 80 mM NaCl; **T_2**:100 mM NaCl; **T_3**: 120 mM NaCl; **T_4**: 140 mM NaCl; **T_5:** 160 mM NaCl

Figure no. 05: Effect of salinity level on number of nodes per plant at *in vitro*

4.1.5.c Combined effect of genotypes and salinity level on number of nodes per plant at *in vitro*

In terms of nodes number, all the genotypes developed their maximum nodes number at salt free MS media and with the increase level of salinity on MS media nodes development was retarded. However, CIP 139, CIP 102, CIP 117, CIP 124, CIP 127 and CIP 111 developed sufficient nodes up to 160 mM salinity level with 9.50, 9.43, 9.43, 9.40, 8.50 and 8.43 respectively **(Table 10).**

On the other hand, CIP 106 emerged as most salinity sensitive genotype showing its tolerance up to 100 mM salinity level with 9.37 nodes and produced the lowest nodes number (6.43) at 160 mM NaCl stress among the all interactions. Whereas, CIP 136 was found tolerant up to 120 mM salinity level with 8.50 nodes. Among the all interactions, CIP 127 developed maximum nodes (15.33) at control which was statistically significant from rest of the interactions. However, an *in vitro* study performed by **Mahmoud et al. (2009)** reported reduced nodes development and growth of potato at 75 mM NaCl stress.

Table no. 10: Combined effect of genotypes and salinity level on number of nodes per plant

Genotypes	Number of nodes per plant					
	T_0	T_1	T_2	T_3	T_4	T_5
CIP 102	12.50 b-d	12.00 c-f	11.50 d-g	11.00 f-h	10.50 g-i	9.43 i-l
CIP 106	12.50 b-d	11.60 d-f	**9.37 j-l**	6.50 n	6.50 n	**6.43 n**
CIP 111	11.50 d-g	11.00 f-h	10.50 g-i	10.43 g-j	9.43 i-l	8.43 lm
CIP 117	12.20 c-e	11.00 f-h	10.50 g-i	10.50 g-j	10.17 h-k	9.43 i-l
CIP 124	12.50 b-d	11.27 e-g	11.17 e-h	11.17 e-h	11.10 f-h	9.40 j-l
CIP 127	**15.33 a**	12.83 bc	11.13 e-h	10.50 g-j	10.50 g-j	8.50 lm
CIP 136	12.50 b-d	9.50 i- l	9.33 kl	**8.50 lm**	7.60 m	7.50 mn
CIP 139	13.37 b	11.50 d-g	11.50 d-g	11.50 d-g	10.50 g-i	9.50 i-l
CV (%)	**6.26**					
LSD (0.05%)	**1.068**					

Where, T_0: Control; **T_1:** 80 mM NaCl; **T_2:** 100 mM NaCl; **T_3:** 120 mM NaCl; **T_4:** 140 mM NaCl; **T_5:** 160 mM NaCl

4.1.6. Effect on length of root (cm) at *in vitro*

4.1.6.a Effect on length of root (cm) as influenced by CIP potato genotypes

Remarkable variation was observed among the genotypes in terms of root length per explant **(Figure 06)** where the longest root length (11.43 cm) was observed in CIP 102 which was statistically significant from rest of the genotypes followed by CIP 136 (9.96 cm). On the other hand, the shortest root length (3.48 mm) was found in CIP 111 followed by CIP 124 (3.99 cm) and CIP 117 (4.24 cm).

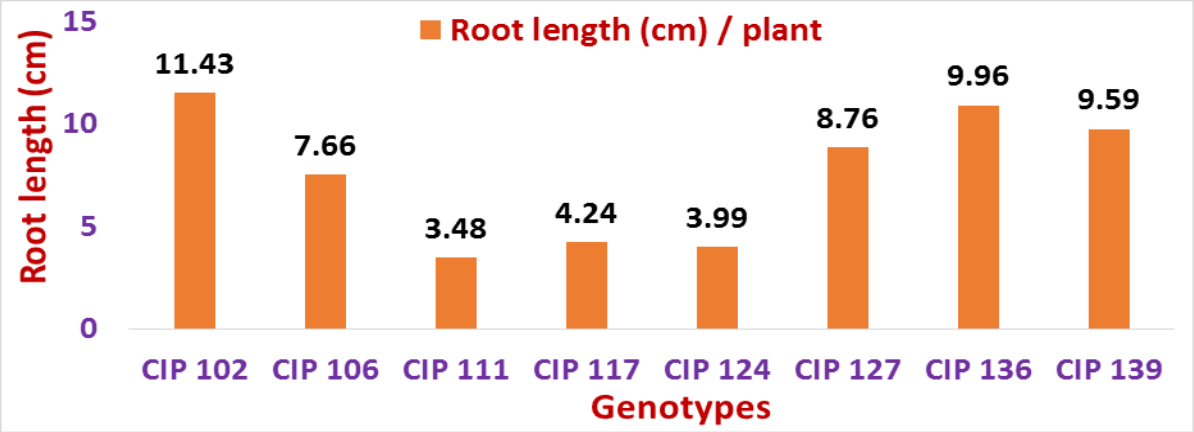


Figure no. 06: Effect on length of root (cm) as influenced by CIP potato

4.1.6.b Effect of salinity level on length of root at *in vitro*

MS media with NaCl caused drastic effect on root length of experimented genotypes. Maximum root length (8.74 cm) was found at control which was statistically similar with 80 mM salinity level (8.32 cm). On the other hand, minimum root length (5.51 cm) was found at 160 mM NaCl salinity level which was statistically significant from all other treatments **(Table 11). Rahman et al. (2008)** also reported reduced plant root length at 75 and 100 mM NaCl.

Table no. 11: Effect of salinity level on length of root (cm) at *in vitro*

Treatment	Root length (cm) / plant
T_0	8.74 a
T_1	8.32 ab
T_2	7.82 b
T_3	7.25 b
T_4	6.45 c
T_5	5.51 d
CV (%)	7.11
LSD $_{(0.05)}$	0.307

Where, T_0: Control; **T_1:** 80 mM NaCl; **T_2:** 100 mM NaCl; **T_3:** 120 mM NaCl; **T_4:** 140 mM NaCl; **T_5:** 160 mM NaCl

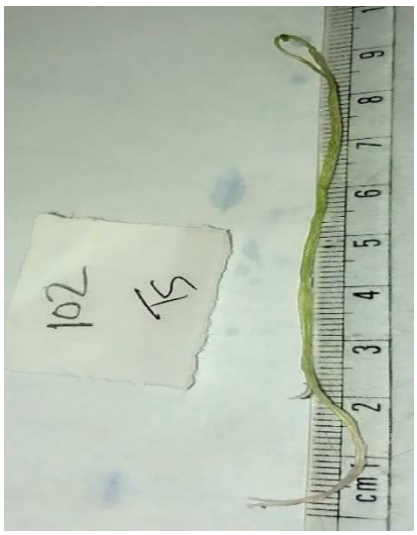
4.1.6.c Combined effect of genotypes and salinity level on root length at *in vitro*

On the basis of combined effect of genotypes and salinity level on root length, tested CIP potato genotypes can be grouped into two broad categories **(Table 12).** First group involved CIP 102, CIP 136, CIP 139, CIP 127 and CIP 106 which were found tolerant up to 160 mM salinity level with 9.50, 9.00, 6.50, 6.50 and 5.00 cm root length respectively. On the other hand, CIP 117, 124 and CIP 111 can be classified into second group showing their highest tolerance up to 100 mM salinity level with 4.50, 4.30 and 3.50 cm root length respectively. Among the all interactions, maximum root length (12.60 cm) was measured in CIP 102 at control and minimum (1.50 cm) in CIP 117 at 160 mM salinity level **(Table 12 and Plate 09). Zaman et al. (2015)** also reported gradual decrease of root length with the increase of salinity level in MS media and found maximum root length (3.1 cm) in potato cultivar Sh-5 followed by Kroda (2.5 cm) up to 60 mM NaCl.

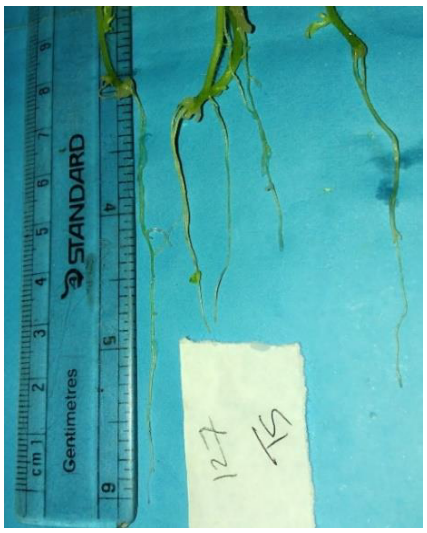
Table no. 12: Combined effect of genotypes and salinity level on root length (cm) / plant at *in vitro*

Genotypes		Root length (cm) / plant					
		T_0	T_1	T_2	T_3	T_4	T_5
01	**CIP 102**	**12.60 a**	12.50 a	11.57 b	11.40 bc	11.00 c	**9.50 h**
02	**CIP 106**	10.10 ef	9.60 gh	8.66 ij	7.50 k	5.10 m	**5.00 n**
03	**CIP 111**	4.50 l-n	3.70 p	**3.50 pq**	3.46 pq	2.70 r	2.50 r
04	**CIP 117**	5.20 m	5.13 mn	**4.50 o**	4.30 m-o	3.50 pq	**1.50 s**
05	**CIP 124**	5.00 n	4.36 op	**4.30 op**	3.50 pq	3.50 pq	3.30 q
06	**CIP 127**	10.50 e	10.00 f	9.70 g	8.10 j	7.50 k	**6.50 l**
07	**CIP 136**	10.50 e	10.50 e	10.33 ef	10.00 f	9.30 hi	**9.00 i**
08	**CIP 139**	11.50 b	10.77 d	10.00 f	9.77 g	9.30 hi	**6.50 l**
CV (%)		**7.11**					
LSD (0.05%)		**0.86**					

Where, T_0: Control; **T_1:** 80 mM NaCl; **T_2:** 100 mM NaCl; **T_3:** 120 mM NaCl; **T_4:** 140 mM NaCl; **T_5:** 160 mM NaCl



9.a CIP 102 at 160 mM NaCl



9.b CIP 127 at 160 mM NaCl

9.c CIP 106 at 160 mM NaCl

9.d CIP 139 at 140 mM NaCl

9.e CIP 111 at 120 mM NaCl

9.f CIP 124 at 100 mM NaCl

9.g CIP 136 at 80 mM NaCl

9.h CIP 102 at control

9.i CIP 117 at 160 mM NaCl

Plate no. 09 (9.a -9.i): Combined effect of genotypes and salinity level on root length (cm) / plant at *in vitro*

4.1.7. Effect on number of roots at *in vitro*

4.1.7.a Effect on number of roots as influenced by potato genotypes at *in*

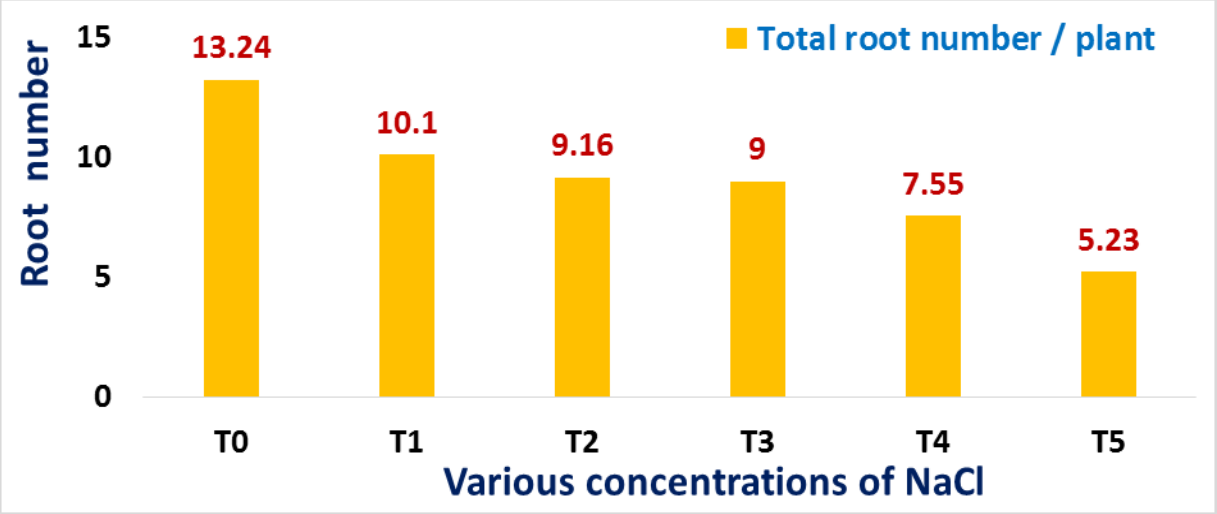
Pronounced variation was found among the genotypes in terms of primary and secondary root number resulting total number of roots per explant **(Table 13).** Considering both primary and secondary roots, CIP 111 responded maximum (10.12) and CIP 102 responded minimum (6.48) on total number of roots where all the genotypes were found statistically significant from one another.

Table no. 13: Effect on number of roots as influenced by CIP Potato genotypes

Genotypes	Total root number / plant
CIP 102	**6.48 f**
CIP 106	9.73 c
CIP 111	**10.12 a**
CIP 117	9.68 c
CIP 124	9.88 b
CIP 127	8.58 d
CIP 136	8.47 e
CIP 139	9.83 b
CV (%)	3.64
LSD (0.05)	0.25

4.1.7.b Effect of salinity level on number of roots at *in vitro*

The NaCl in MS media caused drastic effect on root number i.e. primary root number (PRN), secondary root number (SRN) and total root number (TRN) **(Figure 07)** showing gradual decrease of number of roots with increase of salinity level in MS media. Considering both PRN and SRN, the highest TRN (13.24) was found at control and the lowest (5.23) at 160 mM NaCl salt stress.



Where, T_0 : Control; **T_1**: 80 mM NaCl; **T_2**:100 mM NaCl; **T_3**: 120 mM NaCl; **T_4**: 140 mM NaCl; **T_5:** 160 mM NaCl

Figure no. 07: Effect on salinity level on number of roots at *in vitro*

4.1.7.c Combined effect of genotypes and salinity level on number of roots at *in vitro*

Combined effect of genotypes and salinity level showed that all the genotypes were able to produce root up to 160 mM salinity level but performed better up to 120 mM salinity level. Considering both primary and secondary root, CIP 139 produced highest (8.00) total root number (TRN) at160 mM salinity level followed by CIP 106 with 7.80 TRN at same salinity level. CIP 111, CIP 124, CIP 127 and CIP 117 showed well tolerance up to 140 mM salinity level with 9.0, 9.0, 8.0 and 7.0 TRN respectively. On the other hand, CIP 136 and CIP 102 performed better up to 120 mM salinity level with 8.0 and 6.30 TRN respectively. Among the all interactions, the Highest TRN (18.0) was recorded in CIP 117 at salt free MS media and the lowest (4.50) in CIP 102 at 160 mM salinity level **(Table 14). Sudhersan et al. (2012)** also reported reduced roots plant^{-1} in potato varieties by increasing salt in MS media from 750 - 4000 ppm.

Table no. 14: Combined effect of genotypes and salinity level on number of roots at *in vitro*

Genotypes		Total Root Number					
		T_0	T_1	T_2	T_3	T_4	T_5
1	**CIP 102**	9.30 l-n	7.00 r	6.50 st	**6.30 s**	5.30 t	**4.50 t**
2	**CIP 106**	12.00 gh	11.00 i	10.00 jl	9.70 k-m	8.50 lm	**7.80 op**
3	**CIP 111**	16.30 b	10.30 f-h	10.10 j-i	10.00 j-l	**9.00 m-o**	5.00 t
4	**CIP 117**	**18.00 a**	10.70 ij	8.30 pq	8.10 pq	**8.00 no**	5.00 t
5	**CIP 124**	12.60 de	10.70 ij	10.60 g-i	10.50 g-i	**9.00 m-o**	5.90 s
6	**CIP 127**	12.00 de	10.00 j-l	8.10 pq	8.00 pq	**7.00 r**	6.00 s
7	**CIP 136**	13.00 c	10.30 f-h	8.00 pq	**8.00 pq**	6.30 st	5.33 t
8	**CIP 139**	12.70 de	10.00 j-l	10.70 g-i	9.7 kl	7.30 qr	**8.00 pq**
CV (%)		**3.64**					
LSD (0.05%)		**0.62**					

Where, T_0: Control; **T_1:** 80 mM NaCl; **T_2:** 100 mM NaCl; **T_3:** 120 mM NaCl; **T_4:** 140 mM NaCl; **T_5:** 160 mM NaCl

4.1.8. Effect on fresh weight (mg) and dry weight (mg) of shoot at *in vitro*

4.1.8.a Effect on fresh weight (mg) (FWS) and dry weight (mg) (DWS) of shoot as influenced by CIP Potato genotypes at *in vitro*

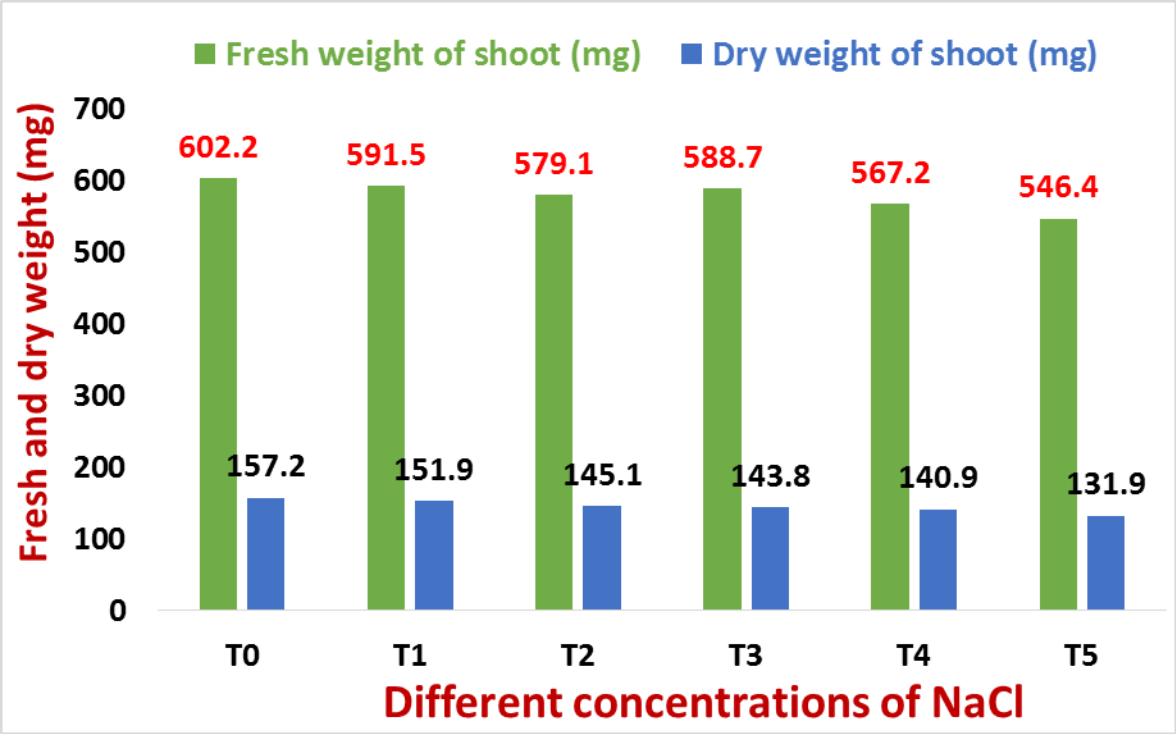
Remarkable variation was observed among the genotypes in terms of fresh weight of shoot per explant. The maximum FWS (638.0 mg) was measured in CIP 127 which was statistically different from all other genotypes followed by CIP139 (609.2 mg). On the other hand, minimum FWS (524.7 mg) was found in CIP 117 which was also statistically significant from rest of the other genotypes **(Table 15).** Likewise, maximum dry weight of shoot (163.1 mg) was measured in CIP 127 and minimum (125.9 mg) in CIP 117 where all the genotypes were found statistically significant from one another **(Table 15).**

Table no. 15: Effect on fresh weight (mg) and Dry weight (mg) of shoot as influenced by CIP Potato genotypes at *in vitro*

Genotypes	Fresh weight of shoot (mg)	Dry weight of shoot (mg)
CIP 102	554.8 g	138.4 e
CIP 106	574.5 e	147.6 d
CIP 111	596.7 c	153.8 c
CIP 117	**524.7 h**	**125.9 f**
CIP 124	556.6 f	126.7 f
CIP 127	**638.0 a**	**163.1 a**
CIP 136	578.8 d	148.9 d
CIP 139	609.2 b	156.8 b
CV (%)	0.37	2.83
LSD $_{(0.05)}$	1.435	2.720

4.1.8.b Effect of salinity level on fresh weight (FWS) (mg) and Dry weight (mg) of shoot (DWS) at *in vitro*

Fresh weight of shoot of *in vitro* produced plant was severely reduced with the increase of salinity level on MS media where the highest fresh weight of shoot (602.2 mg) was measured at MS media free of salt and the lowest (546.4 mg) at MS media supplemented with 160 mM NaCl **(Figure 08).** Similarly, Maximum dry weight of shoot (157.20 mg) was measured at control and minimum (139.90 mg) at 160 mM salinity level **(Figure 08).**



Where, T_0: Control; **T_1:** 80 mM NaCl; **T_2:** 100 mM NaCl; **T_3:** 120 mM NaCl; **T_4:** 140 mM NaCl; **T_5:** 160 mM NaCl

Figure no. 08: Effect of salinity level on fresh and dry weight (mg) of shoot at *in vitro*

4.1.8.c Combined effect of genotypes and salinity level on Fresh weight (mg) (FWS) and dry weight (mg) of shoot (DWS) at *in vitro*

The study revealed significant variation from 783.5 to 492.5 mg for FWS in the combined effect of genotypes and salinity level showing highest FWS (783.5 mg) in CIP 127 at simple MS media which was statistically different from all other interactions. On the other hand, minimum FWS (492.5 mg) was measured in CIP 117 at 160 mM salinity level which was statistically significant from rest of the interactions **(Table 16).** Maximum DWS (205.0 mg) was found in CIP 127 at NaCl stress free MS media which was statistically significant from all other interactions followed by CIP 139 (181.0 mg) at same salinity level. On the contrary, minimum DWS (105.0 mg) was found in CIP 117 at 160 mM salinity level which was statistically similar with CIP 102 (107.5 mg) at same salinity level **(Table 16).** It has also been reported that under salt stress, relatively salt-tolerant potato cultivars accumulated more fresh and dry weights than salt-sensitive cultivars **(Rahnama and Ebrahimzadeh 2004).**

Table no. 16: Combined effect of genotypes and salinity level on fresh weight (mg) and dry weight (mg) of shoot at *in vitro*

Genotypes	Treatment	Fresh weight of shoot (mg)		Dry weight of shoot (mg)	
CIP 102	T_0	593.3	m	157.8	e-g
	T_1	566.2	q	157.5	e-g
	T_2	553.0	r	152.5	g-i
	T_3	550.0	s	150.0	h-j
	T_4	536.1	u	137.7	m-o
	T_5	529.9	v	107.5	uv
CIP 106	T_0	626.8	h	154.7	f-h
	T_1	583.2	n	162.5	de
	T_2	579.7	o	160.0	ef
	T_3	572.3	p	160.0	ef
	T_4	549.0	s	145.0	j-l
	T_5	536.1	u	125.0	qr
CIP 111	T_0	732.8	c	170.0	c
	T_1	616.5	j	152.3	g-i
	T_2	610.0	k	150.0	h-j
	T_3	582.9	no	147.7	i-k
	T_4	519.3	xy	143.0	k-m
	T_5	500.3	\	110.2	uv
CIP 117	T_0	542.0	t	140.0	l-n
	T_1	534.7	u	135.0	no
	T_2	525.0	w	125.2	qr
	T_3	522.7	wx	125.0	qr
	T_4	512.7	z	120.0	rs
	T_5	**492.5**	**]**	**105.0**	**v**
CIP 124	T_0	599.7	l	144.7	j-l
	T_1	580.0	no	135.0	no
	T_2	569.7	pq	127.7	pq
	T_3	541.3	t	122.7	q-s
	T_4	529.7	v	117.3	st
	T_5	519.0	y	113.0	tu
CIP 127	T_0	**783.5**	**a**	**205.0**	**a**
	T_1	715.8	d	180.0	b
	T_2	632.3	g	157.5	e-g
	T_3	631.5	g	150.0	h-j
	T_4	572.3	p	145.0	j-l
	T_5	511.3	z[	141.0	l-n
CIP 136	T_0	637.3	f	164.0	c-e
	T_1	637.2	f	167.5	cd
	T_2	622.0	i	162.5	de
	T_3	542.8	t	155.0	f-h
	T_4	532.8	uv	142.0	k-m
	T_5	501.0	\	132.5	op
CIP 139	T_0	**751.3**	**b**	**181.0**	**b**
	T_1	641.0	e	157.5	e-g
	T_2	622.7	i	147.7	i-k
	T_3	599.7	l	145.1	j-l
	T_4	550.3	rs	144.3	j-l
	T_5	509.0	[	135.0	no
CV (%)		**0.37**		**2.83**	
LSD $_{(0.05)}$		**3.516**		**6.664**	

Where, T_0 : Control; **T_1**: 80 mM NaCl; **T_2**:100 mM NaCl; **T_3**: 120 mM NaCl; **T_4**: 140 mM NaCl; **T_5:** 160 mM NaCl

4.1.9. Effect on fresh weight (mg) and dry weight (mg) of root

4.1.9.a Effect on fresh weight (mg) (FWR) and dry weight (mg) (DWR) of root as influenced by CIP Potato genotypes

Highly significant differences were found among the experimented genotypes for fresh weight of root per explant. The highest FWR (280.7 mg) was found in CIP 139 which was statistically different from all other genotypes and 2nd highest FWR was found in CIP 127 (272.1 mg) which was also statistically different from all other genotypes. On the other hand, minimum FWR (220.5 mg) was measured in CIP 117 which was statistically different from rest of the genotypes **(Table 17).** Concerning the dry weight of root, CIP 139 had the highest value (85.04 mg) as compared to the other genotypes while CIP 117 had the lowest value (46.96 mg) which was statistically different from all other genotypes **(Table 17).**

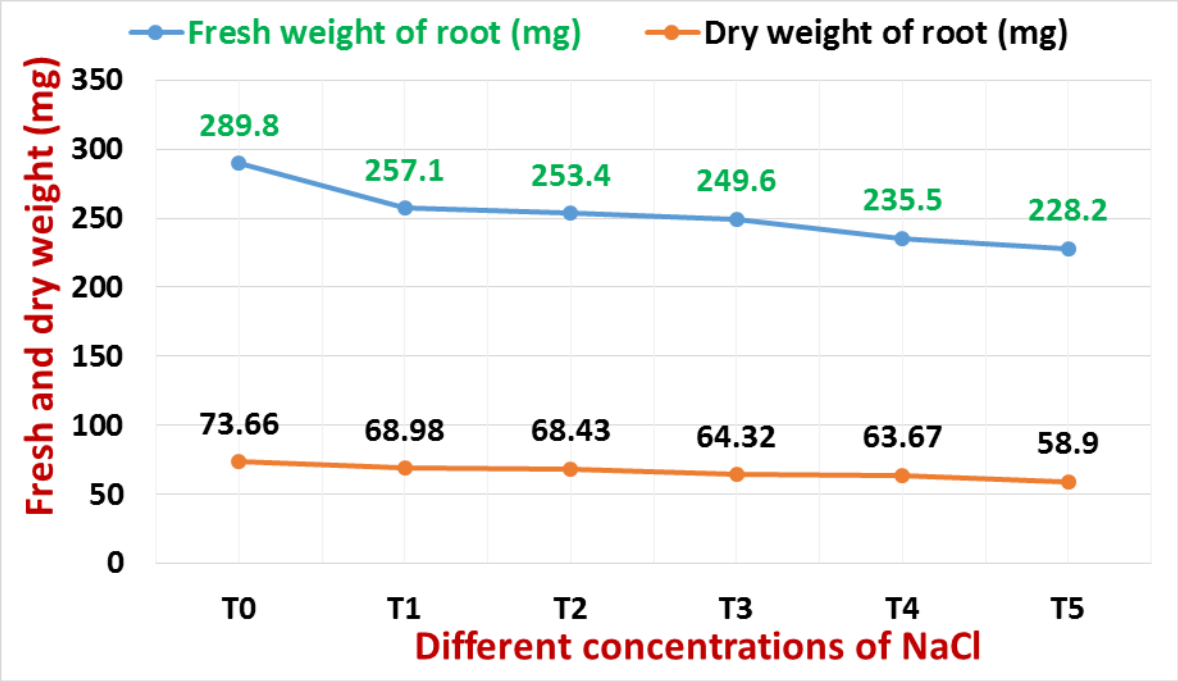
Table no. 17: Effect on fresh weight of root (mg) and dry weight of root (mg) as influenced by CIP Potato genotypes at *in vitro*

Genotypes	Fresh weight of root (mg)	Dry weight of root (mg)
CIP 102	250.9 f	60.83 f
CIP 106	242.3 e	64.09 e
CIP 111	230.6 g	56.63 g
CIP 117	**220.5 h**	**46.96 h**
CIP 124	264.5 c	72.61 c
CIP 127	272.1 b	74.18 b
CIP 136	256.3 d	70.28 d
CIP 139	**280.7 a**	**85.04 a**
CV (%)	3.61	1.02
LSD (0.05)	6.023	0.4463

4.1.9.b Effect of salinity level on fresh weight (FWR) (mg) and dry weight (DWR) (mg) of root at *in vitro*

Application of salt stress in MS media revealed that fresh weight of root of *in vitro* produced plant was severely reduced with the increase level of salinity. At control FWR was measured maximum (289.8 mg) which was statistically significant from all other treatments and at 160 mM salinity level FWR was measured minimum (228.2 mg) which was also statistically significant from rest of the treatments **(Figure 09).**

On the other hand, the highest DWR (73.66 mg) was found at simple MS media, on the contrary, the lowest DWR (58.9 mg) was measured at MS media having 160 mM NaCl where all the treatments were found statistically significant from one another **(Figure 09).**



Where, T_0: Control; **T_1:** 80 mM NaCl; **T_2:** 100 mM NaCl; **T_3:** 120 mM NaCl; **T_4:** 140 mM NaCl; **T_5:** 160 mM NaCl

Figure no. 09: Effect of salinity level fresh weight of root (mg) and dry weight of root (mg) at *in vitro*

4.1.9.c Combined effect of genotypes and salinity level on fresh weight (FWR) and dry weight (DWR) of root (mg)

In terms of the combined effect of genotypes and salinity level, the highest FWR (370.0 mg) was measured in CIP 139 at MS media free from salt which was statistically similar with CIP 127 (370.0 mg) at same media. On the other hand, the lowest FWR (200.0 mg) was found in CIP 117 at 160 mM salt stress which was statistically similar with CIP 111 (205.0 mg) at same treatment **(Table 18).** Likewise, the highest DWR (98.50 mg) was measured in CIP 139 at media free from NaCl stress which was statistically significant from all other interactions and the lowest (40.43 mg) in CIP 117 at 160 mM salt stress which was also statistically significant from rest of the interactions **(Table 18).** Severe loss of root weight in potato cv. Agria was reported by increasing NaCl stress in MS media from 50 to 150 mM by **Askari et al. (2012).**

Table no. 18: Combined effect of genotypes and salinity level on fresh weight (mg) and dry weight (mg) of root at *in vitro*

Genotypes	Treatment	Fresh weight of root		Dry weight of root (mg)	
CIP 102	T_0	240.0	k-o	78.23	i
	T_1	232.8	m-q	90.73	c
	T_2	220.0	q-u	85.80	e
	T_3	215.2	r-v	85.43	ef
	T_4	210.0	t-w	79.50	h
	T_5	210.0	t-w	73.00	m
CIP 106	T_0	315.5	c	84.00	g
	T_1	290.0	de	89.40	d
	T_2	272.8	fg	69.77	n
	T_3	244.4	j-m	66.60	p
	T_4	250.0	i-l	61.87	qr
	T_5	205.6	u-w	49.53	y
CIP 111	T_0	352.8	b	95.77	b
	T_1	300.0	d	77.67	ij
	T_2	292.8	de	69.47	n
	T_3	290.0	de	68.00	o
	T_4	235.6	l-p	50.73	x
	T_5	205.0	vw	48.43	z
CIP 117	T_0	230.0	m-q	60.50	s
	T_1	220.0	q-u	58.40	t
	T_2	235.0	m-p	62.43	q
	T_3	340.0	b	56.60	u
	T_4	270.7	fg	53.40	w
	T_5	**200.0**	**w**	**40.43**	^
CIP 124	T_0	255.0	h-j	55.50	v
	T_1	250.0	i-l	50.43	xy
	T_2	242.7	j-m	45.47	[\
	T_3	242.3	j-n	44.57	\
	T_4	241.7	j-n	43.40	]
	T_5	222.3	p-t	42.37	]
CIP 127	T_0	370.0	a	84.37	fg
	T_1	280.0	ef	77.00	jk
	T_2	265.5	f-h	73.57	lm
	T_3	232.2	m-q	70.43	n
	T_4	232.0	m-q	60.83	rs
	T_5	212.3	s-w	55.60	uv
CIP 136	T_0	280.0	ef	83.37	g
	T_1	227.7	n-r	74.40	l
	T_2	225.6	o-s	61.83	qr
	T_3	225.1	p-s	53.57	w
	T_4	212.8	s-w	46.37	[
	T_5	212.8	s-w	45.47	[\
CIP 139	T_0	**370.0**	**a**	**98.50**	**a**
	T_1	295.6	d	79.43	h
	T_2	263.3	g-i	80.00	h
	T_3	252.8	h-k	76.57	k
	T_4	212.9	s-w	69.50	n
	T_5	205.6	u-w	55.50	v
CV (%)		**3.61**		**1.02**	
LSD $_{(0.05)}$		**14.75**		**1.09**	

Where, T_0 : Control; **T_1**: 80 mM NaCl; **T_2**:100 mM NaCl; **T_3**: 120 mM NaCl; **T_4**: 140 mM NaCl; **T_5:** 160 mM NaCl

Experiment no. 02: *In vitro* root bioassay of different CIP Potato genotypes for salinity tolerance

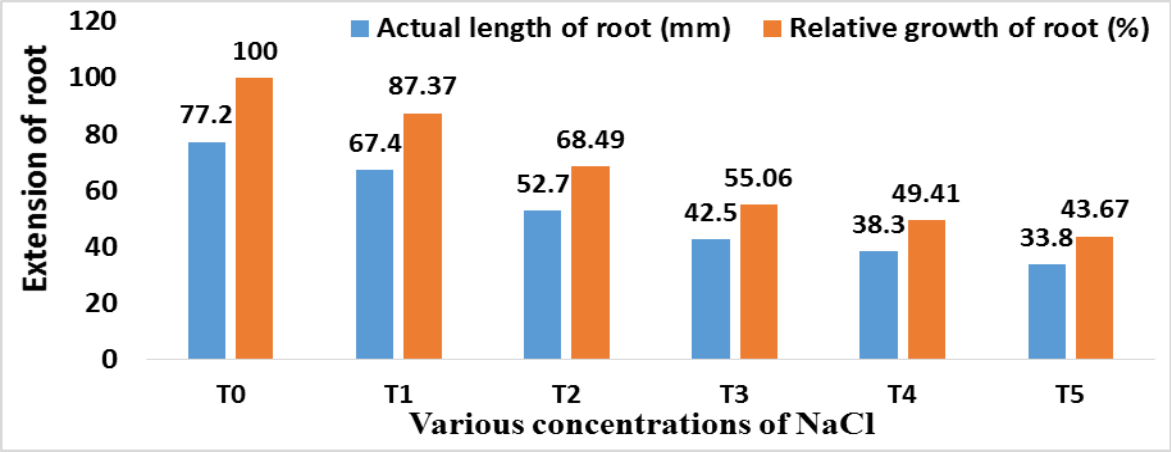
4.2. Effect of salinity level on extension (cm) of root tip segment

Among the five genotypes, CIP 139 responded highest (5.61 cm) in terms of actual root length against different degrees of salinity showing the highest growth (68.31 %) of root in comparison to control followed by CIP 127 (5.29 cm) and CIP 102 (5.23 cm) **(Table 19).** Whereas, the lowest response (4.86 cm) for same parameter was noticed in CIP 120 having 66.31 % growth in comparison to control followed by CIP 106 with 4.99 cm root length having 66.56 % growth comparing to control.

Table no. 19: Response of genotypes on actual extension / length (cm) and relative growth (%) of root tip segments at *in vitro*

Genotypes	Actual extension (length) of root tip segments (cm)	Relative growth of root tip segments (%)
CIP 102	5.23 b	67.22 b
CIP 106	4.99 c	66.56 c
CIP 120	**4.86 d**	**66.31 d**
CIP 127	5.29 b	67.51 b
CIP 139	**5.61 a**	**68.31 a**
CV (%)	4.44	4.17
LSD $_{(0.05)}$	2.35	2.68

In terms of root length, root tips of experimented genotypes showed their highest growth (77.2 mm and 100 %) at MS media lacking NaCl stress and lowest (33.8 mm and 43.67 %) at MS media supplemented with 160 mM salinity level where all the treatments were found statistically significant from one another **(Figure 10).**



Where, T_0 : Control; **T_1**: 80 mM NaCl; **T_2**:100 mM NaCl; **T_3**: 120 mM NaCl; **T_4**: 140 mM NaCl; **T_5:** 160 mM NaCl

Figure no. 10: Effect of salinity level on growth of root tip segments at *in vitro*

Although the combined effect of genotypes and salinity level on root tip segments revealed that root length was gradually decreased with the increased level of salinity **(Table 20)** but not significantly affected up to 120 mM salinity level. Among the experimented genotypes, root tip segments of CIP 139 were found maximum tolerant up to160 mM salinity level with 4.15 cm actual root length having the highest relative root growth (50.55 %) comparing with control. Whereas, root tip segments of CIP 127 and CIP 102 were found tolerant up to 140 mM salinity level with 4.10 cm and 4.01 cm root length indicating 51.57 % and 51.54 % relative growth of root tip segments respectively comparing with control. On the other hand, CIP 120 was found as the most salinity sensitive showing its highest tolerance up to 120 mM NaCl with 3.92 cm root length indicating 55.06 % relative growth of root tip segments comparing with control followed by CIP 106 at same salinity level with 4.11 cm actual root length and 54.56 % relative root growth comparing to control. Among the all interactions, the lowest extension (2.85 cm) of root tip segments was found in CIP 120 at 160 mM NaCl and the highest (8.21 cm) in CIP 139 at MS media free of salt **(Plate 10). Sudhersan et al. (2012)** also reported reduced root length plant^{-1} in potato varieties by increasing salt in MS media from 750 - 4000 ppm.

Table no. 20: Combined effect of genotypes and salinity level on actual extension (cm) and relative growth (%) of root tip segment at *in vitro*

Genotypes	Actual root length (cm)						Relative growth of root (%)					
	T0	T1	T2	T3	T4	T5	T0	T1	T2	T3	T4	T5
CIP 102	7.78 c	7.15 e	5.10 j	4.15 L	4.01 m	3.40 n	100 a	91.90 b	65.55 I	53.34 n	51.54 op	45.21 r
CIP 106	7.51 d	6.32 g	5.06 j	4.11 l	3.51 n	3.35 n	100 a	84.04 d	67.28 G	55.06 l	46.68 q	43.06 T
CIP 120	7.12 e	6.51 f	5.61 h	3.92 M	3.17 o	2.85 p	100 a	91.43 b	78.79 F	54.65 m	44.52 s	40.03 u
CIP 127	7.95 b	6.50 f	5.25 i	4.45 K	4.10 l	3.50 n	100 a	81.76 e	66.04 H	55.97 l	51.57 p	44.03 S
CIP 139	8.21 a	7.20 e	5.32 i	4.62 K	4.50 k	4.15 l	100 a	87.70 c	64.80 J	56.27 k	54.81 m	50.55 o
CV (%)	4.44						4.17					
LSD (0.05)	8.03						9.17					

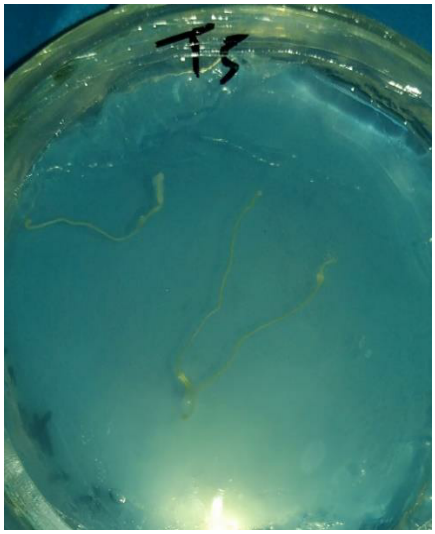
Where, T_0: Control; **T_1:** 80 mM NaCl; **T_2:** 100 mM NaCl; **T_3:** 120 mM NaCl; **T_4:** 140 mM NaCl; **T_5:** 160 mM NaCl

10.a CIP 102 at 80 mM NaCl

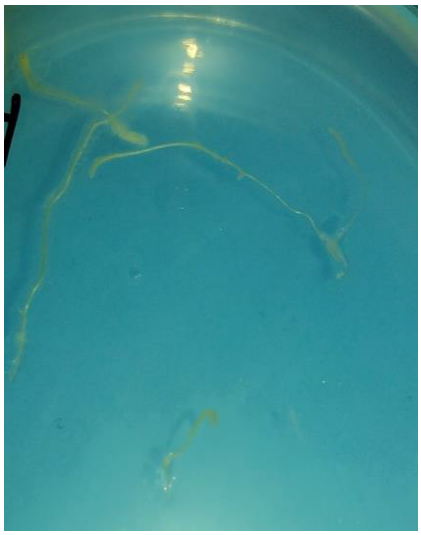
10.b CIP 120 at 100 mM NaCl

10.c CIP 102 at 120 mM NaCl

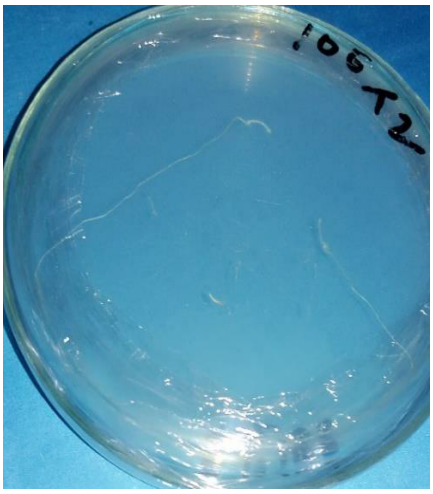
10.d CIP 102 at 140 mM NaCl



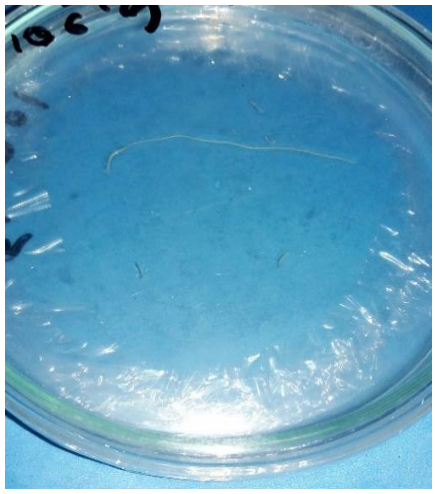
10.e CIP 127 at 140 mM NaCl



10.f CIP 139 at 160 mM NaCl



10.g CIP 106 at 100 mM NaCl



10.h CIP 106 at 160 mM NaCl

10.i CIP 139 at control

Plate no. 10 (10.a – 10.h): Combined effect of genotypes and salinity level on growth of root tip segments at *in vitro*

Experiment no. 03: Effect of different concentrations of IBA on root development of salt tolerant potato genotypes

4.3.1. Effect on days to root initiation and well-developed root at *in vitro*

4.3.1.a Response of genotypes on days to root initiation (DRI) and well-developed root (DWDR)

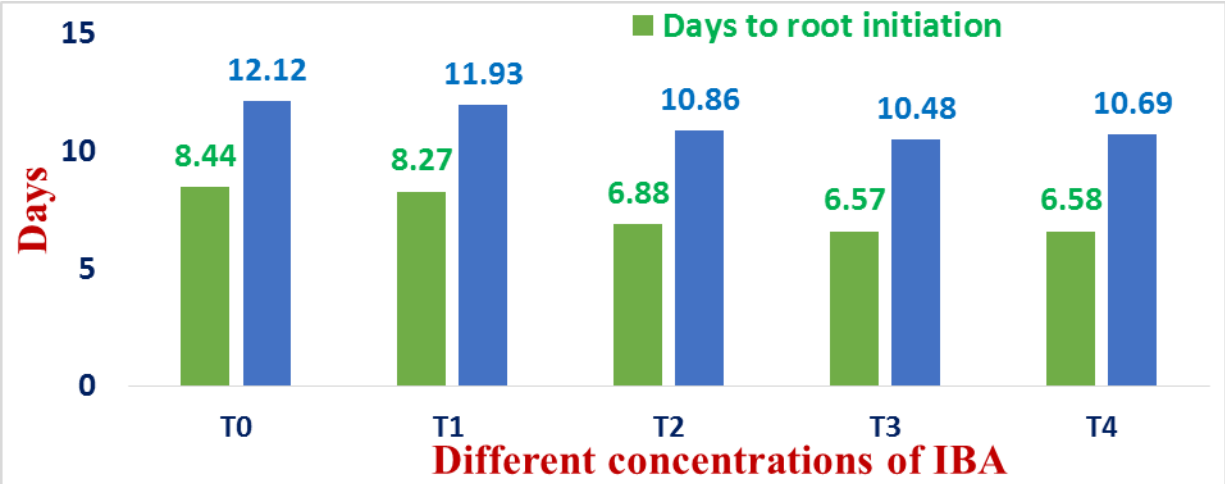
Among the three salt tolerant potato genotypes, CIP 127 required the lowest time for both root initiation (7.00 days) and well development of root (10.68 days). On the contrary, CIP 117 needed highest time for both root initiation (7.55 days) and well-developed root (11.55 days). Here, all the genotypes were found statistically significant from one another (**Table 21**).

Table no. 21: Response of genotypes on days to root initiation and well-developed root

Genotypes	Days to root initiation	Days to well-developed root
CIP 106	7.38 b	11.41 b
CIP 117	7.55 a	11.55 a
CIP 127	7.00 c	10.68 c
CV (%)	2.74	1.55

4.3.1.b Effect of IBA on days to root initiation and well-developed root

Studied potato genotypes responded earlier at MS media supplemented with 1.0 mg IBA / L for both root initiation (6.57 days) and well-developed root (10.48 days). On the other hand, responded lately at fresh MS media lacking IBA for both root initiation (8.44 days) and well-developed root (12.12 days) **(Figure 11).**



Where, T_0 = Control; T_1 = 0.1 mg IBA / L; T_2 = 0.5 mg IBA / L; T_3 = 1.0 mg IBA / L; T_4 = 1.5 mg IBA / L

Figure no. 11: Effect of different IBA conc. on days to root initiation and days to well-developed root

4.3.1. c Combined effect of genotypes and different level of IBA conc. on days to root initiation (DRI) and well-developed root (DWDR)

Combined effect of different level of IBA concentrations and genotypes revealed that DRI varied from 5.77 to 8.53 days and DWDR varied from 9.67 to 12.47 days among the genotypes where CIP 127 needed minimum time for both root initiation (5.77 days) and well-developed root (9.67 days) at 1.0 mg IBA / L treatment. In like manner, CIP 106 required minimum time for both parameters at the same treatment. On the other hand, CIP 117 responded best at 1.5 mg IBA / L for the same parameters. All the genotypes took maximum time at simple MS media responding to DRI and DWDR. According to the findings, MS media supplemented with 1.0 mg IBA / L can be considered best for both DRI and DWDR for the micropropagation of these salt tolerant potato genotypes. However, **Hoque (2010)** found combination of 2.0 mg/L kinetin and IAA best for multiple root regeneration in MS media for five potato varieties.

Table no. 22: Combined effect of genotypes and different level of IBA conc. on days to root initiation and days to well-developed root

Genotypes	Treatment	Days to root initiation	Days to well-developed root
CIP 106	T_0	8.47 a	12.47 a
	T_1	8.20 a	12.17 b
	T_2	7.20 b	11.30 c
	T_3	**6.53 c**	**10.47 e**
	T_4	6.53 c	10.67 de
CIP 117	T_0	8.33 a	12.40 ab
	T_1	8.20 a	12.20 ab
	T_2	7.37 b	11.23 c
	T_3	7.40 b	11.30 c
	T_4	**6.47 c**	**10.63 de**
CIP 127	T_0	8.53 a	11.50 c
	T_1	8.40 a	11.43 c
	T_2	6.17 d	10.03 f
	T_3	**5.77 d**	**9.67 g**
	T_4	6.73 c	10.77 d
CV (%)		2.74	1.55

Where, T_0 = Control; T_1 = 0.1 mg IBA / L; T_2 = 0.5 mg IBA / L; T_3 = 1.0 mg IBA / L; T_4 = 1.5 mg IBA / L

4.3.2 Effect on length of roots (cm) at *in vitro*

4.3.2.a Response of genotypes on length of roots (cm)

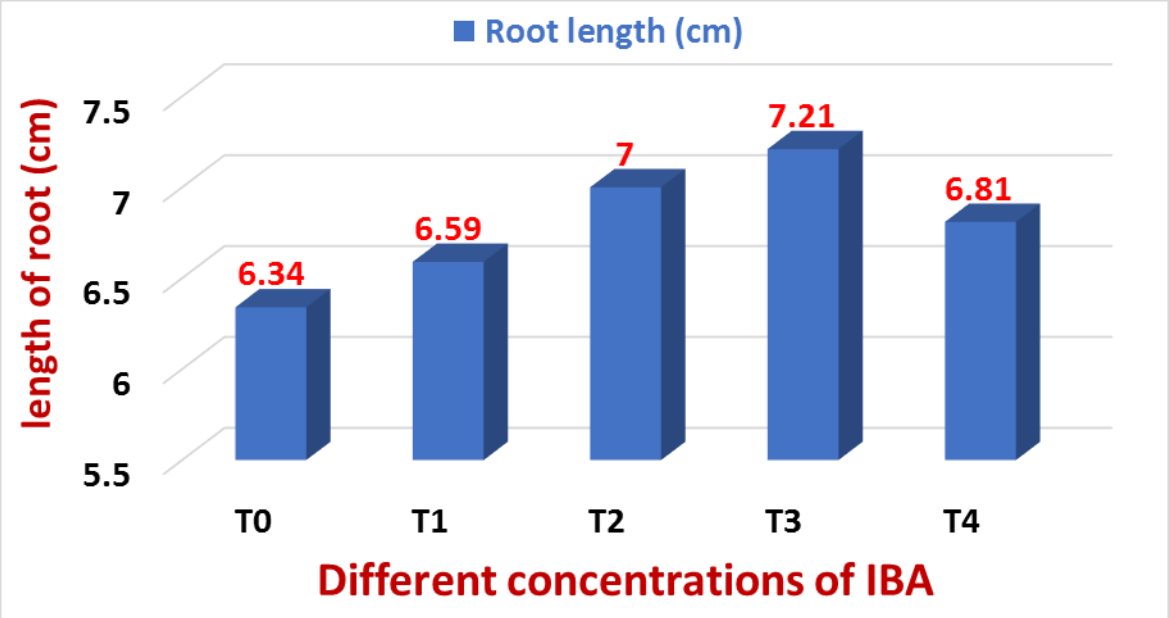
Among the three potato genotypes, the longest root was observed in CIP 127 (8.11 cm) and the shortest root was observed in CIP 117 (5.62 cm) where all the genotypes were statistically significant from one another (**Table 23)**.

Table no. 23: Response of genotypes on length roots (cm) at *in vitro*

Genotypes	Root length (cm)
CIP 106	6.64 b
CIP 117	5.62 c
CIP 127	8.11 a
CV (%)	2.21

4.3.2.b Effect of IBA on length of roots (cm)

The longest root was produced at MS media having 1.0 mg IBA/L with 7.21 cm whereas shortest root was found at control media (6.34 cm). No significant difference was found at IBA 0.1 mg/L and 0.5 mg/L for root length **(Figure 12).**



Where, T_0 = Control; **T_1** = 0.1 mg IBA / L; **T_2** = 0.5 mg IBA / L; **T_3** = 1.0 mg IBA / L; **T_4** = 1.5 mg IBA / L

Figure no. 12: Effect of IBA on primary and secondary root length (cm) at *in vitro*

4.3.2.c Combined effect of genotypes and different level of IBA concentrations on length of roots (cm)

Combined effect of genotypes and different level of IBA conc. revealed that maximum root length was measured in CIP 127 (8.63 cm) at 1.0 mg /L IBA and minimum in CIP 117 (5.47 cm) at control. **(Table 24 and Plate 11).** All the genotypes produced their longest root at 1.0 mg /L IBA conc. and second longest root at 0.5 mg /L IBA. It was noticed that higher conc. of IBA had negative effect on root length.

Hoque (2010) also found negative effect of higher conc. of hormone (KIN and IAA) on root multiplication. On the other hand, **Parveen et al. (2014)** found 0.5 mg /L IBA best for rooting supplemented with half strength PROP medium for three environmental stress tolerant potato varieties viz. Shadaghuti, Challisha and Zaubilai.

Table no. 24: Combined effect of genotypes and different level of IBA conc. on length of root (cm) at *in vitro*

Genotypes	Root length (cm)				
	T_0	T_1	T_2	T_3	T_4
CIP 106	6.05 h	6.60 g	6.90 f	**7.00 f**	6.65 g
CIP 117	**5.47 j**	5.50 j	5.60 i	**6.00 h**	5.55 i
CIP 127	7.50 d	7.67 d	8.50 b	**8.63 a**	8.23 c
CV (%)	2.21				

Where, T_0 = Control; T_1 = 0.1 mg IBA / L; T_2 = 0.5 mg IBA / L; T_3 = 1.0 mg IBA / L; T_4 = 1.5 mg IBA / L



11.a CIP 127 at 1.0 mg IBA / L **11.b CIP 106 at 1.0 mg IBA / L** **11.c CIP 117 at 1.0 mg IBA / L**


11.d CIP 127 at 0.5 mg / L IBA **11.e CIP 127 at 1.5 mg / L IBA**


11.f CIP 106 at 0.1 mg / L IBA **11.g CIP 117 at control**

Plate no. 11 (11.a-11.g): Combined effect of genotypes and different level of IBA concentration on length of roots (cm)

4.3.3. Response on number of roots at *in vitro*

4.3.3.a Response of genotypes on number of roots at *in vitro*

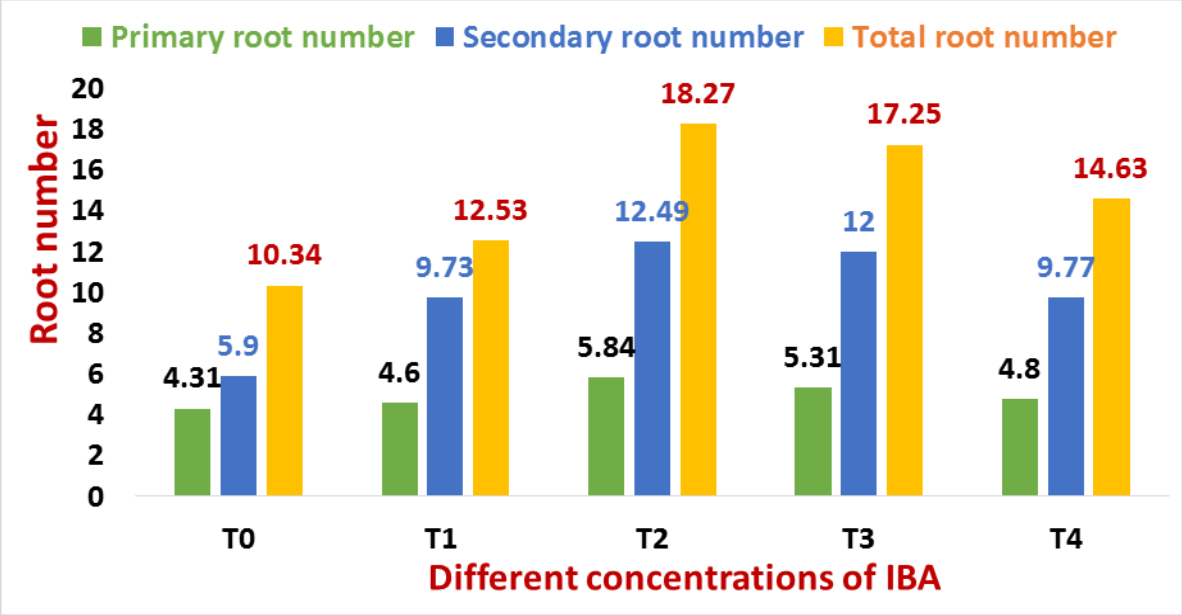
Among the three potato genotypes, CIP 127 produced maximum number of roots (15.23) and CIP 117 produced minimum (12.74) number of roots **(Table 25).** But primary root number was highest in CIP 106 (5.24) and lowest in CIP 117 (4.53). In case of secondary root, highest value was observed in CIP 127 (10.84) and lowest in CIP 117 (8.21).

Table no. 25: Response of genotypes on number of roots at *in vitro*

Genotypes	Primary root number	Secondary root number	Total root number
CIP 106	5.24 a	9.86 b	15.10 b
CIP 117	4.53 c	8.21 c	12.74 c
CIP 127	5.17 b	10.84 a	15.23 a
CV (%)	2.25	2.51	3.88

4.3.3.b Effect of IBA on number of roots at *in vitro*

MS media supplemented with 0.5 mg IBA /L was found best with highest primary (5.84), secondary (12.49) and total root number (18.27) comparing to other treatments. On the other hand, MS media without IBA responded lowest with minimum primary (4.31), secondary (5.90) and total root number (10.34) **(Figure 13).** This is strongly in agreement with **Molla et al. (2011)** who reported IBA 0.5 mg/L best for *in vitro* rooting of potato.



Where, T_0 = Control; T_1 = 0.1 mg IBA / L; T_2 = 0.5 mg IBA / L; T_3 = 1.0 mg IBA / L; T_4 = 1.5 mg IBA / L

Figure no. 13: Effect of IBA on number of roots at *in vitro*

4.3.3.c Combined effect of genotypes and different level of IBA concentrations on number of roots

Combined effect of genotypes and different level of IBA concentrations revealed that maximum (5.80) primary root was produced in CIP 106 at 0.50 mg IBA / L and minimum (4.10) in CIP 117 at MS media lacking IBA concentration. But maximum (14.77) secondary root was noticed in CIP 127 at 0.5 mg IBA /L and minimum (5.27) in CIP 117 at MS media lacking IBA concentration. Considering both primary and secondary root number, highest root (20.03) was found in CIP 127 at MS media supplemented with 0.5 mg IBA /L and minimum (9.37) in CIP 117 at control **(Table 26 and Plate 12).** Here, MS media supplemented with 0.5 mg IBA / L was found best for total root number for all genotypes**.** The finding is different from the finding of **Sarker and mostafa (2002)** who reported 0.1 mg /L IAA best for rooting in potato.

Table no. 26: Combined effect of genotypes and different level of IBA conc. on number of root at *in vitro*

Genotypes	Treatment	Primary root number	Secondary root number	Total root number
CIP 106	T_0	4.40 g	5.80 i	10.20 j
	T_1	4.60 f	8.33 g	12.93 h
	T_2	**5.80 a**	**13.27 b**	**19.07 b**
	T_3	5.20 d	12.53 c	17.73 c
	T_4	5.20 d	8.10 g	13.30 g
CIP 117	T_0	**4.10 h**	**5.27 j**	**9.37 j**
	T_1	5.10 e	5.67 i	10.37 j
	T_2	5.20 d	9.43 f	14.63 f
	T_3	5.13 e	10.27 e	15.40 e
	T_4	5.17 d	10.43 e	15.60 d
CIP 127	T_0	4.20 gh	6.63 h	10.83 i
	T_1	4.33 g	10.20 e	14.53 f
	T_2	**5.53 b**	**14.77 a**	**20.03 a**
	T_3	4.60 f	13.20 b	17.80 c
	T_4	4.23 gh	10.77 d	15.00 e
CV (%)		2.25	2.51	3.88

Where, T_0 = Control; T_1 = 0.1 mg IBA / L; T_2 = 0.5 mg IBA / L; T_3 = 1.0 mg IBA / L; T_4 = 1.5 mg IBA / L

12.a CIP 106 at 0.5 mg IBA / L

12.b CIP 106 at 1.0 mg IBA / L

12.c CIP 117 at 1.5 mg IBA / L

12.d CIP 117 at 0.5 mg IBA / L

12.e CIP 117 at 1.0 mg IBA / L

12.f CIP 117 at 1.5 mg IBA / L

12.g CIP 127 at 0.5 mg IBA / L

12.h CIP 127 at 1.0 mg IBA / L

12.i CIP 127 at 1.5 mg IBA / L

Plate no. 12 (12.a-12.i): Combined effect of genotypes and different level of IBA concentrations on number of roots at *in vitro*

4.3.4. Effect on fresh weight (mg) and dry weight of root (mg) at *in vitro*

4.3.4.a Response of genotypes on fresh weight (mg) and dry weight of root

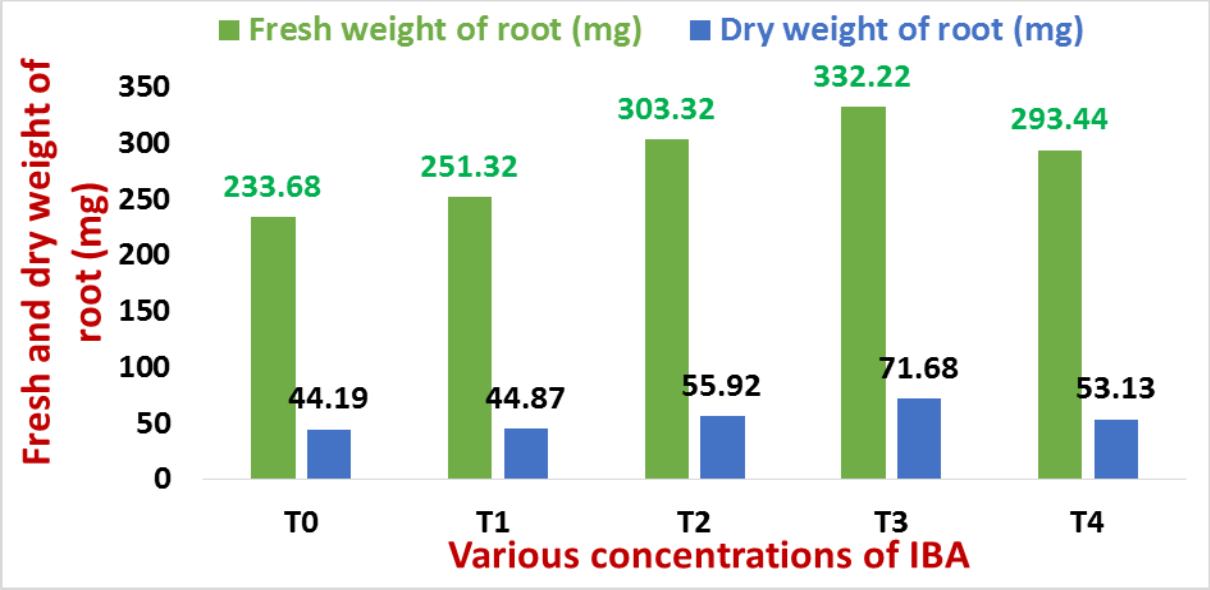
The highest fresh weight of root was measured in CIP 127 (313.19 mg) and the lowest was measured in CIP 117 (264.43 mg). Likewise, the highest dry weight of root was measured in CIP 127 (63.37 mg) and the lowest was measured in CIP 127 (48.96 mg) (**Table 27).**

Table no. 27: Response of genotypes on fresh weight (mg) and dry weight (mg) of root at *in vitro*

Genotypes	Fresh weight of root (mg)	Dry weight of root (mg)
CIP 106	270.77 b	49.55 b
CIP 117	264.43 c	48.96 c
CIP 127	313.19 a	63.37 a
CV (%)	1.38	3.93

4.3.4.b Effect of IBA on fresh weight (mg) and dry weight (mg) of root

Maximum fresh weight (332.22 mg) of experimented potato genotypes was recorded at MS media having 1.0 mg IBA /L and minimum (233.68 mg) at MS media lacking IBA. Likewise, the highest dry weight of root was found at 1.0 mg IBA / L treatment and the lowest at control treatment **(Figure 14).**

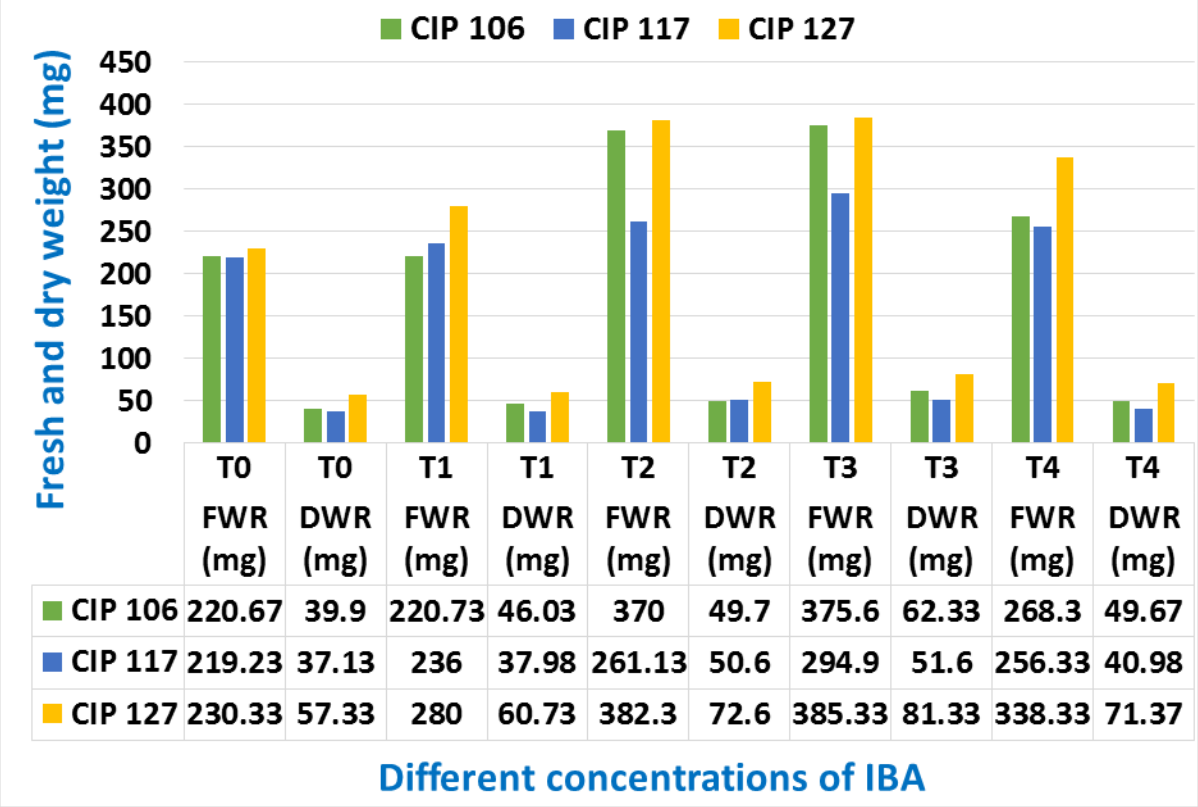


Where, T_0 = Control; **T_1** = 0.1 mg IBA / L; **T_2** = 0.5 mg IBA / L; **T_3** = 1.0 mg IBA / L; **T_4** = 1.5 mg IBA / L

Figure No. 14: Effect of IBA on fresh weight (mg) and dry weight (mg) of root

4.3.4.c Combined effect of genotypes and different level of IBA conc. on fresh weight of root (mg) and dry weight of root (mg)

The study revealed that maximum fresh weight of root was measured in CIP 127 (385.33 mg) at 1.0 mg IBA/ L and minimum was measured in CIP 117 (219.23 mg) at MS media free from IBA. Similarly, maximum dry weight of root was found in CIP 127 (81.33 mg) at 1.0 mg IBA/L and minimum in CIP 117 (37.13 mg) at MS media without supplementation of IBA **(Figure 15).**



	T0 FWR (mg)	T0 DWR (mg)	T1 FWR (mg)	T1 DWR (mg)	T2 FWR (mg)	T2 DWR (mg)	T3 FWR (mg)	T3 DWR (mg)	T4 FWR (mg)	T4 DWR (mg)
CIP 106	220.67	39.9	220.73	46.03	370	49.7	375.6	62.33	268.3	49.67
CIP 117	219.23	37.13	236	37.98	261.13	50.6	294.9	51.6	256.33	40.98
CIP 127	230.33	57.33	280	60.73	382.3	72.6	385.33	81.33	338.33	71.37

Where, T_0 = Control; **T_1** = 0.1 mg IBA / L; **T_2** = 0.5 mg IBA / L; **T_3** = 1.0 mg IBA / L; **T_4** = 1.5 mg IBA / L

Figure No. 15: Combined effect of genotypes and different level of IBA conc. on fresh weight (mg) and dry weight (mg) of root

CHAPTER V
SUMMARY AND CONCLUSION

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SUMMARY AND CONCLUSION

5.1 Summary

The experiment was conducted at the Tissue Culture Laboratory of Tuber Crop Research Center (TCRC), Bangladesh Agricultural Research Institute (BARI), Gazipur-1701, during the period from April 2015 to January 2016. Nine salt tolerant exotic potato genotypes viz. CIP102, CIP106, CIP111, CIP 117, CIP 120, CIP124, CIP127, CIP-136 and CIP-139 were used as experimental materials. Fresh, healthy and diseases free stem segments having 2-3 nodes, single node and root tip segments from *in vitro* regenerated plantlets of these genotypes were used as explants for salinity tolerance.

Basal MS (Murashige and skoog, 1962) media supplemented with different concentrations of NaCl viz. **T_0:** Control; **T_1:** 80 mM; **T_2:**100 mM; **T_3:** 120 mM; **T_4:** 140 mM; **T_5:** 160 mM were used for *in vitro* shoot and root bioassay. MS media supplemented with different concentrations of IBA viz. 0.0 (control), 0.1, 0.5, 1.0 and 1.5 mg /L were used for determining IBA effect on root development. The experiment was conducted at two factorial (Genotypes and Treatment) Completely Randomized Design (CRD) with 5 replications.

The study revealed that the days needed for shoot initiation and well development ranged from 6.37 to 12.40 days and 11.83 to 18.47 days respectively. While, days required for root initiation and well development ranged from 5.46 to 14.27 days and 9.97 to 18.03 days respectively. Basically, days required for shoot and root initiation was increased gradually with the increased level of salinity for all potato genotypes.

In respect of plant height, CIP 139 showed the greatest tolerance (9.67 cm) up to 160 mM salinity level followed by CIP 102 (9.45 cm) and CIP 124 (9.43 cm) at same salinity level. On the other hand, CIP 106 emerged as the most sensitive genotype up to 100 mM salinity level with 8.50 cm tall plantlets followed by CIP 136 (9.50 cm) at same salinity level. CIP 127, CIP 111 and CIP 117 were found vigorous up to 140 mM salinity level with 9.0 cm, 8.51 cm and 8.10 cm plant height respectively.

The study revealed that all the experimented genotypes were able to produce sufficient leaves up to 160 mM salinity level where CIP 139 produced maximum leaves (13.60) at 160 mM salinity level followed by CIP 127 (12.50), CIP 117 (10.50), CIP 111 (10.50), CIP 106 (9.73), CIP 102 (9.73), CIP 124 (9.50) and CIP 136 (9.47) at same level of NaCl stress.

In terms of nodes number, CIP 139, CIP 102, CIP 117, CIP 124, CIP 127 and CIP 111 produced sufficient nodes viz. 9.50, 9.43, 9.43, 9.40, 8.50 and 8.43 respectively up to 160 mM salinity level. On the other hand, CIP 106 was found most susceptible to salinity up to

100 mM salinity level with 9.37 nodes followed by CIP 136 (8.50) up to 120 mM salinity level.

In case of root length, CIP 102, CIP 136, CIP 139, CIP 127 and CIP 106 were found tolerant up to 160 mM salinity level with 9.50, 9.30, 6.50, 6.50 and 5.00 cm root length respectively. On the other hand, CIP 117, 124 and CIP 111 revealed their highest tolerance up to 100 mM salinity level with 4.50, 4.30 and 3.50 cm root length respectively.

Considering both primary and secondary root, CIP 139 and CIP 106 were found tolerant up to 160 mM salinity level with 8.00 and 7.80 total root number respectively. CIP 111, CIP 124, CIP 127 and CIP 117 showed well tolerance up to 140 mM salinity level with 9.0, 9.0, 8.0 and 7.0 total root number respectively. On the other hand, CIP 136 and CIP 102 performed better up to 120 mM salinity level with 8.0 and 6.30 total root number respectively.

The NaCl in MS media caused drastic effect on fresh weight of shoot measuring maximum (783.5 mg) in CIP 127 at MS media free of salt and minimum (492.5 mg) in CIP 117 at 160 mM salinity level. Likewise, Maximum dry weight of shoot (205.0 mg) was measured in CIP 127 at control and minimum (105.0 mg) in CIP 117 at 160 mM salinity level.

Fresh weight of root of *in vitro* produced plant was severely reduced with the increase of salinity where the highest fresh weight of root (370.0 mg) was measured in CIP 139 at MS media free from salt and the lowest (200.0 mg) in CIP 111 at 160 mM salt stress. Similarly, the highest (98.50 mg) dry weight of root was found in CIP 139 at control and the lowest (40.43 mg) in CIP 111 at 160 mM NaCl stress.

In vitro root bioassay also revealed the highest salinity tolerance of CIP 139 (50.55 %) up to 160 mM NaCl, CIP 127 (51.57 %) and CIP 102 (51.54 %) up to 140 mM NaCl in MS media for relative growth of their root tip segments in comparison with control. like *in vitro* shoot bioassay, root tip of CIP 106 showed tolerance (55.06 %) up to 120 mM salinity level and CIP 120 was found as the lowest tolerant (54.65 %) up to 120 mM salinity level for relative growth of root comparing to control.

Among the different concentrations of IBA, 1.0 mg / L was found best for root initiation (6.57 days), well-developed root (10. 48 days), length of root (7.21 cm) and fresh weight of root (332.22 mg) while 0.5 mg/ L was found best for total root number (18.27). It was noticed that, there was no significant differences in 0.5 mg/L and 1.0 mg/L IBA for *in vitro* root induction and development in the experimented CIP potato genotypes.

5.2 Conclusion

From the above result it is concluded that-

1. CIP 139 was found as the most salt tolerant genotype showing excellent performance up to 160 mM (14.61 dSm^{-1}) salinity level at *in vitro* study.

2. CIP 127, CIP 102 and CIP 124 performed better up to 140 mM (12.78 dSm^{-1}) salinity level at *in vitro* condition.

3. CIP 106 showed minimum salinity tolerance up to120 mM NaCl (10.96 ds/m) followed by CIP 136, CIP 117 and CIP 111 at the same salinity level at *in vitro* condition.

4. *In vitro* root bioassay also revealed the highest salinity tolerance of CIP 139 up to 160 mM NaCl, CIP 127 and CIP 102 up to 140 mM NaCl and CIP 120 up to 120 mM salinity level.

5. Considering both the results of *in vitro* shoot and root bioassay, CIP 139 can be considered as the most effective potato genotype for saline belt areas of Bangladesh as well as CIP 127, CIP 102 and CIP 124 can also be chosen for the same purpose.

6. MS media supplemented with 1.0 mg/L IBA showed best performance in root induction and development in CIP potato genotypes.

7. A simple and reliable technique for micropropagation of CIP Potato genotypes is developed.

8. It can be expected that using this protocol a new chapter will be opened for salt tolerant potato cultivars identification and their large scale micropropagation for the better use of land in southern belt of Bangladesh to enhance food security.

CHAPTER VI
RECOMMENDATION

CHAPTER VI
RECOMMENDATIONS

Recommendations:

1. Further study may be conducted with more exotic (CIP) potato genotypes, BARI released potato varieties and indigenous potato varieties.

2. Higher concentrations of NaCl especially more than 160 mM in MS media may be used for *in vitro* shoot and root bioassay.

3. The more experiment may be conducted with root tip segment for salinity tolerance at *in vitro*.

4. Combination of different growth regulators with or without IBA can be used for *in vitro* root and shoot development.

CHAPTER VII

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CHAPTER VIII

APPENDICES

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Appendix 1. Compositions and concentrations used for the preparation of MS medium (Murashige and Skoog, 1962).

Components	Concentration (mg/L)
Macronutrients	
KNO_3	1900
NH_4NO_3	1650
$MgSO_4 . 7H_2O$	370
$CaCl_2 . 2H_2O$	440
KH_2PO_4	170
Micronutrients	
$MnSO_4 . 4H_2O$	22.3
H_3BO_3	6.2
$ZnSO_4 . 7H_2O$	8.6
KI	0.83
$Na_2MoO_4 . 2H_2O$	0.25
$CoCl_2 . 6H_2O$	0.025
$CuSO_4 . 5H_2O$	0.025
Iron Source	
$Fe_2SO_4 . 7H_2O$	27.8
Na_2 EDTA. $2H_2O$	37.5
Organic nutrients	
Myo-inositol	100
Glycine	2.0
Nicotinic acid	0.5
Pyridoxine HCl	0.5
Thiamine HCl	0.1
Sucrose	3000.00
Agar	8000.00

Appendix 2. Analysis of variance on days to shoot initiation, well-developed shoot, root initiation and well-developed root

Source of variation	Degrees of freedom	Mean square			
		Days to shoot initiation	Days to well-developed shoot	Days to root initiation	Days to well-developed root
Potato genotypes (A)	7	14.18*	14.50*	11.357*	13.78*
Salinity level (B)	5	42.40*	48.86*	92.48*	87.42*
Interaction (A×B)	35	2.14*	2.43*	1.76*	1.20*
Error	96	0.07	0.07	0.159	0.22

* Significant at 0.05 level of probability

Appendix 3. Analysis of variance on plant height (cm), number of nodes / plant, number of leaves / plant, length of root / plant and number of roots / plant

Source of variation	Degrees of freedom	Mean square				
		Plant height (cm)	Number of nodes / plant	Number of leaves / plant	Length of root / plant	Number of roots / plant
Potato genotypes (A)	7	36.139*	18.741*	34.774*	189.493*	101.27*
Salinity level (B)	5	83.313*	21.073*	36.538*	23.004*	117.84*
Interaction (A×B)	35	7.468*	6.429*	14.965*	3.868*	9.08 *
Error	96	0.279	0.434	0.259	0.287	0.146

* Significant at 0.05 level of probability

Appendix 4. Analysis of variance on fresh weight of shoot (mg), dry weight of shoot (mg), fresh weight of root (mg) and dry weight of root (mg)

Source of variation	Degrees of freedom	Mean square			
		Fresh weight of shoot (mg)	Dry weight of shoot (mg)	Fresh weight of root (mg)	Dry weight of root (mg)
Potato genotypes (A)	7	22531.68*	3358.68*	7572.176*	2498.186*
Salinity level (B)	5	9535.93*	1855.989*	11040.973*	630.917*
Interaction (A×B)	35	11855.11 *	682.016*	4547.884*	396.280 *
Error	96	4.705	16.905	82.870	0.455

* Significant at 0.05 level of probability

Appendix 5. Analysis of variance on actual root extension (length) (cm) and relative growth (mm) of root

Source of variation	Degrees of freedom	Mean square	
		Actual root length (mm)	Relative root length (mm)
Potato genotypes (A)	4	246.09*	1079.54*
Salinity level (B)	5	4475.23*	6541.91*
Interaction (A×B)	20	163.52*	213.75*
Error	60	6.28	8.18

* Significant at 0.05 level of probability

Appendix 6. Analysis of variance on days to root initiation, days to well-developed root, length of root / plant, number of roots / plant, fresh weight of root (mg) and dry weight of root (mg) under different conc. of IBA

Source of variation	Degrees of freedom	Mean square					
		Days to root initiation	Days to well-developed root	Length of root / plant	Number of roots / plant	Fresh weight of root (mg)	Dry weight of root (mg)
Potato genotypes (A)	2	0.78907*	3.30022*	47.9361*	11.7287*	10544.8*	998.25*
IBA conc. (B)	4	7.81427*	5.14922*	8.7248*	86.575*	14356.9*	1117.15*
Interaction (A×B)	8	0.71090*	0.41939*	6.1095*	27.4034*	20528.6*	146.41*
Error	30	0.04050	0.03039	0.0218	0.3720	15.3	4.50

* Significant at 0.05 level of probability